

Researchers identify a gene that causes male *Drosophila* to produce different courtship songs

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Credit: Howard Hughes Medical Institute

For decades, pinpointing the precise relationship between genes and behavior has been notoriously tricky for biologists. But now, Janelia Research Campus scientists have devised new strategies to drill down into this link. Using new technologies, a group of scientists, led by David Stern, has identified a gene that causes male *Drosophila* to produce

different courtship songs – one sings a high note; the other strikes a low note.

"Although biologists have spent decades trying to track down the genes that cause variation in behavior, there's precious little data to show for that," says Stern, a group leader at Janelia. He hopes the findings and methodology reported on August 10, 2016 in *Nature* will serve as a roadmap for biologists, allowing them to determine other genetic roots of behavior.

The fruit fly song variation, which Stern's group originally mapped between two species, *Drosophila simulans* and *Drosophila mauritiana*, comes down to a mutation in a gene that codes for an ion-channel protein, called slowpoke. The causal mutation is an insertion of a large retroelement into an intron of slowpoke, meaning that the mutation is located in a non-coding region of the gene. "This is one example of how genes evolved to generate variation," Stern says. "But to get general insight into how behavior evolves, we need lots of examples."

Before identifying slowpoke as the gene responsible for the tonal variation, Stern and colleagues at Janelia designed solutions to a handful of obstacles, starting with a custom-built recording studio, tailored to *Drosophila*.

To attract a mate, male fruit flies "sing" by beating their wings, making two noises – pulse song, which sounds like a mini helicopter, and sine song, a continuous hum at a constant frequency. But the songs are very quiet – female flies can hear the call only when they are within about a centimeter of the singing male. So Stern's team created tiny recording booths to eavesdrop on courting couples.

"We call it the courtship-orama," says Stern. "It can record 32 pairs [of flies] simultaneously with an array of 32 microphones. Then, our custom-

built amplifiers boost the sound by 1,000 times, and feed the songs into our software system, which automatically analyzes each feature and tells us what type of song the male is singing."

Paired with a high-throughput sequencing technique, called multiplexed shotgun genotyping, Stern's group initially performed quantitative trait locus mapping, which reveals statistical linkage between behavioral traits (in this case, the tone of sine song) and regions of the genome. They found that variation in the tone of sine song was associated with a stretch of about 1 million base pairs of DNA.

Stern and Yun Ding, a postdoctoral researcher at Janelia, then whittled down that stretch of 1 million base pairs to about 140,000 base pairs through a targeted breeding strategy. Ding saw that progeny flies singing at a lower frequency, typical of *D. simulans*, always carried a 140,000 base pair stretch of DNA from *D. simulans*; flies with this piece of DNA from *D. mauritiana*, however, sang at a higher frequency.

Still, Stern and Ding were not satisfied with the mapping resolution; many genes reside within that 140,000 base pair region, any one of which could have been responsible for the difference in song frequency. To generate higher resolution, the team used CRISPR/Cas9-mediated genome editing to introduce fluorescent markers at specific sites in the genome. They could then pick out flies with recombination events within this specific genomic region by just glancing at the flies' eyes. Using this strategy, they whittled the cause of the song difference down to less than 1,000 [base pairs](#) within the gene *slowpoke*. They then used CRISPR to remove the suspected causal mutation, an intronic retroelement, allowing them to confirm that it caused low frequency songs. Surprisingly, this retroelement was found only in the *D. simulans* strain used for mapping, but not in the other *D. simulans* strains examined, meaning that it's an evolutionarily recent, rare variant in *D. simulans* populations. Further

studies are required to unravel the history of this variant in *D. simulans* populations. This result also indicates that more genes than just *slowpoke* contribute to sine tone variation in *D. simulans* and *D. mauritiana*.

"This study is a real tour de force because it identifies the genetic origin of a behavior variation," says Benjamin Prud'homme, evolutionary and developmental biologist at the Developmental Biology Institute of Marseille, France. "It sets a new standard for genetic mapping analyses, including for species that are not classical genetic model systems. Indeed, most of the genome editing techniques used in this study can be transferred to most, if not all species, amenable to transgenesis."

Currently, Stern and colleagues are expanding their work on courtship songs, harnessing their high-throughput methods to uncover other genes that cause variation in courtship song – like pulse speed. But they're also looking at the bigger picture.

"It's not just identifying the genes that are responsible; it's understanding how these genetic changes alter the function of neurons to generate these variations," says Stern.

"The holy grail of all of this would be to bring together neuroanatomy, neurophysiology, and evolutionary genetics to figure out whether there are general principles that underlie how the brain evolves."

More information: Benjamin J Arthur et al. Multi-channel acoustic recording and automated analysis of *Drosophila* courtship songs, *BMC Biology* (2013). [DOI: 10.1186/1741-7007-11-11](https://doi.org/10.1186/1741-7007-11-11)

Yun Ding et al. Natural courtship song variation caused by an intronic retroelement in an ion channel gene, *Nature* (2016). [DOI: 10.1038/nature19093](https://doi.org/10.1038/nature19093)

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