Journal of Insect Physiology 56 (2010) 73–80

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Homology of olfactory receptor neuron response characteristics inferred from hybrids between Asian and European corn borer moths (Lepidoptera: Crambidae)

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ARTICLE INFO

Article history:
Received 24 July 2009
Received in revised form 14 September 2009
Accepted 15 September 2009

Keywords:
Flight tunnels
Olfaction
Ostrinia
Single-cell electrophysiology
Sex pheromones

ABSTRACT

First generation hybrid males from crosses between the Asian corn borer (ACB), *Ostrinia furnacalis*, and the "univoltine Z-strain" European corn borer (ECB), *Ostrinia nubilalis*, were examined with respect to behavioral and physiological responses to ACB and ECB pheromones. The hybrid males often flew to the pheromone of ECB Z-strain, but very rarely to the ACB pheromone. We mapped the tuning profiles of each ORN of the F_1 hybrids with respect to the relevant pheromone components and a common behavioral antagonist by employing differential cross-adaptation and varying doses of the ligands. In the trichoid sensilla of F_1 hybrid males, the three co-compartmentalized ORNs produced spikes that were very difficult to distinguish by size, unlike the parental populations. Comparing the responses to ACB and ECB components at different doses reveals overlapping profiles similar to males of both parental types, but more responsiveness to the ECB pheromone components. We were unable to detect any differences in the ORN tuning profiles when comparing males with different behavioral phenotypes. While the two ECB pheromone races have similar ORN tuning properties that are different from those in ACB, the spike-amplitude patterns of ECB E-strain and ACB have greater homology when compared to ECB Z-strain.

1. Introduction

Reproductive traits have often been given a primary role in assessing the evolutionary relationships among populations and species, as they provide strong evidence of reproductive isolation, the cornerstone of the Biological Species Concept (Mayr, 1963; Dobzhansky, 1970). Despite the role reproductive isolation is presumed to have played in creating phylogenetic patterns, understanding the mechanisms by which reproductive traits evolve during speciation events continues to be a challenge (Marshall et al., 2008; Smadja and Butlin, 2009). Furthermore, there is ongoing debate as to the relative importance of sexual traits versus ecological forces in the speciation process (Rundle and Nosil, 2005; Sueur et al., 2007; Seehausen et al., 2008).

Ostrinia moths represent a group that has been studied in the context of such multiple evolutionarily significant traits. There are conflicting phylogenies for the group constructed from analyses of morphological traits (Mutuura and Munroe, 1970) and mitochondrial DNA sequences (Kim, 1997). Patterns inferred from such analyses are also difficult to fully reconcile with patterns of reproductive isolating traits across the genus such as pheromone biology, host plant preference differentiation, or the ability to hybridize (Ishikawa et al., 1999; Frolov et al., 2007). Two species within the trilobed uncus group, the European corn borer (ECB), *Ostrinia nubilalis*, and the Asian corn borer (ACB), *Ostrinia furnacalis*, have been most intensely studied with respect to the biochemical and physiological mechanisms of sex pheromone differentiation.

Like most other *Ostrinia* species, ECB uses (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) and (*E*)-11-tetradecenyl acetate (E11-14:OAc) in its pheromone blend (Ishikawa et al., 1999). There are two ECB strains. The E-strain [ECB(E)] has 99% E11-14:OAc and 1% Z11-14:OAc in its blend, whereas the Z-strain [ECB(Z)] uses a reverse 97:3 ratio of Z11- and E11-14:OAc (Kochansky et al., 1975; Glover et al., 1987). The ACB pheromone stands out as unusual among all *Ostrinia*, consisting of variable blends of (*E*)-12-tetradecenyl acetate (E12-14:OAc) and (*Z*)-12-tetradecenyl acetate (Z12-14:OAc) (Klun et al., 1980; Ando et al., 1980; Boo and Park, 1998; Linn et al., 2007). Thus, the lineage leading to the ACB clearly experienced a shift in the production from the Δ-11-tetradecenyl acetates to the Δ-12-tetradecenyl acetates, which appears to have involved the activation of a desaturase gene used by ACB that is present but not active in ECB pheromone production (Roelofs et al., 2002).

Several aspects of the olfactory processing of sex pheromones in these species also have been investigated. Both species have two olfactory receptor neurons (ORNs) that respond to their own pheromone components. The ORNs of ACB and ECB(E) have been shown to regularly respond to the opposing species' pheromone components (Takanashi et al., 2006; Domingue et al., 2007a,b,

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doi:10.1016/j.jinsphys.2009.09.005

2008). Both of these species also have a third ORN that selectively responds to (*Z*)-9-tetradecenyl acetate (Z9-14:OAc), a behavioral antagonist that significantly reduces attraction when co-emitted at very small proportions with the normally attractive pheromone blend (Hansson et al., 1987; Glover et al., 1989; Takanashi et al., 2006; Linn et al., 2007).

First generation hybrid male offspring of ACB and ECB(E) showed overlapping behavioral and physiological response profiles when presented with the pheromones of either parental type, indicating incomplete reproductive isolation (Domingue et al., 2008). The largest spike-size ORN was very broadly tuned in both ACB and ECB(E) and often responded to all of the Δ-11- and Δ-12-tetradecenyl acetates. However, this neuron was most responsive to E11-14:OAc in ECB(E), to E12-14:OAc and Z12:14:OAc equally in ACB, and to all three of the compounds in the F_1 hybrids. There were similar overlapping response profiles on the smallest spike-size ORN of the ACB × ECB(E) F_1 hybrids. This ORN responded to both Z11-14:OAc and E12-14:OAc, which are the respective ligands that stimulate the small spike-size ORN in ECB(E) and ACB males. The medium spike-size ORN remained tuned to Z9-14:OAc in ACB, ECB(E), and F_1 hybrid males.

The ORN profiles of F_1 hybrids between ACB × ECB(E) thus showed a pattern of overlapping response characteristics with respect to the two parental populations. This pattern differs in several respects from that revealed by examinations of F_1 hybrids between the ECB(E) and ECB(Z) populations (Hansson et al., 1987; Roelofs et al., 1987). Similar to ECB(E), ECB(Z) has ORNs tuned to Z11-14:OAc, E11-14:OAc, and Z9-14:OAc, but the ORN tuned to Z11-14:OAc is of larger amplitude than the one tuned to E11-14:OAc. In the ECB(E) × ECB(Z) F_1 hybrids there are three ORNs tuned to Z11-14:OAc, E11-14:OAc, and Z9-14:OAc, but the spike amplitudes are indistinguishable (Hansson et al., 1987). More recently it has been discovered that in males of the parent populations, the glomerular targets of the larger and smaller pheromone-responding ORNs are the same in either species regardless of the ligand-specific tuning properties (Kárpáti et al., 2008). Thus, while the developmental processes causing these olfactory differences between the ECB pheromone strains are not yet fully clear, they do have a different peripheral olfactory arrangement.

To more fully elucidate the relative differences in the olfactory systems of the ECB(E), ECB(Z) and ACB populations, we performed physiological and behavioral analyses of the F_1 hybrids between ECB(Z) and ACB. The goals were to determine whether the spike-size relationships would become obscure for these hybrids as they did when ECB(E) and ECB(Z) were crossed, and to determine whether the ORNs of the hybrids have overlapping response characteristics in a similar pattern to the F_1 hybrids between ECB(E) and ACB. Observations of hybrid male flight to the ECB and ACB sex pheromones were obtained to determine the degree to which these olfactory response characteristics might potentially affect premating isolation.

2. Materials and methods

2.1. Insects

ECB(Z) male moths were obtained from a colony of the "univoltine Z" strain of ECB that has been maintained in the laboratory of W.L. Roelofs in Geneva, NY as previously described (Roelofs et al., 1985). ACB male moths were obtained from another colony briefly kept in Geneva (Linn et al., 2007), and derived from insects provided by Jin Kyo Jung, National Institute of Crop Sciences, South Korea. All moths were maintained at 25 °C, 16:8 L:D photoperiod, using the protocols established in Roelofs et al. (1985). Reciprocal F_1 hybrid crosses were obtained using rearing procedures identical to those used within species. Some males were behaviorally assayed in Geneva, NY, and shipped overnight to State College, PA for physiological analyses. Others that were only physiologically examined were shipped to State College as pupae where they emerged as adults. Details of the handling of moths as they were transported follows previously described protocols (Domingue et al., 2007a).

2.2. Behavioral assay

Males were tested in the sustained-flight tunnel in the Geneva lab during their second to third night as adults, under standard conditions for *Ostrinia* (Glover et al., 1989; Linn et al., 1997). Adult moths were tested individually and a positive response consisted of upwind flight (1.75 m distance) in the odor plume and contact with the rubber septum source. Combinations of Z11-, E11-, Z12-, and E12-14:OAc matching the pheromone blends of ACB (2:1 Z12- to E12-14:OAc), the Z-strain of ECB (97:3 Z11- to E11-14:OAc), and the E-strain of ECB (1:99 Z11- to E11-14:OAc) were used for flight-tunnel lures. During each daily testing period all of the males were tested to the three pheromone blends. Each day the order of presentation was changed. Mixtures were prepared in HPLC-grade hexane and 30 μg of the appropriate blend applied to red rubber septa (Thomas Scientific, Swedesboro, NJ; Glover et al., 1989; Linn et al., 1997).

2.3. Single-cell electrophysiology

Antennal sensilla were tested for ORN responses using the cut sensillum technique (Kaissling, 1974; van der Pers and den Otter, 1978), employed on cut antennae as modified for *Ostrinia* (Domingue et al., 2006, 2007a,b, 2008). The AC signal passed through an amplifier (DAM50, World Precision Instruments, Sarasota, FL, USA) and was recorded using a computer (Syntech Autospike v.32; Syntech, Hilversum, The Netherlands).

In these *Ostrinia* species, most sensilla have three co-compartmentalized ORNs, two of which respond to pheromone components, and another to the behavioral antagonist (Hansson et al., 1987; Takanashi et al., 2006; Domingue et al., 2007a). There is also evidence in the ECB(E) strain that some sensilla, more often those located distally, have fewer ORNs (Hallberg et al., 1994). All the sensilla we sampled were from the basal area of the antenna, where they are more likely to have three co-compartmentalized ORNs.

Dilutions of Z11-, E11-, Z12-, E12-, and Z9-14:OAc in HPLC-grade hexane were used to create odor cartridges containing doses of 1, 10, 30, or 100 μg. The headspaces within such cartridges were pulsed into a humidified airstream leading to the antenna as described previously (Domingue et al., 2006). Syntech software was used to count the number of spikes occurring within 300 ms of the first appearance of a spike. We did not consider spontaneous background activity as it was immeasurably small for ACB, ECB, or F_1 hybrid ORNs (Fig. 1).

In some experiments paired stimulations were performed at 0.3 s intervals to assess the degree to which differential adaptation occurs. In such cases we counted spikes within 300 ms of the first appearance of neuronal activity and then again for the second burst of activity, which was usually approximately 300 ms later. Occasionally, the spike trains associated with these paired stimulations overlapped, which usually involved different ORNs responding to either puff. In such cases, spikes arising from stimulation by the first compound could easily be separated from those associated with the second compound by spike amplitude and/or the tempo of the spike train.

2.4. Mapping ORN response characteristics

The first objective of our electrophysiological analyses was to clarify which compounds stimulated the same ORNs in the F_1

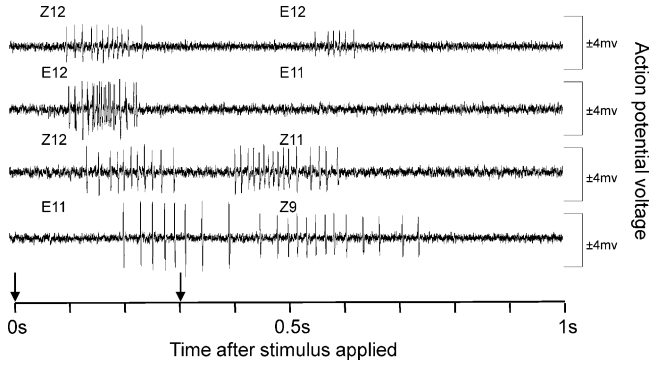

Fig. 1. Example traces of paired stimulations used to determine response profiles of F_1 hybrid male ORNs that have similar spike amplitudes. Arrows indicate the timing of application of either chemical. All examples shown here are from hybrids resulting from ACB males crossed to ECB(Z) females.

hybrids. This information is already known for ACB and ECB(Z) males where response characteristics of the three co-compartmentalized ORNs have been previously described (Hansson et al., 1987; Takanashi et al., 2006; Domingue et al., 2007a). In both parent populations, the pheromone-responsive ORNs have distinct spike amplitudes that facilitate the mapping of their ORN response profiles.

It became apparent from preliminary experiments that the ORNs of ACB × ECB(Z) F_1 hybrids do not have easily distinguished spike-amplitude differences. To map the response profiles of the three co-compartmentalized ORNs in the F_1 hybrids we performed paired-stimulation experiments using the five compounds of interest. For this number of compounds it was impractical to perform paired stimulations of each possible pair in either order. Thus, we performed a more limited array of paired stimulations, focusing primarily on determining which compounds within the ACB and ECB pheromone can cause adaptation that prevents ORN responses to the ECB components or the antagonist. ANOVA with the Tukey's adjustment for multiple paired comparisons was performed to compare spike frequency elicited by the second compound according to which initial compound was used. For statistical analyses total spike count was used, despite our ability to sometimes recognize that one compound was causing responses in two ORNs. This approach was used because the intent of the tests was to determine if one compound could prevent spike activity by another.

2.5. Quantifying relative spike amplitudes

After mapping the ORN response affinities, we also quantified the spike-amplitude differences among the ORNs. The F_1 hybrid paired-stimulation data, described above in the context of assessing differential adaptation, was also used to compare spike amplitudes. For ACB, paired-stimulation experiments had also been previously performed using identical laboratory conditions for E12-, Z12-, and Z9-14:OAc (Domingue et al., 2007a), and analyzed for relative spike amplitudes (Domingue et al., 2008). Additional paired stimulations were performed on ECB(Z) males, using E11-, Z11-, and Z9-14:OAc to allow relative comparison to the ACB and F_1 hybrid populations under the same experimental conditions.

Relative spike amplitudes were calculated using the peak-finder function in Labview as previously described (Domingue et al., 2008). For the F_1 hybrids, the paired-stimulation traces were separated according to which ORN was stimulated by each compound. We used a conservative approach to prevent incorrect classification of spikes. For example, because E12-14:OAc often stimulated two ORNs with similar spike amplitudes, we did not use any data from this compound in our analyses. In other cases clearly extraneous spikes were not considered. Within each population, an ANOVA was performed to test the significance of each comparison. Because the experiment had a nested design with multiple spikes being measured per sampling event, our ANOVA included the factors for *Spike-Size Category* and the *Sampling Event* (nested in *Spike-Size Category*). A Bonferoni correction was used to account for the multiple comparisons employed.

2.6. Dose–response relationships

We obtained dose–response curves for the F_1 hybrids and for the ECB(Z) population. The ECB(Z) population has never been characterized with respect to responsiveness to E12- and Z12-14:OAc in previous studies. Both F_1 hybrid reciprocal crosses and ECB(Z) males were analyzed at similar ages (2–7 days old). Our prior experiences with ACB and both ECB strains have shown that responses to the non-behaviorally active pheromone blend components are weak, and detectable only if presented before the behaviorally active compounds. Thus, for the ECB(Z) and the F_1 hybrids, ACB components were applied before the ECB components because there was already had preliminary behavioral and physiological data indicating that stronger affinity for the ECB pheromone.

For the first population analyzed, ECB(Z)♂ × ACB♀ F_1 hybrids, we used a dose series of 1, 10, and 100 μg. We began with the two ACB components in either order at 1 μg, followed by the 10 and 100 μg doses. When possible we continued stimulating the same sensillum with the ECB components using a similar alternating pattern with respect to E11-14:OAC and Z11-14:OAc at increasing doses. At the end we applied Z9-14:OAc at 100 μg. However, as is typical for both parental species (Domingue et al., 2007a,b), connections were occasionally lost before the protocol could be completed on a single sensillum. Because of the limited availability of F_1 hybrid moths, when contacts were lost after completing all doses for the ACB components, we contacted a new sensillum and began by using only the ECB components and Z9-14:OAc. If possible, we would begin the entire protocol again with the ACB components on another sensillum. The protocol was performed similarly for F_1 hybrids of the opposite ACB♂ × ECB(Z)♀ direction with the exception that a 10, 30, and 100 μg series was used, which was deemed more appropriate after the ECB(Z)♂ × ACB♀ data had been analyzed.

For the ECB(Z) population a similar protocol was used testing ACB components before the ECB components. Because the supply of insects was large, we were able to develop an optimal protocol where Z12- and E12-14:OAc were presented in a 10, 30, and 100 μg series and Z11- and E12-14:OAc in a 1, 10, and 100 μg series. Furthermore, because the moths were plentiful, sensilla were always sampled such that all four desired dose–response

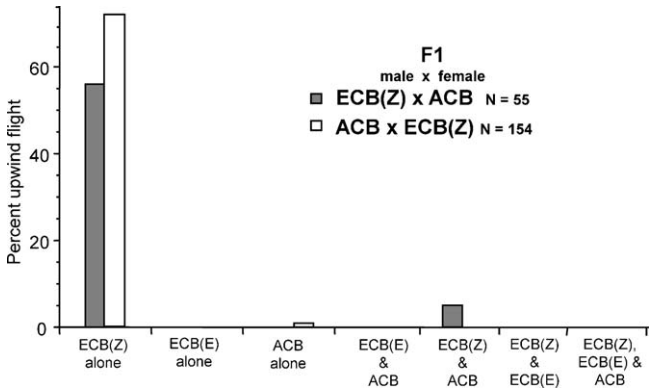

Fig. 2. Phenotypic distribution of F_1 hybrids with respect to whether they flew upwind to the ECB(E), ECB(Z), or ACB pheromone blends or to more than one blend. Percentages are with respect to the total number of males tested in either of the bidirectional crosses.

series were obtained. We did not sample more than one sensillum per antenna in males of this population.

2.7. Behavior–physiology comparisons

Comparisons of the ORN response data were made using the groupings of ACB♂ × ECB(Z)♀ F_1 hybrid males as responders to (1) ECB(Z) blend alone, (2) the ECB(Z) and ACB blends, or (3) none of the pheromone blends presented. The same electrophysiology sampling protocol was used as for the other ACB♂ × ECB(Z)♀ F_1 hybrid males.

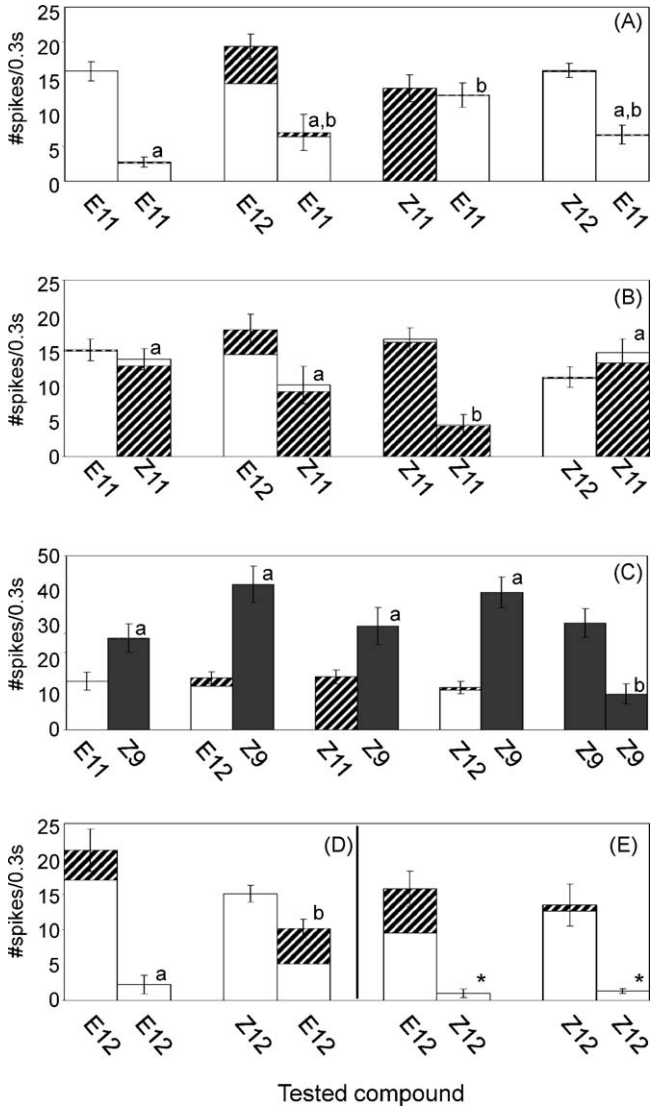

Fig. 3. Spike frequencies of ORN responses upon stimulation with pairs of compounds at a 300 ms interval. Sample sizes as indicated within each part of the figure from left to right performed are as follows: (A) N = 12, 8, 8, and 14; (B) N = 15, 14, 13, and 11; (C) N = 9, 10, 8, 12, and 10; (D) N = 4 and 12; (E) N = 9 and 6. Data are pooled from both sets of reciprocal hybrid crosses. Statistical analyses were performed using total spike count (mean ± SE indicated). Lowercase letters within parts A, B, C, and D indicate pairwise comparisons of the total spike frequencies of the ORNs to the second compound (Tukey's correction, $\alpha = 0.05$). After interpreting these statistical analyses, in conjunction with an analysis of spike-amplitude differences (Fig. 4), the spikes within each observation were assigned to different classes, which are presented by different shading patterns. The bars representing the most broadly tuned pheromone-responsive ORNs in the parent populations are shaded white. Those for the more narrowly tuned pheromone-responsive ORN are shaded with striped patterns. One ORN, shaded black, is always primarily responsive to the antagonist. *For the two observations in part E, the spike frequency of Z12-14:OAc when presented second is usually zero, precluding similar statistical comparisons.

3. Results

3.1. Behavioral assay

For males derived from either reciprocal cross, the most common behavioral outcome was flight to only the ECB(Z) pheromone (Fig. 2). Among the male offspring of the ACB♂ × ECB(Z)♀ cross, 63% flew to only to the ECB(Z) pheromone, while 56% of the offspring of ECB(Z)♂ × ACB♀ did. Only 5% of the ECB(Z)♂ × ACB♀ crosses flew to both the ECB(Z) and ACB pheromones. There was also a rare phenotype (2%) in the ACB♂ × ECB(Z)♀ cross that flew to the ACB pheromone alone. For both reciprocal crosses the remaining 35–39% of male offspring were behaviorally inactive.

3.2. Mapping ORN response characteristics

The ORN responses of F_1 hybrids showed that all of the pheromone components produced spikes with similar amplitudes (Fig. 1). In the case of E12-14:OAc, two overlapping spike trains

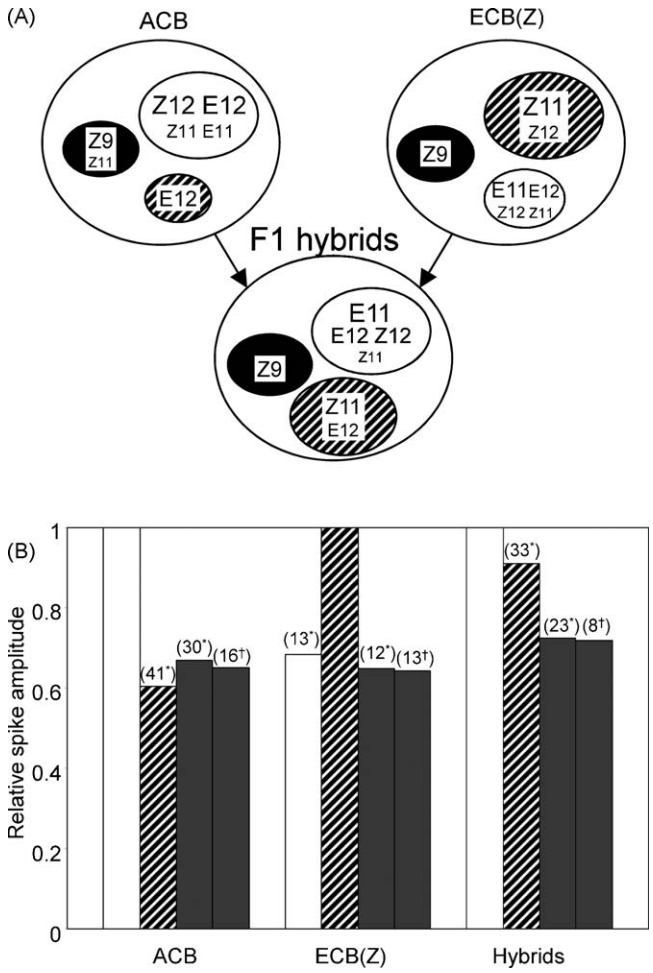

Fig. 4. Spike-amplitude patterns relative to the largest spike-size ORN for ACB, ECB(Z) and their F_1 hybrids, depicted (A) pictorially and (B) graphically. The smaller circles represent the dendrites of each ORN, with their diameters proportional to their relative spike amplitudes. The ligands causing a response by each ORN are abbreviated within these circles, with the font size being proportional to the spike frequency elicited by each ligand. The histograph is similarly shaded with different patterns to indicate the different ORNs, which are consistently used throughout the manuscript. *Sample sizes for comparisons of the smaller spike-amplitude ORNs to the largest spike-amplitude ORN within each population, which are all significant ($p < 0.001$, Bonferoni correction). †Sample sizes for comparisons based on the two smallest spike-amplitude ORNs, which are all not significant ($p > 0.05$, Bonferoni correction).

with similar amplitudes can be observed. Usually, as visible on the last line of Fig. 1, the ORN responding to Z9-14:OAc produced a spike with a noticeably smaller amplitude. An analysis of the paired stimulations (Fig. 3) allowed us to map which ligands stimulated which ORNs. None of the pheromone compounds when presented first caused a significant reduction in the ORN response to the antagonist, Z9-14:OAc (Fig. 3C). Thus, we presume that this ORN is stimulated only by the antagonist. The compound, E11-14:OAc, caused only one ORN to respond. There were no significant differences in spike counts of E11-14:OAc after either E11-, E12-, or Z12-14:OAc were presented (Fig. 3A). However, there was a significantly greater spike frequency to E11-14:OAc when presented after Z11-14:OAc. Likewise, the spike frequency of Z11-14:OAc responses was reduced after a previous puff of Z11-14:OAc but not by E11-, E12-, or Z12-14:OAc (Fig. 3B), indicating that the remaining ORN was primarily responsive to Z11-14:OAc. Thus, it can be inferred that E11-, E12-, or Z12-14:OAc stimulated the same ORN, whereas another was primarily stimulated by Z11-14:OAc.

As noted above, E12-14:OAc often exhibited two ORN responses, both similar in spike amplitude. Since one of these ORNs was commonly stimulated by E12-14:OAc, E11-14:OAc, and Z12-14:OAc, the identity of the other ORN targeted by E12-14:OAc needed to be determined. Presuming the constraints of three co-compartmentalized ORNs, either of the ORNs responsive primarily to Z11-14:OAc or Z9-14:OAc must be involved. The weaker ORN response to E12-14:OAc does not appear to be strong enough to adapt that neuron to prevent a full response to the primary ligand. Similarly, sometimes Z11-14:OAc, and even very rarely E11- or Z12-14:OAc, caused very low frequency responses on a second neuron. To resolve the identities of the ORNs causing these secondary responses it was also instructive to reconsider the spike amplitudes. In each of these cases the two ORNs stimulated by the single pheromone component had very similar spike amplitudes. Since the ORN responding to Z9-14:OAc is characterized by a noticeably smaller spike size, it was presumed that such responses were not on this ORN. Thus, the weaker secondary response to any pheromone component was assigned to the opposite pheromone-responsive ORN. This interpretation is further supported by the cross stimulation experiments involving E12-, and Z12-14:OAc. Repeated stimulations involving E12-14:OAc lead to a reduction in the total number of spikes, including the complete absence of a response by a second ORN (Fig. 3D). However, two ORNs responded to E12-14:OAc at similar frequencies if it was applied after Z12-14:OAc, indicating that Z12-14:OAc only caused adaptation of the ORN more strongly stimulated by E12-14:OAc. In turn both E12-, and Z12-14:OAc caused adaptation of the ORN stimulated by Z12-14:OAc (Fig. 3E).

3.3. Quantifying relative spike amplitudes

Both ACB and ECB(Z) are characterized by a large spike-size ORN that responds to pheromone components, and two other ORNs that are smaller and more similar to each other in spike amplitude (Fig. 4). One of the smaller ORNs is responsive primarily to pheromone components, and the other to the behavioral antagonist. In the F_1 hybrids, the two pheromone components caused spikes of similar size, with the smaller of the two being 94% the size of the other. The action potential of the ORN responding to the antagonist was 72% of the size of that created by the ORN with the largest spike size. Most of the spike-amplitude comparisons were significant with the exception of those marked in Fig. 4. In all such cases where the spike amplitudes were not significantly different, the comparisons were not highly replicated.

3.4. Dose–response relationships

ACB response to the ACB and ECB pheromone components of interest are depicted from a previous study (Fig. 5A). Males from ECB(Z) had high frequency responses to Z11-14:OAc on a large spike-amplitude ORN and to E11-14:OAc on a small spike-size ORN (Hansson et al., 1987) (Fig. 5B). The smaller spike ORN is also

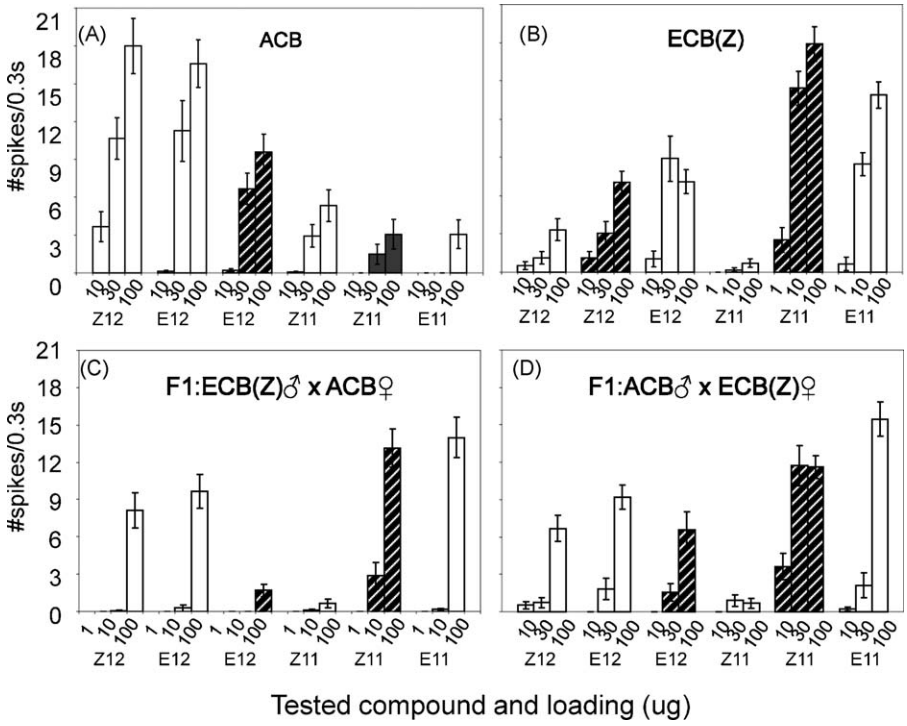

Fig. 5. ORN response (mean + SE) to increasing pipette loadings of Z12-14:OAc, E12-14:OAc, Z11-14: OAc, and E11-14:OAc for (A) ACB males ($N = 21$ for all compounds), (B) ECB Z-strain males ($N = 21$ for all compounds), (C) ECB(Z)♂ × ACB♀ derived F_1 hybrid males ($N = 22$ for E12-/Z12-14:OAc, $N = 20$ for E11-/Z11-14:OAc), and (D) ACB♂ × ECB(Z)♀ derived F_1 hybrid males ($N = 21$ for all compounds). The shading patterns used for the different ORN spike sizes are as outlined in Fig. 4.

responsive to all of the other ECB and ACB pheromone components at varying levels, most strongly to E12-14:OAc. Aside from the expected response to Z11-14:Ac, the large spike-size ORN responded to only Z12-14:OAc.

The F_1 hybrid males, as observed in the paired-stimulation experiments, showed one ORN primarily responsive to Z11-14:OAc, and another primarily responsive to E11-, E12-, and Z12-14:OAc (Fig. 5C and D). Despite the different scale used for the dose–response series, the responses were similar between the two populations resulting from the reciprocal crosses. However, for the ACB♂ × ECB(Z)♀ offspring (Fig. 5D) the secondary responses to E12-14:OAc, which is on the ORN that is highly responsive to Z11-14:OAc, were of greater spike frequency than observed in the reciprocal ECB(Z)♂ × ACB♀ cross (Fig. 5C).

3.5. Behavior–physiology comparisons

All of the groupings of ACB♂ × ECB(Z)♀ F_1 hybrid behavioral responses showed similar ORN response characteristics (Fig. 6). The most common behavioral group, consisting of moths responding to the ECB(Z) pheromone blend (Fig. 6A) had an ORN tuning profile nearly identical to the randomly sampled moths from the population (Fig. 5D). The remaining groups, which included those flying to both the ACB and ECB(Z) blends (Fig. 6B) and those with no flight to recorded pheromones also had similar ORN tuning properties. However, the physiological analysis was not highly replicated in these cases. There is perhaps lower sensitivity to the pheromones overall in the moths flying to ECB(Z) and ACB pheromones (Fig. 6C), but such a low level of replication precludes statistical analyses of any such differences.

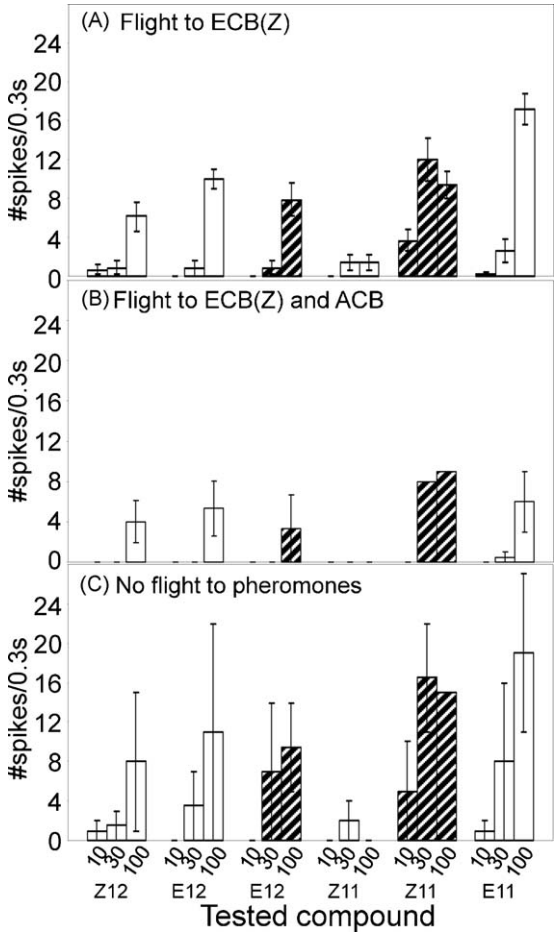

Fig. 6. ORN response (mean + SE) to increasing pipette loadings of Z12-14:OAc, E12-14:OAc, Z11-14:OAc, and E11-14:OAc for behaviorally characterized ACB♂ × ECB(Z)♀ males; (A) responding only to the ECB Z-strain blend ($N = 9$ for all compounds), (B) responding to the ACB and ECB Z-strain blends ($N = 3$ for E12- and Z12-14:OAc, $N = 2$ for E11- and Z11-14:OAc), and (C) not responding to ACB or ECB(Z) blends ($N = 2$ for all compounds). The shading patterns used for the different ORN spike sizes are as outlined in Fig. 4.

4. Discussion

With respect to ECB(Z) and ACB hybrids, flight to the ECB(Z) pheromone is a dominant trait. This dominance is stronger than that previously described in crosses between the ECB(E) and ACB strains (Domingue et al., 2008), which also favored flight to the ECB parental blend. While a similar percentage of the ACB × ECB(E) offspring flew to the ECB(E) pheromone as ACB × ECB(Z) offspring flew to the ECB(Z) blend, in the previous study 20% of ACB × ECB(E) offspring also flew to the ACB blend. The rare occurrences of ACB × ECB(Z) F_1 hybrids flying to the ACB blend were of a similar percentage to that found in the ECB(Z) parental population (Linn et al., 2003).

The male F_1 hybrid offspring of crosses between ECB(Z) and ACB were similar to ECB(E) × ECB(Z) F_1 hybrids (Hansson et al., 1987) in that spike sizes of the different pheromone-responsive ORNs could not be easily distinguished. Considering that there was no such dramatic change in relative spike-size amplitude relationships of ORNs of F_1 hybrids between ECB(E) and ACB (Domingue et al., 2008), the ECB(Z) males appear to have a distinct mode of ORN spike-amplitude patterning versus both ECB(E) and ACB (Fig. 7). It was previously shown in ECB that spike-amplitude patterns are related to dendrite diameter. While the trichoid sensilla of ECB(E) and ECB(Z) males have distinct diameters, the dendrites of the co-compartmentalized ORNs of F_1 hybrid males have similar diameters, which produce indistinguishable ORN spike amplitudes (Hansson et al., 1994). As in the ECB parental strains, the trichoid sensilla of ACB males also have ORN dendrites with distinct diameters (Takanashi et al., 2006). Thus, the F_1 hybrid males from crosses between ACB and ECB(Z), which have three co-compartmentalized ORNs with similar action potential amplitudes likely have dendrites with similar diameters.

Given the changes that would be required for the evolution of the olfactory differences observed among ECB(E), ECB(Z), and ACB, the peripheral olfactory system of ECB(E) might best reflect that of the most recent common ancestral population. ACB and ECB(E) F_1 hybrids showed a nearly perfect overlap of tuning profiles of the two parent species, with relative spike amplitudes changing very little in comparison to both parents. Thus, to allow a population with an olfactory system like ECB(E) to evolve to one like ACB, the only alteration that is required is that the tuning profiles of the ORNs shift from the Δ-11- to the Δ-12-tetradecenyl acetates. However, for a population with an olfactory system like ECB(Z) to evolve into one similar to ACB, in addition to the tuning of the ORNs shifting from the Δ-11- to the Δ-12-tetradecenyl acetates, a change would be required in how the relative action potential relationships are produced. It thus seems likely that the olfactory system of ECB(Z) is more recently derived.

Our inference that the ACB peripheral olfactory system is more similar to ECB(E) differs from of the pattern of homology observed in the biochemical pathways of pheromone production. The reductase system of ACB is more efficient at converting Z11-14-tetradecenoic acid to Z11-14:OAc than E11-14-tetradecenoic acid to E11-14:OAc (Zhao et al., 1995). Further supporting the similarities of ECB(Z) and ACB with respect to pheromone biochemistry, the reductase system of ECB(Z) is able to convert E12-14-tetradecenoic acid to E12-14:OAc, whereas ECB(E) could not convert either of the Δ-12-tetradecenoic acids to the Δ-12-tetradecenyl acetates (Zhu et al., 1996). This conflicting pattern of population differences when considering pheromone biosynthesis

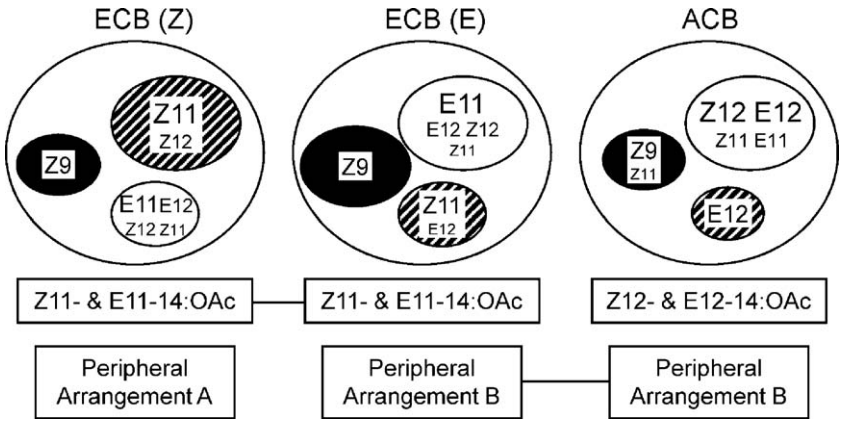

Fig. 7. Summary of ORN differences among males of the ECB pheromone races and the ACB. Each larger circle represents a cross-section of a sensillum. The smaller circles represent the dendrites of each ORN, with their diameters proportional to their relative spike amplitudes. The ligands causing a response by each ORN are abbreviated within these circles. The font size provides a general indication of the relative differences in spike frequency response elicited by each ligand. The ORNs from the different populations shaded similarly have overlapping response characteristic in inter-population hybrids (see also Hansson et al., 1987; Domingue et al., 2008).

versus olfactory perception, suggests that the ancestral species preceding ACB and ECB may have been different from any of the existing populations with respect to these characters.

Comparing the complete tuning profiles for each ORN to both the ACB and ECB pheromone components in ECB(Z) and ECB(E) (Fig. 7) provides further insight into the distinct olfactory systems of these races. Between the two strains, the glomerular targeting of the neurons is constant with respect to spike amplitude, but reversed with respect to Z11-14:OAc versus E11-14:OAc specificity (Kárpáti et al., 2008). Our results indicate that the reversed functional topology observed by Kárpáti et al. (2008) extends beyond the identity of the primary input for each ORN to also include the breadth of the tuning of these ORNs. In ECB(Z), the small spike-size ORN, which is most strongly responsive to E11-14:OAc, is broadly receptive to all the ECB and ACB pheromone components. Conversely, in ECB(E) the large spike-size ORN is similarly tuned to E11-14:OAc, while it is also capable of being stimulated by the other ECB and ACB pheromone components (Domingue et al., 2007b, 2008). The ORN responding best to Z11-14:OAc is more narrowly tuned, regardless of its relative spike amplitude across the two ECB strains. However, the tuning profiles of the ORNs primarily tuned to Z11- or E11-14:OAc are not identical in all respects in ECB(Z) versus ECB(E) (Fig. 7). Thus, other random or selective population processes appear to have affected the secondary tuning properties of these ORNs since the divergence of these strains.

Acknowledgments

We thank Kathy Poole for help in maintaining the ECB and ACB colonies. Andy Myrick of Penn State University assisted in providing technical support for the spike-amplitude calculations. The project was funded by NSF IBN #034340, to WLR and TCB.

References

Ando, T., Saito, O., Arai, K., Takahashi, N., 1980. (Z)- and (E)-12-tetradecenyl acetates: sex pheromone components of oriental corn borer (Lepidoptera: Pyralidae). Agricultural and Biological Chemistry 44, 2643–2649.

Boo, K.S., Park, J.W., 1998. Sex pheromone of the Asian corn borer moth, *Ostrinia furnacalis* (Guenee) (Lepidoptera: Pyralidae) in South Korea. Journal of Asia-Pacific Entomology 1, 77–84.

Dobzhansky, T., 1970. Genetics of the Evolutionary Process. Columbia University Press, New York.

Domingue, M.J., Linn Jr., C.E., Roelofs, W.L., Baker, T.C., 2006. Effects of egg-to-adult development time and adult age on olfactory neuron response to semiochemicals in European corn borers. Journal of Insect Physiology 52, 975–983.

Domingue, M.J., Musto, C.J., Linn Jr., C.E., Roelofs, W.L., Baker, T.C., 2007a. Evidence of olfactory antagonistic imposition as a facilitator of evolutionary shifts in pheromone blend usage in *Ostrinia* spp. (Lepidoptera: Crambidae). Journal of Insect Physiology 53, 488–496.

Domingue, M.J., Musto, C.J., Linn Jr., C.E., Roelofs, W.L., Baker, T.C., 2007b. Altered olfactory receptor neuron responsiveness in rare *Ostrinia nubilalis* males attracted to the *O. furnacalis* pheromone blend. Journal of Insect Physiology 53, 1063–1071.

Domingue, M.J., Musto, C.J., Linn Jr., C.E., Roelofs, W.L., Baker, T.C., 2008. Olfactory neuron responsiveness and pheromone blend preference in hybrids between *Ostrinia furnacalis* and *Ostrinia nubilalis* (Lepidoptera: Crambidae). Journal of Insect Physiology 54, 1261–1270.

Frolov, A.N., Bourguet, D., Ponsard, S., 2007. Reconsidering the taxonomy of several *Ostrinia* species in the light of reproductive isolation: a tale for Ernst Mayr. Biological Journal of the Linnean Society 91, 49–72.

Glover, T.J., Tang, X.H., Roelofs, W.L., 1987. Sex pheromone blend discrimination by male moths from E and Z strains of European corn borer. Journal of Chemical Ecology 13, 143–152.

Glover, T.J., Perez, N., Roelofs, W.L., 1989. Comparative analysis of sex pheromone-response to antagonists in three races of European corn borer. Journal of Chemical Ecology 15, 863–873.

Hallberg, E., Hansson, B.S., Steinbrecht, R.A., 1994. Morphological characteristics of antennal sensilla in the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Tissue and Cell 26, 489–502.

Hansson, B.S., Löfstedt, C., Roelofs, W.L., 1987. Inheritance of olfactory response to sex pheromone components in *Ostrinia nubilalis*. Naturwissenschaften 74, 497–499.

Hansson, B.S., Hallberg, E., Löfstedt, C., Steinbrecht, R.A., 1994. Correlation between dendrite diameter and action potential amplitude in sex pheromone specific receptor neurons in male *Ostrinia nubilalis*. Tissue Cell 26, 503–512.

Ishikawa, Y., Takanashi, T., Kim, C.-G., Hoshizaki, S., Tatsuki, S., Huang, Y., 1999. *Ostrinia* spp. in Japan: their host plants and sex pheromones. Entomologia Experimentalis et Applicata 91, 237–244.

Kaissling, K.-E., 1974. Sensory transduction in insect olfactory receptors. In: Jaenicke, L. (Ed.), Biochemistry of Sensory Functions. Springer, Berlin, pp. 243–273.

Kárpáti, Z., Dekker, T., Hansson, B.S., 2008. Reversed functional topology in the antennal lobe of the male European corn borer. Journal of Experimental Biology 211, 2841–2848.

Kim, C., 1997. Molecular phylogenetic studies on evolution and speciation in the Asian corn borer and its allied species. Ph.D. Dissertation. Tokyo University (in Japanese).

Klun, J.A., Bierl Leonhardt, B.A., Schwarz, M., Litsinger, J.A., Barrion, A.T., Chiang, H.C., Jiang, Z., 1980. Sex pheromone of the Asian corn borer moth *Ostrinia furnacalis*. Life Sciences 27, 1603–1606.

Kochansky, J., Cardé, R.T., Liebherr, J., Roelofs, W.L., 1975. Sex pheromones of the European corn borer in New York. Journal of Chemical Ecology 1, 225–231.

Linn Jr., C.E., Young, M.S., Gendle, M., Glover, T.J., Roelofs, W.L., 1997. Sex pheromone blend discrimination in two races and hybrids of the European corn borer moth, *Ostrinia nubilalis*, 1997. Physiological Entomology 22, 212–223.

Linn Jr., C.E., O'Connor, M., Roelofs, W.L., 2003. Silent genes and rare males: a fresh look at pheromone blend response specificity in the European corn borer moth, *Ostrinia nubilalis*. Journal of Insect Science 3, 15 available online: insectscience.org/3.15.

Linn Jr., C.E., Musto, C.J., Roelofs, W.L., 2007. More rare males in *Ostrinia*: response of Asian corn borer moths to the sex pheromone of the European corn borer. Journal of Chemical Ecology 33, 199–212.

Mutuura, A., Munroe, E., 1970. Taxonomy and distribution of the European corn borer and allied species: genus *Ostrinia* (Lepidoptera: Pyralidae). Memoirs of the Entomological Society of Canada 71, 1–112.

Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge, Massachusetts.

Marshall, D.C., Slon, K., Cooley, J.R., Hill, K.B.R., Simon, C., 2008. Steady Plio-Pleistocene diversification and a 2-million-year sympatry threshold in a New Zealand cicada radiation. Molecular Phylogenetics and Evolution 48, 1054–1066.

Roelofs, W.L., Du, J.-W., Tang, X.-H., Robbins, P.S., Eckenrode, C.J., 1985. Three European corn borer populations in New York based on sex pheromones and voltinism. Journal of Chemical Ecology 11, 829–836.

Roelofs, W.L., Glover, T., Tang, X.H., Sreng, I., Robbins, P., Eckenrode, C.J., Löfstedt, C., Hansson, B.S., Bengston, B.O., 1987. Sex pheromone production and perception in European corn borer moths is determined by both autosomal and sex-linked genes. Proceedings of the National Academy of Sciences USA 84, 7585–7589.

Roelofs, W.L., Liu, W.T., Hao, G.X., Jiao, H.M., Rooney, A.P., Linn, C.E., 2002. Evolution of moth sex pheromones via ancestral genes. Proceedings of the National Academy of Sciences USA 99, 13621–13626.

Rundle, H.D., Nosil, P., 2005. Ecological speciation. Ecology Letters 8, 336–352.

Seehausen, O., Terai, Y., Magalhaes, I.S., Carleton, K.L., Mrosso, H.D.J., Miyagi, R., Van Der Sluijs, I., Schneider, M.V., Maan, M.E., Tachida, H., Imai, H., Okada, N., 2008. Speciation through sensory drive in cichlid fish. Nature 455, 620–626.

Smadja, C., Butlin, R.K., 2009. On the scent of speciation: the chemosensory system and its role in premating isolation. Heredity 102, 77–97.

Sueur, J., Vanderpool, D., Simon, C., Ouvrard, D., Bourgoin, T., 2007. Molecular phylogeny of the genus *Tibicina* (Hemiptera, Cicadidae): rapid radiation and acoustic behaviour. Biological Journal of the Linnean Society 91, 611–626.

Takanashi, T., Ishikawa, Y., Anderson, P., Huang, Y., Löfstedt, C., Tatsuki, S., Hansson, B.S., 2006. Unusual response characteristics of pheromone-specific olfactory receptor neurons in the Asian corn borer moth *Ostrinia furnacalis*. Journal of Experimental Biology 209, 4946–4956.

van der Pers, J.N.C., den Otter, C.J., 1978. Single cell responses from olfactory receptors of small ermine moths to sex-attractants. Journal of Insect Physiology 24, 337–343.

Zhu, J.W., Zhao, C.H., Lu, F., Bengtsson, M., Löfstedt, C., 1996. Reductase specificity and the ratio regulation of E/Z isomers in pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Insect Biochemistry and Molecular Biology 26, 171–176.

Zhao, C.H., Lu, F., Bengtsson, M., Löfstedt, C., 1995. Substrate specificity of acetyltransferase and reductase enzyme systems used in pheromone biosynthesis by Asian corn borer, *Ostrinia furnacalis*. Journal of Chemical Ecology 21, 1495–1510.