EXTRACHROMOSOMAL ELEMENT DELTA IN *DROSOPHILA MELANOGASTER*. IX. INDUCTION OF DELTA-RETAINING CHROMOSOME LINES BY MUTATION AND GENE MAPPING

SUMIO MINAMORI AND KINUE SUGIMOTO

Zoological Laboratory, Faculty of Science, Hiroshima University, Hiroshima, Japan

Manuscript received July 12, 1972
Revised copy received March 13, 1973
Transmitted by E. W. CASPARI

ABSTRACT

[Delta b], symbolized as [δ^b], is retained by S^b chromosome lines and transmitted through the females to their progeny. Transmission through the males is not directly demonstrable (MINAMORI 1969a). [delta r], symbolized as [δ^r], is retained by S^r chromosome lines and transmitted biparentally (MINAMORI 1971). The multiplication of delta is suppressed at low temperature. All descendant lines derived from S^b-carrying or S^r-carrying flies in which the presence of delta cannot be demonstrated gradually accumulate their specific delta factors over many generations (MINAMORI 1969b, 1972). The delta factors and the sensitive chromosomes are inseparably associated. This observation led to the assumption that delta may be a copy of a chromosomal gene or a certain agent integrated into the chromosome (MINAMORI 1972). This assumption was examined in the present study by experiments designed to induce delta-retaining sensitive chromosomes, and to map the gene(s) responsible for delta-retention and/or for sensitivity to the killing action of delta factor. One sensitive chromosome which retained [δ^b] (S^b chromosome) was obtained in the presence of [δ^b] out of 2492 insensitive chromosomes which retained no delta; in addition one S^b chromosome was obtained in the presence of [δ^r] out of 2131 insensitives. The latter finding suggests that S^b might be induced by a mutation caused by [δ^b] or [δ^r], but not by integration of either delta into the chromosome. Four S^b chromosomes and one sensitive chromosome which retained [δ^r] (S^r chromosome) were obtained out of 1970 insensitives when males carrying the chromosome were fed an alkylating mutagen, ethyl methane sulfonate (EMS). The location of delta-retaining genes was examined by crossing-over experiments employing eight S^b and five S^r chromosomes. The genes on these chromosomes were found to be located in the same region or near one another. The gene for [δ^b], symbolized as Da^b, and the gene for [δ^r], symbolized as Da^r, are assumed to be multiple alleles of a locus at 2-24.9. The sensitivity of the chromosomes was modified appreciably by recombination; hence, the genes controlling this trait are assumed to be a polygenic system. The findings obtained in this study lead to the hypothesis that delta may be produced by a chromosomal gene (*Da*) and transmitted extrachromosomally.

CERTAIN extrachromosomal elements have been shown to be infectious or symbiotic microorganisms—e.g., spirochetes responsible for "sex-ratio" con-

ditions in Drosophila (POULSON and SAKAGUCHI 1961; POULSON 1968); bacteria causing the killing characters in Paramecium (BEALE, JULAND and PREER 1969); and viruses for CO_2 sensitivity in *D. melanogaster* (reviewed by L'HÉRITIER 1958, 1970) and scrapie in sheep (PARRY 1962; reviewed by ABINANTI 1967). These microorganisms multiply in the host and are transmitted, generation after generation, through the female side to their progeny. The genetic information carried by these elements may be independent of that carried by the chromosomes, similar to cell organelles such as mitochondria, chloroplasts, or kinetoplasts.

The extrachromosomal element denoted by delta appears virus-like in damaging host chromosomes. It induces frequent recessive lethal mutations in definite regions of chromosome 2 and dysfunctional spermatozoa carrying a specific homolog of the same chromosome (MINAMORI 1970; MINAMORI and ITO 1971). Most lethals (about 82%) induced by delta are located on the right arm, especially in the Lobe region (2–72.0). Delta may have killing action on its carriers; it kills zygotes carrying two sensitive chromosomes at an early embryonic stage, but not those carrying one sensitive chromosome (MINAMORI 1969a).

The presence of delta depends strongly on specific second chromosomes; sensitive chromosomes are symbolized by S^b or S^r and an insensitive chromosome is symbolized by *ID*. The S^b retains [delta b], symbolized as [δ^b], and is sensitive to the killing action of this delta but not to [delta r], symbolized as [δ^r]; S^r retains [δ^r] and is sensitive to both [δ^b] and [δ^r]; *ID* is insensitive to either delta (MINAMORI *et al.* 1970; MINAMORI 1971). [δ^b] is transmitted by S^b-carrying females to their progeny, but transmission through the males cannot be demonstrated. In contrast, [δ^r] is transmitted biparentally at 25°C. The multiplication of delta is suppressed at low temperature, hence, S^b or S^r-carrying flies in which the presence of delta is not demonstrable can be obtained. However, the descendant lines derived from these flies gradually accumulate their specific delta factors over many generations, without exception. This is also true for the descendant lines from S^b-carrying males. The association of delta with the sensitive chromosomes was shown to be inseparable since all attempts to separate them ended in failure (MINAMORI 1969b, 1972). Based on this fact, the entity delta was assumed to be a copy of a chromosomal gene or a certain agent integrated inseparably into the chromosome (MINAMORI 1971, 1972). In this study these alternatives are examined. The first experiment was designed to induce sensitive, delta-retaining chromosomes from an insensitive chromosome which retained no delta. In the second experiment, the location of gene(s) responsible for the retention of delta and/or sensitivity to the killing action of delta was examined by employing various sensitive chromosomes. In this article the results obtained are presented.

MATERIALS AND METHODS

Testing for sensitivity and delta-retention of second chromosomes: Sensitivities of second chromosomes to the killing action of [δ^b] or [δ^r] were examined by scoring viabilities of the chromosomes when heterozygous for an S^b or S^r chromosome and in the presence of [δ^b] or [δ^r]. The S^b lines used were the bw^D chromosome (carrying the gene for brown eyes; MINAMORI

1969a) and the S^b-*9* chromosome (isolated from a natural population in 1965; *loc. cit.*), and the S^r chromosome was S^r-*20* (isolated in 1966; MINAMORI 1971). These sensitive lines steadily retained an appreciable amount of each specific delta; the S^b lines retained [δ^b], and the S^r retained [δ^r]. Males heterozygous for the *Cy* chromosome (Curly wing gene on an insensitive second chromosome which contains large inversions in both arms and retains no delta) and a second chromosome to be tested were mated with *Cy* females heterozygous for one of the sensitive chromosomes; the viability of the non-Curly offspring from this mating was scored. Chromosomes showing a significantly lower viability of non-Curly when heterozygous for bw^D or for S^b-*9*, but not for S^r-*20* were designated as S^b. Chromosomes showing lower viability when heterozygous for each of these S^b and S^r-*20* were designated as S^r.

The retention of delta in sensitive chromosome lines was tested by mating females heterozygous for *Cy* and a tested chromosome with males heterozygous for *Cy* and either of the three standard sensitive chromosomes. Lines showing a significantly lower viability of non-Curly offspring when heterozygous for each of the above chromosomes were presumed to carry [δ^b]. Lines showing lower viabilities when heterozygous for S^r-*20*, but not when heterozygous for either of the S^b chromosomes were presumed to retain [δ^r]. The delta-retention in insensitive chromosome lines was examined by replacing the insensitive chromosome carried by females with the bw^D chromosome, by setting up the mating ♀ *Cy/I* × ♂ *Cy*/bw^D, where *I* represents the insensitive chromosome. The *Cy*/bw^D females obtained would obtain delta, if it had been transmitted to them by the females of the initial insensitive line. The delta-retention in *Cy*/bw^D was tested by mating the females to *Cy*/S^b-*9* males. The viability of bw^D/S^b-*9* offspring of this mating should be lowered in the presence of an appreciable amount of [δ^b].

Induction of sensitive chromosomes: An insensitive second chromosome, symbolized as *I-521*, whose strain retained no delta was employed throughout the experiments. As mutagens, [δ^b] carried by the bw^D line, [δ^r] carried by S^r-*Cy* (sensitive to [δ^b] and [δ^r]), and an alkylating mutagen ethyl methane sulfonate (EMS) were used. The insensitive chromosome *I-521* was examined for mutations induced in the presence of [δ^b] by the mating scheme shown in Figure 1. The *Pm* chromosome used was an insensitive chromosome carrying the eye color gene Plum and the *Pm* strain retained no delta. The *I-521* chromosomes carried by spermatozoa of bw^D/*I-521* F_1 males which had inherited [δ^b] from the *Cy*/bw^D mothers were tested for their viability. In order to test the chromosome, *Cy/I-521* F_2 males were collected, and individually mated to *Cy*/bw^D females which retained [δ^b]. In F_3, the percentage of bw^D/*I-521* flies (expected 33.3%) would become lower if the *I-521* had changed into a sensitive chromosome. Mutagenesis in the presence of [δ^r] was examined by a similar mating scheme employing *I-521* chromosomes carried by spermatozoa of S^r-*Cy/I-521* F_1 males containing [δ^r], instead of the bw^D/*I-521* F_1 males. These S^r-*Cy/I-521* males were obtained by mating S^r-*Cy/L* (L = Lobe eye) females carrying [δ^r] to *Cy/I-521* males.

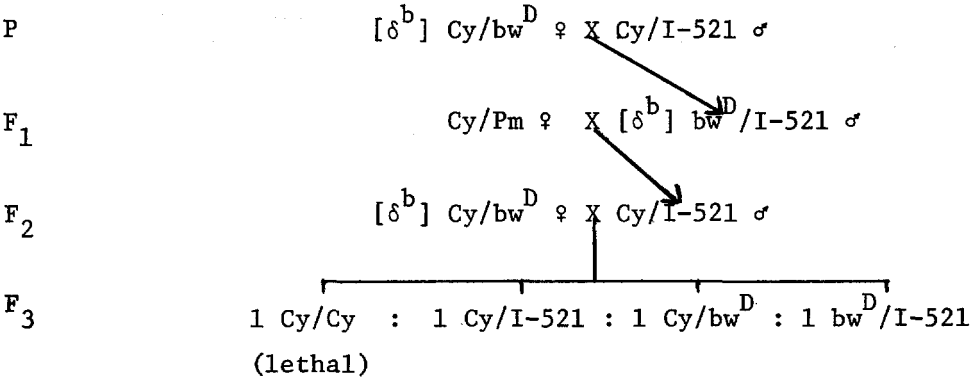

FIGURE 1.—See text.

The mutagenesis by EMS feeding was also attempted (cf. LEWIS and BACHER 1968). Males *Cy/I-521* were fed on kleenex saturated with an 0.025M solution of EMS in sterile 1% sucrose solution for 24 hours. After this period they were shaken into a fresh culture bottle. They were then mated with *Cy/Pm* females for 48 hours, and both males and females were discarded. *Cy/I-521* males recovered from the bottle were crossed individually with Cy/bw^D females, and the viability of bw^D*/I-521* offspring recovered was scored.

Mapping of gene(s) responsible for delta-retention: The location of gene(s) responsible for delta-retention (denoted as "delta-retaining gene") was examined by mapping the sensitivity of the chromosome. Five S^b and four S^r chromosomes which had been isolated from a natural population, *SD-5* chromosome (SANDLER, HIRAIZUMI and SANDLER 1959; S^r, MINAMORI 1971) and three S^b chromosomes induced in this study were used for mapping. The S^r chromosomes used (S^r*-28*, S^r*-32*, S^r*-40*) were detected in S^c lines (MINAMORI *et al.* 1970) by testing them for their sensitivity to [δ^r]. In a preliminary test the gene was shown to be located at the right of the *Sp* locus (Sternopleural, 2–22.0); then, we attempted to determine its location by crossing-over values with respect to three dominant marker genes *S*(Star, 2–1.3), *Sp* and *Bl*(Blistle, 2–54.8). However, this procedure proved to be invalid, as is shown in the RESULTS section.

The location of the gene on S^b*-5* was determined by its crossing-over value with respect to the *Sp* locus. Females S^b*-5/Sp* were crossed to *Cy/Pm* males, and the *Pm/Sp* males recovered were collected. The *Sp* chromosomes (recombinant or nonrecombinant) carried by the males were individually tested for their sensitivity by mating the males with Cy/S^b*-9* females which retained [δ^b]; thus the frequency of sensitive *Sp* chromosomes in the population was obtained. Secondly, the gene location on various sensitive chromosomes was determined by crossing-over values with respect to the locus on the S^b*-5* chromosome. Females heterozygous for a sensitive chromosome and S^b*-5* were crossed with *Cy/Pm* males, and the Plum sons recovered were collected. The non-*Pm* chromosomes carried by the sons were individually tested for sensitivity by mating them individually with Cy/S^b*-9* females. In testing for S^b*-9*, Cy/S^b*-16* females were used.) Thus the frequency of insensitive non-*Pm* chromosome produced by recombination was obtained.

RESULTS

1. *Induction of delta-retaining sensitive chromosomes:* A total of 8,773 insensitive chromosomes, *I-521*, which retained no delta were tested as to whether or not they became sensitive in this experiment, and seven chromosomes were found to be sensitive. One such chromosome was obtained in the presence of [δ^b] together with 2,491 insensitives, and another such chromosome was obtained in the presence of [δ^r] out of 2,131 tested; no sensitive was obtained in 2,180 controls. In contrast, five sensitives were obtained by feeding EMS, in addition to 1,965 insensitives. These induced sensitives were examined further as to their sensitivity to the killing action of [δ^b] carried by Cy/S^b*-9* and of [δ^r] carried by Cy/S^r*-20* females (Table 1). The seven chromosomes varied appreciably in their viabilities (ranging from 0 to about 76%) in heterozygous condition with S^b*-9* and in the presence of [δ^b]. However, six chromosomes shown in the table (S^b*-101–*S^b*-106*) were not sensitive to [δ^r] when heterozygous for S^r*-20*; hence, they were assumed to be S^b chromosomes. In contrast, chromosome S^r*-107*, which had been induced by EMS, was lethal when heterozygous for either S^b*-9* or S^r*-20* in the presence of either delta; hence, this was proved to be an S^r chromosome. It must be stressed that one S^b chromosome, S^b*-102*, was induced in the presence of [δ^r], and that both S^b and S^r chromosomes were induced by EMS.

It has been shown that S^b and S^r lines gradually accumulate their specific deltas over a number of generations in the progeny of males of these lines

TABLE 1

Viabilities of induced sensitive chromosomes in heterozygous condition with S^b-9 *or with* S^r-20 *in reciprocal matings*

Symbol of chromosome	Mutagen	Viability when heterozygous for				Delta retained
		(♀)S^b-9	(♀)S^r-20	(♂)S^b-9	(♂)S^r-20	
S^b-101	[δ^b]	15.71	101.41	7.71	0	[b]
S^b-102	[δ^r]	75.69	105.32	71.65	68.25	[b]
S^b-103	EMS	67.04	109.03	71.58	59.62	[b]
S^b-104	EMS	43.95	97.35	57.76	63.83	[b]
S^b-105	EMS	0	103.32	23.55	19.68	[b]
S^b-106	EMS	34.14	105.38	38.73	42.05	[b]
S^r-107	EMS	0	36.19	101.20	25.66	[r]

The strains Cy/S^b-9 and Cy/S^r-20 employed retained [δ^b] or [δ^r], respectively. *Cy* females heterozygous for induced sensitive chromosomes were sampled from each line during the 10th generation after induction. Standard viability = 100.00.

(MINAMORI 1969b, 1971, 1972). Therefore, the induced sensitives were maintained for ten generations at 25°C, and then the amount and kind of accumulated delta were determined by mating *Cy* heterozygous females of these lines to males of Cy/S^b-9 (test for [δ^b]) and Cy/S^r-20 (test for [δ^r]). The viabilities of offspring heterozygous for each of the six induced S^bs and either S^b-9 or S^r-20 were appreciably reduced (8%–72%). This was also true for chromosome S^b-102, which was induced in the presence of [δ^r]. The viability of offspring carrying the induced S^r chromosome, S^r-107, was only lowered when it was heterozygous for S^r-20, and not for S^b-9. Therefore, it is most likely that the induced S^b lines accumulated [δ^b] and the S^r line accumulated [δ^r]. Hence, it may be concluded that each of the sensitive chromosomes retained its specific delta in the induction.

2. *Identification of the gene(s) responsible for sensitivity and for delta-retention:* The finding noted above suggests that a single event may induce both sensitivity and delta-retention, since these two traits were induced simultaneously by mutation. This assumption was further tested by examining delta-retention in insensitive recombinant chromosomes derived from S^b-5/*Sp Bl L* (the *Sp Bl L* is quasi-insensitive) females which carried [δ^b]. The heterozygous females were mated to Cy/S^b-9 males. Among the offspring of this mating, heterozygotes for S^b-9 and recombinants carrying sensitive gene(s) would mostly be eliminated, since they are inviable in the presence of [δ^b]. The non-Curly males carrying some of the dominant markers (S^b-9/recombinant) which emerged were collected (discarding wild-type and *Sp Bl L* flies) and again tested for their sensitivity to [δ^b] by mating the males with Cy/bw^D females carrying [δ^b]. Recombinants which showed significantly lower viabilities in this mating were discarded, and those recombinants which showed normal viability (insensitive) were tested for delta-retention. Curly females heterozygous for the insensitive recombinant were individually examined as to whether or not they carried [δ^b] by the procedure described in the previous section. No insensitive recombinant which retained [δ^b] was found among a total of the 436 lines which carried the follow-

TABLE 2

Frequency distribution of non-Curly recombinants recovered from females S Sp Bl/*sensitive which carried [δ^b] or [δ^r] and males* Cy/S^b-9 *or* Cy/S^r-32

Recombinants recovered	Original sensitive chromosome			
	S^b-5	S^b-16	S^r-20	S^r-28
S Sp Bl	1,496	575	1,341	720
+	384	191	395	208
Sp	739	475	655	431
S Bl	44	21	41	14
Sp Bl	342	183	363	256
S	65	28	71	36
S Sp	110	52	133	39
Bl	8	0	19	0
Total	3,188	1,525	3,018	1,704

Location of markers: *S* 1.3; *Sp* 22.0; *Bl* 54.8.

ing marker gene(s): *Sp, Bl L, Sp L,* or *Sp Bl.* Thus, it may be concluded that the sensitivity of chromosomes is not expressed in the absence of the delta-retaining gene on the same chromosome in these experiments.

3. *Determination of location of delta-retaining gene:* We tried to determine the locations of the delta-retaining genes on various sensitive chromosomes by determining the crossing-over values with respect to three dominant genes, *S, Sp* and *Bl.* The gene had been previously shown to be located on the left arm of chromosome 2. Non-Curly flies which emerged from mating *S Sp Bl*/sensitive females retaining delta to *Cy*/S^b-9 males were classified into eight classes according to their phenotypes. The frequency distributions of the eight phenotypes were very similar in different sensitive lines. As an example, the results obtained with two S^b and two S^r lines are shown in Table 2. The frequency of *Sp* (heterozygous of *Sp* recombinant and S^b-9) was markedly higher than that of its reciprocal recombinant *S Bl* (heterozygous for *S Bl* recombinant and S^b-9); therefore, most *Sp* recombinants appear to be fully viable and most *S Bl* recombinants appear to be inviable. Accordingly, most *Sp* recombinants do not carry the delta-retaining gene, and most reciprocal recombinants *S Bl* do carry this gene; hence, the gene appears to be located near *Sp.* More *Sp Bl* recombinants were recovered than *S Sp* recombinants in spite of a longer distance between *Sp* and *Bl* (32.8 unit) than between *S* and *Sp* (20.7 unit). This finding suggests that the gene may be located between *Sp* and *Bl,* the right of *Sp.* This conclusion was borne out in all the S^b and S^r lines examined. The frequency of wild-type flies among non-Curly offspring was much higher than expected, assuming that the flies recovered are heterozygous for an insensitive recombinant and S^b-9. It was shown in the experiment noted below that the sensitivities of chromosomes are modified appreciably by recombination. Therefore, some proportion of the wild-type flies may carry the delta-retaining gene, and the crossing-over value estimated from the data was considered to be incorrect.

Next, the location on the S^b-5 chromosome was determined from the crossing-over value with the *Sp* locus. Males *Cy*/S^b-5 were mated to *Cy/Sp* (*Sp* chromo-

some is insensitive) females which carried no delta, then the S^b-5/*Sp* daughters of this mating were mated with *Cy/Pm* males. The *Pm/Sp* males recovered were collected and individually mated with *Cy*/S^b-*9* females carrying [δ^b], and the viability of non-Curly offspring (*Sp*/S^b-*9*) was scored. Among a total of 732 *Sp* recombinants tested, 21 were sensitive. Thus, the crossing-over value between *Sp* and the delta-retaining gene on S^b-5 was estimated to be 2.9%, by assuming that the *Pm/Sp* males sampled were equally viable irrespective of whether or not they carried the delta-retaining gene. Thus, this gene may be located at 24.9 on chromosome 2, since it was recognized to be located on the right of the *Sp* gene (22.0).

The localization of the delta-retaining genes on various sensitive chromosomes was determined by their crossing-over values with respect to the locus on S^b-5. For the estimation, seven S^b and five S^r were selected. Plum males heterozygous for recombinants between S^b-5 and each of the chromosomes tested were assumed to be equally viable, irrespective of the recombinant they carried, sensitive or insensitive. To test for the sensitivity of recombinants, the Plum males were mated individually with *Cy*/S^b-*9* (or *Cy*/S^b-*16*) females which carried [δ^b]. Although each of the sensitive chromosomes isolated from wild flies produced no viable flies when heterozygous for S^b-*9* (or for S^b-*16*), some proportion of the recombinants produced viable flies in varying numbers when heterozygous for the tester chromosome (Table 3). In contrast, some proportion of the recombinants derived from induced sensitives and S^b-5 produced no viable heterozygotes,

TABLE 3

Frequencies of sensitive and insensitive chromosomes recovered from recombination between S^b-5 *and each of twelve sensitive chromosomes*

Symbol of chromosome	Sensitivity*	Sensitive		Insensitive	Total	Percent insensitive	Source
		Complete lethal	Semi-lethal or subvital				
S^b-*7*	0	131	438	0	569	0	
S^b-*9*	0	312	245	0	557	0	isolated from
S^b-*16*	0	50	488	5	543	0.92	wild flies
S^b-*18*	0	645	93	6	744	0.81	
S^b-*101*	15.71	405	150	1	556	0.18	induced
S^b-*104*	43.95	397	191	0	588	0	by
S^b-*106*	34.14	430	223	5	658	0.76	EMS
S^r-*20*	0	264	444	3	711	0.42	
S^r-*28*	0	49	471	7	527	1.33	isolated from
S^r-*32*	0	103	472	9	584	1.54	wild flies
S^r-*40*	0	129	396	3	528	0.57	
SD-5†	0	106	599	10	715	1.40	from SANDLER, HIRAIZUMI and SANDLER (1959)

* Expressed by heterozygous viability for S^b-*9* or for S^b-*16* in the presence of [δ^b]. Normal = 100.00.

† An S^r chromosome (MINAMORI 1971).

even though the original sensitives had produced viable heterozygotes. Recombinants producing a significantly smaller number of progeny when heterozygous for the tester chromosome were assumed to carry the delta-retaining gene. All of the twelve sensitive chromosomes tested gave rise to sensitive recombinants which produced no progeny, or a small number of viable flies, in heterozygous condition. The frequency of recombinants which produced viable flies varied appreciably depending on the original chromosome employed. The frequency of insensitive recombinants was very low for each wild sensitive chromosome tested, being less than 1% through the S^b lines, and less than 1.54% through the S^r lines. It was also very low in induced sensitives. If the insensitives are due to crossing over between the delta-retaining gene on S^b-5 and that on the other chromosome, it is likely that the genes are located at the same locus or at closely-linked loci on the S^b and S^r chromosomes.

DISCUSSION

It has been shown that delta is always accompanied by a sensitive chromosome, and that the association is inseparable (*loc. cit.*). In this study, the relationship between delta and the chromosomes was examined to determine whether delta is a gene product or a copy of an agent integrated into the chromosome. The answer seems to be that some delta-retaining sensitive chromosomes have been obtained by mutation from an insensitive chromosome which retained no delta. Both S^b and S^r chromosomes were induced by $[\delta^b]$ and $[\delta^r]$, and by EMS, and these chromosomes were demonstrated to retain $[\delta^b]$ or $[\delta^r]$. Moreover, induction of an S^b chromosome by $[\delta^r]$ permits us to reject the second assumption that delta may be a copy of an integrated agent. If this assumption were correct, $[\delta^r]$ should induce an S^r chromosome retaining $[\delta^r]$. The S^b chromosome noted above may, most plausibly, have been induced by mutagenesis caused by $[\delta^r]$, and not by the integration of this delta into the chromosome. This may also be true for the S^b chromosome induced by $[\delta^b]$. The delta-retaining gene on the S^b-5 chromosome was shown to map at 24.9 in chromosome 2, and those carried by other S^b chromosomes were found to map on the same locus, or close to it, within 0.92 map units. Since more exact identification of these genes appears to be impossible with the methods used in this study, the genes responsible for $[\delta^b]$ are tentatively assumed to be located at the same site on the chromosomes. The genes responsible for $[\delta^r]$ on various S^r chromosomes were also found to map close to the above locus; thus, the gene for $[\delta^r]$ is assumed to be located at the same locus as the gene for $[\delta^b]$. These two genes may be called multiple alleles, since each retains and possibly produces its specific delta. Hereafter, the allele for $[\delta^b]$ will be symbolized as Da^b and the allele for $[\delta^r]$ as Da^r. As far as we know, no gene-produced extrachromosomal element is as yet known in multicellular organisms. The success in induction of delta-retaining sensitive chromosomes by mutation and the mapping of the *Da* gene lead to the revised hypothesis that delta may be a gene product. The possibility that delta may be an infectious agent and may selectively infect flies carrying Da^b or Da^r has been discussed in a previous article (MINAMORI 1972) and excluded for the reasons that (1) the

phenomenon of gradual accumulation of delta over many generations is hard to interpret as being due to gradual infection or propagation of an infectious agent over generations, and (2) delta is not eliminated from S^b or S^r lines by feeding certain antibiotics for many generations. The last finding appears to favor the speculation that delta may be a DNA particle rather than a virus produced by the *Da* gene. GERSHENSON (1965) found in *D. melanogaster* that most lethals induced by feeding calf-thymus DNA were distributed on the right arm of chromosome 2, especially close to the Lobe region. This finding agrees remarkably with the distribution of lethals induced by [δ^b] on the same chromosome (MINAMORI and ITO 1971). Such a similarity might offer some clue for the elucidation of the physical nature of delta.

A number of instances of genotype-dependent susceptibility to the deleterious action of an extrachromosomal element are known—e.g., gene *k* and the kappa particle in *Paramecium aurelia* (reviewed by SONNEBORN 1959); gene *ms* and a specific cytoplasm in onion (JONES and CLARKE 1943) and in pepper (PETERSON 1958; SHIFRISS and FRANKEL 1971). In these organisms, homozygotes for the gene suffer from the deleterious action of the extrachromosomal element; however, the heterozygotes do not. This situation was also established in the present case; flies carrying two doses of the *Da* allele on sensitive chromosomes were, in general, inviable in the presence of delta, but heterozygotes were viable. The sensitivity varied appreciably, from chromosome to chromosome, from complete lethality to quasinormal viability. It was shown in this study that this variation could be greatly extended by recombination between sensitive chromosomes. This finding appears to indicate that the sensitivity may be due to polygenes. In an earlier study, an insensitive second chromosome which retained delta, symbolized as *ID*, was found, and it was suggested that this chromosome may have originated from a delta-retaining sensitive chromosome by accumulation of polygenes for resistance (MINAMORI *et al.* 1970). Most sensitive chromosomes may carry a number of sensitivity genes, while the *ID* chromosome may carry a number of resistance genes which take the place of the sensitivity genes. The induction of sensitive chromosomes from insensitive chromosomes may be interpreted as follows: the latter may originally carry some sensitivity polygenes whose action is expressed in the presence of two doses of *Da*, but not in the presence of a single dose. Thus, the insensitive chromosome would become sensitive simultaneously with the induction of *Da* on the chromosome.

As noted earlier, [δ^b] induces frequent recessive lethal mutations; hence, *Da* may be considered as a mutator gene. The locations of the lethals induced were highly concentrated near the Lobe region (72.0). This locus is separate from the *Da* locus in which a low mutation rate was induced by delta. It is apparent, therefore, that the region where frequent mutations are induced is not identical with the region where delta may be produced. The Lobe region would mutate in every generation in the presence of [δ^b], a product of Da^b. Hence, this region may be an unstable or mutable region in the presence of [δ^b]. It has been known that unstable lines can be induced by various mutagens. According to the findings obtained in this study, some instances of genetic instability reported previously

might have been produced by a similar mechanism; namely, (1) a mutation is induced; (2) the mutated allele produces some substance which may or may not be transmitted extrachromosomally; (3) the substance induces mutations in a specific region of a chromosome; and (4) thus, mutations are induced in every generation in the progeny of the original mutated line. This hypothesis will be examined by further studies.

The transmission of a gene-product from mothers to their offspring may be a universal phenomenon in the animal and plant kingdom since the egg cytoplasm is formed under the control of the mother's genotype—maternal inheritance. Although this transmission is usually confined to one successive generation, that of the extrachromosomal element extends over many generations, and in most instances, the elements appear to carry genetic information independent of the chromosomal gene. According to the hypothesis presented above, the information for delta is transmitted in a dual way—chromosomally and extrachromosomally. The chromosomal transmission is considered to be very stable since *Da* may produce delta at a definite rate in each generation. The extrachromosomal transmission may be unstable and the amount of delta transmitted has been known to vary appreciably according to genotype, sex, and environmental factors. Such dual transmission may produce the unique phenomenon that the expression of *Da* gradually changes over generations (MINAMORI 1969a, 1971, 1972). This is apparently an instance of dauermodification, as discussed in a previous paper (MINAMORI 1972).

We are most grateful to DR. E. W. CASPARI for his comments on an earlier draft of this paper and we would like to thank DR. Y. NAKAO for his comments and MR. R. KAKIGI for his help during the preparation of the manuscript.

LITERATURE CITED

ABINANTI, F. R., 1967 The possible role of microorganisms and viruses in the etiology of chronic degenerative disease of man. Ann. Rev. Microbiol. **21**: 467–497.

BEALE, G. H., A. JULAND and R. A. PREER, 1969 The classes of endosymbiont of *Paramecium aurelia*. J. Cell Sci. **5**: 65–91.

GERSHENSON, S., 1965 Induction of lethal mutations in *Drosophila melanogaster* by DNA. Genet. Res., Camb. **6**: 156–161.

JONES, H. A. and A. E. CLARKE, 1943 Inheritance of male sterility in the onion and the production of hybrid seed. Proc. Am. Hort. Sci. **43**: 189–194.

LEWIS, E. B. and F. BACHER, 1968 Methods of feeding ethyl methane sulfonate (EMS) to Drosophila males. Drosophila Inform. Serv. **43**: 193.

L'HÉRITIER, P., 1958 The hereditary virus of Drosophila. Advan. Virus Res. **5**: 195–245. ——, 1970 Drosophila viruses and their role as evolutionary factors. Evolution. Biol. **4**: 185–209.

MINAMORI, S., 1969a Extrachromosomal element delta in *Drosophila melanogaster*. I. Gene dependence of killing action and of multiplication. Genetics **62**: 583–596. ——, 1969b Extrachromosomal element delta in *Drosophila melanogaster*. II. Transmission through male parents. Japan. J. Genetics **44**: 347–354. ——, 1970 Extrachromosomal element delta in *Drosophila melanogaster*. III. Induction of one-sided gamete recovery from male and female heterozygotes. Genetics **66**: 505–515. ——, 1971 Extrachromosomal element delta in *Drosophila melanogaster*. V. A variant inherited biparentally at 25°C. Japan. J. Genetics

46: 169–180. ——, 1972 Extrachromosomal element delta in *Drosophila melanogaster*. VIII. Inseparable association with sensitive second chromosomes. Genetics **70:** 557–566.

MINAMORI, S., N. FUJIOKA, K. ITO and M. IKEBUCHI, 1970 Extrachromosomal element delta in *Drosophila melanogaster*. IV. Variation and persistence of delta-associating second chromosomes in a natural population. Evolution **24:** 735–744.

MINAMORI, S. and K. ITO, 1971 Extrachromosomal element delta in *Drosophila melanogaster*. VI. Induction of recurrent lethal mutations in definite regions of second chromosomes. Mutation Res. **13:** 361–369.

PARRY, H. B., 1962 Scrapie: A transmissible and hereditary disease of sheep. Heredity **17:** 75–105.

PETERSON, P. A., 1958 Cytoplasmically inherited male sterility in Capsicum. Am. Naturalist **92:** 111–119.

POULSON, D. F., 1968 Nature, stability, and expression of hereditary SR infection in Drosophila. Proc. XII Intern. Congr. Genet. **11:** 91–92.

POULSON, D. F. and B. SAKAGUCHI, 1961 Nature of the "sex-ratio" agent in Drosophila. Science **133:** 1489–1490.

SANDLER, L., Y. HIRAIZUMI and I. SANDLER, 1959 Meiotic drive in natural populations of *Drosophila melanogaster*. I. The cytogenetic basis of Segregation-Distortion. Genetics **44:** 233–250.

SHIFRISS, C. and R. FRANKEL, 1971 New sources of cytoplasmic male sterility in cultivated peppers. J. Heredity **62:** 254–256.

SONNEBORN, T. M., 1959 Kappa and related particle in Paramecium. Advan. Virus Res. **6:** 229–356.