GAMETIC FREQUENCY OF SECOND CHROMOSOMES OF THE *T-007* TYPE IN A NATURAL POPULATION OF *DROSOPHILA MELANOGASTER* IN TEXAS[1]

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ABSTRACT

The *T-007* second chromosome, which was isolated from a natural population of *Drosophila melanogaster* in south Texas in 1970, is known to show, when made heterozygous in males with a standard *cn bw* second chromosome, a transmission frequency (k) of 0.35—much lower than the theoretically expected 0.5. Natural populations of this species in Texas contain second chromosomes that, against the standard *cn bw* genetic background, are associated with distorted transmission frequencies comparable to that of the *T-007* chromosome. In order to explain how such chromosomes can persist in natural populations in nontrivial frequencies, it has been postulated that, although such chromosomes show reduced k values when tested under the genetic background of a laboratory stock such as *cn bw*, they may show, on the average, k values larger than 0.5 under natural genetic backgrounds. If this were true, the frequency of chromosomes of the *T-007* type (*T* chromosomes) should be higher in male than in female gametes under natural genetic backgrounds. The present study was conducted to examine this possibility. The results clearly showed that the frequency of such chromosomes was much higher among male than among female gametes, and that the transmission frequency of this type of chromosome was higher than 0.5 under natural genetic backgrounds. These results suggest that *T* chromosomes behave like Segregation Distorter (*SD*) chromosomes in natural populations of this species in Texas. A possible relationship between *T-007* and *SD* chromosomes is suggested.

IT has been shown repeatedly that one of the second chromosomes in a natural population of *Drosophila melanogaster* in Texas, named *T-007*, shows, when made heterozygous in males with standard laboratory second chromosomes such as *cn bw*, a transmission frequency much smaller (about 0.35 when heterozygous with *cn bw*) than the expected 0.5 (HIRAIZUMI 1971, 1977, 1979; MATTHEWS *et al.* 1978). In order to simplify descriptions in this report, the transmission frequency of any particular chromosome or element from a heterozygous parent to its progeny will be referred to as k. In the above example, k for the *T-007* chromosome from a *T-007/cn bw* male is 0.35. Estimation of transmission frequency of the *T-007* chromosomes from females is rather difficult because a high frequency of recombination in females will generate a large number of recombinant chromo-

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somes, and only a small fraction of the chromosomes transmitted to the progeny will be the parental types. Nevertheless, an estimate of k can be obtained by making the *T-007* chromosome heterozygous with a multiply marked chromosome *2* and choosing only those progeny carrying either of the two parental type chromosomes. Such an experiment was conducted in this laboratory, and the result suggested that k for the *T-007* chromosome from the female parent was roughly 0.5. *T-007* also shows, when heterozygous with the *cn bw* or other laboratory chromosomes, male recombination (HIRAIZUMI 1971), reduced male fertility (HIRAIZUMI 1977) and, under a specific genotype situation, drastically reduced fertility at 28° or higher (MATTHEWS and GERSTENBERG 1979), although it shows nearly normal fertility in the temperature range of 23–24°.

Further investigations on natural populations of *D. melanogaster* in Texas revealed that *T-007* was not the only exceptional chromosome *2* (MATTHEWS, in preparation and this report). A small percent of the second chromosomes sampled from Texas populations, when made heterozygous with *cn bw* chromosomes in males, give k values at the low level characteristic of the *T-007* chromosome, *i.e.*, about 0.35. This is a puzzling observation. How are chromosomes like *T-007* that show selective disadvantage for several traits able to remain in natural populations in consistently nontrivial frequencies?

As an explanation of those contradictory observations, it has been postulated that *T-007*-like chromosomes (hereafter, *T* chromosomes) may show an average transmission frequency from males larger than 0.5 under a natural genetic background, and that this may work as one of the selective forces to maintain this type of chromosome in natural populations. Assuming this to be true, we would expect to obtain different frequency estimates of the *T* chromosomes, depending upon whether the second chromosomes are isolated from male or from female parents. The present experiment was conducted to test this line of reasoning.

MATERIALS AND METHODS

Stocks of *D. melanogaster* used in this study were as follows:

cn bw: a standard chromosome *2* line marked with two recessive eye-color mutants, *cn* (cinnabar, *2R*-57.5) and *bw* (brown, *2R*-104.5).

Tokyo: a standard wild-type strain that was established from a collection of flies from a natural population in Tokyo more than 20 years ago. Since then, this strain has been maintained in this laboratory in culture vials through mass transfer.

C(1)DX, In(1)dl-49/In(1)sc^{8}, y f; cn bw: a strain containing an attached-*X* chromosome marked with *y* (yellow body color, *1*–0.0) and *f* (forked bristles, 1–56.7). Other chromosome pairs, including the *Y* chromosome, are those from the standard *cn bw* strain. Being bobbed-deficient, *C(1)DX* females must carry a *Y* chromosome to survive. This strain will be abbreviated as $\overline{XX}/Y;cn\ bw$ in this report.

BiT-1 and *BiT-19*: two wild-type isofemale lines, each established from a naturally inseminated female captured in July, 1979, at *B*rownsville *i*n *T*exas. Since then, these lines have been maintained in this laboratory by mass transfer every 2 weeks. The cultures were 7 months (14 generations) old when the present experiment was initiated. These two isofemale lines were chosen for present study after a preliminary survey of a total of 28 isofemale lines established at the same time. This survey suggested that the *Bit-1* line contained the *T* chromosomes in high frequency; whereas, the *BiT-19* line showed an absence, or at most a low frequency, of such

chromosomes, although this line still contained the "minor" elements (MATTHEWS *et al.* 1978) causing effects similar to those of the *T-007* chromosome, but to a greatly reduced degree.

In most cases, matings were performed by placing two females, 2–4 days of age, in a culture vial with one male, 1–5 days of age. They were kept together for three days, then discarded. Under these experimental conditions, practically all of the F_1 progeny eclosed by the 17th day from the date when mating was initiated; therefore, progeny counts were terminated at the 17–19th day. A standard corn meal medium was used, and the temperature was 23–24° throughout the investigation.

Analyses of data were made following the sign test for paired comparisons and the Mann-Whitney *U* test for unpaired comparisons.

RESULTS

A preliminary survey: A total of twenty-eight isofemale lines established from females captured in July, 1979, at Brownsville, Texas, were investigated for the presence of *T* second chromosomes. Three males, A. B and C, were chosen from each of the isofemale lines and individually crossed to two standard *cn bw* females. A single F_1 male was chosen from each mating and backcrossed to two *cn bw* females. Up to ten F_2 males heterozygous with the *cn bw* chromosomes were chosen from each F_1 male mating and were individually crossed to two *cn bw* females to examine the *k* values for wild second chromosomes. The results are summarized in Table 1.

As mentioned earlier, the *T-007* chromosome shows, when made heterozygous in males with the *cn bw* chromosome, an average *k* value of approximately 0.35. We shall use this value as characteristic *k* of *T* second chromosomes. There are two isofemale lines, *BiT-1* and *BiT-17*, in Table 1 that appear to contain *T* chromosomes. One other line, *BiT-28*, might also contain this type of chromosome. Considering the fact that each isofemale line started with four potentially different second chromosomes, a minimum estimate of the *T-007*-type chromosome in this population is 2/112–3/112, or 1.79%–2.68%. This frequency is probably a considerable underestimate because some *T* chromosomes, which were originally present in some of the isofemale lines when they were established, might have been lost or reduced in frequency in the cultures during the more than ten generations under laboratory conditions, so that they were not represented among three parental males examined. Also, as will be discussed later, this population appeared to contain suppressors of *k* located in chromosomes *3* and/or *4*. These would tend to make the *k* somewhat larger than 0.35, even if the *T* chromosomes were in fact included in some of the parental males tested. Nevertheless, one may conclude that the frequency of the *T* chromosomes in this population was at least 2%, and perhaps much higher than this.

After completing this preliminary survey, two lines, *BiT-1* and *BiT-19*, were chosen as representative lines containing and not containing the *T* chromosome, respectively. It should be noted here that the *BiT-19* line, although the *T* chromosome appeared to be absent in it, seemed to contain the "minor" elements (MATTHEWS *et al.* 1978) that showed effects similar to, but much weaker than that of *T-007*.

Chromosome 2 sampled from males: Six males from *BiT-1* and five males

TABLE 1

A list of k *values for each isofemale line in a preliminary survey for the presence of* T-007*-type chromosomes*

Line	$\bar{k}$	n^*	Line	$\bar{k}$	n^*	Line	$\bar{k}$	n^*	Line	$\bar{k}$	n^*
BiT-1—A	0.379	9	*BiT-8*—A	0.533	10	*BiT-15*—A	0.424	8	*BiT-22*—A	0.499	10
B	0.448	9	B	0.403	10	B	0.519	9	B	0.409	10
C	0.330	7	C	0.505	10	C	0.488	10	C	0.482	10
2—A	0.466	10	*9*—A	0.519	10	*16*—A	0.554	4	*23*—A	0.488	10
B	0.492	8	B	0.470	10	B	0.513	10	B	0.523	10
C	0.481	10	C	0.469	10	C	0.538	10	C	0.474	7
3—A	0.485	9	*10*—A	0.435	10	*17*—A	0.480	10	*24*—A	0.505	10
B	0.495	10	B	0.461	10	B	0.314	7	B	0.510	10
C	0.528	10				C	0.443	10	C	0.460	10
4—A	0.445	10	*11*—A	0.449	6	*18*—A	0.514	10	*25*—A	0.491	10
B	0.471	10	B	0.519	10	B	0.536	10	B	0.451	10
C	0.541	10	C	0.443	10	C	0.513	10	C	0.452	10
5—A	0.550	10	*12*—A	0.522	10	*19*—A	0.476	10	*26*—A	0.407	10
B	0.456	9	B	0.486	9	B	0.515	10	B	0.516	10
C	0.517	10	C	0.472	9	C	0.489	10			
6—A	0.429	10	*13*—A	0.529	10	*20*—A	0.484	10	*27*—A	0.463	7
B	0.452	10	B	0.442	10	B	0.474	10	B	0.494	10
C	0.420	10	C	0.438	10	C	0.493	10	C	0.526	10
7—A	0.514	10	*14*—A	0.503	10	*21*—A	0.508	10	*28*—A	0.535	10
B	0.533	10	B	0.542	10	B	0.493	9	B	0.386	10
C	0.536	10	C	0.498	10	C	0.504	10	C	0.435	10

See text for a detailed explanation of mating scheme.
* Number of F_2 males tested.

from *BiT-19* were crossed individually to *cn bw* females, and about twenty F_1 males for each mating were chosen and crossed individually to two *cn bw* females in order to measure k for the *BiT* chromosomes. Ten F_2 males were then randomly chosen from each F_1 male line and crossed individually to two *cn bw* females to measure k for *BiT* chromosomes from each of the F_2 males.

Another independent set of experiments similar to those described above was also conducted by choosing four males from both the *BiT-1* and *BiT-19* stocks and repeating the backcross matings until F_5 heterozygous males were obtained. As before, 24 F_1 male lines were established for each parental male. During the four generations of backcross matings, 14 of the initial 96 F_1 male lines for the *BiT-1* stock were lost, either accidentally or due to extremely reduced transmission frequencies of the *BiT-1* chromosomes from the *cn bw* heterozygous males (such chromosomes are often associated with reduced male fertility), so that only 82 F_1 male lines were left when the F_5 male tests were performed. Only three of the initial 92 F_1 male lines were lost accidentally for the *BiT-19* stock. Five (for some, less than five) F_5 males were examined for k for each F_1 male line. Results are summarized in Table 2.

The mean k values for the *BiT-1* chromosomes in F_2 males are consistently lower than those in F_1 males. This may also be true for the *BiT-19* group, although the results are not so clear.

As was suggested in the preliminary survey, the *BiT-1* isofemale line contained *T* chromosomes in a high frequency; whereas, such chromosomes were absent or rare in the *BiT-19* isofemale line. The difference in k values between the F_1 and F_2 males in the *BiT-1* group (and possibly in the *BiT-19* group) suggests the presence of a system modifying the transmission frequency. Because any cytoplasmic factors, the *X* chromosomes and second chromosomes were all from the *cn bw* stock, and because the *Y* chromosomes were from the same isofemale line in both the F_1 and the corresponding F_2 males, a simple explanation for the decrease in k in F_2 males is to assume an autosomal modifier (suppressor) or modifiers in the *BiT-1* stock associated with either chromosome *3* and/or *4*. Through one generation of backcross matings to the standard *cn bw* females, the frequency of such modifier chromosomes in the F_2 males will be reduced to ½ of that in the F_1 males. It should be pointed out that, although "suppression" of the deviant k values in the F_1 males of the *BiT-1* group is clear, they are in all cases smaller than that of the standard, *Tokyo/cn bw* heterozygous males ($k = 0.529$). Hence, the effect of "suppression" is incomplete. The variation in k among the 20 or so F_1 males within each line was rather low, suggesting that the frequency of modifiers in the *BiT-1* stock was very high—they might have been fixed in this stock. The overall mean k of F_1 males in the *BiT-19* group, 0.503, is somewhat smaller than, but close to, the control value of 0.529. This also decreased slightly in the F_2 males ($= 0.483$). The overall k value for the *BiT-1* group at the F_5 generation, 0.355, is somewhat smaller than but nearly the same as that at the F_2 generation (0.362). This is a rather unexpected result, since the frequency of modifier autosomes should be much reduced through an additional three generations of backcrossing to the standard *cn bw* stock; therefore the overall mean k value for

TABLE 2

A list of k *values for the* BiT-1 *and* BiT-19 *second chromosomes from F_1, F_2 and F_5 males heterozygous with the* cn bw *chromosomes*

Line		F_1		F_2		F_5			
		k	n_1*	k	n_2**	k	n_3†	χ_1^2‡	p
BiT-1	A	0.455	24	0.397	9.5	—	—	4.17	<0.05
	B	0.425	21	0.311	7.8	—	—	21.00	<0.01
	C	0.463	20	0.352	8.0	—	—	16.20	<0.01
	D	0.384	24	0.338	8.0	—	—	6.00	<0.05
	E	0.476	20	0.359	8.6	—	—	16.20	<0.01
	F	0.457	17	0.415	8.2	—	—	4.76	<0.05
Mean		0.442	21.0	0.362	8.4				
	G	—	24 (22)	—	—	0.388	4.1		
	H	—	24 (20)	—	—	0.372	4.3		
	I	—	24 (18)	—	—	0.332	4.1		
	J	—	24 (22)	—	—	0.327	4.0		
Mean			24.0			0.355	4.1		
BiT-19	A	0.520	19	0.481	8.9	—	—	0.47	>0.05
	B	0.495	24	0.423	9.3	—	—	10.67	<0.01
	C	0.492	20	0.510	9.5	—	—	1.60	>0.05
	D	0.504	24	0.501	9.9	—	—	0.00	>0.05
	E	0.505	22	0.502	9.6	—	—	0.18	>0.05
Mean		0.503	21.8	0.483	9.4				
	F	—	24 (23)	—	—	0.503	4.7		
	G	—	24 (24)	—	—	0.465	4.7		
	H	—	20 (20)	—	—	0.442	4.3		
	I	—	24 (22)	—	—	0.510	4.7		
Mean			23.0			0.480	4.6		

Sampling of the second chromosomes was made from male parents of each isofemale line.

* Number of F_1 male lines chosen for each parental male. Figures in parentheses are the number of F_1 male lines that could be tested in the F_5 generation.

** Number of F_2 males tested for each F_1 male line.

† Number of F_5 males tested for each F_1 male line.

‡ χ_1^2 value based on the sign test for the difference in k between the F_1 and F_2 generations.

the F_5 males has to be considerably smaller than the k value for the F_2 males. However, as mentioned earlier, a considerable number of F_1 male lines were lost before the F_5 males could be obtained, as a result of the extremely low k values and reduced male fertility. The mean k value obtained, 0.355, therefore, is an overestimate, and the true value should be smaller. This point was further examined by repeating some of the above experiments, as follows.: Twelve males were randomly chosen from the *BiT-1* stock culture and individually crossed to two *cn bw* females. A single F_1 male was selected from each of these matings and backcrossed to two *cn bw* females. Several heterozygous F_2 males from each of the F_1 matings were chosen and crossed to a group of *cn bw* females to obtain *BiT-1/cn bw* heterozygous F_3 males. These F_3 males, ten for each of the 12 F_1 male lines, were then individually crossed to two *cn bw* females to measure the k values. This time, experiments were conducted very carefully, so that no lines were lost during

these procedures. The mean k value among the 12 lines was 0.338, which is close to the expected value for the F_3 generation $[0.362 - (0.442 - 0.362)/2 = 0.322]$.

In the above description, the word "suppression" was used to describe the phenotypic effects of the modifier chromosomes, since they "suppress" the deviant transmission ratios of *BiT-1* chromosomes from males heterozygous with *cn bw* chromosomes. The term is therefore operationally defined for a male of the specific genotype, *BiT-1/cn bw*. Actually, the function of modifier chromosomes may simply be to increase the transmission frequencies of *BiT-1* chromosomes from heterozygous male parents such that, under certain genotypic conditions, k values for the *BiT-1* chromosomes may increase to levels even higher than the expected 0.5. In such cases, the modifier chromosomes may be termed "enhancers," since their presence will result in larger deviant transmission frequencies.

Chromosome 2 sampled from females: Four females from both the *BiT-1* and *BiT-19* lines, were crossed individually to two *cn bw* males. On the average, about 20 F_1 males from each mating were chosen and crossed individually to two *cn bw* females to measure k for *BiT* chromosomes from the F_1 males. Ten F_2 males from each of the F_1 male matings were chosen, and they were crossed individually to two *cn bw* females to measure k for the F_2 males.

Another independent set of experiments similar to those described above was also conducted by choosing four females from both the *BiT-1* and *BiT-19* lines and repeating the backcross matings to *cn bw* females until F_5 heterozygous males were obtained. On the average, 20 F_1 male lines were established from each parental female, and five F_5 males were tested for k for each of the F_1 male lines. Six of the 92 *BiT-1* F_1 male lines and one of 80 *BiT-19* F_1 male lines were accidentally lost before the F_5 males could be obtained. Results are summarized in Table 3.

It should first be pointed out that genotypes of the second chromosomes sampled from a single female could be different from one another due to crossing over; whereas, only two second chromosome types (except for occasional possible male recombinants) are expected when the chromosomes are sampled from a single male parent. Therefore, when k is under the control of a polygenic system associated with chromosome *2*, a single female parent will generate many more genotypes in her progeny than will a single male parent. However, if one assumes that the population (stock) has approximately the same zygotic frequency distribution between males and females, the value of k of F_2 males, taking an overall avergae for all male or female parents separately, will remain the same, irrespective of whether the second chromosomes are sampled from male or female parents.

Table 3 shows that the k values for the *BiT* second chromosomes in F_1 males were very close to the control value of 0.529 for both *BiT-1* and *BiT-19*. In the F_2 males, the second chromosomes in the *BiT-19* group showed an overall average k value somewhat smaller than, but close to that, of the F_1 males; whereas, those in the *BiT-1* group showed a corresponding k of 0.441, which was distinctly smaller than that of the F_1 males. It was, however, much larger than the corresponding k ($= 0.362$) of F_2 males in this group when the second chromosomes

TABLE 3

A list of k *values for the* BiT-1 *and* BiT-19 *second chromosomes from F_1, F_2 and F_5 males heterozygous with the* cn bw *chromosomes*

Line		F_1		F_2		F_5		χ_1^2‡	p
		k	n_1*	k	n_2**	k	n_3†		
BiT-1	P	0.517	23	0.451	9.0	—	—	7.35	<0.01
	Q	0.527	23	0.418	9.1	—	—	15.70	<0.01
	R	0.492	24	0.473	9.5	—	—	0.00	>0.05
	S	0.489	22	0.423	8.8	—	—	11.64	<0.01
Mean		0.506	23.0	0.441	9.1				
	T	—	23 (22)	—	—	0.474	4.8		
	U	—	23 (21)	—	—	0.405	4.6		
	V	—	23 (22)	—	—	0.418	4.7		
	W	—	23 (21)	—	—	0.434	4.6		
Mean			23.0			0.433	4.7		
BiT-19	P	0.510	22	0.468	8.5	—	—	8.91	<0.01
	Q	0.524	24	0.517	9.3	—	—	0.17	>0.05
	R	0.501	21	0.522	9.0	—	—	0.43	>0.05
	S	0.503	20	0.511	9.3	—	—	0.20	>0.05
Mean		0.510	21.8	0.505	9.0				
	T	—	20 (20)	—	—	0.512	4.8		
	U	—	20 (20)	—	—	0.508	4.7		
	V	—	20 (20)	—	—	0.436	4.9		
	W	—	20 (19)	—	—	0.517	5.0		
Mean			20.0			0.493	4.9		

Sampling of the second chromosomes was made from female parents of each isofemale line.

* Number of F_1 male lines chosen for each parental female. Figures in parentheses are the numbers of F_1 male lines that could be tested in the F_5 generation.

** Number of F_2 males tested for each F_1 male line.

† Number of F_5 males tested for each F_1 male line.

‡ χ_1^2 value based on the sign test for the difference in k between the F_1 and F_2 generations.

were sampled from male parents (six k values of F_2 males in Table 2 and four in Table 3 were subjected to the Mann-Whitney U test, and the probability was calculated to be 0.0095).

The consistent difference in k between the F_1 and F_2 males in the *BiT-1* group seen in Table 3 again suggests the presence of a modifier system for deviant transmission frequency. It should be noted that the overall average k of F_1 males in the *BiT-1* group (= 0.506) is significantly larger than the corresponding k (= 0.442) of F_1 males in the same group when the second chromosomes were isolated from male parents ($p = 0.0095$ calculated in the same way as shown above). This indicates that, in addition to the probable autosomal modifiers, there is another modifying factor(s) associated with either the X chromosome or the cytoplasm, or both. A more interesting observation in Table 3, however, is that the overall average k value of F_2 males of the *BiT-1* group is distinctly larger than the corresponding k value shown in Table 2. The overall average k value for the *BiT-1* group at the F_5 generation (= 0.433) was somewhat smaller than that at the F_2

generation (= 0.441), but much larger than the corresponding k value of F_5 shown in Table 2 (= 0.355). The same trend, though less striking, can also be seen in the *BiT-19* groups. Because the two F_2 male groups should have the same genetic constitution, including cytoplasmic factors, but not for the *Y* chromosomes and since (as will be shown later) the *Y* chromosome from the standard *cn bw* stock and the *BiT-1* or *BiT-19* isofemale line behave in the same way, the cause of the difference in k values between the two sets of F_2 males, or between the two sets of F_5 males, may be traced to the very first parental generation. For some reason yet unknown, the second chromosomes transmitted from male parents show more reduced k values than do the second chromosomes transmitted from female parents when tested under the standard *cn bw* background.

Effect of the Y *chromosome*: As shown in the previous sections, the overall average k value of F_2 (and F_5) males in the *BiT-1* group was smaller when second chromosomes were sampled from male parents than when they were sampled from female parents. The only genotypic difference between those two male groups is that the former carries the *Y* chromosomes from the *BiT-1* line; whereas, the latter carries the chromosomes from the standard *cn bw* stock. There is, therefore, a possibility that the *Y* chromosomes from the *BiT-1* line "enhance" the activity of elements that cause deviant transmission frequency. In order to examine this possibility, F_1 males from the *BiT-1* group, in which the second and the *Y* chromosomes were sampled from male parents, were mated, for each male parental line separately, to $\overline{XX}/Y$; *cn bw* females that carried all chromosome pairs other than the *X* from the *cn bw* stock, which is known to contain no demonstrable modifiers for deviant transmission frequency. The progeny females of the matings, $\overline{XX}/Y$; *cn bw*, were crossed to some of the F_4 *BiT-1/cn bw* males, the grandsons of the F_2 males shown in the previous section (in these F_4 males, the cytoplasm, *X* and *Y* chromosomes and 15/16 of the third and fourth chromosomes were from the *cn bw* stock). Progeny males from this mating, *BiT-1/cn bw*, were then mated individually to *cn bw* females, and their progeny were scored for k. Since the tested males contained on the average, only 5/32 or 0.15625 (= 1/8 + 1/32) of the possible "suppressor" third and fourth chromosomes from the *BiT-1* stock, the overall average k among them should be smaller than 0.362 (*i.e.*, the overall average k for F_2 males shown in Table 2; the *Y* and ¼ or 0.25 of the third and fourth chromosomes are from the *BiT-1* stock), if in fact the *Y* chromosomes in the *BiT-1* stock have enhancing effects. A similar set of experiments was also conducted for the *BiT-19* Y chromosomes. The results are summarized in Table 4.

It is clear that there is no enhancing effect of demonstrable magnitude associated with the *Y* chromosomes from either the *BiT-1* or *BiT-19* stock.

Transmision frequency in BiT-1/BiT-19 *heterozygous males*: It was shown above that the second chromosomes transmitted from males of the *BiT-1* line showed, in the later generation, lower k values than did those transmitted from females when both were tested as heterozygotes with the *cn bw* chromosomes. This is what we would expect if the transmission frequencies of *T* chromosomes from males of natural genetic background are, in fact, higher than the Mendelian expectation of 0.5. There remains a possibility, however, that the frequency of

TABLE 4

Effects of Y *chromosomes from* BiT-1 *and* BiT-19 *isofemale lines on* k *values for the second chromosomes sampled from* BiT-1 *females*

Chromosome line no.		k_1*	*Y* from *BiT-1*	*Y* from *BiT-19*
			k	k
BiT-1	P-16	0.425 (10)	0.397 (6)	0.499 (7)
	21	0.509 (7)	0.447 (3)	0.530 (6)
	22	0.438 (9)	0.430 (5)	0.322 (6)
	Q-3	0.486 (10)	0.398 (7)	0.390 (5)
	22	0.397 (10)	0.469 (1)	0.463 (6)
	R-3	0.524 (10)	0.436 (5)	0.539 (7)
	4	0.442 (10)	0.414 (7)	0.510 (4)
	S-7	0.351 (9)	0.504 (4)	0.413 (7)
	8	0.480 (10)	0.448 (4)	0.495 (6)
	18	0.409 (9)	0.415 (4)	0.400 (7)
Overall mean		0.446	0.436	0.456

Figures in parentheses indicate the number of males tested for each corresponding F_1 chromosome line of *BiT-1* group (see text for detailed explanation).
* The k values observed in F_2 males.

T chromosomes is higher in *BiT-1* male zygotes than in females. This situation could be due to differential survival rates between the two sexes in the stock when they carry the *T* chromosomes. This possibility seems to be unrealistic since, in order to explain the observed results, it is necessary to assume a drastic difference in genotypic frequency between the two sexes in the stock. Nevertheless, it was thought worthy to examine this point further. Accordingly, the following experiment was conducted. A single female chosen from the *BiT-1* line was crossed to a single male from the *BiT-19* line. They were kept in a culture vial for three days and then discarded. A total of six replicate pair matings were made. Another six pair matings were also made for the reciprocal cross, *i.e.*, *BiT-19* female × *BiT-1* male. A single F_1 *BiT-1/BiT-19* was chosen from each parental pair mating and individually crossed to two *cn bw* females. On the average, about 20 F_2 males heterozygous with *cn bw* were chosen arbitararily from each of the F_1 matings and individually crossed to two *cn bw* females. A single F_3 male heterozygous with *cn bw* was chosen from each of the F_2 matings. It was again backcrossed to the *cn bw* females. The backcross procedure was continued until F_5 heterozygous male progeny were obtained. During this procedure, only three lines were lost accidentally from an original total of 270 F_2 males. Therefore there was practically no experimental bias caused by loss of F_2 male lines. Results are summarized in Table 5.

The difference in k between the two reciprocal parental mating groups is clear ($p = 0.0022$ after the Mann-Whitney U test). Since the *Y* chromosomes in both the *BiT-1* and *BiT-19* lines do not influence k, the difference in the two average k values should be due to differences between the two reciprocal matings in either the parental, the F_1 or both genertaions. As was shown in Table 3, most of the

TABLE 5

A list of average k *values for the second chromosomes transmitted from* BiT-1/BiT-19 *heterozygous* F_1 *males*

Parental mating		k	n_1*	n_2**
BiT-1 ♀ × *BiT-19* ♂	A	0.456	22	9.4
	B	0.509	21	9.6
	C	0.459	22	9.1
	D	0.498	23	9.6
	E	0.385	22	8.3
	F	0.481	24	9.0
	Mean	0.465	22.3	9.2
BiT-19 ♀ × *BiT-1* ♂	A	0.379	21	8.8
	B	0.368	24	8.5
	C	0.315	22	8.1
	D	0.368	24	7.4
	E	0.349	22	7.4
	F	0.364	20	8.4
	Mean	0.357	22.2	8.1

Characterization of each transmitted chromosome was made for *cn bw* heterozygous F_5 males after repeated backcrosses to standard *cn bw* females.

* Number of F_2 male lines chosen for each of the F_1 males, A through F.

** Number of F_5 males tested for each F_2 male line.

second chromosomes from the parental *BiT-1* females will be those that show k values close to 0.5. Most of the F_1 males from the *BiT-1* female × *BiT-19* male mating would receive *BiT-1* second chromosomes showing such high k values. The other second chromosomes in those F_1 males came from the *BiT-19* male parents and, as shown in Table 2, the majority of these second chromosomes show relatively high k values. Thus, a large fraction of the second chromosomes in the F_1 males of this parental mating type will show relatively high k values when heterozygous with *cn bw* chromosomes in males. In a similar way, it can easily be seen that a large fraction of the F_1 males of the reciprocal parental mating type, *BiT-19* female × *BiT-1* male, will contain one chromosome *2* from the *BiT-1* male parent, which is associated with a low k value, and the other from the *BiT-19* female parent, which is associated with a high k value.

It is perhaps worthy to mention at this point that here we can have a reasonably good expectation of the genotypes of the F_1 males from this parental mating with respect to the k values. The majority of them should be "low k"/"high k" heterozygotes, but not "low k"/"low k" homozygotes. If a Mendelian segregation ratio is assumed, we would expect to observe about 50% of the descendent second chromosomes from these F_1 males to show "low k" and the other 50% "high k." As mentioned earlier, the k value of the F_5 males in Table 2 might be an overestimate, therefore not very dependable. From Table 2, the amount of decrease in the overall average k between the F_1 and F_2 males in the *BiT-1* group is 0.080. Assuming that this decrease is due to decreasing frequency of the modifier chro-

mosomes by 50% per backcross generation and assuming the effects of the modifier are additive, the expected k value for F_4 males, when the *BiT-1* chromsomes are sampled from male parents of this stock, is calculated to the 0.302. The mean k for F_4 males when the *BiT-19* chromosomes are sampled from female parents of this stock will be slightly larger than the mean k for F_5 males shown in Table 3, 0.493, but we shall use this value as close approximation. Noting that, with respect to the number of generations backcrossed to the standard *cn bw* females, the F_4 generation in those matings corresponds to the F_5 generation of the *BiT-19* female × *BiT-1* male mating under consideration, the expected overall average k for the F_5 males will be $(0.302 + 0.493)/2$ or 0.398. This is certainly larger than the observed value of 0.357 shown in Table 5, suggesting that the transmission frequencies of "low k" second chromosomes from "low k"/"high k" heterozygous F_1 males are generally much larger than the expected 0.5.

DISCUSSION

The present observations strongly suggest that the *T* chromosomes behave like the Segregation Distorter (*SD*) chromsomes (see HARTL and HIRAIZUMI 1976 for review) in natural populations of *D. melanogaster* in Texas. It is interesting to note at this point that many of the wild populations of this species have been found to contain *SD* chromosomes in frequencies of a few percent; whereas, no *SD* chromosomes were found among more than 600 second chromosomes isolated from several wild populations in Texas (MATTHEWS, in preparation).

SLATKO and HIRAIZUMI (1975) and MATTHEWS *et al.* (1978) showed that the *T-007* chromosome isolated from a natural population of *D. melanogaster* in Texas carried a "major" element near the centromere region, which was largely responsible for the male recombination and transmission ratio distortion, and many "minor" elements distributed along the chromosome *2* arms, which had effects similar to that of the major element, but in a much reduced degree. Further analysis of the position of the major element was conducted by SLATKO (1977), who found that the element actually consisted of two or three elements, all of which were located close to and around the centromere region, which, in fact, is the region where the elements of the *SD* system are also located. Recently, HIRAIZUMI, MARTIN and ECKSTRAND (1980) reported, beside the two basic elements *Sd* and *Rsp*, the presence of a modifier locus *M(SD)* near this region, suggesting that the transmission frequency of a given chromosome from a male could be larger than, equal to or smaller than the theoretical 0.5, depending upon the genotypes of males with respect to the *Sd*, *Rsp* and *M(SD)* loci. Putting this information together, it may be that the *Sd* allele is in fact present, and segregation distortion is occurring in natural population of this species in Texas, but the combinations of alleles among the three loci mentioned above are such that the direction of distortion is reversed (k becomes smaller than 0.5) under the standard *cn bw* genetic background.

A number of questions arise, such as what is the counterbalancing selective force against k values larger than 0.5 that maintains a polymorphic status of *T* chromosomes in natural populations, and what is the specific mechanism of

"distorted transmission frequency?" Obviously, the presently available information is too limited to answer these questions, which are left for future studies. However, this study has established that the frequency of *T* chromosomes is higher among male than among female gametes under a "natural" genetic background, which suggests that the transmission frequency of this type of chromosome from heterozygous male parents is higher than the theoretical 0.5.

As shown in Tables 2 and 3, the *BiT-1* and *BiT-19* lines appear to contain chromosomes carrying only the minor (but not the major) elements described earlier. Therefore, during the course of the present investigation, some of the *T* chromosomes from the *BiT-1* line might have been heterozygous with chromosomes carrying such minor elements. Since the transmission ratios for the T chromosomes from heterozygous males under the natural genetic background were uniformly high, it can be suggested that segregation distortion takes place in favor of the *T* chromosomes (both the major and minor elements are present and therefore may behave as stronger distorters) in males heterozygous with the chromosomes carrying only the minor elements (*i.e.*, they may behave as weak distorters).

Although no evidence is available at this time, we may speculate that these minor elements cause sperm dysfunction similar to that reported for the *SD* system (HARTL, HIRAIZUMI and CROW 1967; NICOLETTI, TRIPPA and DEMARCO 1967; TOKUYASU, PEACOCK and HARDY 1977) and for the *T-007* chromosome (MATTHEWS 1981), but in much reduced frequencies. If a normal sperm maturation process requires that most genes be "turned off" during this process, any loci that are accidentally "turned on" may disturb the course of sperm maturation. The probabilities of causing such accidental events, and the resulting effects, may differ depending upon the loci, and a locus that shows an extremely strong effect will be classified as a "major" element.

Perhaps a word should be given to a possible relationship between the present observation and the so-called *P-M* system of hybrid dysgenesis in *D. melanogaster* (SVED 1976; KIDWELL and KIDWELL 1976). Following the terminology of the *P-M* system, the *cn bw* stock could be classified as *M* type and the *BiT-1* stock as *P* type, although it is not yet quite clear whether the reduced *k* values characteristic of the *T* chromosomes in this study are in fact associated with the *P-M* system. It should be pointed out here, however, that, although the reduced *k* value for the *BiT-1* chromosomes was detected under the standard *cn bw* genetic background (*i.e.*, *P* chromosomes under *M* background), this was done simply as a technique to characterize two or more types of chromosomes in nature that otherwise might be indistinguishable from one another. Obviously, the finding presented in this report has no direct relationship with hybrid dysgenesis itself, because the phenomena reported here are those occurring under the natural genetic background of, at least for the *BiT-1* line, *P* type.

Finally, the present results raise a basic question of the appropriateness of assuming that the gene frequencies in the gametes are approximately the same between the two sexes.

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