OM MUTATIONS IN *DROSOPHILA ANANASSAE* ARE LINKED TO INSERTIONS OF A TRANSPOSABLE ELEMENT

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ABSTRACT

It has been hypothesized that *Om* mutability in *Drosophila ananassae* (involving spontaneous mutation at 20 loci, resulting in semidominant, nonpleiotropic eye morphology defects) was due to insertion of a transposable element, *tom*. One particularly unstable *X*-linked *Om* allele produced several derivatives, one of which has a more extreme *Om* phenotype and was accompanied by a singed bristle mutant, sn^{9g}. DNA probes from the *sn* locus of *D. melanogaster* were used to clone the homologous region of *D. ananassae*. Analysis of sn^{9g} DNA detected a 6.5-kb insert. Genomic Southern blotting and *in situ* hybridization techniques showed that this insert is repetitive and dispersed. The existence of the *tom* element is supported by genetic mapping that established homology between the 6.5-kb sn^{9g} insert and *Om* mutants at the four *X*-linked loci tested.

THE optic morphology (*Om*) mutability system of *Drosophila ananassae* (HINTON 1984) is an example of unusual genetic phenomena found in this species. Previously *D. ananassae* was shown to exhibit male crossing over (KIKKAWA 1938; MORIWAKI 1940; HINTON 1970; MATSUDA, IMAI and TOBARI 1983) and hypermutability (KIKKAWA 1938; HINTON 1979, 1981). Evidence for association between hypermutability and male crossing over has also been found in some strains (HINTON 1983; MATSUDA, IMAI and TOBARI 1983). HINTON (1984) first observed the *Om* system in a newly constructed stock, *ca*; *px*. He noticed the frequent occurrence of a class of mutations having the following properties: (1) semidominant effects on eye morphology, (2) few if any pleiotropic effects, (3) genetic localization to at least 20 loci (C. W. HINTON, personal communication) throughout the genome and (4) absence of dosage compensation of those mutants at the *X*-linked loci. Although there is some phenotypic similarity of mutants at the same locus, there is considerable overlap of phenotype among the loci.

Based on genetic analysis, HINTON (1984) concluded that the mutator activity

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required either homozygosity of the *X* chromosome from the original stock or heterozygosity of that *X* chromosome and the presence of self-replicating, non-chromosomal factors he had previously identified as being involved in other forms of hypermutability (HINTON 1983). HINTON (1984) hypothesized that *Om* mutability involved a transposable element (*tom*) with insertion site specificity for sequences shared by a set of genes coordinately expressed during eye morphogenesis.

In this report we describe the cloning of a DNA sequence from a mutant derived from the *Om* mutability system and present evidence that this sequence is homologous to *tom*. The *Om* mutator rarely produces any other type of mutant than the characteristic *Om*. An exception was a *singed* (sn^{9g}) mutant that HINTON (1984) recovered simultaneously with a more extreme *Om* derivative of *Om(1D)9*, called *Om(1D)9g*. Utilizing DNA probes from the *singed* locus of *D. melanogaster*, we cloned the *singed* region of *D. ananassae*. An insert of 6.5 kb was identified in sn^{9g}. Sequences homologous to this insert were found at sites of all tested *Om* mutations.

MATERIALS AND METHODS

Routine molecular techniques described in MANIATIS, FRITSCH and SAMBROOK (1982) were used unless otherwise specified. Drosophila genomic DNA was prepared according to the method of BINGHAM, LEVIS and RUBIN (1981). Lambda libraries of *D. ananassae* were constructed as follows: High molecular weight Drosophila DNA was digested in serial dilutions of *Mbo*I for 15 min at 37° (1 μg of DNA in 10 μl of 167 mM Tris-Cl, pH 7.4, 17 mM $MgCl_2$, 25 mM DTT, 25 mM spermidine, 25 μg/ml gelatin and 0.5–0.01 units of restriction enzyme). The digests were immediately placed at 65° for 15 min. During this period 1 μl of each digest was electrophoresed on a 1% agarose gel. At the end of 15 min at 65° the digests were cooled, 1 unit of calf intestinal alkaline phosphatase (Boehringer Mannheim) was added and the mixtures were incubated at 37° for 30 min. The digests were then returned to 65° for 15 min. During this period the electrophoretic gel was examined to determine the degree of *Mbo*I digestion of each sample. The digestion/dilution with the minimum detectable digestion was chosen for cloning. To ligate the digested DNA into lambda vectors, 7 μl of λEMBL4/*Bam*HI DNA [digested, phenol-extracted and ethanol-precipitated and resuspended in 10 mM Tris, 1 mM EDTA, pH 7.2, at 400 μg/ml (FRISCHAUF *et al.* 1983)], 1 μl 10 mM ATP and 1 unit of T4 ligase were combined with the *Mbo*I-digested *D. ananassae* DNA. This ligation reaction was incubated overnight at 12°. The ligation reaction was packaged according to the protocol of SCALENGHE *et al.* (1981), using 100 μl of freeze-thaw lysate and 25 μl of sonicated extract.

The localization of DNA sequence homologies by *in situ* hybridization to salivary gland chromosomes followed PARDUE and GALL (1975), with the following modifications as suggested by J. LIM. Dissected glands were suspended in one part lactic acid, two parts water and three parts acetic acid for 4–5 min before being squashed. Slides were then kept at 4° overnight before removing the coverslips. Probe DNAs were labeled with biotinylated dUTP (LANGER, WALDROP and WARD 1981; LANGER-SAFER, LEVINE and WARD 1982). Hybridization of probes to chromosomes was detected using the ABC Kit of Vector Laboratories (HSU, RAINE and FANGER 1981). All *D. ananassae* stocks were obtained from C. W. HINTON, and the mutants are described by HINTON (1980, 1984). Flies were cultured at 25° in 25 × 95-mm glass vials containing standard cornmeal and agar medium. The mapping of *Om* mutants and sites of *in situ* hybridization of the putative *tom* element began with females heterozygous at one of four *X*-linked *Om* loci and at two or more linked recessive visible loci. In most cases, previous data

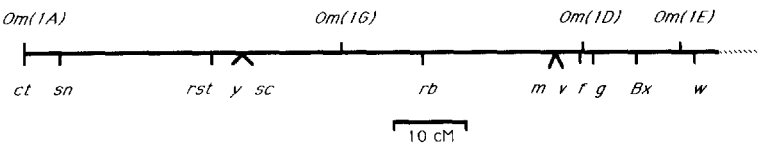


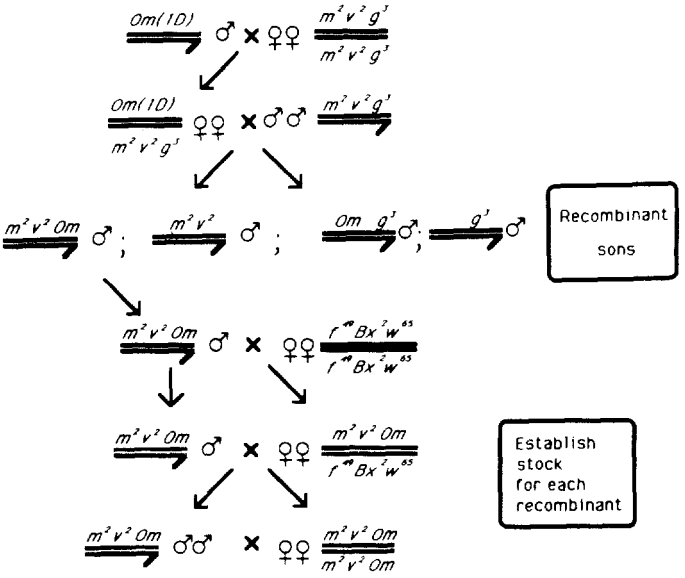
FIGURE 1.—Genetic map of *X* chromosome of *D. ananassae* showing positions of *Om* and marker mutants used in this study. The dashed extension indicates that the *XR* terminus has not been genetically defined.

of C. W. HINTON (personal communications) allowed us to choose recessive markers on opposite sides of the particular *Om* locus (Figures 1 and 2). Male progeny recombinant for the flanking recessive marker loci were crossed to females from a second marker stock carrying mutant alleles at other loci in the same region. Their daughters were either backcrossed to the recombinant male father or were crossed to their brothers. In the next two generations, appropriate homozygous females were crossed to male sibs to produce a stock homozygous for the region of interest for each of the recombinants (see Figure 2). Each recombinant stock was then scored for *Om* and by *in situ* hybridization for the putative *tom* element.

RESULTS

Cloning of sn^{9g}: To determine whether sn^{9g} was the result of an insert (perhaps *tom*), we first cloned sequences from the *singed* region of *D. ananassae*. K. O'HARE, H. ROIHA and G. RUBIN kindly gave us three DNA probes from the *singed* region of *D. melanogaster* (CS*sn*2, CS*sn*8 and CS*sn*12 from Canton-S). The white-tawny (w^t) stock was used as a source of a *D. ananassae* wild-type *singed* allele. A λEMBL4/*Bam*HI//*D. ananassae-*w^t/*Mbo*I library was screened using the *D. melanogaster singed* probes. Hybridization and final washes were performed under reduced stringency conditions (37°). Figure 3 shows the restriction maps of the inserts in the four phages recovered and how they align with the three CS *sn* probes. *In situ* hybridization to *D. ananassae* polytene chromosomes with these clones showed that they shared homology with a single site on *XL* at position 3A that is consistent with the known genetic position of *singed* (Figure 1). By *in situ* probing to *D. melanogaster* chromosomes, it was shown that these *D. ananassae* clones also shared homology with a single site at the known cytogenetic location of *singed*. From H. ROIHA, K. O'HARE and G. RUBIN (unpublished results) we learned that the spacing of sequences homologous with the *D. melanogaster* clones (CS*sn*2, 8 and 12) is similar to that found in *D. melanogaster*, as is the position of the insertion (within 1 kb of the predominant target region in *D. melanogaster*; H. ROIHA, K. O'HARE and G. RUBIN, unpublished results). These considerations led us to conclude that we had cloned most, if not all, of the *singed* region in *D. ananassae*. The probing of genomic digests of sn^{9g} *Om(1D)9g* vs. various sn^+ stocks with these recombinant phages enabled us to conclude that sn^{9g} is, in fact, due to the insertion of additional DNA sequences. Figure 4 shows the Southern blot of *Om(1D)9* and *Om(1D)9* sn^{9g} DNAs probed with λ*sn*12. Evidence for a 6.5-kb insertion is the increased size of the 10.4-kb *Sal*I fragment.

Two subclones of λ*sn*6, p*sn*6R1 and p*sn*6R3 (see Figure 3) were used to screen a λEMBL4/*Bam*HI//sn^{9g} *Om(1D)9g*-male/*Mbo*I library. Males were used



Om Mutant	First marker stock	Recombinants	Second marker stock
Om(1A)24	$ct^5\ y\ f^{49}\ w^{65}$	ct^5	$sn^{67}\ rst$
		$y\ f^{49}\ w^{65}$	ct^5
Om(1D)5 *Om(D)30* *Om(1D)48*	$m^2\ v^2\ g^3$	$m^2\ v^2$	$f^{49}\ Bx^2\ w^{65}$
		g^3	$f^{49}\ Bx^2\ w^{65}$
Om(1E)53	$f^{49}\ Bx^2\ w^{65}$	$f^{49}\ Bx^2$	$sc^{33}\ m^{62}\ w^t$
		w^{65}	$sc^{33}\ m^{62}\ w^t$
Om(1G)52	$sn^k\ sc^{24}\ rb^2$	$sn^k\ sc^{24}$	$m^2\ v^2\ g^3$
		rb^2	$m^2\ v^2\ g^3$

FIGURE 2.—Mating schemes used to isolate recombinant *Om* lines for *in situ* hybridization. (*Above*) Shows the marker stocks used in the experiments with the various *Om* loci. (*Below*) An example of the mating scheme used to isolate *Om(1D)* alleles from sites of homology to the 6.5-kb insert. Individual *Om(1D)* males from each of three mutant stocks were mated to virgin $m^2\ v^2\ g^3$ females; their F_1 daughters were mated to $m^2\ v^2\ g^3$ males, and $m^2\ v^2$ and g^3 recombinant sons were recovered. These males were individually mated to virgin $f^{49}\ Bx^2\ w^{65}$ females and were then backcrossed with their daughters. The progeny of both sexes that shared the phenotype of their fathers were pair-mated (10 pair matings per line, to ensure recovery of a pure breeding line of the correct genotype). Sufficient male progeny from one such pair were examined to ensure that it was homozygous for the *X* chromosome.

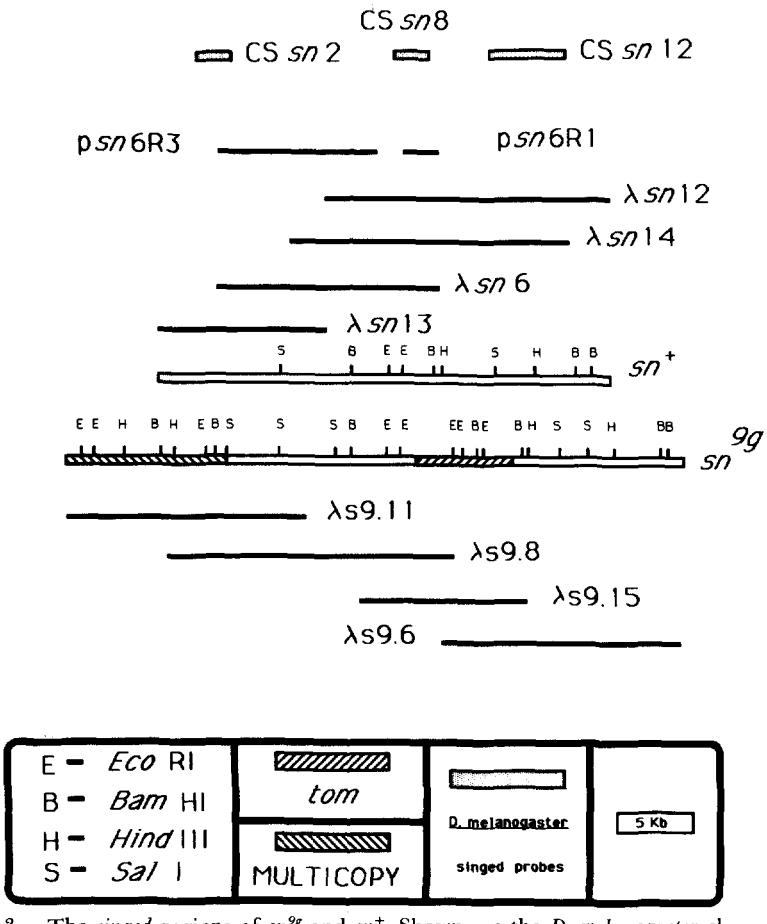


FIGURE 3.—The *singed* regions of sn^{9g} and sn^{+}. Shown are the *D. melanogaster* clones from the *singed* region: CS*sn*2, 8 and 12. Below these are the sn^{+} clones w^{t} stock of *D. ananassae* (plasmids: p*sn*6R1, p*sn*6R3; phages: λ*sn*12, 14, 6 and 13) and their composite restriction map. Next is the map of the sn^{9g} *singed* region, with the regions of multicopy sequence indicated. Below this are shown the phage clones of this region, λs9.11, 9.8, 9.15 and 9.6.

because sn^{9g} females are sterile but have wild-type bristles and cannot be distinguished from their heterozygous [*Om(1D)9g* sn^{+}/*Om(1D)9g* sn^{9g}] sisters. sn^{9g} males were separated from their sn^{+} brothers prior to the isolation of their DNA. Eight recombinant phages were isolated and used to construct a restriction map of sn^{9g} (Figure 3). A comparison of sn^{9g} and wild-type restriction maps showed that the sn^{9g} contains a 6.5-kb insert in the 1.6-kb *Eco*RI/*Bam*HI fragment present in p*sn*6R1 (see Figures 3 and 4). The exact location of the insert within this fragment is uncertain, but since single copy *singed* sequences were found on both sides of the insert within the 1.6-kb *Eco*RI/*Bam*HI fragment (data not shown), the 6.5-kb insert may be centrally located.

The restriction maps of sn^{9g} and sn^{+} from the w^{t} stock also differ at the left end (Figure 3). Genomic Southern blots and *in situ* hybridization to salivary gland chromosomes with sn^{9g} clones indicated that the sn^{9g} males carry another

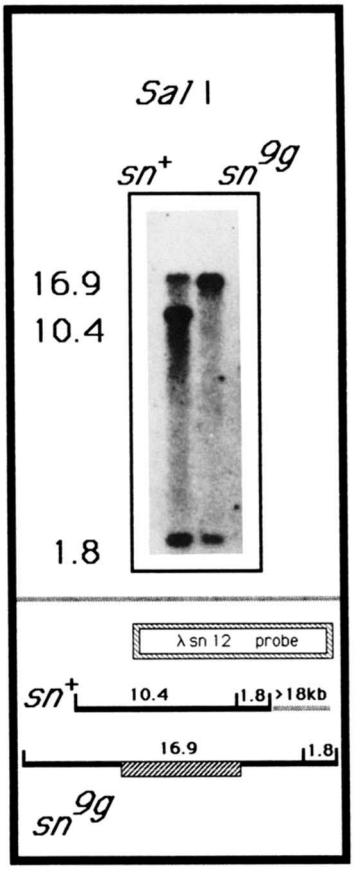


FIGURE 4.—sn^{9g} is associated with a 6.5-kb insertion, as indicated in this Southern blot of sn^+ and sn^{9g} probed with λ*sn*12. The 10.4-kb *Sal*I fragment in *Om(1D)9* sn^+ (lane 1) is replaced by a larger (~16 kb; lane 2) fragment in *Om(1D)9g* sn^{9g}. The restriction maps below indicate the interpretation.

repetitive sequence in the left end of the cloned region. Genomic DNA from sn^{9g} males and their sn^+ brothers was digested with *Bam*HI and *Eco*RI, electrophoresed in 0.8% agarose gel, blotted onto nitrocellulose and probed with λ*sn*12 and λ*sn*13. The hybridization patterns were the same when probed with λ*sn*13, indicating that sn^{9g} and sn^+ males have the same restriction site pattern at the left end of the cloned *singed* region, *i.e.*, they both carry the repetitive sequence at the left end. However, the two genotypes differ when probed with λ*sn*12, indicating that the 6.5-kb insert in the 1.6-kb *Bam*HI/*Eco*RI fragment of the sn^+, rather than the repetitive sequence to the left, produces the sn^{9g} phenotype. The next step was to determine if the 6.5-kb insert showed any homology to *Om* sites.

A preliminary *in situ* hybridization survey of various laboratory stocks of *D. ananassae* showed that the *ca*, *px* and *Om* stocks had from 6 to 14 sites of hybridization to λs9.6 (containing part of the 6.5-kb insert), whereas others

TABLE 1

Site of λs9.6 and λs9.15 labeling in experimental *X* chromosomes of *D. ananassae*

X chromosomes	Cytological locations												
Om(1A)24	1A[d]			9A	9C		10B			11C					
Om(1D)5			8B	9A		10A				11C	12A	13A[p]			16A
Om(1D)30			8B	9A			10B			11C	12A	13A[p]		15B	
Om(1D)48				9A			10B		11A	11C		13A[p]		15B	16A
Om(1E)53				9A		(10A)	(10B)		(11A)	11C			14C[d]		16A
Om(1G)52		7A					10B		11A	11C				15B	16C
$ct^5 yf^{49} w^{65}$															
$m^2 v^2 g^3$															
$f^{49} Bx^2 w^{65}$															
$sn^k sc^{24} rb^2$															
$sn^{67} rst$															
ct^5															
$sc^{33} m^{62} w^1$															

[d] = Centromere distal portion of cytological section.
[p] = Centromere proximal portion of cytological section.
( ) = Site segregating in stock.
Underline = Site showing completed linkage to the particular *Om* mutant.

(non-*Om*) had hybridization to the *singed* site and, sometimes, one or two other sites (data not given). Preliminary data indicated four to nine sites of hybridization on the *X* chromosomes in the *X*-linked *Om* stocks (see Table 1) that precluded an unambiguous determination of association with the *Om* mutations. To prove such an association we recovered recombinants from the regions around four of the *X*-linked *Om* sites [*Om(1A)*, *Om(1D)*, *Om(1E)* and *Om(1G)*] as indicated in Figures 2 and 3. The nonconforming phenotype of *Om52* made its original assignment to the *Om(1C)* locus dubious (HINTON 1984), and HINTON (personal communication) has mapped it to a new locus designated *Om(1G)*. As described in the MATERIALS AND METHODS and Figure 2, an *Om* mutant was crossed to a stock carrying flanking recessive visible markers. Recombinants between these flanking markers were recovered and made homozygous (*e.g.*, the specific crosses for the three alleles at *Om(1D)* are shown in Figure 2). These were then scored for *Om* phenotype and for sites of homology to λs9.6 or λs9.15 (λs9.6 and λs9.15 bear most of the 6.5-kb insert and some *singed* region sequences; see Figure 3).

As is shown in Tables 1 and 2 and in Figure 5, a single site of *in situ* hybridization of λs9.6 or λs9.15 was found to be completely linked to each of the *Om* mutants. Of the 168 recombinant lines examined (Table 2), 72 were *Om* and labeled at the site indicated in Table 1. As shown in Table 1, 96 were Om^+ and failed to label at the indicated sites. For example, *Om(1A)* was already known to be tightly linked to the *cut* locus at the genetic tip of *XL*. All 21 ct^+ *y* recombinants from *Om(1A)/ct y* females were *Om* in phenotype and labeled the polytene tip of *XL* when probed with λs9.6 or λs9.15. We had previously determined by *in situ* hybridization with *D. melanogaster* probes (data not shown) that sequences homologous to the *cut* locus of *D. melanogaster* are

TABLE 2

The results of mapping of four *X*-linked *Om* loci and homology to the 6.5-kb *singed* insert

	Recombinant lines	No. of lines		Polytene *Om* site
		Tested	Labeled	
Om(1A)24	ct^5 *Om24* y^+			
	ct^5 Om^+ *y*	21	0	
	ct^+ *Om24* y^+	21	21	1A
	ct^+ Om^+ *y*			
Om(1D)5	v^2 *Om* g^+	3	1	13A
	v^2 Om^+ g^+	1	0	
	v^+ *Om* g^3	3	3	13A
	v^+ Om^+ g^3	3	0	
Om(1D)30	v^2 *Om* g^+	1	5	13A
	v^2 Om^+ g^+	1	0	
	v^+ *Om* g^3	3	7	13A
	v^+ Om^+ g^3	3	0	
Om(1D)48	v^2 *Om* g^+	5	3	13A
	v^2 Om^+ g^+	7	0	
	v^+ *Om* g^3	7	7	13A
	v^+ Om^+ g^3	7	0	
Om(1E)53	Bx^2 *Om53* w^+	12	12	14C
	Bx^2 Om^+ w^+	12	0	
	Bx^+ *Om53* w^{65}	5	5	14C
	Bx^+ Om^+ w^{65}	11	0	
Om(1G)52	sc^{24} *Om52* rb^+	8	8	9A
	sc^{24} Om^+ rb^+	13	0	
	sc^+ *Om52* rb^2	4	4	9A
	sc^+ Om^+ rb^2	17	0	

located at the tip of *XL* in *D. ananassae*. All 21 *ct* y^+ recombinants were Om^+ and failed to label at the tip of *XL* with λs9.15. A specific cytological site of hybridization to the 6.5-kb *singed* insert was mapped for each of the six *X*-linked *Om* mutants investigated (see Tables 1 and 2). *Om(1A)24*, *Om(1G)52*, the three *Om(1D)* alleles and *Om(1E)53* mapped to positions: distal 1A, 7A, proximal 13A and distal 14C, respectively (HINTON and DOWNS 1975). For all four *Om* loci investigated, the remaining ten *X*-linked sites labeled by the 6.5-kb *singed* insert-bearing phages (λs9.6 and λs9.15) were genetically separable from the *Om* mutation (see Tables 1 and 2).

The complete linkage between sites of hybridization to the 6.5-kb *singed* insert and *Om* mutant phenotype establishes that either part or all of the 6.5-kb *singed* insert is found at *Om* mutant loci. λs9.6 and λs9.15 were used interchangeably as probes, and since they share 1.8 kb of *singed* sequence, there must formally remain the possibility that this *singed* sequence is present at *Om* sites, rather than the 6.5-kb insert. However, λ clones from the *singed* region of w^t hybridized to only one site and appeared to be single copy in genomic Southern blots, so this possibility seems unlikely.

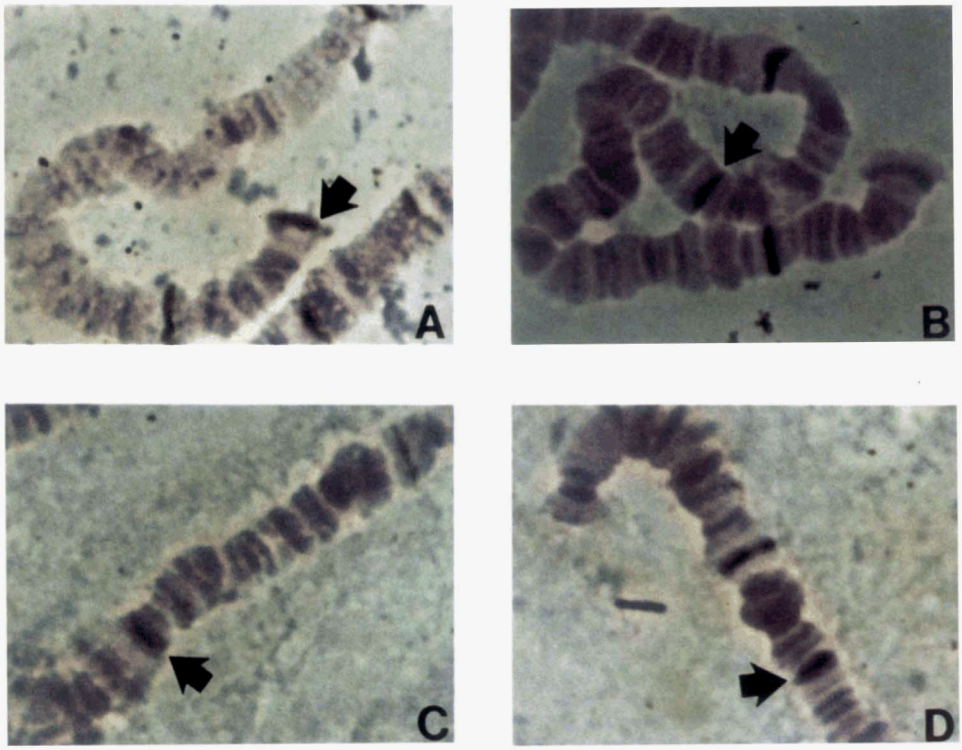


FIGURE 5.—*In situ* hybridization of *tom*-bearing probe λs9.6 to salivary gland chromosomes of four *Om* stocks [A, *Om(1A)24*; B, *Om(1G)52*; C, *Om(1D)48*; and D, *Om(1E)53*]. In each panel the appropriate arm of the *X* chromosome is shown. An arrow indicates the labeling site found to be linked to the *Om* mutation (see text).

DISCUSSION

In a comparatively short time the generally accepted view of a largely rigid, immobile genome has been changed by the discovery and study of mobile genetic elements. Although MCCLINTOCK (1956) originally saw significant developmental roles for such elements and, more recently, some authors have speculated about their importance in cancer, evolution and speciation, the only biological phenomenon that is consistently associated with mobile genetic elements is mutation. The developmental and site specificities of the *Om* mutability system make it particularly relevant to these other suspected functions of transposable elements.

The vast majority of mutations recovered by HINTON from the *ca*; *px* stock and its derivative *Om* mutator stocks are *Om* in phenotype and map to 20 sites in the *D. ananassae* genome. This association of a particular mutant phenotype with specific sites is unique among eukaryotic transposable element systems. In this report we have presented evidence that one of the rare non-*Om* mutations recovered by HINTON, sn^{og}, is due to an insertion of a 6.5-kb sequence of repetitive DNA into the *singed* region. This mutation was recovered in a single male whose paternal *Om(1D)9* allele had simultaneously mutated to the more

extreme allele, *Om(1D)9g*. This coincidence indicated that the insert in the *singed* region might have homology to the *tom* element, the putative cause of the various *Om* mutations. Our genetic analysis has demonstrated that the 6.5-kb insert in the *singed* region has substantial homology to sequences present at the sites of all *Om* mutations studied. The wild-type loci showed no homology to this insert. The most reasonable interpretation of these results is that *Om* mutations are associated with the presence (presumably via insertion) of multicopy sequences with substantial homology to the 6.5-kb insert at sn^{9g}. We do not know if the entire 6.5-kb insert is present in *Om* mutations or if other sequences are also inserted at *Om* mutations. Further analysis will determine whether the 6.5-kb insert represents the complete *tom* element, as proposed by HINTON (1984). But it does seem that *Om* mutability is associated with a mobile genetic element.

HINTON (1984) speculated that *Om* mutants arise from the insertion of a transposable element, *tom*, the target site of which is found within a control sequence shared by structural genes coordinately expressed during eye morphogenesis. An alternative hypothesis is that *tom* itself carries the *Om* determinant and is limited by target site availability or has a phenotype expressed only at the 20 *Om* sites. Interactions between the environs of particular target sites and *tom* could account for the differences in *Om* mutant phenotypes. We have not been able to distinguish between these alternatives. Detailed molecular analysis of inserts and target sites of both *Om* and non-*Om* regions should answer some of these interesting questions about the biology of the *Om* mutability system in *D. ananassae* and its associated transposable element, *tom*.

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