EXTRACHROMOSOMAL ELEMENT DELTA IN *DROSOPHILA MELANOGASTER*. VII. RELATION TO FERTILITY IN A SECOND-CHROMOSOME LINE

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ABSTRACT

The *ID^b-45* chromosome line usually carries an appreciable amount of delta b, but it is not susceptible to the killing action of this delta. The fertility of this line was examined when it carried various amounts of delta b. More than one third of the *Cy/ID^b-45* males and females tested became sterile when they carried cytoplasm of the *Cy/Pm* stock which is assumed to carry no delta b. The number of progeny is smaller when flies of this line are raised at 25°C than at 28°C at which temperature the multiplication of delta is accelerated. The progeny number was appreciably reduced when the flies were raised at 18°C at which temperature the multiplication of delta is suppressed. This line could not be maintained at that temperature, since both males and females became sterile. Thus, the conclusion may be drawn that the presence of an appreciable amount of delta b is necessary for the gametogenesis of the *Cy/ID^b-45* flies.

VARIOUS extrachromosomal elements carried by higher organisms were found because of their defective or injurious expression in the carriers. The element denoted by delta in *Drosophila melanogaster* is no exception to the above. It was found because it kills flies carrying two sensitive second chromosomes (MINAMORI 1969a). Moreover, it does damage to the host chromosomes; it induces frequent lethal mutations (MINAMORI and ITO 1971) and dysfunctional gametes carrying counterpart chromosomes of the second chromosome in certain heterozygous males and females (MINAMORI 1970). Two variants of delta are known, delta b and delta r; each multiplies in the presence of a specific sensitive chromosome (MINAMORI 1971). The multiplication is accelerated at higher temperatures and suppressed at lower temperatures. An appreciable amount of delta b is transmitted through females and an undemonstrable amount through males of the sensitive lines. The association of delta with the sensitive lines is inseparable (MINAMORI 1969b, 1972). Based on several lines of evidence, the senior author postulated that delta may be a copy of a chromosomal gene(s) or of a certain agent which is integrated inseparably into the chromosome; possibly a particulate DNA or RNA which multiplies autonomously and may be transmitted extrachromosomally (*loc. cit.*).

Wild second chromosomes were surveyed as to their retention of delta, and an

insensitive second chromosome, symbolized by *ID*, was found to retain delta b (MINAMORI *et al.* 1970). Thus, delta appeared not to be harmful to the carrier of *ID* chromosomes as far as its killing action is concerned. The frequency of *ID* chromosomes in a natural population surveyed in 1968 was approximately 40%. Therefore, a polymorphism has become established between *ID* chromosomes and insensitive chromosomes carrying no delta. In the course of the experiment estimating mutation rate, it was found that the fertility of an *ID* chromosome line heterozygous for a *Cy* chromosome (insensitive; carrying the dominant Curly wing gene and a lethal gene) was extremely reduced when the flies carried the cytoplasm of a *Cy/Pm* stock (*Pm*, Plum eye gene and a lethal gene on a sensitive chromosome; no delta b retained). This phenomenon seemed to suggest that the existence of an appreciable amount of delta may be necessary for the fertility of the *Cy/ID* line. In this study the phenomenon was analyzed.

METHODS AND RESULTS

Reduction in fertility of Cy/ID^b-45 flies carrying Cy/Pm cytoplasm: An *ID* chromosome line, denoted by *ID^b-45*, isolated from a natural population in 1967 was employed for this study. The chromosome was combined with the cytoplasm of a *Cy/Pm* stock by mating males of the line *ID^b-45* with *Cy/Pm* females. The fertility of male and female *Cy/ID^b-45* offspring of this mating was individually tested by backcrossing them with females or males of *Cy/Pm*. In the present report, the *Cy/ID^b-45* offspring is symbolized as $[\pm]$ *Cy/ID^b-45*, in contrast to $[\delta^b]$ *Cy/ID^b-45* for the original strain which retained an appreciable amount of delta b. The results of fertility tests made for a total 3,226 flies of the $[\pm]$ *Cy/ID^b-45* condition are shown in Table 1. It is evident that both males and females of this condition show reduced fertility; more than one third of the flies tested produced no offspring when they carried the cytoplasm of *Cy/Pm*.

The number of progeny recovered from individual vials which contained four pairs of *Cy/ID^b-45* males and females was counted. The *ID^b-45* chromosome used in this experiment carried a lethal gene, and was denoted as *o-9*. This line had been maintained for about one year after establishment, as a balanced strain

TABLE 1

Fertility of males and females of the original $[\delta^b]$ Cy/ID^b-45 strain and of the $[\pm]$ Cy/ID^b-45 strain carrying cytoplasm of Cy/Pm stock

Strain	Sex	Number of flies tested	Number of sterile flies	Sterile/Total (percent)
$[\delta^b]$ <i>Cy/ID^b-45</i>	♀	421	62	14.73
	♂	1,326	85	6.41
	Pooled	1,747	147	8.41
$[\pm]$ <i>Cy/ID^b-45</i>	♀	713	250	35.06**
	♂	2,513	975	38.80**
	Pooled	3,226	1,225	37.97**

** Differs from $[\delta^b]$ *Cy/ID^b-45* condition at the 1% level of significance.

TABLE 2

Number of progeny recovered from Cy/ID^b-45 and Cy/S^b-5 flies in heterozygous condition with two different Cy chromosomes and with different amounts of delta

Genotype and delta	Number of vials counted	Number of progeny				
		Range	Total	Mean		
[δ^b] Cy/ID ^b -45 (original strain)	47	20-232	4,249	90.4	**	**
[δ^b] Cy*/ID ^b -45	24	135-286	5,256	219.0	**	]
[$\pm$] Cy*/ID ^b -45	30	8-87	1,191	39.7		
[δ^b] Cy/S ^b -5 (original strain)	76	22-241	11,978	157.6	**	**
[δ^b] Cy*/S ^b -5	32	52-192	3,553	111.0	**	]
[$\pm$] Cy*/S ^b -5	29	174-303	6,618	228.2		

[δ^b] carries an appreciable amount of delta b; [$\pm$] carries a non-demonstrable amount of delta b.

Cy*: Cy chromosome derived from Cy/Pm stock.

** Difference at the 1% level of significance.

with a Cy chromosome. Progeny counts were made for: 1) flies from the original strain (control), [δ^b] Cy/ID^b-45; 2) flies carrying the Cy chromosome of the Cy/Pm stock and the cytoplasm of the original [δ^b] Cy/ID^b-45 strain; and 3) flies carrying the same genotype as the second, but carrying the cytoplasm of Cy/Pm (the same condition as [$\pm$] Cy/ID^b-45). The flies for 2) were obtained by mating [δ^b] Cy/ID^b-45 females with Cy/Pm males, and the flies for 3) by the reciprocal mating. For comparison, a sensitive line Cy/S^b-5 was also examined for its progeny number by the same procedure. The S^b-5 chromosome had been maintained for about five years as balanced strain with a Cy chromosome, and had been extensively employed in earlier studies. This chromosome line carried a lethal gene and retained delta b steadily. All the vials were kept in an incubator at 25°C. The results obtained are shown in Table 2. The number of offspring obtained from [δ^b] Cy/ID^b-45 flies in which the Cy chromosome was replaced with that from Cy/Pm stock (Cy*) was higher than that of the original stock, being about twice the latter. However, it was markedly lower in the [$\pm$] Cy*/ID^b-45 condition as compared with both [δ^b] Cy/ID^b-45 conditions. These findings suggest that the fertility reduced by the ID^b-45 chromosome may vary appreciably according to the genetic condition of the homologous chromosome 2, and that is reduced by a reduction in the amount of delta b.

The number of progeny recovered from Cy/S^b-5 flies was generally greater than that recovered from the Cy/ID^b-45 flies. The number of progeny from flies carrying the cytoplasm of the original [δ^b] Cy/S^b-5 strain and the Cy chromosome from the Cy/Pm stock was significantly smaller than that from flies sampled from the original strain. However, it was increased when the flies carried the Cy chromosome and cytoplasm of the Cy/Pm stock. This increase in progeny number is interpreted as due to a reduction in mortality of the Cy/S^b-5 zygotes in

TABLE 3

Frequency of S^r -Cy/ S^b -5 progeny from the mating, Cy/S^b -5 ♀ × S^r -Cy/ L ♂, in which the Cy/S^b -5 females inherited delta from Cy/ID^b -45 and the mothers were raised at 18° or 28°C

Temperature	S^r -Cy/ S^b -5	Cy/L	L/S^b -5	(S:Curly) ratio
18°C	1,600	1,197	1,451	133.67
28°C	108	1,067	972	10.12

(S:Curly) ratio: the number of S^r -Cy/ S^b -5 progeny divided by the number of Cy/L progeny, expressed percent.

** Difference at the 1% level of significance.

the presence of reduced amount of delta b. It may be stated that the sensitive and the ID chromosome lines differ from each other in the reproductive response to the presence of various amounts of delta b.

Fertility of Cy/ID^b -45 flies raised at various temperatures: The fertility was examined in the presence of various amounts of delta by raising flies at 28°, 25° or 18°C. The amount of delta carried by individual flies is usually measured by the magnitude of segregation distortion in matings employing sensitive lines. However, this method could not be used for the estimation of the amount of delta b carried by Cy/ID^b -45 flies, since the ID^b -45 chromosome is insensitive to the killing action of delta. For this estimation, the ID^b -45 chromosome carried by the line was replaced by a S^b -5 chromosome (sensitive) by mating Cy/ID^b -45 females with L/S^b -5 males (L , Lobe eye gene). The Cy/S^b -5 daughters obtained from this mating were crossed with S^r -Cy/ L males, where S^r -Cy is a sensitive chromosome carrying Cy and lethal gene, and retaining delta r (MINAMORI 1971). The segregation in this mating is expected to be 1 S^r -Cy/ S^b -5 : 1 Cy/L : 1 L/S^b -5, since S^r -Cy/ Cy zygotes are inviable. However, the frequency of the S^r -Cy/ S^b -5 offspring would be lower if the Cy/S^b -5 females inherit an appreciable amount of delta b from their Cy/ID^b -45 mothers. As shown in Table 3, the ratio of S^r -Cy/ S^b -5 to Cy/L offspring (S:Curly ratio in MINAMORI 1969b, 1970) was larger when the Cy/ID^b -45 females were raised at 18°C, and smaller when the females were raised at 28°C. Accordingly, it is most likely that the amount of delta obtained by multiplication is greater when the females are raised at higher than at lower temperatures.

The number of progeny recovered from four pairs of males and females of the Cy/ID^b -45 line raised at 28°C was counted. The progeny of these flies were raised at 25°C during their preadult stage. For comparison, the number of progeny recovered from Cy/S^b -5 flies was examined by the same procedure. As shown in Table 4, the number of progeny recovered from Cy/ID^b -45 flies raised at 28°C was significantly greater than that from flies raised at 25°C (cf. Table 2). In sharp contrast, the number in Cy/S^b -5 flies was significantly smaller when the parents were raised at 28°C. This decrease may be due to an increase in the mortality of the zygotes which inherited more delta from their parents; the increase in the progeny number of Cy/ID^b -45 flies raised at 28°C may be due to an improvement in fertility of the flies in the presence of higher amounts of delta.

TABLE 4

Number of progeny recovered from [δ^b] Cy/ID^b-45 and of [δ^b] Cy/S^b-5 flies which were raised at 28°C

Strain	Number of vials counted	Number of progeny		
		Range	Total	Mean
[δ^b] Cy/ID ^b -45	60	31-215	6,685	111.4*
[δ^b] Cy/S ^b -5	80	58-209	9,608	120.1**

* Differs from the progeny number obtained in flies raised at 25°C at the 5% level of significance.

** Differs from the progeny number obtained in flies raised at 25°C at the 1% level of significance (cf. Table 2).

The fertility of Cy/ID^b-45 was also examined in flies raised for successive generations at 18°C. Forty sublines of the Cy/ID^b-45 strain were established by sampling four pairs of males and females in every generation from each line. The flies raised from each generation were divided into two groups: one was raised at 18°C and the other was returned to 25°C, and their progeny number was scored. This procedure was repeated for four generations. The results obtained are shown in Table 5. The flies (o-9) raised for one generation produced no progeny in all the sublines established. The same experiment was also carried out with the other line, γ -10, of Cy/ID^b-45. Although the number of progeny of this line was greater than that of the o-9 line and survived more generations at 18°C, all the 40 sublines became ultimately extinct at the fifth generation after establishment. The progeny number from cultures returned to 25°C did not differ from that of cultures raised at 18°C in every generation. Accordingly, it may be said that the progeny number is affected by the temperature at which the parental flies but not the progeny were raised. The findings obtained in this experiment may lead to the conclusion that the fertility of Cy/ID^b-45 flies is reduced at low temperature at which temperature the multiplication of delta is suppressed.

The fertilities of males and females of Cy/ID^b-45 (o-9 line) raised at 18°C for one generation were individually examined. A single fly emerging from each

TABLE 5

Number of progeny (average) recovered from Cy/ID^b-45 flies which were raised for successive generations at 18°C

Line	Temperature for progeny (°C)	Generations raised at 18°C			
		1	2	3	4
o-9	18°	0	—	—	—
	25°	0	—	—	—
γ -8	18°	5.9	15.0	3.4	0
	25°	33.5	0	0	0

culture at 18°C was crossed with four *cn bw/cn bw* (*cn*, cinnabar eye; *bw*, brown eye) mates, and the progeny were raised at 25°C during the preadult stage. It was observed that the mating behavior of the flies raised at this temperature was the same as that of flies raised at 25°C. After confirming the occurrence of copulation, flies were allowed to lay eggs for three days. The males and females which produced no offspring in the above test were treated as sterile. Most flies were found to be sterile; only 5 males out of 92, and 61 females out of 90 produced progeny. Therefore, it appears that male fertility may be affected more strongly than female fertility by low temperature. According to microscopic observation, few or no moving spermatozoa were found in the genital organs of most *Cy/ID^b-45* males raised at 18°C.

DISCUSSION

The reduction in fertility or appearance of sterility in *Cy/ID^b-45* flies was observed only when they carried cytoplasm of the *Cy/Pm* stock or when they were raised at low temperature; i.e., when the flies retained a minute amount of delta b. The data obtained show that the reduction in number of progeny result from reduced fertility in both male and female flies, but not from reduced viability of the offspring during the preadult stage. Therefore, it is most likely that the existence of an appreciable amount of delta b may be necessary for the gametogenesis in this strain, and that an insufficient amount may cause reduction in fertility and eventual sterility of the fly. Thus, it may be said that delta is not harmful with respect to viability of *Cy/ID^b-45* flies and, moreover, that it must be involved in normal development of gametogenesis of the fly. In an earlier report (MINAMORI *et al.* 1970), the origin of ID chromosomes was interpreted as having originated from sensitive chromosomes by accumulating polygenes which control resistance against the killing action of delta b. However, there may be an alternative interpretation. As noted earlier, the association of delta with sensitive chromosomes was inseparable; thus, delta was postulated to be a copy of a chromosomal gene(s) or of a certain agent which is integrated inseparably into the chromosome. This notion might lead to the interpretation that delta may be involved in the normal development of insensitive flies carrying delta; however, it may express injurious effects to the carrier of a certain mutant gene(s) on the sensitive chromosome. Such a situation has also been postulated by HUEBNER *et al.* (1970) for C-type RNA tumor viruses: these viruses may be produced by a virogene, and the virogene may be a necessary and useful normal gene in normal cells, but it may express oncogenic activity when the oncogene is activated. The findings described in this report appear to substantiate the above notion for delta.

A sharp contrast was observed between the fertilities of *Cy/S^b-5* and *Cy/ID^b-45* flies in response to temperature variation. The number of progeny was reduced in *Cy/S^b-5* flies and increased in *Cy/ID^b-45* flies when these flies were raised at higher temperatures. This contrast may be a consequence of the difference in sensitivity to the killing action of delta b and of the chromosome-delta interaction in the effect on fertility of these two chromosomal strains. With regard to changes

in fertility due to temperature variation, *Cy/ID^b-45* appears to be favored in warm environments in which delta thrives, in contrast to the greater fertility of the *Cy/S^b-5* in cooler environments. Some difference in temperature adaptation between local races of the same species have been known; for example, in local races of the leopard frog, *Rana pipiens*, from northern and southern localities of North America (MOORE 1939), or those of a cobitid fish, *Cobitis taenia striata*, from various streams in Japan (MINAMORI 1957). If *Cy/ID^b-45* and *Cy/S^b-5* flies are randomly sampled from different localities and compared as to their fertility at different temperatures, they may appear to be samples of two local races differing in temperature adaptation. Although such a variation caused by extrachromosomal elements has not yet been found, its presence in actual populations is hardly excluded.

The extrachromosomal element carried in races or species occurring in the wild does not appear to be injurious to its carriers, but it becomes harmful when it is associated with foreign genomes which may result in the hybrids becoming inviable or sterile. An example is the hybrid male sterility observed in crosses of local strains of the *Drosophila paulistorum* complex (EHRMAN 1960; DOBZHANSKY and PAVLOVSKY 1967). WILLIAMSON and EHRMAN (1967) and EHRMAN and WILLIAMSON (1969) could obtain sterile non-hybrid sons from females which were injected with a homogenate from flies of the other strain. Evidently the determinant does not appear to be injurious to its carrier, but causes male sterility when associated with a genome of the other strain. However, an additional factor may be involved in the hybrid inviability or sterility: absence, in the maternal strain, of some element which is carried by the paternal strain and indispensable for the existence of the strain. The distinction between these two mechanisms had not previously been demonstrated; however, the possibility of this mechanism cannot be overlooked in view of the results obtained in this study.

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