Genetic Mapping of the *bss* Mutation in *Drosophila Melanogaster*

Nathaniel Tussey, Professor Christopher Jones
Biology, Moravian College, 1200 Main Street, Bethlehem, PA 18018

Abstract

The bang senseless mutation, *bss,* is a well documented member of bang-sensitive mutants in *Drosophila* that become paralyzed upon mechanical shock. Previous recombination mapping has limited *bss* to the 14B2 – 14B9 region on the X chromosome corresponding to the markers *sd* (scalloped) and *f* (forked). The dominant nature of *bss* precludes deletion mapping. We are currently constructing new triple point test crosses that will narrow the breadth of the suspected area. We are also investigating possible recessive traits of the *bss* mutation.

Introduction

The *bss* mutation is one of several bang-sensitive *Drosophila* mutants characterized by seizure and paralysis upon mechanical shock *(2)*. This family of mutants is believed to result from mutations of membrane transporters necessary for maintenance of ionic gradients *(4)*. These gradients are responsible for the relay of electrical impulses between the neurons in our nerves which control movement. It is thought that knowledge of the nervous system gained by study of *Drosophila* mutants can be applied to the study of mammalian nervous systems, in particular epileptic seizures *(4)*.

First believed to be allelic to the mutation bang sensitive (*bas*), *bss* was named bas^{MW1} under the notion that it was a recessive mutation *(2)*. Further study correctly identified the mutation as having a semi-dominant nature and was renamed bss^{MW1} along with a previously identified mutant, PC75, which was allelic to bas^{MW1} and renamed bss^{2} *(5)*. *bss* is characterized by a stereotyped sequence of initial muscle spasm, paralysis, delayed spasm, and recovery *(3)*. The two spasms are typified by collapse of the body, high frequency wing flapping, leg extension, and fully curved abdomen (Figure 1) *(3)*. The recovery phase has been designated as the refractory period during which a mechanical shock is not effective in evoking seizure or paralysis.

bss is believed to lie in the region encompassed by 14B2 – 14B9 of the X chromosome (Figure 2). It has been mapped to position 54.0 by recombination relative to *sd* (scalloped) and *f* (forked) *(2)*. Recombination between *bss* and *eas* (easily-shocked) flies places *bss* 0.8 centimorgans proximal from *eas* (*2*). The exact positioning has yet to be determined due to complication arising from its semi-dominant nature.

Experimental Method

In lieu of the semi-dominant nature of *bss*, traditional mapping techniques proved inconclusive. To this effect the method of triple point test crosses was employed (Figure 3). This technique involves mapping a mutation using three phenotypically visible markers; two flanking the suspected area of mutation and a central marker used for positioning. Recombination would result in differentially marked progeny depending on the positioning of the mutation. The flanking markers used included *f*, *sd*, *os* (out-stretched), *na* (narrow abdomen), and white eyes. The central marker was inserted P-elements resulting in orange colored eyes whose position on the chromosome is known to the exact base pair..

The other experimental focus was finding a recessive trait associated with *bss*. Any such trait could potentially be used in conjuction with traditional deletion mapping to narrow the range of possible *bss* location. The first trait tested was differential temperature-sensitive paralysis. Flies tested included three wild-type strains (OR (Oregon-R), CS (Canton-Special), and BePA2 (Bethlehem Pennsylvania 2)), *bss*/*bss* homozygotes, and *bss*/wild-type heterozygotes. Temperatures ranging between 5-10°C were tested. Any differentiation in paralysis between bss homozygotes, bss heterozygotes, and wild-type strains could be used as a recessive trait in deletion mapping.

Length of paralysis was another trait tested for differential result. Flies tested included the three wild-type strains, *bss*/*bss*, *bss*/wildtype, *bss*/deletions in the range encompassing *bss*, and bss^{2}/wildtype. Paralysis was induced by vortexing five or less flies in an empty vial for ten seconds. Time of paralysis was recorded from cessation of vortexing till first movement. Any differentiation in length of paralysis between *bss* homozygotes, *bss* heterozygotes, and wild-type strains could be used as a recessive trait in deletion mapping.

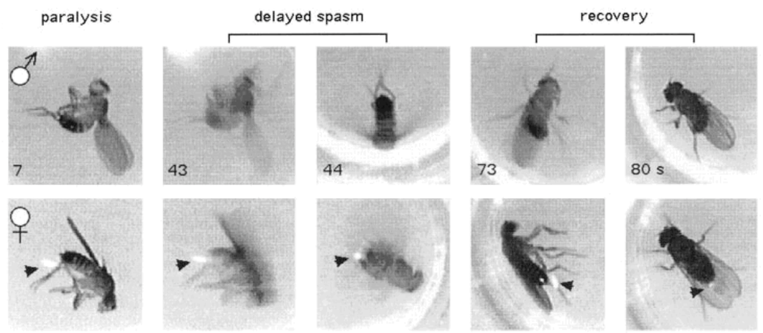

Figure 1. Pictorial representation of *bss* paralysis, spasm, and recovery. Arrow in female photographs indicates laying of egg *(3)*.

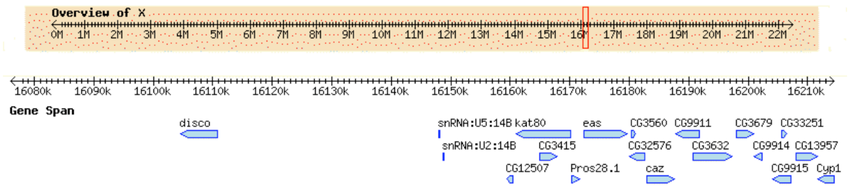

Figure 3. Representation of the *Drosophila* X chromosome (salmon). Pictured in blue are all known or suspected genes residing in the 14B2 – 14B9 region, the range suspected of containing the mutated gene responsible for *bss (1)*.

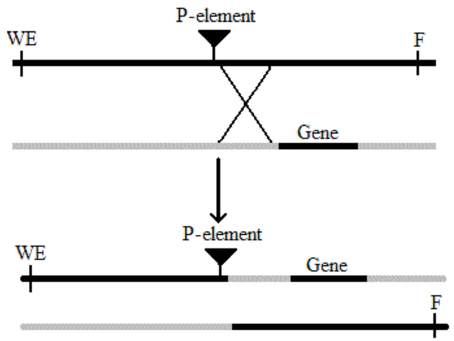

Figure 3. Example of possible triple point test cross: Two chromosomes, one with the phenotypically visible markers WE, F (*f*), and a P-element, and one with the gene of interest (mutation), undergo recombination. The black lines constituting the X represent the lines of recombination. The resulting chromosome with the gene of interest and P-element but lacking F indicates that the gene of interest is to the right of the P-element, whose exact position is known.

Results and Discussion

Testing for differential paralysis, either temperature or vortex induced, did not result in a reliable means of phenotypic identification. Temperature paralysis was tested every degree from 5-10°C. At 5°C, all flies became paralyzed and above 10°C no flies experienced paralysis. Temperatures in-between these extremes resulted in no differentiation of paralysis. Length of paralysis was tested after vortexing. Of the flies that did experience paralysis the longest time recorded was 28 seconds, abnormally short in comparison to the average time of paralysis for a *bss* homozygote which spans minutes. No discernable differentiation in the time of paralysis was detected.

Testing of the triple point test crosses was also unsuccessful. The construction of the chromosomes with the three markers was not completed. As the markers and the gene of interest become closer to one another on the chromosome, the chance of recombination between them diminishes, in some cases resulting in a successful recombination in less than 1% in all progeny.

While the range of potential *bss* location was not narrowed, valuable characteristic information was obtained from the experiments. The semi-dominant nature of *bss* was confirmed and reports of temperate-sensitive paralysis and differential length of paralysis were disproved. The next phase in locating *bss* would involve the technique of reverse rescue. This involves isolating a gene thought to contain the *bss* mutation and placing it into wildtype flies to see if the *bss* phenotype occurs.

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