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Mini-review

Sex in the night: Fatty acid-derived sex pheromones and corresponding membrane pheromone receptors in insects

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ABSTRACT

The moth sex pheromone communication is one of the most striking examples of chemical communication in the animal kingdom. Investigating the molecular mechanisms of pheromone biosynthesis in the female pheromone gland and of pheromone reception in the male antennae not only defines new concepts in signalling research but also opens new perspectives for insect control. In this mini-review, we use the cotton leafworm *Spodoptera littoralis* as a guideline to illustrate the recent advances gained in the understanding of moth sex pheromone communication.

1. Introduction

The insect environment is full of chemicals that animals use efficiently for food and oviposition substrate choice, mating partner identification and danger avoidance [1–3]. The most striking example of such chemical communication is the moth sex pheromone communication. The sensitivity and the specificity of this sexual communication offer an interesting system to define new concepts in signal recognition via membrane bound receptors. Moths are night active Lepidoptera, and sex pheromone communication is crucial for conspecifics to meet in the night where visual cues are less informative or non-existing. Typically, the female moth when sexually ready emits a species-specific signal biosynthesized in an epidermal gland located on the abdomen. The species-specificity of the signal is ensured by the time window of emission, the blend composition and the ratio of the different components. To the specificity of the female message corresponds a highly sensitive and specific reception system in male antennae. Thus, both males and females contribute to species isolation. After signal integration by brain centres, activated males take off for a stereotype zigzag flight to the source. The first sex pheromone bombykol was purified and identified in the silkmoth *Bombyx mori* [4]. Since then, pheromones have been identified in several thousands of insect species [5](http://www.pherobase.com/), mainly due to their potential as attractants or repellents in crop protection strategies.

In this mini-review, we use the cotton leafworm *Spodoptera littoralis* to illustrate the recent advances gained in the understanding of moth pheromone biosynthesis and pheromone reception. *S. littoralis* is commonly known as the Egyptian armyworm and provokes considerable damage on cotton and vegetables in Europe, Africa and Asia. Many insecticide resistant strains have emerged and alternative strategies to pesticides are urgently needed. Several attempts to control this pest using synthetic pheromone blends have been published [6–9] but the success is minor compared to the damage. Thus, this species has been chosen by diverse laboratories to conduct both basic and applied researches, involving molecular, physiological, neuroanatomical and behavioural approaches. A better knowledge on the molecular mechanisms of the sex pheromone communication in this species and in moths in general will help designing innovative ways to fight against crop pest targeting pheromone-driven behaviours in an environment friendly manner.

The first part of this review focuses on sex pheromone biosynthesis and its regulation. The second part illustrates how transcriptomic approaches allowed us to identify a large array of chemosensory genes in a species without a sequenced genome. The third part relates the unexpected discovery we made in *S. littoralis* that the sex pheromone is also used by larvae.

Abbreviations: ORN, olfactory receptor neuron; OBP, odorant-binding protein; OR, olfactory receptor; PBAN, pheromone biosynthesis activating neuropeptide; PBP, pheromone-binding protein; PDE, pheromone-degrading enzyme; PR, pheromone receptor; SNMP, sensory neuron membrane protein.

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2. Sex pheromone biosynthesis and regulation

In most species of moths, the sex pheromone is a blend of several components consisting of fatty acid derivatives, usually alcohols, aldehydes, or acetates [10,11]. Fatty acid precursors such as stearic and palmitic acid are processed by various combinations of key enzymes, mainly chain-shortening enzymes, desaturases, reductases, acetyl transferases and oxidases [12–14]. Limited chain-shortening enzymes reduce the number of carbon atoms and desaturases introduce one or more double bonds at specific locations along the carbon chain, whereas reductases, acetyl transferases and oxidases are responsible for the functional group carried by the pheromone component. Among these enzymes, the desaturases have been extensively studied as they represent excellent models to study several aspects of enzymatic desaturation: in contrast to most desaturases found in the animal kingdom, moth desaturases generate unique unsaturated fatty acyl-CoA esters of variable chain lengths, different positions of unsaturations and either the ordinary Z or the unusual E double bond geometry.

The sex pheromone blend of *S. littoralis* was first described by Nesbitt et al. (1973) [15] and further completed by Campion et al. (1980) [16] and Martinez et al. (1990) [17]. In addition to the major pheromone component, Z9,E11-14:OAc, female glands have been shown to contain, depending on the strains, other minor components: Z9,E12-14:OAc, 14:OAc, E11-14:OAc, Z11-14:OAc, Z9-14:OAc, E10,E12-14:OAc [18]. Each of these chemicals is constituted by a linear C14 chain containing one or two unsaturations and an ester-type functional group. Their biosynthesis has been depicted [17,19,20] and revealed a complex system of desaturases, with the evidence of a multi-catalytic Δ11 desaturase (Fig. 1). Biosynthesis of the pheromone components occurs by combined chain-shortening and desaturation reactions of acyl-CoA intermediates: Δ11 desaturation of palmitic acid to Z,11-16:acid and that of myristic acid to both Z and E,11-14:acids, Δ9 desaturation of E,11-14:acid to Z9,E11-14:acid and Δ10,12 desaturation of Z,11-14:acid to E10,E12-14:acid, followed by reduction and acetylation to generate the acetate function (Fig. 1). Heterologous expression in a yeast system revealed that the Δ11 desaturase of *S. littoralis* is a bifunctional enzyme with both Δ11 and Δ10,12 desaturation activities. Such multi-functional desaturases have been evidenced in other moths and represent a new sub-family of lepidopteran desaturases [13].

Moth sex pheromone biosynthesis follows a daily rhythm. *S. littoralis* females usually emit their pheromone 2–3 h into the scotophase [21], presumably under the regulation of a circadian clock [22]. As in other moths, the pheromone biosynthesis is controlled by a neurohormone, the *pheromone biosynthesis activating neuropeptide* (PBAN) [13], and the gene encoding the PBAN pre-prohormone has been cloned in *S. littoralis* [23]. This neurohormone is produced by the sub-oesophageal ganglia and its rhythmic release controls the rhythm of pheromone production. PBAN activates pheromone biosynthesis through binding with its receptor, a G-protein coupled receptor expressed at the membrane of pheromone gland cells and classified with the vertebrate subfamily of neuromedin U receptors [24]. The PBAN receptor activation leads to up-regulation of key enzymes in the biosynthesis pathway (for review see Ref. [25]). In diverse species, PBAN has been proposed to control pheromone biosynthesis by regulating a step in or prior to fatty acid biosynthesis [26,27], but in other species including *S. littoralis*, PBAN has been shown to stimulate the reductase step [17,28]. Although no PBAN receptor has been yet identified in *S. littoralis* pheromone gland, a gene encoding a functional PBAN receptor-like protein has been cloned from *S. littoralis* larvae, suggesting that PBAN-like neuropeptides, like those encoded by the pre-pro PBAN hormone, may regulate numerous physiological events, such as melanization and diapause, during the larval stages [29].

3. Molecular mechanisms of pheromone reception in male moth antennae

3.1. The pheromone-sensitive sensilla are specialized in pheromone detection

Pheromones are airborne molecules and are thus detected by the insect olfactory system: the antennae (Fig. 2). Antennae carry innervated and perforated hair-like structures called sensilla that house the olfactory receptor neurons (ORNs). Some of these sensilla are specialized in pheromone detection, and such pheromone-sensitive sensilla are typically the most abundant and the longest ones on the male antennae (with the exception of some scarce long mechanosensory sensilla), improving the detection of minute amounts of sexual signals. Pheromone-sensitive sensilla

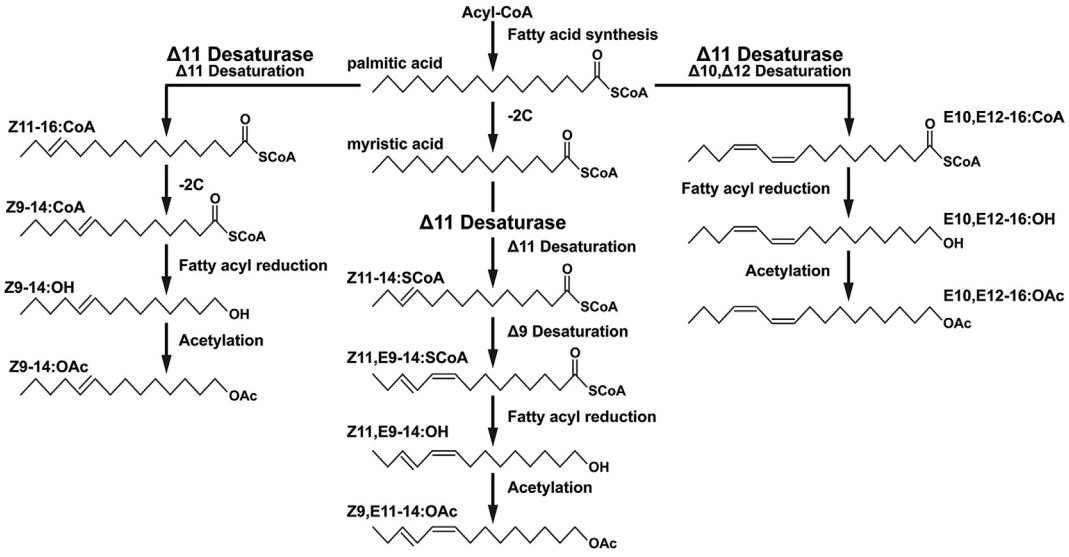

Fig. 1. Biosynthesis of Z9,E11-14:OAc, Z9-14:OAc and E10,E12-16:OAc, three components of the *Spodoptera litoralis* pheromone blend, via a multi-functional Δ11 desaturase (Adapted from Ref. [13]).