THE MEIOTIC ORIGIN OF SPONTANEOUS CROSSOVERS IN *DROSOPHILA ANANASSAE* MALES

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THE immediate fitness of a species depends on a certain uniformity of genotype, whereas long term survival depends on production of adequate genetic variability. The over-all recombination rate must therefore be maintained at an optimum (BODMER and PARSONS 1962). This adjustment has been attained in the genus *Drosophila* by abolition of crossing over in males (DARLINGTON 1939). *D. ananassae* however, stands apart from all other species of the genus studied so far in having spontaneous male crossing over in some strains. This phenomenon, reported thirty years ago (MORIWAKI 1937, KIKKAWA 1938), still needs attention with respect to the nature and distribution of the factors responsible for the event and especially to the precise identification of the cell stages in which the spontaneous exchanges occur. The cellular origin of these crossovers has its own evolutionary importance since it is now known that a single exchange in a pre-definitive spermatogonium undergoing cambial division can liberate a very large, even infinite, number of new variants in the progeny, whereas a similar exchange in a spermatocyte can at the most give rise to two crossovers. Secondly, if it can be established that gonial crossing over is of general occurrence in the genus *Drosophila*, it will help to explain the tolerance of high mutation rates found in the group, since lethal alleles will be eliminated due to their homozygosis in gonial cells.

The first attempt to identify the cell type releasing the spontaneous crossovers in the males of *D. ananassae* was made by MUKHERJEE (unpublished), using a third chromosome double recessive stock, *px pc*. Inequality of the reciprocal recombinants and wide individual variation in recombination values led him to conclude that crossing over occurs in the apical spermatogonial stage. RAY-CHAUDHURI and KALE (1966) on the other hand, observed equality of complementary classes and a random distribution of exchanges in the tested region and are of the opinion that the recombinants originate in the spermatocytes. However, according to WHITTINGHILL (personal communication), these data contain extensive and strong evidence for spermatogonial crossing over. More precise experiments and correlated cytological studies were therefore performed with larger samples of males using newly collected wild strains and daily broods. The results are presented in this paper.

MATERIALS AND METHODS

The only available suitable mutant culture with two marked regions in this species is a second

chromosome recessive stock, *cu b se* (*cu*, curled wings; *b*, black body; *se*, sepia eye), prepared and described by RAY-CHAUDHURI *et al.* (1959). The wild strains have been collected from distant places in India (a6, Calcutta; a7, Port Blair; a9, Bombay; a11, Nagpur). The strain a6 was used for earlier studies on male crossing over and has been recently inbred by full sib matings for 15 generations. The other three strains are recent collections.

Triple recessive virgins were crossed to wild males and the heterozygous males were collected within eight hrs after eclosion to avoid age variation. A sample of 50 males, heterozygous for the chromosomes of the four wild stocks (*cu b se*/wild-a6, a7, a9, a11) was collected and was aged for nearly 36 hr. The males were then mated individually for 13 days at a rate of five to eight virgins per day. Thirteen 1-day broods were thus scored.

For salivary chromosome studies recessive virgins were individually mated to a single wild male. 10–15 such pairs were examined for each of the four wild strains taking five larvae from each pair. All slides were permanent acetocarmine preparations. Only second chromosomes were examined for the presence of heterozygous inversions.

RESULTS

The four strains differ significantly in the total recombination frequency ($P < 0.005$). In region I, the crossover values from the combination *cu b se*/a9 are much higher than those from the other strains. Similar observations have been made from scores of the female recombination data belonging to these four strains. Presence of inversion heterozygosity was, therefore, suspected and the salivary chromosomes of the four combinations were examined.

These observations revealed that three out of the four combinations, (*cu b se*/a6, *cu b se*/a7 and *cu b se*/a11), carry a heterozygous inversion in the left arm of the second chromosome. This inversion has been described from an Indian population of *D. ananassae* by RAY-CHAUDHURI and JHA (1966) and is probably identical with *Inv II L* reported by KIKKAWA (1938) from Japan. This is longer than any other inversion reported so far in this species with its distal break just near the tip, giving it a subterminal appearance. The inverted segment covers more than half of the left arm. Recombination values from females heterozygous for different wild strains suggest that it suppresses crossing over very strongly in region I (between *cu* and *b*) but does not affect region II. Almost all preparations from these three combinations were heterozygous for this inversion. However, all the 90 larvae from the 20 pair matings belonging to *cu b se*/a9 were homozygous for this arrangement. These individuals are thus free from any heterozygous inversion in the second chromosomes. Map distances calculated from the females *cu b se*/a9, testcrossed to recessive males are: between *cu* and *b* approximately 40 map units and between *b* and *se* nearly 46 map units. This map is thus more reliable than that of RAY-CHAUDHURI *et al.* (1959), where the strain a6 was used which then carried the arrangements for the second chromosome both in heterozygous condition.

Extensive scores from 179 individual males, heterozygous for *cu b se* and the four wild strains in the thirteen daily broods are kept on file with the editor of this journal. These data are analyzed statistically for the balance of complementary types, interstrain and intrastrain variation in recombination frequency, homogeneity of recombination in the two regions and for the effect of parental age on crossing over in the males.

Homogeneity in the recombination values between the thirteen broods was tested by means of χ^2 . Variation is highly significant in all four experiments (Table 1). In order to see if there is any trend in this variation, the linear regression of the crossing over values (in percent) on the broods was tried. The coefficients are negative and the regression is significant for three of the stocks. In stock a11, however, the regression is not significant suggesting irregular variation. Therefore, regression of the percentage of positive males on the broods was fitted. This regression is significant for the stock a11 also. There is thus a decrease in the number of crossover males in the later broods. In other words, the crossover values and the percentage of positive males in a sample decreases with increase in age of the tested males.

The total recombination frequency in the four strains appears very variable. Region I is free from the inversion in the stock a9, while it is present in the three others. However, the crossover production of these three strains is widely different (a6-43/36,232; a7-16/28,845; a11-43/60,105). Similar variation is apparent in the second region which is inversion free in all four stocks (a6-92/36,232; a7-36/28,845; a9-204/37,784; a11-132/60,105). The differences in total recombination values are quite wide and may result from differences in the genetic constitutions of the strains.

The balance of reciprocal recombinants produced by individual males in separate broods was studied by FISHER's method used by HINTON and WHITTINGHILL (1950). Instead of calculating the probabilities on the basis of equality considered by these authors, the proportion observed in the data itself was used to account for the viability differences in the two complementary types. Probabilities for obtaining the observed distributions of the reciprocal recombinants in each family in each brood were calculated and the respective $-\log_e$ values were referred. These $-\log_e$ values are additive and when multiplied by 2 give χ^2 values. The overall probabilities based on these χ^2 values are given in Table 2. Observations on the individual probabilities reveal that out of the 460 samples examined statistically for the balance of reciprocal classes, only three deviate from equality. One of the three distributions is 5:0, $P = 0.018$. The other two are 0:8, $P = 0.006$ and 9:12, $P = 0.016$. Because of the latter two unbalanced samples, the broods containing them also deviate from equality. The additive χ^2 values obtained from the rest of the 102 broods do not indicate inequality of the complementary cross-

TABLE 1

Relationship between the spontaneous recombination values recovered from males of D. ananassae, heterozygous for cu b se/wild and the thirteen laying periods

Strain	Homogeneity test		Regression of percent crossing over on broods			Regression of percent positive males on broods		
	χ^2	P<	b	t	P<	b	t	P<
a6	208.054	.005	-.125	-5.682	.001	-3.267	-3.594	.005
a7	100.084	.005	-.050	-2.083	.050-.100	-0.839	-2.387	.050
a9	193.635	.005	-.207	-4.224	.005	-2.061	-1.922	.050-.100
a11	67.626	.005	-.018	-1.385	.100-.200	-2.352	-5.036	.001

TABLE 2

χ^2 analysis for testing the equality of spontaneous complementary crossovers within the testcross progeny of individual males of *D. ananassae*, heterozygous for *cu b se/wild*

Strain Brood	$-\log_e$	^{a6} P>	$-\log_e$	^{a7} P>	$-\log_e$	^{a9} P>	$-\log_e$	^{a11} P>
Region I								
1	0.543	.50	1.165	.25	13.667	.25	1.399	.50
2	3.752	.50	0.849	.25	17.712	.30	5.715	.75
3	5.718	.75	1.165	.25	17.002	.50	6.068	.75
4	3.643	.50	0.000	.99	14.774	.70	5.042	.50
5	3.042	.50	0.375	.50	9.552	.50	1.701	.25
6	0.870	.25	1.539	.50	5.962	.50	0.583	.50
7	0.000	.99	1.599	.75	2.426	.50	0.000	.99
8	2.610	.50	0.000	.99	0.000	.99	1.521	.50
9	0.000	.99	0.375	.50	2.786	.10	0.000	.99
10	0.870	.25	0.000	.99	9.114	.75	0.000	.99
11	0.000	.99	0.000	.99	5.846	.75	0.000	.99
12	1.413	.50	1.108	.25	5.086	.25	0.000	.99
13	2.499	.75	0.000	.99	1.605	.50	2.215	.50
Region II								
1	5.093	.50	2.479	.25	7.596	.50	6.787	.25
2	8.109	.50	1.911	.50	8.392	.75	5.731	.75
3	9.780	.50	0.697	.25	9.097	.75	4.116	.75
4	5.929	.50	0.750	.25	18.034	.30	3.535	.50
5	8.244	.75	1.387	.50	12.478	.50	6.661	.75
6	3.349	.75	3.585	.50	8.693	.50	2.025	.50
7	0.785	.25	2.425	.50	3.302	.50	6.484	.50
8	0.610	.50	0.000	.99	1.200	.50	12.338	<.02
9	0.702	.25	0.000	.99	7.615	.50	1.510	.50
10	0.702	.25	0.750	.25	9.772	.50	2.001	.25
11	0.785	.25	0.000	.99	8.022	.10	2.087	.50
12	2.004	.25	0.750	.25	8.293	.50	0.755	.25
13	2.964	.25	0.000	.99	6.394	.50	4.890	<.05

The $-\log_e$ values are obtained by adding three values from individual males of the respective brood.

over classes. The statistical analysis thus clearly demonstrates the production of equal reciprocal recombinants by almost all individuals with only two exceptions.

The statistical significance of the variation in recombination frequencies from individual males obtained separately in the thirteen broods was tested by fitting Poisson distributions, because of the low incidence of success in most families. The Poisson distribution is a limiting form of the binomial with low probabilities and therefore this method can be applied in such cases. This application has further been strengthened by KONDO's (1961) theoretical considerations. According to this theory, the number of observed radiation induced mutant clusters should not deviate from the Poisson series if the mutants are of independent origin. In the present data all samples from *cu b se/a6* follow a Poisson distribution (Tables 3, 4 and 5). A few broods in other experiments are however non-

TABLE 3

Spontaneous crossover values in the thirteen daily broods of males heterozygous for cu b se/a6 and a7, individually testcrossed to cu b se females of D. ananassae
Fitted Poisson distribution. Test of significance

Strain Brood	Number of males with indicated number of crossovers per male a6							a7							P>	
	0	1	2	3	4	P>	0	1	2	3	4	5	6	7		8
Region I																
1	14	1	..	..	..	.90	17	1	..	..	..	..	..	..	..	.95
2	9	2	2	1	..	.25	35	..	1	..	..	..	..	..	..	.25
3	16	8	1	..	..	.75	29	1	..	..	..	..	..	..	..	.95
4	29	2	1	1	..	.10	34	..	..	..	..	..	..	..	..	.99
5	28	3	1	..	..	.75	27	1	..	..	..	..	..	..	..	.95
6	31	1	..	..	..	.95	25	2	..	..	..	..	..	..	..	.90
7	29	..	..	..	..	.99	24	2	1	..	..	..	..	..	..	.50
8	25	3	..	..	..	.90	29	..	..	..	..	..	..	..	..	.99
9	38	..	..	..	..	.95	34	1	..	..	..	..	..	..	..	.95
10	39	1	..	..	..	.95	29	..	..	..	..	..	..	..	..	.99
11	28	..	..	..	..	.99	33	..	..	..	..	..	..	..	..	.99
12	37	2	..	..	..	.95	24	..	..	..	1	..	..	..	..	.25
13	33	4	..	..	..	.90	26	..	..	..	..	..	..	..	..	.99
Region II																
1	14	4	1	1	..	.50	17	..	1	..	..	..	1	..	..	<.05
2	9	5	4	..	..	.50	35	3	..	..	..	..	..	..	..	.90
3	16	4	5	2	1	.05	29	..	1	..	..	..	..	..	..	.25
4	29	6	1	..	..	.90	33	1	..	..	..	..	..	..	..	.95
5	28	6	4	1	..	.10	27	2	..	..	..	..	..	..	..	.90
6	31	4	..	1	..	.25	25	2	..	1	..	..	..	..	1	<.05
7	28	1	..	..	..	.95	24	2	..	1	..	..	..	..	..	.25
8	25	1	..	..	..	.95	29	..	..	..	..	..	..	..	..	.99
9	37	..	1	..	..	.25	35	..	..	..	..	..	..	..	..	.99
10	39	..	1	..	..	.25	29	..	..	..	..	..	..	..	..	.95
11	27	1	..	..	..	.95	33	..	..	..	..	..	..	..	..	.99
12	37	1	1	..	..	.50	24	1	..	..	..	..	..	..	..	.95
13	33	2	1	..	..	.75	26	..	..	..	..	..	..	..	..	.99
Total		62	24	7	1			20	4	2	1	0	1	0	1	

Poisson (2 broods of stock a7, 3 broods of a11 and 5 broods of a9). It can be noted that in stock a9 where the number of exchanges is highest, the number of broods deviating from the Poisson series is also high.

Observations on the size of recombinant samples recovered in various broods reveal that (see totals of Tables 3, 4 and 5) amongst the total number of 460 exchanges scored, 388 samples (84%) contain either single or two crossovers; 44 samples contain 3 or 4 recombinants and the remaining 28 contain more than 4 recombinants. The majority of the exchanges thus produced solitary crossovers.

Total recombination frequency of individual males with more than five recombinants in their progeny was tested for homogeneity of crossover production in the two possible regions (Table 6). Of the 20 high males belonging to the strains a6, a7 and a11, eleven males deviate from such equality. All these eleven

TABLE 4

Spontaneous crossover values in the thirteen daily broods of males heterozygous for cu b se/a9, individually testcrossed to cu b se females of D. ananassae
Fitted Poisson distribution. Test of significance

Brood	0	1	Number of males with indicated number of crossovers per male															P>
Region I																		
1	12	3	4	1	1	1	..	1	..	..	2	..	..	..	..	..	<.005	
2	16	6	5	2	2	..	1	..	..	..	..	..	..	..	..	1	<.005	
3	15	10	2	3	1	1	..	1	1	..	..	..	..	..	..	..	<.025	
4	12	8	6	1	1	..	..	1	1	..	..	..	..	..	..	..	.10	
5	22	7	1	1	2	..	..	..	..	..	..	..	..	..	..	..	.25	
6	21	5	2	..	1	..	..	..	..	..	..	..	..	..	..	..	.25	
7	23	2	..	..	..	..	1	..	..	..	..	..	..	..	..	..	.10	
8	15	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.99	
9	19	..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	.25	
10	18	8	3	..	..	..	..	..	..	..	..	..	1	..	..	..	.25	
11	16	5	3	..	..	..	..	..	..	..	..	..	..	..	..	..	.50	
12	19	3	..	..	..	1	..	..	1	..	..	..	..	..	..	..	<.02	
13	15	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	.10	
Region II																		
1	12	6	2	1	..	..	..	..	..	..	..	..	..	..	..	..	.75	
2	16	8	3	..	..	..	..	..	..	..	..	..	..	..	..	..	.95	
3	15	8	4	..	..	..	..	..	..	..	..	..	..	..	..	..	.75	
4	12	8	5	1	1	1	1	..	..	..	..	..	..	..	..	..	.10	
5	22	11	4	..	..	..	..	..	..	..	..	..	..	..	..	..	.95	
6	21	7	4	..	..	..	..	..	..	..	..	..	..	..	..	..	.25	
7	23	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	.50	
8	15	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	.90	
9	19	5	2	1	1	..	..	..	..	..	..	..	..	..	..	..	.25	
10	18	8	2	1	..	..	..	..	..	1	..	..	..	..	..	..	.25	
11	16	1	3	..	..	1	1	..	..	..	..	..	..	..	..	..	<.02	
12	19	4	4	1	..	..	..	..	..	..	..	..	..	..	..	..	.05	
13	15	6	2	..	..	..	..	..	..	..	..	..	..	..	..	..	.90	
Total	133	66	14	10	5	4	3	3	1	2	0	1	0	0	1			

males except one show a preference for region II. In strain a9, however, region I is preferred by 6 out of the 7 males deviating from equality. Thus most of the males tabulated in the Table 6 are high for both regions and those deviating from equality collectively prefer either one of the two regions depending on the strain to which they belong. In other words, none of the four strains has two types of males, some preferring region I and others region II.

In the first experiment of the present study, there is no individual variation in crossover values in any of the broods. The reciprocal recombinants are balanced in all tested males and most high males produced an equal number of crossovers in the two regions. This experiment thus shows no evidence in favour of dependence among crossovers. However, deviations from such exclusive evidence have been recorded in the other three experiments. Although broods with

TABLE 5

Spontaneous crossover values in the thirteen daily broods of males heterozygous for cu b se/a11, individually testcrossed to cu b se females of D. ananassae
Fitted Poisson distribution. Test of significance

Brood	0	Number of males with indicated					number of crossovers per male					10	11	P>
		1	2	3	4	5	6	7	8	9				
Region I														
1	32	2	..	..	..	..	..	..	..	..	..	..	..	.90
2	26	7	1	..	..	..	..	..	..	..	..	..	..	.90
3	24	8	1	..	..	..	..	..	..	..	..	..	..	.90
4	31	3	2	..	1	..	..	..	..	..	..	..	..	.05
5	29	1	..	1	..	..	..	..	..	..	..	..	..	.25
6	36	1	..	..	..	..	..	..	..	..	..	..	..	.95
7	32	..	..	..	..	..	..	..	..	..	..	..	..	.99
8	31	1	1	..	..	..	..	..	..	..	..	..	..	.50
9	35	..	..	..	..	..	..	..	..	..	..	..	..	.99
10	35	..	..	..	..	..	..	..	..	..	..	..	..	.99
11	34	..	..	..	..	..	..	..	..	..	..	..	..	.99
12	33	..	..	..	..	..	..	..	..	..	..	..	..	.99
13	32	3	..	..	..	..	..	..	..	..	..	..	..	.90
Region II														
1	32	4	1	2	..	..	..	..	..	..	..	..	..	.10
2	26	7	..	1	..	..	..	..	..	..	..	..	..	.95
3	24	3	3	..	..	..	..	..	..	..	..	..	..	.05
4	31	5	..	..	..	..	..	..	..	..	..	..	..	.75
5	29	5	2	2	..	..	..	..	..	..	..	..	..	.10
6	36	3	..	..	..	..	..	..	..	..	..	..	..	.90
7	32	3	1	..	1	..	..	..	2	..	..	..	..	<.005
8	31	2	1	..	1	..	..	..	1	..	..	1	..	<.005
9	35	2	..	..	..	..	..	..	..	..	..	..	..	.25
10	34	1	..	..	..	..	1	..	..	..	..	..	..	.05
11	33	2	1	..	..	..	..	..	..	..	..	..	..	.50
12	30	1	..	..	..	..	..	..	..	..	..	..	..	.95
13	32	1	..	..	..	..	..	..	..	..	..	..	..	.01
Total		65	14	6	3	0	1	0	3	0	0	2		

large samples also follow a Poisson distribution, there are a few broods which deviate from it. The maximum number of such broods belongs to stock a9 which has the maximum number of exchanges. These broods may be suspected to contain gonial crossovers. On the basis of random cell death following a gonial exchange resulting in inequality, nearly half of the samples in these broods should be unbalanced. However, only two such samples have been found, the rest of these, even large ones, being balanced. Further, in all these three experiments males deviating from equal crossover production in the two regions preferred only one of the two regions, depending on the stock. In sum, distributions apparently derived from gonial exchanges are too few to permit a conclusion in favour of spermatogonial crossing over in *D. ananassae* and therefore may be treated as exceptional events. Moreover, studies on induced crossing over in

TABLE 6

Comparison of total observed spontaneous crossovers recovered from of D. ananassae, with more than five recombinants in their progeny in the two regions

Stock	Number of high crossover males			Total
	With preference for region I	With preference for region II	With preference for neither region	
<i>cu b se/a6</i>	0	1	7	8
<i>cu b se/a7</i>	0	2	1	3
<i>cu b se/a11</i>	1	7	1	9
<i>cu b se/a9</i>	6	1	26	33

Drosophila have shown that exchanges in spermatogonia are always accompanied by a few cases of apical gonial crossing over when a sizable sample of males is treated. Absence of such predefinitive exchanges in *D. ananassae* also indicates absence of gonial crossing over.

DISCUSSION

Although crossing over is essentially a meiotic process in sexually reproducing animals, a premeiotic origin of crossovers has been reported in *Drosophila* males. However, of the several reports on the induction of male crossing over in *D. melanogaster*, only a few have satisfactorily identified the cellular origin of all the recovered crossovers. Nevertheless, a few criteria have been laid down to distinguish between the cell types affected by inducing agents. Broods homogeneous for crossover production by individual males with balanced reciprocal recombinants and with random exchanges in the tested chromosomes are considered to have arisen from independent exchanges in the spermatocytes or from the terminal secondary spermatogonia (HANNAH-ALAVA 1965). MUKHERJEE (unpublished) applied these criteria to identify the cell types producing the spontaneous crossovers in males of *D. ananassae*. Great individual variation in crossover values accompanied by unequal reciprocal recombinants lead him to suggest the occurrence of apical spermatogonial crossing over. However, it has now been observed by MUKHERJEE (personal communication) that one of the two markers utilized in these studies behaves like a semidominant mutant. Also, the imbalance of the complementary classes in these data consistently showed an abundance of only one type of crossover class in all tested males which is unexpected on the basis of random death of one of the two cells each carrying one crossover type. Further, continuation of crossovers in several later broods, the only positive evidence for exchanges in apical cells, was not observed.

RAY-CHAUDHURI and KALE (1966) studied spontaneous crossing over in the second chromosomes of *D. ananassae* using a triple recessive stock. They observed equality of reciprocal recombinants and evenness of recombination frequency in the two tested regions as noted from the total crossover values. It was, therefore, suggested that crossing over occurs in the spermatocytes. However, wide individual variation in recombination frequencies was reported and although most of the positive males produced crossovers in the two regions, many of these were

not high in both regions. Some showed a significant preference for the first region whereas some others preferred the second region. According to WHITTINGHILL (personal communication), these data indicate spermatogonial origin of spontaneous crossovers. These experiments, were however performed in a somewhat unorthodox manner with four day broods each containing a two day rest period. The individual variation was calculated from total recombination frequencies in both regions without using the Poisson distribution for separate broods. Moreover, strain a6, which has now been inbred by full sib matings for fifteen generations, then contained both the AL/AL and AL/ST arrangements (RAY-CHAUDHURI and JHA 1966), which could have produced both homozygous and heterozygous individuals when crossed to the recessive stock. The present work which is more precise and extensive gives clear evidence for a spermatocytic origin of crossovers. Recent studies by MORIWAKI (personal communication) also indicate meiotic origin of these crossovers. SUZUKI, BAILLIE and PARRY (1966) suggested a two step mechanism for the origin of rare multiple recombinants. This mechanism consists of crossing over occurring both in gonial cells and their daughter meiocytes carrying crossover chromosomes. In the present experiments very few double crossovers were scored which were solitary. However, it is possible that the largest samples scored originated in spermatocytes carrying chromosomes weakened in gonial cells as suggested by WHITTINGHILL (1955).

Effects of parental age on recombination in females of *D. melanogaster* has been reported by BRIDGES (1929), PLOUGH (1917), STERN (1926) and by WHITTINGHILL and HINTON (1950). The drop in recombination in earlier laying periods was interpreted in two different ways. Whereas BRIDGES (1929) suggested the use of earlier broods for chromosome mapping, according to WHITTINGHILL and HINTON (1950), earlier broods may contain gonial crossovers and therefore are not suitable for this purpose. In any case, the importance of this variable in crossing over studies is clear. However, LEVINE and LEVINE (1955) were unable to confirm the age effect in *D. pseudoobscura*, and opined that the earlier observations may have been due to other factors, such as culture conditions. In *D. ananassae* RAY-CHAUDHURI and KALE (1966) observed a much more pronounced drop in spontaneous male crossover values with increase in age of the males. It was suggested that the effect may be a genetic characteristic associated with factors responsible for male crossing over. Present work extends the evidence to three more strains and although there is a significant decrease in the amount of crossing over in later broods, the response is different in the four strains. It appears, therefore, that the age effect may be strain specific.

Occurrence of meiotic and mitotic autosomal chiasmata in considerable frequency was reported by COOPER (1949). Since crossing over does not occur in males of *Drosophila*, he believed that chiasmata are not related to crossing over. It was however suggested that these chiasmata represent 'competent targets' for induction of crossing over in males of *D. melanogaster*. HINTON (1968) was unable to make a positive identification of autosomal chiasmata in males of *D. ananassae*. However, crossover variability in males of this species should also be considered. The phenomenon is observable only in some strains with considerable

individual variation in recombination values. Even in positive males exchanges may occur only at a certain stage of the life cycle and in limited regions of tested chromosomes. Moreover since genetic crossing over has been detected in males, existence of such competent targets, susceptible to induction of crossing over may be suspected.

Evidence in favour of such genetic agents responsible for crossing over in *Drosophila* comes from studies on the genotypic control of the phenomenon. Such evidence has been provided by GOWEN (1933), PARSONS (1958), GREEN (1959), LAWRENCE (1963) in *D. melanogaster*; by LEVINE and LEVINE (1955) in *D. pseudoobscura* and by MORIWAKI (1937, 1940), MORIWAKI and TOBARI (1967), KIKKAWA (1938), MUKHERJEE (1961) and KALE (1968) in *D. ananassae*. Variable spontaneous male crossing over arising in different strains reported in the present work suggests genotypic control of the phenomenon.

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SUMMARY

Spontaneous crossing over in males of *D. ananassae* was studied in four Indian strains. The majority of the daily broods (94 out of 104) followed a Poisson distribution indicating homogeneity in crossover production by the individuals. Most of the large recombinant samples (177 in 180), including those from the heterogeneous broods, were balanced for the reciprocal classes. Positive males produced either equal numbers of crossovers in the two marked regions or preferred either one of the regions depending on the strain. These observations indicate a spermatocytic origin of the recombinants. A few exchanges appear to have originated in spermatogonial cells. However, such incidents are too few to permit a conclusion in favour of a gonial origin of crossovers and may be treated as exceptional events. Crossovers decrease significantly with age of the males and this age effect appears to be strain dependent. Variable crossing over arising in different strains extends the evidence in favour of a genotypic control of crossing over in males of *D. ananassae*.

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