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Chemical Communication between Male and Female Sex of *Bombyx mori*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Communication involves the systematic and symbolic exchange of information between entities. In insects, the most significant form of communication occurs between members of the same species. Silkworms primarily utilize pheromones for communication. Bombykol, the sex pheromone released by the female silk moth Bombyx mori, was first extracted by Adolf Butenandt. The biosynthesis of bombykol occurs in the pheromone gland, where various enzymes and genes participate in the biosynthesis process through an influx of extracellular Ca^{2+}. Bombykol-sensitive ORNs are located on the antennae of male moths, which perceive the bombykol released by female moths. These ORNs are so sensitive that even a single pheromone molecule can generate an electric signal in the male moth. Once the male moth detects the bombykol molecule, the Pheromone Binding Protein plays a crucial role in enabling it to bind to the receptor without disruption. Mori, B. The

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substance is rapidly eliminated from its receptor site and rendered inactive by phenomenol-degrading enzymes and alcohol oxidase genes. Numerous studies and tests conducted on B. mori's sex pheromone have yielded important insights into the molecular and genetic aspects for the chemical interactions with male and female silkworms, which including the synthesis, behavioral reaction, and the perception of bombykol.

Keywords: Communication; Bombyx mori; bombykol; sex pheromone; olfactory receptor neuron.

1. INTRODUCTION

Communication constitutes the systematic and symbolic exchange of information between entities [1]. The most important communication in insects takes place between the members of the same species; they use different signals to find mates, give warnings about danger, notify about food sources and to ward off predators or attract prey. The different types of insect communication are visual communication, tactile communication, auditory communication and chemical communication.

1.1 Chemical Communication

Chemical communication is possibly the most extensive communication mechanism among insects. In this type of communication, the emitter distributes chemical substances at the environment which are detected by other organisms. Semiochemical is any chemical that transmits information between organisms. Law and Regnier (1971) were the first to suggest the word semiochemical. The word comes from the Greek word "semeion," which meaning signal or sign. Allelochemicals (as defensive systems or alarm signals), pheromones (for locating a partner), and other chemical substances are among the various kinds utilized for communication.

1.2 Pheromone

Pheromones are chemical substances that is usually produced by an animal and serves especially as a stimulus to other individuals of the same species for one or more behavioral responses. The term “pheromone” was first coined by Peter Karlson and Martin Luscher (1959). The name is derived from the Greek words, *pherein* - “to transfer” and *horman* - “to excite”. Adolf Butenandt (1959) was the first to identify and synthesize Bombykol, the pheromone utilized by the female silkmoth *Bombyx mori* to attract a mate.

2. SEX PHEROMONES

“Sex pheromones are released by one sex only and trigger behaviour patterns in the other sex that facilitate in mating. They are most commonly released by females but may be released by males also. Around 150 female insect species have been discovered to release sex pheromone. Males roughly of 50 species have been discovered to release sex pheromones” [2]. “In Lepidoptera, sex pheromonal system is highly evolved. The male and female sex pheromones differ in their property and action. The female sex pheromone acts at a longer range, excites males to copulate and by and large is species specific. The male sex pheromones on the other hand act at short range and only lower the female’s resistance to mating. Insect sex attractants are the pheromones that have been studied the most” [3,4] International Silkworm Genome Consortium [5]. This is partly because the prospective application of attractants for pest management has a large financial value. Field research using attractant traps have produced some encouraging results, at least in certain regions. Interestingly, these pheromones are present in other insect orders as well as social insects. As a result, they will be treated according to the insect orders, beginning with the Lepidoptera, which have been studied the most [6].

3. SEX PHEROMONE OF SILKWORM

The sex pheromone of the silkmoth, *Bombyx mori* was the first pheromone to be chemically identified in 1959 and is considered as the most important semiochemical used in pest management. Adolph Butenandt and his colleagues set out to isolate and identify the chemical that causes attraction in the commercial silkworm moth *B. mori* in 1939. After 20 years of research, 12 mg of a chemical derivative was recovered from half a million virgin females, and the active pheromone was found in 1959 (Butenandt et al., 1959) but it took only one year to figure out its structure. “By using chemical analysis, Butenandt was able to discover the

molecular formula of bombykol to be $C_{16}H_{30}O$. The scientists determined the presence of an alcohol group using the relatively new technology of infrared spectroscopy, while other infrared absorptions revealed that the UV spectrum indicated the existence of conjugated double bonds. The identity of acetyl alcohol, CH3(CH2)15OH, was verified by Butenandt, formed via catalytic hydrogenation of bombykol, by mixing it with an actual sample and demonstrating that the melting point was unaffected. He then esterified bombykol with 4'-nitroazobenzene-4-carboxylic acid and used oxidative degradation with $KMnO_4$ to determine the position of the double bonds in the ester, resulting in the formation of ethane-1,2-dioic acid (oxalic acid), butanoic acid, and the 4'-nitroazobenzene-4-carboxylic acid ester of -hydroxydecanoic acid (identified as its methyl ester)" [7].

3.1 Behavioral Response of Female Prior to the Release of Bombykol

According to Biram Saheb et al., [8] males and females of the *B. mori* moth are nonfeeding in nature, they are unable to fly and are very anxious to mate immediately after emerging from the cocoon. Soon after the eclosion, the female moth fully expands their wings and protrudes the paired scent glands from the hindmost abdominal segment, exposing the actual odoriferous surface and then accomplishes the task of quick and efficient chemical substance liberation. After being touched or approached by a male, the female immediately retrieves the glands into their body.

3.2 Behavioral Response of Male after Perception of the Pheromone

Males are anatomically attracted to the source of pheromone when they are stimulated. The responding male displays typical alertness behaviours, such as cleaning its antennae, lifting its head, and vibrating its wings in smaller to bigger amplitudes to create a strong air current that runs parallel to the body's longitudinal axis in order to find the pheromone source. "An actively dancing male has been seen to be able to draw air from a distance of 4 cm from the frontal region of its head, while wing movements draw air from the sides, boosting the antennae's efficacy" [9]. Males who are highly excited and extremely energetic run-in circles, semicircles, and zigzag paths, with a clearly bent abdomen, coming into contact with the female genitalia and ultimately taking on an oriented turn. "Throughout the procedure, the male is led in the direction of the female by distinctive dancing motions, and he touches any area of the female body while dancing, eventually establishing contact with the female genital area and succeeding in copulation by trial and error" (Samson and Saheb, 1990). "The locking of the male genitalia with the female is sufficiently firm that disengagement requires manually sliding out or pulling back the male from the female. Male moths with intact antennae and blackened eyes were found to find females more quickly than males without antennae" [10]. Significant reduction in searching ability was seen when both the antennae of the males were removed [11]. Copulation took a much longer duration and copulates only when placed in closer proximity [12]. In the absence of air current, the normal males can locate the female-only up to a distance of 4 cm otherwise the female can be detected from a distance of 25 - 150 cm with moving air current. Searching activity cannot be elicited in odor-free air currents. The males with intact stretched and fully expanded wings can locate the female within a few seconds (25 ± 10) even from a distance of 10 cm under normal atmospheric conditions, a much longer time is required to locate the female for the males with defective wings [13].

3.3 Mechanism of Perception of Sex Pheromone

"Pheromone molecules are spread-out on-air currents as female moths flutter their wings. After picking up the scent, the male flies up the plume of the female's pheromone in a zigzag pattern, moving towards the direction of increasing pheromone concentration" [7]. "The silk moth *B. mori* possesses the simplest sex pheromone system known to science, in which a single pheromone ingredient unleashes programmed pheromone-source searching. While the other moth species, have a more complicated mechanism where several pheromone compounds must be blended for behavioral responses in males. Bombykol [10,12-(*E*, *Z*)-hexadecadien-1-ol] and bombykal [10,12-(*E*, *Z*)-hexadecadien-1-al] are the two pheromone components produced by the female silk moth in a ratio of 10:1 in the pheromonal gland" [14,15]. "Bombykol, on the other hand, is sufficient to cause male moths to seek out pheromone sources on their own" [16]. Researchers can easily correlate molecular and neural functions with behavioral responses because to a simple input-output relationship between bombykol and pheromone-source searching behavior. Internal

factors can also influence pheromone-source searching behavior along with non-bombykol odorant stimuli [17], circadian rhythms, and related serotonin levels in the brain. [18]. “Moths use a pair of antennae on their heads to detect odorants. Moth antennae often modify their form in order to detect odorants, particularly in males. The silk moth's bipectinate antenna is composed of two pairs of side branches that branch out of the antennal stalk. The antennae's cuticular specialization, the olfactory sensillum, is home to the olfactory receptor neurons (ORNs). Their dendrites extend into the sensillum, while their axons project into the antennal lobe, the brain's first olfactory region (AL). On the inner side of the antennal branches, most olfactory sensilla are organized in a regular array” [19]. “This structure acts as a molecular filter, catching pheromone molecules as they pass over the antenna” [20]. There are 25,000 olfactory sensilla on the male antenna, which are divided into four morphological types: long trichodea, medium trichodea, basiconica, and coeloconica. The majority (75%) are long trichodea sensilla [19], which contain a pair of ORNs tuned to detect bombykol and bombykal [14]. ORNs tuned to general odorants, such as plant-derived odorants, are housed in other types [21]. “Bombykol-sensitive ORNs are so sensitive that even a single pheromone molecule can elicit an electrical signal, according to electrophysiological studies done by using radiolabeled bombykol” (Kaissling, 1978). “However, these receptor neurons have a high degree of selectivity, responding to only one pheromone component for most instances” [22]. “Sex pheromone receptors detect pheromones released at the dendritic membrane of ORNs. Sex pheromone receptors are members of the insect OR family, according to the first identification of moth sex pheromone receptors from *B. mori”* [23]. “In the almost entirely sequenced genome of *B. mori*, 66 OR genes were identified” [5,24]. “Five of these ORs (BmOR1, 3, 4, 5, 6) belong to the mentioned cluster and are expressed solely or predominantly in male adult antennae” [25]. “BmOR1 and BmOR3 has been identified as sex pheromone receptors in *B. mori*, suggesting that the precise molecular recognition of BmOR1 and BmOR3 enables the particular detection of pheromone components by corresponding ORNs” [26].

3.4 Biochemical Pathway of Synthesis of Bombykol

Most of the components of moth pheromones are produced in the pheromone gland (PG) through modifications of fatty acid biosynthetic pathways, starting from acetyl-CoA. The pheromone biosynthesis activating neuropeptide (PBAN), which is a neurohormone produced by a cephalic organ called the suboesophageal ganglion, stimulates the synthesis of sex pheromones in the PG by increasing the influx of extracellular Ca2+. The wide variety of small molecules used by Lepidoptera insects as chemical signals for sexual maturation reflects the incredible diversity of these insects. Females often use these compounds to attract males of the same species. The precursor of most lepidopteran sex pheromones is produced in the Pheromone Gland (PG), a specialized organ situated between the eighth and ninth abdominal segments, from acetyl-CoA through fatty acid synthesis, which is a specialized organ located between the eighth and ninth abdominal segments. The species specificity of these complex sex pheromones is determined by the structural and compositional variations in the pheromone blends, which are generated through enzymatic processes that modify the chain length, degree of unsaturation, and/or carbonyl carbon reduction (1-3)” [27].

3.5 Key Components Essential to silkworm Sex Pheromone Biosynthesis

A. Pheromone Gland:

“Moth PGs are functionally differentiated organs that originate in the intersegmental membrane between the eighth and ninth abdominal segments, they are fundamentally uniform in their location and histological composition regardless of species” [28]. In B. mori, the PG can be recognized as a pair of sacs on the ventrolateral side that can be turned inside out. In the PG cells of B. mori, multiple cellular events occur at the same time before and after eclosion, leading to the creation of the sex pheromone, bombykol (also referred to as E, Z-10,12-hexadecadien-1-ol). This series of events is known as "pheromonogenesis" [29].

B. Hormonal Regulation of Moth Sex Pheromone Production:

Since mating is often limited to a specific phase of the photoperiod, the biochemical processes that comprise sex pheromone biosynthesis must be precisely regulated. In most moth species, these processes are regulated by PBAN, a neuropeptide consisting of 33–34 amino acids

with the core C-terminal pentapeptide, FSPRL amide initially purified and sequenced from the corn earworm, *Helicoverpa zea* [30], and the silk moth, *B. mori* [31]. In *B. mori*, the pheromonotropic effects of PBAN are mediated by a G protein-coupled receptor (GPCR), termed PBAN receptor (PBANR), that is predominantly expressed in the PG cells.

C. Requirement of Extracellular Ca2+

Extracellular Ca^{2+} is essential for transforming the PBAN signal into the biological response of sex pheromone production. The PBAN stimulus causes an influx of extracellular Ca^{2+} into PG cells. Moreover, the pheromonotropic effects of PBAN have been successfully reproduced using ionomycin in *B. mori*. Additionally, the inorganic Ca^{2+} channel blocker, La^{3+}, hampers sex pheromone production in *B. mori* [32].

D. Bombykol Biosynthesis Enzymes:

1. Fatty-acyl desaturase (Bmpgdesat1): In the process of bombykol biosynthesis, palmitate undergoes two desaturation steps and fatty acyl reduction to ultimately form bombykol (Ando et al., 1988). Unlike most other Type I pheromone biosynthetic pathways, the biosynthesis of bombykol is relatively straightforward and does not involve chain-shortening or additional modification of the terminal hydroxyl group. The initial desaturation step seems to be a typical process, most likely facilitated by a Z11 desaturase enzyme that is often seen in the pheromone biosynthetic pathways of different moth species. On the other hand, the second desaturation step is less frequent, as it creates a conjugated diene system by removing two hydrogen atoms from the allylic positions of the double bond in the Z11-monoene C16 intermediate through a 1,4-elimination process.

2. PG-specific fatty-acyl reductases (PG FAR): The biosynthesis of Type I pheromones involves a crucial enzyme called FAR. This enzyme is responsible for producing oxygenated functional groups by converting fatty acyl pheromone precursors into alcohols. Depending on the species of moth, these alcohols can be further modified through acetylation or oxidation, leading to the formation of corresponding aldehydes. A study conducted on *B. mori* PG FAR revealed that it possesses the consensus N-terminal NAD(P)H binding motif, which is commonly observed in other reductases. Moreover, this enzyme demonstrates a remarkable substrate specificity for the bombykol precursor fatty acid, known as (E, Z)-10,12-hexadecadienoic acid [2].

3.6 Process of Bombykol Synthesis

"Bombykol is produced from acetyl-CoA through the C-16 fatty acyl palmitoyl-CoA. Palmitoyl-CoA undergoes a series of steps involving desaturation and reductive modification of the carbonyl carbon to convert into bombykol. Unlike other Type I pheromones, the biosynthesis of bombykol does not require chain-shortening or modification of the terminal hydroxyl group. The gene Desat1 encodes an enzyme called Bmpgdesat1 (Desat1), which produces the monoene (11Z)-hexadecenoyl-CoA and the diene (10E,12Z)-10,12-hexadecadienoyl-CoA. This desaturase enzyme is the only one needed to catalyze these two consecutive desaturation steps. The bombykol acyl precursor (10E,12Z)-10,12-hexadecadienoate is mainly present as a triacylglycerol ester in the cytoplasmic lipid droplets of the pheromone gland cells in the moth. When adult females emerge from pupae, the neurohormone PBAN (pheromone biosynthesis-activating neuropeptide) triggers signaling events that regulate the lipolysis of the stored triacylglycerols, releasing (10E,12Z)-10,12-hexadecadienoate for its final reductive modification. The exact mechanism of releasing (10E,12Z)-10,12-hexadecadienoate from triacylglycerols through lipolysis is not fully understood, but potential lipase-encoding genes have been identified" [33].

3.7 Genes Involved in Biosynthesis of Bombykol

The main sex pheromone component in the silk moth, *Bombyx mori*, (E, Z)-10,12-hexadecadien-1-ol (bombykol), is actively generated and released shortly after adult emergence (Ando *et. al*., 1998) from the cocoon. Just below the endocuticle, there is a single layer of epidermal cells that make up the PG's pheromone-producing cells [34]. "In these cells, acetyl-CoA-derived palmitate (C16: Acyl) is converted stepwise to bombykol by the sequential actions of a bifunctional desaturase and a fatty acyl reductase; genes corresponding to each have recently been cloned and characterized as *B.*

mori ΔPG Z11/ 10,12 desaturase (*Bmpgdesat1*) and PG fatty acyl reductase (*pgFAR*), respectively" [35,36]. The bombykol biosynthetic pathway is regulated by a molecular interaction between the neurohormone pheromone biosynthesis-activating neuropeptide (PBAN) and its cognate G protein-coupled receptor (GPCR), a candidate of which has also been cloned [37] as is the case with most lepidopteran sex pheromone biosynthetic pathways (Rafeli, 2002); [38]. Pheromonogenesis in *B. mori* PG cell is a dynamic period marked by cytosolic lipid droplet accumulation [34,39], lipolysis of stored triacylglycerols (TG) from the lipid droplets [40], and up-regulation of numerous PG-specific genes such as PG acyl-CoA-binding protein (*pg*ACBP), midgut ACBP (*mg*ACBP), *pg*FAR, B*mpgdesat*1 and PBAN receptor (PBANR) [35,36,37] Yoshiga et al., 2000; [41]. Despite these breakthroughs, the particular chemical pathways behind the generation of sex pheromones were still unknown. In addition, the functional roles of the genes currently associated with biosynthetic pathways have yet to be shown in vivo [42]. Therefore, a study was conducted to stimulate the influx of extracellular calcium that activates an enzymatic cascade, resulting in the terminal reduction of bombykol production. PBAN interacts with its corresponding receptor in *B. mori* to generate an influx of extracellular calcium, which activates an enzyme cascade that governs the terminal reduction step of bombykol production. pgFAR, Bmpgdesat1, mgACBP, pgACBP, and PBANR are a few of the genes that have been discovered in recent years and are expected to play essential roles in the bombykol biosynthesis pathway. Although molecular analysis of these genes revealed capabilities compatible with involvement in the bombykol biosynthesis pathway, the functional relevance of these gene products in vivo has yet to be demonstrated. Previous research has shown that 1 day before adult emergence, mRNA transcripts corresponding to these genes increase. It was reasoned that if these gene products are actually critical components of the bombykol biosynthetic pathway, then injecting pupae with the corresponding dsRNAs would result in reduced bombykol synthesis. Pupae from various developmental stages were injected with 5 g of dsRNA corresponding to pgACBP, mgACBP, pgFAR, or Bmpgdesat1 and kept under normal conditions until adult emergence to test this hypothesis. After decapitation, overnight elimination of endogenous PBAN, and injection of 5 pmol of synthetic PBAN, we evaluated bombykol production in RNAi-treated females and compared it to control pupae injected with DEPC-treated H_2O. The disruption of the targeted genes had no influence on pupal development or adult emergence, but it did affect bombykol production, with the most significant effects seen 1 day after the larval–pupal moult [42]. From this it was concluded that *pg*ACBP, *mg*ACBP, *pg*FAR, B*mpgdesat*1 and PBANR were the genes involved in the biosynthesis of bombykol.

3.8 Process of Pheromone-binding Protein Mechanism

"The male moth's antennae are covered with 17,000 hairs (sensillae), each with 3,000 pores. Around half of the sensillae in each moth specialize in detecting bombykol, resulting in over 20 million pores through which bombykol can be diffused. After passing through one of these pores, the bombykol molecule must pass through the sensillar lymph, a watery fluid that surrounds the pheromone receptor cells. However, bombykol is hydrophobic due to its long carbon chain, therefore it requires assistance to reach the receptor site. This is accomplished through the use of a 'pheromone binding protein' (PBP). This protein has a lot of charged groups on the outside that attract water molecules, making it water-soluble" [7]. "Pheromone Binding Proteins (PBPs) are small, soluble proteins produced by two of the three accessory cells and secreted in large quantities into the sensillum lymph at concentrations of up to 10mM" [43]. "Olfactory Receptor Neurons (ORNs) in distinct sensilla can be surrounded by lymphatic fluid containing various molecular constituents because the sensillum lymph is isolated between each sensillum by accessory cells. The expression of Odorant-binding protein (OBP) in *B. mori* is linked to the morphological type of sensillum: BmPBP1 is found in the lymph of pheromone-sensitive long sensilla trichodea (s. trichodea), but other sensillum types including long s. trichodea in females tuned to general odorants express different OBPs" [44,45]. "BmPBP2 and 3 transcripts have been found in cells that do not overlap with BmPBP1 cells, indicating that they are involved in sensilla other than long s. trichodea" [26]. "BmPBP1's pheromone binding and release mechanisms are among the best-studied biochemical and structural examples of OBP function. When the pH becomes more acidic, as is expected near a cell membrane, BmPBP1 experiences a conformational shift, which could result in the release of bound pheromones onto the ORN's

dendritic membrane" [46]. "This idea is backed by structural investigations, which show that at acidic pH, conformational changes cause BmPBP1 to release bound bombykol from BmOBP1". "BmPBP1 has six helices, according to an X-ray diffraction analysis of a BmPBP1 crystal complexed with bombykol grown at neutral pH. Four antiparallel helices form the binding pocket in the center of the protein, entirely enclosing a bombykol molecule" (Sandler et al., 2000). The unliganded BmPBP1 Nuclear Magnetic Resonance (NMR) structure was then determined at acidic (pH 4.5) [47] and neutral (pH 6.5) pH [48]. "At the C-terminal ends, the structural changes are most noticeable. At acidic pH, the C-terminal dodecapeptide forms a 7th helix that binds to bombykol, whereas at neutral pH, the C-terminal end forms a flexible loop structure. This conformational transformation has been proposed as one of the mechanisms for ligand release near a cell membrane. The presence or absence of ligands, as well as their structures, affects BmPBP1 pH-dependent conformational change" [49]. "The pH of the transition midpoint in the presence of bombykol (pH 5.37) is much lower than in the absence of ligands (pH 7.25) and slightly lower than in the presence of structurally related ligands (pH 5.75), implying that BmPBP1 has a higher affinity for bombykol than related ligands at low pH binding" [26]. The following model for the preferential transport of bombykol to the sex pheromone receptor has been proposed based on the pH and ligand-dependent conformational transition:

(1) Bombykol is preferentially uptaken by BmPBP1 in areas with lower pH near the olfactory pore;

(2) Bombykol is protected from degradation by Odorant Degrading Enzyme (ODE) in the sensillum lymph by binding to the cavity of BmPBP1 during transport;

(3) The acidic pH around the membrane allows bombykol to be released from BmPBP1, resulting in its reception by the sex pheromone receptor protein BmOR1.

A nerve impulse is delivered to the brain as a result of this contact, which causes an electrical change in the receptor. The function of Bombykol is done once it has delivered its message to the receptor. Pheromone degradation enzymes immediately remove the molecule from the receptor site and deactivate it.

3.9 Terminal Pathway or Enzymatic Degradation of Pheromone

The monitoring of intermittent pheromone stimuli with high temporal resolution is essential for efficient orienting of male moths to conspecific females. Pheromone molecules must be promptly degraded into non-active substances after activation of the pheromone receptors to avoid prolonged activation of the pheromone receptors (Sakurai et al., 2014). Alcohol oxidase (AO) is revealed to be associated in the transformation of fatty alcohol to similar aldehyde pheromone [50,51]. According to studies, there were 25 AOs found in the Pheromone Gland (PG) transcriptomes, with six genes showing differential expression between domestic and wild silkworms, five of the six Differentially Expressed Genes (DEGs) were upregulated in the domestic silkworm, with BmorAO1, 2, and 3 being the most strongly expressed. In the diverse tissues of female adults, expression profiles of all six differently expressed AO genes were found. BmorAO1, 2, and 4 were found to be expressed particularly in PGs and abdomens (without pheromone glands). BmorAO3 was expressed at high levels in PGs but at low levels in the other five tissues. In the domestic silkworm PGs, however, the differentially expressed BmorAO5 and 6 expression signals were not identified. BmorAO1, 2, 3, and 4 began to be expressed in the PGs in day 6 or 7 pupae and were down-regulated following sexual mating, according to our findings. BmorAO1, 2, 3, and 4 may be the putative causal genes in the process of *B. mori* catalysing bombykol to bombykal when taken together. Phylogenetic study revealed that AOs diverged more among moths, while ARs and AOXs remained relatively constant throughout evolution. When it came to *B. mori* PGs, BmorAR1 expression varied between domestic and wild silkworms, with RPKM = 582.61. Wang et al. (2021) suggest that BmorAR1 may be the main gene responsible for the bombykal decrease of the pheromone gland. The tissue and developmental expression analysis of profiles suggested that BmorAO1, BmorAO2, BmorAO3, BmorAO4, BmorAR1, and BmorAOX5 were likely causal genes associated with the silkworm's final pathway of bombykal synthesis and metabolism [52].

3.10 Factors Affecting Pheromone Production

According to the findings of [53] a combination of mechanical stimulation from mating and

insemination greatly suppressed sex pheromone production in *B. mori*, and in a few moths of *B. mori*, changes in female reproductive activities were documented in response to a variety of stimuli. Mating with a castrated male, causes a stronger suppression [54,55]. The transection of N1 nerves, which innervate these sensory hairs, was reported to have no effect on bombykol production [56]. Mating with a pr-male or tactile stimulation of the abdominal tip has been demonstrated to suppress the activity of neurosecretory cells that release Pheromone Biosynthesis Activating Neuropeptide (PBAN) [57]. The presence of viable sperm in the vestibulum is sufficient to speed up egg-laying, but it is insufficient to decrease pheromone synthesis, when artificial insemination was combined with tactile stimulation, only females who delivered fertilized eggs shown a significant decrease in bombykol production [53]. Mating signals in females are transmitted via a humoral or neurological pathway. According to Ando et al. (1996) and Ichikawa et al. (1996) [56], there is no involvement of the humoral pathway in any of the stages of the subsequent decline of bombykol synthesis in B. mori, which is dependent on the brain pathway.

4. CONCLUSION

Bombykol is the first insect pheromone to be discovered in *Bombyx mori*. The various studies and experiments done on bombykol revealed a lot about the chemical communication between the male and female silkworm. These studies revealed a better understanding of the way of communication, synthesis, behavioural response and perception of bombykol in genetic as usual as molecular levels.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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