Smells like a new species: Gene duplication at the periphery

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The remarkable long-distance sexual communication system of moths has fascinated and puzzled biologists and chemists since bombykol was identified and the term "pheromone" was coined 51 years ago. The pheromone signal is a bouquet of volatile chemicals wafting through the night air, synthesized and released by the female's pheromone gland and detected and decoded by the male's antenna and central nervous system. The sensitivity and selectivity of this chemical code poses a dilemma in explaining how changes could evolve, because strong reciprocal stabilizing selection between signaler and responder would seem to allow little scope for change. The genetic study of Gould et al. in PNAS (1) shows the importance of gene duplication in generating the variation in peripheral sensory physiology that could enable a change in male preference from one species' pheromone blend to another.

This scenario fits satisfyingly into the "asymmetric tracking" hypothesis (2) of pheromone evolution, which posits weaker selection for signaling fidelity on the limiting sex, females, than on males. Some sort of saltational mechanism is assumed to produce initially random, nonadaptive variants in female pheromone production, which are then "tracked" by a subpopulation of males with "broadened tuning" of some of their olfactory receptor neurons (ORNs) (3). As the genes altering female signaling are likely different from those changing male response, it is still unclear what mechanism maintains an association between them in the early stages; and there is no general agreement as to whether this process can drive speciation or can only proceed once reproductive isolation due to other factors has already occurred (4). *Heliothis virescens* (Hv) and *H. subflexa* (Hs) serve as a useful model system for studying this process. Although distinct in many respects, the two species are similar enough so that laboratory hybrids may represent some features that were present in the early stages of divergence.

Gould et al. sought to map QTL (quantitative trait loci) for the response to species-specific attractants, by marking all chromosomes and looking for correlations with male flight behavior in interspecific backcrosses. Both species produce the major component *Z*-11-hexadecenal (Z11-16:Ald). Hv also produces *Z*-9-tetradecenal (Z9-14:Ald), which is critical to male Hv attraction; Hs instead produces *Z*-9-hexadecenal (Z9-16:Ald), *Z*-11-hexadecenol (Z11-16:OH), and *Z*-11-hexadecenyl acetate (Z11-16:OAc) which attract Hs males; the latter inhibits Hv males. The behavioral assay required presentation of the species-specific attractants in just the right ratios to elicit a stronger response from F_1 males than Hv or Hs. Active flight was required to show that an intact signal-processing pathway capable of discriminating among pheromone blends was functional in hybrids. Gould et al. found that attraction to either Z9-14:Ald or Z9-16:Ald was strongly correlated with the presence of chromosome 27 (C27) from Hv or Hs, respectively; the deterrent effect of Z11-16:OAc on Hv males was also reduced by presence of C27 from Hs (Hs-C27). Chromosomal replacement of Hv-C27 with Hs-C27 by introgression produced responses similar to pure Hs. Only the Hs requirement for Z11-16:OH showed no effect of C27 or any of the other 29 autosomes. The region of Hv-C27 responsible for these differences contains four similar, tightly linked odorant receptor (OR) genes expressed in male antennae (HR6, HR14, HR15, and HR16). Given the importance of highly specific interactions between ORs and their pheromone ligands, this implicates variation in peripheral sensory physiology rather than central nervous system processing in governing differences in attraction.

Tandem gene duplication by unequal crossing-over may immediately create a novel phenotype.

Could this variation explain the evolutionary transition from an ancestral species like Hv to a derived species like Hs, or vice versa? At least two steps would be required: first a switch in the relative preference for Z9-14:Ald vs. Z9-16:Ald, and then a change in the aversion to the acetate. These transitions should occur in the antennal sensilla, each embracing two ORNs with highly specific responses to pheromone components and projections onto the antennal lobe of the brain. Three types of sensilla have been studied in both species and hybrids; expression of some ORs in specific ORNs of Hv is also known (reviewed in ref. 5). Type A sensilla with ORNs expressing HR11 and HR13 (6) respond similarly to both species' major pheromone component; no changes would seem to be required here. Type B sensilla from the two species have ORNs with similar projection patterns but different spiking responses to the species-specific attractants; one ORN responds in Hv to Z9-14:Ald but in Hs more strongly to Z9-16:Ald (7). This difference also correlates with the inheritance of C27 (1); thus, species-specific structural variation in ORs expressed in type B ORNs affecting the relative affinity for the two attractants could be responsible. These ORs are suspected to be HR6 and HR15 (5). A mutation in the Hv OR increasing its affinity for Z9-16:Ald could broaden the tuning of its ORN and represent the first step in making the male more responsive to the component that is now the Hs-specific stimulus. One copy of the mutation would suffice because F_1 males will fly toward a blend with that component (1).

The third, type C sensilla, is more complicated, because the correspondence between the electrophysiological responses of ORNs and their projection patterns onto glomeruli of the antennal lobe differs (7, 8). In Hv, one ORN responds strongly to the Hv antagonist Z11-16:OAc and projects onto the AM (anteriomedial glomerulus); the second ORN responds to Z11-16:OH and projects onto the VM (ventromedial). These associations are reversed in Hs. In Hv, ORN responses are due to expression of HR14 in the first type and HR16 in the second (9). OR expression patterns in ORNs of Hs are not known, but a switch in HR14 and HR16 expression with no change in ORN projection patterns is one possibility. Recordings from type C sensilla showed that Hs-C27 suppressed the overall firing response to Z11-16:OAc (1), corresponding to the Hs-C27 reduction of the acetate's inhibitory effect on Hv flight behavior. A

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Table 1. Linkage of ORs and FARs in the genome of *Bombyx mori*

		Antennal expression (16)				Distance	
Chromosome	OR	Male	Female	Larva	FAR	Physical (MB)	Genetic (cM)
1	BmOR1	+	—	—	BGIBMGA000659	3.3	5.8
10	BmOR54	+	+	+	BGIBMGA006569	1.2	0.0
12	BmOR6	+	—	—	pgFAR (BAC79425)	2.0	8.3
23	BmOR26	+	+	+	BGIBMGA011217	2.2	3.7

For genome of *Bombyx mori*, see ref. 17. MB, megabases; cM, centimorgans.

reduced efficiency of acetate detection by HR14 of Hs, combined with an exchange of expression of HR14 and HR16 in the two ORNs, could result in an attenuated response projecting to the VM rather than an antagonistic response projecting onto the AM. This could represent the second step in interconverting the Hv and Hs patterns, by reducing the aversion to acetates. This would be tightly linked genetically to the first change, because both would transpire in a gene cluster already generated by tandem gene duplication. Another possibility is that expression of a different OR in an ORN could alter its glomerular targeting as it does in mammals; it would be interesting to determine whether projections of type C ORNs are reversed in the Hv moths with Hs-C27.

The involvement of a cluster of OR genes in generating differences in male behavior highlights the role of gene duplication in the evolution of pheromone signaling systems. Tandem gene duplication by unequal crossing-over may immediately create a novel phenotype, if it makes a chimeric protein with altered odorant affinity or puts a coding sequence downstream of a different promoter causing expression of an OR in a different ORN. If instead an identical copy of an existing gene is produced, more time will be required for enough mutations to accumulate for functional divergence. When the original and postduplication versions of an OR gene cluster coexist, a subpopulation of males may be capable of responding to a different pheromone blend, as required by the asymmetric tracking model. Cloning the entire C27 OR cluster in both Hv and Hs would determine whether either has extra copies resulting from a duplication polymorphism in the ancestral species.

If gene duplication of pheromone biosynthetic enzymes such as desaturases, fatty-acyl reductases (FARs), and acetyltransferases plays a complementary role in generating signal variability in females, could this also provide a mechanism for an association between pheromone signal and response? The distribution of OR gene clusters in moth genomes might provide opportunities for "preadaptive genetic linkage" to novel duplicated pheromone synthesis genes in the early stages of signal divergence. A survey of the *Bombyx mori* genome reveals four cases of tight linkage between ORs and FARs (Table 1). Some are directly involved in the pheromone system; BmOR1 is the receptor for bombykol (10), and BmOR6 likewise is expressed only in male antennae; pgFAR catalyzes the last step in the synthesis of bombykol (11). A homologous FAR controls the production of opposite ratios of E11-14:OAc and Z11-14:OAc in females of the E and Z strains of *Ostrinia nubilalis* (12). Given the high degree of synteny among *Bombyx* and other Lepidoptera (13), some of these linkages might be ancient features of lepidopteran genomes.

However, there is no evidence for present-day linkage in *Heliothis*. QTL mapping of pheromone production in interspecific crosses has revealed 15 QTLs in nine chromosomes, affecting nine compounds produced by the pheromone gland (14). There is also intraspecific genetic variation in acetate production by Hs (15). However, no major QTL affecting active pheromone components maps to C27. Compared with male response, female pheromone production seems to have diverged more, consistent with the asymmetric tracking model, but making present-day Hv–Hs differences likely to be less representative of the earliest stages of speciation. The enigma of coordinated divergence in signaler and receiver persists, and additional species pairs will need to be investigated to determine whether preadaptive genetic linkage is a viable explanation.

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