Proc. Natl. Acad. Sci. USA
Vol. 74, No. 7, pp. 2973–2975, July 1977
Genetics

Genetic instability in *Drosophila melanogaster*: Putative multiple insertion mutants at the singed bristle locus

(mutable genes/back mutation/spontaneous mutation)

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Communicated by Dan L. Lindsley, May 2, 1977

ABSTRACT **A series of eleven independent mutants at the *X* chromosome singed bristle (*sn*) locus of *Drosophila melanogaster* is described. All mutants descend from flies caught in the wild and bred in the laboratory. On the basis of their inordinately high spontaneous mutation frequency, ten of the mutants are classified as putative insertion mutants. Reversions to wild type occur at frequencies of 10^{-4}–10^{-3}. Some reversions appear to be losses of the inserted element, others appear (by analogy with prokaryotes) to be changes in the orientation of the inserted elements. Consistent with the insertion hypothesis, some *sn* mutants generate what are interpreted to be deletions at the *sn* locus. In their mutational properties, the *sn* mutants are analogous to insertion sequence (IS) elements and bacteriophage Mu of *Escherichia coli*, but the precise nature of the insertion remains unknown.**

A compelling albeit circumstantial case can be made for the occurrence of insertion mutations in *Drosophila melanogaster*. Thus particular spontaneous mutations are presumed to be putative insertion mutations if they fulfill one or more of the following genetic features: revert spontaneously to wild type at inordinately high rates; revert at an increased frequency under the influence of mutagens that make deletions; generate an unusually high frequency of spontaneous deficiencies that map to the site of mutation; and decrease the frequency of interallelic crossing over. By invoking these criteria, the existence of putative insertion mutations can be inferred at a number of *X* chromosome loci (1).

By and large the occurrence in *D. melanogaster* of putative insertion mutations has been infrequent and sporadic. Consequently, the recovery of a series of independent, presumptive insertion mutants at one locus is of more than passing interest. We here report the recovery of a group of functional alleles at the *X*-linked, recessive, singed bristle (*sn*) locus of *D. melanogaster*. All are independent and spontaneous in origin and all exhibit inordinate mutability consonant with the mutability of insertion mutations.

Origin of the *sn* Mutants. All mutants stem from phenotypically wild-type *D. melanogaster* males collected at Tashkent (1973) and Krasnodar (1974), U.S.S.R. These males were brought into the laboratory and crossed to homozygous *Basc* females. [*Basc* is a multiply inverted *X* chromosome marked with the mutants Bar eye (*B*), white-apricot (w^a), and scute (*sc*).] Spontaneous *X*-linked recessive lethal mutations were scored in the F_2 after crossing individual F_1 *Basc*/+ females to their brothers. The several *sn* mutants were recovered among the otherwise wild-type F_2 males; mutants *77-27* and *63-15* came from Tashkent, the remainder from Krasnodar. In Table 1 the *sn* mutants are listed according to their numerical designation, phenotype, and origin in the F_2. Because bristle phenotype is rather subjective, the mutants were arbitrarily put into three classes characterized as follows. The phenotype designated s in Table 1 means slight, with the bristles exhibiting a wavy appearance. Phenotype m means moderate and the bristles appear hooked or mildly twisted with the hairs not affected. Finally, phenotype e means extreme, with the bristles distinctly twisted and the hairs also affected. All mutants were fully penetrant. Each mutant was tested to a known *sn* mutant and its functional allelism was confirmed. So far as phenotypes are concerned, each *sn* mutant listed in Table 1 has its counterpart among the conventional *sn* mutants already described (2).

Mutability of the *sn* Mutants. Subsequent to its discovery each *sn* mutant was crossed to attached-*X* females and a stock was established. Examination of the male progeny in each stock revealed the presence of phenotypically wild-type (sn^+) males, suggesting that the *sn* mutants were reverting at inordinately high frequencies. Therefore, a systematic study of the mutability of a number of the *sn* mutants was undertaken. A simple crossing procedure was adopted. For each *sn* mutant 20 to 25 newly enclosed males were crossed individually to harems of six females homozygous for the recessive *X* chromosome mutants y^2 *w spl* sn^3(cf. ref. 2 for a description of the mutants). Exceptions were scored among the F_1 females on the basis of their bristle phenotype. Two classes of exceptions were sought: (*i*) reversions to wild type in which the F_1 females exhibited a normal (sn^+) bristle phenotype; (*ii*) mutation to a more extreme *sn* mutant identified as a bristle phenotype of F_1 females whose departure from wild type is greater than that of their sisters.

The results of testing the mutability of 11 separate *sn* mutants are given in Table 2 and merit a few comments. Among 11 mutants tested, 10 were mutable. On the basis of their phenotype, three classes of mutants were detected. One class, designated sn^+ in Table 2, represents reversions to wild-type bristles. The reversion chromosome produces a wild-type phenotype in compound with either sn^3 or an *X* chromosome carrying a cytologically visible deletion of the *sn* locus. A second class, designated sn^{su} (singed subliminal) in Table 2, represents those revertants that appeared wild type in compound with sn^3 but manifest a slight but distinct wavy bristle phenotype in compound with the *sn* deletion. The third class, designated sn^{ex} in Table 2, represents mutation to a phenotypically more extreme allele. These could not always be objectively identified, especially when the *sn* mutant under study evoked an extreme bristle phenotype.

It is obvious from the data of Table 2 that the several *sn* alleles mutate at inordinately high rates. As recorded, many mutants occur in clusters, suggesting that some, perhaps most, mutants are premeiotic in occurrence. If all the mutants of a cluster are assumed to stem from one premeiotic mutational event, then the mutation frequencies range between 10^{-4} and 10^{-3}. It is of relevance to note here that in addition to producing mutations transmitted through the germ line, some alleles were also somatically unstable. The allele *77-27* was especially noteworthy in this respect and genotypically *77-27* males and fe-

Table 1. Summary of the phenotypic properties of the *sn* mutants

Allele designation	*sn* phenotype: Bristles*	*sn* phenotype: Female fertility†	Found in F_2 as:
26-7	m	f	1♂
33-13	m	f	3♂
42-5	e	f	3♂
50-18	e	s	1♂
63-15	s	f	1♂
77-27	e	s	1♂
79-15	m	f	1♂
79-22	e	s	1♂
84-6	e	f	3♂
88-9	s	f	1♂
90-9	m	f	1♂

* s = slight, m = moderate, e = extreme. See *text* for full description.

† Homozygous females fertile (f) or sterile (s).

males were frequently found with patches of *sn*$^+$ bristles amid phenotypically *77-27* bristles. These were most readily detected on the head and thorax. When such mosaic males were bred, some bred as germinally *77-27* but others bred as gonadal mosaics transmitting both *sn*$^+$ and *77-27*-bearing *X* chromosomes.

Mutability of the *sn*$^+$ Revertants. Studies on the mutability of wild-type revertants originating from mutable (insertion) genes at other *X* chromosome loci in *D. melanogaster* suggest that in the main they are mutationally stable (1). However, there is at least one case on record where the revertant is unstable and mutates back to the original mutant state (3). With these results in mind, we examined for mutability a sample of *sn*$^+$ revertants recorded in Table 2 and originating from different *sn* mutants. The test protocol involved crossing 20 to 25 revertant males individually to harems of 10 *cm ct*^{6} *sn*^{3} females and scoring the F_1 females for *sn* mutants. In Table 3, representative results are given for *sn*$^+$ revertants from two different *sn* mutants, *77-27* and *63-15*. These results are essentially self-explanatory and demonstrate that the *sn*$^+$ revertants fall into two classes. The *sn*$^+$ revertant of *77-27* is mutationally unstable, mutating back to a phenotypically extreme *sn* mutant, whereas the revertant derived from *63-15* seems to be mutationally stable. Subsequent tests, which will be reported in detail elsewhere, demonstrate that other *sn*$^+$ revertants of *63-15* are mutationally unstable. Our results with other revertants follow a similar pattern. Thus, for example, in parallel experiments *sn*$^+$ revertants derived from *90-9*, *42-5*, *26-7*, and *42-5* were stable, while a revertant from *50-18* was unstable.

Table 3. Mutability of *sn*$^+$ and *sn*^{su} revertants

Revertant	No. males tested	No. *sn* mutants recovered	Total chromosomes scored
sn$^+$ (*77-27*)	23	8 (2 × 1, 2, 2 × 4, 14, 15, 34)	13,359
sn$^+$ (*63-15*)	24	0	9,660
sn^{su} (*79-22*)	23	13 (9 × 1, 2 × 2, 3, 7)	7,339
sn^{su} (*79-22*)	21	0	6,741

The mutants derived from the *77-27 sn*$^+$ merit a brief discussion here. In phenotype they cannot be separated from the original mutant *77-27* because all exhibit the extreme bristle phenotype and are female sterile. However, mutationally two classes may be distinguished. One class behaves just like the original *77-27*, reverting to *sn*$^+$ both somatically and germinally. A second class is mutationally stable and in extensive tests failed to revert. This latter class will be considered further below.

Mutability of the *sn*^{su} Revertants. The summary remarks bearing on the mutability of the *sn*$^+$ revertants apply equally well to the *sn*^{su} revertants. Although our mutational analysis of *sn*^{su} mutants is not extensive, it is clear that two classes occur,

Table 2. Spontaneous mutability of the *sn* mutants in males

sn mutant tested	No. males tested	No. of mutations recovered*: *sn*$^+$	*sn*^{su}	*sn*^{ex}	Total chromosomes scored
26-7	22	3 (2 × 1, 2)	3 (1, 3, 6)	4	10,789
33-13	23	5 (2 × 1, 2 × 2, 3)	3 (1, 3, 4)	0	7,973
42-5	21	2	0	—	5,929
50-18	20	10 (6 × 1, 2 × 2, 2 × 3)	1	—	7,328
63-15	25	16 (5 × 1, 3 × 2, 4 × 3, 2 × 5, 2 × 6)	5 (1, 2, 3, 8, 49)	3 (2 × 1, 2)	9,588
77-27	25	5 (4, 5, 7, 13, 118)	0	—	12,284
79-15	25	0	0	0	6,224
79-22	22	8 (4 × 1, 2 × 2, 2 × 4)	2	—	4,470
84-6	23	3	2 (1, 6)	—	12,059
88-9	25	0	0	8	6,680
90-9	23	2	0	0	12,550

* The numbers in parentheses represent the mutations recovered per male. Thus, 2 × 1 means 2 males each gave a single mutant, 2 × 2, 2 males each produced two identical mutants, etc. A number without parentheses means all mutants occurred singly.

mutationally stable and mutationally unstable. Table 3 includes results from two different sn^{su} mutants derived from *sn* mutant *79-22* and shows one to be stable, one to be mutable. Similar results were obtained when sn^{su} mutants originating from *63-15* were tested.

Mutability of the sn^{ex} Mutants. As recorded in Table 2, exceptions producing a sn^{ex} phenotype were recovered from three different *sn* mutants. All sn^{ex} mutants bred true and were, on the basis of bristle phenotype, easily distinguishable from the mutants from which they arose. Furthermore, all sn^{ex} exceptions as homozygous females proved to be female sterile even though each arose from a female-fertile *sn* mutant. A further test of the sn^{ex} mutants entailed assaying their mutability. For this purpose two sn^{ex} exceptions that arose from *63-15* were studied rather extensively. In each case single sn^{ex} males were tested for revertability as described above. For each sn^{ex} approximately 8000 chromosomes were scored and no mutations were found either to sn^+ or to an intermediate phenotype. Thus, in comparison to the mutant from which they arose, both sn^{ex} exceptions appear to be mutationally stable. Precisely the same results were obtained with one class of *sn* mutants that arose from wild-type revertants of *77-27*. Individual males were tested for mutability and among almost 20,000 chromosomes scored no mutants were found. Thus, compared to $sn^{77\text{-}27}$, the sn^{ex} derivative is refractory to mutation.

Discussion. On the basis of the diagnostic genetic criterion of inordinately high mutation rate, the *sn* mutants we have described are presumed to be insertion mutations. While we are not in position to specify what is inserted, we infer in part by analogy with proved insertion mutants, e.g., in *Escherichia coli* (4), that "foreign" DNA is inserted. The "foreign" DNA inserted could be analogous to insertion sequence (IS) DNA or to phage DNA such as bacteriophage Mu (5) and lead to the mutational instability we have found. It should be noted that the genetic properties of presumptive *Drosophila* insertion mutants and *E. coli* insertion mutants are similar but not identical. Thus, in the case of presumed *Drosophila* insertions, spontaneous reversion is inordinately high and deletion at the insertion site frequent, but considerably less frequent than reversion. The converse seems to be the case for *E. coli* insertion mutants. Furthermore, at the functional level it is not possible to make a clear-cut comparison between *Drosophila* and *E. coli* insertion mutants. At this time the distinction in *Drosophila* between structural and regulatory mutants cannot be objectively made. Thus, whether or not the mutants are polar, as is the case in *E. coli*, is not clear.

What is of more than passing interest is that we trace the origin of the *sn* insertion mutants to flies caught in the wild and bred in the laboratory. Consideration and testing of this situation is deferred to the succeeding paper (6), in which the basis for the origin of these mutations will be postulated and tested.

At this juncture it is appropriate to consider the kinds of mutational events we have recorded. On the basis of their subsequent mutability, we have found two types of reversions to wild type. One class of sn^+ reversions is mutationally stable. These we infer are associated with excision of the inserted element with a concomitant restoration of the wild-type gene and accordingly mutational stability. The second class of reversions found is mutationally unstable and is characterized by frequent mutations—as judged by phenotype—to the original *sn* mutant from which the reversion arose. These reversions we infer are analogous to the changes in orientation of IS elements recorded in *E. coli* (7). In one orientation the inserted element evokes a mutant phenotype, in the alternative orientation the wild-type phenotype occurs. Evidence that these wild-type reversions do retain an insertion is provided by the fact that they can mutate to a state that evokes the extreme *sn* phenotype, and that is mutationally stable. As we shall argue shortly, we believe these mutations are deletions produced by the faulty excision of the inserted material. Production of deletions in this way is a demonstrated property of insertion mutations.

In addition to reversions, we found that three *sn* mutants produced mutants which were phenotypically classified as sn^{ex} and exhibited a bristle phenotype that is the greatest departure from wild type. All sn^{ex} mutants that are female sterile arose from female-fertile mutants. Those sn^{ex} mutants tested for mutability proved to be stable. Taken together, these facts suggest that the sn^{ex} mutants are deletions in which a loss of the *sn* locus occurred coincidental with excision of the inserted element. Deletion induction in association with excision of inserted elements has been documented in *Drosophila* (8, 9) and in *E. coli* for IS (10), Mu (4), and phage P2 (11). For the present we cannot define the cytological extent of this loss except to note that in the polytene chromosomes of sn^{ex} mutants the *sn* locus appears to be unchanged.

Finally, we have noted the occurrence of two types of sn^{su} mutants: those mutationally stable, those unstable. The nature of the sn^{su} mutants is at present unclear, and rather than indulge in further speculation we shall defer discussing them until additional cytogenetic studies are completed.

We thank G. Lefevre for the cytological analysis reported herein, and anonymous reviewers for constructive comments. M.M.G. was supported by U.S. Public Health Service Grant GM 22221.

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