New insights into how genes control courtship and aggression

April 29 2020

Researchers studied how sex-determining genes affect neurons known to control courtship (shown in orange on the left) and aggression (shown in blue on the right) in fly brains. Credit: Salk Institute

Fruit flies, like many animals, engage in a variety of courtship and fighting behaviors. Now, Salk scientists have uncovered the molecular mechanisms by which two sex-determining genes affect fruit fly behavior. The male flies' courtship and aggression behaviors, they showed, are mediated by two distinct genetic programs. The findings, both published in *eLife* on April 21, 2020, demonstrate the complexity of the link between sex and behavior.

"Courtship and aggression seem to be controlled somewhat separately by these two genes," says Kenta Asahina, an assistant professor in Salk's

Molecular Neurobiology Laboratory and senior author of the two papers. "Having behaviors controlled by different genetic mechanisms can have some benefits in terms of evolution." In other words, he explains, a fly population that is under evolutionary pressure to compete more—perhaps due to limited resources—can evolve aggressive behaviors without affecting courtship.

Male fruit flies' aggression is primarily toward other males, while their courtship behaviors—which involve a series of movements and songs—are toward female flies. Both behaviors are reinforced by evolution over time, because the ability of male flies to compete with other males and attract females directly affects their ability to mate and pass on their genes.

Researchers already knew which neurons in the brain are important for controlling aggression and courtship. In general, studies had suggested that specialized brain cells called P1/pC1 neurons, promote both courtship and aggression while Tk-GAL4FruM neurons promote aggression specifically. They also knew that the two sex-determining genes fruitless (fru) and doublesex (dsx) played key roles in this behavior. But the connection between the genes and the behaviors hadn't been clear.

In the new study, Asahina and his colleagues raised Drosophila fruit flies that contained light-activatable versions of the courtship and aggression neurons. The team could turn the neurons on at any time by shining a light on the flies. The researchers next altered the fru or dsx genes in some of these male flies. They then developed an automated system using machine-learning to analyze videos of the flies and count how often they carried out aggressive or courtship behaviors.

"We made a computer system to capture aggressive behaviors and courtship behaviors to more quickly and accurately count actions," says

Salk postdoctoral fellow Kenichi Ishii, co-first author of both of the new papers. "Getting the program to work was actually difficult and time-consuming but in the end, it made it easier for us to get good data."

The team found that dsx was required for the formation of courtship-inducing neurons: when the fruit flies had the female version of dsx, the courtship neurons were no longer present. On the other hand, fru played a different role—without this gene, flies could still be coaxed to perform courtship behaviors by activating courtship neurons but the courtship was directed at both males and females, suggesting that fru was required for flies to differentiate between the sexes. For aggression, however, the findings were the opposite: fru but not dsx was required for the activation of aggression neurons to cause fighting in male flies.

"This is an important example of the neurobiological differences between sexes and what kind of approaches we can use to study sexually-linked behaviors," says Asahina, who holds the Helen McLoraine Developmental Chair in Neurobiology.

"I think the interesting part of this is understanding that sex is really not a binary thing," says UC San Diego doctoral student Margot Wohl, co-first author of both of the new papers. "A lot of factors come together to control behaviors that differ between the sexes."

Since sex determination in flies is very different than in humans—fruit flies don't have sex hormones, for instance—the new findings don't carry over to how biological sex may impact behavior in people. But Asahina says his approach—the combination of optogenetics and sex-linked gene manipulation—may be useful in understanding behaviors that vary by sex in other animals.

More information: Kenichi Ishii et al, Sex-determining genes distinctly regulate courtship capability and target preference via sexually

dimorphic neurons, *eLife* (2020). DOI: 10.7554/eLife.52701

Margot Wohl et al. Layered roles of fruitless isoforms in specification and function of male aggression-promoting neurons in Drosophila, *eLife* (2020). DOI: 10.7554/eLife.52702

Provided by Salk Institute