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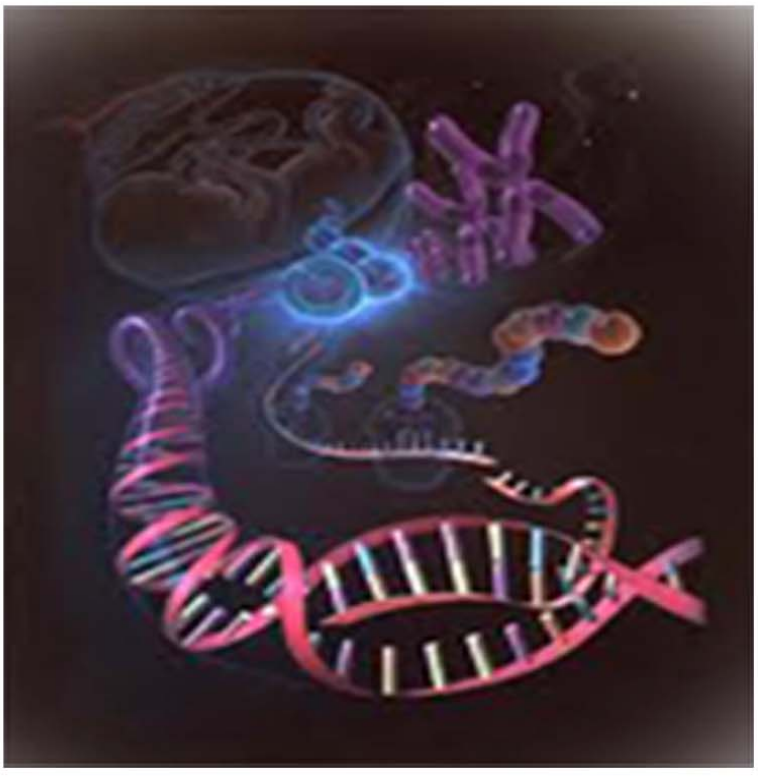

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Research Paper

GENE SEQUENCE OF THE ADIPOKINETIC HORMONE OF THE MANGO LEAF WEBBER, *ORTHAGA EXVINACEA*

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Adipokinetic hormones form a major group of neuropeptides, which regulates physiological homeostasis. They are members of a large family of structurally related peptides and known under the acronym AKH/RPCH family. AKHs are released from the neurosecretory cells of corpora cardiaca, neuroendocrine glands connected to the brain. The present study was undertaken with the objectives of amplification, sequencing, characterization of adipokinetic gene sequence of the *O. exvinacea*, a mango leaf webber. Here we report the partial DNA sequence of the AKH gene (GenBank Accession No. HQ269419) and its phylogenetic status. The results showed that the sequence obtained have a high homology to that of AKH II of *B. mori*. The study also attempts to demonstrate the systematic position and relationships of lepidopteran species, based on amino acid sequences of adipokinetic neuropeptides, by constructing phylogenetic trees.

Keywords: Neuropeptide, *Orthaga exvinacea*, Polymerase chain reaction, Phylogeny

INTRODUCTION

Neuropeptides represent the largest single class of regulatory compounds in vertebrates, as well as in invertebrates. The mobilization of stored fuels during episodes of flight or locomotion is controlled by peptides belonging to adipokinetic hormone/red pigment concentrating hormone (AKH/RPCH) family. They are the members of a large family of structurally related peptides which are found in crustaceans and insects (Gaede, 1996). They affect the release of diglycerides from the fat body and also stimulate the flight muscles to use them as an energy source. Besides these, AKHs exert other physiological functions: they acts on the fat body to mobilize stored lipids and carbohydrates, activate glycogen phosphorylase, accumulate cAMP (Goldsworthy, 1983) and inhibit the synthesis of proteins (Carlisle and Loughton, 1979; Kodrik, 2008), lipids (Gokuldas *et al*., 1988), and RNA (Kodrik and Goldsworthy, 1995).

Neuropeptides are typically derived from larger precursor molecules, which undergo post-translational processing and sometimes modifications to yield mature peptides. A single

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neuropeptide precursor molecule can give rise to a single neuropeptide, multiple distinct neuropeptides, multiple copies of a single neuropeptide, or any combination thereof. As an additional mechanism to increase neuropeptide complexity in mammals, a single precursor molecule can be differentially cleaved to yield different sets of peptides in different cell types (Salio *et al*., 2006). An AKH precursor begins with a signal peptide that is followed by a single AKH of 8-10 amino acids in length and then an AKH-precursor-related peptide (APRP). Prior to secretion, the AKH region is enzymatically cleaved from APRP and modified at the amino termini by a pyroglutamic acid residue and carboxy termini by an amide residue (Gaede and Auerswald, 2003).

Recent development in genome studies of different insect species have allowed for the discovery of novel genes coding for bioactive peptides. Genome sequences have been available for the fruit fly *Drosophila melanogaster* (Adams *et al*., 2000), two mosquitoes, *Anopheles gambiae* (Holt *et al*., 2002) and *Aedes aegypti* (Nene *et al*., 2007). Genomic studies on neuropeptides and their receptors in these insects have been highly successful and have provided crucial information about their development, physiology, behavior and evolutionary relationships (Coates *et al*., 2000; Hewes and Taghert, 2001; Park *et al*., 2002; Riehle *et al*., 2002; Hummon *et al*., 2006; Zitnan *et al*., 2007; Hauser *et al*., 2008; Li *et al*., 2008).

The present investigation was carried out to elucidate the adipokinetic gene sequence of *Orthaga exvinacea* by conducting genomic DNA extraction, PCR, and sequencing. Furthermore, the phylogenetic analysis of *O. exvinacea* with other lepidopteran species was studied by constructing a phylogenetic tree.

MATERIALS AND METHODS

Insect Rearing

Larvae of *Orthaga exvinacea* (Lepidoptera: Pyralidae) were collected from their natural habitat, mango trees, and were transferred to plastic basins and reared in the insectary by feeding mango leaves. The colony was maintained at 27+ 2^{o}C and 70%-80% relative humidity. Sixth instar larvae were separated from the colony and used for experiments. The insects of both sexes were used for genomic DNA extraction and amplification.

GENOMIC DNA EXTRACTION

The intestinal tissues (50 mg) of *O. exvinacea* were weighed out and the DNA was extracted using Quick Extract DNA extraction solution (Epicentre Biotechnologies, USA) as per the manufacturer's instruction. The tissue was ground using a mortar and pestle with 0.5 mL of Quick Extract DNA extraction solution. The mixture was vortexed for 15 s and transferred the tube to 65^{o}C and incubated for 6 min. After incubation, the mixture was vortexed again and mixed for 15 s. The tubes were incubated at 98^{o}C for 2 min. A sample of the extracted DNA (3 µL) was used for PCR amplification.

AMPLIFICATION OF AKH GENE

The available sequences for adipokinetic hormone from different species of Lepidoptera were used for primer designing. The genomic DNA was amplified for AKH gene using the forward primer with DNA sequence 5'-AACAACAGCA GAGTTCGCGG-3' and reverse primer with DNA sequence 5'-ATGCGCTTCGACTCTGCGCT-3'.

All the primers used in the study were synthesized by Integrated DNA Technologies, Inc. USA. The PCR reaction mixture contained 3 µL of genomic DNA from *O. exvinacea*, 1 µL of each primer (100 pmol/µL), 2 µL 10 mM deoxy-ribonucleoside triphosphate, 5 µL 10xPCR buffer containing $MgCl_2$, and 1 µL of 5 U/µL Taq DNA polymerase. The PCR was conducted with the initial denaturation at 94°C for 2 min followed by denaturation at 94°C for 45 s, annealing at 60°C for 60 s and elongation at 72°C for 2 min. These cycles were then followed by 34 cycles of denaturation, annealing and elongation was followed by an extended final elongation step at 72°C for 10 min.

The PCR product was electrophoresed in a 1% (w/v) agarose gel, stained with ethidium bromide and observed on a UV transilluminator. The amplicon was excised from the gel and the DNA was eluted from the gel slice by using the Nucleo Spin column DNA Gel extraction kit (Macherey-Nagel, Germany) according to the manufacturer's specifications.

SEQUENCING AND SEQUENCE ANALYSIS

Sequencing was done using the big dye terminator kit (Applied Biosystems) in 3730XL DNA Analyser. The PCR product was sequenced and the sequences were analyzed online using BLAST.

EVOLUTIONARY STUDIES

Phylogenetic analysis of AKHs of Lepidoptera was conducted using MEGA4 software (Tamura *et al*., 2007). We used amino acid sequences of adipokinetic neuropeptides reported for other lepidopteran insects for constructing phylogenetic tree. To reveal the evolutionary relationship of *O. exvinacea* adipokinetic peptide with other adipokinetic peptide sequences, 8 putative AKH sequences (*B. mori* AKH-I and II, *M. sexta*, *H. zea*, *M. cinxia*, *S. frugiperda* AKH-I, II and III) were collected from previous works for multiple alignment by ClustalW and phylogenetic analysis. The results of our mass spectrometric studies revealed that the primary structure of *O. exvinacea* is pELTFTSSWG-amide (Umadevi *et al*., 2013).

RESULTS

Amplification and Sequencing

The amplification of genomic DNA with degenerate primers, forward AKHF and reverse AKHR yielded an amplicon of approximately 400 bp at an annealing temperature of 60°C (Figure 1). The sequenced PCR product was analyzed using BLAST. Sequence alignment was carried out using ClustalW and dendrogram shows the phylogenetic relationship of *O. exvinacea* AKH gene with *B. mori* AKH-II mRNA, *B. mori* neuropeptide receptor-A26 (NGR-A26) mRNA and *B. mori* neuropeptide receptor-A21 (NGR-A21) mRNA. From the results it is clear that *O. exvinacea* AKH gene identified is a novel one and it had 100% similarity with *B. mori* AKH-II mRNA and *B. mori* NGR-A26 mRNA and 92% similarity with *B. mori* NGR-A21 mRNA, indicating that they belong to the same cluster (Figure 2).

The comparison of nucleotide sequence information with the known gene sequence from Genbank indicated that this gene could encode AKH. Gene sequence containing 184 bp was obtained from the genomic DNA and sequence analysis revealed partial homology with adipokinetic hormone gene in *B. mori*. This result

confirms the unique nature of this AKH gene as it shows variability among other AKH genes. The amino acid sequence obtained, 1 to 14 (PVFKGNLLVAESQR) showed high homology to that of AKH II of the silkworm *B. mori*. The AKH gene sequence was submitted to NCBI GenBank and the sequence was accepted and put in the public database under the accession number HQ269419.

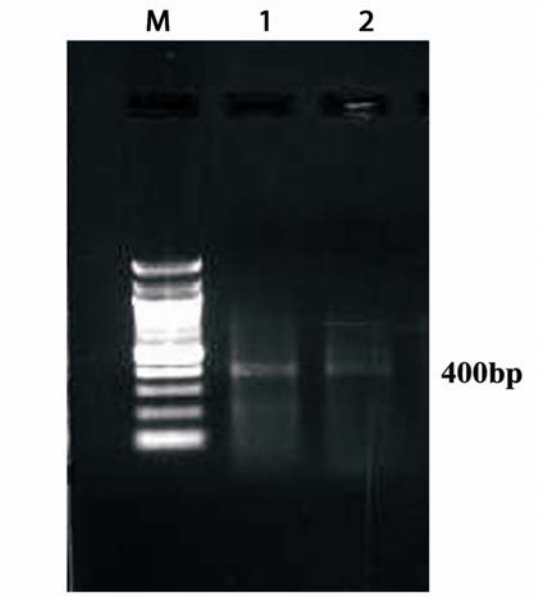
EVOLUTIONARY TREE CONSTRUCTION USING PEPTIDE SEQUENCE

Phylogenetic relationship of AKHs of Lepidoptera was studied using MEGA4 software. The systematic position of lepidopteran species were demonstrated in the cladogram of AKHs identified from the studied species (Table 1) of Lepidoptera. The phylogenetic tree was generated using neighbor joining method and in a rectangular format (Figure 3). Phylogenetically, *M. sexta*-AKH and AKH I of *B. mori* and *S. frugiperda* are the nearest relatives of *O. exvinacea* AKH peptide.

DISCUSSION

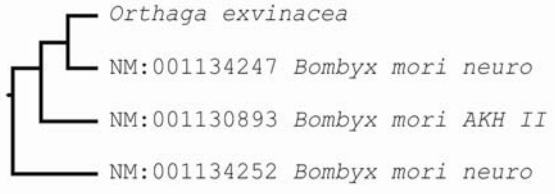
The data obtained from the gene sequencing studies revealed that the sequence obtained "PVFKGNLLVAESQR" shows high homology to that of AKH II of *B. mori*. The peptide BLAST of the conceptual peptide of the nucleotide sequence of AKH gene of *O. exvinacea* showed 100% similarity with *B. mori* AKH II mRNA and *B. mori* NGR-A26 mRNA whereas 92% similarity obtained with *B. mori* NGR-A21 mRNA. The result confirms that there exist ancestral forms of AKH and suggests that the genes coding for this AKHs are conserved during the course of evolution. The BLASTp analysis indicates that the AKH gene identified is a novel one. The adipokinetic hormone

Figure 1: PCR analysis of the genomic DNA extracted from the intestinal tissues of *O. exvinacea*. Agarose gel separation of PCR products yielded an amplicon of 400 bp



Note: M = 100 bp ladder; 1 and 2 = sample duplicates

Figure 2: Dendrogram showing the phylogenetic relationship of *O. exvinacea* AKH gene with *B. mori* AKH-II gene and with other two neuropeptide hormone receptor (AKHR) genes



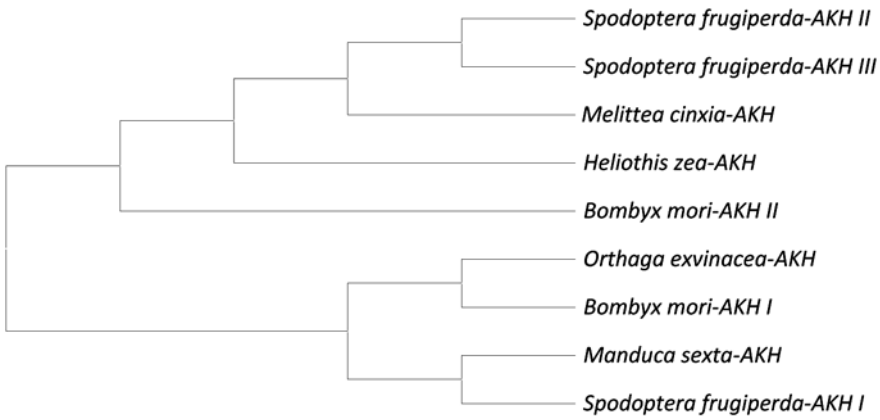
gene sequence of *O. exvinacea* obtained can be used as a molecular barcode of the species.

Cloned AKH gene sequences will facilitate studies on the synthesis of AKH peptides and provide insight into molecular evolution. With a cloned AKH structural gene sequence, we can examine the cellular events leading from AKH transcript modulation to the release of a

Table 1: Adipokinetic Peptides of Lepidoptera

Peptide name	Peptide sequences	Reference
Manduca sexta-AKH (Manse-AKH)	pELTFTSSWG-amide	Zeigler *et al*, 1985
Heliothis zea-AKH (Helze-HrTH)	pELTFSSGWGN-amide	Jaffe *et al*., 1988
Bomby mori-AKH I (Bommo-AKH I)	pELTFTSSWG-amide	Ishibashi *et al*., 1992
Spodoptera frugiperda-AKH I(Spofr-AKH I)	pELTFTSSWG-amide	Abdel-Latief and Hoffmann, 2007
Spodoptera frugiperda-AKH II(Spofr-AKH II)	pELTFSSGWGN-amide	Abdel-Latief and Hoffmann, 2007
Spodoptera frugiperda-AKH III(Spofr-AKH II)	pELTFSSGW-amide	Abdel-Latief and Hoffmann, 2007
Bombyx mori-AKH II(Bommo-AKH II)	pELTFTPGWGQ-amide	Roller *et al*., 2008
Melittea cinxia-AKH (Melcin-AKH)	pELTFSSGW-amide	Gaede *et al*., unpublished data
O. exvinacea-AKH	pELTFTSSWG-amide	Umadevi *et al*., 2013

Figure 3: Phylogenetic tree plotted using neighbor joining method in rectangular format to study the evolutionary relationship of AKH sequences of Lepidoptera



biologically active insect neuropeptide. However, many more questions remain unanswered in insect studies that may be addressed by additional investigations at different biochemical, physiological and molecular levels. The cDNAs encoding AKH precursors in Lepidoptera have been cloned from *M. sexta* (Bradfield and Keeley, 1989) and from *S. frugiperda* (Abdel-latief and Hoffmann, 2007). The primary structure of *M. sexta*-AKH was identified as 'QLTFTSSWG' (Zeigler *et al.*, 1985). In *B. mori*, a nonapeptide identical with *M. sexta*-AKH have been reported (Ishibashi *et al*., 2002) while *B. mori* -AKH II is identified as a decapeptide (Roller *et al*., 2008). *M. sexta*-AKH is also present in *S. frugiperda*. (Abdel-latief and Hoffmann, 2007). The primary structure of *O. exvinacea* AKH elucidated by our mass spectrometric studies revealed that the sequence obtained is identical with that present in *M. sexta*. The peptide structure of *H. zea* (Jaffe *et al*., 1988) and *S. frugiperda* AKH-II (Abdel-latief and Hoffmann, 2007) are reported to be similar,

'QLTFSSGWGN' indicating their phylogenetic relationship.

The primary structures of peptides from the AKH/RPCH family have been used as additional data to aid in the construction of phylogeny in insect orders. Even though the peptide structures of AKH family have been characterized from representatives of many insect orders, little is known about the evolutionary connections of AKH structures and functions within insect orders or even between insects and animals from other phylogenetic levels. The studies on the evolutionary relationships of AKH peptides from insects indicate that there exists a family or order specificity. The first attempt to demonstrate phylogenetic relationships to structural variations of AKHs was made for cockroaches (Gaede, 1989). Phylogenetic analyses were also conducted with some species belonging to the orders Odonata (Gaede and Marco, 2005) and the Orthoptera: Ensifera (Gaede *et al*., 2003) and Caelifera (Gaede and Marco, 2009). The lepidopteran database encompasses their phylogenetic relationship even though the biochemical, structural and functional studies conducted indicate the similarities in their function and peptide sequence.

CONCLUSION

Insect hormones and interventions in the insect endocrine regulated processes have been propagated as possible pest management tools ever since investigations on insect neuroendocrinology have been initiated. Confronted with the problem of developing insect resistance to conventional pesticides, there is a critical need for developing new concepts and alternative approaches in controlling pest insects. New, selective control measures may be developed in designing metabolically stable mimics of those neuropeptides that actively inhibit or overstimulate the functions regulated by them, resulting in sustained disruption of the internal insect homeostatic environment. Neuropeptide receptors have been identified and characterized in *D. melanogaster*, *B. mori*, *M. sexta* and similar receptors are being targeted in other insects considered to be economically detrimental pests in agriculture and forestry**.** Defining neuropeptide action or effects in different insect systems has been more challenging and as a result, identifying unique targets for potential pest control is also a challenge. The application of molecular biology techniques to transform insects with neuropeptide or neuropeptide receptor genes, or knockout genes to identify potential pest control targets, is a relatively new area that offers promise to insect control (Bendena, 2010). Insect immune systems can also be manipulated through neuropeptides which in turn can aid in compromising the insects ability to defend against foreign invasion. Since neuropeptides regulate critical physiological processes including metabolism, homeostasis, development, behavior and reproduction, they would appear to be ideal candidates for the pest management strategies. Insect specific neuropeptides offer insect target specificity and environmental compatibility.

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REFERENCES

1. Abdel-latief M and Hoffmann K (2007), "The adipokinetic hormones in the fall armyworm, *Spodoptera frugiperda*: cDNA cloning, quantitative real time RT-PCR analysis, and gene specific localization", *Insect Biochem. Mol. Biol*, Vol. 37, pp. 999-1014.
2. Adams e*t al.* (2000), "The genome sequence of *Drosophila melanogaster*", *Science*, Vol. 287, pp. 2185–2195.
3. Bendena W G (2010), "Neuropeptide physiology in insects". Advances in Experimental Medicine and Biology, Vol. 692, pp. 166-191.
4. Bradfield J Y and Keeley L L (1989), "Adipokinetic hormone gene sequence from *Manduca sexta*". *J. Biol. Chem,* Vol. 264, pp. 12791-12793.
5. Carlisle J and B G Loughton (1979), "Adipokinetic hormone inhibits protein synthesis in *Locusta*", *Nature*, Vol. 282, pp. 420-421.
6. Coates D, Siviter R and Issac R E (2000), "Exploring the *Caenorhabditis elegans* and *Drosophila melanogaster* genomes to understand neuropeptide and peptidase function", *Biochem. Soc. Trans*, Vol. 28, pp. 464-469.
7. Gaede G (1989), "The hypertrehalosaemic neuropeptide of cockroaches: a phylogenetic study", *Gen. Comp. Endocrinol,* Vol. 75, pp. 287-300.
8. Gaede G (1996), "The peptide revolution in insects illustrated by the AKH/RPCH family of peptides", *Z. Naturforsch*, Vol. 51, pp. 607-617.
9. Gaede G and Auerswald L (2003), "Mode of action of neuropeptides from the adipokinetic hormone family". *Gen. Comp. Endocrinol*, Vol. 132, pp. 10-20.
10. Gaede G and Marco H G (2005), In: Atta-ur-Rahmann (Eds.), *Studies in Natural Product Chemistry (Bioactive Natural Products),* Elsevier Science Publishers, Netherlands, Vol. 33, pp. 69-139**.**
11. Gaede G and Marco H G (2009), "Peptides of the adipokinetic hormone/red pigment-concentrating hormone family with special emphasis on Caelifera: primary sequences and functional considerations contrasting grasshoppers and locusts", *Gen. Comp. Endocrinol,* Vol. 162, pp. 59-68.
12. Gaede G, Marco H G and Dessutter-Grandcolas L (2003), "A phylogenetic analysis of adipokinetic neuropeptides of Ensifera"*, Physiol. Entomol*, Vol. 28, pp. 283-289.
13. Gokuldas M, Hunt P A and D J Candy, (1988), "Inhibition of lipid synthesis *in vitro* in the locust, *Schistocerca gregaria*, by a factor from *Corpora cardiaca*", *Physiol. Entomol,* Vol. 13, pp. 43-48.
14. Goldsworthy G J (1983), In: Berridge, M J, Treherne J E and V B Wigglesworth, (Eds.), *Advances in Insect physiology,* Academic Press, New York, pp. 149-204.
15. Hauser *et al* (2008), "A genome-wide inventory of neurohormones GPCRs in the red flour beetle", *Tribolium castaneum*. *Front. Neuroendocrinol,* Vol. 29, pp. 142-165.
16. Hewes R S and Taghert P H (2001), "Neuropeptides and neuropeptide receptors

in the *Drosophila melanogaster* genome", *Genome Res*, Vol. 11, pp. 1126-1142.

17. Holt et al (2002), "The genome sequence of the malaria mosquito, *Anopheles gambiae*", *Science,* Vol. 298, pp. 129-149.

18. Hummon *et al* (2006), "From the genome to proteome: uncovering peptides in the *Apis*", *Brain Science*, Vol. 314, pp. 647-649.

19. Ishibashi J, Kataoka H, Nagasawa H, Isogai A and Suzuki A (1992), "Isolation and identification of adipokinetic hormone of the silkworm, *Bombyx mori*", *Biosci. Biotech. Biochem,* Vol. 56, pp. 66-70.

20. Jaffe H, Raina A K, Riley C T, Fraser B A, Bird T G, Tseng C M, Zhang Y S and Hayes D K (1988), "Isolation and structure of a neuropeptide hormone from *Heliothis zea* with hypertrehalosaemic and adipokinetic activities", *Biochem. Biophys. Res. Commun,* Vol. 155, pp. 344-350.

21. Kodrik D (2008), "Adipokinetic hormone functions that are not associated with insect flight", *Physiol. Entomol*, Vol. 33, pp. 171-180.

22. Kodrik D and Goldsworthy G J (1995), "Inhibition of RNA synthesis by adipokinetic hormones and brain factor (s) in adult fat body of *Locusta migratoria*", *J. Insect Physiol*, Vol. 41, pp. 127-133.

23. Li et al (2008), "Genomics, transcriptomics and proteomics of neuropeptides and protein hormones in the red flour beetle, *Tribolium castaneum*", *Genome Res*, Vol. 18, pp. 113-122.

24. Nene et al (2007), "Genome sequence of *Aedes aegypti*, a major arbovirus vector", *Science*, Vol. 316, pp. 1718–1723.

25. Park Y, Kim Y J and Adams M E, (2002), "Identification of G protein coupled receptors for *Drosophila* PRXamide peptides, CCAP. Corazonin and AKH supports a theory of ligand receptor co-evolution", *Proc. Natl. Acad. Sci.* USA, Vol. 99, pp. 11423-11428.

26. Riehle M A, Garczynski S F, Crim J W, Hill C A and Brown M R (2002), "Neuropeptides and peptide hormones in *Anopheles gambiae*", *Science*, Vol. 298, pp. 172-175.

27. Roller L, Yamanaka N, Watanabe K, Daubnerova I, Zitnan D, Kataoka H and Tanaka Y (2008), "The unique evolution of neuropeptide genes in the silkworm *Bombyx mori*", *Insect Biochem. Mol. Biol*, Vol. 38, pp. 1147-1157

28. Salio C, Lossi L, Ferrini F and Merighi A (2006), "Neuropeptides as synaptic transmitters", *Cell Tissue Res,* Vol. 326, 583–598. Abstract Article

29. Tamura K, Dudley J, Nei M and Kumar S (2007), "MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0", *Molecular Biology and Evolution*, Vol. 24, pp. 1596-1599.

30. Umadevi D, K U M A Rafeeq, Ajaykumar A P, K M Ismail, K A Rashed and Gokuldas M (2013), "Identification and structural characterization of a hyperlipemic neuropeptide from the mango leaf webber, *Orthaga exvinacea* Hampson", *American journal of Biochemistry and Molecular biology*, DOI:103923/ajbmb, pp 1-10.

31. Ziegler R, Eckart K, Schwarz H and Keller R (1985), "Amino acid sequence of

Manduca sexta adipokinetic hormone elucidated by combined fast atom bombardment (FAB) tandem mass spectrometry", *Biochem. Biophys. Res. Comm*, Vol. 133, pp. 337-342.

32. Zitnan D, Kim Y J, Zitnanova I, Roller L. and Adams M E, (2007), "Complex steroid peptide-receptor cascade controls insect ecdysis", *Gen. Comp. Endocrinol*, Vol. 153, pp. 88-96.

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