SPONTANEOUS MALE CROSSING-OVER OF FREQUENT OCCURRENCE IN *DROSOPHILA ANANASSAE* FROM SOUTHEAST ASIAN POPULATIONS

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Received January 13, 1973

Spontaneous occurrence of male crossing-over was first observed by Moriwaki (1937, 1938, 1940) and Kikkawa (1937) in *D. ananassae* independently. They concluded that the events were under genetic control. Lately, as to this phenomenon several authors published data which demonstrated that the male crossing-over in this species was controlled by several genes or polygenes and mainly occurred in meiotic stage (Mukherjee 1961, Kale 1969, Moriwaki *et al.* 1970, Hinton 1970). As stated above, in *D. ananassae*, in contrast with other species of *Drosophila*, male crossing-over has rather often been observed. However, little is known about proportion of chromosomes responsible for spontaneous male crossing-over in natural populations.

In the present experiment frequencies of chromosomes which produced spontaneous male crossing-over in *D. ananassae* of Southeast Asian populations were examined.

MATERIALS AND METHODS

From May to September in 1971, a research group presented by Dr. K. Wakahama of Shimane University explored many localities over Southeast, Mideast Asia and Africa, collecting *Drosophila* species. By the member's aid, several isofemale lines of *D. ananassae* from each locality were made and shipped by air mail to Tokyo. Thanks are due to their help.

The isofemale lines of *D. ananassae* used in this study were established from single inseminated females collected in nature from six localities as follows: Taiwan (A), Kota Kinabaru, Borneo (B), Kuala Lumpur, Malaysia (C), Chian Mai, Thailand (D), Calcutta, India (E) and Colombo, Ceylon (L) (Fig. 1). Five isofemale lines from each locality, but three in E, were examined in male crossing-over frequencies within several generations after collection. Five males from each line were singly mated with double recessive females, *b se* (black sepia) on the second chromosome. One day old single F_1 male (*b se*/++) recovered from each cross was backcrossed with *b se* virgin females.

1) This work was supported by a grant from the Scientific Research Fund of the Ministry of Education, Japan, No. 50801.

Fig. 1. Localities from which *D. ananassae* were examined for male crossing-over.

After a three or four day egg laying period, parents were transferred to new vials successively twice. Thus three 3-4 day broods were provided. Progenies recovered from each brood of each family were scored. The experiments were performed in the constant temperature room at 25°C.

RESULTS

The data of three broods were pooled to calculate the recombination values in males between *b* and *se*. The results were summarized in Table 1. As shown in the table, the male crossing-over occurred in 133 chromosomes (95%) of 140 tested. In the four populations, A, B, C and E, among six, all chromosomes tested were observed to show male crossing-over. In most of the lines of six localities great variations from male to male were observed in recombination value. These values ranged from zero to about 20%. Significant differences in recombination values were detected among lines but not among localities by the analysis of variance.

The mean recombination value of all 140 chromosomes from six localities amounted to about 4.0%, while in females it was 48.8%. In other words, the male recombination value between *b* and *se* was about 8% of the female one.

The equality of complementary crossover classes in each family was examined following Fisher's method used by Hinton and Whittinghill (1950). In the Appendix Table, probabilities of obtaining the observed distributions of crossover classes in each family and respective $-\log_e$ value are presented. The $-\log_e$ value is equal to $\chi^2/2$ associated with 2 degrees of freedom and additive in character. Only two out of 125 families studied are significantly deviated from equality. Therefore it will be concluded that the equality of complementary crossover classes is demonstrated in almost all families with only two exceptions.

Correlation between recombination frequency and *k* value was computed, where $k = (\text{wild type} + 1/2 \text{ recombinant}) / \text{total progeny}$. The recombination values were used

Table 1. Recombination values between *b* and *se* in males, calculated for each line of six localities

Locality	Line	No. of chromosome tested	No. of chr. with male c.o.	Progeny				N	Mean R.V. (%)	Range of R.V. (%)
				Non-recom- binant <i>b se</i>	+ 	Recom- binant <i>b se</i>				
TAIWAN	A3	5	5	716	689	35	26	1466	3.90	2.29- 5.62
	A5	5	5	784	944	31	26	1785	3.12	2.05- 4.25
	A31	5	5	930	1176	36	26	2168	2.67	0.17- 6.06
	A36	5	5	1115	1334	37	38	2524	2.98	1.20- 4.39
	A75	5	5	931	1135	49	46	2161	4.57	1.74- 6.74
	Total	25	25	4476	5278	188	162	10104	3.45	
BORNEO	B13	5	5	467	538	15	20	1040	3.42	1.59- 5.65
	B15	5	5	407	436	17	6	866	2.67	1.79- 3.65
	B18	5	5	408	574	40	35	1057	7.61	4.87-11.97
	B42	5	5	532	547	16	16	1111	2.35	0.55- 5.07
	B45	5	5	558	596	14	14	1182	2.42	0.61- 4.39
	Total	25	25	2372	2691	102	91	5256	3.69	
MALAYSIA	C104	5	5	504	560	45	33	1142	6.54	2.91-16.07
	C105	5	5	325	404	17	18	764	4.48	2.17- 7.38
	C106	5	5	700	739	38	39	1516	5.44	3.49- 9.52
	C107	5	5	1064	1198	47	31	2340	3.34	2.91- 4.77
	C123	5	5	989	1091	43	44	2167	4.28	0.79- 8.67
	Total	25	25	3582	3992	190	165	7929	4.82	
THAILAND	D20	5	5	517	678	31	36	1262	5.14	2.58- 9.00
	D21	5	5	410	552	33	33	1028	6.39	3.42-10.43
	D23	5	5	567	625	18	17	1227	2.49	0.62- 4.01
	D25	5	4	347	399	9	10	765	2.48	0 - 5.84
	D28	5	4	417	475	24	23	939	5.62	0 -13.91
	Total	25	23	2258	2729	115	119	5221	4.42	
CALCUTTA	E22	5	5	408	495	9	7	919	1.84	0.97- 2.83
	E23	5	5	359	384	15	13	771	3.99	0.74-10.66
	E24	5	5	495	534	25	21	1075	4.52	1.75- 6.38
	Total	15	15	1262	1413	49	41	2765	3.45	
COLOMBO	L2	5	5	321	371	10	14	716	3.29	1.34- 6.33
	L5	5	5	412	513	12	8	945	1.82	0.47- 3.57
	L7	5	2	350	354	9	8	721	1.82	0 - 7.33
	L8	5	5	322	447	51	33	853	11.28	1.95-20.47
	L10	5	3	438	500	10	4	952	1.44	0 - 3.80
	Total	25	20	1843	2185	92	67	4187	3.93	
		140	133							

after arcsine transformation. Slightly significant positive correlation was detected ($r=0.170$, $t=2.034$, $0.05>p>0.025$). In Fig. 2, recombination value of each family after arcsine transformation was plotted at the corresponding k value.

DISCUSSION

The equality of complementary crossover classes was demonstrated, suggesting the meiotic origin of the event. Wide individual variation and significant differences of recombination values among lines may attribute to polygenic systems (Moriwaki *et al.* 1970).

Fig. 2. Distribution of recombination values plotted against k values of males in *D. ananassae*.

Hiraizumi (1971) reported highly significant negative regression of recombination frequency on the k value in *D. melanogaster*. In contrast with that, the present experiment represented positive correlation between recombination and k values. However, meanings of the property are not explained at this moment.

In the male of *Drosophila* there is normally no crossing-over. In the female inversion heterozygosity leads to extensive crossing-over reduction. Consequently they prevent recombination in a balanced gene complex. One way to maintain balance between immediate fitness and future flexibility of *Drosophila* in natural populations is the storage and release of potential genetic variability which are controlled by the amount of inversion heterozygosity and extreme difference in recombination between sexes (Bodmer and Parsons 1962).

In the present study amazingly large proportion of chromosomes in Southeast Asian populations was observed to produce spontaneous male crossing-over. In laboratory stocks originated from various localities of *D. ananassae* about 65% of chromosomes were found to have male crossing-over (Moriwaki *et al.* 1970). Therefore, in *D. ananassae* populations male recombination may not a little contribute to the release of the populations. More extensive survey over the range of species distribution with regards to male crossing-over and distribution of inversions may provide a mode of compromise between immediate fitness and future flexibility of the species.

SUMMARY

Drosophila ananassae from six geographical localities in Southeast Asia were examined in the recombination frequencies in the male. The investigation reveals that male crossing-over may be very common. At least in the second chromosome from these populations, almost all males can be expected to carry some genetic elements to cause male recombination, although in general the recombination value in the male was not always so high as that in the female.

The equality of complementary crossover classes in each family was observed suggesting the meiotic origin of the event.

Positive correlation between recombination and k values was obtained.

ACKNOWLEDGMENTS

The authors wish to express their thanks to Miss S. Serizawa for her technical assistance.

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Appendix Table. Familial distribution of recombinants in offspring from *b se/+* males and Chi square analysis for testing the equality of complementary crossovers within families

	<i>b</i>	<i>se</i>	<i>p</i>	-Log _e		<i>b</i>	<i>se</i>	<i>p</i>	-Log _e
A 3 -1	10	9	0.500	0.693	B13-1	1	3	0.313	1.161
2	8	4	0.194	1.640	2	1	3	0.313	1.161
3	6	1	0.063	2.765	3	5	1	0.109	2.216
4	9	10	0.500	0.693	4	3	4	0.500	0.693
5	2	2	0.500	0.693	5	5	9	0.212	1.551
A 5 -1	4	5	0.500	0.693	B15-1	3	2	0.500	0.693
2	7	6	0.500	0.693	2	3	1	0.313	1.161
3	7	7	0.500	0.693	3	3	0	0.125	2.080
4	10	7	0.315	1.155	4	3	1	0.313	1.161
5	3	1	0.313	1.161	5	5	2	0.227	1.483
A31 -1	6	6	0.500	0.693	B18-1	9	8	0.500	0.693
2	1	0			2	4	5	0.500	0.693
3	14	14	0.500	0.693	3	12	12	0.500	0.693
4	14	6	0.058	2.847	4	4	6	0.377	0.975
5	1	0			5	11	4	0.059	2.830
A36 -1	9	16	0.115	2.163	B42-1	5	10	0.151	1.890
2	7	4	0.274	1.295	2	1	0		
3	3	2	0.500	0.693	3	1	1	0.500	0.693
4	7	7	0.500	0.693	4	8	5	0.291	1.234
5	11	9	0.412	0.887	5	1	0		
A70 -1	9	7	0.402	0.911	B45-1	0	1		
2	8	6	0.395	0.929	2	5	1	0.109	2.216
3	3	3	0.500	0.693	3	3	7	0.172	1.760
4	14	13	0.500	0.693	4	2	1	0.500	0.693
5	15	17	0.430	0.844	5	4	4	0.500	0.693
C104-1	24	21	0.383	0.960	D20-1	5	5	0.500	0.693
2	5	3	0.363	1.013	2	2	4	0.344	1.067
3	7	1	0.035	3.352*	3	2	5	0.227	1.483
4	5	3	0.363	1.013	4	5	12	0.072	2.631
5	4	5	0.500	0.693	5	17	10	0.124	2.087
C105-1	3	2	0.500	0.693	D21-1	12	10	0.416	0.877
2	2	3	0.500	0.693	2	3	9	0.073	2.617
3	4	7	0.274	1.295	3	3	2	0.500	0.693
4	7	4	0.274	1.295	4	12	9	0.332	1.103
5	1	2	0.500	0.693	5	3	3	0.500	0.693
C106-1	2	4	0.344	1.067	D23-1	5	3	0.363	1.013
2	6	9	0.304	1.191	2	6	6	0.500	0.693
3	9	8	0.500	0.693	3	1	0		
4	8	7	0.500	0.693	4	1	1	0.500	0.693
5	13	11	0.419	0.870	5	5	7	0.387	0.950
C107-1	11	6	0.166	1.796	D25-1	1	3	0.313	1.167
2	12	9	0.332	1.103	2	3	0	0.125	2.080
3	4	5	0.500	0.693	3	4	5	0.500	0.693
4	12	6	0.119	2.129	4	0	0		
5	8	5	0.291	1.234	5	1	2	0.500	0.693
C123-1	10	9	0.500	0.693	D28-1	4	2	0.344	1.067
2	14	16	0.428	0.849	2	7	10	0.315	1.155
3	15	9	0.154	1.871	3	4	4	0.500	0.693
4	3	7	0.172	1.760	4	9	7	0.402	0.911
5	1	3	0.313	1.161	5	0	0		
E22 -1	4	2	0.344	1.067	L 2-1	2	0	0.250	1.386
2	1	2	0.500	0.693	2	1	3	0.313	1.161
3	1	2	0.500	0.693	3	2	4	0.344	1.067
4	1	1	0.500	0.693	4	4	6	0.377	0.975
5	2	0	0.250	1.386	5	1	1	0.500	0.693
E23 -1	7	9	0.402	0.911	L 5-1	0	1		
2	1	1	0.500	0.693	2	4	1	0.188	1.671

Appendix Table. (continued)

	<i>b</i>	<i>se</i>	p	−Log _e		<i>b</i>	<i>se</i>	p	−Log _e	
	3	2	2	0.500	0.693	3	6	5	0.500	0.693
	4	1	1	0.500	0.693	4	0	1		
	5	4	0	0.063	2.765	5	2	0	0.250	1.386
E24	−1	9	4	0.133	2.017	L 7−1	0	0		
	2	2	3	0.500	0.693	2	0	0		
	3	4	3	0.500	0.693	3	7	7	0.500	0.693
	4	7	2	0.090	2.408	4	2	1	0.500	0.693
	5	3	9	0.073	2.617	5	0	0		
						L 8−1	3	3	0.500	0.693
						2	14	15	0.500	0.693
						3	16	10	0.164	1.808
						4	4	0	0.063	2.765
						5	14	5	0.032	3.442*
						L10−1	3	1	0.313	1.161
						2	3	2	0.500	0.693
						3	0	0		
						4	4	1	0.188	1.671
						5	0	0		

p: Probabilities of obtaining the observed distribution of complementary crossover classes in each family.

The $-\log_e$ is equal to $\chi^2/2$ associated with 2 degrees of freedom.