Commentary

Mechanism for saltational shifts in pheromone communication systems

Thomas C. Baker*

Department of Entomology, Iowa State University, 411 Science II, Ames, IA 50011

The specificity and sensitivity involved in moth sex pheromone communication are remarkable and renowned. Males are exquisitely sensitive and narrowly tuned to the female-emitted conspecific sex pheromone blend, which usually consists of two or three components (1, 2). Slight changes in the ratios of these components can shut off the upwind flight attraction response of males of one species and turn on the response of males from a different species. For years, it had been assumed that blends are under a very high degree of stabilizing (symmetrical male–female) selection that would strongly resist any shifting off of the species' norm (1, 2). Recently, however, this view has been questioned (3, 4), and evidence has accumulated that an asymmetry exists in the strength of selection pressures on female senders and male receivers. The asymmetry, with lower pressure exerted on females' signaling systems, should allow for much higher variance in the females' blends than had been assumed (3, 4). The correspondingly higher selection pressure on males' response systems also should select for some subset of the male population to follow major variations in females' blends over generations by adjusting their olfactory response spectrum through asymmetric tracking (4, 5). Until now (6), there had been no solid evidence from one pair of species to support this process, nor was there evidence for how a departure from the normal female blend might arise to initiate asymmetric tracking.

Males are exquisitely sensitive and narrowly tuned to the female-emitted conspecific sex pheromone blend.

In this issue of PNAS, Roelofs *et al.* (6) now provide compelling evidence that a saltational event occurred in an ancestral *Ostrinia* moth population and gave rise to a new pheromone and new species, the Asian corn borer, *Ostrinia furnicalis*. A pseudogene that had been unexpressed for millions of years in the genome of *Ostrinia* and that controlled desaturation of pheromone molecules, suddenly became expressed. As a result, the position of the double bond shifted in the pheromone of females expressing the gene. A convincing case is made (6) that the shift to the new pheromone was accomplished by ancestral males via asymmetric tracking of females emitting the new blend. Evidence comes from a very few broadly tuned unusual males found in their European corn borer, *O. nubilalis*, (Fig. 1) laboratory population that were capable of flying upwind to the new pheromone blend molecules emitted by *O. furnicalis* females as well as to those emitted by their own females.

It is only fitting that this stunning report (6) comes from the Roelofs laboratory. Their pioneering work had earlier helped show how the majority of moth pheromone blend molecules can be explained by the activities of a handful of enzymes in the female pheromone gland that sequentially modify fatty acyl chains (7, 8). Most moth pheromone molecules are monoenes of long-chain fatty acyl moieties that are converted into either alcohols, acetate esters, or aldehydes that are emitted into the air by females to attract males (1, 2). During pheromone biosynthesis in the female pheromone gland, members of one class of enzyme, the desaturases, create a double bond in a particular location in the fatty acyl chain by removing two hydrogen atoms. Members of another class shorten the fatty acyl chain by two carbons. Members of a third class, the reductases, reduce the fatty acid portion to create an alcohol. Alcohols are sometimes emitted by females as pheromone components but, more commonly, they are acetylated to form acetates or oxidized to form aldehydes.

Simply reversing the order in which a particular pair of desaturase and chain-shortening enzymes works can lead to an entirely different set of double bond positions in fatty acyl chains (7, 8). Also, either of these same enzymes might perform not just one but two or more rounds of activity on fatty acyl chains and by doing so create a still greater variety of molecules that are shorter, doubly desaturated, or both. The affinity of reductases and acetylases for particular substrates is the final determinant of pheromone blend composition. For example, some reductases do not reduce fatty acids that are more than 14 carbons in length (9). Other reductases preferentially select fatty acyl chains with (*Z*) double bonds for reduction over those having an (*E*) configuration (10).

The desaturases, however, have received most of the research attention by pheromone biochemists, because different kinds of desaturases, which place double bonds in recognizable positions, leave obvious and easily interpreted biochemical and genetic signatures of evolutionary importance. Before the recent findings of Roelofs *et al.* (6), it was known (11) that *O. nubilalis*, and all of the other *Ostrinia* species studied thus far except *O. furnicalis*, use the common lepidopteran Δ11-desaturase (7, 8). This desaturase acts on a chain-shortened 14-carbon fatty acyl chain to produce the pheromone blend comprised of (*Z*)- and (*E*)-11-tetradecenyl acetate. The work of the Löfstedt pheromone group and collaborators (9, 12) also had shown that the lone *Ostrinia* species deviating from this plan, *O. furnicalis*, uses Δ14-desaturase. The Δ14-desaturase acts on a 16-carbon fatty acid and, after a subsequent round of chain shortening, produces a pheromone blend comprised of (*Z*)- and (*E*)-12-tetradecenyl acetate.

One revelation provided by the Roelofs *et al.* findings (6) is that the gene controlling production of the *O. furnicalis* Δ14-desaturase had been present as a pseudogene in the *Ostrinia* genome for millions of years, but it was not expressed in ancestral females until relatively recently. Additionally, they show that the gene controlling the *O. nubilalis* Δ11-desaturase is present, but not expressed, in *O. furnicalis* females, indicating that expression of this gene has

See companion article on page 13621.

*E-mail: tcbaker@iastate.edu.

www.pnas.org/cgi/doi/10.1073/pnas.222539799

Fig. 1. Females of the European corn borer, *O. nubilalis* (*Left*), carry the gene for producing the unusual pheromone of the Asian corn borer but do not express it (6). A very small proportion of European corn borer males (*Right*) are capable of responding to the Asian corn borer pheromone as well as to their own pheromone (6). Image courtesy of Marlin Rice, Iowa State University.

been switched off. Roelofs *et al.* (6) placed their findings in the framework of other previous morphology-based phylogenetic analyses, showing that *O. furnicalis* had been derived from ancestral *O. nubilalis* populations. They also performed molecular phylogenetic reconstructions by using desaturase genes from across the Lepidoptera to estimate the time course of events.

I suggest that the greatest impact of the Roelofs *et al.* discovery (6) will be that it may explain the evolution of moth pheromone communication systems in general. The door has now been opened for us to contemplate the intriguing possibility that the evolutionary mechanism they described to explain a major blend shift may also have been responsible for creating all of the many more minor pheromone component blend-ratio differences that abound in moth pheromone systems. Alterations in the activities of any of the three major types of pheromone biosynthetic enzymes are capable of causing evolutionarily significant changes in the emitted pheromone blend. Researchers have tended to focus on the most severe blend alterations, because these are the most definitive in providing proof for the evolutionary mechanisms involved. However, more subtle shifts, such as in the emitted ratios of two isomers used by two congeners, might be caused by a new mutant reductase that has different substrate preferences than originally. Such a blend-ratio shift might not seem major, but it might be just as critical in orchestrating divergence via asymmetric tracking as would be the more major change documented for *Ostrinia* (6). In both examples, the expression of one gene is replaced by the expression of another. How major these changes are is ultimately determined by the olfactory resolution abilities built into the responding males' olfactory systems.

An example of such a blend-ratio shift involves a mutation in the gene controlling production of a chain-shortening enzyme in the cabbage looper, *Trichoplusia ni* (13). Females expressing the mutant enzyme emitted a ratio of pheromone components that was strikingly different from the ratio emitted by normal females (14, 15). The blend-ratio shift was so great, and differed so much from the normal wild-type blend, that *T. ni* males were poorly attracted to the mutant female blend. After ≈40 generations of laboratory breeding, however, mutant *T. ni* males broadened their spectrum of response so that they were now attracted equally well to both the new mutant *T. ni* blend ratio and the old one. Asymmetric tracking (4, 5) of the mutant female blend by males would seem to be supported by these results.

Another impact of the Roelofs *et al.* results (6) is that they reemphasize the need to understand male moth olfaction, now with regard to the ability of males' pheromone olfactory response systems to be sufficiently broadly tuned to respond to odd molecules or to changes in the blend ratios of the existing pheromone blend molecules. These two different kinds of shifts in pheromone blends might require different kinds of corresponding changes to occur in males' olfactory systems. For males to be responsive to novel molecules in female emissions, receptor neurons must be present that are capable of being stimulated by the new molecules. Thus, for the rare *O. nubilalis* males (6), we might expect to find broadened tuning exhibited by the pheromone receptor neurons themselves. Pheromone receptor neurons that had broadened their acceptance spectrum to include the new molecules would be at an advantage compared with completely new types of neurons, because old broadly tuned receptor neurons would already have the necessary synaptic connections through the appropriate glomeruli of the olfactory lobe to result in attraction. However, moth pheromone receptors (and the intimately associated perireceptor environment that includes various binding proteins to ferry molecules across to dendritic membrane-bound receptors) are notoriously strict in requiring that the pheromone ligand have correct space-filling and electron-distribution properties (16). It would seem that, although such broadly tuned pheromone receptor neurons should be found only infrequently, their occurrence should coincide with alterations in the pheromone blend that have occurred via changing the molecules themselves (e.g., the Δ11- changed to the Δ12-tetradecenyl acetates).

Although it has been uncommon to find even a slightly broadly tuned pheromone-component receptor neuron in moths, the few examples that do exist are instructive and thus far seem to coincide with apparent major chain-shortening or desaturation-enzyme-related alterations in species' pheromone emissions (17–21). A good example comes from extensive research on the European ermine moth species complex, genus *Yponomeuta* (3, 17). Only a single species, *Yponomeuta rorellus*, of the many sympatric species in this group, does not use either (*Z*)- or (*E*)-11-tetradecenyl acetate as the major component of its pheromone blend (17). Rather, it uses the saturated analog tetradecan-1-ol acetate (tetradecyl acetate) as its only pheromone component. It is logical to speculate that in ancestral populations giving rise to *Y. rorellus,* the Δ11-desaturase enzyme in some females apparently became nonfunctional (similar to the mutant *T. ni* system). Possibly the Δ11-desaturase gene suddenly became a pseudogene, as it now seems to be in *O. furnicalis* (6).

For desaturase-lacking females of *Y. rorellus* to successfully mate (and pass on the mutation), some proportion of males in the population would have to be capable of responding to the saturated acetate with their existing olfactory apparatus, beginning with receptor neurons that were able to be stimulated by this compound. Male *Y. rorellus* antennae do have an abundance of receptor neurons that are stimulated by tetradecyl acetate (21). What sets this particular type of neuron apart from the typical lepidopteran sex pheromone receptor neuron, however, is that it is at least as responsive to the (*E*)- and (*Z*)-11-tetradecenyl acetate molecules emitted by other sympatric *Yponomeuta* species as to its own saturated tetradecyl acetate pheromone (21). This neuron is also very sensitive to sev-

eral other pheromone-like compounds, including (*E*)-6-tetradecenyl acetate and, most interestingly, with regard to the Roelofs *et al.* report (6), to (*E*)- and (*Z*)-12-tetradecenyl acetate.

This type of *Y. rorellus* pheromone receptor neuron is thus unusually broadly tuned and strikingly also exhibits exactly the kind of acceptance of the Δ12-tetradecenyl acetate isomers expected in ancestral *O. nubilalis* males' pheromone receptor neurons (and perhaps also in those of present-day rare broadly responding *O. nubilalis*). Comparative studies on receptor neuron breadth of tuning of "rare" males as well as "normal" males in these two *Ostrinia* species need to be performed and include not only Δ11- and Δ12-tetradecenyl acetates but also tetradecyl acetate to determine whether there are any similarities to the *Y. rorellus* neurons.

One process left somewhat unexplained in the asymmetric tracking scenario described for the divergence of *O. furnicalis* from *O. nubilalis* (6) was the mechanism that might accentuate males eventually abandoning their upwind flight responsiveness to the ancestral pheromone blend. Selection against the old blend might occur if the proliferation of the new blend types has proceeded to such a degree that a linkage disequilibrium develops between the new sender and receiver loci (22), causing assortative mating. Strong female-biased genetic linkages, however, have not been demonstrated thus far in moth pheromone systems (3). There are, however, examples of attraction to pheromone blends of other species being impaired by the excitation of antagonistic neurons. These neurons seem to function solely for detecting components emitted by nonconspecific females and to thus stop males from flying upwind to these blends (2, 3, 18, 19, 21). Such behavioral antagonist neurons typically exhibit relatively broad response spectra to a variety of pheromone-related molecules (18). One might hypothesize that the asymmetric tracking process involves these antagonistic neurons becoming even more broadly tuned to now accommodate also the old pheromone molecules; their stimulation would prevent mating mistakes with females emitting the old molecules.

It is noteworthy that for *Y. rorellus*, an antagonistic neuron does in fact respond to (*Z*)- and (*E*)-11-tetradecenyl acetate. The activity of this neuron has been conjectured to be the factor preventing these two compounds from attracting *Y. rorellus* males despite the ability of these compounds to excite the tetradecyl acetate pheromone receptor neuron (21). Neither (*Z*)- nor (*E*)-12-tetradecenyl acetate activates the antagonistic neuron, but, as mentioned, both compounds do excite the tetradecyl acetate pheromone receptor neuron. Thus, *Y. rorellus* males readily fly upwind to traps containing the Δ12-tetradecenyl acetate (nonpheromone) compounds due to lack of antagonistic input. The report by Roelofs *et al.* (6) certainly gives us a fresh way of looking at such results and possibly also a new explanation for how moths developed such a great variety of sex pheromone blends with so few genes.

1. Cardé, R. T. & Baker, T. C. (1984) in *Chemical Ecology of Insects*, eds. Bell, W. J. & Cardé, R. T. (Chapman & Hall, London), pp. 355–383.
2. Baker, T. C. (1989) *Experientia* **45,** 248–262.
3. Löfstedt, C. (1993) *Philos. Trans. R. Soc. London B* **240,** 167–177.
4. Phelan, P. L. (1992) in *Insect Chemical Ecology*, eds. Roitberg, B. D. & Isman, M. B. (Chapman & Hall, New York), pp. 265–314.
5. Phelan, P. L. (1997) in *Insect Pheromone Research*, eds. Cardé, R. T. & Minks, A. K. (Chapman & Hall, New York), pp. 563–579.
6. Roelofs, W. L., Liu, W., Hao, G., Jiao, H., Rooney, A. P. & Linn, C. E., Jr. (2002). *Proc. Natl. Acad. Sci. USA* **99,** 13621–13626.
7. Bjostad, L. B., Wolf, W. A. & Roelofs, W. L. (1987) in *Pheromone Biochemistry*, eds. Prestwich, G. D. & Blomquist, G. L. (Academic, New York), pp. 77–120.
8. Jurenka, R. A. & Roelofs, W. L. (1993) in *Insect Lipids: Chemistry, Biochemistry and Biology*, eds. Stanley-Samuelson, D. W. & Nelson, D. R. (Univ. of Nebraska Press, Lincoln), pp. 353–388.
9. Zhao, C.-H., Fang, L., Bengtsson, M. & Löfstedt, C. (1995) *J. Chem. Ecol.* **21,** 1795–1810.
10. Zhu, J. W., Zhao, C. H., Lu, F., Bengtsson, M. & Lofstedt, C. (1996) *Insect Biochem. Mol. Biol.* **26,** 171–176.
11. Ma, P. W. K. & Roelofs, W. L. (2002) *Zool. Sci.* **19,** 501–511.
12. Zhao, C.-H., Löfstedt, C. & Wang, X. (1990) *Arch. Insect Biochem. Physiol.* **15,** 57–65.
13. Jurenka, R. A., Haynes, K. F., Adlof, R. O., Bengtsson, M. & Roelofs, W. L. (1994) *Insect Biochem. Mol. Biol.* **24,** 373–381.
14. Haynes, K. F. & Hunt, R. E. (1990) *J. Chem. Ecol.* **16,** 509–519.
15. Haynes, K. F. (1997) in *Insect Pheromone Research*, eds. Cardé, R. T. & Minks, A. K. (Chapman & Hall, New York), pp. 525–534.
16. Liljefors, T., Bengtsson, M. & Hansson, B. S. (1987) *J. Chem. Ecol.* **13,** 2023–2040.
17. Löfstedt, C., Herrebout, W. M. & Menken, S. B. J. (1991) *Chemoecology* **2,** 20–28.
18. Todd, J. L. & Baker, T. C. (1999) in *Insect Olfaction*, ed. Hansson, B. S. (Springer, Berlin), pp. 67–96.
19. Cossé, A. A., Todd, J. L. & Baker, T. C. (1998) *J. Comp. Physiol. A* **182,** 585–594.
20. Vickers, N. J., Christensen, T. A., Mustaparta, H. & Baker, T. C. (1991) *J. Comp Physiol. A* **169,** 275–280.
21. Löfstedt, C., Hansson, B. S., Dijkerman, H. J. & Herrebout, W. M. (1990) *Physiol. Entomol.* **15,** 47–54.
22. Butlin, R. & Trickett, A. J. (1997) in *Insect Pheromone Research*, eds. Cardé, R. T & Minks, A. K. (Chapman & Hall, New York), pp. 548–562.