NUCLEOCYTOPLASMIC RELATIONS IN A MUTATOR-SUPPRESSOR SYSTEM OF *DROSOPHILA ANANASSAE*

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ABSTRACT

A partially characterized mutator-suppressor system, previously identified in the *ca*; *stw* stock of *Drosophila ananassae*, was shown to exist in the *ca* ancestral stock; it consists of a clastogenic mutator of sperm chromosomes and a supressor that functions in the oöcyte soon after fertilization. Transmission of these components was monitored by Minute mutation frequencies produced by the progeny of recurrently backcrossed hybrid females derived from reciprocal outcrosses of the *ca* stock. In this way, the mutator was shown to be an extrachromosomally transmitted element whose propagation depends upon nuclear genes. Suppressivity was found to be determined by nuclear genes, some of which are expressed only after a delay of several generations. Neither the mutator nor its suppressor appear to be infectious. Measurement of dominant lethal frequencies showed that the suppressor is completely effective in repair of premutational lesions induced by the mutator. The properties of this mutator-suppressor system were compared with those of hybrid dysgenesis in *Drosophila melanogaster*.

OF the two mutator-suppressor systems previously reported (HINTON 1979) to exist in laboratory stocks of *Drosophila ananassae*, one was tentatively characterized by a mutator component responsible for lesions in chromosomes of sperm and a suppressor, acting in the egg soon after fertilization, that cancelled the effects of the mutator on paternal chromosomes. Thus, outcrosses of males from the stock carrying both the mutator and suppressor to females from a stock lacking both components resulted in high mutation frequencies among the progeny; whereas, neither the reciprocal outcross nor intrastock matings exhibited any sign of unusual mutability. Initial observations on the backcross progeny of hybrid males and females indicated that both mutator and suppressor components are transmitted extrachromosomally through the egg cytoplasm. In this report, new observations are described and analyzed to expand the definition of this mutator-suppressor system, particularly with respect to both the transmission pattern of each component and the mode of suppression.

MATERIALS AND METHODS

The mutator-suppressor system was initially identified in a stock bearing the mutants *ca* (claret eye color, chromosome *2*) and *stw* (straw-colored bristles and cuticle, chromosome *3*). In addition to this double-mutant stock, one of its antecedents marked with *ca* and a stock marked with *px* (plexus wing venation, chromosome *3*) were used. Polytene chromosome analyses of

these stocks showed homozygosity for standard sequences in all arms, with the exception of *2L*, which was homozygous for *In(2L)A* in the *px* stock. All flies were reared at 25° in 25 × 95 mm glass vials containing 10 ml of a medium composed of cornmeal, molasses, dried yeast, agar and propionic acid and inoculated with live yeast. Most experimental crosses involved two or three virgin parents of each sex per vial to maximize mating success and scoring efficiency. The data from individual vials, as well as those from replicates of the same cross performed at different times, were pooled for analysis. Chi-square tests were used to determine the statistical significance of differences between replicates or between crosses.

Mutation was monitored primarily by the frequency of progeny having a dominant Minute phenotype characterized by small bristles and frequently by one or another additional phenes such as frail body, pale pigmentation, rough eyes, delayed development and sterility. The Minute phenotype in *Drosophila ananassae*, as in *D. melanogaster*, is referable to point mutations at numerous loci throughout the genome, or it may be associated with chromosome rearrangements and with monoploidy for the largely heterochromatic chromosome *4*; all Minutes are lethal as homozygotes, as are *X*-linked Minutes in hemizygotes. Some putative Minute mutations, approximately 15% of the total scored, were detected as thoracic mosaics with large sectors of Minute and wild-type tissue. To demonstrate translocations arising from chromosome breakage, males heterozygous for the *ca* and *px* markers were individually testcrossed to *ca*; *px* females, and their progenies were scored with respect to sex and the autosomal markers; pseudolinkage between any two of these markers indicated translocation heterozygosity in the tested male, and this was confirmed by polytene chromosome analysis in subsequent generations. To determine dominant lethal mutation frequencies, the adaptation of Falcon 3040 Micro Test II tissue culture plates for scoring female sterility (ENGELS and PRESTON 1979) was further modified. Instead of placing medium into each of the 96 culture wells, the culture plate lid was filled with a continuous layer of grape juice agar onto which females, previously inseminated in mass matings and then isolated one per culture well, oviposited; each well was provided with a small hole for gas exchange. At the end of a convenient egg collection period, the entire assembly was immersed in CO_2 to immobilize the females while replacing the agar plate with a fresh one for an additional egg sample. Each agar plate was incubated for an additional 26 to 30 hr before scoring the embryos as hatched or lethal.

RESULTS AND ANALYSIS

Presence of the mutator-suppressor system in the ca *stock*: The *ca* and *stw* antecedents of the *ca*; *stw* stock produced only low frequencies of Minute mutations (0.0009 and 0.0004, respectively) in intrastock matings (HINTON 1979). However, in the interest of determining which, if either, antecedent might have contributed to the mutator-suppressor system in their derivative, both were outcrossed to the same *px* stock previously used in analysis of the *ca*; *stw* stock. For the *ca* stock, these outcrosses and subsequent backcrosses showed that its behavior is at least qualitatively equivalent to that of the *ca*; *stw* stock (Table 1). First, there is a highly significant difference in Minute frequencies between the reciprocal parental outcrosses in which *ca* males produced approximately 1% Minute progeny, but *ca* females produced hardly more Minute progeny than the low background frequencies of the parental stocks. When F_1 males from either reciprocal cross were backcrossed to *ca* females, the yield of Minutes was consistently low, but when backcrossed to *px* females, there was again a highly significant difference in which the sons of *px* females showed low mutability (0.0009 Minutes) in contrast to the 0.0063 Minute yield by sons of *ca* females.

This difference in behavior of F_1 males might be related to differences in the

TABLE 1

Minute frequencies observed in the px *and* ca *stocks, their reciprocal outcrosses and in backcrosses of their hybrids*

Matings		Progeny scored	Minutes		Matings		Progeny scored	Minutes	
Female	Male		Number	Frequency	Female	Male		Number	Frequency
px	*px*	23212	13	0.0006	*ca*	*ca*	12557	11	0.0009
px	*ca*	24046	250	0.0104	*ca*	*px*	22857	26	0.0011
ca	F_1	9814	3	0.0003	*ca*	F_1	8864	8	0.0009
px	F_1	11446	10	0.0009	*px*	F_1	14473	91	0.0063
F_1	*px*	8986	4	0.0005	F_1	*px*	12047	4	0.0003
F_1	*ca*	14882	61	0.0041	F_1	*ca*	18904	20	0.0011
px	X_1*	7911	12	0.0015	*px*	X_1	7676	29	0.0038
X_1	*ca*	6124	34	0.0056	X_1	*ca*	10975	9	0.0008

* X_1 parents were derived from the immediately preceding backcrosses of F_1 females to *ca* males.

parental sources of their sex chromosomes or of their cytoplasm. To distinguish these possibilities, chromosomally identical F_1 females from the reciprocal crosses were backcrossed to *ca* males to produce X_1 sons whose *Y* chromosomes originated from the *ca* stock, whose *X* chromosomes were derived equally from both parental stocks, but whose maternal cytoplasmic sources differed; in backcrosses to *px* females, the X_1 males whose cytoplasm derived from the *ca* stock produced more Minutes (0.0038) than did those having mothers with *px* stock cytoplasm (0.0015 Minutes). These results indicate that the mutator activity exhibited by males is attributable, as a first approximation, to an extrachromosomal factor transmitted to them by their mothers.

Despite their transmission of the mutator to their sons, both *ca* females and their F_1 *ca/+; +/px* daughters produced very few Minutes in crosses to either *px* or F_1 *+/ca; px/+* males. By itself, this result could mean that the action of the mutator is restricted to males, but the equally low yields of Minutes from these same females when mated to either *ca* or F_1 *ca/+; +/px* males suggest the existence of a suppressor function in their eggs that prevents the recovery of mutator-induced lesions present in the sperm of these males. Such a suppressor in oöcytes might similarly mask the effects, if any, of the co-existing mutator on maternal chromosomes. This suppressor function also appears to be transmitted extrachromosomally because chromosomally equivalent F_1 daughters from reciprocal outcrosses produced very different frequencies of Minute progeny in crosses to *ca* males: F_1 daughters of *ca* females had only 0.0011 Minute progeny, as compared to 0.0041 Minutes from F_1 daughters of *px* females. Furthermore, this difference persisted between the X_1 female progeny of these matings. Those with *ca* grandmothers produced 0.0008 Minutes; whereas, those with *px* grandmothers yielded a Minute frequency of 0.0056. This transmission pattern of the suppressor from mother to daughter implies that it should also be transmitted from mother to son. If that is true, it follows that the suppressor must be

ineffective in males. It is conceivable, based on the evidence presented so far, that the mutator and suppressor functions are sexually alternative expressions of a single, extrachromosomally transmitted entity.

That the mutator-suppressor systems identified in the *ca*; *stw* and *ca* stocks are equivalent except for minor quantitative differences (see Table 6, HINTON 1979) is supported by the results of crosses between them (Table 2). Neither of the parental crosses nor their backcrossed F_1 daughters produced significantly elevated frequencies of Minutes, indicating that the suppressor of each stock is effective against the mutator of the other. The F_1 sons of both reciprocal matings produced essentially the same high Minute frequencies in tests with *px* females to demonstrate the presence of the mutator in both stocks. A second confirmation of the identity of the mutator components in the two stocks is that the lesions induced by both are clastogenic. Of 205 non-Minute F_1 +/*ca*; *px*/+ males test-crossed to *ca*; *px* females, five were shown to be heterozygous for reciprocal translocations between their paternal chromosomes, one *T(Y;3)* and four *T(2;3)*s. This induced translocation frequency of 2.4% is comparable to the 2.2% yield among similar F_1 sons of *ca*; *stw* males (HINTON 1979), and the polytene chromosome analysis of this translocation sample, as in the previous one, showed that none of the autosomal breakpoints were in chromocentral heterochromatin.

Transmission of the mutator-suppressor system in recurrent backcrosses: Whereas the foregoing transmission analysis indicated an extrachromosomal basis for the mutator-suppressor system, this explanation does not account for certain quantitative variations in Minute yields. In particular, reference to Table 1 shows that the effect of the mutator declines from parental *ca* males (0.0104 Minutes) to F_1 *ca*/+; +/*px* males (0.0063 Minutes), and further to X_1 sons (0.0038 Minutes) of F_1 *ca*/+; +/*px* females; if the mutator were a strictly autonomous extrachromosomal element, this decline would not be expected. At the same time, F_1 +/*ca*; *px*/+ females and their X_1 daughters exhibited more suppressor activity (0.00041 and 0.0056 Minutes, respectively, in crosses to *ca* males) than their *px* stock cytoplasm alone would accommodate (*px* females produced 0.0104 Minutes in crosses to *ca* males). These variations suggest the existence of nuclear genome effects on the mutator-suppressor system; this possibility was explored extensively in two series of recurrent backcrosses.

In the first backcross series, the dependence of the mutator and suppressor

TABLE 2

Minute yields in reciprocal crosses between the ca *and* ca; stw *stocks and in backcrosses of their F_1 progeny*

Matings		Progeny scored	Minutes		Matings		Progeny scored	Minutes	
Female	Male		Number	Frequency	Female	Male		Number	Frequency
ca; stw	*ca*	3329	1	0.0003	*ca*	*ca; stw*	2084	0	0
F_1	*ca*	3390	3	0.0009	F_1	*ca; stw*	2513	1	0.0004
px	F_1	3074	19	0.0062	*px*	F_1	2618	18	0.0069

TABLE 3

Minute frequencies observed in successive generations produced by recurrent backcrosses to px *stocks males, beginning with* F_1 ca/+; +/px *females*

Assays for mutator activity in males					Assays for suppressor activity in females				
Matings		Progeny scored	Minutes		Matings		Progeny scored	Minutes	
Female	Male		Number	Frequency	Female	Male		Number	Frequency
px	X_1	6578	22	0.0033	X_1	*ca*	10975	9	0.0008
px	X_2	6818	12	0.0018	X_2	*ca*	7406	21	0.0028
px	X_3	9586	6	0.0006	X_3	*ca*	6993	15	0.0021
px	X_4	5095	4	0.0008	X_4	*ca*	6469	26	0.0040
px	X_5	2303	1	0.0004	X_5	*ca*	3784	14	0.0037
px	X_6	1017	1	0.0010	X_6	*ca*	7803	44	0.0056
px	X_7	3347	1	0.0003	X_7	*ca*	5718	52	0.0091
					X_8	*ca*	1594	11	0.0069
					X_9	*ca*	1642	17	0.0104

components on the *ca* stock genome was examined by backcrossing females of each generation to *px* stock males, beginning with F_1 *ca/+; +/px* females carrying the mutator-suppressor system; in each backcross generation, daughters were assayed for the suppressor in matings to *ca* males and sons were assayed for the mutator in matings to *px* females (Table 3). The second backcross series utilized males from the *ca* stock as recurrent parents, and was initiated with F_1 *+/ca; px/+* females having *px* stock cytoplasm; again, assays for suppressivity in females and mutability in males were conducted in each generation (Table 4). Recombination in the backcrossed females should have been unrestricted except for the segment associated with *In(2L)A* and marked by ca^+; several comparisons of Minute frequencies produced by *In(2L)A* homozygotes (identified by polytene analysis) from backcrosses to the *px* stock, or by *ca/ca* segregants in the *ca* backcross series, with those of their X_n *In(2L)A, +/ca* cohorts revealed

TABLE 4

Minute frequencies observed in successive generations produced by recurrent backcrosses to ca *stock males, beginning with* F_1 +/ca; px/+ *females*

Assays for mutator activity in males					Assays for suppressor activity in females				
Matings		Progeny scored	Minutes		Matings		Progeny scored	Minutes	
Female	Male		Number	Frequency	Female	Male		Number	Frequency
px	X_1	14127	22	0.0016	X_1	*ca*	21150	62	0.0029
px	X_2	12280	24	0.0020	X_2	*ca*	11026	46	0.0042
px	X_3	8170	9	0.0011	X_3	*ca*	9131	22	0.0024
px	X_4	10403	16	0.0015	X_4	*ca*	8061	17	0.0021
px	X_5	10275	10	0.0010	X_5	*ca*	5025	17	0.0034
px	X_6	6827	12	0.0018	X_6	*ca*	4974	10	0.0020
px	X_7	5217	9	0.0017	X_7	*ca*	3842	4	0.0010
px	X_8	3387	7	0.0021	X_8	*ca*	3597	0	0
px	X_9	9296	13	0.0014	X_9	*ca*	3382	2	0.0006

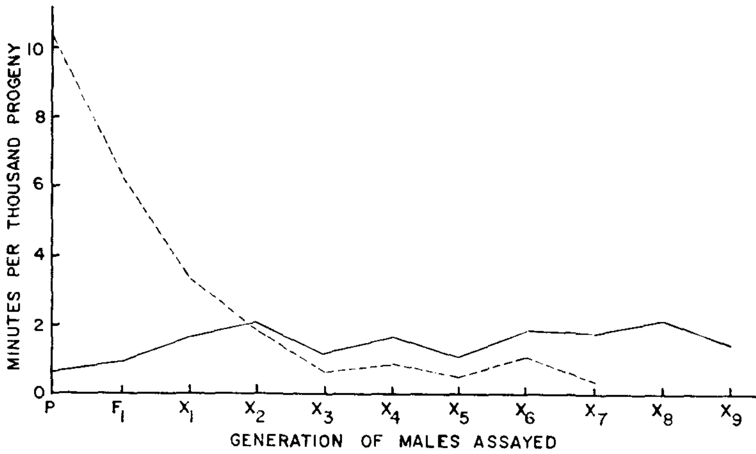

FIGURE 1.—Minute yields in assays for mutator activity of males mated to *px* females. The dashed line connects points from *ca* stock males, F_1 *ca*/+; +/*px* males, and the X_n sons from recurrent backcrosses of F_1 *ca*/+; +/*px* females and their X_{n-1} daughters to males from the *px* stock (Tables 1 and 3). The solid line connects points from *px* stock (P) males, F_1+/*ca*; *px*/+ males, and the X_n sons from recurrent backcrosses of F_1 +/*ca*; *px*/+ females and their X_{n-1} daughters to males from the *ca* stock (Tables 1 and 4).

no significant influence of this segment on either mutability or suppressivity. To facilitate comparisons, the numerical results in Tables 3 and 4 from assays of mutator activity in males have been combined into Figure 1 and the assays for suppressor activity in females are graphically represented in Figure 2; both figures include data points from Table 1 for parental stocks and their outcrosses.

In the *px* backcross series, the initial decline in mutability previously noted

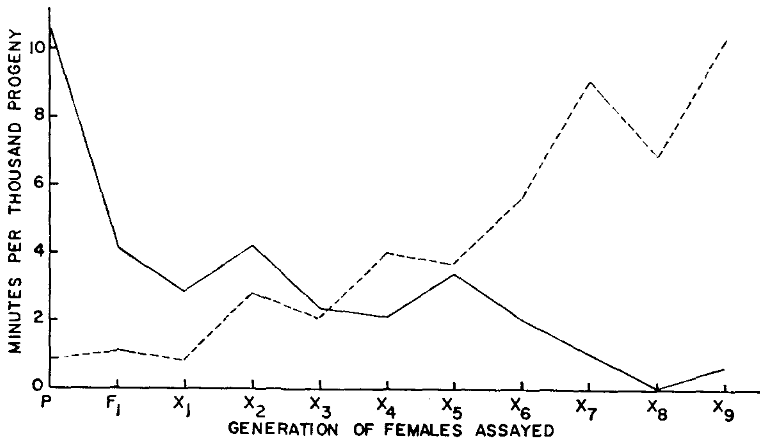

FIGURE 2.—Minute yields in assays for suppressor activity of females mated to *ca* males. The dashed line connects points from *ca* females (P), F_1 *ca*/+; +/*px* females and the X_n daughters from recurrent backcrosses of F_1 *ca*/+; +/*px* females and their X_{n-1} daughters to *px* stock males (Tables 1 and 3). The solid line connects points from *px* females (P), F_1 +/*ca*; *px*/+ females, and the X_n daughters from recurrent backcrosses of F_1 +/*ca*; *px*/+ females and their X_{n-1} daughters to males from the *ca* stock (Tables 1 and 4).

for the F_1 and X_1 generations continued through the X_3 generation when the background level of Minutes was reached and then was maintained through the X_7 generation (Figure 1). In each of the first four generations, the Minute frequency was approximately halved, as was the remaining fraction of the *ca* genome. The possibility that the *px* genome is resistant to the mutator has not been tested directly, but the following comparison strongly discounts it. Whereas only 25% of the genome of the X_1 sons of F_1 *ca*/+; +/*px* females and *ca* males (Table 1) was of *px* stock origin, 75% of the genome of the X_1 sons of these females and *px* males (Table 3) was derived from the *px* stock; nevertheless, these two groups of males produced nearly equal frequencies of Minutes (0.0038 and 0.0033, respectively). An alternative interpretation, that the decline in mutability was due to decreasing mutator activity in the *ca* genome itself, is negated by the results of the *ca* backcross series in which replacement of the *px* genome should have caused acquisition of full mutator activity.

As shown in Figure 1, there was an initial small increase in Minute frequencies that persisted through the X_9 generation, but this residual mutability associated with the *ca* genome was clearly not comparable to that characteristic of males from the parental *ca* stock. On the other hand, failure to restore mutability to the level exhibited by the *ca* stock would be expected if the mutator is an extrachromosomally replicating element that does not arise *de novo* from the ca genome, at least not with a frequency detectable in these backcrosses. According to this view of the mutator, its decline in the recurrent backcrosses to the *px* stock may be ascribed to the loss of some component of the *ca* genome essential to its steady-state propagation.

In each generation of the recurrent backcrosses to the *ca* stock, females lacking the mutator were in contact with males carrying it. Since these females, at least in the later generations, were genetically capable of supporting the mutator, it is apparent that the mutator is not infectious by his route. A supplementary test for infectious transfer of the mutator between other stages of the life cycle utilized the *ca*; *stw* mutator stock and a mutator-null *ca* stock established by inbreeding the X_9 generation from the *ca* backcross series. Inseminated females from each stock were placed together for oviposition, and their progeny developed under crowded conditions in the same medium. Upon eclosion, the first generation of co-cultured progeny was temporarily separated by means of the *stw* marker to insure homogamic matings, after which the two types of inseminated females were placed together again to produce a second generation co-cultured without crowding. From this generation X_9 *ca* males were tested for acquisition of mutability in matings to *px* females, and produced 0.0015 Minutes among 7265 progeny as compared to 0.0038 Minutes among 2599 offspring of co-cultured *ca*; *stw* males; at the same time, control males taken directly from X_9 stock cultures had 0.0014 Minutes (N=5105). Thus, it appears that the extrachromosomal mutator element is not ordinarily infectious.

Inspection of Figure 2 reveals that suppressor activity in females gradually declined (Minute frequencies increased) and ultimately disappeared as the *ca* genome was replaced by the *px* genome; whereas, substitution of the *px* genome

by that of the *ca* stock resulted in full development of the suppressor function. It is clear that some causal relation exists between the *ca* genome and suppression but, as previously indicated, that relation is probably complex. The contradiction of the simplest expectation that frequencies of Minutes produced by genetically equivalent F_1 females from reciprocal outcrosses should be equal leads to the alternative suggestion of a maternal effect, in which the maternal genotype is expressed in the next generation. The equivalence of suppressor activity between F_1 *ca*/+;+/*px* females and their *ca* mothers is in accord with this model, but the significantly lower Minute frequencies produced by F_1 +/*ca*; *px*/+ females than by their *px* mothers is diagnostic of zygotic expression, albeit incomplete, of the *ca* genome in these heterozygotes. Furthermore, the observation that even X_3 females with 15/16 of their genomes from the *ca* stock still had no more suppressor activity (0.0024 Minutes) than their X_3 counterparts (0.0021 Minutes) with only 1/16 of their genomes from the *ca* stock shows that full expression of the *ca* genome is delayed for several generations, rather than just one. The difference between Minute frequencies for X_4 females is only marginally significant, and the difference between the two backcross series became clearly established only in the sixth generation.

The eventual appearance of full suppression in X_{7-9} females of the *ca* backcross series would eliminate the possibility that suppression is associated with an exogenous extrachromosomal factor unless these females could acquire suppressor activity by infection from their *ca* mates. This particular mode of transmission, however remote, is not readily dismissed, although a test for suppressor infectivity at other stages of the life cycle was negative. Inseminated females from *ca* and *px* stock cultures were placed together, and their progeny developed in the same medium. In test matings to *ca* males, co-cultured *px* females produced 0.0076 Minutes among 6584 progeny, which was equivalent to the 0.0076 Minutes recovered among 6800 offspring of *px* control females that were not co-cultured; co-cultured *ca* female controls produced only one Minute among 3090 progeny. This failure to find acquired suppressor activity in the co-cultured *px* females could reflect an inappropriate host genotype for susceptibility to, or expression of, an infectious agent. If there is no infectious agent involved, the delayed expression of the *ca* genome in the suppression phenomenon may be the result of intervention of some other form of extrachromosomal factor, perhaps a normal cell organelle.

Dominant lethality: Since the mutator itself is not transmitted by males, it has been inferred that mutator-induced damage to their chromosomes must occur prior to the completion of spermiogenesis; however, the induced damage must be labile (premutational), because its expression depends upon the presence or absence of the suppressor in the fertilized oocyte cytoplasm. The reduction of Minute frequencies, by which the suppressor has been recognized, might be due either to diversion of the premutational lesions into an unrecoverable class of zygotes (dominant lethals) or to reversion of the lesions into a recoverable, but nonmutant, class of zygotes (HINTON 1979). These alternatives have been dis-

tinguished by assaying dominant lethality among embryos. Preliminary observations of embryos from intrastock matings showed the incidence of lethality to range from about 15% for both the *px* and *ca* stocks to about 30% for the *ca*; *stw* stock; such high frequencies of lethal embryos are not unexpected in inbred stocks that may have accumulated a variety of recessive alleles causing defects in gametogenesis or embryogenesis. In order to minimize confusion from such nonspecific sources of lethality, the experimental design relied on F_1 heterozygotes from reciprocal crosses between the *px* and *ca* or *ca*; *stw* stocks and, as shown by the first pair of entries in Table 5, in which both parents lacked the mutator, at least 95% of the F_2 zygotes successfully completed embryonic development. Similarly, in matings of females carrying the mutator to males lacking it, only a small fraction of embryos failed to hatch. On the other hand, matings of F_1 females expected to have only partial suppressor activity to F_1 males carrying the mutator resulted in 21–24% dominant lethals, as predicted by the previously inferred clastogenic nature of the mutator that, in concurrent control observations, induced 0.0020 and 0.0031 Minutes among adult progeny of these parents. Finally, the critical fourth pair of matings between females having full suppressor activity to males with the mutator produced only control levels of dominant lethal embryos and of Minute adults. The extensive mutator-induced lesions manifested as dominant lethals in the third pair of matings must have been repaired by the suppressor in females of the last set of matings in such a way as to leave no detectable effect.

DISCUSSION

In *Drosophila melanogaster*, increased mutability is a commonly observed feature of the hybrid dysgenesis syndrome displayed by progeny from one of the reciprocal outcrosses between a variety of stocks, usually females from a laboratory tester or balancer strain and males from a wild type recently extracted from a natural population (for reviews of hybrid dysgenesis, see KIDWELL, KIDWELL

TABLE 5

Dominant lethal and Minute mutations assayed in F_2 generations from reciprocal crosses between the px *and* ca *or* ca; stw *stocks*

Matings: Females	Matings: Males	Embryos: Lethals/Total = Frequency	Adults: Minutes/Total = Frequency
F_1 +/*ca*; *px*/+	F_1 +/*ca*; *px*/+	114/2168 = 0.0526	1/4657 = 0.0002
F_1 +/*ca*; *px*/*stw*	F_1 +/*ca*; *px*/*stw*	96/377 = 0.0284	—
F_1 *ca*/+; +/*px*	F_1 +/*ca*; *px*/+	34/1276 = 0.0266	2/4537 = 0.0004
F_1 *ca*/+; *stw*/*px*	F_1 +/*ca*; *px*/*stw*	91/2307 = 0.0394	—
F_1 +/*ca*; *px*/+	F_1 *ca*/+; +/*px*	417/1992 = 0.2093	38/12274 = 0.0031
F_1 +/*ca*; *px*/*stw*	F_1 *ca*/+; *stw*/*px*	544/2250 = 0.2418	6/2957 = 0.0020
F_1 *ca*/+; +/*px*	F_1 *ca*/+; +/*px*	68/2105 = 0.0323	2/8943 = 0.0002
F_1 *ca*/+; *stw*/*px*	F_1 *ca*/+; *stw*/*px*	73/2493 = 0.0293	1/3381 = 0.0003

and SVED 1977; THOMPSON and WOODRUFF 1978; SVED 1979; BREGLIANO *et al.* 1980). In the most extensively explored systems of hybrid dysgenesis, crossing over in males and female sterility, rather than mutation, have been the monitored phenes, but where these and other aspects of the hybrid dysgenesis syndrome occur simultaneously, their transmission patterns are correlated. The contribution of the paternal parent stock (P) to dysgenic hybrids is chromosomally linked; whereas, the maternal stock (M) contribution is a cytoplasmic state that initially exhibits matrilineal inheritance, but is ultimately specified by chromosomal genes. These briefly summarized properties of hybrid dysgenesis indicate considerable similarity with those of the *D. ananassae* mutator-suppressor system analyzed here, so that a somewhat more detailed comparison of these phenomena is warranted.

Studies of hybrid dysgenesis have emphasized its manifestation, not by the parental stocks, but by the F_1 M/P hybrids, and this has been specifically demonstrated for elevated rates of recessive lethal mutations (KIDWELL, KIDWELL and IVES 1977), as well as meiotic chromosome breakage (HENDERSON, WOODRUFF and THOMPSON 1978), *X–Y* translocations (ENGELS 1979a) and aberrations of the *X* chromosome (BERG, ENGELS and KREBER 1980). These M/P *D. melanogaster* hybrids are thus comparable to the mutagenic *D. ananassae* F_1 *ca/px* males; similarly, F_1 P/M and *px/ca* males are comparable in that both are nonmutagenic. However, in apparent contrast to their F_1 P/M counterparts, the F_1 *px/ca* progeny themselves exhibit significantly increased frequencies of Minutes and reciprocal translocations that originate in their fathers' sperm. It would be of interest to confirm this difference by assays for dominant mutations in F_1 P/M hybrids as has been done in *D. ananassae*, but evidence from analysis of other phenes of the syndrome (*e.g.*, ENGELS 1979a) that dysgenesis originates in the germ line of the hybrids themselves points to a real, rather than technically trivial, difference with respect to mutagenicity of the *ca ananassae* and P *melanogaster* stocks.

In the *D. ananassae* mutator-suppressor system, the transmission patterns observed in recurrent backcrosses were sufficiently disparate to justify the conclusion that mutability and suppressivity are separately determined, rather than being sexually alternative expressions of the same entity. These two components appear to parallel those of hybrid dysgenesis in *D. melanogaster*; more specifically, the mutator corresponds to the P factors, and the absence of the suppressor function corresponds to the M contribution to dysgenic hybrids. According to the results of the recurrent backcrosses, the *D. ananassae* mutator is most readily interpreted as a noninfectious, matrilinearly and extrachromosomally transmitted element whose continued propagation depends upon one or more alleles present in the *ca* stock genome. This mode of transmission clearly distinguishes the mutator from the chromosomally linked transmission of P factors in hybrid dysgenesis, although these are also unorthodox in exhibiting properties expected of transposable insertion sequences (PICARD 1976; GREEN 1977; ENGELS 1979c).

If, as recently speculated by BERG, ENGELS and KREBER (1980), "nomadic" sequences of moderately repetitive DNA are involved in hybrid dysgenesis, then the difference between the *D. melanogaster* P factors and the *D. ananassae* mutator might correspond to the chromosomal and free circular forms of such sequences. The mutator transmission pattern also characterizes a suppressor of male crossing over in *D. ananassae* (HINTON 1974), in addition to such traits as CO_2 sensitivity, maternal sex ratio and male sterility analyzed in other Drosophila species (reviewed by PREER 1971). A clastogenic mutator of paternal chromosomes in *D. robusta* was shown by LEVITAN (1962, 1963; LEVITAN and SCHILLER 1963) to be extrachromosomally transmitted although, unlike the *D. ananassae* mutator, it apparently functions in the oocyte after fertilization and proliferates independently of nuclear genes (LEVITAN and WILLIAMSON 1965). An alternative interpretation of the *D. robusta* case allows it to conform with the *D. ananassae* system (HINTON 1979).

Little is known of the mode of action of the *D. ananassae* mutator beyond its induction of numerous premutational lesions that are present in sperm of mutagenic males and are recovered as Minute mutants, chromosome rearrangements or dominant lethal embryos. Although the polytene breakpoints of recovered rearrangements involving the large autosomes suggest a nonrandom disposition of the lesions (HINTON 1979), they do not appear to be so highly restricted as those described for the *X* chromosome rearrangements produced by dysgenic *D. melanogaster* males (BERG, ENGELS and KREBER 1980). No precise test for clustering of mutants among progenies of single mutagenic males has been performed to exclude the possibility that the mutator functions premeiotically as in dysgenic *D. melanogaster* hybrids, but mutant flies appear to be randomly distributed among matings that involve more than one male parent. It should also be noted that casual experience with newly recovered *D. ananassae* mutants provides no cause for suspecting instability of the sort implicating transposable elements, but the mutants successfully established in stocks may comprise a selected sample or have phenotypes inappropriate for the detection of instability.

Inheritance of the suppressor component of the mutator-suppressor system is consistent with the rules established for the M component of hybrid dysgenesis by very different kinds of observations (ENGELS 1979b; BUCHETON and PICARD 1978). In both species there are alternative cytoplasmic states with which chromosomes of paternal origin interact, and these cytoplasmic differences are specified by chromosomal genes, some of which are expressed only after several generations of backcrossing. The mechanism of delayed or cumulative expression of the genotype remains unidentified, but the intervention of an extrachromosomally replicating entity subject to qualitative or quantitative regulation by nuclear genes is compatible with the observations in both species. ENGELS' analysis of the P–M system of hybrid dysgenesis showed that females were bimodally distributed with respect to their cytoplasmic state (P or M cytotype); whereas, the I–R system studied by BUCHETON and PICARD exhibits continuous variation

among females with respect to their cytoplasmic reactivity. The *D. ananassae* observations permit no conclusion in this regard because the frequency of mutation is so low as to preclude classification of single females according to degree of suppression. The stock recovered from the X_9 generation of the *ca* backcross series and characterized by full suppressor, but no mutator, activity is analogous to the neutral strains diagnosed among *D. melanogaster* stocks assayed for hybrid dysgenesis.

No specific model has been proposed for the participation of the cytotype in hybrid dysgenesis; ENGELS (1979b) viewed the M cytotype as either having a factor necessary for the development of hybrid dysgenesis or lacking a suppressor of hybrid dysgenesis. On the other hand, the observations on dominant lethality in the *D. ananassae* system clearly identify the suppressor function to be the reversal of premutational lesions induced by the mutator. Formally, the suppressor function implies error-free repair of DNA lesions, but whether the specific molecular mechanisms known to exist in *D. melanogaster* (*e.g.*, BAKER *et al.* 1976) are involved here remains to be established. Specific identification of the suppressor function would be of added significance in suggesting the nature of the lesions induced by the mutator. The failure of the suppressor to function in *D. ananassae* males is in contrast to the P cytotype of hybrid dysgenesis, which is expressed in the germ lines of both sexes, at least in the PM system. This difference provides an obvious basis for the previously noted difference in mutagenicity of outcrossed males from P *D. melanogaster* and *ca D. ananassae* stocks.

The *ca, stw* and *px* mutants were recovered in the F_2 generations produced by single inseminated *D. ananassae* females trapped from a natural population in Calcutta more than 20 years ago (RAY-CHAUDHURI *et al.* 1959), and the double mutant *ca; stw* stock was probably constructed soon thereafter; since 1966, these stocks have been reproductively isolated in this laboratory. Thus, the presence in both the *ca* stock and its *ca; stw* derivative of the mutator-suppressor system shows it to be stable, at least under laboratory conditions. Whereas the adaptive value of the suppressor component is apparent, the origin and the maintenance of the mutator remain entirely obscure. That neither component is generally present in stocks derived from the Calcutta population is shown by the behavior of the *px* stock, and preliminary observations (HINTON, unpublished) of the *stw* stock show that, although it exhibits an incompletely characterized, probably chromosomal, mutator, it does not carry the suppressor present in the *ca* stock, because 0.0087 Minutes were observed among 8041 progeny of *stw* females and *ca* males. It is also of interest that a stock of the *pc* (peacock wings) mutant, of the same provenance as the *ca, stw* and *px* mutants, lacks the *ca* stock's suppressor of mutability, but has an extrachromosomally transmitted suppressor of male crossing over (HINTON 1974, and unpublished). The alternatives that the special properties of these stocks reflect those existing in the Calcutta population at the time of its sampling or that they developed subsequently in the laboratory appear to parallel those for the origin of hybrid dysgenesis in *D. melanogaster*, as briefly considered by KIDWELL (1979), SVED (1979), BREGLIANO *et al.* (1980) and

ENGELS and PRESTON (1980). In either view of their origin, the existence of these mechanisms in addition to the previously described chromosomal mutator-suppressor system (HINTON 1979) indicates an enormous and quite diverse potential for the control of genetic diversity.

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