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Evidence of olfactory antagonistic imposition as a facilitator of evolutionary shifts in pheromone blend usage in *Ostrinia* spp. (Lepidoptera: Crambidae)

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Abstract

Olfactory receptor neuron (ORN) response was measured to assess why some males ("rare males") of the Asian corn borer (ACB), *Ostrinia furnacalis*, have a broad behavioral response to fly upwind to both the ACB and the European corn borer (ECB), *Ostrinia nubilalis*, pheromone blends. We performed single-sensillum electrophysiological recordings on ACB males that had been behaviorally assessed for upwind flight response to the ACB blend [60:40 (*Z*)-12-tetradecenyl acetate (Z12-14:OAc) to (*E*)-12-tetradecenyl acetate (E12-14:OAc)], as well as to ECB (Z-strain) and ECB (E-strain) blends [3:97 and 99:1 (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) to (*E*)-11-tetradecenyl acetate (E11-14:OAc)]. Sensilla from all types of males had large- and small-spike-sized ORNs responding strongly to Z12- or E12-14:OAc, but weakly to Z11- and E11-14:OAc. In the majority of males ("normal males") that flew upwind only to the ACB blend, Z11-14:OAc elicited responses in an intermediate spike-sized ORN associated with behavioral antagonism that is mainly tuned to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc). In the rare-type ACB males that flew to both the ACB and ECB pheromone blends, Z11-14:OAc did not stimulate this ORN. Increased responsiveness to ancestral pheromone components by ORNs associated with behavioral antagonism could be instrumental in reproductive character displacement, or in reinforcement and reproductive isolation during speciation by helping to increase assortative mating between males and females in derived populations that use novel sex pheromone blends.

Keywords: Olfactory receptor neurons; *Ostrinia nubilalis*; *Ostrinia furnacalis*; Asymmetric tracking; Pheromone shift; Behavioral antagonism

1. Introduction

Pheromone specificity can be a powerful means of reproductive isolation, being associated with species specificity in mating and/or aggregation behavior across a wide array of taxa (Roelofs and Comeau, 1969; Lanier and Wood, 1975; Costa et al., 1997; Kotani et al., 2001; Lemaster and Mason, 2003). Historically, it had often been assumed that pheromone systems involve strong stabilizing selection on emitters and receivers (Cardé and Baker, 1984). Theoretically, such stabilizing pressure would only allow small, incremental changes in pheromone systems (Paterson, 1980). However, investigations have revealed that there are large within- and between-species shifts in pheromone-related traits that tend to involve a simple genetic basis (Klun and Maini, 1979; Hansson et al., 1987; Roelofs et al., 1987; Löfstedt, 1990; LaForest et al., 1997; Roelofs et al., 2002; Domingue et al., 2006).

It was later proposed that many of these patterns might be explained better in the context of differential selection on the pheromone-emitting and receiving sexes with respect to their parental investment (Phelan, 1992, 1997). This model, called asymmetric tracking (Phelan, 1992, 1997),

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predicts that the non-limiting sex (usually males) experiences stronger selection and would more strongly track changes that occur in the limiting sex (usually females), regardless of which sex is the emitter. For female pheromone emission systems, this asymmetry allows high between-individual variation in the emitted pheromone blend quality and quantity (Löfstedt, 1990, 1993). At the same time, male responses tend to possess little heritable variation (individual variation), and all males stereotypically broadly bracket the emission ratios emitted by all the females in the population. Shifts in pheromone blends, therefore, are predicted to be initiated by males in the population that have broadened response profiles to include mutant or rare female pheromone blends as well as the normal female blends, rather than by immediately changing specificity to a new blend. The plausibility of this process has been confirmed for the cabbage looper moth, *Trichoplusia ni* (Liu and Haynes, 1994). When confronted with a mutant phenotype consisting of highly skewed, disparate ratios of female-emitted pheromone components, normal males in the population, over the course of 40 generations, succeeded in broadening their behavioral response profiles to include both the wild-type and mutant-type female blends (Liu and Haynes, 1994).

It is inherently difficult to find situations in which one can observe and characterize the genetic and olfactory changes that might be involved in dramatic shifts in pheromone blends implied by the diverse communication systems in taxa such as Lepidoptera (Baker, 2002). Additionally, most of the study systems described above that support the asymmetric tracking model involve intraspecific pheromone races where divergence has already occurred. In such cases, it will always remain uncertain whether genetic properties of the population are the cause or result of speciation processes. For example, where major genetic loci have been implicated in controlling pheromone blends between pheromone races, there is also the presence of a second level of interacting genetic variation (Zhu et al., 1996; Domingue et al., 2006). In this context, it is possible for initial divergence to be more incremental, with major genes arising later.

Recently revealed aspects of two *Ostrinia* species, the European corn borer (ECB), *Ostrinia nubilalis*, and the Asian corn borer (ACB), *Ostrinia furnacalis*, suggest a system wherein shifts in sex pheromone communication have occurred that are directly relevant to the plausibility of the asymmetric tracking process. ECB has pheromone races utilizing either a 97:3 or a 1:99 ratio of (*Z*)- to (*E*)-11-tetradecenyl acetate (Z11/E11-14:OAc) (Kochansky et al., 1975). ACB produce blends of (*Z*)- to (*E*)-12-tetradecenyl acetate (Z12/E12-14:OAc) ranging from 1:1 (Klun et al., 1980) to 2:1 (Ando et al., 1980). A mechanism has been uncovered (Roelofs et al., 2002) demonstrating that pheromone production between these species is controlled by the expression or lack of expression of two desaturase genes (plus other enzymes) that cause a simple divergence in the biosynthetic pathway to result in either a Z12/E12 or a Z11/E11 system.

A key co-factor of the mechanism for facilitating a shift to the divergent blend has also been discovered in the observation that there are "rare males" (Roelofs et al., 2002) in both species that fly upwind and are attracted to the cross-specific ECB or ACB pheromone blend. In wind tunnel tests, 3–5% of Z-strain or E-strain ECB males flew upwind and located an ACB pheromone source in addition to exhibiting the same response to their own blend (Linn et al., 2003). Similarly, 70% of ACB males flew upwind and located an ACB blend source, whereas 3–4% of ACB males also flew upwind and located either ECB(Z) or ECB(E) pheromone sources (Linn et al., 2007a). Also, 1% of ACB males flew upwind in response to both ECB blends as well as to the ACB blend. This pattern is consistent with the asymmetric tracking model because it shows the potential of individual males to be able to broadly respond to a wide variety of female-emitted compounds. These observations, though, present the question of what aspects of the olfactory perception system might account for the response behavior of the broadly responding rare males in such populations.

The peripheral olfactory system in the ECB has been well described, consisting of three olfactory receptor neurons (ORNs). Using single-cell electrophysiology, it has been observed that two co-compartmentalized ORNs in every trichoid sensillum respond to the pheromone components (Z11/E11-14:OAc) (Hansson et al., 1987, 1994; Hallberg et al., 1994; Cossé et al., 1995). There is also a third ORN co-compartmentalized in each sensillum that responds to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), and, at least in ECB (Z-strain), also responds to (*Z*)-11-hexadecenal (Z11-16:Ald) (Linn et al., 2007b). These latter compounds act as behavioral antagonists to ECB attraction (Klun and Robinson, 1971; Struble et al., 1987; Glover et al., 1989; Gemeno et al., 2006).

Asian corn borers have sensilla containing two co-compartmentalized ORNs responsive to the two pheromone components of this species (Takanashi et al., 2006). However, in this case, one ORN responds to both Z12-14:OAc and E12-14:OAc. The second, small-spike-amplitude ORN is most sensitive to E12-14:OAc, but some also exhibit a weak response to Z12-14:OAc. As with ECB, there is a third co-compartmentalized ORN that is highly responsive to Z9-14:OAc. It is presumably through the input of this ORN that Z9-14:OAc acts as a behavioral antagonist in ACB (Takanashi et al., 2006; Linn et al., 2007a). Takanashi et al. (2006) also found that the ECB pheromone components (Z11/E11-14:OAc), were both capable of stimulating the attraction-related ORN in ACB males that is stimulated by Z12-14:OAc and E12-14:OAc, and importantly these ECB components also stimulate the neuron associated with behavioral antagonism tuned to Z9-14:OAc. They noted that the antagonistic response could be useful for avoiding cross-specific mating with numerous sympatric *Ostrinia* species in