

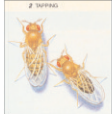
# Behavioral characterization of the *Drosophila* bang-sensitive mutant *slamdance (sda)*

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## Courtship

Courtship has a long history as a behavioral assay in *Drosophila*. The process of courtship may be broken down into 6 stages:

Upon recognizing the female fly, the male orients toward her, positioning himself in front of her to visually stimulate her.



The male then moves to the female's side and taps her abdomen with his foreleg; this allows the male to determine via her cuticular pheromones whether the female is receptive to mating.

He next vibrates whichever of his wings is closer to the female's head. The resulting species-specific "song" functions to acoustically stimulate the female and to identify the male as a conspecific.



The male then extends his proboscis and "licks" the female's genitalia to further stimulate her and to taste her pheromones.

After this, the male attempts to copulate: he mounts the female from behind and curls his abdomen beneath hers until their genitals touch.



Finally the male copulates with the female.

## Procedures

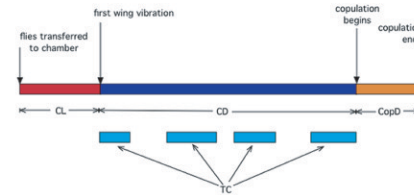
We are interested in whether mutations in the gene *slamdance (sda)* have any effect on this complex behavior. Both mutations in *sda* identified to date result in a bang-sensitive phenotype: following strong electrical or mechanical stimulation, *sda* mutant flies seize and become paralyzed. This paralysis typically lasts for about 30 seconds, after which the flies recover completely. The two mutations described to date appear to be recessives with incomplete penetrance: only a subset of homozygous mutant flies will seize upon mechanical agitation of their container. The *sda* gene product very closely resembles human aminopeptidase N, a membrane-bound enzyme which cleaves neutral or basic amino acids from the N-terminus of small peptides and has been implicated in a wide variety of biological processes, including digestion, collagen degradation, antigen presentation, and peptide activation (described in Zhang et al., *Genetics* 162: 1283 (2002)).

Courtship assays were conducted in a specially-constructed apparatus: a 0.25-cm thick piece of Plexiglass with 1.27-cm diameter holes drilled through it. This was sandwiched between glass, so that each assay chamber had a Plexiglass wall and a glass floor and ceiling.

We used the *iso<sup>7-8</sup>* allele of *sda* for all courtship assays reported here. Oregon-R (OR) and *sda* flies outcrossed to an OR background were raised on standard cornmeal-agar-molasses medium, collected under light CO<sub>2</sub> anesthesia, and housed en masse (by gender) in standard food vials. Homozygous *sda* flies were tested for bang sensitivity several days after eclosion; those which became paralyzed were then separated from their siblings which didn't. All flies were assayed one week after eclosion.

In scoring courtship, five different measurements were recorded:

- **Courtship Latency (CL):** Courtship latency was measured as the time that passed between the introduction of the two flies into the mating chamber and the execution of the male's first wing vibration.
- **Courtship Duration (CD):** Courtship duration was measured as the time from when the male first began courting the female by executing an initial wing vibration to the onset of copulation.
- **Time Courting (TC):** Time courting was measured as the time within the CD that the male fly spent actively courting the female. Behaviors that were scored as active courtship consisted of orienting, chasing, wing vibrating, licking, curling, and attempting copulation.
- **Courtship Index (CI):** Courtship index was calculated as the percentage of time of courtship duration that the male spent actively courting the female. This percentage was calculated by dividing TC by CD.
- **Copulation Duration (COPD):** Copulation duration was measured as the time the two flies spent copulating.



We also noted the Fraction Courting (FC) and Fraction Copulating (FCop): The percentage of males in each experimental group which initiated courtship within 10 minutes and the percentage of those which copulated within 20 minutes.

Trials in which the male didn't initiate courtship within 10 minutes were discontinued, as were trials in which the male didn't copulate within 20 minutes.

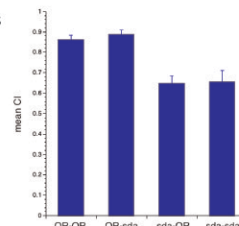
## Results

We tested OR and *sda* males with both OR and *sda* females, for a total of four experimental groups. For convenience, we abbreviate each group with the male genotype followed by the female genotype; thus OR-*sda* indicates OR males courting *sda* females.

**FC:** no significant differences among groups (Row x Column test for independence (Sokal & Rohlf, 1981),  $G(\text{adj}) = 2.69$ ,  $0.25 < p < 0.50$ )

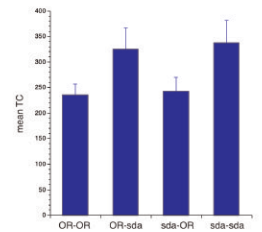
**CL:** extremely significant differences among groups (Kruskal-Wallis test;  $p < 0.0001$ )

[All pairwise comparisons significant (Dunn's Multiple Comparisons test;  $p < 0.0001$ ) except OR-OR vs. OR-*sda* and *sda*-OR vs. *sda*-*sda*, indicating that female genotype does not contribute to the observed differences in CI values.]



**TC:** significant differences among groups (Kruskal-Wallis test;  $p = 0.0212$ )

[No pairwise comparisons significant (Dunn's Multiple Comparisons test;  $p > 0.05$ ). If we pool these data by female genotype, there is a very significant difference (Mann-Whitney two-tailed  $p = 0.0026$ ), suggesting *sda* females are perceived differently from OR females as courting objects.]



**CD:** extremely significant differences among groups (Kruskal-Wallis test;  $p = 0.0002$ )

[Three pairwise comparisons significant (Dunn's Multiple Comparisons test;  $p < 0.0001$ ): OR-OR vs. *sda*-*sda* ( $p < 0.001$ ), OR-OR vs. *sda*-OR ( $p < 0.05$ ), and OR-*sda* vs. *sda*-*sda* ( $p < 0.05$ ).]

**FCop:** extremely significant differences among groups (Row x Column test for independence (Sokal & Rohlf, 1981),  $G(\text{adj}) = 48.17$ ,  $p < 0.001$ ); groups with OR males are homogeneous ( $G = 2.13$ ,  $0.10 < p < 0.25$ ) as are those with *sda* males ( $G = 0.51$ ,  $0.25 < p < 0.50$ ), again indicating that female genotype is not relevant to the observed differences.

**CopD:** nearly-significant differences among groups (Kruskal-Wallis test;  $p = 0.0725$ )

[If we pool these data by male genotype, the raw data show a significant difference (unpaired t test with Welch correction,  $p = 0.0346$ ), and reciprocally-transformed data show a very significant difference (Mann-Whitney two-tailed  $p = 0.0084$ ).]

## Conclusion

In summary, a normal number of *sda* males will court (FC), but have an abnormal courtship pattern (CI). Most of this arises due to differences in CD: *sda* males spend a little more time courting than do OR males, but these courting bouts are spread out over a much larger time (CD). Thus *sda* males seem to lack "motivation" in their courtship bouts. This is consistent with the observation that significantly fewer *sda* males than OR males copulate within 20 minutes (FCop). Those *sda* males who do copulate do so for a nearly-normal period of time (COPD).

We are continuing this work by looking at whether our results are due to differences in peripheral (visual, olfactory, locomotor) behaviors between OR and *sda* flies. We also plan to evaluate the courtship behavior of *sda* flies which aren't paralyzed upon mechanical agitation, as well as at the courtship of OR/*sda* heterozygotes. We also plan to examine the effects of *sda* mutations on associative learning and memory using conditioned courtship.



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