Interspecies Sex and Taste

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Different species of fruit flies share habitats but are believed to mate with each other only rarely. In this issue, Fan et al. show that interspecies mating is inhibited by the taste receptor Gr32a (Gustatory receptor 32a) and a neural circuit in which it functions.

Individuals of a species breed productively with each other, but not with other species. Much is known about pheromones and other sensory cues that promote mating within species. Little is known about mechanisms that prevent mating between species. For example, sex between fruit flies of the species *Drosophila melanogaster* has been studied intensively by generations of prurient investigators (Greenspan and Ferveur, 2000). However, *D. melanogaster* encounters many other fruit fly species in its natural habitat. How does it recognize that another fly is of another species and that attempts to mate with it would be futile? In this issue of *Cell*, Nirao Shah and colleagues (Fan et al., 2013) elegantly reveal that a set of chemical signals, a chemoreceptor, and a defined neural circuit are required to prevent interspecies courtship among fruit flies.

Drosophila mating is preceded by a courtship ritual that allows evaluation of potential mates. A male tracks and pursues a female, generates a courtship song by vibrating his wing, taps her abdomen with his forelegs, and extends his proboscis to contact her abdomen. Taste sensilla on his forelegs and proboscis allow sensation of chemical cues. Fan et al. begin by asking whether these taste organs are required by *D. melanogaster* to distinguish between females of the same species (conspecifics) and females of other *Drosophila* species (heterospecifics). A series of surgical ablation and sensory deprivation experiments show that input via the forelegs, but not olfactory, visual, or other gustatory input, is essential for inhibiting courtship toward females of another species, *D. virilis.*

What exactly do the male forelegs sense on females of other species? Different *Drosophila* species contain different hydrocarbons in the waxy cuticle that covers their bodies. Fan et al. use an ingenious approach (Billeter et al., 2009), in which a genetically engineered *D. melanogaster* female depleted of cuticular hydrocarbons (CHs) can be coated with individual CHs. When z-7-tricosene (7T), which is found in *D. simulans* and *D. yakuba* (as well as in *D. melanogaster* males), is applied to this female, it suppresses courtship displays by *D. melanogaster* males. Likewise, z-9-tricosene (9T) and z-11-pentacosene (11P) from *D. virilis* suppress *D. melanogaster* male courtship. Thus specific CHs seem to inhibit interspecies courtship via the forelegs of *D. melanogaster* males (Figure 1).

What is the cellular and molecular basis of the recognition? In mice, species recognition seems to operate through a large number of chemoreceptors, each housed in a different set of neurons (Isogai et al., 2011; Papes et al., 2010). Intriguingly, flies seem to use a single set of neurons to detect females of widely divergent *Drosophila* species. These neurons coexpress two receptors of the Gr family, Gr32a and Gr33a. Both of these receptors are required to detect bitter compounds, and they are also required to suppress conspecific male-male courtship (Miyamoto and Amrein, 2008; Moon et al., 2009). However, only Gr32a is required to prevent *D. melanogaster* males from courting heterospecific females. Thus, a single receptor and a single set of neurons mediate response to phylogenetically diverse *Drosophila* species.

Are Gr32a^{+} foreleg neurons part of a previously characterized neural circuit? The Fru^{M} transcription factor is required for many aspects of male sexual behavior. A subset of Fru^{M+} neurons (designated P1) triggers courtship behavior when the male forelegs contact a conspecific female (Kohatsu et al., 2011). In contrast, another subset of Fru^{M} neurons (designated aDT6) within the subesophageal ganglion, a region where Gr32a^{+} foreleg neurons synapse with higher order neurons, is required for the suppression of interspecies courtship behavior. Hence, Gr32a^{+} foreleg neurons and aDT6 neurons are likely to be components of a neural circuit that suppresses interspecies courtship. Moreover, the results suggest that Fru^{M} acts in the specification of distinct neural circuits that translate different kinds of sexual encounters into different behaviors.

The current study adds a new dimension to our understanding of mate recognition. Differences in CH profiles among species have long been suggested to play a key role in mate discrimination (Ferveur, 2005), and a previous study showed that one CH, 7,11-heptacosadiene (7,11HD), allows *D. melanogaster* males to positively identify conspecific females (Billeter et al., 2009). Fan et al. have now uncovered a complementary mechanism that allows males to negatively identify heterospecific females. This wide-ranging study reveals the receptor, sensory neurons, and neural circuit underlying this mechanism.

These remarkable findings raise fascinating questions about species discrimination in insects. First, how does this mechanism operate in males of other *Drosophila* species? One would expect males of other species to respond to CHs such as 7T, 9T, and 11P differently from *D. melanogaster.* Given that Gr32a is more conserved among *Drosophila* species than most Grs, one might ask whether Gr32a is the CH-binding protein or whether it is an obligate coreceptor for another receptor that is evolving

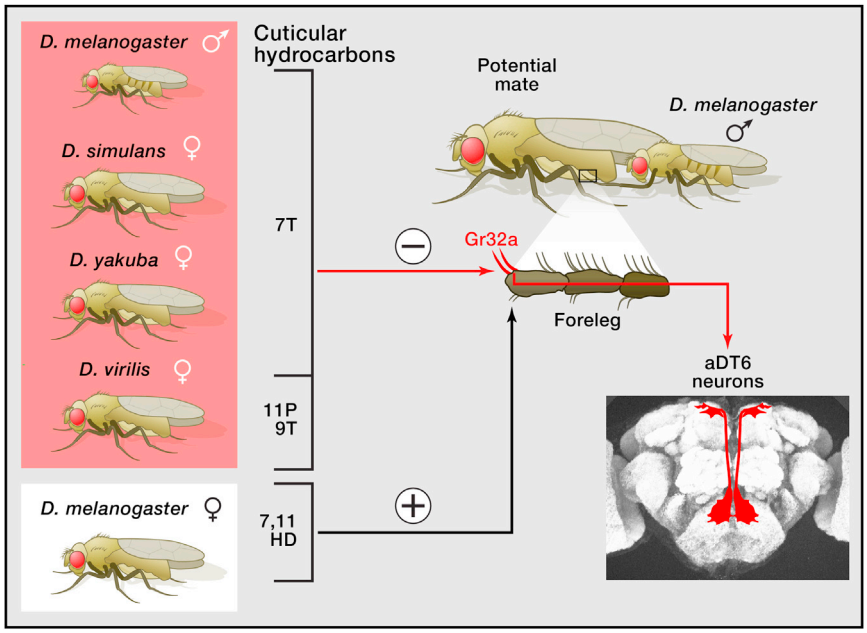

Figure 1. Detection of Cuticular Hydrocarbons Underlies Mate Discrimination
The foreleg of a *D. melanogaster* male makes contact with potential mates and detects key CHs. The indicated CHs of *D. melanogaster* males (7T) and *D. simulans*, *D. yakuba*, and *D. virilis* females (7T, 11P, or 9T) inhibit mating (red arrow) via Gr32a. Gr32a^{+} neurons may communicate indirectly with aDT6 (Fru^{M+}) neurons in the brain to inhibit courtship behavior. Another CH, 7,11HD, on conspecific females, has previously been shown to trigger male courtship behavior (green arrow; Billeter et al., 2009).

more rapidly. Second, has the signal reception machinery coevolved with the corresponding signal generation machinery as *Drosophila* species have diverged? In one possible scenario, as the CH profiles of each species diverge, Gr32a and its associated signal reception machinery in the *D. melanogaster* male may evolve to avoid detecting the CHs of conspecific females, whereas the signal generation machinery that synthesizes CHs in the *D. melanogaster* female may diverge from other species such that her CH profile is compatible with conspecific males. One candidate component of the signal generator is the hydrocarbon desaturase, DesatF, whose evolution has been correlated with changes in CH profiles across *Drosophila* species (Shirangi et al., 2009). Third, what are the limits of this system? There are many species of *Drosophila*, many other kinds of flies, and an extraordinary diversity of other insects in the natural habitat of *D. melanogaster*. How many different CHs and how many other species can *D. melanogaster* males detect via Gr32a and its neurons, and how many other insect species use a similar mechanism?

This study provides a major advance in understanding of reproductive isolation. Interspecies breeding can be prevented by pre- and postfertilization mechanisms in a wide variety of animals. Anatomical, physiological, or geographical factors can impose barriers to reproduction, and Fan et al. now provide a molecular and cellular basis for an intriguing behavioral mechanism. It will be interesting to see whether this work will lead eventually to a molecularly defined systematics of mating compatibility.

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REFERENCES

Billeter, J.-C., Atallah, J., Krupp, J.J., Millar, J.G., and Levine, J.D. (2009). Nature *461*, 987–991.

Fan, P., Manoli, D.S., Ahmed, O.M., Chen, Y., Agarwal, N., Kwong, S., Cai, A.G., Neitz, J., Renslo, A., Baker, B.S., and Shah, N. (2013). Cell *154*, this issue, 89–102.

Ferveur, J.F. (2005). Behav. Genet. *35*, 279–295.

Greenspan, R.J., and Ferveur, J.F. (2000). Annu. Rev. Genet. *34*, 205–232.

Isogai, Y., Si, S., Pont-Lezica, L., Tan, T., Kapoor, V., Murthy, V.N., and Dulac, C. (2011). Nature *478*, 241–245.

Kohatsu, S., Koganezawa, M., and Yamamoto, D. (2011). Neuron *69*, 498–508.

Miyamoto, T., and Amrein, H. (2008). Nat. Neurosci. *11*, 874–876.

Moon, S.J., Lee, Y., Jiao, Y., and Montell, C. (2009). Curr. Biol. *19*, 1623–1627.

Papes, F., Logan, D.W., and Stowers, L. (2010). Cell *141*, 692–703.

Shirangi, T.R., Dufour, H.D., Williams, T.M., and Carroll, S.B. (2009). PLoS Biol. *7*, e1000168.