Identification and Tissue Localization of Olfactory Proteins in the Antenna and Head of *Glossina* Species

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A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in Biochemistry in the Jomo Kenyatta University of Agriculture and Technology

2011

ABSTRACT

Tsetse flies use olfaction in search for food, mates and larviposition sites. Olfactory proteins [(odorant binding proteins (OBPs), pheromone binding proteins (PBPs), chemosensory protein (CSPs), odorant degrading enzymes (ODEs) and odorant receptors (Ors)], located within the antennae, play key role in this process. In this work, presence of olfactory proteins was investigated by constructing and sequencing cDNA libraries from *Glossina pallidipes* Austen, antennae; *Glossina palpalis gambiensis* Vanderplank, head and *Glossina tachinoides* Westwood, head. The Expressed sequence tags (ESTs) were clustered using cDNA Annotation™ Software (CAS) and annotated by blast searches. ESTs were generated from the antennal (1127) and head (906 for *G. p. gambiensis* and 830 for *G. tachinoides*) libraries, composed of 296 clusters (18 contigs and 278 singletons for *G. pallidipes* antennae), 305 clusters (36 contigs and 269 singletons for *G. p. gambiensis* head) and 232 clusters (54 contigs and 178 singletons for *G. tachinoides*). The analyses implicated ten (10) sequences in olfaction (2 OBPs from *G. pallidipes*, 5 OBPs from *G. p. gambiensis*, 2 OBPs and 1 CSP from *G. tachinoides*). Clustal alignment revealed a diverse multigene family while phylogenetic analysis supports the existence of different olfactory protein subfamilies.

To identify Dipteran orthologs, the three *Glossina* ESTs (*G. pallidipes* antennae, *G. p. gambiensis* head and *G. tachinoides* head) were clustered using StackPACK, then compared to *G. morsitans morsitans, Drosophila melanogaster*, *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus* proteomes. A total of 663 clusters for *G. pallidipes* (45 contigs and 618 singletons), 930 clusters for *G. p. gambiensis* (43 contigs and 387 singletons) and 444 clusters for *G. tachinoides* (40 contigs and 404 singletons) were generated. Nine putative OBPs (*G. pallidipes*: 2, *G. p, gambiensis*: 5, *G. tachinoides*: 2) and one putative CSP (*G. tachinoides*) were

identified by BLAST search against the dipteran protein databases. Multiple sequence alignments revealed a diverse OBP gene family and a conserved CSP.

Phylogenetic analysis revealed a closely related multigene family that could have evolved separately along different evolutionary time. Reverse Transcription Polymerase Chain Reaction (RT-PCR) screening of male and female *G. pallidipes* tissues (antennae, head, thorax and abdomen) for presence of OBPs and CSPs homologs identified in *G. pallidipes*, *G. tachinoides* and *G. p. gambiensis*, and similar ones (putative OBPs from *G. m. morsitans*), revealed 7 none sex specific (tissue dependent), and 2 sex (male) specific *G. m morsitans* OBP homologues, one specific to the thorax tissue (Gmm_cn14014) and the other to both thorax and abdomen tissues (Gmm_GLAAS20TVB). Two putative OBPs identified in *G. pallidipes* and *G. p. gambiensis* (Gpacontig266 and Gphcontig184) were localised to the antennae tissue. Alignment of the sequenced amplicons revealed a diverse OBP and conserved CSP gene families. These results indicates that olfactory process in tsetse is a complex and interactive process involving established olfactory and non olfactory tissues, suggesting that tissues, other than antennae should also be targeted/considered in development of novel odor based technologies for tsetse control.