FURTHER EVIDENCE THAT THE CHROMOSOME BREAKAGE FACTOR IN *DROSOPHILA ROBUSTA* INVOLVES A MATERNAL EFFECT

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FORTY spontaneous chromosomal aberrations in *Drosophila robusta* in the laboratory were observed over a 15-month period (LEVITAN, 1962). All except two of the aberrations were found in crosses involving several generations of a certain laboratory stock. Wherever discrimination was possible, it was noted that the aberrations appeared in crosses involving females from this stock but not in crosses involving males from it, suggesting that induction of the aberrations depended on a maternal effect.

In the next 15 months, 425 additional new aberrations have appeared, most of them during a series of crosses designed to test this hypothesis more accurately. The results of these experiments, to be described in this paper, confirm the maternal effect projected from the initial data.

METHODS

These experiments followed the same basic methodology as previous work, namely, testcrossing individuals of unknown karyotype to homokaryous stock-flies and studying salivary gland smears of their larval offspring. In this series, however, the identification of aberration inducers and the rate of aberration induction was to be the primary aim of the experiment, identification of unknown karyotypes secondary. It was hoped to avoid thereby the following defects which impaired the usefulness of the earlier crosses (LEVITAN 1962) for building a strong hypothesis concerning the aberration inducing property: (1) The small numbers and heterogeneity of the tested individuals (the unknown karyotype). These consisted of small samples from a number of artificial and widely separated natural populations, hence the possibility existed that the sample crossed to stock females was accidentally biased toward certain susceptible genotypes. (2) A variable number of stock flies were used per analysis cross. As many as five stock flies were used per cross when many were available, whereas other crosses each received but a single stock fly. The records indicated only which stocks, not how many flies from the stock, were used in the crosses, hence the possibility existed that more flies were consistently used in the crosses using stock females. (3) Analyses were generally stopped at the minimum number of larvae needed to determine the karyotype of the "unknown" parent. From some crosses cells from two larvae were seen, whereas in others ten or more were studied, with obvious oppor-

tunity for unpremeditated bias. As a result, a large series of crosses using uniform procedures for both the male and the female analyses was desirable to test the indicated hypothesis.

In an attempt to ensure a large supply of stock flies, ten subdivisions of the "inducer" (ST_y -A) stock were established by brother-sister matings. Later in the experiment additional derivative lines were established, in a similar manner, from some of the initial substrains. Ten larvae were smeared from each cross establishing a new substrain to detect whether any new aberrations had arisen in the parents or progeny. Flies from these stocks (referred to as " ST_y substrains" in the rest of the paper) were then mated *individually* to flies of unknown karyotype from two laboratory (cage) populations in progress ("Experimental" section of Table 1). As a "control," about 300 individuals from these cages were mated to flies from stocks apparently lacking aberration inducing ability.

The two cage populations had been started reciprocally from the same two strains. One initial population had consisted of virgin females from a Tsala Apopka, Florida strain (TA) and males from a Chadron, Nebraska strain (CH), the other, CH virgin females $\times$ TA males. The cages will be referred to in this paper as FN and NF, respectively. The karyotypes present may be inferred from the contents of the founder strains (LEVITAN 1962).

Table 1 shows the numbers of crosses that were made. The "FN-A" and "NF-A" were the ninth-generation adults collected from the cages, after the food cups were removed, by attracting the flies into a food vial. Since the females had to be despermated, as in wild samples (LEVITAN 1955), they were mated to a later generation of stock flies than were the males. The "FN-H" and "NF-H" samples were flies that "hatched" in the removed food cups, which had been attached to stoppered glass cylinders. Being the progeny of the mature "A" flies, the "H" samples represented the tenth cage-generation. These flies were aged at least one week before mating, some of the males considerably longer because of a temporary shortage of stock virgin females.

TABLE 1
Mating scheme in this study. Details in the text

	Suspected aberration inducers	Cage sample (Source of fly with unknown karyotype)	Number of crosses	
			Using female suspected inducer	Using male suspected inducer
Experimental	Substrains of homokaryous stock ST_y *	FN-A	98	258
		NF-A	101	241
		FN-H	200	201
		NF-H	203	200
Total			602	900
Control	BF-5, ST_{p1} -A and TA homokaryous stocks*	FN-H	84	100
		NF-H	46	97
Total			130	197

* Origin and contents of the stocks are described by LEVITAN (1962).

When larvae appeared, an effort was made to study the salivary glands of eight to ten of them, even when the karyotype of the cage parent became known from the first two or three. The analyses of cage females heterozygous for arrangement in both arms of the second chromosome often extended to 25 or more larvae in order to measure crossing-over (as in CARSON 1953 and LEVITAN 1958).

RESULTS

D. robusta pair matings are commonly unsuccessful in our laboratory, probably because the long virginity period and egg-to-egg generation time of the species allow molds to gain foothold in the culture- and storage-vials, thus adding to the usual problems of mating flies compatibly, transferring them, and smearing larval salivary glands in a large series of crosses. Comparison of the number of crosses made in Table 1 with the corresponding "crosses studied" cell in Table 2 shows that some information was obtained from about 60 percent of the crosses attempted. The proportion of successes in the "control" series is not significantly different than for the matings involving ST_y flies, however, indicating no greater sterility or lower longevity among the ST_y flies.

In 11.3 percent of the crosses, data were obtained from less than the eight larvae thought desirable for uniform comparability, but the results (in terms of aberrations per larva) of these smaller samplings were found to fit the general pattern of the data. They underscore, therefore, the strength of the inducing effect and give it a statistical validity, where effective, independent of the sampling procedure.

The grand total of 11,101 larvae examined in the 1,072 crosses contributing data contained 261 new chromosomal aberrations. (If an aberration had been seen in a preliminary survey of the ST_y stock or its substrains, it was not considered

TABLE 2

Comparative encounter of new chromosomal aberrations in crosses using stock females and those using stock males

Stock used	Cage sample	Crosses using stock females			Crosses using stock males		
		Crosses studied	Crosses with at least one new aberration		Crosses studied	Crosses with at least one new aberration	
			Newly induced	Transmitted*		Newly induced	Transmitted
ST_y substrains	FN-A	66	17	1(3)	110	0	3
	NF-A	54	19	1(0)	87	0	2
	FN-H	136	57	1(2)	149	0	4
	NF-H	135	65	2(4)	147	0	4
	ST_y total	391	158	5(9)	493	0	13
Not ST_y (control)	FN-H	32	0	0(0)	58	0	0
	NF-H	29	0	0(0)	69	0	1†
Control total		61	0	0(0)	127	0	1†

* Figures in parentheses are cases where new transmitted aberrations were detected in crosses producing newly induced aberrations as well.

† Questionable; probably aberrant crossovers rather than typical aberrations (see text).

"new" when encountered in these crosses; similarly, an aberration that appeared in several different larvae or in several different crosses was not "new" after the first detection.) The two second-chromosome aberrations found in several larvae from the cross of an NF-H female to a BF-5 stock male may belong in a different category than the others. They appeared to be left arm deficiencies and duplications and, unlike all other aberrations encountered in several larvae from the same cross, were not seen again in analyses of their adult siblings. The probability is strong that they represent crossover products rather than typical rearrangements, the female having been heterozygous for the overlapping inversions *2L-1* and *2L-3* (CARSON 1958) and for inversions in four other chromosome arms. The point should not be labored, for such crossovers are so rare in *D. robusta* that their production here may also represent an "aberration." It is interesting, incidentally, that BF-5 also provided the only "exception" in the earlier series (LEVITAN 1962).

All of the other new aberrations appeared in the crosses involving ST_y stock flies, the vast majority from those using ST_y females. The 3,444 larval offspring of 391 ST_y females accounted for 244, or 93.5 percent, of the new aberrations, only 15, 5.7 percent of the total, appearing in the 5,864 larvae examined from 493 crosses to ST_y males.

There was a sharp qualitative difference in the detection of aberrations from the two sexes as well. Of the new aberrations in the crosses to ST_y females, 227, or 93 percent were seen in but a single larva each, whereas none of the aberrations encountered in the crosses to ST_y males were of this character. *Every one* of the latter appeared in two or more larvae, as did the other 17 aberrations first seen in the crosses to ST_y females. Several of these "repeated" aberrations appeared in more than one cross, resembling in this some aberrations first detected during the preliminary survey of the substrains. Now, every one of the aberrations seen in two or more larvae involved Standard chromosome arms, the arrangements of the stock parent. Aberrations found in but a single larva, on the other hand, involved non-Standard arrangements, that is, the arrangements of the unknown karyotype parent, in every instance where identification of the arrangements was possible; some illustrations of this phenomenon are given in Figure 2 of the previous report (LEVITAN 1962). This rule even operated conversely: if there was a choice, and an aberration was found to involve the Standard arrangement, it could be predicted that it would recur in other larvae, but if it involved non-Standard, it would not recur; the prediction was correct in every case. (Identification of the arrangement involved was easier in this experiment than in some of the natural-population studies because the cage flies differed from Standard in almost every chromosome arm.)

The conclusion is inescapable that the aberrations appearing in but a single larva each were induced in that zygote or in the particular gamete producing it, whereas the aberrations appearing in more than one offspring of a cross had developed in a previous generation, either in the stock fly crossed or one of his or her progenitors in the ST_y stock. In Table 2 this distinction is made by referring to the aberrations appearing in but one larva as "newly induced," those appearing

more than once as "transmitted." Wherever less than six larvae had been smeared from a cross, adult siblings of the larva with an aberration were analyzed (by brother-sister matings or by crosses to noninducer stocks) to make certain that a "repeated" aberration had not been missed by chance.

Table 2 suggests that aberration production was not uniform in all parts of the experiment. Newly induced aberrations appeared in nearly half of the crosses to NF-H males, for example, but in only about a fourth of the experiments using FN-A males. Do such differences indicate differences in susceptibility, or are they determined by differences in "inducing power" of the females responsible?

Table 3 examines this question by comparing the rate of aberration induction of the females from the various ST_y substrains. Substrain 1, failing to develop a strong stock, was not used at all, and Substrain 5 produced no aberrations in one successful cross. Since the matings with the rest also had to be done in an opportunistic manner, not all the substrains are represented in the cells of the table. The summaries, or grouped populations, do include most lines, and are more suitable for comparisons. They indicate no significant susceptibility differences between the FN and NF populations as a whole. The rate of induction was higher, however, for the newly eclosed (H) group of the two populations than for the older adult (A) males tested. The difference is not significant with any single substrain (though *P* is close to five percent for Substrain 4, the one with the best comparative data), but it is significant for the total data (chi-square of 6.2, *P* < 0.02 percent).

While thus apparently supporting the differential susceptibility hypothesis, Table 3 also suggests that the derivation of the stock female used may be a more significant factor. For many of the crosses of "H" males had been delayed because of a lack of sufficient stock virgins, and more new aberrations seemed to occur in the later crosses. Unfortunately, the laboratory records do not identify exactly the substrain transfer from which a given fly was obtained; but, consideration of the mating and analysis dates indicates subdivision of the first hundred numbers from the second hundred in each "H" male sample approximates the generation differences among the females used. After this division was made, its validity was strengthened by the observation that many of the "2nd 100" males had been mated to derivative sublines of ST_y strains 3, 4, and 10 that were not yet existent when the earlier males were mated.

These data, the "H subdivided" grouping in Table 3, confirm the impression that the later crosses tended to have a higher aberration rate than the earlier ones. Although individual strains are equivocal, the tendency is quite uniform, so that the difference in the total data is highly significant (chi-square of 9.6, *P* close to .001). Most noteworthy are the similar aberration rates for the contemporaneously mated "1st 100 H" and the "A" samples, 5.0 and 5.7, respectively. Were age of the male the important factor, the "A" rate would be expected to resemble more closely that for the "2nd 100 H" males, since the latter had to be aged about a month longer awaiting the next generation of stock virgins. Apparently some of the stocks became "stronger" inducers with time, and the fortuitous mating of fe-

TABLE 3
Rate of aberration induction, per 100 larvae, by females from various substrains of ST_y stock. The number of larvae examined is indicated by "n"

ST _y substrains																		
	2		3		4		6		7		8		9		10		All	
	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate	n	Rate
Male sample																		
FN-A	9	0.	10	0.	375	2.1	116	7.8	0	..	0	..	76	11.8	10	0.	606*	4.3
NF-A	0	..	0	..	253	2.8	0	..	0	..	0	..	144	10.4	38	10.5	435	6.0
FN-H	0	..	185	4.3	389	4.4	0	..	183	3.8	12	0.	370	8.4	66	28.8	1205	6.8
NF-H	45	8.9	184	3.8	237	4.2	46	13.0	294	6.8	40	7.5	225	11.1	127	14.2	1198	7.8
Total	54	7.4	379	4.0	1254	3.4	162	9.3	477	5.7	52	5.8	815	9.8	241	17.0	3444*	6.6
Summaries:																		
All FN	9	0.	195	4.1	764	3.3	116	7.8	183	3.8	12	0.	446	9.0	76	25.0	1811*	6.0
All NF	45	8.9	184	3.8	490	3.5	46	13.0	294	6.8	40	7.5	369	10.8	165	13.3	1633	7.3
All A	9	0.	10	0.	628	2.4	116	7.8	0	..	0	..	220	10.9	48	8.3	1041*	5.0
All H	45	8.9	369	4.1	626	4.3	46	13.0	477	5.7	52	5.8	595	9.4	193	19.2	2403	7.3
H subdivided:																		
1st 100	0	..	20	0.	458	3.5	0	..	336	6.3	0	..	441	8.4	32	0.	1287	5.7
2nd 100	45	8.9	349	4.3	168	6.5	46	13.0	141	4.3	52	5.8	154	12.3	161	23.0	1116	9.1

* Includes ten larvae from a cross to Substrain 5.

males from these to H males, rather than susceptibility differences among the males, was mainly responsible for the differences between the H and A aberration frequencies.

Whenever possible a stock was established, by mass mating the progeny, from each cross producing new aberrations. After several transfers the content of these stocks was investigated by methods similar to those described in METHODS for the formation of the ST_y substrains and the investigation of their contents prior to making the matings. Irrespective of its complexity, each of the original aberrations transmitted by a ST_y male has been found to be well established in the stock, but no additional new aberrations have been encountered in the 320 larvae observed to date. In contrast, investigation of the stocks derived from the crosses to ST_y females have turned up 43 additional new aberrations in 650 larvae.

DISCUSSION

Unlike the earlier data (LEVITAN 1962), this series uncovered new aberrations in crosses to inducer-stock (ST_y) males as well as to females. In every case, however, the aberrations appearing in crosses to ST_y males proved to be aberrations that had arisen in the stock male himself or in a progenitor of his in the stock. Newly induced aberrations, those confined to individual progeny of the cross, appeared only when the stock parent was a female. The data confirm, therefore, that this ability to induce aberrations is exclusive to the maternal parent.

The data show that the ST_y males differ from their sisters in yet another way. Each of the males carrying a new aberration, the ones responsible for the data in the "Transmitted" column of Table 2, must have had an ancestor (his mother, usually), with inducing power. Yet his daughters and grand-daughters do not show aberration inducing ability. The comparable progeny of his female siblings, on the other hand, are generally as strong (even stronger, in some cases) inducers as their mothers were. Apparently these males not only lack the power to induce aberrations, but they are also unable to pass on the trait from their maternal ancestors to their daughters. This is the strongest evidence to date that transmission of inducing power depends on more than the nuclear genome.

If it is true that this aberration-inducing property depends on a cytoplasmic inclusion, the observed steady increase in its power (Table 4) is particularly interesting. Apparently the inclusion is increasing with time and can be selected to establish "high" and "low" lines. Table 3 suggests that some such selection may have been achieved, unwittingly.

SUMMARY

A large controlled series of crosses were made to study a spontaneous chromosome breakage factor which arose about two years ago in a laboratory strain of *Drosophila robusta*. The data confirm that only the females from this strain are able to induce new chromosomal aberrations in their progeny, and certain data indicate that only the females are able to transmit the inducing trait to later gen-

TABLE 4

Increase in aberration inducing power of ST_y females since the trait was discovered

Approximate chronology	Series	Source	New aberrations observed			
			Larvae studied	Newly induced	Transmitted	Rate*
Nov. 1960–Aug. 1961	“Unclassified” ST _y	LEVITAN 1962	1450†	14	2	1.0†
Aug. 1961–Feb. 1962	ST _y -A	LEVITAN 1962	600†	11	0	1.8†
April–June 1962	NF and FN: A and early H	This report	2328	125	9	5.4
June–July 1962	NF and FN: late H	This report	1116	102	8	9.1

* “Newly induced” aberrations per 100 larvae examined.

† Approximation based on a random sampling average of nine larvae studied per cross. A more exact figure would be misleading, since detecting new aberrations was not the primary consideration in reading the smears.

erations. The results suggest a cytoplasmic basis for the aberration-producing factor.

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