EXTRACHROMOSOMAL ELEMENT DELTA IN *DROSOPHILA MELANOGASTER* V. A VARIANT INHERITED BIPARENTALLY AT 25°C

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The extrachromosomal element denoted by delta in *Drosophila melanogaster* was found and studied in our laboratory. The results obtained were reported on in a series of papers under the same title as this one. The main conclusions from the studies relevant to the present report are given briefly below: a) Delta induces a segregation distortion in crosses of certain second chromosome lines called sensitive chromosome. The distortion was considered as due to the differential mortality of zygotes to the killing action of delta. Zygotes carrying two sensitive chromosomes were more susceptible than those carrying one or no sensitive chromosome (Minamori 1969a). b) The multiplication of delta is controlled by a certain gene or genes on a sensitive chromosome symbolized by S^b and is appreciably affected by temperature. c) The ratio distortion is produced in crosses employing females of S^b chromosome lines and not in crosses employing females of a sensitive line symbolized by S^c . Hence, S^c chromosome lines are not considered to be carrying delta. However, these S^c chromosome lines can retain delta for several generations when delta is introduced into them (Minamori *et al.* 1970). d) Delta is transmitted steadily, generation after generation, through S^b -carrying females to their progeny. It is also transmitted through S^b -carrying males, although the amount transmitted is considered to be lower than a certain threshold amount at and above which the killing action appears (Minamori 1969b). e) Delta is accumulated in an S^b line derived from S^b males and S^c females extending over many generations, and reaches an equilibrium which is expressed by the original S^b line at around the tenth generation after establishment. f) Delta appears to be associated inseparably with S^b chromosome lines, since no S^b line has been obtained which is missing delta. g) Delta induces frequent recessive lethal mutations in three definite regions of the second chromosome, possibly at an early embryonic stage of the carrier (Minamori and Ito 1970). h) Delta causes recoveries of only Curly progeny from S -Cy/ S -5 heterozygous males and females (S -Cy, S^c chromosome carrying Curly wing gene; S -5, S^b chromosome). The one-sided recovery of Curly flies from the heterozygotes occurred in the presence of an appreciable amount of delta. Data showed that delta may cause damage only to the S^b -5 chromosome at an early embryonic stage of the carrier in such a manner that the gametes receiving this chromosome may become dysfunctional (Minamori 1970).

As noted earlier, the ratio distortion was not observed in crosses employing females of S^c chromosome lines. However, it was recently found that certain S^c chromosome

lines produced a ratio distortion in matings between any two of these lines. Also, the *S-Cy/S^c* heterozygous males and females obtained in the mating produced only Curly progeny, as did *S-Cy/S-5* heterozygotes. These traits were inherited biparentally when the parental flies were derived from a line maintained at 25°C. In this study, the observations were analyzed to decide whether they were associated with an extra-chromosomal element. Data presented below suggests that the observed phenomena may be due to a certain agent which appears to be a variant of delta. Based on the results obtained so far, a working hypothesis is presented as to the origin of delta.

MATERIALS

Three *S^c* chromosome lines in the sense noted in c) of the preceding section were examined in this study. All the three carried, respectively, a recessive lethal gene. The designation and origin of these lines are as follows.

1. *S-Cy*: found in *Cy* chromosomes of the *Cy/L* stock (*L*, Lobe eye) which was supplied by Dr. C. Oshima of the National Institute of Genetics, Japan (Minamori 1969a).
2. *S-20*: isolated from a natural population in 1966 (Minamori *et al.* 1970).
3. *SD-5*: carries the Segregation-Distorter gene (*SD*; Sandler, Hiraizumi and Sandler 1959), supplied also by Dr. Oshima. The *SD* gene causes a non-random progeny recovery from *SD/SD⁺* males.

In order to determine whether the *S-Cy*, *S-20*, and *SD-5* lines carry any extra-chromosomal element, differently conditioned females of each line were used for crossings. Females were sampled from the original stock which had been maintained at 25°C since the establishment (25°C line), from a stock maintained successively at 18°C for more than twenty generations (18°C line), and from offspring of matings of males of these *S^c* lines and females of an insensitive line (*I-521*) which was recognized as not carrying delta (male passing line). The offspring obtained by matings of these chromosome lines were raised during the preadult stage either at 25° or at 18°C.

METHODS AND RESULTS

1. *Inviabilities of heterozygotes from females of an S^b line and males of the sensitive lines*

The segregation ratios in the reciprocal matings between *Cy/S-5* (*S-5*, *S^b* chromosome, Minamori 1969a) and each of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* were examined by employing females and males of the original strain maintained at 25°C (25°C line). The progeny of the mating were raised during the preadult stage at 25°C. It was reported in an earlier paper (*loc. cit.*) that there was a striking difference in the segregation ratio between the reciprocal matings of *Cy/S-5* and *S-Cy/L* when 25°C lines were employed. The segregation of this mating is expected to be 1 *S-Cy/S-5* : 1 *Cy/L* : 1 *L/S-5*, since *S-Cy/Cy* is lethal. However, the frequency of *S-Cy/S-5* among the total offspring was extremely low in the mating employing *Cy/S-5* females, and it was

Table 1. Segregations in the reciprocal matings between *Cy/S-5* and any of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* when females and males of 25°C lines were employed

Cross	Curly		Non-Curly	S-Curly ratio or non-Curly ratio (%)
	(<i>S-Cy/S-5</i>)	(<i>Cy/L</i>)		
<i>Cy/S-5</i> ♀ × <i>S-Cy/L</i> ♂	35	1,444	1,221	2.12
Reciprocal cross	1,583	1,058	1,280	149.62
<i>Cy/S-5</i> ♀ × <i>Cy/S-20</i> ♂	1,305		5	# 0.38
Reciprocal cross	1,225		630	# 33.96
<i>Cy/S-5</i> ♀ × <i>Cy/SD-5</i> ♂	1,365		0	# 0
Reciprocal cross	1,431		697	# 32.75

S-Curly ratio = $S-Cy/S-5 \div Cy/L$, # Non-Curly ratio = non-Curly $\div$ total

appreciably high in the reverse mating employing *S-Cy/L* females, as shown in Table 1. The low frequency in the former may be due to higher mortality of the *S-Cy/S-5* zygotes caused by the killing action of delta (*loc. cit.*). The high frequency in the latter may be due to lower viability of *Cy/L* zygotes in contrast to the normal viability of the *S-Cy/S-5* zygotes in the presence of minute amount of delta. To express the magnitude of ratio distortion, the number of *S-Cy* heterozygous progeny was divided by the number of *Cy/L* progeny, and expressed in per cent (S-Curly ratio) in individual matings. The segregation in the mating, *Cy/S-5* × *Cy/S-20*, is expected to be 2 Curly: 1 non-Curly flies, since *Cy/Cy* is lethal. However, the frequency of non-Curly flies among the total, denoted as non-Curly ratio, was extremely low nearly zero in the mating employing *Cy/S-5* females, and nearly normal in the reverse mating employing *Cy/S-20* females (Table 1). Similar extreme ratio distortion and sharp contrast between the reciprocal matings were also observed in the mating, *Cy/S-5* × *Cy/SD-5*. No non-Curly fly was recovered in the mating employing *Cy/S-5* females, while normal recovery in a ratio of 2 Curly: 1 non-Curly were obtained in the reverse mating. Thus, the chromosome bearing the *SD* gene, *SD-5* chromosome, may be included together with *S-Cy* and *S-20* into the category of *S*^c chromosome in the sense noted earlier, and these three lines are considered not to be carrying delta which is carried by *S-5* chromosome line.

2. Inviabilities of heterozygotes from matings between any two of the sensitive lines when females of 25°C lines were used

The segregation in the reciprocal matings between any two of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* of 25°C lines are shown in Table 2. The offspring were raised during preadult stage either at 25°C or at 18°C. The frequencies of *S-Cy* heterozygotes in both reciprocal matings of *S-Cy/L* with either *Cy/S-20* or *Cy/SD-5* were extremely low; the S-Curly ratios were usually lower than 10 percent, irrespective of raising temperatures for offspring. In the reciprocal matings between *Cy/S-20* and *Cy/SD-5*, no non-Curly fly (*S-20/SD-5*) was obtained through the matings, also irrespective of raising temperatures for offspring. It is clear that zygotes carrying any two of these three chromosomes are semilethal or lethal in the present condition. No marked difference was found in the S-Curly ratio between the reciprocal matings of any two lines,

Table 2. Segregations in the reciprocal matings between any two of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* when females of 25°C lines were employed: Offspring were raised either at 25°C or at 18°C

Cross	Raising temperature for offspring	Curly		Non-Curly	S-Curly ratio or non-Curly ratio (%)
		(<i>S-Cy/S</i>)	(<i>Cy/L</i>)		
<i>S-Cy/L</i> ♀ × <i>Cy/S-20</i> ♂	25°	82	1,629	2,061	5.03
	18°	135	1,874	2,556	7.20
Reciprocal cross	25°	89	1,878	2,049	4.74
	18°	215	3,365	3,984	6.39
<i>S-Cy/L</i> ♀ × <i>Cy/SD-5</i> ♂	25°	109	1,521	658	7.17
	18°	138	1,714	777	8.05
Reciprocal cross	25°	258	2,532	1,249	10.19
	18°	210	1,749	864	12.01
<i>Cy/S-20</i> ♀ × <i>Cy/SD-5</i> ♂	25°	1,894		0	# 0
	18°	2,135		0	# 0
Reciprocal cross	25°	2,207		0	# 0
	18°	1,802		0	# 0

* Significant at the 5% level, ** at the 1% level in χ^2 test: # Non-Curly ratio

although the difference in the *S-Cy/L* × *CySD-5* crosses was significant. This finding presents a sharp contrast to the marked reciprocal differences in the mating of *Cy/S-5* and each of these lines (cf. Table 1). The chromosomes *S-20* and *SD-5* were discriminated from each other when heterozygous for *L* chromosome. The number of *L/S-20* offspring was more than that of *Cy/L* offspring in both reciprocal matings, *S-Cy/L* × *Cy/S-20*. On the other hand, the number of *L/SD-5* offspring was less than that of *Cy/L* flies in both reciprocal matings, *S-Cy/L* × *Cy/SD-5*. The difference between chromosomes *S-20* and *SD-5* was consistently observed through the crosses made in this study.

3. Biparental transmission of the inviable trait at 25°C

To test whether there is any matroclinous inheritance as to the inviability of heterozygotes, females of a male passing line derived from males of 25°C lines and females of an insensitive line were employed for crossings, and the segregations were examined. Males used for these matings were sampled from 25°C lines. The females and their progeny were raised at 25°C during the preadult stage. It was expected that if the inviability of heterozygotes was due to some maternally inherited agent, the viability of heterozygotes from the present mating might be improved, since their mothers carried cytoplasm of an insensitive line. However, the results presented in Table 3 do not appear to support this expectation. The S-Curly ratios obtained were not so markedly higher than those obtained in the corresponding mating employing females of 25°C lines. However, the ratio was significantly higher in the matings, *S-Cy/L* ♀ × *Cy/S-20* ♂ and *Cy/SD-5* ♀ × *S-Cy/L* ♂, when compared respectively with the corresponding mating shown in Table 2. In the reciprocal matings between *Cy/S-20* and *Cy/SD-5*, no non-Curly fly (*S-20/SD-5*) was obtained either, as in the crosses employing females of 25°C lines. A similar mating experiment was made by using females of a male passing line obtained by repeated backcrosses of males of these lines

Table 3. Segregations in the reciprocal matings between any two of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* when females of male passing lines were employed: Females and their offspring were raised at 25°C

Cross	Curly		Non-Curly	S-Curly ratio or non-Curly ratio (%)
	(<i>S-Cy/S</i>)	(<i>Cy/L</i>)		
<i>S-Cy/L</i> ♀ × <i>Cy/S-20</i> ♂	157	1,841	2,619	8.53**
Reciprocal cross	103	1,746	2,206	5.90
<i>S-Cy/L</i> ♀ × <i>Cy/SD-5</i> ♂	144	1,987	1,068	7.25
Reciprocal cross	196	1,516	812	12.93**
<i>Cy/S-20</i> ♀ × <i>Cy/SD-5</i> ♂	2,576		0	# 0
Reciprocal cross	1,925		0	# 0

** Differs at the 1% significant level from the ratio in the corresponding mating shown in Table 2 (25°C line), # Non-Curly ratio

to females of the same insensitive line (*I-521*), or by using females of a male passing line which carried cytoplasm of various insensitive lines. However, no marked improvement of the heterozygous viability was obtained in these matings. Thus, it may be concluded that the inviable trait may be transmitted biparentally at 25°C.

4. Improvement of heterozygous viabilities in matings employing females of 18°C lines

In an earlier paper (Minamori 1969a), it was reported that the multiplication of delta might be suppressed at a low temperature, since the segregation was not distorted in crosses employing *Cy/S^b* females raised at 18°C. To examine whether such a modification in heterozygous viabilities of these lines is obtained, females of a line maintained at 18°C (18°C line) were used for crossings. The offspring of the crosses were raised either at 25°C or at 18°C during the preadult stage. Males used for crosses were sampled

Table 4. Segregations in the reciprocal matings between any two of *S-Cy/L*, *Cy/S-20*, and *Cy/SD-5* when females of 18°C lines were employed: Offspring were raised either at 25°C or at 18°C

Cross	Raising temperature for offspring	Curly		Non-Curly	S-Curly ratio or non-Curly ratio (%)
		(<i>S-Cy/S</i>)	(<i>Cy/L</i>)		
<i>S-Cy/L</i> ♀ × <i>Cy/S-20</i> ♂	25°	1,991	1,520	2,051	130.99
	18°	2,158	1,712	2,230	126.05
Reciprocal cross	25°	1,794	1,461	1,929	122.79
	18°	1,554	1,205	1,726	128.96
<i>S-Cy/L</i> ♀ × <i>Cy/SD-5</i> ♂	25°	1,721	1,242	1,133	138.57
	18°	2,108	1,714	1,619	122.99
Reciprocal cross	25°	1,705	1,326	1,221	128.58
	18°	2,095	1,518	1,497	138.01
<i>Cy/S-20</i> ♀ × <i>Cy/SD-5</i> ♂	25°	2,112		987	# 31.85
	18°	2,231		1,083	# 32.68
Reciprocal cross	25°	2,993		1,452	# 32.67
	18°	3,601		1,786	# 33.15

* Significant at the 5% level in χ^2 test, # Non-Curly ratio

from 25°C lines. As is shown in Table 4, the S-Curly ratios in the reciprocal matings between *S-Cy/L* and *Cy/S-20* were markedly higher, irrespective of breeding temperatures for progeny, than those in the corresponding cross employing females of 25°C lines (cf. Tables 2 and 3). A similar improvement in viabilities was found in the *S-Cy/SD-5* heterozygotes when they were derived from *S-Cy/L* or *Cy/SD-5* females of 18°C lines. The non-Curly ratios in the reciprocal matings between *Cy/S-20* and *Cy/SD-5* in the present experiment were appreciably high, also irrespective of breeding temperatures for progeny, and close to normal segregation ratio. The ratios did not deviate significantly from the expected 33.3 per cent, respectively. It is most plausible that the heterozygous inviability is due to certain extrachromosomal inherited agent which may be produced or multiplied at 25°C but not or only minutely at 18°C.

The heterozygous viabilities noted above were extremely lowered when flies of 18°C lines were raised at 25°C for one generation, and reached the normal level shown by 25°C lines, within two or three generations by raising successively at 25°C. In contrast, the viabilities increased appreciably when flies of 25°C lines were raised at 18°C for one generation, and become nearly normal within three or four generations by raising successively at 18°C.

5. *One-sided recovery of Curly progeny from S-Cy heterozygotes*

As noted earlier, it was found that only Curly progeny were recovered from *S-Cy/S-5* males and females when the S-Curly ratios of the broods were appreciably low (Minamori 1970). The *S-Cy/S-20* and *S-Cy/SD-5* heterozygotes were also examined as to whether or not they produce such a one-sided recovery of progeny. A single male or female heterozygote was sampled from individual broods of the matings and crossed with four *cn bw/cn bw* flies (*cn*, cinnabar eye; *bw*, brown eye; *cn bw* gives white eye phenotype). In Figs. 1 and 2, the percentage of Curly flies among the total offspring recovered from a single heterozygote is plotted against S-Curly ratio of the brood from which the heterozygote was sampled. The progeny recoveries from male and female heterozygotes sampled from broods showing lower than 24 per cent S-Curly ratio are presented in Fig. 1, and those from broods showing S-Curly ratios higher than 24 per cent are shown in Fig. 2. In the latter figure, the differences due to sexes of the heterozygotes and to reciprocal matings were ignored. As is known from the figures the phenomenon of one-sided progeny recovery was also observed in these heterozygotes. By inspection of the figures, the results obtained may be summarized in the following manner. a) The frequency of Curly flies among the total was either 100 per cent, or it did not deviate significantly from expected 50 per cent when non-Curly flies were recovered. b) The one-sided recovery occurred in both male and female heterozygotes. c) The one-sided recovery was observed in a total of 175 heterozygotes which belonged to the broods showing less than 74 per cent S-Curly ratios. Above 74 per cent, the recovery was always normal. d) The normal recovery also occurred in some broods which showed extremely lower S-Curly ratios, and the lower limit of the ratio for normal recovery appeared to vary with kinds of heterozygotes. e) The frequencies of Curly progeny from a total of 100 normal recoveries ranged from 41.6 to 56.6 per cent and the unweighted average was 49.32 per cent. These findings coincide well with those obtained in *S-Cy/S-5* heterozygotes except for one point. The point is that one-sided recovery

Fig. 1. Relationship between recoveries of Curly progeny from *S-Cy* heterozygotes and *S-Curly* ratios (lower than 24%) of the brood from which the heterozygote was sampled. Bracket... the mother of the heterozygote; Solid circle... female; Cross... male.

Fig. 2. Relationship between recoveries of Curly progeny from *S-Cy* heterozygotes and *S-Curly* ratios (higher than 24%) of the broods from which the heterozygote was sampled. Open triangle *S-Cy/S-20*; Open circle *S-Cy/SD-5*.

from the present *S-Cy* heterozygotes occurred for members of the brood showing higher *S-curly* ratios in contrast to the recovery in *S-Cy/S-5*. The upper limit of the *S-Curly* ratio in the former (74 per cent) was appreciably high in contrast with 17 per cent in the *S-Cy/S-5*.

The four *S-Cy* heterozygotes shown in Fig. 1 appeared to be discriminated from each other by a difference in the occurrence of normal recovery. The normal recovery

occurred more often in *S-Cy/SD-5* (12/108) than in *S-Cy/S-20* (7/77), and more in heterozygotes derived from *S-Cy/L* females (17/83) than in those derived from *Cy/S-20* or *Cy/SD-5* females (2/102). The difference in the latter was significant ($\chi^2=13.93$, $P<0.01$). Thus, some difference in the recovery may be recognized between the reciprocal hybrids, although it was not as prominent as that observed between those of the *S-Cy/L* $\times$ *Cy/S-5* matings.

DISCUSSION

The inviabilities of heterozygotes for any two of the *S-Cy*, *S-20*, and *SD-5* chromosome were observed in crosses employing females of 25°C lines, and not in the crosses employing females of 18°C lines. The mortality was not appreciably affected by temperature at which the male parents or the heterozygotes were raised. This finding seems to indicate that the mortality of these heterozygotes is due to a certain agent, transmitted maternally in these chromosome lines. Also, the multiplication of the agent may be delayed at 18°C. If this interpretation is valid, the inviability observed may not be due to an allelic lethal or semilethal gene carried by these chromosomes. Females of male passing lines produced inviable heterozygotes when the females were raised at 25°C. This finding suggests that a minute amount of the agent may be transmitted through male flies and multiply in the offspring at 25°C in such a quantity that exceeds the threshold for the killing action to the heterozygotes. Such a rapid multiplication within one generation contrasts sharply with the gradual accumulation of delta in *Cy/S-5* lines extending over many generations (Minamori 1969b). As has been already mentioned, the heterozygous viability was not modified by breeding temperatures for the heterozygotes themselves or their male parents. Therefore, it is plausible that the mortality depend solely on the quantity of the agent transmitted from mothers, but not on the quantity transmitted from father flies, or multiplied in the offspring. This leads to the interpretation that the death of the heterozygotes might be caused at an early embryonic stage, as observed in the delta-carrying heterozygotes (Minamori 1969a).

The one-sided recovery of Curly progeny observed in Figs. 1 and 2 may be induced by the agent when sibs of the same genotype were mostly inviable. The phenomenon may be induced when the agent exceeds a certain threshold amount. The progeny from these heterozygotes were either only Curly flies or Curly and non-Curly flies in a 1:1 ratio. These findings completely coincide with those obtained in *S-Cy/S-5* heterozygotes. The occurrence of this phenomenon in *S-Cy/S-5* gave rise to the interpretation that delta may cause damage to *S-5* chromosome at an early embryonic stage in heterozygotes, possibly before or immediately after the nuclear migration into polar plasm, in such a manner that gametes receiving this chromosome may become dysfunctional (Minamori 1970). The same may be applied to the present case; the agent may cause damage to the *S-20* and *SD-5* chromosome in the same manner as delta does.

The comparison of the present agent with delta carried by *S^b* lines may be sum-

Table 5. A list of sensitive second chromosomes

Symbol	Carrying delta	Sensitivity	Lines
S^b	delta b	sensitive to delta b when S^b/S^b or S^b/S^r	<i>S-5</i> , <i>S-7</i> , <i>S-9</i>
S^r	delta r	sensitive to delta b when S^b/S^r , and to delta r when S^r/S^r	<i>S-Cy</i> , <i>S-20</i> , <i>SD-5</i>
S^c	—	sensitive to delta b when S^b/S^c	<i>S-15</i> , <i>S-28</i> , <i>S-33</i>

marized in the following manner. The agent is similar to delta in: a) gene dependency of its multiplication, b) delayed multiplication at a low temperature, c) transmission through both female and male flies, d) killing certain sensitive/sensitive heterozygotes, and e) induction of one-sided recovery of Curly progeny from *S-Cy* heterozygotes. However, the agent differs from delta in: a) rapid multiplication at 25°C, and b) producing viable *S-5* heterozygotes for *S-Cy*, *S-20*, or *SD-5*. Thus, it may be suggested that the agent carried by these chromosome lines is a variant of delta. This agent is denoted hereafter as delta r, and the original delta carried by S^b chromosome lines is denoted as delta b. The notation of S^c chromosome was initially given to the chromosomes which are sensitive to delta b but accompany no delta b under usual conditions. However, the *S-Cy*, *S-20*, and *SD-5* chromosomes differ from the other S^c chromosomes in retention of delta r. Then, these three are symbolized as S^r . In Table 5, a list of sensitive chromosomes is presented.

It has been found that some extrachromosomal elements in animals are symbiotic microorganisms; *e. g.* sigma virus responsible for CO₂ sensitivity trait in *Drosophila melanogaster* (reviewed by L'Héritier 1958), certain spirochetes responsible for "sex-ratio" conditions in several species of *Drosophila* (Poulson and Sakaguchi 1961; Poulson 1968) or bacteria responsible for the killing trait in *Paramecium* (Beale and Juland 1969). Although it appears to be early to argue the entity of delta, the problem is outlined below based on the results obtained so far. The first point is that the multiplication of delta is controlled by a certain gene or genes in the S^b or S^r chromosome. Such gene dependency in multiplication of an extrachromosomal element has been known in *Paramecium aurelia* (reviewed by Sonneborn 1959). The kappa particle multiplies only in the presence of *K* gene. However, it can be eliminated from *K*-bearing individuals, and the individuals become sensitive when they do not retain kappa. In order to dissociate delta from the sensitive chromosome lines, repeated backcrosses of *Cy/S-5* males to *Cy/Pm* females, and successive raising of the line at a low temperature were made. However, these attempts ended in failure. Secondly, it must be pointed out that delta is transmitted through male and female flies. Only two instances of biparental transmission of an extrachromosomal element or virus in *Drosophila* have been found, except for the present case. Sigma is inherited through males of sensitive lines, but not from males through two successive generations (*loc. cit.*). The other instance is the extrachromosomal inherited melanotic Freckled trait in *Drosophila melanogaster* (Barigozzi and Sari Gori 1968). However, both sigma and Freckled are not so strongly gene dependent as in delta. The third point is that delta resembles a virus in its

action on chromosomes of the carrier. It causes some damage to chromosomes and induces frequent mutations, as with the sigma virus (Baumiller and Lewis 1968), Rous sarcoma virus (Burdette and Yoon 1967, Yoon 1969) or viruses carried by other insects (Gershenson 1969) which induce mutations of *Drosophila melanogaster*.

The characteristics noted above lead to the conclusion that delta may be a biparentally inherited virus whose multiplication is controlled by a chromosomal gene of the fly. However, this notion appears to be insufficient in explaining the nature of the inseparable association of delta with the S^b or S^r chromosome line. The author postulated in an earlier paper (Minamori 1969b) that delta might harbor on the S^b chromosome and send out its replicates into the extrachromosomal space of the cell. The tentative hypothesis presented here is that delta may be a copy of a chromosomal gene or genes, or of a certain agent which integrates inseparably into the chromosome of the fly; possibly a particulate DNA or RNA which multiplies autonomously and is transmitted extrachromosomally. A similar hypothesis has been presented for the C-type RNA tumor viruses (*e. g.* Rous sarcoma virus, murine leukemia virus). Huebner and Todaro (1969) postulated that the viral information (the virogene), including that portion responsible for transforming normal cell into a tumor cell (the oncogene), is most commonly transmitted from animal to progeny animal and from cell to progeny cell in a covert form. The viral genome is presumed either a) to be integrated in the chromosomes of host cell, or b) to be a part of the host genetic material located in the chromosome. Serra (1968) presented a model for the extrachromosomal determinants originating from the chromosomal gene. Viewed in the light, it appears not unreasonable to postulate the chromosomal origin of delta.

The Segregation-Distorter gene *SD* (Sandler, Hiraizumi and Sandler 1959) produces a ratio distortion in the recovery of progeny from *SD/SD*⁺ heterozygous males. A male produces dysfunctional *SD*⁺-bearing spermatozoa, and thus *SD* are recovered in great excess in the progeny. This phenomenon differs from the one-sided progeny recovery noted in the present report in two points; a) both *SD* and *SD*⁺-bearing progeny are recovered in distorted ratio in contrast to only Curly progeny recovery in the present case, and b) the recovery occurs only in male heterozygotes in contrast to the occurrence in both male and female heterozygotes in the latter. No confirmatory evidence is known as to whether the *SD* induced segregation distortion is concerned with any extrachromosomal element. To examine the relation of delta to this phenomenon, the progeny recovery from *S-Cy/cn bw* and *S-20/cn bw* males was tested by mating with *cn bw/cn bw* females (*cn bw* is used as an *SD*⁺ line for routine *SD*-test). However, the progeny recovery in these males were normal; *S-Cy* vs. *cn bw*, or *S-20* vs. *cn bw* were recovered in nearly 1:1 ratio (unpublished). Since these *S-Cy* and *S-20* bearing males should carry delta r, it may be said that delta r is not, or not directly concerned with the *SD* induced segregation distortion phenomenon.

SUMMARY

1. A variant, denoted by delta r, of the extrachromosomal element delta (delta b) in *Drosophila melanogaster* was examined. The variant was carried by certain second

chromosome lines, symbolized *S-Cy*, *S-20*, and *SD-5* (Sandler, Hiraizumi and Sandler, 1959).

2. These three chromosomes were inviable when heterozygous for any of the other two in matings employing females of a line maintained at 25°C, or in matings employing daughters from males of these lines and females of an insensitive chromosome line (carrying no delta). Thus, delta r appeared to be transmitted biparentally at 25°C.

3. However, the heterozygous viabilities were normal when females of a line maintained at 18°C were used for matings. It is most likely that the multiplication of delta r may be delayed at 18°C.

4. Male and female heterozygotes for *S-Cy* and either *S-20* or *SD-5* produced only Curly phenotyped progeny in the presence of an appreciable amount of delta r.

5. A working hypothesis was presented that delta may be a copy of a chromosomal gene or genes, or of a certain agent which integrates inseparably into the chromosome of the fly.

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