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. Lindley, 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 $10^{-4}$ to $10^{-3}$. 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 (1975) 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 (wa*), and secur (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 $y^a$ 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$^{ss}$ (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$^{ss*}$ 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 $10^{-5}$. 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-
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Table 1. Summary of the phenotypic properties of the sn mutants

<table>
<thead>
<tr>
<th>Allele designation</th>
<th>Bristles*</th>
<th>Female fertility†</th>
<th>Found in F2 as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>26-7</td>
<td>m</td>
<td>f</td>
<td>1♂</td>
</tr>
<tr>
<td>33-13</td>
<td>m</td>
<td>f</td>
<td>3♂</td>
</tr>
<tr>
<td>42-5</td>
<td>e</td>
<td>f</td>
<td>3♂</td>
</tr>
<tr>
<td>50-18</td>
<td>e</td>
<td>s</td>
<td>1♂</td>
</tr>
<tr>
<td>63-15</td>
<td>s</td>
<td>f</td>
<td>1♂</td>
</tr>
<tr>
<td>77-27</td>
<td>e</td>
<td>s</td>
<td>1♂</td>
</tr>
<tr>
<td>79-15</td>
<td>m</td>
<td>f</td>
<td>1♂</td>
</tr>
<tr>
<td>79-22</td>
<td>e</td>
<td>s</td>
<td>1♂</td>
</tr>
<tr>
<td>84-6</td>
<td>e</td>
<td>f</td>
<td>3♂</td>
</tr>
<tr>
<td>88-9</td>
<td>s</td>
<td>f</td>
<td>1♂</td>
</tr>
<tr>
<td>90-9</td>
<td>m</td>
<td>f</td>
<td>1♂</td>
</tr>
</tbody>
</table>

* 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 harem of 10 cm ct snW females and scoring the F1 females for sn mutants. In Table 3, revertant mutants derived from 90-9, 42-5, 26-7, and 42-5 were stable, while a revertant from 50-18 was unstable.

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

Table 2. Spontaneous mutability of the sn mutants in males

<table>
<thead>
<tr>
<th>sn mutant tested</th>
<th>No. of males tested</th>
<th>No. of mutations recovered*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>sn+</td>
</tr>
<tr>
<td>26-7</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>(2 × 1, 2)</td>
<td></td>
<td>(1, 3, 6)</td>
</tr>
<tr>
<td>33-13</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>(2 × 1, 2 × 2, 3)</td>
<td></td>
<td>(1, 3, 4)</td>
</tr>
<tr>
<td>42-5</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>50-18</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>(6 × 1, 2 × 2, 2 × 3)</td>
<td></td>
<td>(1, 2, 3, 8, 49)</td>
</tr>
<tr>
<td>63-15</td>
<td>25</td>
<td>16</td>
</tr>
<tr>
<td>(5 × 1, 3 × 2, 4 × 3, 2 × 5, 2 × 6)</td>
<td></td>
<td>(1, 2, 3, 8, 49)</td>
</tr>
<tr>
<td>77-27</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>(4, 5, 7, 13, 118)</td>
<td></td>
<td>(1, 2, 3, 8, 49)</td>
</tr>
<tr>
<td>79-15</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>79-22</td>
<td>22</td>
<td>8</td>
</tr>
<tr>
<td>(4 × 1, 2 × 2, 2 × 4)</td>
<td></td>
<td>(1, 6)</td>
</tr>
<tr>
<td>84-6</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>88-9</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>90-9</td>
<td>23</td>
<td>2</td>
</tr>
</tbody>
</table>

* 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.

Table 3. Mutability of sn+ and snXX revertants

<table>
<thead>
<tr>
<th>Revertant</th>
<th>No. males tested</th>
<th>No. sn+ mutants recovered</th>
<th>Total chromosomes scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>sn+ (77-27)</td>
<td>23</td>
<td>8</td>
<td>13,359</td>
</tr>
<tr>
<td>(2 × 1, 2, 2 × 4, 14, 15, 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sn+ (63-15)</td>
<td>24</td>
<td>0</td>
<td>9,660</td>
</tr>
<tr>
<td>sn&quot; (79-22)</td>
<td>23</td>
<td>13</td>
<td>7,339</td>
</tr>
<tr>
<td>(9 × 1, 2 × 2, 3, 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sn&quot; (79-22)</td>
<td>21</td>
<td>0</td>
<td>6,741</td>
</tr>
</tbody>
</table>
mutationally stable and mutationally unstable. Table 3 includes
results from two different sn° mutants derived from sn mutant
79-22 and shows one to be stable, one to be mutable. Similar
results were obtained when sn° mutants originating from
63-15 were tested.

Mutability of the sn° Mutants. As recorded in Table 2,
exceptions producing a sn° phenotype were recovered from
three different sn mutants. All sn° mutants bred true and
were, on the basis of bristle phenotype, easily distinguishable
from the mutants from which they arose. Furthermore, all sn°
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° mutants entailed assaying their muta-
bility. For this purpose two sn° exceptions that arose from
63-15 were studied rather extensively. In each case single sn°
males were tested for revertability as described above. For each
sn° 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° 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. Indi-
vidual males were tested for mutability and among almost
20,000 chromosomes scored no mutations were found. Thus,
compared to sn° 77-27, the sn° 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 re-
version. The converse seems to be the case for E. coli inser-
tion 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 objec-
tively 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 situa-
tion is deferred to the succeeding paper (6), in which the basis
for the origin of these mutations will be postulated and test-
ed.

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 ele-
ment 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°
and exhibited a bristle phenotype that is the greatest departure
from wild type. All sn° mutants that are female sterile arose
from female-fertile sn mutants. Those sn° mutants tested for
mutability proved to be stable. Taken together, these facts
suggest that the sn° 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 in-
serted 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 polynucle chromosomes of sn° mutants the sn locus
appears to be unchanged.

Finally, we have noted the occurrence of two types of sn°
mutants: those mutationally stable, those unstable. The nature of
the sn° mutants is at present unclear, and rather than in-
dulge 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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