Revelations on the Regulatory Mechanisms in Moth Sex-Pheromone Signals

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Abstract The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis, but the application of this knowledge in pest management has had only marginal success, possibly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Sexual encounters in moths are initiated by the release of a unique blend of volatile organic compounds, the sex pheromones, by one sex, to attract conspecifics and signal receptivity for mating. After mating, pheromone biosynthetic activity in females is reduced, calling behavior ceases and oviposition is enhanced. Both post-mating responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. This review examines the research trend concerning mating behavior in moths by appraising the available information revealed by molecular, genomic, phylogenetic and transcriptomic studies on the mechanisms that up-regulate sex-pheromone production in receptive females and down-regulate after mating. The review concludes by examining future research directions needed to enhance our present-day knowledge concerning these regulatory mechanisms so as to reach a level of understanding that will facilitate its utilization for pest management.

1 Overview

The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis, but the application of this knowledge in pest management has had only marginal success, possibly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Sexual encounters in moths are initiated by the release of a unique blend of volatile organic

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compounds, the sex pheromones, by one sex, to attract conspecifics and signal receptivity for mating. After mating, pheromone biosynthetic activity in females is reduced, calling behavior ceases and oviposition is enhanced. Both post-mating responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. This review examines the research trend concerning mating behavior in moths by appraising the available information revealed by molecular, genomic, phylogenetic and transcriptomic studies on the mechanisms that up-regulate sex-pheromone production in receptive females and down-regulate after mating. The review concludes by examining future research directions needed to enhance our present-day knowledge concerning these regulatory mechanisms so as to reach a level of understanding that will facilitate its utilization for pest management.

2 Moth Behavior and Regulation of Pheromone Signals

Insects as a group have demonstrated evolutionary resilience through their phenomenal reproductive success. Understanding the behavioral adaptations of their mating and post-mating responses would contribute considerably to our knowledge concerning the evolutionary significance of these processes and how we can intervene to disrupt mating for use in pest management. The understanding of chemical communication in Lepidoptera, particularly in moths, has advanced greatly over the last half-century including sex-pheromone identification and synthesis but the application of this knowledge in pest management has had only marginal success, mostly for the use in population monitoring and perception-disruption, the latter only for a select number of pests (Welter et al. 2005). In many of the species, sexual encounters are initiated by the release of a unique blend of volatile organic compounds, the sex pheromones, to attract conspecifics and signal receptivity for mating. In Lepidoptera, pheromone release is characterized by calling behavior in which the female extrudes the ovipositor tip exposing the pheromone gland to release the sex pheromone blend. In most moths this blend is derived from downstream products of fatty acid biosynthesis in the pheromone gland which is located between the ultimate and penultimate terminal segments of the abdomen (see reviews Rafaeli 2002, 2009, 2011).

The driving force behind reproductive isolation and species differentiation in insects, particularly those highly dependent on sex-pheromone components for mate location, lies within the variation in pheromone components. This diversity is reflected in a variation of the biosynthetic pathways and the enzymes that are involved. In addition, further complexity and diversification of sex-pheromone communication is attained through multiple component systems integrated with stereoisomer composition (Bjostad et al. 1987; Abad et al. 2001). A major class of sex-pheromones produced by female moths is the C_{10}–C_{18} unsaturated, acyclic, aliphatic compounds that contain an oxygenated functional group, such as aldehydes, alcohols or acetate esters. These pheromone components are synthesized *de novo* in

the pheromone gland, from acetyl-CoA involving acetyl-CoA carboxylase (ACCase) and fatty acid synthetase producing fatty acids (Jurenka 2003; Rafaeli and Jurenka 2003; Tsfadia et al. 2008). The production of fatty acids is followed with the double bond positioning as a result of the action of unique desaturases to make mono- and di-unsaturated fatty acids (Tillman et al. 1999). Chain shortening through chain-shortening enzymes that make the specific chain-length fatty acid and, depending on the functional group of the pheromone, a fatty-acyl reductase, an acetyl-transferase or an alcohol oxidase will produce the final pheromone blend. The order in which these enzymes act and the stereo-specificity of the enzymes involved determines the final pheromone components produced (Jurenka 2003). Using comparative transcriptomics and EST development from cDNA libraries, followed by BLAST searches (NR and Swissprot databases), a large number of candidate genes in the Lepidopteran pheromone biosynthetic pathways have been identified but many await further exploration by functional expression studies and/or RNAi technology (Strandh et al. 2008; Vogel et al. 2010; Gu et al. 2013; Jung and Kim 2014).

After mating, pheromone biosynthetic activity in females is reduced and calling behavior ceases. Females do not re-mate for the remainder of the night in some cases, and permanently in others (see reviews Rafaeli 2011; Hanin and Rafaeli 2014). In addition, oogenesis and the rate of ovulation and oviposition increase in mated females (Soller et al. 1999; Jin and Gong 2001). The absence of released pheromone effectively terminates male orientation to females, indicating female non-receptivity. Both responses i.e. reduced receptivity and increased oviposition, can be theoretically visualized as systems that could be manipulated to the advantage for pest management. If females receive the signal for non-receptivity or oviposition prematurely and permanently, mating will not occur and viable eggs will not be laid, in effect unfertilized eggs will be aborted and for those insects with one vitellogenic cycle, essentially the affected female will not be able to produce progeny.

3 Revelations from Molecular, Genomic, Phylogenetic and Transcriptomic Studies

3.1 PBAN & PBAN-Receptors: Up-Regulation

Regulatory mechanisms of sex-pheromone biosynthesis involving endocrine and gene regulation have progressed relatively slowly due to the complexity of the biosynthetic cascades that result in the production of the pheromone components. Over 20 years ago, a neurohormone, Pheromone Biosynthesis Activating Neuropeptide (PBAN) was identified, using classical endocrine methodology, as a regulatory hormone in the biosynthetic pathway of some moth sex-pheromones (Raina and Klun 1984). Since the discovery of PBAN, a steep rise in publications on regulation of pheromone production was witnessed for the next 10 years but after that it has

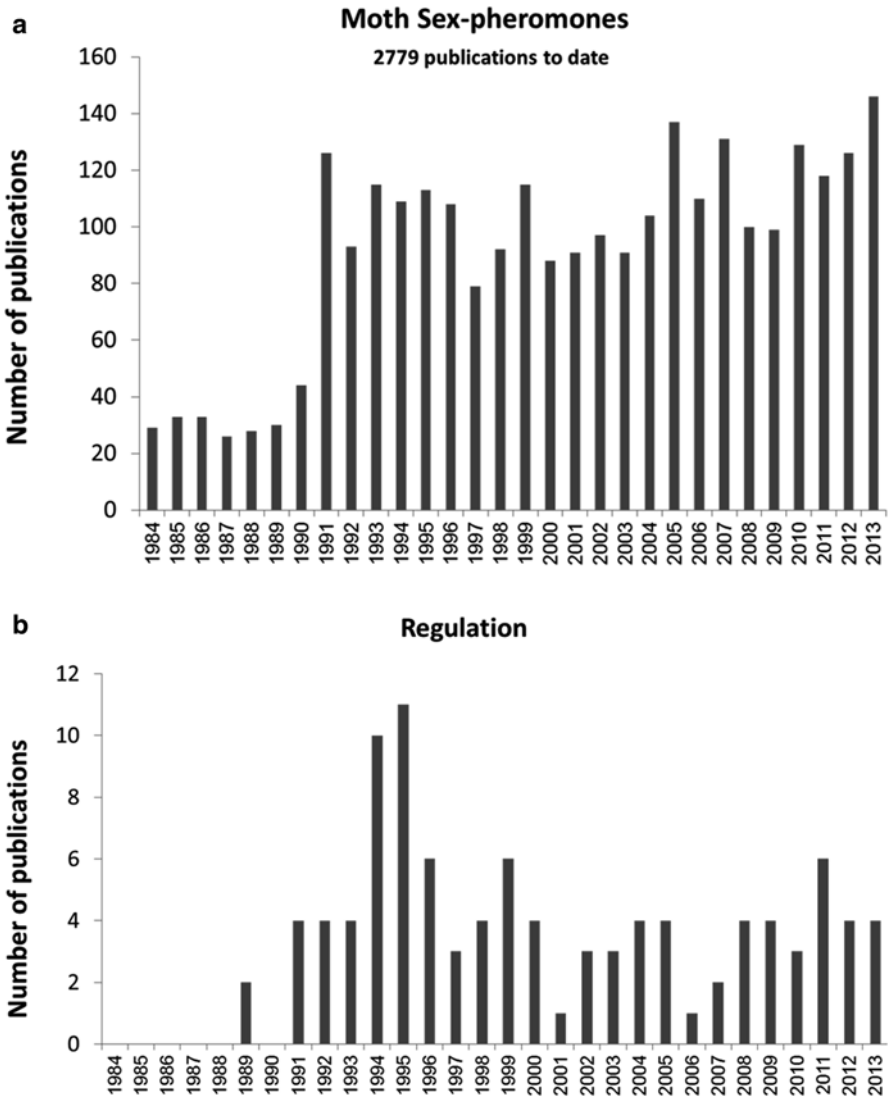

Fig. 1 Number of publications in the past 30 years, after the discovery of PBAN, on moth sex pheromones (**a**) and regulation of moth pheromones (**b**) (Search performed via ISI Web of Science, 2014)

declined to a steady state despite rapid advances in moth genomics. Such a decline however, was not observed in publications concerning sex pheromones in moths in general (Fig. 1) and probably reflects the complex nature of these regulatory mechanisms.

In *Helicoverpa armigera*, PBAN up-regulates the production of malonyl coenzyme A from acetate by the action of acetyl coenzyme A carboxylase (ACCase)

(Eliyahu et al. 2003; Tsfadia et al. 2008; Hanin et al. 2008). Additionally, ACCase inhibitors and commercial, grass-selective herbicides such as 2-aryloxyphenoxypropionate (e.g., diclofop) and cyclohexandione oxime (e.g., tralkoxydim) inhibit the PBAN-stimulation of pheromone production in several moth species (*H. armigera*: Eliyahu et al. 2003; Hanin et al. 2008; *H. armigera & Plodia interpunctella*: Tsfadia et al. 2008; *Cydia pomonella*: Kleinman 2008; Fig. 2), particularly the stimulation of malonyl-CoA incorporation into the main pheromone component (Tsfadia et al. 2008).

The above studies provide irrefutable support to the hypothesis that the PBAN-induced rate-limiting step for sex-pheromone biosynthesis lies within the activation of this enzyme. Moreover, the results indicate that moth pheromone biosynthesis may also be targeted by these herbicides and suggest that they may be used as a novel means of manipulating moth pest populations of important agricultural crops. Future research, directed at formulating methods of dissemination of these compounds amongst targeted pest populations, will have to be undertaken.

PBAN is photoperiodically released from the corpora cardiaca into the hemolymph during scotophase in response to circadian cues (Fabrias et al. 1994; Jacquin et al. 1994; Nagalakshmi et al. 2007; Bloch et al. 2013). Circulating PBAN stimulates pheromone gland cells directly to produce and release sex pheromone. Its pheromonotropic role has been well elucidated in many Lepidoptera (Rafaeli 2011). PBAN, a 33-amino acid *C*-terminal amidated peptide was subsequently shown to be

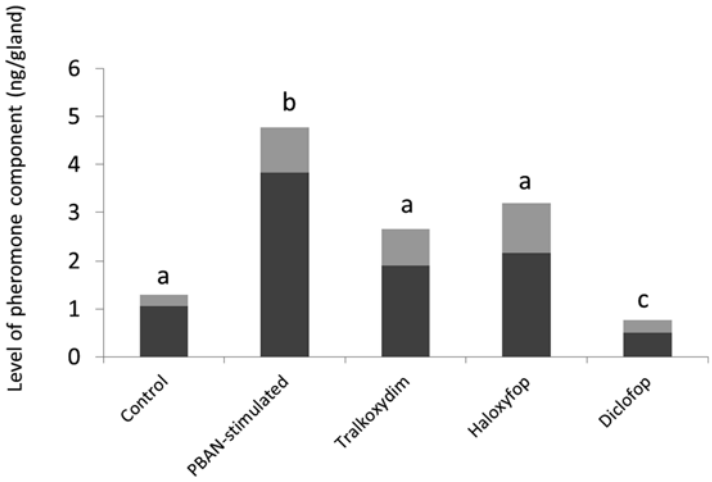

Fig. 2 Reduction in levels of the main pheromone component of *Cydia pomonella* females, (E,E)-8,10 dodecadienol (codlemone), after inhibition with various herbicides. Decapitated (24 h) females were injected with either Control = water; PBAN-stimulated = synthetic HezPBAN (5 μM); or PBAN + herbicide (100 μM Tralkoxydim, Haloxyfop or Diclofop). Pheromone titers were quantified using GC and tridecenyl acetate as internal standard. *Light shaded areas* show extent of SEM, n = 4–27 replicates. *Different letters* indicate a statistically significant difference (two tailed ANOVA, Fisher protected least significance difference test $P \leq 0.05$) (Unpublished data, Kleinman 2008)

a member of the pyrokinin (PK)/PBAN family of peptides characterized by a common *C*-terminal FXPRL amide motif (X = G/S/T/V), the minimum sequence necessary to elicit activity. It is produced by neurosecretory cells within the subesophageal ganglion and the gene encoding PBAN has post-translational processing sites that could produce four additional PBAN-gene neuropeptides: PGN-24 (pyrokinin-like/diapause hormone), PGN-18, PGN-8 and PGN-7 all having the FXPRLamide motif. Moreover, it was shown to be widespread throughout insects where it has diverse functions (Rafaeli 2011). In the moth *H. armigera*, in addition to its presence in pheromone glands, PBAN-R protein and gene transcript are detected in membranes of neural tissues and therefore indicate a possible neural function, perhaps as neurotransmitter (Rafaeli et al. 2007).

PBAN activity on pheromone gland cells causes an influx of extracellular calcium that promotes the production of intracellular cyclic-AMP through the involvement of G proteins, indicating its association with a G-protein coupled receptor (GPCR) (Rafaeli and Gileadi 1996; Rafaeli 2002). GPCRs belong to the largest superfamily of membrane-bound receptors and have in common a topology based on seven-transmembrane α-helical domains, coupling to heterotrimeric G proteins (Gαβγ). We were the first to identify a PBAN-receptor from moths (HezPBAN-R) (Choi et al. 2003). At that time, due to the lack of genomic information on moth model species, our strategy involved homology comparisons of the ligand PBAN, assuming that ligand and receptor co-evolve. PBAN's similarity to the vertebrate neurohormone, neuromedin U suggested that the vertebrate neuromedin U receptor could be used as a basis for the search for the PBAN-R in moths. The gene was thus identified based on sequence identity to a group of GPCRs from the *Drosophila* genome that is homologous to neuromedin U receptors in vertebrates.

The full-length PBAN receptor was subsequently cloned and expressed in Sf9 insect cells and shown to mobilize calcium in response to PBAN. Subsequent RNAi silencing of the PBAN-R gene transcript in *H. armigera* females significantly reduced the level of pheromone produced by females during their peak pheromone production (7th hour during the scotophase) (Fig. 3), however, preliminary tests performed in small cages did not successfully demonstrate a reduction in mating (Hanin and Rafaeli, unpublished). This may be the result of close encounters between males and females in the small cages. Thus, demonstration of the effectiveness of RNAi technology on mating disruption in the field is yet to be resolved.

Since its discovery, PBAN was known to be present in the suboesophageal ganglion of both males and females but its role in the males remained a mystery. The identification of the gene transcript for the PBAN-R in the aedeagus of males, a tissue homologous in position to the female pheromone gland initiated a study aimed at elucidating PBAN's role in the male moth. The aedeagus is connected to lateral valvae containing hair-pencils that are displayed during courtship and are implicated in the dissemination of male putative sex-pheromone components (Birch 1974). Male hair-pencil complexes contain fatty-acid components and alcohol components that bear similarity to the female sex-pheromone components. We demonstrated a distinct diel periodicity in a number of these components and showed the influence of PBAN on their levels (Bober and Rafaeli 2010). In addition, gene

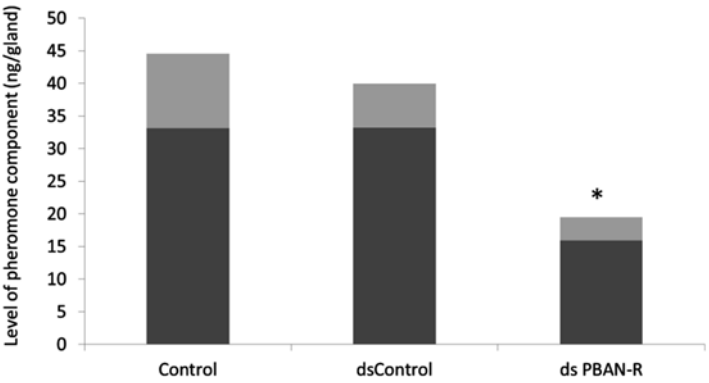

Fig. 3 Reduction in levels of the main pheromone component, Z-11 hexadecenal of *H. armigera* females after silencing of the PBAN-R gene transcript. Females were injected with either Control = diethyl polycarbonate-treated water injected females; dsControl = λphage DE3ea59 dsRNA; dsPBAN-R = PBAN-R dsRNA during the first photophase (photophase after emergence). Pheromone titers were quantified during the 7th hour of the second scotophase as published previously (Hanin et al. 2012). *Light shaded areas* show extent of SEM, n = 7–12 replicates. *Asterix* indicates a statistically significant difference (two tailed ANOVA, Fisher protected least significance difference test $P \leq 0.05$) (Unpublished data, Hanin and Rafaeli)

expression of the PBAN-R transcript in male hair-pencil complexes was shown to be differentially up-regulated after emergence of the adult moth from the pupal stage (Bober et al. 2010). Subsequently RNAi knockdown of the PBAN-R gene transcript, revealed its function in regulating the levels of key male sex-pheromone components (Bober and Rafaeli 2010) thus associating PBAN as a regulatory neuropeptide of male hair-pencil sex-pheromone components, as was shown in females.

Since the identification of the HezPBAN-R, a considerable number of additional receptors in this family have been sequenced based on homology studies or identified from genome sequencing projects (Bober and Rafaeli 2009). Thus, with the advances in sequence technologies and the emergence of Lepidoptera as model organisms, the physiological role of PBAN; its target site; its G-protein coupled receptor and the signal transduction involved in the induction of downstream enzymatic events have been elucidated. Although GPCRs in insects are regulators in many essential functions involved in survival and propagation and are therefore an attractive target for insecticide design, the rational designing of potent antagonists has been hampered by the lack of structural information on receptor-bound ligands. Some progress has been attained on the development of peptidomimetic analogs of the PK family of peptides with enhanced biostability and bioavailability and with the potential to disrupt the reproductive process in insect pests of agricultural importance (Nachman 2014). However, the peptides in the PK family do not exhibit species specificity and experiments have shown that all of the functions listed can be stimulated by more than one peptide. Therefore, due to their ubiquitous and multifunctional actions, the PK family of peptides and their receptors are unlikely candidates for future targeted application against insect pests since they bear the possibility of also affecting beneficial insects.

3.2 Sex-Peptide (SP) and SP-Receptors: Down-Regulation

During copulation male insects transfer sperm and seminal fluid to females. Within the seminal fluid are peptides (Acps) produced by the male accessory glands that are implicated in influencing the behavior of females after mating (Kingan et al. 1995; Wolfner 2009; Kubli 2003; Gillott 2003). In *Drosophila*, where this aspect of reproductive biology has been most thoroughly investigated, mating-induced changes in females, from sperm and seminal fluid transfer during copulation, induce both short- and long-term effects (Ottiger et al. 2000). Of particular importance in eliciting post-mating non-receptivity and increased fecundity in *Drosophila* is sex-peptide (DrmSP or Acp70A) (Chen et al. 1988).

DrmSP is a 36-amino-acid peptide produced in the male *Drosophila* accessory gland. During mating the peptide is transferred with the seminal fluid and passes from the female reproductive tract into the hemolymph, ultimately acting directly on targets in the nervous system of females (Kubli 2003; Hasemeyer et al. 2009; Yang et al. 2009). Ectopic expression of DrmSP and injection of purified peptide into virgin females decreased receptivity to males and stimulated egg production for 1–2 days in *Drosophila melanogaster*, indicating its major role in both short- and long-term post-mating effects (Chen et al. 1988; Nakayama et al. 1997; Aigaki et al. 1991). The long-term actions of DrmSP require the presence of sperm and involve binding to sperm via *N*-terminal sequences and subsequent cleavage, releasing the *C*-terminus of the peptide that then enters the hemolymph (Chapman et al. 2003; Liu and Kubli 2003; Peng et al. 2005). The mode of action of DrmSP has been partially determined and has been shown to be allatotropic in *D. melanogaster*, stimulating the release of juvenile hormone (JH-III-bisepoxide) from the corpora allata thereby modulating oocyte maturation and egg-laying (Moshitzky et al. 1996; Soller et al. 1997, 1999).

Similar to its allatotropic effect in *D. melanogaster*, synthetic DrmSP also stimulates JH-II production in isolated corpora allata from virgin female *H. armigera in vitro* in a dose dependent manner (Fan et al. 1999, 2000). These observations are significant in light of previous reports demonstrating the increase in JH titers in females after mating and the involvement of JH in mating-induced suppression of pheromone production (Ramaswamy et al. 1997; Delisle et al. 2000). Consistent with these findings is the observation that JH as well as fenoxycarb (a JH analog) inhibit pheromone production in female *H. armigera* and reduce transcript and protein levels of the PBAN-receptor (PBAN-R) (Rafaeli and Bober 2005; Bober et al. 2010). Nagalakshmi et al. (2007) showed that PBAN levels in the hemolymph of virgin *H. armigera* females are drastically reduced after mating. Significantly, synthetic DrmSP and truncated fragments of DrmSP injected into the hemolymph of virgin female *H. armigera* lead to the termination of PBAN-stimulated pheromone production (Fan et al. 1999, 2000) and inhibition of calling behavior by female moths (Hanin et al. 2012).

The male accessory glands of *H. armigera* contain proteins that result in the termination of pheromone production when injected into the hemolymph of virgin

females as crude extracts. Some of these accessory gland proteins are immunoreactive with antibodies against DrmSP and have been shown to be pheromonostatic in *H. armigera* (Nagalakmish et al. 2004). Despite the presence of DrmSP-like activity and immunoreactivity in the moth and after many attempts to identify the SP-like factor from the moth accessory glands, SP has only been identified in *Drosophila*, notwithstanding the large body of genomic data available to date in several species of insects.

Nagalakshmi et al. (2007) reported that different sets of DrmSP-like immunoreactive peptides (HeaSP) in the moth are up-regulated during scotophase in male accessory glands and the central nervous system (CNS) of mated females. These findings suggested that target receptors for these seminal-peptides reside in the pheromone gland as well as the CNS of females. Indeed, a receptor for DrmSP (SP-R, CG16752) was identified in the reproductive tract and nervous system of *D. melanogaster* (Yapici et al. 2008). Mutants that lacked this receptor failed to respond to DrmSP and continued to show virgin behavior even after mating. Comparative genomic studies that followed the latter discovery, have revealed the presence of SP-R genes in several *Drosophila* species, *Aedes* and *Anopheles* mosquitoes, *Tribolium* and the moth *Bombyx*. On the basis of sequence homologies deposited in the GenBank, we identified a putative SP-R in *H. armigera* (HeaSP-R) with 99 % homology to the *Bombyx mori* SP-R (Hanin et al. 2011).

To determine whether this receptor is involved in mating behavior, we conducted a differential expression study of this receptor comparing gene expression levels in relation to different photoperiods, sex and mating status of the moth. Photoperiod and mating influence SP-R gene expression levels and sexual dimorphic changes were observed in neural tissues due to the different physiological states. After mating SP-R transcript levels in female neural tissues and pheromone glands are up-regulated. Physiological studies *in vivo* confirm the up-regulation of gene expression levels in pheromone glands isolated from mated females (Hanin et al. 2011). Thus, these studies confirmed that the SP-R in the moth plays a role in mating behavior.

Recent studies (Kim et al. 2010; Poels et al. 2010), showed that heterologously expressed *D. melanogaster* SP-R responded to *D. melanogaster* myoinhibitory peptides (MIPs). MIPs are pleiotropic peptides that belong to the $W(X)_6W$ amide peptide family that were initially named based on their ability to inhibit spontaneous muscle contractions of insect gut and oviduct but have also been shown to suppress ecdysteroid production in the prothoracic gland, and they control salivary gland activity in ticks (see review Hanin and Rafaeli 2014). Due to the response to MIPs, Kim et al. (2010) claim that the SP-R is the ancestral receptor for MIPs whilst SP, that contains a similar conserved sequence of tryptophan residues but with different spacing and positioning ($W(X)_8W$), adopted this receptor in the course of evolution. However, MIPs failed to mimic the SP post-mating responses *in vivo* when tested either through generating a transgene or through the injection of varying concentrations of synthetic MIPs into the hemocoel of virgin females (Kim et al. 2010).

Utilizing RNAi technology we were able to silence the moth HeaSP-R expression by 50–60 % which effectively prevented *in vivo Drm*SP-suppression of pheromone production and calling behavior. However, sex pheromone production by

mated, silenced females remained low, comparable to mated, normal females, thereby indicating the probable involvement of additional factors in the suppression of sex pheromone production after mating in the moth. None-the-less, mated, silenced females failed to increase their oviposition rates as is normally observed in mated females, and their behavior in that respect did not differ from that of virgin females indicating that the SR-R plays a crucial role in the post-mating behavioral changes in the female moth that influence oviposition.

4 Future Research Directions and Applications in Pest Management

As discussed above, after mating the female moth undergoes drastic behavioral changes in particularly pertaining to reproductive behavioral changes. In this context, pheromone biosynthesis, receptivity and calling behavior are terminated and newly mated females actively reject subsequent attempts of males to mate with them. Concurrently, oogenesis and the rate of ovulation and oviposition increase in mated females. As observed in many insect species during copulation, males transfer seminal peptides (Acps) that are implicated in these post-mating responses. It is probable that these processes are regulated by more than one factor (Rafaeli and Hanin 2013) but it is unclear how they are associated. Several Acps have been identified to various degrees in diverse insect species. A recent study demonstrated that allatotropic activity in males influences the production and consequent transfer of juvenile hormone to females during copulation (Hassanien et al. 2014). Not many studies have been undertaken focusing on defining the physiological, biochemical and molecular effects of these Acps and their precise roles in the manifestation of the observed behavioral changes in the mated female. Moreover, the interactions of seminal peptides with the regulatory peptides controlling receptivity and the biosynthesis of pheromones have not been studied. I believe that with the present day rapid progress in genomic techniques, we will be able to identify and elucidate the roles of several of these seminal peptides in model insects that are serious agricultural pests.

A number of well-established strategies for insect population suppression such as the Sterile Insect Technique (SIT) are based on understanding and exploiting aspects of the reproductive behavior of the target insect (Knipling 1979). The SIT is based on introducing large numbers of sterile males to reduce the overall growth of a target population. Wild, fertile females, once mated to sterile males will have reduced reproductive output depending upon aspects of their reproductive behavior. The impact of mating with a sterile male is most effective when females mate only once, which effectively removes the female permanently from the population. Where females mate multiple times, implementing the SIT becomes more demanding since a single sterile mating only temporarily reduces the effective population size and subsequent mating with wild fertile males will result in progeny. Some of

the most successful SIT programs are directed against insects whose females have low re-mating frequencies, e.g. *Ceratitis capitata* and *Pectinophora gossypiella*. If the frequency of female re-mating can be reduced or eliminated in polyandrous species then the SIT could be applied and would be more effective and efficient. Understanding the molecules and mechanisms responsible for these post-mating behaviors is therefore important if reproduction-based control strategies such as the SIT are to find more widespread use.

Exploitation of the mating process for transmitting desired control molecules into females could be envisioned as the "next generation" of insect pest strategies. Transgenic insect technology is a mature and robust technology whose application is limited largely only by the amenability of insects to embryo-microinjection and post-injection manipulation (O'Brochta and Handler 2008). With current transgenic technologies there are few, if any, genetic and biochemical barriers to integrate genes into insect genomes (O'Brochta and Handler 2008). Given the maturity of transgenic insect technology, it is not surprising to find multiple examples of the technology being applied to insect population control and eradication efforts. For example, the control of *P. gossypiella* by the SIT is being enhanced through the release of sterilized insects containing a dominant visible transgene (Simmons et al. 2007) and transgenic *Aedes aegypti* are being used to suppress wild populations of this species (Enserink 2010; Miller 2011). Improved transgenic technologies to facilitate the creation of males that have a potent mixture of accessory gland proteins (and conceptually transmitting other physiologically active specific compounds) capable of strongly inhibiting female mating behaviors or female fitness will provide solutions for the manipulation of pest species in the future, once transgenic methods are accepted and validated.

5 Concluding Remarks

This review endeavored to assess the available literature concerning up- and down-regulation of Lepidopteran sex-pheromone production. Targeting up-regulatory pathways involves the possible use of herbicides that will influence enzymatic function; the use of RNAi silencing or peptide mimics. Difficulties in the success rate for RNAi silencing have been reported, particularly for the Lepidoptera, although in our research we have not encountered such difficulties. This could be attributed to our preliminary developmental expression studies in order to determine the ideal timing for silencing. On the whole, targeting PBAN or its receptor, is unlikely to produce specific effects on pest species alone due to the fact that it is multifunctional, it is present in most insect species, and its presence in both females and males. On the other hand, the unique presence of SP in *Drosophila* that shows activity in moths, whilst contrasting with the widespread presence of its receptor, presents a unique opportunity for its exploitation as a target against moth pest species. In this review, I have suggested the consideration of integrating insect transgenesis,

the sterile insect technique and SP for the development of an effective method to down-regulate the reproductive potential of pest moths.

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