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Journey in the *Ostrinia* World: From Pest to Model in Chemical Ecology

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Abstract The European corn borer *Ostrinia nubilalis* (ECB; Lepidoptera: Crambidae) is a widely recognized pest of agricultural significance over much of the northern hemisphere. Because of the potential value of pheromone-based control, there has been considerable effort devoted to elucidation of the ECB chemical ecology. The species is polymorphic regarding its female-produced pheromone. Partly because of this feature, over the years the ECB has become a model to study pheromone evolution. This review should assist in identifying new areas of pheromone research by providing an overview of the literature produced on this subject for the ECB since the late 1960's.

Key Words *Ostrinia nubilalis* · Moth · Pheromone · Polymorphism · Biosynthesis · Olfaction · Behavior · Genetics

Introduction

The European corn borer *Ostrinia nubilalis* (Hübner) (ECB; Lepidoptera: Crambidae) has long been recognized as an economically important insect pest. The earliest records of the economic significance of this species in Europe date back to 1835 (Caffrey and Worthley, 1927). The ECB attacks a variety of cereals and other crops in Europe, North Africa, and North America (Fig. 1) where it was accidentally introduced with shipments of broomcorn (*Sorghum vulgare*) imported from Central Europe and Italy between 1909 and 1914 (Caffrey and Worthley, 1927). Nowadays, the pest is distributed throughout nearly all the major corn producing regions of North America east of the Rocky Mountains (Klun and Cooperators, 1975). In Canada and the U. S., losses resulting from ECB damage and control costs exceed $1 billion annually (www.ent.iastate.edu/pest/cornborer).

Because of the economic importance of the ECB, there has been considerable effort devoted toward the elucidation of its chemical ecology, which has been envisioned as a source of new control methods. At the same time, efforts made to understand ECB chemical communication have resulted in the accumulation of a tremendous amount of information, elevating this pest to the level a model organism in the field of chemical ecology, especially regarding how elements of the signaling system are inherited and how new communication systems evolve.

Here, I provide a comprehensive review on the status of our current knowledge concerning the ECB pheromone communication.

Female Sex Pheromone: Identification

Pheromones are defined as "substances secreted to the outside of an individual and detected by other individuals of the same species in which they elicit a definite behavior or physiological change" (Karlson and Lüscher, 1959). In most species of moths, females typically emit pheromones that attract conspecific males at long range (Wyatt, 2003). In addition, when males of *O. nubilalis* detect pheromone in the vicinity of a female, they respond by taking part in a characteristic precopulatory dance, with the wings extended up and vibrating, the genitalia expanded, followed by a

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Fig. 1 Approximate distribution of *Ostrinia nubilalis*. The European corn borer has been observed in most Eurasia as well as North Africa and North America. Because of a certain degree of confusion concerning the taxonomic status of *O. scapulalis* and *O. nubilalis* (Frolov et al., 2007) the Eastern limit of the distribution may be underestimated

clasper response (Klun, 1968). This observation provided a reliable bioassay for the presence of pheromone in extracts of female corn borers. The sex pheromone produced by female ECB was extracted for the first time in 1968 from 10,000 females (Klun, 1968). The active sex pheromone was isolated as a single component that could elicit male response. The male sex stimulant was identified as (*Z*)-11-tetradecenyl acetate (Z11-14:OAc) (Klun and Brindley, 1970), a compound that since has been found to be part of the pheromone blend of more than 300 moth species (El-Sayed, 2010). Synthetic Z11-14:OAc was shown to possess biological properties identical to the natural pheromone in a close-range bioassay (Klun and Brindley, 1970). In the first field trials, the presence of the geometrical isomer (*E*)-11-tetradecenyl acetate (E11-14:OAc) was found to have a negative impact on trap catches (Klun and Robinson, 1971). However, contradictory results were reported from other field sites where males were specifically attracted to E11-14:OAc, Z11-14:OAc attracting comparatively few males in these locations (Roelofs et al., 1972). The hypothesis advanced then was that the distinct catches would correspond to different strains of corn borer that employed a different pheromone system (Roelofs et al., 1972). The pheromone polymorphism of the ECB had just been discovered.

When geometrically pure isomers could be synthesized and made available for field assays, previous conclusions regarding ECB pheromone communication could be revisited and modified. Male ECB were, indeed, weakly attracted to pure Z11-14:OAc (Klun et al., 1973). The addition of a small fraction of pure E11-14:OAc, however, dramatically increased trap captures, and the requirement of the E isomer for optimum attraction became obvious from field tests (Klun et al., 1973). Ultimately, the composition of female abdominal tips was scrutinized to identify pheromone components of the two behavioral strains, and females were found to produce either 97:3 Z/E11-14:OAc or 1:99 Z/E11-14:OAc (Kochansky et al., 1975). Tetradecyl acetate (14:OAc) also was found in gland extracts but had no effect on attraction (Kochansky et al., 1975). Similarly, trace amounts of (*Z*)-11-hexadecenyl acetate (Z11-16:OAc) were detected in extracts from E-strain females, but this compound was without noticeable behavioral effects (Peña et al., 1988).

Reproductive Isolation Between the Strains

It was shown through analyses of the sex pheromone of female moths that two strains of corn borer exist. The occurrence in nature of Z and E pheromonal phenotypes of the ECB was established through an extensive survey conducted in the mid-1970's. In this survey, males at different geographic sites were found to respond to opposite isomeric blends of 11-14:OAc (Klun and Cooperators, 1975). Studies also revealed that the most prevalent ECB pheromonal phenotype in nature is the 97:3 Z/E11-14:OAc; the E strain having a more restricted distribution in Europe and North America probably because it may lack certain adaptive features possessed by Z strain individuals (Klun and Cooperators, 1975; Anglade and Stockel, 1984). This would explain the limited part of North America that was effectively colonized by the E strain, essentially close to the sites of introduction. Apart from the pheromone, there seem

to be no fixed differences between types, nor are there any morphological characters that permit differentiation.

In regions where sympatric populations of the Z and E strains were detected, males responding to a 1:1 blend occasionally were captured, and it was suggested that these males could be hybrid individuals produced from crosses between the two pheromonal types (Klun and Cooperators, 1975; Cardé et al., 1978). An alternative explanation may lie in a broader window of response in certain populations caused by genetic variability within these populations; these males would be a mixture of individuals that respond predominantly to Z or E blend (Klun and Cooperators, 1975; Cardé et al., 1978). Nevertheless, viable hybrids can be obtained under laboratory conditions (Liebherr and Roelofs, 1975), and interbreeding of opposite pheromonal types is not unlikely because aggregation of males and females of both strains occurs in similar grassy habitats (Showers et al., 1976; Dalecky et al., 2006). The inter-strain crosses, when successfully completed, give progeny showing heterosis, with the portion of individuals surviving each life stage being significantly higher (Liebherr and Roelofs, 1975). Similarly, F2 sibling crosses show 100% viable matings, and females do not exhibit a reduced fecundity (Liebherr and Roelofs, 1975). However, the hybrid crosses that occur under confined conditions are much less frequent than intra-strain crosses, and laboratory choice tests suggest that a degree of premating reproductive isolation exists between the two strains (Liebherr and Roelofs, 1975). A plausible explanation would be that a large proportion of the males are inhibited from attempting mating by the detection of the incorrect sex pheromone isomer ratio (Liebherr and Roelofs, 1975). Since an increase in the amount of the respective minor component eliminates male attraction, it was hypothesized that this presumably would cause reproductive isolation in the field and, therefore, support separate species status for the two strains (Kochansky et al., 1975).

Because of the relative rarity of inter-strain matings under laboratory conditions, it might be expected that such matings would be even rarer in nature. In addition, the few hybrids produced may not be able to mate at all (Liebherr and Roelofs, 1975). Analysis of the Δ11-tetradecenyl acetate isomeric composition of wild female moths indicated that sympatric pheromonal types mate assortatively with only a low level of hybridization detected (Klun and Maini, 1979; Roelofs et al., 1985; Klun and Huettel, 1988). Evidence that the Z and E pheromone strains of *O. nubilalis* are not freely interbreeding in areas of coexistence first came from allozyme data (Cardé et al., 1978). Although small, the genetic divergence detected between the Z and E strains is suggestive of ongoing speciation, and justifies that the strains could be considered as semispecies (populations that have acquired some attributes of species rank for which the speciation process is partially complete) or sibling species (Cardé et al., 1978). This was confirmed by a subsequent study that involved 30 loci corresponding to regulatory and non-regulatory enzymes in which the values of genetic distance obtained were indicative of taxa at the beginning of divergence (Cianchi et al., 1980). Areas in North America are secondary contact zones of recent origin where introgression could occur, though at a low level (Cardé et al., 1978).

The behavioral response of hybrid males was characterized to define precisely the response profile of hybrid males, which is crucial to understanding the interaction between sympatric Z and E individuals. Glover et al. (1991) reported that many hybrid males from reciprocal crosses failed to complete the behavioral sequence to locate a female, regardless of the blend they were exposed to. In addition, the few males that responded did not exhibit a preference for any particular blend but, rather, were attracted to a broad range of blends (Glover et al., 1991). Given that most hybrid males do not respond to any blend, and that the remaining ones are not tuned to any particular blend, F1 hybridization should be seen as a transient stage with a limited possibility of creating recurrent gene flow between the strains. However, the actual fate of hybrid individuals in the field remains obscure.

Pheromone Biosynthesis and Its Regulation

The pheromone components, E11-14:OAc and Z11-14:OAc, are produced through the fatty acid cycle. Palmitic acid is shortened to a 14-carbon intermediate through one cycle of β-oxidation. Desaturation then is accomplished by a Δ11-desaturase to give the (*E*)- and (*Z*)-11-tetradecenoyl precursors, which are subsequently reduced and acetylated to form the pheromone compounds (Roelofs et al., 1987) (Fig. 2). In both strains, the Δ11-desaturase produces the same ratio of the (*E*)- and (*Z*)-11-tetradecenoyl intermediates (Roelofs et al., 1987; Wolf and Roelofs, 1987). The enzyme has been characterized and expressed in a heterologous system, thus confirming its capacity to produce both geometric isomers of Δ11-tetradecenoyl from myristic acid (Roelofs et al., 2002). *In vivo* experiments that involve the use of labeled precursors have helped to determine that substrate selectivity occurs during the reduction step (formation of fatty alcohol from the acyl precursors); no selectivity of the acetylation step was noticed (Jurenka and Roelofs, 1989; Zhu et al., 1996b).

As in other moth species, the presence of lipid droplets in the pheromone gland cells has been reported in the ECB (Ma and Roelofs, 2002). These lipid droplets consist of triacylglycerols, the main pool of non-membrane lipids (Ma and Roelofs, 2002). Fatty acyl pheromone analogues, (*Z*)-

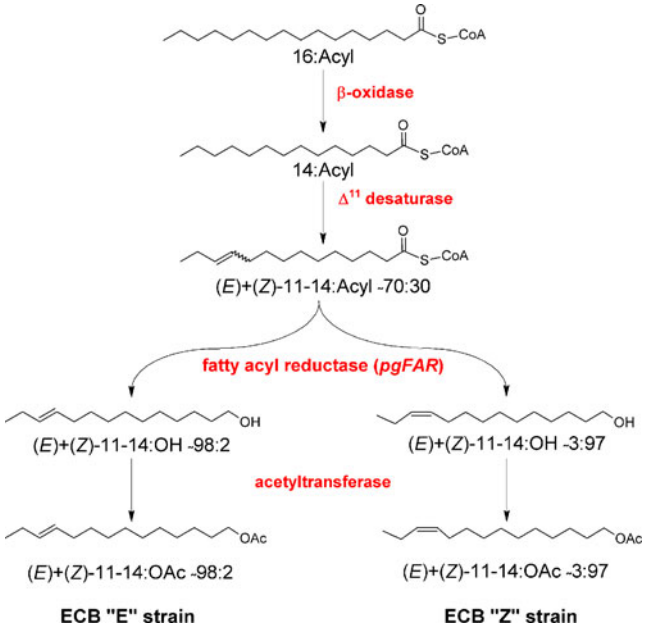

Fig. 2 Biosynthetic pathway towards the female *Ostrinia nubilalis* pheromone. *De novo* biosynthesis of (*Z*)- and (*E*)-11-tetradecenyl acetates starts from palmitoyl-CoA. One cycle of β-oxidation generates myristoyl-CoA, which is a substrate for a fatty acyl Δ11 desaturase. The (*E*)- and (*Z*)-11-tetradecenoyl moieties produced are converted into the corresponding alcohols by a fatty acyl reductase *pgFAR*. Finally, an acetyltransferase catalyzes the conversion of the fatty alcohol precursors into acetates

and (*E*)-11-tetradecenoate, are found predominantly in the triacylglycerols (Foster, 2004). The physiological role of these lipid deposits in the sex pheromone biosynthesis is two-fold. First, they seem to function as a sink for excess fatty acyl precursors, especially the direct precursor of the minor component (Foster, 2004). Second, they may be a source for the biosynthesis of pheromone, especially of tetradecanoate (14:Acyl), precluding the necessity for *de novo* biosynthesis of this acid during the period of pheromone production (Foster, 2004).

The sex pheromone production in female corn borers exhibits a cyclic pattern throughout the photoperiod, with a peak during the scotophase, and a valley with no pheromone during the photophase (Foster, 2004). The pattern changes slightly as females age, with the peak observed towards the end of the scotophase in young females, an apparent constant titer during the scotophase in 2 to 3-day-old females, and a peak in the early scotophase in older females (Foster, 2004; Kárpáti et al., 2007). This pattern apparently correlates with the mating activity as observed under laboratory conditions (Kárpáti et al., 2007). Pheromone biosynthesis is regulated by the neuropeptide pheromone biosynthesis activating neuropeptide (PBAN) (Raina and Klun, 1984; Raina et al., 1989; Ma and Roelofs, 1995b). PBAN is produced in three discrete sets of neurosecretory cells in the subesophageal ganglion of adult ECB females (Ma and Roelofs, 1995a, c). The subesophageal ganglion establishes a physical connection between the brain and the ventral nervous system (Ma and Roelofs, 1995a), and it had been suggested that, in some species, PBAN could be transported via the ventral nerve cord to its target site (Teal et al., 1989). However, surgical section of the ventral nerve cord in ECB females does not lead to a decrease in pheromone production, nor does a complete removal of the nerve cord. This suggests that a physical connection between the site of PBAN production and its target, the pheromone gland, is not required (Ma and Roelofs 1995c). Hence, no neuronal elements connect the ventral nerve cord to the sex pheromone gland in *O. nubilalis* (Ma and Roelofs 1995c). The fact that PBAN can be delivered by injection in the abdomen and restore pheromone production in decapitated females or stimulate pheromone production in isolated pheromone glands supports the idea that PBAN is released into the hemolymph, and that it acts directly on the pheromone gland to trigger the production of sex pheromone (Ma and Roelofs, 1995b). The corpora cardiaca have been implicated in the control of pheromone biosynthesis (Ma and Roelofs 1995c) but a precise mechanism has not been determined.

PBAN interacts with a G protein-coupled receptor (Choi et al., 2003) and promotes the opening of ion channels leading to an influx of extracellular calcium ion into the ECB pheromone gland cells (Ma and Roelofs, 1995b). The increase of cytosolic calcium leads to the activation of secondary messengers such as cAMP that in turn activate kinases and/or phosphatases, which stimulate the pheromone biosynthesis pathway (Rafaeli, 2009). From investigations conducted in different moth species, it has been shown that various enzymatic steps are under the influence of PBAN, from the control of fatty acid synthesis to the metabolism of fatty acid precursors (Tillman et al., 1999). In *O. nubilalis*, PBAN affects pheromone biosynthesis during the reduction step, that is the conversion of (*Z*)- and (*E*)-11-tetadecenoyl precursors into the corresponding alcohols (Eltahlawy et al., 2007).

Genetic Basis of Pheromone Polymorphism

The finding that viable hybrids could be obtained when crossing the two pheromone strains has made possible a full series of investigations aimed at unraveling the genetic basis of polymorphism in the corn borer communication system, notably the genetics that regulates the geometric composition of the sex pheromone.

Hybrid females from reciprocal crosses have an isomeric complement that approximates 65:35 E/Z, which is intermediate from that of either of the parent strains (Klun and Maini, 1979). The difference in female pheromone production is

controlled primarily by one autosomal locus with two alleles under Mendelian inheritance (Klun and Maini, 1979; Roelofs et al., 1987). Recently, a genetic map based on crosses between the two strains confirmed that the pheromone production trait is encoded by a single autosomal locus (Dopman et al., 2004).

The E/Z ratio produced by females from two successive backcrosses is variable to a higher degree than predicted (Löfstedt et al., 1989). This additional variation in the blend of pheromone components could not be explained directly under the basic "1-locus-2-alleles" model and it was, therefore, considered that independently segregating modifier genes that affect the exact ratio produced by heterozygous females could be present in some populations (Löfstedt et al., 1989; Zhu et al., 1996a). It has been proposed that the basic model can be extended based on the idea that there are two variant alleles in the Z strain for the major pheromone production locus, while the E strain carries one allele at this locus (Zhu et al., 1996a). The genetic variation noticed in the Z strain could result from another locus than the major production locus itself, although the data available so far suggest that, if it exists, this locus would be in linkage disequilibrium with the major production locus (Zhu et al., 1996a).

The autosomal gene in question has been proposed to encode for a factor that affects the specificity of the final reduction step, because a specific change in this critical step would produce various pheromone blends of acetate components (Roelofs et al., 1987; Zhu et al., 1996a, b). Indeed, the last reduction step is certainly decisive for the determination of the final pheromone ratio since both parental strains, and F1 females possess a similar ratio of geometric isomers of the unsaturated 14-carbon intermediates (Roelofs et al., 1987). *In vivo* labeling experiments were used to demonstrate that the reductase system differs between the two strains (Zhu et al., 1996b). Recent molecular investigations have confirmed the existence of two fatty acyl reductase alleles with strain-specific substrate specificities (Lassance et al., 2010). A genetic mapping approach was used to confirm the link between the genotype at the fatty acyl reductase *pgFAR* locus and the phenotype for pheromone production (Lassance et al., 2010). The variation unraveled by Zhu et al. (1996a) can be interpreted as the consequence of polymorphism existing within each strain at the *pgFAR* locus. Future experiments should aim at determining the level of variation existing for the *pgFAR* gene in natural populations of ECB.

Location of Production Site

In the adult female *O. nubilalis*, nine abdominal segments can be distinguished; seven of them are covered with scales, whereas the remaining terminal segments form the ovipositor that normally remains telescoped within the preceding segments (Ma and Roelofs, 2002). When describing the isolation of the sex pheromone, Klun (1968) reported that it could be isolated from the fused 9th and 10th abdominal segments. Furthermore, the sex pheromone was localized on the surface of the ovipositor, which is characteristic of many moth species (Klun and Maini, 1979). Details on the ECB sex pheromone gland ultrastructure revealed that the gland is formed by a single layer of hypertrophied epidermal cells that are located in the dorsal fraction of the intersegmental membrane between the 8th and 9th/10th segments, thus forming a half-ring gland (Ma and Roelofs, 2002).

The cells that form the gland have some characteristic features such as the presence of numerous mitochondria, the presence of lipid droplets both in the cells and the overlying cuticle, apical plasma membrane foldings, and smooth endoplasmic reticulum (Ma and Roelofs, 2002). Gas chromatographic analysis has confirmed the presence of both the pheromone components and their immediate fatty acyl precursors in the dorsal intersegmental membrane (Ma and Roelofs, 2002). *In situ* hybridizations using the Δ11-desaturase as probe were performed in the sister species *O. scapulalis*, and revealed that the enzyme producing the fatty acyl precursors is expressed only in the dorsal part of the terminal abdominal segments. The molecular observations thus corroborate the biochemical results (Fukuzawa et al., 2006). Similar results are to be expected for *O. nubilalis.*

Male Behavioral Response to the Pheromone

In nature, the females release sex pheromone that attracts potential mates from a distance. The detection of pheromone by males results in a well-defined stereotypic behavioral sequence composed of successive steps: orientation towards the pheromone source, upwind flight, and location of the odor source (Glover et al., 1987). In the close vicinity of the pheromone source, males of *O. nubilalis* respond by taking part in a characteristic precopulatory courtship display (Klun, 1968).

As mentioned earlier, the identification of the sex pheromone was tightly linked to the understanding of the associated male behavioral response. Precopulatory behavior was used in a close-range bioassay to demonstrate the activity of Z11-14:OAc in the Z strain (Klun, 1968). This behavioral response could be elicited in the complete absence of the minor component of the pheromone (Webster and Cardé, 1984), suggesting that this behavior does not require a complete pheromone blend to be elicited or that the close-range bioassay used did not allow for a

determination of the behavioral function of the minor component. However, the results of both field-trapping and flight tunnel studies have shown the importance of the natural pheromone blend compared to the use of the single major component for orientation, upwind flight, and source location (Klun et al., 1973; Klun and Cooperators, 1975; Glover et al., 1987). Indeed, when males were flown to a series of pheromone sources that varied in the percentage of the minor isomer, very few insects completed the sequence of behaviors when none of the minor component was applied (Glover et al., 1987; Linn et al., 1997). The flight tunnel assay is very discriminating because males must fly upwind to the chemical source for a reasonable distance (1 m to 2 m in general), and show close-range behaviors that include landing close to the source and displaying. This type of bioassay established that the males show limited to no attraction to the blend of the opposite strain (Glover et al., 1987, 1990) (Fig. 3).

In a close-range bioassay, Z, E, and F1 hybrid males responded more intensively to the isomeric blend that corresponds to sibling females (Klun and Maini, 1979). However, in each male type, some males also responded to other isomeric combinations, with E males being the least specific in their response preferences (Klun and Maini, 1979). Among field-captured individuals, some males showed a broad response window, and exhibited a response to isomer ratios well outside that produced by their respective females (Klun and Huettel, 1988). The behavioral response of males was evaluated in wind tunnel assays, and revealed the ability of males to discriminate blend quality during flight. Typically, ECB males display increased specificity with successive steps in the flight sequence (Linn et al., 1997). Males of the two strains, as well as their hybrids, exhibited different levels of specificity for ratios: Z males were quite discriminating with fewer insects performing a full behavioral sequence when exposed to blends other than the Z blend (Roelofs et al., 1987; Glover et al., 1990; Linn et al., 1997). On the other hand, E males had a broader window of response, with some rare males even being attracted to the blend produced by Z females, which may indicate that E males are less canalized in their behavioral response compared to Z males (Roelofs et al., 1987; Glover et al., 1990; Linn et al., 1997) (Fig. 3). The variability reported for some males may help explain how individuals belonging to different pheromonal types can closely interact, thus leading to interbreeding in the field. As a rule of thumb, the peaks of male response (corresponding to source contact in the flight tunnel) are centered on the natural female-produced ratio (Linn et al., 1997). Hybrid males exhibit high levels of upwind flight and source contact to a wide range of E:Z ratios (Fig. 4) indicating that, unlike their parents, they are not tuned to a specific ratio; they are, however, characterized by a relative lack of response to any dose of the 99:1 ratio typically attractive to E males (Roelofs et al., 1987; Linn et al., 1997). Interestingly, an appreciable number of hybrid males (10%) can exhibit source contact with sources mimicking a 98:2 ratio (Linn et al., 1997).

The survey conducted by Klun and his collaborators in the 1970's indicates that the sex-attraction response specificities of populations of males vary somewhat from generation to generation and from one geographic location to another (Klun and Cooperators, 1975). In addition to the established pheromone polymorphism, populations may differ in their voltinism, the number of generation per year, with univoltine and bivoltine populations having been reported (Roelofs et al., 1985). In the U. S., Z strain populations can be either uni- or bivoltine, whereas the E strain is bivoltine (Roelofs et al., 1985). The two Z populations have different sensitivities for responding to the ratio of the minor component, with bivoltine Z borers requiring less E isomer to complete the behavioral sequence (Glover et al., 1987). The temporal and spatial heterogeneity of male responses can be seen as an expression of intraspecific genetic variability within the populations and strains. However, because of the somewhat broad window of response exhibited by males, pheromone traps cannot be used to accurately estimate hybridization level or assign the

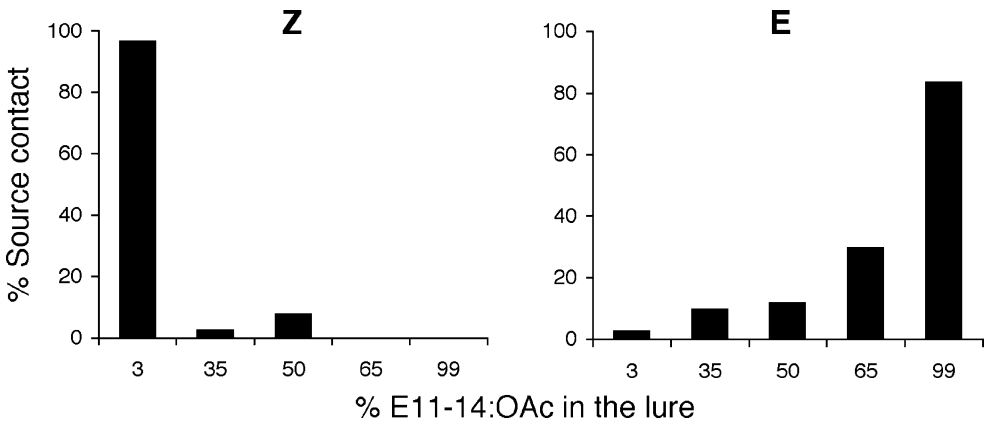

Fig. 3 Flight tunnel responses for pure *Ostrina nubilalis* pheromone races exposed to different blends of Δ11-14:OAc. Profiles represent the percentage of males that flew upwind and touched the pheromone source (adapted from Linn et al., 1997)

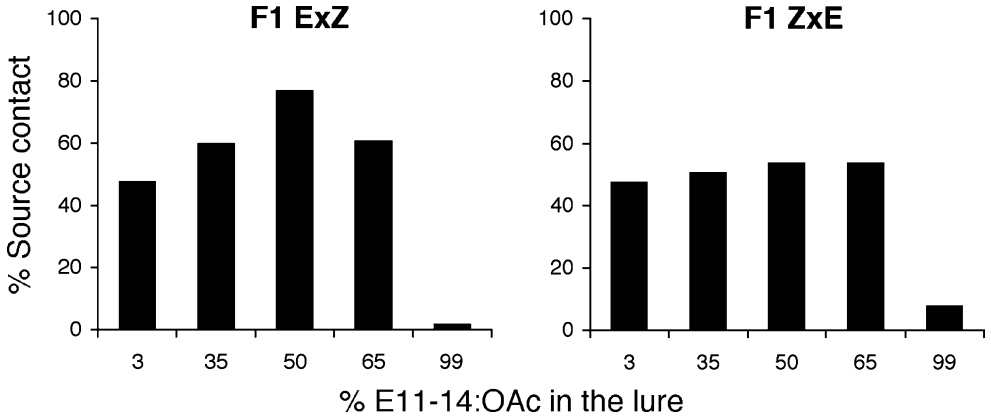

Fig. 4 Flight tunnel responses for male hybrids of *Ostrina nubilalis* pheromone races from reciprocal crosses exposed to different blends of Δ11-14:OAc. Profiles represent the percentage of males that flew upwind and touched the pheromone source (adapted from Linn et al., 1997)

pheromone-response genotype of captured males (Glover et al., 1990), and future surveys should include the use of appropriate molecular markers.

Behavioral Antagonists

Some compounds, when added to the sex pheromone blend can, dramatically affect its attractiveness. Such a molecule is said to be an antagonist. Here, I will not consider responses to chemical analogues of the sex pheromones, although studies devoted to explore this issue have been conducted; only naturally occurring compounds produced by species more or less related to the ECB are considered.

The isomer of the ECB pheromone components (*Z*)-9-tetradecenyl acetate (Z9-14:OAc) is the most studied ECB behavioral antagonist. This compound is part of the pheromone used by two other extant species from the genus, namely *O. zealis* and *O. zaguliaevi* (Huang et al., 1998; Ishikawa et al., 1999a, b). Indeed, the ECB most likely evolved from an ancestral species that used a blend of Z9-14:OAc and Z/E11-14:OAc as pheromone components (Ishikawa et al., 1999b). Early data showed clearly that the addition of Z9-14:OAc reduced the attraction of ECB males to their sex pheromone blend (Klun and Robinson, 1972; Struble et al., 1987; Glover et al., 1989). Electrophysiological recordings suggested that males possess receptors that respond to Z9-14:OAc (Struble et al., 1987). It was first hypothesized that behavioral antagonists such as Z9-14:OAc would interact with the same chemoreception sites as the pheromone components (Nagai et al., 1977). However, single sensillum recordings demonstrated the existence of a specific detection channel in both E and Z strains for Z9-14:OAc (Hansson et al., 1987). The recent identification of an olfactory receptor from *O. scapulalis* that is primarily tuned to E11-14:OAc but inhibited by Z9-14:OAc opens the possibility for a direct action of the antagonist in distorting the firing rates of olfactory receptor neurons (ORN) expressing olfactory receptors (OR) that respond to the pheromone components (Miura et al., 2010). The antagonists may interfere through two distinct mechanisms: via the antagonist pathway itself, or via interferences with the agonist pathway. It is noteworthy that the behavioral antagonism-related ORN sometimes can respond to an agonist component, a phenomenon reported for the Asian corn borer, *O. furnacalis* (Takanashi et al., 2006; Domingue et al., 2007a). Investigations of the genetics of male response to pheromone components have revealed that the spike amplitude of cells that respond specifically to Z9-14:OAc do not appear linked to the factor that determines the development of Z and E11-14:OAc-responding ORNs (Cossé et al., 1995).

Other compounds have been reported for their antagonism of ECB pheromone, with the addition of as little as 1% in the pheromone blend leading to antagonism: (*E*)-9-tetradecenyl acetate (E9-14:OAc), (*E*)-and (*Z*)-9-dodecenyl acetate (E9-12:OAc and Z9-12:OAc, respectively) (Klun and Robinson, 1972; Klun et al., 1979; Struble et al., 1987; Glover et al., 1989), as well as (*Z*)-11-hexadecenal (Z11-16:Ald) (Gemeno et al., 2006; Linn et al., 2007a). These compounds have not been found as part of the pheromone blend of any *Ostrinia* species (Ishikawa et al., 1999b). As they have been found in other moth species, one could hypothesize that they have evolved under the private channel hypothesis, thus lowering the probability of cross attraction and, therefore, preventing male mistakes (Löfstedt, 1993; Cardé and Haynes, 2004). On the other hand, the antagonistic activity of some of these compounds may have no adaptive origin (no selective pressure for the emergence of the antagonism), but may be purely coincidental; these molecules may be able to interact with the

olfactory receptors implicated in the antagonist pathway because of their structural similarity to Z9-14:OAc (Linn et al., 2007a).

Smelling the Pheromone: The Male Olfactory System

The demonstration that the antennae of the ECB male are involved in the sex pheromone response came from a study by Klun in the late 1960's (Klun, 1968). This was consistent with observations reported earlier that established the antennae as the primary interface of olfactory reception in moths, and insects in general (Schneider, 1957, 1962). The antennae in both sexes are filiform, with about sixty flagellomeres, but the basal diameter of the antenna in the male is larger than in the female (Hallberg et al., 1994). The response resulting from the detection of a recognized odorant can be measured as an action potential, and electroantennograms (EAG) represent recordings of the summed electrical potentials being sent to the brain by ORNs for which odor stimulants elicit a response (Schneider, 1962, 1969; Mayer et al., 1984). The action potentials are transmitted so as to increase the response in the proximal direction only (Nagai, 1981). Typically, EAG potentials are visualized as negative change in the background potential.

The morphology of the antenna has been described, highlighting particular features of the antenna of male corn borers (Hallberg et al., 1994). Along the filiform antenna of corn borer males, the olfactory sensilla occupy the ventral surface, the dorsal surface being covered by scales (Cornford et al., 1973; Hallberg et al., 1994). Different sensillum types have been characterized, with the sensilla trichodea representing the most abundant type of sensillum found on the antenna (Hallberg et al., 1994). It was noticed that the highest EAG response was obtained when the ventral side of the antenna faced the air stream conveying the pheromonal stimulus (Nagai et al., 1977). This suggested that the sensilla housing olfactory receptor neurons sensitive to Z and E11-14:OAc are localized mainly on the scale-free ventral part of the male antenna and could, therefore, be the sensilla trichodea, given that these are more numerous on male antenna as opposed to female antenna (Cornford et al., 1973). Electrophysiological recordings from single sensilla trichodea indicated that the olfactory receptor neurons that respond to pheromone are housed within this type of sensilla (Hansson et al., 1987). In total, three subtypes of sensilla trichodea have been described on the ECB antenna: one longer type that is innervated by three sensory cells (trichodea A), and two shorter types innervated by two or one single sensory cell (trichodea B and C, respectively) (Hallberg et al., 1994). Note that in the female, only trichoid sensilla with two or three cells have been observed, and the trichoid sensilla appear shorter in females than in males (Hallberg et al., 1994). It was shown that the EAG response to the pheromone components is proportional to the length of the antenna that is stimulated, indicating that olfactory receptor neurons tuned to pheromone components occur regularly throughout the entire antenna (Nagai, 1981). Similarly, the fact that the ratio between the response to E and Z11-14:OAc remained constant, regardless of the length stimulated, suggested that the olfactory receptor neurons that respond to the major and minor pheromone components are distributed evenly along the antenna (Nagai, 1981). Therefore, the number of sensilla trichodea was predicted to be uniform per unit of surface area; this was confirmed by direct measurements (Nagai, 1981). Interestingly, the distribution of the various subtypes of sensilla trichodea along the antenna is uneven. The type A (three cells) is the most common type in the basal part, whereas in the distal part the majority of the sensilla are of the type B (two cells). In contrast, the type C sensilla (one cell) has an even distribution, and constitutes a large proportion of the sensilla present towards the tip of the antenna (Hallberg et al., 1994). The adaptive significance of this particular arrangement remains to be clarified.

Nagai et al. (1977) attempted to establish a link between antennal response recorded by EAG and the behavioral response of male exposed to the female sex pheromone by comparing the electrophysiological responses to Z and E11-14:OAc of Z and E strain males as well as to hybrid individuals. For individuals of the Z strain, Z11-14:OAc always gave a significantly larger EAG response than E11-14:OAc. Although the amplitude of the antennal response decreased throughout the lifespan of the preparation, the response E/Z ratio varied little over time; the E isomer eliciting a response about 70% of that elicited by identical amount of the Z isomer. On the other hand, no difference in the EAG response to the geometrical isomers could be detected when testing individuals of the E strain. Hybrids from reciprocal crosses were intermediate in their responses, although they appeared closer to the E strain males. However, other authors have reported that the E strain individuals have a stronger EAG response to E11-14:OAc than to Z11-14:OAc, making possible a distinction between the strains via EAG recordings (Linn et al., 1999). The amount used to stimulate the antenna during these tests appears critical, and this is certainly a factor that needs to be considered with particular attention.

Single sensillum recordings have revealed important differences between the subtypes of sensilla trichodea, and demonstrated that the strains are highly distinguishable in their electrophysiological responses (Hansson et al., 1987; Hallberg et al., 1994). In type A, one cell responds to the major pheromone component, the second cell to the minor

component, and the third to the behavioral antagonist. Type B sensilla houses cells that respond to the pheromone components only, whereas the single cell present in type C sensilla responds to either the major pheromone component or the behavioral antagonist (Hallberg et al., 1994). Typically, extracellular recordings of male antenna exhibit high-amplitude spike responses from an olfactory cell responding to the major pheromone component, and a low-amplitude spike from an olfactory cell responding to the minor component (Hansson et al., 1987; Hallberg et al., 1994). Furthermore, there is a positive correlation between the spike amplitude and the spike frequency of olfactory receptor neurons (Cossé et al., 1995). The existence of a correlation between spike size produced by stimulated pheromone-responding olfactory receptor neurons and the diameter of the neurons' dendrites has been demonstrated by morphometric measurements (Hansson et al., 1994). Thus, spikes with large amplitude are produced by receptor neurons that are larger than the receptor neurons that elicit spikes of small amplitude. One possibility is that a larger dendrite diameter may be facilitating the presence of more receptor sites specific for the pheromone component to which the olfactory receptor neuron is tuned; the consequence being a higher sensitivity to that component and an action potential of higher amplitude (Hansson et al., 1994; Cossé et al., 1995). Similarly, the outer dendritic segments taper distally in the sensillum (Hallberg et al., 1994). This gradual narrowing of the dendrite diameter towards the tip theoretically optimizes the charge transfer to the dendritic root while minimizing the dendrite volume (Cuntz et al., 2007).

The response profiles obtained from electrophysiological recordings of male corn borer antenna, as well as those for other species, suggested that the main pheromone component always elicits response from OR neurons displaying large spike amplitude, and that the behavioral response may be influenced by the spike amplitude and frequency evoked when these cells are stimulated. However, the results reported by Cossé et al. (1995) indicate that the behavioral response is not necessarily elicited by the component that evokes responses from the large spike amplitude neuron in olfactory sensilla because F2 hybrid males possessing Z-like antennae from an electrophysiological viewpoint responded behaviorally to the E blend. To test whether or not the antennal phenotype is a controlling factor in pheromone blend discrimination, Linn et al. (1999) used an antennal transplant technique to produce individuals with mixed phenotypes for antennal and behavioral responses. Their results were in agreement with the study of Cossé et al. (1995), and suggested that the sex pheromone preference of males is independent of the make-up of the peripheral sensilla and is, therefore, determined at a higher level. However, because the actual projection pattern of the ORNs is unknown, no firm conclusion can be drawn.

The investigations conducted to identify the female sex pheromone of the ECB have revealed also that the olfactory system of the male is organized such that it can detect small variations in the geometric composition of the attractant, and that each strain responds optimally to different geometrical proportions of Δ11-tetradecenyl acetate (Klun et al., 1973). Although the major sex pheromone component is Z or E11-14:OAc, the insect is obviously exquisitely sensitive to small amounts of the opposite isomer (Klun et al., 1973). No increase in EAG response was found when antennal preparations were stimulated with mixtures at the ratios found to be best in field trials (Nagai et al., 1977). Single sensillum responses from olfactory sensilla on the male antenna clearly indicated that the two pheromone-components are detected by different specialized receptors (Hansson et al., 1987; Löfstedt et al., 1989). Also, electrophysiological recordings from single sensilla indicated that, irrespective of the strains, all males have sensory cells sensitive to both pheromone compounds (Hansson et al., 1987). Taken together, these results can be interpreted as further indication that the pheromonal signal is integrated at a higher echelon than the peripheral level. The central nervous system input from the cells appears to be differentially interpreted to give the various phenotypic behavioral responses observed (Roelofs et al., 1987).

Understanding the central nervous processing is necessary when trying to link the processes that occur at the periphery with behavioral responses. Sex pheromone receptor neurons project into the antennal lobe, the first-order olfactory brain area, through the antennal nerve (Hansson, 1995). The antennal lobe comprises a number of glomeruli in which synaptic contacts are made between receptor neurons, projection neurons, and interneurons (Hansson, 1995). Each receptor neuron arborizes in a single glomerulus within the antennal lobe (Anton et al., 1997). Based on three-dimensional reconstructions that allow high resolution of the antennal lobe neuroanatomy, the number of glomeruli in the antennal lobe of Z strain male and female individuals was estimated to be approximately 66 and 64, respectively (Karpati et al., 2008). Located at the entrance of the antennal nerve of male moths, a few enlarged glomeruli dedicated to the reception of pheromonal information constitute the macroglomerular complex (MGC). The number of glomeruli that form the MGC compartments is variable between species and can be an indicator of the number of sex pheromone components, as well as pheromone antagonists detected by highly specialized olfactory receptor neurons. In *O. nubilalis*, the MGC of both E and Z strain males consists of three enlarged glomeruli (Anton et al., 1997; Karpati et al., 2008). Two

large highly convoluted and interdigitated glomeruli of variable shape and dimension receive information from E11 or Z11-14:OAc-responding ORNs, and the third glomerulus, located posteriorly to the two others, is dedicated to Z9-14:OAc, a behavioral antagonist (Anton et al., 1997; Karpati et al., 2008).

The two strains are indistinguishable on the basis of the neuroanatomy of their MGC: in both strains, ORNs that respond to the major pheromone component arborize in the largest medial glomerulus of the MGC, whereas the minor pheromone component-specific ORNs arborize in a smaller lateral glomerulus (Karpati et al., 2008). Olfactory output is made by projection neurons that have dendritic branches in the MGC and axons projecting through the inner antennocerebral tract to the calyces of the mushroom bodies and the lateral protocerebrum (Hansson, 1995; Anton et al., 1997). In the ECB, neurons are classified according to the component eliciting response at the lowest level of abundance, namely E or Z. E+Z-blend neurons respond equally to the same levels of Z and E isomers and mixtures of the two isomers (Anton et al., 1997). Recordings and staining of projection neurons responding to E or Z11-14:OAc show that they arborize only in the E or Z11-14:OAc-specific compartment of the MGC, respectively (Karpati et al., 2008). Similarly, projection neurons that exhibit a larger sensitivity to a blend rather than to either of the pheromone component alone arborize in both pheromone-sensitive MGC glomeruli (Karpati et al., 2008). Finally, neurons described in Anton et al. (1997) as generalist are most likely local interneurons arborizing in most if not all glomeruli (Karpati et al., 2008). Interestingly, the type of blend neurons that discriminate strain-specific blends is apparently consistent with the behavioral profile of each type of male (Anton et al., 1997). This might explain, in part, the ability of insects to discriminate behaviorally between the pheromone ratios of the different strains (Anton et al., 1997). However, it is still unclear whether these neurons are essential in the behavioral response that follows detection of different ratios of pheromone components (Karpati et al., 2008). Irrespective of their type, the antennal lobe neurons differ in their absolute sensitivity (Anton et al., 1997). It is worth mentioning that both receptor and antennal lobe neurons respond to both pheromone isomers when stimulated at high concentrations, while specific responses of the neurons are observed only at lower concentrations (Anton et al., 1997). Therefore, it is important to bear in mind that the pheromone concentrations and ratios used in electrophysiological experiments should be within the natural range that males may encounter.

The males of both pheromone strains and reciprocal hybrids exhibit the same neuron types, but the abundance of these types differs between the types of males, which indicates the existence of strain-specific characteristics at the antennal lobe level (Anton et al., 1997). The two strains have an identical MGC morphology accompanied by a reversed functional topology, with both olfactory receptor neurons and projection neurons displaying opposite innervation patterns (Karpati et al., 2008). Karpati et al. (2008) proposed that the occurrence of an interchange of olfactory receptors between pheromone receptor neurons within the same sensillum would explain the finding of a reversed functional topology, while olfactory receptor neuron and projection neuron arborization patterns in the MGC remained unchanged. The pheromone receptor neurons would always arborize to the same location in the MGC but the ORs expressed within the membrane of their dendrites would be tuned to a different pheromone component, thus leading to an opposite behavioral response. One may argue that such a receptor swap occurs when antennae are transplanted from one strain to the other, such as reported by Linn et al. (1999), who observed maintenance of the behavioral response of the implanted strain, which is not according to the prediction. However, because of a lack of information concerning the neuroanatomy of individuals that undergo antennal transplantation and, in particular, how the axonal targeting of olfactory receptor neurons is influenced, no definitive conclusion can be drawn from this type of experiment. Further studies are required to elucidate what factors determine the type of receptor expressed in a particular olfactory receptor neuron, and the arborization path followed by the sensory axon of that olfactory receptor neuron. Recently, a series of ORs responding to pheromone components has been identified in the ECB (Wanner et al., 2010). ORs appear to be narrowly and broadly tuned, with some responding nonspecifically to (*E*) and (*Z*)-11- or -12-tetradecenyl acetate, as found in *O. scapulalis* (Miura et al., 2010; Wanner et al., 2010); the latter E and Z12-14:OAc constitute the pheromone components of a close relative of the ECB, the Asian corn borer *O. furnacalis* (Ishikawa et al., 1999b). Previous electrophysiological recordings of ORNs indicated that ECB males possess ORNs responsive to E and Z12-14:OAc, and that these ORNs are, indeed, the same large- and small-spiking ORNs responsive to the ECB pheromone components (Domingue et al., 2006, 2007b). Some rare ECB males may be attracted to the Asian corn borer blend (Linn et al., 2003, 2007b); this alteration in the normal behavior might be caused by a firing ratio of ORNs responsive to E and Z12-14:OAc close to the ratio observed in response to the attractive ECB blend (Domingue et al., 2007b).

Many advances have been made during the last 30 years, and we have a much better understanding of

the ECB male olfactory system. Nevertheless, the precise mechanism leading to blend discrimination remains unknown. Most studies have used neurophysiological approaches, and the molecular bases of pheromone detection in ECB are yet to be unraveled. For example, the actual diversity of ORs in ECB, and what factors determine on which sensory dendrites they are expressed, remain essentially unexplored.

Genetics of Male Response

As the genetics underlying ECB sex pheromone differences was investigated, the heredity of male sexual response preferences toward specific mixtures of Δ11-tetradecenyl acetate was also scrutinized. Two types of response are distinguishable: the physiological response at the level of male antennae, and the behavioral response of males.

Electrophysiological recordings from single olfactory sensilla on male antenna showed that the reaction patterns of males from the two strains are distinguishable (Hansson et al., 1987; Roelofs et al., 1987). Males from the first filial generation were characterized as intermediate by displaying two types of olfactory cells that gave similar spike amplitudes to the E and Z isomers (Hansson et al., 1987). The inheritance of the response profiles is determined by a single autosomal gene with two alleles (Hansson et al., 1987; Roelofs et al., 1987). Because the locus controlling female pheromone production also was found to be autosomal, the possible linkage of the two loci or the existence of a single locus that determines both phenotypes came to be questioned. Evidence showed that even if the inheritance of pheromone production and the electrophysiological response of pheromone receptor cells are most often coupled in a complementary fashion, so that production and response are coordinated, their coordination can be uncoupled in matings among individuals of opposite types (Klun and Huettel, 1988). The experiments conducted by Löfstedt et al. (1989) demonstrated unambiguously the absence of a close linkage between the autosomal genes that determine the sex pheromone production and the organization of olfactory receptors in *O. nubilalis*. That was the first time where the degree of genetic linkage between characteristics of the sender and responder in a pheromone communication system had been investigated.

In terms of behavioral response profile, the F1-generation hybrid males from reciprocal crosses exhibited similar response profiles with about 50% of the males being attracted to different ratios of geometrical isomers (97:3, 65:35, 50:50, 35:65 Z/E), with the noticeable exception of the 1:99 Z/E blend that seldom attracted males (Roelofs et al., 1987; Glover et al., 1990; Linn et al., 1997). The remaining 50% of the F1 progeny do not respond to any blend (Roelofs et al., 1987; Glover et al., 1990). In Lepidoptera, males are the homogametic sex, usually denoted ZZ, and the females are the heterogametic sex, denoted ZW. The observation that paternal backcrosses to Z or E strains gave individuals with response profiles similar to that of the pure Z or E parent males indicated that the inheritance of the behavioral response is determined by a major sex-linked factor (Roelofs et al., 1987). It is not known if this major factor consists of a single gene or whether it consists of a set of closely linked genes. The demonstration that male behavioral response is determined by a locus present on the sex chromosome was made by using triose phosphate isomerase (TPI) an allozyme marker for the Z chromosome (Glover et al., 1990). A perfect match between the phenotype at TPI and the response profile of males indicated a complete linkage of the TPI locus and the locus controlling response to sex pheromone, thus confirming the sex linkage of male behavior (Glover et al., 1990). There was evidence for little recombination between the allozyme marker locus and the male behavior locus (Glover et al., 1990). It is worth mentioning that the phenotype at TPI may not always differ between populations of the E and Z strains, as reported by Cianchi et al. (1980). Genetic mapping of male response showed that TPI and the locus responsible for the difference in male behavioral response are not tightly linked, and a factor other than response to pheromone may maintain the linkage disequilibrium between TPI and response (Dopman et al., 2004, 2005).

Since the locus controlling male behavioral response is sex-linked, and the locus determining male electrophysiological response is autosomal, it appears evident that these loci are not linked and may segregate independently. For this reason, certain crosses between the E and Z strains should produce unusual males that respond behaviorally to one blend despite possessing antennae that respond electrophysiologically to the opposite blend. A study was undertaken to examine whether or not these unusual males possessing the wrong antennae can perform a complete behavioral sequence in a wind tunnel experiment. Males were produced through F2 crosses between the two races and the analysis of the spike amplitudes obtained with recordings of E-behavioral responders revealed that some males with Z-like antennae can fly to the E pheromone source (Cossé et al., 1995). This provided further support for the idea that the sex-linked factor associated with behavioral response affects the way an incoming signal from the antenna is processed in the central nervous system of male moths. The sex-linked factor that controls the behavioral response profile of males may, therefore, influence the architecture of the antennal lobe. The pattern

of neuron types found in the antennal lobes of hybrid or pure strain males coincide to some extent with the behavioral profile (Anton et al., 1997). Unfortunately, the limited number of recordings made did not allow the authors to draw any firm conclusion, despite striking coincidence. A study that focuses on males obtained from paternal backcrosses (EZxZ and ZExE), which should possess to a large extent only Z- or E-blend neurons, should give a clue regarding the link between discrimination of different ratios of pheromone components at the level of the antennal lobe and the behavioral response profiles of males, thus providing a physiological basis for the differences in behavior. The finding that the functional topology of the MGC in Z and E strain males is reversed (Karpati et al., 2008) indicates that further research is still required.

Given that the loci for sex pheromone production, male electrophysiological response, and male behavioral response are on different pairs of chromosomes, the response and the signal are not expected to be physically linked in this pheromone system.

Evidence for A Male-Produced Pheromone

Laboratory tests conducted under confined conditions have indicated that the two ECB strains are not freely interbreeding, which may result from the action of a chemical governing sexual behavior at close range (Liebherr and Roelofs, 1975; Pélozuelo et al., 2007). The detection of the incorrect substance may inhibit a large proportion of the females from accepting the mating overture (Liebherr and Roelofs, 1975). Males possess on both sides of the claspers and at the intersection between the 7th and 8th sternites two tufts of differentiated scales called hairpencils. The first clear evidence for the existence of a male pheromone in the ECB came from the observation that males deprived of their hairpencils have a decreased mating success (Royer and McNeil, 1993).Recently, the pheromone from hairpencils was demonstrated to play an crucial role in female choice, and was identified in the Z strain as a mixture of 16-carbon acetates, namely (*Z*)-9-hexadecenyl acetate, (*Z*)-11-hexadecenyl acetate, (*Z*)-14-hexadecenyl acetate, and hexadecanyl acetate (Lassance and Löfstedt, 2009). The bouquet produced by males of the E strain was found to be of similar composition but lacked (*Z*)-11-hexadecenyl acetate in most individuals (Lassance and Löfstedt, 2009). The behavioral significance of this difference in composition remains to be firmly demonstrated.

Males produce compounds that are structurally similar to those used by females, and both sexes appear to rely on the same genes to produce their pheromones. This finding raises interesting questions concerning the coevolution of the two traits, and especially for the coevolution of the two detection systems.

Concluding Comments

One of the purposes of investigating the European corn borer chemical ecology was to provide new tools to monitor and control the pest. Trapping systems that rely on synthetic sex pheromone have been used to catch the attracted males and monitor adult activity (Pélozuelo and Frérot, 2007). However, the information gathered from such pheromone-baited traps may be taken with circumspection as it is still unclear how well moth phenology correlates with trapping data and, while only males are captured, their mating status is unknown (McNeil, 1992; Pélozuelo and Frérot, 2007). The obvious potential of semiochemicals should not be questioned, but increasing our knowledge of the factors that influence their efficacy and how to interpret what we observe is necessary.

One line of ECB research that has not been sufficiently pursued is host plant selection by females. Whereas the ECB owes its name to the use of maize as a host, the insect can thrive on more than 200 plants species (Caffrey and Worthley, 1927). Little is known about what criteria determine the suitability of a host, in particular, what are the cues that make a host attractive to gravid females. The two pheromone strains may differ in their host preference, as exemplified by studies conducted in Canada, the U. S., and France (McLeod, 1981; Straub et al., 1986; Eckenrode and Webb, 1989; Bontemps et al., 2003; Thomas et al., 2003; Pelozuelo et al., 2004). The use of transgenic maize that express *Bacillus thuringiensis* toxin, and the associated strategies that prevent the emergence of resistance, require a better knowledge of the host range actually used by ECB in the field. Is *O. nubilalis* a truly opportunistic polyphagous species, or is it a mosaic of host-plant races hidden under the same name?

The two strains of *O. nubilalis* are sufficiently isolated to be considered as sibling species but still compatible enough from a genetic point of view to produce fertile offspring. As such, the ECB has become a model to study the evolution of sex pheromone communication (Smadja and Butlin, 2009). A series of studies have elucidated the genetic bases of the polymorphism observed in this sex pheromone communication system. While the gene responsible for the differences in female-produced sex pheromone has been characterized, those involved in the differences in male behavior and antennal responses are still unidentified. The two pheromone strains of *O. nubilalis* constitute a unique system to investigate how speciation affects different parts of the genome.

The apparent simplicity of the ECB communication system, as it turns out, has ample hiden complexity, and, as some who spent some years investigating the ECB chemical ecology say, "the more answers we obtain with the ECB, the more questions we get in return". The recent identification of the male-produced pheromone, a trait acting as a reproductive barrier between the strains, opens a new dimension of ECB research. Fascinating discoveries may be just around the corner, and corn borers probably have many more secrets to reveal.

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References

ANGLADE P, and STOCKEL J. 1984. Intraspecific sex-pheromone variability in the European corn borer, *Ostrinia nubilalis* Hbn (Lepidoptera, Pyralidae). *Agronomie* 4:183–187.

ANTON S, LÖFSTEDT C, and HANSSON BS. 1997. Central nervous processing of sex pheromones in two strains of the European corn borer *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Exp. Biol.* 200:1073–1087.

BONTEMPS A, BOURGUET D, PÉLOZUELO L, BETHENOD M-T, and PONSARD S. 2003. Managing the evolution of *Bacillus thuringiensis* resistance in natural populations of the European corn borer, *Ostrinia nubilalis*: host plant, host races and pherotype of adult males at aggregation sites. *Proc. R. Soc. B-Biol. Sci.* 271:2179–2185.

CAFFREY DJ, and WORTHLEY LH. 1927. A progress report on the investigations of the European corn borer. United States Department of Agriculture Bulletin 1476:155 pp.

CARDÉ RT, and HAYNES KF. 2004. Structure of the pheromone communication channel in moths. pp 283–332 In Carde RT, Millar JG, (eds.). Advances in Insect Chemical Ecology. Cambridge University Press, New York.

CARDÉ RT, ROELOFS WL, HARRISON RG, VAWTER AT, BRUSSARD PF, MUTUURA A, and MUNROE E. 1978. European corn borer: pheromone polymorphism or sibling species? *Science* 199:555–556.

CHOI M-Y, FUERST E-J, RAFAELI A, and JURENKA RA. 2003. Identification of a G protein-coupled receptor for pheromone biosynthesis activating neuropeptide from pheromone glands of the moth *Helicoverpa zea*. *Proc. Natl. Acad. Sci. USA* 100:9721–9726.

CIANCHI R, MAINI S, and BULLINI L. 1980. Genetic distance between pheromone strains of the European corn borer, *Ostrinia nubilalis:* Different contribution of variable substrate, regulatory and non regulatory enzymes. *Heredity* 45:383–388.

CORNFORD ME, ROWLEY WA, and KLUN JA. 1973. Scanning electron microscopy of antennal sensilla of the European corn borer *Ostrinia nubilalis*. *Ann. Entomol. Soc. Am.* 66:1079–1088.

COSSÉ AA, CAMPBELL MG, GLOVER TJ, LINN CE JR., TODD JL, BAKER TC, and ROELOFS WL. 1995. Pheromone behavioral responses in unusual male European corn borer hybrid progeny not correlated to electrophysiological phenotypes of their pheromone-specific antennal neurons. *Experientia* 51:809–816.

CUNTZ H, BORST A, and SEGEV I. 2007. Optimization principles of dendritic structure. *Theor. Biol. Med. Model*. 4:21.

DALECKY A, PONSARD S, BAILEY RI, PÉLISSIER C, and BOURGUET D. 2006. Resistance evolution to Bt crops: predispersal mating of European corn borers. *PLoS Biol.* 4:e181.

DOMINGUE MJ, ROELOFS WL, LINN JCE, and BAKER TC. 2006. Effects of egg-to-adult development time and adult age on olfactory neuron response to semiochemicals in European corn borers. *J. Insect Physiol.* 52:975–983.

DOMINGUE MJ, MUSTO CJ, LINN CE, ROELOFS WL, and BAKER TC. 2007a. Evidence of olfactory antagonistic imposition as a facilitator of evolutionary shifts in pheromone blend usage in *Ostrinia* spp. (Lepidoptera: Crambidae). *J. Insect Physiol.* 53:488–496.

DOMINGUE MJ, MUSTO CJ, LINN JR CE, ROELOFS WL, and BAKER TC. 2007b. Altered olfactory receptor neuron responsiveness in rare *Ostrinia nubilalis* males attracted to the *O. furnacalis* pheromone blend. *J. Insect Physiol*. 53:1063–1071.

DOPMAN EB, BOGDANOWICZ SM, and HARRISON RG. 2004. Genetic mapping of sexual isolation between E and Z pheromone strains of the European corn borer (*Ostrinia nubilalis*). *Genetics* 167:301–309.

DOPMAN EB, PÉREZ L, BOGDANOWICZ SM, and HARRISON RG. 2005. Consequences of reproductive barriers for genealogical discordance in the European corn borer. *Proc. Natl. Acad. Sci. USA* 102:14706–14711.

ECKENRODE CJ, and WEBB DR. 1989. Establishment of various European corn borer (Lepidoptera, Pyralidae) races on selected cultivars of snap beans. *J. Econ. Entomol*. 82:1169–1173.

EL-SAYED AM. 2010. The Pherobase: database of insect pheromones and semiochemicals (updated: April 2010). <http://www.pherobase.com>.

ELTAHLAWY HS, BUCKNER JS, and FOSTER SP. 2007. Regulation of pheromone biosynthesis in the "Z strain" of the European corn borer, *Ostrinia nubilalis*. *Arch. Insect Biochem. Physiol.* 65:29–38.

FOSTER SP. 2004. Fatty acid and sex pheromone changes and the role of glandular lipids in the Z-strain of the European corn borer, *Ostrinia nubilalis* (Hübner). *Arch. Insect Biochem. Physiol*. 56:73–83.

FROLOV AN, BOURGUET D, and PONSARD S. 2007. Reconsidering the taxonomy of several *Ostrinia* species in the light of reproductive isolation: a tale for Ernst Mayr. *Biol. J. Linn. Soc*. 91:49–72.

FUKUZAWA M, FU X, TATSUKI S, and ISHIKAWA Y. 2006. cDNA cloning and *in situ* hybridization of Δ11-desaturase, a key enzyme of pheromone biosynthesis in *Ostrinia scapulalis* (Lepidoptera: Crambidae). *J. Insect Physiol*. 52:430–435.

GEMENO C, SANS A, LOPEZ C, ALBAJES R, and EIZAGUIRRE M. 2006. Pheromone antagonism in the European corn borer. *J. Chem. Ecol*. 32:1071–1084.

GLOVER TJ, TANG X-H, and ROELOFS WL. 1987. Sex pheromone blend discrimination by male moths from *E* and *Z* strains of European corn borer. *J. Chem. Ecol*. 13:143–151.

GLOVER TJ, PEREZ N, and ROELOFS WL. 1989. Comparative analysis of sex-pheromone-response antagonists in three races of European corn borer. *J. Chem. Ecol*. 15:863–873.

GLOVER TJ, CAMPBELL M, ROBBINS P, and ROELOFS WL. 1990. Sex-linked control of sex pheromone behavioral responses in European corn-borer moths (*Ostrinia nubilalis*) confirmed with TPI marker gene. *Arch. Insect Biochem. Physiol*. 15:67–77.

GLOVER TJ, CAMPBELL MG, LINN CE, and ROELOFS WL. 1991. Unique sex chromosome mediated behavioral response specificity of hybrid male European corn borer moths. *Experientia* 47:980–984.

HALLBERG E, HANSSON BS, and STEINBRECHT RA. 1994. Morphological characteristics of antennal sensilla in the European cornborer Ostrinia nubilalis (Lepidoptera: Pyralidae). *Tissue and Cell* 26:489–502.

HANSSON BS. 1995. Olfaction in Lepidoptera. *Experientia* 51:1003–1027.

HANSSON BS, LÖFSTEDT C, ROELOFS WL. 1987. Inheritance of olfactory response to sex pheromone components in *Ostrinia nubilalis*. Naturwissenschaften 74:497–499.

HANSSON BS, HALLBERG E, LÖFSTEDT C, and STEINBRECHT RA. 1994. Correlation between dendrite diameter and action potential amplitude in sex pheromone specific receptor neurons in male Ostrinia nubilalis (Lepidoptera: Pyralidae). *Tissue and Cell* 26:503–512.

HUANG Y, HONDA H, YOSHIYASU Y, HOSHIZAKI S, TATSUKI S, and ISHIKAWA Y. 1998. Sex pheromone of the butterbur borer, *Ostrinia zaguliaevi*. *Entomol. Exp. Appl*. 89:281–287.

ISHIKAWA Y, TAKANASHI T, and HUANG Y. 1999a. Comparative studies on the sex pheromones of *Ostrinia spp*. in Japan: the burdock borer, *Ostrinia zealis*. *Chemoecology* 9:25–32.

ISHIKAWA Y, TAKANASHI T, KIM C-G, HOSHIZAKI S, TATSUKI S, and HUANG Y. 1999b. *Ostrinia* spp. in Japan: their host plants and sex pheromones. *Entomol. Exp. Appl*. 91:237–244.

JURENKA RA, and ROELOFS WL. 1989. Characterization of the acetyltransferase used in pheromone biosynthesis in moths: Specificity for the Z isomer in Tortricidae. *Insect Biochemistry* 19:639–644.

KARLSON P, and LÜSCHER M. 1959. 'Pheromones': a new term for a class of biologically active substances. *Nature* 183:55–56.

KÁRPÁTI Z, MOLNÁR B, and SZŐCS G. 2007. Pheromone titer and mating frequency of E-and Z-strains of the european corn borer, Ostrinia nubilalis : Fluctuation during scotophase and age dependence. *Acta Phytopathol. Entomol. Hung.* 42:331–341.

KARPATI Z, DEKKER T, and HANSSON BS. 2008. Reversed functional topology in the antennal lobe of the male European corn borer. *J. Exp. Biol*. 211:2841–2848.

KLUN JA. 1968. Isolation of a sex pheromone of the European corn borer. *J. Econ. Entomol*. 61:484–487.

KLUN JA, and BRINDLEY TA. 1970. *cis*-11-tetradecenyl acetate, a sex stimulant of the European corn borer. *J. Econ. Entomol*. 63:779–780.

KLUN JA, and ROBINSON JF. 1971. European corn borer: sex attractant and sex attraction inhibitors. *Ann. Entomol. Soc. Am*. 64:1083–1086.

KLUN JA, and ROBINSON JF. 1972. Olfactory discrimination in European corn borer and several pheromonally analogous moths. *Ann. Entomol. Soc. Am*. 65:1337–1340.

KLUN JA, and COOPERATORS. 1975. Insect sex pheromones: Intraspecific pheromonal variability of *Ostrinia nubilalis* in North America and Europe. *Environ. Entomol*. 4:891–894.

KLUN JA, and MAINI S. 1979. Genetic basis of an insect chemical communication system: the European corn borer. *Environ. Entomol*. 8:423–426.

KLUN JA, and HUETTEL MD. 1988. Genetic regulation of sex pheromone production and response: interaction of sympatric pheromonal types of European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Chem. Ecol*. 14:2047–2061.

KLUN JA, CHAPMAN OL, MATTES KC, WOJKOWSKI PW, BEROZA M, and SONNET PE. 1973. Insect sex pheromones: minor amount of opposite geometrical isomer critical to attraction. *Science* 181:661–663.

KLUN JA, MAINI S, CHAPMAN OL, LEPONE G, and LEE GH. 1979. Suppression of male European corn borer sex attraction and precopulatory reactions with (E)-9-tetradecenyl acetate. *J. Chem. Ecol*. 5:345–352.

KOCHANSKY J, CARDÉ RT, LIEBHERR J, and ROELOFS WL. 1975. Sex pheromone of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), in New York. *J. Chem. Ecol*. 1:225–231.

LASSANCE J-M, and LÖFSTEDT C. 2009. Concerted evolution of male and female display traits in the European corn borer, *Ostrinia nubilalis*. *BMC Biol*. 7:10.

LASSANCE J-M, GROOT AT, LIÉNARD MA, ANTONY B, BORGWARDT C, ANDERSSON F, HEDENSTRÖM E, HECKEL DG, and LÖFSTEDT C. 2010. Allelic variation in a fatty-acyl reductase gene causes divergence in moth sex pheromones. *Nature* 466:486–489.

LIEBHERR J, and ROELOFS WL. 1975. Laboratory hybridization and mating period studies using two pheromone strains of *Ostrinia nubilalis*. *Ann. Entomol. Soc. Am*. 68:305–309.

LINN CE, YOUNG MS, GENDLE M, GLOVER TJ, and ROELOFS WL. 1997. Sex pheromone blend discrimination in two races and hybrids of the European corn borer moth, *Ostrinia nubilalis*. *Physiol. Entomol*. 22:212–223.

LINN CE, POOLE K, ZHANG A, and ROELOFS W. 1999. Pheromone-blend discrimination by European corn borer moths with inter-race and inter-sex antennal transplants. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol*. 184:273–278.

LINN CE, O'CONNOR M, and ROELOFS WL. 2003. Silent genes and rare males: A fresh look at pheromone blend response specificity in the European corn borer, *Ostrinia nubilalis*. *J. Insect Sci*. 3:15.

LINN C, DOMINGUE M, MUSTO C, BAKER T, and ROELOFS W. 2007a. Support for (Z)-11-hexadecanal as a pheromone antagonist in *Ostrinia nubilalis* : flight tunnel and single sensillum studies with a New York population. *J. Chem. Ecol*. 33:909–921.

LINN C, MUSTO C, and ROELOFS W. 2007b. More rare males in *Ostrinia* : response of Asian corn borer moths to the sex pheromone of the European corn borer. *J. Chem. Ecol*. 33:199–212.

LÖFSTEDT C. 1993. Moth pheromone genetics and evolution. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* 340:167–177.

LÖFSTEDT C, HANSSON BS, ROELOFS WL, and BENGTSSON BO. 1989. No linkage between genes controlling female pheromone production and male pheromone response in the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Pyralidae). *Genetics* 123:553–556.

MA PWK, and ROELOFS WL. 1995a. Anatomy of the neurosecretory cells in the cerebral and subesophageal ganglia of the female European corn borer moth, *Ostrinia nubilalis* (Hübner)(Lepidoptera: Pyralidae). *Int. J. Insect Morphol. Embryol*. 24:343–359.

MA PWK, and ROELOFS WL. 1995b. Calcium involvement in the stimulation of sex pheromone production by PBAN in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Insect Biochem. Mol. Biol*. 25:467–473.

MA PWK, and ROELOFS WL. 1995c. Sites of synthesis and release of PBAN-like factor in the female European corn borer, *Ostrinia nubilalis*. *J. Insect Physiol.* 41:339–350.

MA PWK, and ROELOFS WL. 2002. Sex Pheromone Gland of the Female European Corn Borer Moth, Ostrinia nubilalis (Lepidoptera, Pyralidae): Ultrastructural and Biochemical Evidences. *Zool. Sci*. 19:501–511.

MAYER MS, MANKIN RW, and LEMIRE GF. 1984. Quantitation of the insect electroantennogram: Measurement of sensillar contributions, elimination of background potentials, and relationship to olfactory sensation. *J. Insect Physiol*. 30:757–763.

MCLEOD DGR. 1981. Damage to sweet pepper in Ontario by 3 strains of European corn borer, *Ostrinia nubilalis* (Lepidoptera, Pyralidae). *Proc. Entomol. Soc. Ont*. 112:29–32.

MCNEIL, JN. 1992. Evolutionary perspectives and insect pest control: an attractive blend for the deployment of semiochemicals in management programs. pp 334–351 In Roitberg BD, Isman MB, (eds.). Evolutionary Perspectives on the Chemical Ecology of Insects. Chapman and Hall, New York.

MIURA N, NAKAGAWA T, TOUHARA K, and ISHIKAWA Y. 2010. Broadly and narrowly tuned odorant receptors are involved in female sex pheromone reception in Ostrinia moths. *Insect Biochem. Mol. Biol*. 40:64–73.

NAGAI T. 1981. Electroantennogram response gradient on the antenna of the European corn borer, *Ostrinia nubilalis*. *J. Insect Physiol*. 27:889–894.

NAGAI T, STARRATT AN, MCLEOD DGR, and DRISCOLL GR. 1977. Electroantennogram responses of the European corn borer, *Ostrinia nubilalis*, to (*Z*)- and (*E*)-11-tetradecenyl acetates. *J. Insect Physiol.* 23:591–597.

PÉLOZUELO L, and FRÉROT B. 2007. Monitoring of European corn borer with pheromone-baited traps: review of trapping system basics and remaining problems. *J. Econ. Entomol*. 100:1797–1807.

PELOZUELO L, MALOSSE C, GENESTIER G, GUENEGO H, and FRÉROT B. 2004. Host-plant specialization in pheromone strains of the European corn borer *Ostrinia nubilalis* in France. *J. Chem. Ecol.* 30:335–352.

PÉLOZUELO L, MEUSNIER S, AUDIOT P, BOURGUET D, and PONSARD S. 2007. Assortative mating between European corn borer pheromone races: beyond assortative meeting. *PLoS One* 6: e555.

PEÑA A, ARN H, BUSER H-R, RAUSCHER S, BIGLER F, BRUNETTI R, MAINI S, and TÓTH M. 1988. Sex pheromone of European corn borer, *Ostrinia nubilalis*: polymorphism in various laboratory and field strains. *J. Chem. Ecol.* 14:1359–1366.

RAFAELI A. 2009. Pheromone biosynthesis activating neuropeptide (PBAN): Regulatory role and mode of action. *Gen. Comp. Endocrinol.* 162:69–78.

RAINA AK, and KLUN JA. 1984. Brain factor control sex pheromone production in the female corn earworm moth. *Science* 225:531–533.

RAINA AK, JAFFE H, KEMPE TG, KEIM P, BLACHER RW, FALES HM, RILEY CT, KLUN JA, RIDGWAY RL, and HAYES DK. 1989. Identification of a neuropeptide hormone that regulates sex pheromone production in female moths. *Science* 244:796–798.

ROELOFS WL, CARDÉ RT, BARTELL RJ, and TIERNEY PG. 1972. Sex attractant trapping of the European corn borer in New York. *Environ. Entomol*. 1:606–608.

ROELOFS WL, DU JW, TANG XH, ROBBINS PS, and ECKENRODE CJ. 1985. Three European corn borer populations in New York based on sex pheromones and voltinism. *J. Chem. Ecol.* 11:829–836.

ROELOFS WL, GLOVER TJ, TANG X-H, SRENG I, ROBBINS P, ECKENRODE C, LÖFSTEDT C, HANSSON BS, and BENGTSSON BO. 1987. Sex pheromone production and perception in European corn borer moth is determined by both autosomal and sex-linked genes. *Proc. Natl. Acad. Sci. USA* 84:7585–7589.

ROELOFS WL, LIU W, HAO G, JIAO H, ROONEY AP, and LINN CE. 2002. Evolution of moth sex pheromones via ancestral genes. *Proc. Natl. Acad. Sci. USA* 99:13621–13626.

ROYER L, and MCNEIL JN. 1993. Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): Impact on female longevity and reproductive performance. *Funct. Ecol.* 7:209–215.

SCHNEIDER D. 1957. Electrophysiological investigation on the antennal receptors of the silk moth during chemical and mechanical stimulation. *Cell. Mol. Life Sci*. 13:89–91.

SCHNEIDER D. 1962. Electrophysiological investigation on the olfactory specificity of sexual attracting substances in different species of moths. *J. Insect Physiol*. 8:15–30.

SCHNEIDER D. 1969. Insect Olfaction: Deciphering System for Chemical Messages. *Science* 163:1031–1037.

SHOWERS WB, REED GL, ROBINSON JF, and DEROZARI MB. 1976. Flight and sexual activity of the European corn borer. *Environ. Entomol*. 5:1099–1104.

SMADJA C, and BUTLIN RK. 2009. On the scent of speciation: the chemosensory system and its role in premating isolation. *Heredity* 102:77–97.

STRAUB RW, WEIRES RW, and ECKENRODE CJ. 1986. Damage to apple cultivars by races of European corn borer (Lepidoptera, Pyralidae). *J. Econ. Entomol*. 79:359–363.

STRUBLE DL, BYERS JR, MCLEOD DGR, and AYRE GL. 1987. Sex-pheromone components of an Alberta population of European corn borer, *Ostrinia nubilalis* (HBN) (Lepidoptera, Pyralidae). *Can. Entomol*. 119:291–299.

TAKANASHI T, ISHIKAWA Y, ANDERSON P, HUANG YP, LOFSTEDT C, TATSUKI S, and HANSSON BS. 2006. Unusual response characteristics of pheromone-specific olfactory receptor neurons in the Asian corn borer moth, *Ostrinia furnacalis*. *J. Exp. Biol*. 209:4946–4956.

TEAL PEA, TUMLINSON JH, and OBERLANDER H. 1989. Neural regulation of sex pheromone biosynthesis in *Heliothis* moths. *Proc. Natl. Acad. Sci. USA* 86:2488–2492.

THOMAS Y, BETHENOD M-T, PELOZUELO L, FRÉROT B, and BOURGUET D. 2003. Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hübner. I. Sex pheromone, moth-emergence timing, and parasitism. *Evolution* 57:261–273.

TILLMAN JA, SEYBOLD SJ, JURENKA RA, and BLOMQUIST GJ. 1999. Insect pheromones-an overview of biosynthesis and endocrine regulation. *Insect Biochem. Mol. Biol*. 29:481–514.

WANNER KW, NICHOLS AS, ALLEN JE, BUNGER PL, GARCZYNSKI SF, LINN CE JR, ROBERTSON HM, and LUETJE CW. 2010. Sex Pheromone Receptor Specificity in the European Corn Borer Moth, *Ostrinia nubilalis*. *PLoS ONE* 5:e8685.

WEBSTER RP, and CARDÉ RT. 1984. Role of (*Z*)- and (*E*)-11-tetradecenyl acetate pheromone components in the sexual behavior of the (Z) strain of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Chem. Ecol.* 10:9–15.

WOLF WA, and ROELOFS WL. 1987. Reinvestigation confirms action of Δ11-desaturases in spruce budworm moth sex pheromone biosynthesis. *J. Chem. Ecol*. 13:1019–1027.

WYATT TD. 2003. Pheromones and Animal Behaviour: Communication by Smell and Taste. P. 391. Cambridge University Press, Cambridge.

ZHU J, LÖFSTEDT C, and BENGTSSON BO. 1996a. Genetic variation in the strongly canalized sex pheromone communication system of the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera; Pyralidae). *Genetics* 144:757–766.

ZHU JW, ZHAO CH, BENGTSSON M, and LÖFSTEDT C. 1996b. Reductase specificity and the ratio regulation of *E/Z* isomers in the pheromone biosynthesis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Insect Biochem. Mol. Biol*. 26:171–176.