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Balanced Olfactory Antagonism as a Concept for Understanding Evolutionary Shifts in Moth Sex Pheromone Blends

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Abstract In the sex pheromone communication systems of moths, both heterospecific sex pheromone components and individual conspecific pheromone components may act as behavioral antagonists when they are emitted at excessive rates and ratios. In such cases, the resulting blend composition does not comprise the sex pheromone of a given species. That is, unless these compounds are emitted at optimal rates and ratios with other compounds, they act as behavioral antagonists. Thus, the array of blend compositions that are attractive to males is centered around the characterized female-produced sex pheromone blend of a species. I suggest here that the resulting optimal attraction of males to a sex pheromone is the result of olfactory antagonistic balance, compared to the would-be olfactory antagonistic imbalance imparted by behaviorally active compounds when they are emitted individually or in other off-ratio blends. Such balanced olfactory antagonism might be produced in any number of ways in olfactory pathways, one of which would be mutual, gamma-aminobutyric-acid-related disinhibition by local interneurons in neighboring glomeruli that receive excitatory inputs from pheromone-stimulated olfactory receptor neurons. Such mutual disinhibition would facilitate greater excitatory transmission to higher centers by projection interneurons arborizing in those glomeruli. I propose that in studies of moth sex pheromone olfaction, we should no longer artificially compartmentalize the olfactory effects of heterospecific behavioral antagonists into a special category distinct from olfaction involving conspecific sex pheromone components. Indeed, continuing to impose such a delineation among these compounds may retard advances in understanding how moth olfactory systems can evolve to allow males to exhibit correct behavioral responses (that is, attraction) to novel sex-pheromone-related compositions emitted by females.

Keywords Sex pheromone · Moths · Lepidoptera · Attractant · Behavioral antagonist · Olfaction · Evolution · Asymmetric tracking · Speciation · Reproductive character displacement

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Introduction

A major unresolved question in the study of sex pheromone communication in moths revolves around the conundrum that if species specificity of sex pheromone blends is high and tightly regulated, then how is it possible for new blends to evolve? A solution to this specific problem could lead also to a better understanding of the evolution of species-specific communication pathways in insect and animal groups in general, including those that use other communication modalities.

Sex pheromone blends of moths are usually comprised of a species-specific blend of several components emitted by females that strongly attracts and otherwise mediates the behavior of conspecific males. In such systems, the components of the sex pheromone blends must be shown to be emitted by females, and to elicit behavioral responses from males (attraction). Constituents of the pheromone gland or its volatile emissions that do not evoke any significant behavioral effect individually or as part of the blend, are *not* pheromone components, but rather, should be called pheromone gland, or volatile emission, *constituents*.

Some compounds emitted as part of the sex pheromone blend of a particular species can cause reduction in or cessation of upwind flight when they are admixed with the pheromone blend of another species. Such compounds, originally termed behavioral "inhibitors", have more recently come to be known as heterospecific behavioral "antagonists" because they interfere with the attraction of heterospecific males. Thus, these *behavioral* antagonists have been considered to act differently from conspecific pheromone components, which are sometimes termed behavioral "agonists" (c.f., Linn et al. 2003; Baker and Heath 2004). However, one critical factor that usually has been overlooked is that pheromone components themselves have the ability to decrease or even eliminate attraction of conspecifics, depending on their amounts in a blend relative to other components. When emitted at the wrong rates relative to other components, the resulting blends no longer comprise the "pheromone" of that species, and it follows that the compounds, in terms of the responses that they elicit, can be both pheromone components and behavioral antagonists, with the behavioral outcome dependent upon the ratio in which they are presented. Here, I propose that the underlying mechanism that results in these various behavioral outcomes is olfactory antagonistic balance, a concept that may facilitate our understanding of the evolution of moth pheromone systems.

Odorants, Odors, and Pheromone Components

It is important to recognize the difference between odorants and odors. Odorants are single volatile compounds; the use of odorants as stimuli allows us to understand neurophysiological and behavioral responses at a simple chemosensory level. Whereas a particular odorant may cause neurophysiological activity in designated olfactory receptor neurons (ORNs), behavioral activity may not result from exposure to this odorant. In contrast, odors are blends of odorants, and odors often produce behavioral responses only when presented in a limited range of blend ratios. Pheromones are odors, inasmuch as they are blends of odorants (i.e., the individual pheromone components), presented in the "correct" blend ratios for a particular species.

Olfactory Antagonistic Balance

I suggest that we should rethink the way we have traditionally distinguished pheromone components as being distinct from heterospecific pheromone antagonists, in terms of olfactory processing. I propose that *all* sex-pheromone-related compounds (including heterospecific antagonists) that mediate behavioral responses should be considered to act as antagonists through olfactory pathways, resulting in a continuum of various levels of attraction. When the antagonistic olfactory inputs are not balanced the compounds causing the imbalance, even including the compounds that at the optimal ratios would have comprised the pheromone, result in reduced attraction. Olfactory balance with regard to sex-pheromone-related compounds is consonant with the concept of "combinatorial coding" (c.f., Hildebrand and Shepard 1997; Vickers et al. 1998; Christensen et al. 2000) that has prevailed since the days of Vincent Dethier (1971) when it was called "across-fiber patterning". For sex-pheromone-related compounds, however, an advantage of the olfactory antagonistic balance concept is that it eliminates heterospecific, behavioral antagonist compounds from being viewed as acting differently on olfaction and subsequent behavioral responses than do conspecific pheromone components.

Consider, for instance, two-component sex pheromones that involve specific ratios of the components in *Yponomeuta* spp. moths [(i.e., (*E*)-11-tetradecenyl acetate and (*Z*)-11-tetradecenyl acetate; (E11-14:OAc and Z11-14:OAc)] (Löfstedt et al. 1990, 1991). For a given *Yponomeuta* species (Fig. 1), when one component is present in too high a ratio, attraction is diminished, or eliminated altogether. In such cases, the blend can no longer be considered to comprise that species' sex pheromone, and the out-of-range "pheromone component" now acts as a behavioral antagonist. For example, when E11-14:OAc is emitted in excessive amounts relative to Z11-14:OAc in any of the species in Fig. 1, creating an off-blend ratio, it acts antagonistically to stop or reduce attraction. Similarly, too much Z11-14:OAc that creates an off-ratio also stops or reduces attraction, and thus Z11-14:OAc can also act antagonistically.

Furthermore, it can be seen that blending the two pheromones of *Yponomeuta cagnagellus* and *Yponomeuta plumbellus*, which share only these same two 'components' but at widely disparate ratios, will result in the blended component ratios from the two species exerting behavioral antagonism on males of the other species, to the extent that little or no attraction will occur. Thus, pheromone components are only components of the pheromone odor when they are emitted at optimal ratios for the pheromone blend of that species. Otherwise, these olfactory antagonists also act as behavioral antagonists.

The only time the two components do not seem to act as behavioral antagonists is when they produce olfactory inputs, relative to the other pheromone-related odorants, that are centered around that species' pheromone blend ratio. I propose that in the behaviorally optimal pheromone blend, pheromone components could appropriately be viewed as balancing each others' olfactory antagonism at a particular position in that species' defined sex pheromone

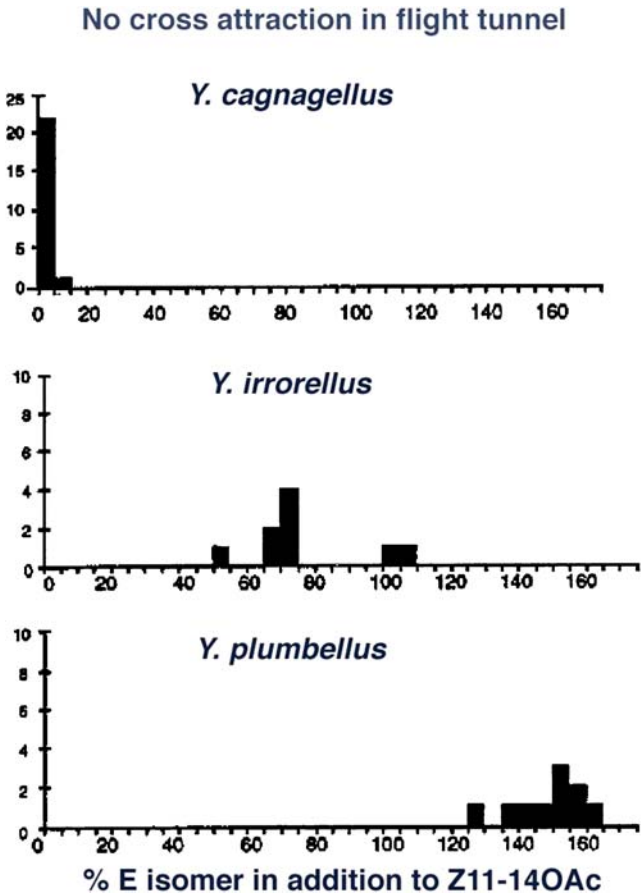

Fig. 1 Attraction of three *Yponomeuta* species that share the same host-plant (European spindle tree) to blends of their pheromone components in the wind tunnel (Löfstedt et al. 1991). There is no overlap in attraction to any of the cross-specific blends. Löfstedt et al. (1991) hypothesized that the species-specific blends arose as a result of blend interference between these sympatric and synchronic species that share the same host plant

"odor space" (Hallem and Carlson 2006). To use an analogy, opposing muscle groups are termed 'antagonists', and it is the balance of the forces exerted by each muscle group that results in various limb positions. Similarly, olfactory neuronal elements may be considered to act antagonistically, producing optimal behavioral outcomes only when their antagonism is balanced.

All pheromone-related inputs to the brain from the ORNs on the antennae, even those tuned to heterospecific behavioral antagonists, are transmitted in the form of excitatory action potentials to glomeruli in the macroglomerular complex (MGC). Among the first synaptic transmissions from the axon terminals of ORNs in the glomeruli are the excitation of gamma-aminobutyric acid (GABA)-ergic local interneurons, as well as direct synapses of ORN terminals with the dendrites of projection interneurons (Anton and Homberg 1999). There is evidence from neurophysiological studies of such synapses (Christensen et al. 1993) that the GABA-transmitted inhibition of one local interneuron can disinhibit a second local interneuron, resulting in excitation of projection interneurons that exit that glomerulus. These projection interneurons then send their excitatory outputs to higher centers such as the mushroom body and lateral protocerebrum (De Belle and Kanzaki 1999). Here, two negatives, through mutual disinhibition, can produce a positive, and this might be one of the neuronal bases for balanced olfactory antagonism. There may be other neurophysiological contributing factors as well. Excitation and further possible balanced antagonism in higher centers, such as the lateral protocerebrum and mushroom body, could also contribute to the ultimate behavioral outcome, that is, olfaction-stimulated, visually mediated flight that results in attraction to the correct blend.

I also propose that with regard to sex pheromone olfaction, the addition of a heterospecific behavioral antagonist to the pheromone blend is no different than the addition of excessive amounts of one of the sex pheromone components to the blend. In both instances, the new blend falls outside an optimal olfactory odor space, and the off-ratios result in an olfactory antagonistic imbalance due to an excessive amount of a given compound. The increased level of inhibition alters the balance of mutual disinhibition-related excitatory outputs, and ultimately results in reduction or even elimination of attraction.

This concept of olfactory antagonistic balance of sex-pheromone-related mediators of behavior does not require changing our definition of sex pheromones or of heterospecific behavioral antagonists. Rather, it provides a different way of thinking about the olfaction-related effects of the individual chemical components, and how they might influence sex pheromone olfaction-related pathways in ways that result in optimal or suboptimal attraction to the odor source.

Another reason that it makes sense not to label one set of pheromone component molecules as agonists (pheromone components, attractants) and another set the other way (antagonists) is because pharmacologically and neurophysiologically, they all act in the same way, as *agonists* to the odorant receptor (OR) that receive them on the ORNs. The ORNs all produce various frequencies of excitatory action potentials both in response to pheromone components and heterospecific antagonists. General odorants can produce both excitatory and inhibitory effects on action potential outputs of the ORNs on which the specific ORs are expressed (c.f., Hallem et al. 2004), but the odorants' initial interactions with their ORs are as pharmacological agonists, and the ORNs' outputs are still various levels of excitatory action potential frequencies.

In pheromone systems comprised of two or more pheromone components, attraction of males can occur in response to a partial blend or even a single component of the blend, but such partial blends are not optimal for

attraction (Linn et al. 1986, 1987). Even when the complete blend *is* emitted, impairment of a component-specific olfactory pathway through habituation or sensory adaptation due to pre-exposure to that single component can temporarily shift the balance of olfactory inputs to create a new, optimal blend ratio and render the true pheromone blend ratio as suboptimal (Linn and Roelofs 1981). In other cases that involve sensory impairment but with no pre-exposure, if the optimal blend ratio is emitted but at an excessively high rate such that upwind flight ceases, especially near the pheromone source, the underlying cause of suboptimal attraction can be found to be due to a suboptimal balance of inputs due to sensory adaptation. That is, the ORNs for one of the component-specific olfactory pathways can become differentially adapted to the excessive flux of that component in the blend, whereas the other ORNs tuned to the remaining two components retain their fidelity in reporting the flux specific to those components, thus creating an imbalance in the antagonistic interactions (Baker et al. 1989; Hansson and Baker 1991).

If we continue to compartmentalize the olfaction of pheromone components and heterospecific antagonists in our minds, instead of considering them as elements of an olfactory continuum, it will hamper progress in understanding the olfactory basis for shifts in male behavioral responses to novel sex pheromone blends, and our understanding of the evolution of divergent blends. With our ever-advancing knowledge of olfactory pathways, it is time to unify our thinking regarding the underlying processes involved in pheromone component and heterospecific antagonist olfaction. A unified model of pheromone and antagonist olfaction also may enhance and unify discourse between those who work on the evolution of sex pheromone olfaction and those who study the molecular and evolutionary aspects of olfaction of general odorants. For example, host-fruit odors attractive to one species of tephritid fruit fly cause a cessation of upwind flight by a second tephritid species when they are added to the latter species' preferred blend (Linn et al. 2005). Labeling the first species' host-fruit volatiles as being behaviorally antagonistic to the second species' is certainly appropriate, and yet I suggest that progress in understanding the evolution of these responses will be slowed if we structure our thinking along the lines of searching for special "antagonist"- or "agonist"-related *olfactory* pathways in such general odorant systems.

Asymmetric Tracking

In the concept of sex pheromone component antagonistic balance, a relaxation of the antagonism that would have otherwise occurred in response to excessive amounts of one compound, which we termed "olfactory antagonistic release" (Domingue et al. 2007), can be envisioned as a way for pheromone blends to shift away from their mean ratio in a first stage of reproductive character displacement, as particular types of "rare" males "track" the widely disparate blends of odd females, while retaining their responsiveness to the majority of females emitting blends centered around the norm of the population (Löfstedt 1990, 1993; Löfstedt et al. 1991; Phelan 1992, 1997). Such a scenario might explain the broadening of behavioral response profiles of male *Trichoplusia ni* (Liu and Haynes 1994; Haynes 1997). That is, after more than 40 generations of laboratory breeding, male *T. ni* subsequently were attracted as readily to the off-ratio blends produced by mutant females as they were to the blend of normal females. At the outset of these breeding experiments, males were attracted to mutant females' blends only at very low response rates, well below their level of attraction to the normal blend (Liu and Haynes 1994; Haynes 1997).

To illustrate this scenario in another way, the assortment of Z11/E11-14:OAc ratios (Fig. 1) seen in the three species of *Yponomeuta spp.* briefly discussed above were suggested to be the result of adaptive responses to "blend interference" from the pheromone blends of sympatric species sharing the same host plant (Löfstedt et al. 1991). We might view the three ratios, each of which is optimal for its respective species, as the result of an optimal antagonistic olfactory balance that is specific to that species. One of many ways we can hypothesize this end result as having occurred is that all three species' blends were originally closer to a 50:50 E/Z ratio (Fig. 2a). A broadening of males' responses then could have occurred in two of the species to include responses to rare (mutant) females that emit off-ratios different from the original blends. This broadening would have been facilitated by a relaxation ("olfactory antagonistic release") (Domingue et al. 2007) of the original degree of antagonism that otherwise would have been imposed in response to excessive amounts of either component. Olfactory antagonistic release could have occurred due to a desensitization or reduction in the number of neuronal elements somewhere along the ORN or central nervous system (CNS) pathway specific for carrying information initiated by the excitation of one of the two ORN types that respond to either Z11- or E11-14:OAc (Fig. 2b, left and right response curves, respectively).

Reproductive Character Displacement and Reinforcement

On the other hand, "olfactory antagonistic imposition" (strengthening of antagonism) (Domingue et al. 2007) would subsequently be involved in moving the shift in mean male responsiveness further away from the former mean, with the strength of antagonism on the other isomer's olfactory pathway being increased through increased ORN

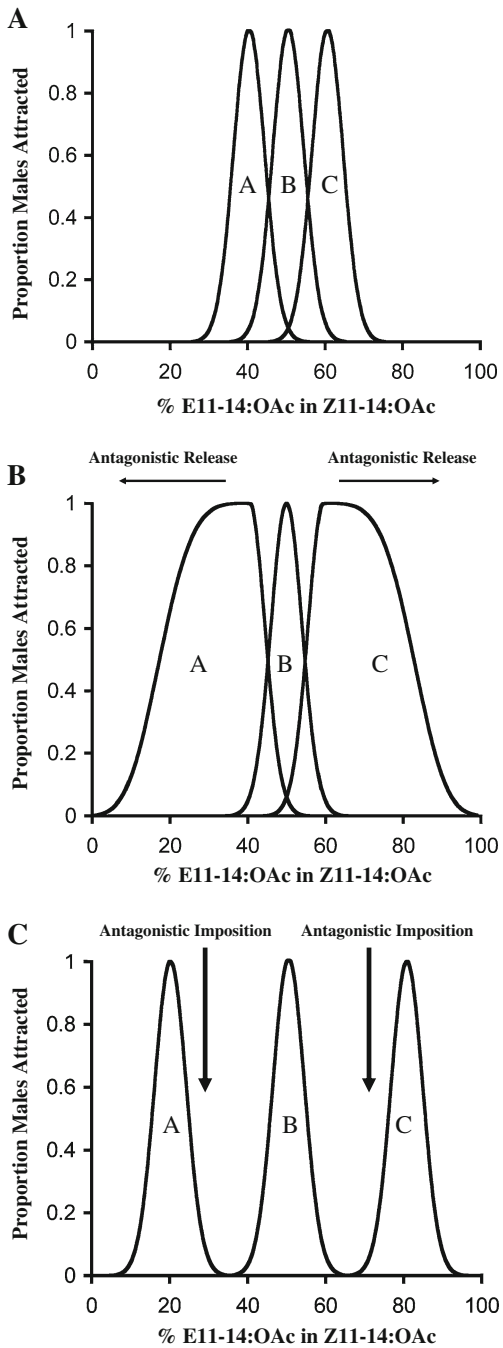

Fig. 2 Illustration of antagonistic release and imposition of changes in blend ratios that elicit male attraction in three hypothetical species that utilize Z11- and E11-14:OAc. **a** Original populations, in which there is a narrow range of Z11- and E11-14:OAc ratios to which males will be attracted. **b** After antagonistic release has occurred in males, the blend ratios to which males can respond are expanded in either direction to include off-ratios that may be emitted by rare mutant females in the population. The males' ranges of responsiveness include these variant blends, but males retain responsiveness to ratios in the previous norm so that such males can respond to both ancestral and derived pheromone blends. **c** After antagonistic imposition has occurred, males respond to new, tighter blend ratios in the derived populations, and thus assortative mating occurs in the new populations

or CNS sensitivity to that second isomer (Fig. 2c). If such a shift occurred as a response to blend interference (Löfstedt et al. 1991) between already existing species, then reproductive character displacement would result (Butlin and Ritchie 1989; Butlin and Trickett 1997). Alternatively, if this two-part process occurred during a speciation event, the imposition of increased antagonism would be implicated in reinforcement and subsequent reproductive isolation that is favored by selection (as the fitness of hybrids between the derived and ancestral populations decreases) so that males and females from the two populations can avoid fitness-related mating mistakes (Phelan 1992, 1997).

The addition of a third component to the sex pheromone blend can also impart species specificity to the new blend through this same two-part process. *Yponomeuta padellus*, a derived species of *Yponomeuta*, may have diverged in this way (Löfstedt et al. 1991), and its three-component blend sets it apart from two other *Yponomeuta* species that have similar two-component E11- and Z11-14:OAc blend ratios (Fig. 3). The process by which the third component might have been added, thus creating a three-component blend that males tracked, is hypothetically depicted in Fig. 3.

We hypothesize that there was first a broadening of responsiveness in rare males to include behaviorally beneficial olfactory antagonistic input from Z11-16:OAc, so they could respond to and track rare females emitting small amounts of this compound in addition to the original Z11- and E11-14:OAc blend (antagonistic release). This would have been followed by olfactory antagonistic imposition to keep *Y. padellus* males in the derived population from being attracted to the ancestral blends of *Yponomeuta viginctipunctatus* or *Yponomeuta evonomellus* females, which were emitting *only* ratios of the original two components, without Z11-16:OAc. These latter females, therefore, will have had antagonistically excessive amounts of this two-component blend in their emissions (Fig. 3).

Similar evolutionary scenarios for shifts in balanced antagonism can likely be constructed for the well-investigated pheromone systems in the Tortricidae and in heliothine moths. Two- and three-component pheromones in sympatric tortricid species, as in yponomeutids, often rely on tightly regulated, differing ratios of E11- and Z11-14:OAc, as well as on various additional components (Cardé et al. 1977; Roelofs and Brown 1982). In the sex pheromone systems of heliothine moths, although male behavioral response profiles to varying 2-component blend ratios are not as tightly regulated as in tortricids and yponomeutids, there is at least some degree of ratio specificity (Vickers et al. 1991; Vickers 2002). Also, upwind flight is inhibited in response to single "components" (Vickers et al. 1991), and by addition of heterospecific compounds to otherwise optimal pheromone component blends (Vickers and Baker 1997; Baker et al. 1998; Quero and Baker 1999). Notably,

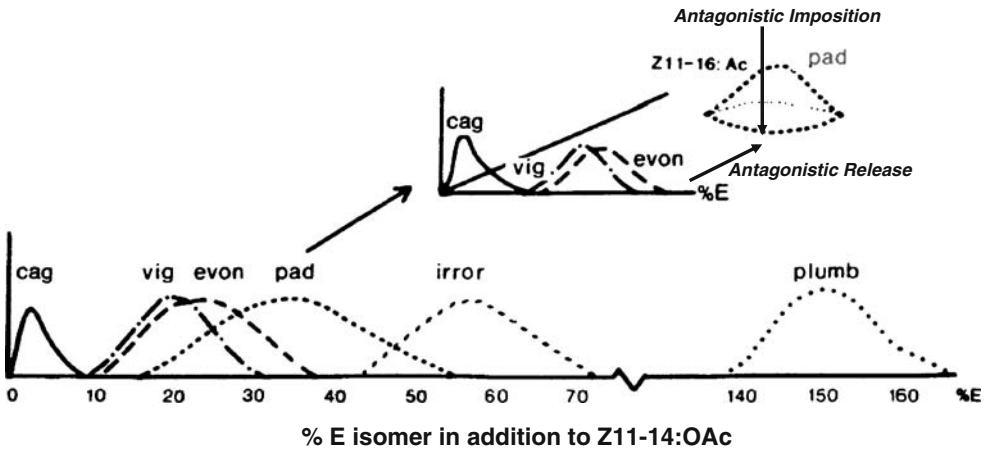

Fig. 3 Illustration of the pattern of antagonistic release, followed by antagonistic imposition, in the *Yponomeuta* species depicted by Löfstedt et al. (1991). *Y. padellus*, a derived species, has added a third pheromone component, Z11-16:OAc, to its blend, along with Z11- and E11-14:OAc. First, we hypothesize that antagonistic release has occurred to allow expansion of male responsiveness to this new, three-component blend. Then, we hypothesize that antagonistic imposition evolved to reinforce the assortative mating that will occur because of decreased fitness of hybrids from matings between the derived and ancestral populations, through increased sensitivity to blends emitting now-excessive amounts of the Z11- plus E11-14:OAc's (lacking Z11-16:OAc)

as in all other moth species in which antennal lobe neuroanatomy has been studied (Hansson and Christensen 1999), the glomeruli in heliothine males that receive inputs from ORNs tuned to heterspecific antagonists occur together in the MGC in close association with glomeruli that receive inputs from ORNs tuned to conspecific pheromone components (Christensen et al. 1995; Vickers et al. 1998; Berg et al. 1998, 2005; Vickers and Christensen 2003; Lee et al. 2006a, b). Alterations in balanced antagonism due to the mutual-inhibition (disinhibition) activities of GABA-ergic local interneurons would be most pronounced in this typical lepidopteran arrangement of closely spaced glomeruli for conspecific and heterospecific pheromone components in the MGC.

Single-component Pheromones and Balanced Antagonism

A saltational pheromone shift (Baker 2002; Roelofs et al. 2002) seems to have occurred in another derived species of yponomeutid, *Yponomeuta rorellus* (Löfstedt et al. 1986, 1990, 1991). This species' pheromone odor appears to be solely comprised of the unusual pheromone component, tetradecyl acetate (14:OAc). One of the types of ORN known to be involved in attraction in *Y. rorellus* responds with high activity to 14:OAc, but it also responds to E11- and Z11-14:OAc that are emitted by other sympatric *Yponomeuta* species in the environment. Attraction to other *Yponomeuta spp.* females that emit the ancestral blends containing Z11- or E11-14:OAc is prevented because of the activity of an ORN involved in behavioral antagonism that is stimulated by both E11- and Z11-14:OAc (Löfstedt et al. 1990, 1991). We view the broadened activity of this ORN that accepts either E11- and Z11-14:OAc as ligands as an example of olfactory antagonistic imposition (Domingue et al. 2007). Importantly, this behaviorally antagonistic ORN pathway is not merely responsive to any generic type of pheromone-related compound. Its lack of response to 14:OAc, as well as to the odd compounds (*E*)-6-tetradecenyl acetate and (*E*)-12-tetradecenyl acetate (E12-14:OAc) is apparently responsible for allowing equally high levels of attraction of *Y. rorellus* males to all three of these single compounds, all of which cause high firing rates in the attraction-related ORN (Löfstedt et al. 1990), despite the fact that only 14:OAc is the *Y. rorellus* pheromone component.

Single-component pheromone blends are rare in the Lepidoptera, but I maintain that they still fit the concept of attraction being due to balanced olfactory antagonism. First of all, even single component pheromones must be considered to be *blends* at the neurophysiological level because of the combinatorial coding process that compares the ratio of inputs into a single-component glomerulus (and out through projection interneurons) relative to the lack of activity occurring in other glomeruli in the absence of other pheromone-like odorants. Thus, even for single-component pheromones, inputs to the MGC are analyzed by the CNS as patterns of stimuli, i.e., "blends", with parts of the pattern consisting of the absence as well as the presence of activity in various glomeruli and along the rest of the pathways in the system. To make an analogy, in the auditory realm, the combinatorial code that produces recognition of single musical notes, as it does for chords, depends as much on the absence of many notes as it does on the presence of others.

Olfactory Pathways

Studies of *Drosophila* ORNs that respond to general odorants have shown that ORN activity is determined almost exclusively by whatever OR is expressed on that ORN (Hallem et al. 2004). In pheromone olfaction, modulating perireceptor factors such as binding proteins seem to affect the presentation of the pheromone component ligand to the OR more than in general odorant systems (Du and Prestwich 1995; Leal et al. 2005). The effects of degradative enzymes specific for pheromone components also seem to affect the time-course of ORN excitation more than in general odorant systems (Syed et al. 2006). Nevertheless, olfactory antagonistic release and imposition in moth sex pheromone systems will depend to a large degree on the up- or downregulation of OR gene expression on particular pheromone-component sensitive ORNs. Coexpression of two ORs on single ORNs is also possible in insects (Dobritsa et al. 2003; Goldman et al. 2005) and in moths might potentially contribute to shifts that broaden behavioral responsiveness to a wider array of pheromone blends (Baker et al. 2006), that affect both reinforcement and assortative mating.

Alternatively, a single OR itself might possibly have biochemical cross-affinities to two or more structurally related pheromone odorants, just as many ORs for general odorants do (c.f., Hallem and Carlson 2006). There are many examples in moths of ORNs that are tuned to a particular sex pheromone component, but they are also highly responsive to other molecules, even including totally synthetic analogs of the pheromone component that could not possibly comprise part of a sex pheromone blend of any species (c.f., Grant et al. 1989; Löfstedt et al. 1990; Berg et al. 1995).

It is difficult to imagine that there are OR genes in the genome specific for producing ORs having specific affinities for every one of such odd, non-naturally occurring molecules, and that they have suddenly become coexpressed on the dendrites of pheromone-component-responsive ORNs to cause this cross-responsiveness. Rather, cross-reactivity to structurally similar compounds by a single, broadly accommodating pheromone-component-tuned OR might provide a more logical explanation for such phenomena. Such ORs might be viewed as being preadapted to respond to odd conspecific females emitting such compounds, should such rare occurrences ever happen. Thus, such pheromone-odorant-related ORs that (perhaps serendipitously) accommodate structurally similar compounds might provide the basis for broader sex-pheromone-related ORN responsiveness (Linn et al. 2007a), which can result in either broader or narrower behavioral responsiveness, depending upon on which ORNs the ORs reside. Learning more about ORN coexpression of two ORs versus single expression of broadly tuned ORs should help guide our interpretations of the evolution of sex pheromone blends, particularly as they relate to shifts in sex-pheromone-related behavior.

Because the glomerular projection addresses of ORNs in insects do not change with whatever ORs are expressed on the ORNs (Dobritsa et al. 2003; Hallem et al. 2004; Goldman et al. 2005), shifts in the olfactory balance that change behavioral response specificity to certain sex pheromone blend ratios, therefore, might begin with ORN response profile shifts. Ultimately, the response profiles of ORNs, both chemically and temporally, rely on the expression of ORs that dictate stereotypical temporal spike-train characteristics and odorant tuning profiles of the ORNs (Hallem et al. 2004). Of course, changes in synaptic connectivity in the antennal lobe, such as the number, types, and targets of synapses in particular glomeruli, could affect the amount of inhibitory local interneuron activity as well as projection interneuron output to the mushroom body and lateral protocerebrum (Anton and Homberg 1999; De Belle and Kanzaki 1999; Hansson and Christensen 1999). Thus, shifts in olfactory antagonistic balance are not necessarily entirely dependent upon pheromone odorant OR gene expression and levels of ORN excitation.

Nevertheless, the predominance of OR gene expression in insects in determining both the overall activity levels and temporal characteristics of ORN action potential output (Hallem et al. 2004) indicates that the study of the response profiles of ORNs (DeBruyne et al. 1999, 2001) may to a large extent explain what occurs during shifts in pheromone-related olfactory pathways and in optimal attraction to various blends. This perspective has been supported by the neuroethological studies that concern geographic variation in moth sex pheromone communication systems, as exemplified in *Agrotis segetum* (c.f. Löfstedt 1990, 1993; Hansson et al. 1990). Further neuroethological studies on species that utilize highly "unusual" (from an anthropomorphic perspective) sex pheromone blend components should be particularly instructive for understanding how shifts in olfactory antagonistic balance might explain the evolution of sex pheromone blend shifts.

Possible Examples With *Ostrinia spp.*

Recent studies with *Ostrinia furnacalis*, the Asian corn borer (ACB), and *Ostrinia nubilalis*, the European corn borer (ECB), have implicated a mechanism involving ORNs on male antennae that explains how a few "rare" males (Roelofs et al. 2002; Linn et al. 2003, 2007b) in the population are attracted to both their own ECB or ACB sex pheromone blend, as well as to the entirely different blend of the other species (Domingue et al. 2007; Linn et al. 2007b). The ACB is considered to be a derived species

(Ishikawa et al. 1999), and is the only *Ostrinia* species that uses ~ 1:2 to 1:1 E:Z blends of E12-14:OAc and (*Z*)-12-tetradecenyl acetate (Z12-14:OAc) as its sex pheromone. The vast majority of ACB males are attracted only to their own ACB blend, and Takanashi et al. (2006) and Domingue et al. (2007) found that these "normal" ACB males, as with the rare males, have attraction-related ORNs that respond to both the Z11-/E11-14:OAc components of ECB and also to the Z12-/E12-14:OAc ACB components. However, normal ACB males have an ORN tuned to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc) that is involved in heterospecific behavioral antagonism and which responds also to Z11-14:OAc (Takanashi et al. 2006; Domingue et al. 2007), preventing attraction to the ECB blend (Domingue et al. 2007). This heterospecific behavioral antagonism-related ORN in *rare* ACB males does not respond to Z11-/E11-14:OAc, and thus it does not impede attraction to the ECB blend, nor does it impede attraction to the ACB blend due to its lack of activity to Z12-/E12-14:OAc (Domingue et al. 2007).

Domingue et al. (2007) considered the rare ACB males as being similar to the type of male that existed when ACB diverged from Z11/E11 species, during the first stage of asymmetric tracking (Phelan 1992, 1997). In this context, broadly tuned males would be able to be attracted to both the unusual ACB females emitting Z12- and E12-14:Oac, while retaining their responsiveness to the ancestral pheromone blend comprised of Z11- and E11-14:OAc. This stage of divergence of pheromone blends in the Lepidoptera had been previously noted as producing 'asymmetrical reproductive isolation" (Löfstedt et al. 1991) (as represented by the rare ACB males in our study) because males from the derived population (Fig. 2b) could respond to both the derived and the ancestral females, whereas ancestral population males could only respond to ancestral females.

The second stage of asymmetric tracking (Phelan 1992, 1997) involves the occurrence of assortative mating between females that emit the new blend and the derived males that respond to it. In a speciation event, the impetus for assortative mating would be a fitness disadvantage that arises in hybrids resulting from matings between the ancestral population females and males from the derived population (Phelan 1992, 1997). Ancestral females then should be selected to reject derived males for mating, and such males should subsequently be selected to not be attracted to these females because of the (ultimately) fruitless mating encounters with these females. The lack of responsiveness in derived males to the ancestral blend (here represented by the normal ACB males) could be accomplished by the emergence of behavioral antagonism to the old blend. Domingue et al. (2007) suggested that the responsiveness of the Z9-14:OAc behaviorally antagonistic pathway-related ORNs to Z11-/E11-14:OAc in these normal ACB males is evidence for this second step. After olfactory antagonistic imposition has occurred (Fig. 2c), full premating reproductive isolation would result (Löfstedt et al. 1991; Phelan 1992, 1997).

There are species of *Ostrinia* in Asia that have three-component pheromone blends comprised of Z11- and E11-14:OAc plus Z9-14:OAc (Ishikawa et al. 1999). These appear to have been derived from ancestral populations that use only Z11- and E11-14:OAc (Ishikawa et al. 1999). The scenario for the addition of this third component may have been the same as outlined above for the addition of Z11-16:OAc into the *Y. padellus* blend (Fig. 3).

Other studies are emerging that support the concept of olfactory antagonistic balance, with OR gene expression being a predominant factor (Hansson et al. 2007). The ECB "E"- and "Z"- pheromone strains' ORN glomerular projection destinations in MGC glomeruli, the MGC morphologies, and the projection interneuron sex pheromone component tuning profiles were investigated. The only difference between the blend-specific behavioral responsiveness of males of the two ECB strains was shown to be due to a swapping of the expression of ORs specific for Z11-14:OAc and E11-14:OAc in one strain onto the opposite colocalized ORNs (in the same sensillum) in the other strain (Hansson et al. 2007).

The specificities of the two ORNs thus seem to have been switched to respond now to the opposite isomer, without a concomitant switch in the glomeruli of the MGC to which the ORNs project. The new olfactory antagonistic balance that allows for the potential responses to the two widely disparate blends of the ECB Z-strain and E-strain females apparently has occurred only by a swapping of expression of the ORs, leaving the rest of the pathways unchanged to impart the same balanced olfactory inputs in the antennal lobe and farther up in the CNS, but now through reversed ORN specificities of response.

A similar interpretation that uses antagonistic olfactory balance also can be made regarding the study of Cossé et al. (1995) on F2 hybrids between the E- and Z-strains of ECB. This study showed that as predicted (W.L. Roelofs, unpublished), a significant portion of the F2s in one cross that were behaviorally E-strain males would readily fly upwind to the E-strain pheromone blend, even though neurophysiological studies on these males showed that they had Z-strain ORN architecture (spike-size relationships). The large-spiking ORNs in the antennal sensilla of this portion of the F2 males responded to Z11-14:Oac, and the co-compartmentalized small-spiking ORN responded to E11-14:OAc. Normally, E-strain behavioral responders have the reverse situation, with the large-spiking ORN responding to E11-14:OAc and the small-spiking ORN responding to Z11-14:OAc.

As in the study by Hansson et al. (2007), the results of Cossé et al. (1995) lend themselves to the interpretation that the ORs specifically responsive to each of the two pheromone components in these males have been swapped onto the neighboring, colocalized ORN, perhaps due to regulatory genes (Clyne et al. 1999; Endo et al. 2007; Ray et al. 2007). Because the same olfactory antagonistic balance has been maintained, even though the balance is now the result of a different ratio of components, strong upwind flight behavior can occur. The two MGC glomeruli receiving input from the same two ORNs cannot discern that the inputs are now coming from these ORNs responding to the geometric isomers opposite to those than they normally do. No changes in CNS wiring or CNS integration of these inputs need to have occurred in order for optimal attraction to continue to take place. Rather, the fact that strong behavioral responses are elicited provides strong evidence that the remainder of the pathway has remained unchanged, and that only a switch of ORs has occurred.

Summary

I propose this new model of olfactory antagonistic balance of sex-pheromone-related compounds as a possible way of unifying our thinking and discussions about the sex-pheromone-mediated behaviors that we observe, the olfactory pathways involved, and the evolution of sex pheromones. I suggest that the concept of olfactory antagonistic balance can revitalize our thinking and suggest new possibilities for research on the evolution of divergent sex pheromone blends, and on the combinatorial coding that is involved in the positive or negative behavioral outcomes displayed by individual male moths based on their olfactory discrimination abilities.

The upwind flight behaviors of male moths that are elicited or not elicited by correct or incorrect blends of moth sex pheromone "components" are directly related to reproductive success. Thus, a detailed knowledge of such behaviors provides a foundation for understanding speciation, reproductive character displacement, and hence the evolution of insect communication systems and olfaction in general. Studies of shifts in sex-pheromone related ORN response profiles and their regulation by expression, coexpression, or shifts in expression of putative OR genes, may uncover general principles that govern how such factors might cause evolutionarily important olfactory and behavioral shifts in response to general odorants, such as the host cues that mediate feeding and oviposition in insect general odorant olfactory systems (c.f. Linn et al. 2005; Olsson et al. 2006a, b). Finally, I suggest that studies of possible shifts in sex-pheromone-related ORN response profiles that are related to shifts in behavior and to pheromone OR gene expression will complement and augment continuing advances with identified OR/ORN systems such as those in *Drosophila, Anopheles, Aedes*, and *Culex spp*.

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