Mate Recognition: Should Fly Stay or Should Fly Go?

Recent studies have shown that male fruit flies use close-range olfactory cues to assess the status of potential mating partners. The presence of a volatile, male-derived pheromone can suppress the default male behavioral state of courting females.

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To propagate their genes all sexually reproducing animals need to be able to recognize individuals that are members of the same species, of the opposite sex, and that have the capability to produce healthy offspring. Courtship is a multi-sensory experience. Studies with male and female fruit flies indicate that these animals use vision, hearing, touch, taste and smell to assess each other before performing the sexual act [1]. How varied stimuli such as courtship song, body movement and bodily scents are processed to cause survival-promoting behavioral output has yet to be clarified. Although it has been known for over a century that cuticular hydrocarbons found on female flies act as important attractive cues for males [2], receptors for these substances have only emerged in the last few years. Conversely, genetic evidence has shown that the male-specific receptor Gr68a, which is restricted to the forelegs of males, is necessary for the progression of the courtship ritual, but the pheromonal ligand of this receptor is still not known [3].

Three recent studies [4–6], two [4,5] reported in this issue of *Current Biology*, have begun to assemble the peripheral pieces of circuitry involved in mate recognition in *Drosophila melanogaster*, shedding light on one pheromone in particular, (Z)-11-octadecanyl acetate, or cis-vaccenyl acetate (cVA), which is detected by olfactory sensory neurons in the antenna [5–9]. cVA is the first olfactory pheromone in *Drosophila* to be fully characterized, in the sense that the ligand, its receptor and behaviors that it induces are now all described. Male fruit flies produce cVA, which induces social aggregation behavior in both male and female flies [8,10]. However, the *Drosophila* cVA story does not end here. It turns out that cVA acts not only as an aggregation pheromone, but also as an anti-aphrodisiac sex pheromone. Males produce cVA and transfer it to females in the seminal fluid during mating [11]. The attraction of males to females who have been mated or to virgin females who have been anointed with cVA is drastically reduced [12,13]. Thus, this male pheromone acts to mark the female as fertilized and unavailable for mating with other males. How can one substance induce both attractive (aggregation) and repulsive (courtship inhibition) behavior in male flies?

The answer may lie in the existence of multiple receptors and circuits for cVA detection. Recording directly from olfactory hairs on the antenna, Van der Goes van Naters and Carlson [5] found not one, but two odorant receptors that respond to cVA. Purified cVA, extracts of male genitalia or mated female genitalia, but not virgin female genitalia, activate odorant receptors Or67d and Or65a [5]. This result is partially consistent with a previous report from Ha and Smith [9] that implicated only Or67d in cVA detection. Are these pheromone receptors redundant, both supporting aggregation as well as mating discrimination? According to Ejima *et al.* [4], this is not the case. By selectively silencing cells with tetanus toxin, they found that only olfactory sensory neurons expressing Or65a, but not Or67d, are necessary for cVA-mediated courtship inhibition. Therefore it seems that one ligand, cVA, activates two peripheral receptors that are starting points for separate neural circuits.

It is interesting to note that cVA activates both Or67d and Or65a, but the receptors have different response spectra to cVA-related compounds, and Or67d is more sensitive to cVA than Or65a [5]. We know that Or65a olfactory sensory neurons project their axons to the DL3 glomerulus of the antennal lobe in the brain, a different area of the antennal lobe than where Or67d olfactory sensory neurons projections connect with the brain (DA1) [14,15]. One interpretation of these results is that Or67d mediates cVA-induced aggregation while Or65a mediates cVA-induced courtship inhibition. The results of Kurtovic *et al.* [6], contradict those results of Ejima *et al.* [4], in showing that Or67d is involved in courtship inhibition. Future studies will be required to reconcile these different models.

Regardless of whether one or two receptors mediate courtship inhibition in males, there must be additional factors interacting with the cVA response pathways, which add contextual information about the specific situation facing the fly. For example, when cVA is detected in the absence of nearby flies, a fly may respond by moving toward the odor source. If cVA is then detected in combination with a close encounter with male cuticular hydrocarbons, the male fly curbs courtship behavior (Figure 1).

Van der Goes van Naters and Carlson [5] further explored peripheral pheromone detection by asking which olfactory neurons respond to male and female extracts. They limited their analysis to neurons associated with trichoid sensilla. These specialized sensory hairs are well-described in other insects as exclusively tuned to pheromones, so it was a likely bet that *Drosophila* trichoid-associated olfactory neurons would also detect pheromones. In an exhaustive survey of all trichoid-associated neurons and odorant receptors expressed in these neurons, van der Goes van Naters and Carlson [5] concluded that other trichoid odorant receptors on the fly antenna, such as Or47b and Or88a, respond to male and female-derived fly odors, but that none of the trichoid odorant receptors tested were specifically tuned to female odors.

As the authors suggest [5], the non-cVA pheromone receptors could be providing information about the presence of a fly, activating a default behavioral condition of courtship. If cVA is detected in addition to other fly odors, the default courtship behavior is inhibited. This scenario makes sense for a male fly, but what neural pathways does cVA turn on in females? cVA appears to activate Or65a and Or67d neurons similarly in male and female antennae [5], but there is no male courtship behavior to be inhibited in females. Recent evidence shows that Or67d mediates female sexual receptivity in response to cVA [6]. Sexual dimorphism of some kind must occur downstream of the olfactory sensory neurons, whether it manifests itself physiologically or neuroanatomically. The sexual dimorphism is likely mediated by *fruitless*, an important sex determination gene. Manipulations of the *fruitless* gene have been shown to eliminate sexual dimorphism of the size of antennal lobe glomeruli that are innervated by pheromone receptors [16], and *fruitless* expression promotes development of male-specific circuitry in the brain [17].

What are the sex pheromones produced by both males and females that promote courtship behavior? Thus far, the only sexual pheromones identified in *Drosophila* have been long-chain cuticular hydrocarbons, which are not volatile [2]. Either these large substances are detected by physical antennal contact or there are additional volatile pheromones that have yet to be characterized. The idea of physical contact between pheromones and antennal olfactory neurons cannot be discounted, because with the exception of Or67d cVA-responsive cells, van der Goes van Naters and Carlson [5] only saw activation of pheromone-responsive sensilla with physical contact or stimulation less than half a millimeter away from the antenna. These are very close quarters indeed!

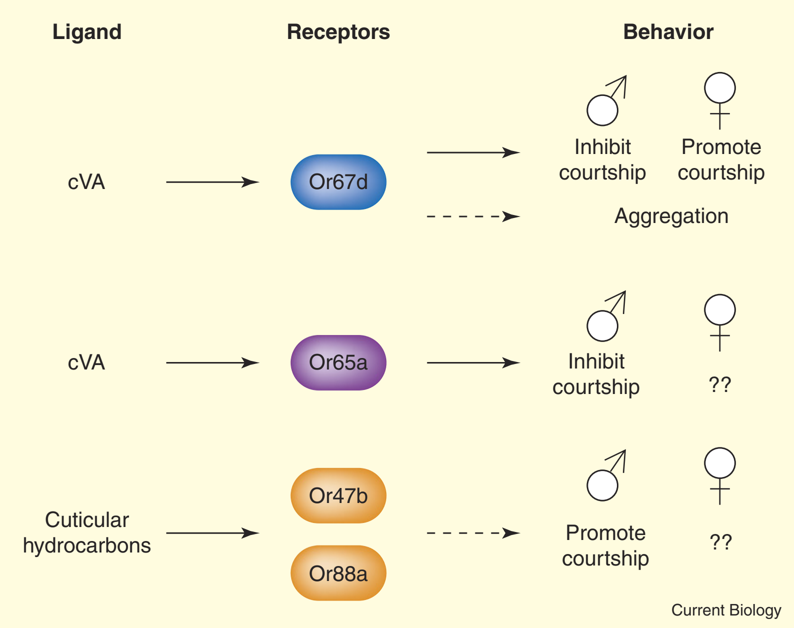

Figure 1. Cis-vaccenyl acetate (cVA) induces two separate behaviors in the male fly, aggregation and inhibition of courtship.

Both Or67d and Or65a odorant receptors respond to cVA electrophysiologically [5], and both Or65a and Or67d have been shown to be involved in courtship inhibition [4,6]. Or47b and Or88a respond to both male and female cuticular hydrocarbons [5], possibly promoting courtship by males. Or67d has been implicated in female receptivity [6], whereas the functions of Or65a, Or47b, and Or88a in the female are unknown.

Evidence from behavioral experiments reinforces the idea that positive sexual pheromones require fly-to-fly contact. During associative courtship learning, an aversive unconditioned stimulus, cVA, can be several millimeters away from the courting male, but there must be a female in physical contact with the male during training to suppress courtship of subsequent females [4]. This points to a contact pheromone as being the positive conditioned stimulus for this associative conditioning paradigm. The cuticular hydrocarbon 7-pentacosene has been shown to be a major contributor to the conditioned stimulus in this type of learning [18]. Future studies will be needed to definitively identify the ligands for the remaining orphan pheromone receptors. Classical chemical ecology methods to fractionate fly extracts and apply individual components to the antenna to look for specific neuronal excitation may provide a means to this end.

Social behaviors in fruit flies, such as courtship and aggregation at feeding sites, can serve as excellent models for studying sensory integration. A fly must be extremely versatile to produce different behaviors in response to different combinations of odors and tastes. In fact, *Drosophila* males can use a variety of stimuli to mediate courtship learning, including cVA [4], benzaldehyde [19] or quinine [20] as aversive cues. It will be interesting to see how pheromone receptors directly mediate courtship plasticity, as this was not directly addressed in the papers discussed here. Understanding how the fly nervous system efficiently processes information to produce evolutionarily advantageous behavior adds to our understanding of how neural circuits work in general.

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DOI: 10.1016/j.cub.2007.02.014

Animal Behavior: Tolerant Primates Cooperate Best

Chimpanzees and bonobos show different cooperative tendencies when the prize is easily monopolizable food: bonobos cooperate more than their less socially tolerant relatives.

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When faced with a task that can only be solved by two individuals working together, who are we most likely to cooperate with? Although reciprocal altruism might explain cases where some individuals appear to cooperate for no apparent reward in the short term, intuitively, cooperation seems most likely to occur between partners who would share the gains of the cooperative effort. A recent study of chimpanzees [1] showed that individuals who co-fed peacefully were more likely to cooperate on a task that required simultaneous pulling on ropes to drag in food rewards. Now, as they report in this issue of *Current Biology* [2], the same research team has extended this line of investigation to compare social tolerance and aptitude for cooperation between chimpanzees and their closest phylogenetic neighbors, bonobos. The results suggest that cooperation may occur more in societies in which selection has occurred for emotional reactions that limit social intolerance in potentially competitive situations.

To structure their study, Hare *et al.* [2] set up two opposing hypotheses based on known facts about behavior in these two species of apes. The 'emotional reactivity hypothesis' predicts greater cooperative ability in bonobos because of these apes' more relaxed social relationships, including fewer and less intense conflicts over access to resources such as food [3]. The 'hunting hypothesis', in contrast, emphasizes the fact that only chimpanzees have been observed hunting cooperatively for mammalian prey in the wild [4]; such behavior has never been reported in bonobos. This latter hypothesis therefore leads to the prediction of greater cooperative success in chimpanzees.

The first experiment by Hare *et al.* [2] confirmed that bonobos were more likely than chimpanzees to co-feed peacefully on freely available food; this was especially true when the food (pieces of fruit) was clumped. In the second experiment, pairs of individuals in both species were presented with a simple, but ingeniously designed task requiring cooperation. Originally developed by Satoshi Hirata [5] of the Great Ape Research Institute at Hayashibara, Japan, the apparatus consists of two food dishes on a platform beyond the subjects' reach. A length of rope is threaded through two loops attached to the platform, and each end of the rope is left within reach of a subject. If only one subject pulls, then the other end of the rope retreats away from the partner and eventually comes unthreaded. The end result is no food. But if both subjects pull together on their respective rope ends, then the platform and the food dishes can get dragged within reach.