Effects of chromosomal interaction on male mating activity in *Drosophila melanogaster*

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ABSTRACT

The epistatic interaction between the second and third chromosomes of *D. melanogaster* was quantified in male mating activity under no competition among males. The mean mating activity of the two-chromosome homozygote was 0.517 ± 0.064 . The expected value under a multiplicative model was 0.509 ± 0.054 . Coefficient of interaction, i , was calculated to be 0.008 ± 0.129 . k statistic, a new measure of epistasis devised by Seager and Ayala (1982), was 0.029 ± 0.027 , which is not significantly different from zero. It is concluded that there is no interchromosomal interaction between two major autosomes in male mating activity.

1. INTRODUCTION

To extend our understanding of the fitness function, a large body of experiment has been carried out for various fitness components (Dobzhansky *et al.* 1963; Malogolowkin-Cohen *et al.* 1964; Spassky *et al.* 1965; Crow 1968; Mukai 1969; Temin *et al.* 1969; Kosuda 1971, 1972, 1980; Clark and Feldman 1981; Sharp 1984; Seager and Ayala 1982; Seager *et al.* 1982). It has become apparent that the male reproductive component of fitness may play a more important role than fitness variables in preadult stages and female fertility, in estimating the net fitness differentials in *Drosophila* (Anderson 1969; Sved and Ayala 1970; Bundgaard and Christiansen 1972; Marinković and Ayala 1975a, b; Petit *et al.* 1980; Brittnacher 1981; Sharp 1984; Kosuda 1983). However, no studies have been conducted on the interchromosomal epistatic interaction for mating activity in a chromosomally homozygous condition. In the present study I have examined if there is an epistasis between two chromosomes of *Drosophila melanogaster* in male mating activity under no competition among males for their mates.

2. MATERIALS AND METHODS

A natural population of *Drosophila melanogaster* collected in Katsunuma, Yamanashi, Japan, were employed for the examination. Single pairs of the second and third chromosomes were simultaneously extracted from each collected male, and were made homozygous by means of a complete marked

Fig. 1. Mating scheme to obtain the double chromosome homozygotes and each of single chromosome homozygotes for the second and third chromosomes, and the double heterozygotes.

inversion method using *Cy/Pm*; *Ubx/Sb* (Fig. 1). The dominant inversion-linked markers, *Cy* (Curly) and *Ubx* (Ultra-bithorax) on chromosome 2 and 3, respectively, are lethal when homozygous, and the linked complicated inversions suppress recombination in respective chromosomes. *Pm* and *Sb* are also dominant marker genes on chromosome 2 and 3, respectively. Males showing both *Cy* and *Ubx* were crossed singly with several *Cy/Pm*; *Ubx/Sb* females in the next generation (G₂). In generation 3, several pairs of *Cy/+*; *Ubx/+* genotypes were sib-mated. Flies showing both *Cy* and *Ubx* in G₄ are doubly heterozygous for the two autosomes from a natural population (Fig. 1). *Ubx* or *Cy* individuals are single homozygotes for either second or third chromosome, whereas wild type flies are double homozygotes for both chromosomes (Fig. 1).

Wild-collected chromosomes which are lethal or semilethal were excluded from experimental materials in the present study. Sterile chromosomes or chromosomes with low fertility were also excluded from materials. Twenty-three homozygous lines having no lethal or sterility genes were finally established.

Non-competitive male mating activity was measured for the 23 homozygous lines thus established as follows. Males were individually placed into a 3×8 cm mating vial with food containing 10 virgin females of a standard laboratory strain designated "2SG", aged 3–4 days. They were kept at 25°C under continuous light. After 24 hours, males were discarded, and females were dissected to examine the sperm insemination in ventral receptacles or spermathecae under the compound microscope. About 20 to 25 replicates were made for each homozygous line. Male mating activity was measured by the

ratio of inseminated females.

Mating activity of heterozygous males was measured in a similar way, using males produced by 21 crosses between *Cy; Ubx* females and males each derived from 19 different lines among the 23 established lines above. Thus, the wild type males in these controls have two different second and two different third chromosomes ($F=0$).

To evaluate the nature and the magnitude of interchromosomal interaction in male mating activity, two indices were employed: i statistic used by Spassky *et al.* (1965), Temin *et al.* (1969) and Kosuda (1971), and k statistic devised and used by Seager and Ayala (1982) and Seager *et al.* (1982). i is defined as $i=1 - \text{observed mating activity/expected mating activity}$. i is rewritten as $i=1 - wXYZ/Wxyz$, where W, X, Y, Z are the male mating activity in heterozygous condition ($F=0$) and w, x, y, z are the mating activity in homozygous condition ($F=1$) of *Cy; Ubx*, *Ubx*, *Cy* and wild type, respectively (Table 1). I statistic used in Kosuda (1971) can be replaced by $I=1-i$. When there is no epistatic interaction, *i.e.*, if the chromosomes are independent in fitness effect, $i=0$. If positive epistasis (reinforcing or synergistic epistasis, Crow and Kimura, 1970) exists, *i.e.*, if male mating activity of double chromosome homozygote is smaller than expected, positive i will be obtained. A larger-than-expected mating activity for the double chromosome homozygote gives $i<0$ (negative or diminishing epistasis). The range of i is, $-\infty < i \leq 1$. i statistic has defective properties that it is not symmetrical about zero and it responds considerably to random fluctuations in the estimation of mating activity. In comparison with viability estimation, mating activity is measured from a relatively limited number of observations, and experimental chromosome lines are restricted. Therefore, i statistic is not an appropriate measure in

Table 1. *The method of estimation of the relative male mating activity of the double chromosome homozygotes and each of single homozygotes for the second and third chromosomes to the double heterozygotes*

Genotype	<i>Cy/+;Ubx/+</i>	<i>+/+;Ubx/+</i>	<i>Cy/+;+/+</i>	<i>+/+;+/+</i>
Male Mating Activity				
F=0	W	X	Y	Z
F=1	w	x	y	z
Estimators				
The relative mating activity of the second chromosome homozygotes to double heterozygotes;			W _x /wX	
The relative mating activity of the third chromosome homozygotes to double heterozygotes;			W _y /wY	
The relative mating activity of the double chromosome homozygotes to double heterozygotes;			W _z /wZ	
<i>i</i> statistic;			1-wXY _z /W _{xy} Z	

variable characters such as male mating activity. To overcome this difficulty, Seager *et al.* (1982) devised another statistic, k , correlation between heterozygosity and homozygosity for the two chromosomes. If the fitness effect of homozygosity for one chromosome do not depend upon the homozygosity in the other chromosome, there will be no correlation, and k will be zero. If two chromosomes interact, *i.e.*, if there is an association of fitness effects for homozygosity of the two chromosomes, there will be a correlation, the magnitude of which is a measure of the strength of the fitness interaction. k statistic is symmetric around 0 and its range is $-1 \leq k \leq 1$. The sign of k is the same for i statistic. k is calculated by the following formulae;

$$k = -r = -\frac{d - (b+d)(c+d)}{\{[b+d - (b+d)^2][c+d - (c+d)^2]\}^{1/2}},$$

where a, b, c and d are the standardized relative mating activity of Cy ; Ubx , Ubx , Cy , and wild type, respectively, so that they add up to 1.0 (for further details, see Seager and Ayala 1982).

3. RESULTS

Male mating activity in homozygous and heterozygous conditions is summarized in Table 2. According to the method presented in Table 1, the relative mating activity of the double homozygotes and each of single homozygotes for the second and third chromosomes to the double heterozygotes are calculated to be 0.517 ± 0.064 , 0.726 ± 0.042 , 0.695 ± 0.050 , respectively. The heterozygous effects of marker chromosomes are thus taken into consideration in the calculation of homozygous male mating activity (Table 1). The results show that more deleterious genes were concealed in a heterozygous condition in the third chromosomes than the second chromosomes. These

Table 2. *Male mating activity of the four different genotypes at F=0 and F=1*

Genotypes		$Cy/+;$ $Ubx/+$	$+/+;$ $Ubx/+$	$Cy/+;$ $+/+$	$+/+;$ $+/+$	Total
F=0	Total No. of Females Mated	959	1019	796	868	3642
	Total No. of Females Tested	1276	1293	1086	1148	4803
	Male Mating Activity	.7516	.7881	.7331	.7561	
F=1	Total No. of Females Mated	986	757	647	509	2899
	Total No. of Females Tested	1561	1576	1524	1583	6244
	Male Mating Activity	.6316	.4803	.4245	.3215	
	Relative Mating Activity*	1	.726 $\pm .042$	.695 $\pm .050$	.517 $\pm .064$	.517 $\pm .064$

* Calculatd as shown in Table 1.

Table 3. *Observed and expected mating activities of double chromosome homozygotes and two indices of epistasis, i and k*

Line No.	Observed Mating Activity	Expected Mating Activity	k	i
001	0.565	0.581	0.007	20.08
002	0.870	0.435	-0.167	-1.000
003	0.534	0.394	-0.074	-0.355
006	0.612	0.551	-0.026	-0.111
007	0.385	0.536	0.077	0.282
008	0.232	0.619	0.213	0.625
009	0	0.121	0.245	1.000
010	0.349	0.353	0.003	0.011
011	0.947	0.671	-0.086	-0.411
012	0.221	0.461	0.159	0.521
013	0.488	0.186	-0.212	-1.624*
014	1.293	0.959	-0.074	-0.348
016	0.520	1.140	0.189	0.544
017	0.179	0.218	0.040	0.179
018	0.696	0.347	-0.168	-1.006*
021	0.261	0.392	0.090	0.334
024	0.255	0.497	0.147	0.487
031	0.886	0.714	-0.054	-0.241
032	0.474	0.549	0.036	0.137
033	0.091	0.257	0.184	0.644
034	0.700	0.925	0.069	0.243
035	0.790	0.600	-0.068	-0.317
036	0.086	0.201	0.141	0.571
Mean	0.517	0.509	0.029	0.008
S. E.	0.064	0.054	0.027	0.129

* When these two values of $i < -1.0$ are moderately fixed at $i = -1.0$, mean $i = 0.036 \pm 0.116$ (see text).

chromosomes, however, were not a random sample from a natural population, since lethal and sterility chromosomes were excluded from experimental materials. The values for k and i statistics are -0.003 and -0.013 , respectively. These figures suggest that there is no or almost no epistasis between chromosomes in male mating activity.

Table 3 shows the observed and expected mating activity of the double chromosome homozygotes individually for 23 lines, together with two different indices of epistasis. The expected mating activity is the products of the mating activity of the single homozygotes under a multiplicative model. Eleven out of 23 double chromosome homozygotes had the mating activity greater

than 0.50. The mean observed male mating activity was 0.517 ± 0.064 , whereas the mean expected mating activity was calculated to be 0.509 ± 0.054 . These two values do not statistically differ, indicating no or almost no epistatic interaction between chromosomes.

As shown in Table 3, the mean k value was 0.029 ± 0.027 . It does not significantly differ from zero ($P > 0.05$). Negative k values were obtained in 9 cases out of 23. The mean value of i statistic was 0.008 ± 0.129 . It is also not statistically different from zero. Since the range of i is $-\infty < i \leq +1.0$, the mean value of i is not so meaningful. The median value of i was 0.137. Since i is not symmetrically distributed about zero, there remains the possibility that chromosomal interaction is underestimated toward negative epistasis due to the disproportionate contribution of a few large negative i values. If the negative i statistic lower than -1.0 is moderately fixed at $i = -1.0$, the mean i increases to 0.036 ± 0.116 , which is not significantly different from zero. Taken together it may be concluded that there is no significant epistatic interaction between chromosomes in the non-competitive male mating activity in *D. melanogaster*.

4. DISCUSSION

In an attempt to examine whether different gene loci have independent fitness effect, egg-to-adult viability of individuals simultaneously homozygous for two non-homologous chromosomes in *Drosophila* has been compared with that of individuals homozygous for one or another chromosomes (Spassky *et al.* 1965; Temin *et al.* 1969; Kosuda 1971). The conclusion derived from these studies is that, in general, there exists a weak positive or synergistic interaction, *i.e.*, the double homozygotes viability is slightly lower than that expected from single homozygotes. These studies have only examined egg-to-adult viability as a measure of fitness, and fitness components other than viability have been ignored in this kind of approach to the problem. In fact, however, a body of evidences has accumulated that male mating activity has much greater effects on total fitness than viability (Anderson 1969; Sved and Ayala 1970; Prout 1971; Bundgaard and Christiansen 1972; Marinković and Ayala 1975a, b; Petit *et al.* 1980; Brittnacher 1981; Sharp 1982; Kosuda 1983).

Seager *et al.* (1982) found a conspicuously strong negative epistasis for total fitness in cage population under a highly competitive condition ($k = 0.352 \pm 0.210$). If the negative synergistic interaction in fitness predominates in natural populations, a greater number of polymorphisms can be accounted for by the selective maintenance. If the strong diminishing epistasis exists in total fitness, and if there is a slight reinforcing epistasis or no interaction in egg-to-adult viability, a larger magnitude of negative epistatic interaction is expected in male mating activity. However, any evidence for diminishing epistasis in male mating activity could not be detected in the present investi-

gation. The discrepancy may result from the differences in experimental conditions and methods. Extremely severe competition would be suspected to occur in larval and/or adult stages in cage populations. Epistatic interaction may increase as competition becomes stronger.

Clark and Feldman (1981) investigated interactions between genes on specific inversions marked by *Cy* and *Pm* in the second chromosome of *D. melanogaster* in a heterozygous condition for various fitness components. For male mating success, they could not detect consistent epistasis as in the present study. Interaction was significantly positive in one case and was significantly negative in another case, but it was insignificant in other 4 cases.

To assess the intrachromosomal epistatic interaction, another kind of research has been carried out in several species. If different gene loci have independent fitness effects, then the relationship between the degree of inbreeding and the fitness depression on logarithmic scale should be linear (Morton *et al.* 1956). For the competitive male mating activity as a measure of fitness, Latter and Robertson (1962) found no significant departure from lineality between the inbreeding coefficient and the reduction in mating activity of *D. melanogaster*, in a wide range of inbreeding coefficient. Furthermore, Sharp (1984) has recently showed the linear relationships under an additive fitness model. In this type of experiments, however, continuous mating is inevitable. Therefore, the coefficient of inbreeding would be overestimated, particularly in the later stages of continuous inbreeding, and positive epistatic interaction would be underestimated (Kosuda 1972). The results in the present experiment are thus not inconsistent with these studies.

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