

MODIFICATION OF ABNORMAL GAMETIC RATIOS IN DROSOPHILA.
II. EVIDENCE FOR A MARKED SHIFT IN GAMETIC RATIOS
IN EARLY VS. LATER SPERM BATCHES FROM A-TYPE
BAR-STONE TRANSLOCATION MALES¹

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FOLLOWING the finding of NOVITSKI and I. SANDLER (1957) that complementary gametic types from *Drosophila* males carrying the Bar translocation of Stone, T(1;4)*B*^s, are recovered with grossly unequal frequencies, it was reported by ZIMMERING (1960) that such inequalities were subject to considerable variation. It was shown that otherwise ordinary Y chromosomes and major autosomes derived from different common laboratory stocks were responsible for the variation, for in the presence of A ("abnormal ratios") Y autosome combinations marked inequalities are observed, while in the presence of E ("equal ratios") Y autosome combinations, the aberrant ratios tend to disappear. Males exhibiting the former kind of behavior were designated as the A type, and those exhibiting the latter kind as the E type.

The present paper concerns itself with evidence of an "age effect," in that while the first sperm released by young Bar-Stone males of the A type give clear evidence of aberrant ratios, those released later by the same males show more nearly equal ratios.

MATERIALS AND METHODS

It may be well at this point to describe the translocation components of the *B*^s male. Such a male possesses, as one pair of homologues, a fourth chromosome attached to a large distal piece of the X chromosome (*X*^D), and the normal minute-sized fourth chromosome (IV), and as another pair, the remaining proximal piece of the X chromosome (*X*^P) and the longer Y chromosome (Y). Four kinds of gametes are produced, namely, *X*^D + *X*^P, IV + Y, *X*^D + Y, and IV + *X*^P. The gametic types *X*^D + *X*^P, IV + Y, and IV + *X*^P are recovered from crosses with attached-X females usually carrying in addition a normal Y chromosome (*XX*/Y), and the types IV + Y, *X*^D + Y, and IV + *X*^P are recovered from crosses with attached-X females carrying *X*^P in place of the normal Y chromosome (*XX*/*X*^P). From the values representing the relative frequencies of recovery of the four gametic types, the over-all frequency of each translocation component

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may then be extracted and comparisons made between the recovery of X^D and IV, and between X^P and Y.

All B^s males employed in the present experiments were taken from a newly derived stock (June 1960) designated as an A type, since the males from this stock gave marked distortions in segregation ratios in each of two tests run previously. The results from each of these tests are given in tabular form and are compared with those from males taken from a stock designated as an E type.

	X^D	IV	X^P	Y	n
Test 1—A type	41.5	58.5	68.7	31.3	2086
Test 2—A type	40.5	59.5	67.0	33.0	2299
E type	52.6	47.4	49.1	50.9	3856

With reference to the results from the crosses involving males from the A-type stock, the following points are worth mentioning. The initial tests were made in a manner identical to previous tests; that is, by mating, on a small scale, a single male with a single tester female, and transferring the parents to fresh food at three-day intervals through the ninth day. Now, in these experiments, as in those conducted previously, while the *grand total* of offspring was sufficiently large to provide a fairly firm basis for calculating over-all frequency values, comparisons of frequency values *between* broods gave results of doubtful significance since the number of flies scored in each brood was small. On the other hand, a basis for comparison of gametic frequencies in different broods was provided from larger scale experiments to be described below.

Results from the first experiment: In the two experiments to be described, the males were a maximum of 24 hours in age when mated and the tester females were about 3–4 days old. The experiments were carried out at a temperature of about 25°C.

In the first experiment, single males from the A-type stock were mated each with three tester females. The parents were then simply transferred to fresh food every three days to make up three 3-day broods. The results from this experiment, as shown in Table 1, are only those obtained from males which produced offspring in all three broods. The data from the first three-day brood give convincing evidence of highly abnormal gametic ratios, apparent from the fact that IV is recovered about 50 percent more frequently than is X^D , and X^P is recovered about 100 percent more frequently than is Y. However, the data from the second and third broods are clearly out of line with those from the first. It is evident that there was a sizeable change toward a more nearly equal recovery of the gametic types $X^D + Y$ and $IV + X^P$ from the first to the third brood, the difference in recovery rate between these two classes dropping from an initial 172 percent in the first brood to only 33 percent in the third brood. This change is reflected in the over-all recovery rate of the translocation components as evidenced by the decrease in the difference between X^D and IV from 44 percent in the first brood to virtually no difference in the third, and by the decrease in the difference between X^P and Y from 100 percent in the first brood to 34 percent in the third. Although changes in the relative frequencies of the gametic types

TABLE 1

Progeny from three 3-day broods derived from matings of single males from the A-type B^S stock, approximately 12–24 hours old, with three tester females

	X ^D + X ^P	IV + Y	X ^D + Y	IV + X ^P	
Brood 1	400	287	(235)	641	
	(450)	323	245	668	
	850	610	480	1309	
	26.2	18.8	14.8	40.3	
Brood 2*	1071	861	(814)	1293	
	(709)	570	597	948	
	1780	1431	1411	2241	
	25.9	20.8	20.5	32.7	
Brood 3	780	578	(659)	880	
	(451)	334	384	513	
	1231	912	1043	1393	
	26.9	19.9	22.8	30.4	
Over-all frequency of recovery of each translocation component					
Brood	X ^D	IV	X ^P	Y	n
1	41.0	59.1	66.5	33.6	3249
2	46.4	53.5	58.6	41.3	6863
3	49.7	50.3	57.3	42.7	4579

* Broods 2 and 3 were made up by simply transferring the parents successively from Brood 1. The data are only from males which produced progeny in all broods.

X^D + X^P and IV + Y are not apparent in these results, this is not surprising since these two classes, even in the first brood, were recovered in frequencies so nearly equal that changes of a few percent tending to make them somewhat more equal would probably by chance alone not be demonstrable in this experiment.

Results from the second experiment: More precise information about this apparent gametic shift came from the results of the following experiment. Single males were supplied with three tester females every two days for a period of six days, thus making up three 2-day broods. After separation from the male, the females were transferred to fresh food and allowed to oviposit for an additional 3–4 days. The results from this experiment as shown in Table 2 were obtained only from males which gave offspring in all three broods. The following features of these results may be pointed out. (1) The gametic ratios are highly aberrant in the first brood, but (2) while the difference in the recovery rate between the gametic type X^D + Y and IV + X^P is about 190 percent in the first brood, it drops to only seven percent in the third. The somewhat more precipitous drop found in this experiment as compared with that found in the first is probably related to the breeding procedure, with that employed in this experiment permitting a greater precision in the delineation of successive sperm batches.

Briefly, then, each experiment has the following characteristics, namely, that while there is evidence of clearly aberrant gametic ratios in the first brood, there is a distinct trend toward more nearly equal ratios in subsequent broods.

TABLE 2

Progeny from three 2-day broods derived from matings of single males from the A-type B^S stock, approximately 12-24 hours old, with three tester females. Each brood was made up by crossing such males with three virgin tester females. The results are only from males which produced progeny in all broods

	X ^P + X ^P	IV + Y	X ^P + Y	IV + X ^P
Brood 1	370	249	(182)	528
	(190)	134	110	320
	569	383	292	848
	27.2	18.3	14.0	40.5
Brood 2	1277	1060	(1218)	1534
	(779)	647	707	890
	2056	1707	1925	2424
	25.3	21.0	23.7	29.9
Brood 3	1358	1199	(1318)	1409
	(717)	633	710	759
	2075	1832	2028	2168
	25.6	22.6	25.0	26.8

Over-all frequency of recovery of each translocation component					
Brood	X ^P	IV	X ^P	Y	n
1	41.2	58.8	67.7	32.3	2092
2	49.0	50.9	55.2	44.7	8112
3	50.6	49.4	52.4	47.6	8103

The question of heterogeneity in the population of parental males employed: The argument could be made that while on the whole the males employed in these experiments gave markedly abnormal gametic ratios in the first brood, some proportion of them were in fact of the E type. If the A-type males were, for example, less fertile in the long run and left considerably fewer offspring in the second and third broods than did the E-type males, this would result in a swamping of the A effect and give rise to what appeared to be a rather drastic shift in gametic ratios toward normality.

In partial answer to this argument, the over-all frequency of recovery of the translocation components from the first brood gives evidence of markedly abnormal ratios, and may be shown to be in at least fair agreement with those from previously published experiments which gave the *most* aberrant ratios thus far obtained from B^S males. This is illustrated in a comparison with the data from NOVITSKI and SANDLER (1957).

	X ^P	IV	X ^P	Y
Expt. I, Brood 1	41.0	59.1	66.5	33.6
Expt. II, Brood 1	41.2	58.8	67.7	32.3
NOVITSKI and SANDLER	38.1	61.9	69.3	30.7

Thus, while there is an indication of somewhat more highly distorted ratios from the experiments of NOVITSKI and SANDLER, it would seem justified to suggest that

no large proportion of the males in the present experiments would be suspected of being of the E type.

As another approach to this question, the following analysis was carried out. Since the complementary gametic types $X^p + Y$ and $IV + X^p$ show the most marked shifts in their relative frequencies and thus serve as a sensitive measure of the extent of the shift from brood to brood, the ratio of these gametic types was calculated from the results of NOVITSKI and SANDLER (1957). The value of this ratio turns out to be approximately 27 percent. Consequently, on the basis of the data from the crosses of males with XX/X^p females (from which these two types are recoverable) a selection was made of cultures where the value of this ratio in the first brood did not exceed 27 percent. Then, the value of the ratio was calculated for the second and third broods and compared with the corresponding ones derived from the offspring of all males used in these crosses. These results are shown from the selected cultures (s.c.) and from the population of males as a whole (p.w.).

Expt. I	Brood 1	Brood 2	Brood 3
s.c.	44/268 = 16.4%	220/398 = 55.3%	151/223 = 67.7%
p.w.	188/366 = 36.6%	481/764 = 63.0%	318/425 = 74.8%
Expt. II			
s.c.	19/146 = 13.0%	228/303 = 75.2%	273/316 = 86.4%
p.w.	110/320 = 34.4%	709/892 = 79.5%	712/761 = 93.6%

Inspection of the table shows that the selected cultures in the first experiment gave a value of 16.4 percent in the first brood, as compared with 36.6 percent for the population as a whole, while the values from the second and third broods from the selected cultures were in rough agreement with those from the population as a whole—the comparisons being 55.3 percent (s.c.) *vs.* 63.0 percent (p.w.), and 67.7 percent (s.c.) *vs.* 74.8 percent (p.w.). The selected cultures in the second experiment gave a value of 13.0 percent in the first brood as compared with 34.4 percent for the population as a whole, and those from the second and third broods from the selected cultures were also in rough agreement with those from the population as a whole—the comparisons being 75.2 percent (s.c.) *vs.* 79.5 percent (p.w.) and 86.4 percent (s.c.) *vs.* 93.6 percent (p.w.). It may be suggested, therefore, that there does appear to be a real change in gametic ratios from early to later broods since the values in the later broods from the selected cultures were not only markedly higher than those in the first, but were higher than the value from the first brood based on the population as a whole, and were in at least rough agreement with the values derived from the population as a whole in the later broods.

As was indicated previously, it appears also that some males, initially used, may indeed have been of the E type since not only is there a consistently higher value in the second and third broods from the population as a whole as compared with those from the selected cultures, but the values in the first broods of 36.6 percent and 34.4 percent for the first and second experiments, respectively, are

higher than the value calculated from the data of NOVITSKI and SANDLER, namely, 27 percent.

The probable major source of the E-type males: Since the second experiment was conducted in a considerably more precise fashion than the first in that shorter brood periods were analyzed, and the males were supplied with fresh females for each brood, only the data from the second experiment were analyzed with the view of determining the major source of the E-type males. The following breakdown of the data provides a basis for a reasonably satisfactory answer to this question. Each of the 40 males employed in the crosses with XX/X^P females was characterized according to the total number of offspring representing the recovery of the gametic types $X^D + Y$ and $IV + X^P$ which were recovered in all three broods combined, and then simply arranged in ascending order according to this criterion. The males were then divided arbitrarily into two groups, one group representing that fraction of males which individually gave the fewer number of offspring, the other fraction the larger number of offspring. The kinds of divisions made were as follows: 1/2:1/2, 3/4:1/4, 4/5:1/5, 7/8:1/8, and 9/10:1/10. This breakdown is given in Table 3 with the associated values of the ratio $X^D + Y/IV + X^P$.

Simple inspection of the results in this table is sufficient to show that the ratio remained remarkably constant at a value of about 30 percent (not far out of line with the value of 27 percent discussed above), and was found ultimately to represent the average value for 36 of the 40 males used in this experiment. The remaining four males gave a high value of 71 percent in the first brood and probably represented the major source of the E-type males in this experiment. The ratio of the two gametic types for each of the three broods for the two fractions,

TABLE 3

Groupings of males from the second experiment based on the total number of offspring in all three broods representing the recovery of the gametic types $X^D + Y$ and $IV + X^P$, with the associated values of the ratio $X^D + Y/IV + X^P$

Proportions*	n	(1) $X^D + Y$	(2) $IV + X^P$	(1)/(2)
F 1/2	1105	32	104	.308
L 1/2	2399	78	216	.361
F 2/3	1711	54	182	.297
L 1/3	1793	56	138	.406
F 3/4	2184	61	205	.297
L 1/4	1320	49	115	.426
F 4/5	2428	71	226	.314
L 1/5	1076	39	94	.415
F 7/8	2809	76	265	.287
L 1/8	695	34	55	.620
F 9/10	2944	90	292	.308
L 1/10	560	20	28	.714

* F signifies that proportion of males yielding individually the fewer number of offspring, and L signifies that proportion giving the larger number of offspring.

i.e., 9/10 and 1/10, and those from the population as a whole are shown as follows.

9/10	90/292 = 30.8%	573/762 = 75.2%	565/654 = 86.4%
p.w.	110/320 = 34.4%	709/892 = 79.5%	712/761 = 93.6%
1/10	20/28 = 71.4%	134/128 = 105%	145/105 = 138%

It is clear that a pronounced change occurred in the gametic ratios from the first to the third broods in the 9/10 fraction, and that the extent of the change is in excellent agreement with that found from the highly selected males (s.c.) referred to above which gave a value of only 13 percent in the first brood. The suggestion is therefore justified that the small number of highly selected males (10 of 40) and those representing 9/10 (36 of 40) of all males analyzed came from a population of genetically similar males.

DISCUSSION

The following observations have been made: (1) sperm released by young B^S males of the A type are characterized by marked distortions in the segregation ratios of the members of homologous pairs of chromosomes, in that IV is recovered about 50 percent more frequently than X^D , and X^P is recovered about 100 percent more frequently than Y; while (2) sperm released 4–6 days later by the same males are characterized by a virtual absence of distorted ratios, in that X^D is recovered almost as frequently as IV, and Y is recovered almost as frequently as X^P . In a formal sense, then, disjunction appears to be more normal in those groups of spermatocytes which are destined to form sperm used by the male about 5–7 days after hatching (or somewhat earlier), than in those groups of spermatocytes which form sperm used by the male about 1–3 days after hatching. This may be the result of some physiological difference(s) between these groups of cells, or between the groups of cells antecedent to them, such that in one instance, the expression of the A-type genetic factors is strong, and in the other, relatively weak or virtually absent.

Other examples of rapid changes with age may be noted here. These include such cases as (1) the relationship between the age of the male and the frequency of spontaneous mutations as reported by MULLER (1945) where newly hatched *Drosophila* males gave rise to a proportion of mutant offspring 2–3 times as high as did the same males tested a week or more later, and (2) the precipitous drop in the frequency of crossing over in the *Drosophila* female in approximately the first six days, from an initial high value of about 60–80 percent above the mean on the first day to a value of about 20–40 percent below the mean on the sixth day (SCHULTZ and REDFIELD 1951).

Finally, some speculation may be made about the possible bearing of these results on the finding by NOVITSKI and L. SANDLER (1956) that the change in the secondary sex ratio in humans with change in the age of the parents is more closely correlated with increasing age of the father than that of the mother. They conjectured that the simplest interpretation to account for this would be that the

relative frequencies of X- and Y-bearing sperm shift with the increase in age of the father so that the greater probability of younger fathers having sons is reversed in the case of older fathers. If the suggestion of a shift in gametic ratios as is indicated by the B^s data has validity for human populations, this finding would lend support to that idea, since at least superficially, the shorter homologue in each case (the Y chromosome in man, and for example, X^p in the B^s case) tends to be recovered more frequently from younger males than are the longer homologues (the X chromosome in man, and Y in the B^s case) while from older males both homologues tend to be recovered with more nearly equal frequencies.

SUMMARY

Males of *Drosophila melanogaster* which carry the Bar translocation of Stone, $T(1;4)B^s$, possess as one pair of homologues a fourth chromosome attached to a large distal piece of the X chromosome (X^p) and the normal minute-sized fourth chromosome (IV), and as another pair, the remaining proximal piece of the X chromosome (X^p) and the longer Y chromosome. Evidence is presented which suggests that (1) sperm released by young B^s males of the A type are characterized by marked distortions in the segregation ratios, in that IV is recovered about 50 percent more frequently than X^p , and X^p is recovered about 100 percent more frequently than Y; while (2) sperm released 4–6 days later by the same male (provided the males are permitted to expend sperm in the meanwhile) are characterized by a virtual absence of distorted ratios in that all four translocation components are recovered with approximately equal frequencies. Conjecture is made that this may be the result of physiological differences between the groups of cells destined to give rise to the different sperm batches. Some speculation is made as to the possible bearing of the present results on the suggestion by NOVITSKI and SANDLER that that portion of the change in the secondary sex ratio in humans related to the change in parental age could be most simply interpreted as a shift in the relative frequencies of X- and Y-bearing sperm with a change in the age of the father.

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