IDENTIFICATION OF *GLOSSINA MORSITANS MORSITANS* ODORANT BINDING PROTEINS GENES IN *GLOSSINA FUSCIPES FUSCIPES*: A PRELIMINARY STUDY

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Abstract
Tsetse flies are vectors of trypanosome parasites, causative agents of Trypanosomiasis in humans and animals. Odorant Binding Proteins (OBPs) are critical in insect olfaction as they bind volatile odours from the environment and transport them to receptors within olfactory receptor neurons for processing providing critical information for host identification. *Glossina morsitans morsitans* primers designed for conventional PCR were used to screen OBP genes in *Glossina fuscipes fuscipes* in female head, thorax, abdomen and leg tissues. A total of 31.8% of OBPs were identified in *G. f. fuscipes* head while 18.2% were detected in the thorax. The abdomen had 22.7% OBPs identified and the legs revealed 18.2% OBPs. OBP5 and OBP6 were the most predominant since they were detected in the head, thorax and abdomen of *G. fuscipes fuscipes* which may provide the need to identify their functions in both *G. morsitans morsitans* and *G. fuscipes fuscipes.* This study confirms genetic differences between OBPs from riverine (*G. f. fuscipes*) and savanna (*G. m morsitans*) species which may be key in understanding the role of olfaction in enhancing vector competence of *G. m. morsitans* and *G. f. fuscipes*. Such information will be critical in designing better vector control strategies based on olfactory mediated behavior.

Key words: Trypanosomiasis, olfaction, tsetse flies, odorant binding proteins

1.0 Introduction

Olfaction is an important sensory modality in insects which play a crucial part in mating and oviposition site finding as well as in detection of food sources (Kaissling, 2001). Insect sense the volatile chemicals from the environment through the antennae **(**Shanbhag *et al.,* 1999). However other additional organs like maxillary pulps also detect odors in many insect species. The organs of olfaction are covered with sensilla each possessing dendrites of a few olfactory receptor neuron (Allison and John, 2011). There are several classes of olfactory sensilla depending on their morphological formation; the long, single walled and short, double walled sensilla (Hunger and Steinbrech, 1998). The number of sensilla and ORNs per antenna vary greatly among species with the moth *Manduca sexta* contains >100,000 antennal sensilla housing >250,000 ORNs, whereas *D. melanogaster* has approximately 400 sensilla carrying 1,200 ORNs (Allison and John, 2011).

Odorant binding proteins (OBPs) and Pheromone Binding Proteins (PBPs) are highly diverse group of olfactory proteins even in insects of the same species. They are antennal specific proteins containing about 130 - 150 amino acids residues. They are small (14-20 kda) proteins with a signal peptide at the N-terminal and six conserved cysteines (Prestwich, 1993; Biessmann *et al.,* 2002). OBPs bind general odors while PBPs bind pheromones (Pelosi, 2006). There are three types of chemically identified insect released pheromones. These include: those which cause sexual attraction, alarm behavior and recruitment. Sex pheromones are released by the female insect to cause response to sexual behavior. This makes the male insect to be attracted to the female and attempt to copulate with the female that has released the sex pheromone. As a way of survival and continuity this explains why insects are rather sensitive and selective to sex pheromone of their species. However the insects show far less sensitivity and chemo specificity for alarm pheromones (Fred and John, 1968).

In *Drosophila* thirty five OBP genes have been identified in complete genome sequence (Vogt *et al.,* 1991). While in *Glossina morsitans morsitans* a total of twenty two OBPs have been reported (Liu *et al.,* 2010). *A. gambiae* has 60 putative OBP genes (Biessmann *et al*., 2005), Culex mosquito has two identified OBP genes (Ishida *et al.,* 2002**,** Sengul and Tu, 2008**),** *A. mellifera* has 21 OBPs **(**Sylvain and Ryszard, 2006) while *Aedes aegypti* has 22 OBPs (Sha *et al*., 2008). Clearly, OBPs are essential components of the chemosensory system based on the high number reported in different insect species (Plettner *et al*., 2000).

The olfactory defects associated with loss of an OBP shows that these proteins are required for normal olfactory behavior at the point of odor detection. The potential roles for these proteins has been reported to include solubilizing or concentrating odorants in the sensillum lymph, mediating odorant removal or acting as co-ligand at the receptor (Pelosi, 1994). In mosquitoes the OBPs are used to identify and

discriminate hosts by temperature and chemical sensation (Dekker *et al*., 2002). Volatile compounds released from human skin attract female *Anopheles gambie*. (Dekker and Takken, 1998).

Glossina species use OBPs to find their suitable hosts (Liu *et al.,* 2010). The uniqueness of their ability to detect their suitable hosts makes them not necessarily to feed on the hosts that happen to be in the same habitat. This phenomenon was observed by Clausen *et al*., (1998) who indicated that common animals, such as zebra and wildebeest are not hosts of all tsetse species suggesting that each Glossina species has a specialist range of hosts for example *Glossina pallidipes* is known to feed on ox, buffalo, kudu and human but not monitor lizard while *G. f fuscipes* prefers monitor lizard to other hosts (Liu *et al.*, 2010).

2.0 Materials and Methods

2.1 Study Site

Tsetse flies of species *Glossina fuscipes fuscipes* were collected from Mbita in Homabay county Kenya situated at 0° 25' 0" south and 34° 12' 0", on the shores of Lake Victoria.

2.2 Tsetse Fly Collection

The study tsetse flies were collected using biconical traps which were placed strategically at the shore of Lake Victoria. The traps were colored blue and black which are colors seen to attract tsetse flies. For this species the biconical traps (Figure 1) were baited with acetone (Brightwell *et al.,* 1991), cages were emptied after every 4 hours catch for three days. A total of 235 tsetse flies were collected of which 111 were males and 124 females.

2.3 Tsetse Species Identification and Preparation

Tsestes flies were identified based on their morphological features; in this case the males are known to have smaller abdomens from the females. In this study, 51 female *G. f. fuscipes* were utilized out of which 23 were used during protocol optimization. The insects were then preserved in absolute ethanol before being stored in liquid nitrogen then transported to the laboratory where they were kept at -80 ºC freezer until the time of use (FAO, 1982**).**

2.4 DNA Extraction

In this study, DNA from female *G. f. fuscipes* was extracted by first dissecting under a light microscope to separate the different body part tissues into their respective labeled 1.5 ml eppendorf tubes. DNA was extracted from the legs, head, abdomen and thorax of the selected Glossina species, using phenol chloroform as described by Abdel- Hamid *et al.,* 1999. Briefly, the tissues (head, thorax, abdomen and legs) were separated to different sterile eppendorf tubes. The tissues were then washed in 200 µl of 20 mM Tris EDTA (T.E) buffer of p*H* 8.5 and centrifuged at 17000 g for 5

min at room temperature. This was repeated twice to ensure that all the ethanol was washed off. This was then followed by homogenizing the tissues in the eppendorf, vortexed then added 25 µl of 10% SDS and centrifuged at 17000 g for 5 minutes at room temperature, 15 µl of 10 mg/mL proteinase K and 15 µl of concentration 10 mg/mL RNase were added then mixed well. The contents were incubated overnight in a water bath at 38ºC while ensuring that the eppendorf tubes were well immersed. Equal volume**s** of phenol/chloroform was added twice and centrifuged as above. An equal volume of isoamyl/chloroform was then added and again centrifuged as above. A tenth of the sample volume of 3M potassium acetate was then added followed by 2.5 times of ice cold absolute ethanol to precipitate the DNA. DNA extracted from each tissue had varying concentration and purity as indicated in Table 1.

2.5 DNA Quantification

UV spectrophotometer was used to quantify extracted DNA. Briefly, 900 µl of Tris EDTA (T.E) buffer at *pH* 8 was added to a cuvette and the UV spectrophotometer calibrated at 260 nm and 280 nm. 10 µl of each sample was added and mixed well, T.E buffer was used to blank the spectrophotometer (Paul and Reject, 2012).

2.6 Genomic Gel Electrophoresis

For both genomic and PCR product gel, 0.8% of agarose was dissolved in X1 Tris base EDTA (1XTBE) then boiled for 4 minutes. The contents were then cooled to 45 ºC then 3 µl of ethidium bromide was added. The gel was then allowed to polymerize in a casting plate making sure that the combs do not touch the bottom of the plate. The gel was immersed in the electrode dish while still in the casting plate. 5 µl of the DNA sample mixed with 5 µl bromophenol blue was loaded in the specific wells for each tissue and the electrode dish connected to power. The gel was then run for 40 minutes then placed in the UV chamber where the DNA observed and recorded and recorded**.**

2.7 PCR Amplification

The extracted DNA was used as template for PCR amplification of the target genes. Primers were designed for 22 *G. m. morsitans* OBPs downloaded from NCBI (Liu *et al*., 2010) using Primer3 V0.4.0 shown in Table 2**.** Amplification by PCR was carried out in a total volume of 20 µl containing 8 µl of PCR master mix, 8 µl of PCR H_2O, 1 µl each of reverse and forward primer then 2 µl of the template. The respective tissue DNA concentration was diluted further to 50 ng per 20 µl of PCR reaction. The amplification was carried out in a DNA thermal cycler (MiniCycler, MJ Research, Watertown, MA). The reaction mixtures was initially heated at 95 ºC for 1 min and then subjected to 33 cycles at 95 ºC for 1 min, 55 ºC for 1 min, and 72 ºC for 1 min. Finally, an elongation step at 72ºC for 8 mins was included (Liu *et al*., 2010).

3.0 Results

3.1 DNA Extraction and Quantification from G. *F. Fuscipes* Body Parts

The DNA extracted from different body part tissues (Head, Thorax, Abdomen and Legs) of female *G. f. fuscipes* revealed the same banding pattern (Figure 2). The quantity of extracted DNA from the body parts was between 20 – 80µM/ml with the head having a concentration of 20 µM/ml, 80 µM/ml in the abdomen, 74 µM/ml for the thorax and 53 µM/ml from the legs tissues. Extracted DNA purity values ranged between 1.83 and 2.02 (Table 1), where the head 260/280 ratio of 1.83, 2.02 in the abdomen, 2.01 in the thorax and 1.83 in the leg tissues.

3.2 PCR Amplification of OBP Genes in Four *G. F. Fuscipes* Body Parts

This study focused on screening for the presence of *G. m morsitans* in riverine *G. f. fuscipes* species. The proportion of body parts amplified varied proportionally with most primers amplifying *G. f. fuscipes* head (31.8%) and Abdomen (22.7%). The thorax and legs were amplified in the same proportion (18.2%) (Figure 2). Amplification of Glyceraldehyde phosphate dehydrogenase (GAPDH) gene in *G. f. fuscipes* head, thorax, legs and abdomen with internal control primer, GAPDH of molecular size of 377bp **(**Figure 3)**.** Out of the 22 *G. m. morsitans* OBP primers, only 10 (45.4%) amplified *G. f. fuscipes* body parts while the other 12 (54.5%) did not amplify any of the OBP in any *G. f. fuscipes* body part**.** Considering the OBP distribution across the four body parts understudy, 22.7% OBPs were identifies in the abdomen, 18.2% in the thorax, 31.8% in the head and 18.2% in female tsetse legs (Figure 5). *G. m. morsitans* OBPs 2 and 13 amplified both head and leg with band size of 247bp and 276bp respectively. The thorax and abdomen were amplified specifically with *G m. morsitans* OBPs 6 and 9 with molecular weight of 326bp and 322bp respectively. The two *G m. morsitans* OBPs that amplified head, thorax and abdomen were OBPs 4 and 5 with amplicon of 320bp and 241bp respectively. *G. m. morsitans* OBP 3 amplified the head, legs and abdomen with molecular size of 345bp while *G. m. morsitans* OBP 1 showed a band size of 221bp on the legs and head. *G. m. morsitans* OBPs 8 and 19 amplified thorax and leg respectively (Figure 3).

4.0 Discussion and Conclusion

Glossina m. morsitans and *G. f. fuscipes* are vectors of Trypanosomiasis with *G. m. morsitans* being savanna species while *G. f fuscipes* being a riverine species. They both have different host range based on the ecological niche they occupy. Olfaction plays an important role in identification of hosts that the tsetse fly feed on. Odorant binding proteins have been identified in *G. m. morsitans* and reported to be expressed at different stages (Liu *et al*., 2010).

DNA extraction showed that there was similar proportion of the sample DNA from different Tsetse body parts; this could be an indication that despite the tissue difference, the molecular weight of DNA in the same species will always remain the same. The study did show that there were several *G. m morsitans* OBP genes found

in *G. f. fuscipes* which could probably mean that they have conserved functions despite having different hosts range and mates.

Ten *G. m. morsitans* OBP genes were identified in *G. f. fuscipes* while 12 *G. m. morsitans* genes were absent giving a probable indication that the genes that were absent are *G. m. morsitans* species specific. This has also been reported for mosquitoes where some *Anopheles gambie* OBPs were lacking in *Aedes aegypti* **(**Bill and Marcus, 2011). The male *A. gambie* are known to feed on various nectar and while the female rely on blood meal to provide nutrient for the eggs to mature. On the other hand, *A. aegypti* are known to be generalized feeder with *A. gambie* preferring to feed on humans than other animals. These mosquitoes exhibit diversity in the olfactory proteins and mechanism that aid then to identify their host (Bill and Marcus, 2011).

The distribution of OBPs in different body parts of *G. f. fuscipes* may pinpoint that some OBPs are involved in functions not related to olfaction. This had been observed in insects such as Hawk moth *Manduca sexta* (Lepidoptera: Sphingidae) and *Anopheles gambiae* (Diptera: Culicoidae) **(**Liu *et al.,* 2007; Vosshall and Stocker, 2007).

OBP genes could be playing a critical role in enabling *G. fuscipes fuscipes* host and specialization shifting in consistent with alteration in the odour detecting machinery. This is because insects are known to use peripheral olfactory system to responds to environmental changes by binding odors that play a critical role in conveying information on specific cues that bring about innate behaviors and discrimination of a given odor among the broad chemical composition that can be learnt and guide adaptive behavior (Bill and Marcus, 2011).

Identification of more OBP genes in the head compared to other body parts could indicate the richness of olfactory sensillia in the head expressing many OBPs. This need to be investigated further to determine their precise role as reported by Bruyne and Baker, (2008) in *A. gambie*. It is known that the head of the tsetse fly play a critical role in terms of olfaction to the environmental, physiological and ecological changes (Rutzler and Zwiebel, 2005).

There was variability in the presence of OBP genes in different parts of the tsetse tissues. This could be as a result of function specificity in different body parts. For example the head which has the antennae could be having only those OBPs that are associated with olfaction especially those associated with mate identification, oviposition places and host detection, these OBPs could be absent in the legs and other parts of the body since they have not evolved in performing those functions. While OBPs in the legs could be specific for detecting safe landing places which could not be the function of the head and other body parts.

Also considering the fact that only female *G. f fuscipes* were used in this study the *G. m morsitans* OBP genes that were identified in this study could be sex specific. This is consistent with the findings of Sengul and Tu, (2008) where studies of OBP1 and OBP 2 reported that they are specific in females only. The same OBP orthologs were studied in *Anopheles gambiae* by Biessmann *et al*. (2005) and Li *et al.* (2005). This underscores the fact that some OBP are sex specific, however to acertain this it will be necessary to study the same OBPs identified in female *G. f fuscipes* in male *G. f fuscipes* and *G. morsitans morsitans*.

From this study we conclude that there exist different OBP genes within tsetse flies species for example between *morsitans* and *palpalis* with the OBPs playing a critical role in discriminating mates, sites of oviposition and their hosts providing the need for further investigation to determine expression levels and their probable functions. This may provide important information to be used in designing tsetse control strategy based on olfaction mediated behavior.

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Table Legend

Table 1: The genomic DNA of different tsetse fly body parts tissues depicting their quantity in µl/ml and purity. 260A= Absorbance at the wavelength of 260 and 280A= Optical density at 280 wavelength

Table 2: Primers from *G. m. morsitans* OBP genes as designed by primer3 plus version 0.4.0 that gave positive amplification.

Figure Legend

Figure 1: The biconical tsetse fly trap being set up at the shores of Lake Victoria, Mbita Point

Figure 2: Genomic gel photo of the denaturing gel electrophoresis as taken under UV, in this figure FH stands for tsetse head, FTH thorax, FAB abdomen and FL tsetse legs

Figure 3: Control gel, *G. f fuscipes* body parts amplified with GAPDH primer (377bp). L = 1kb base pair; -C = negative control; +FAB = Female abdomen; +FL = female legs; +FT = female thorax; +FH = Female head

Figure 4:Distribution of OBP genes in different body parts, *G. f fuscipes* PCR products amplified with OBP primers. L = 1 kb ladder; FL = female legs; FAB = female abdomen; FTH = female thorax; –C = negative control

Figure 5: Percentage OBP distribution across different body parts in *Glossina f fuscipes*

Figure 1: The biconical tsetse fly trap being set up at the shores of lake Victoria, Mbita point

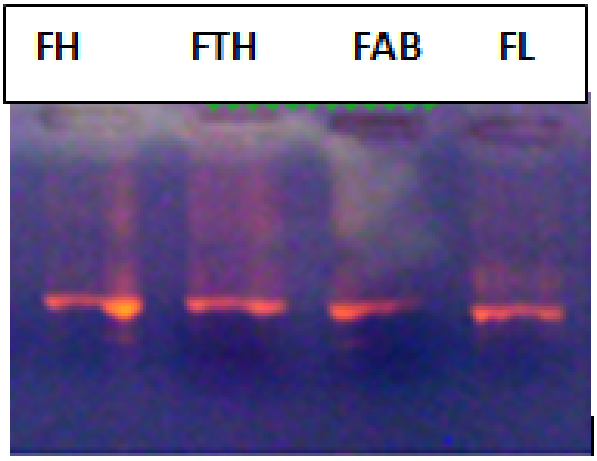


Figure 2: Genomic gel, DNA extracted from different tsetse body parts tissues; FH=Female head, FTH= Female thorax, FAB= Female abdomen and FL= Female legs

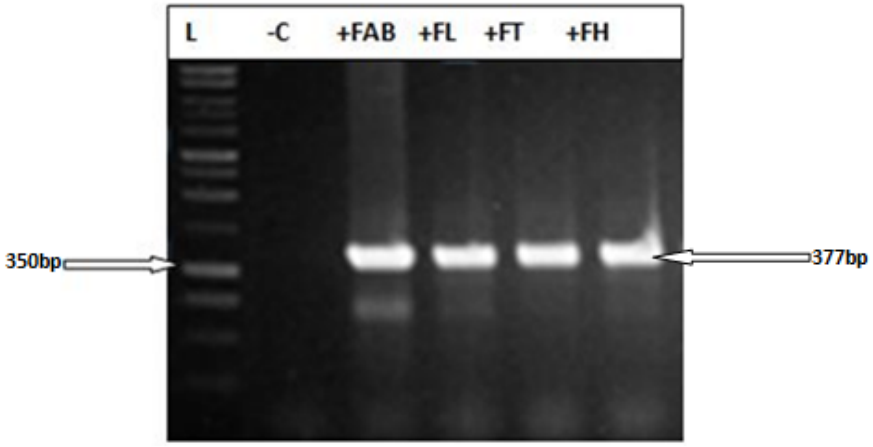
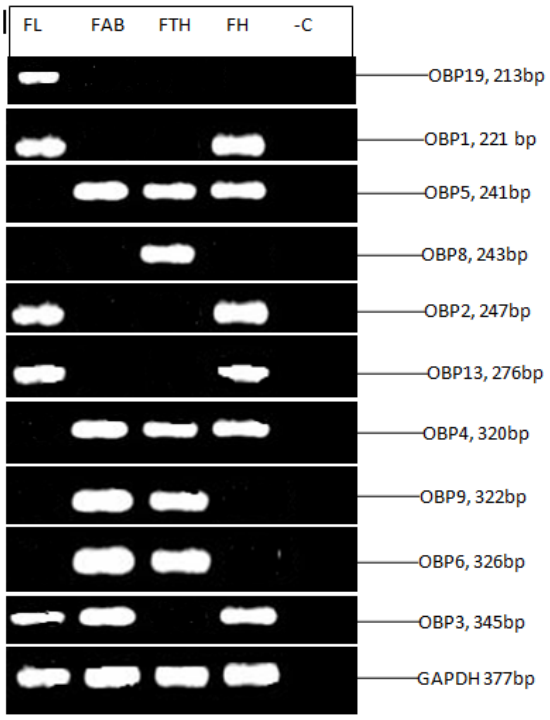


Figure 3: Internal control PCR product gel, G. f fuscipes body parts amplified with GAPDH primer of band size 377bp. Ladder (L) = 1kb base pair; -C = negative control; +FAB = Female abdomen; +FL = female legs; +FT = female thorax; +FH = Female head



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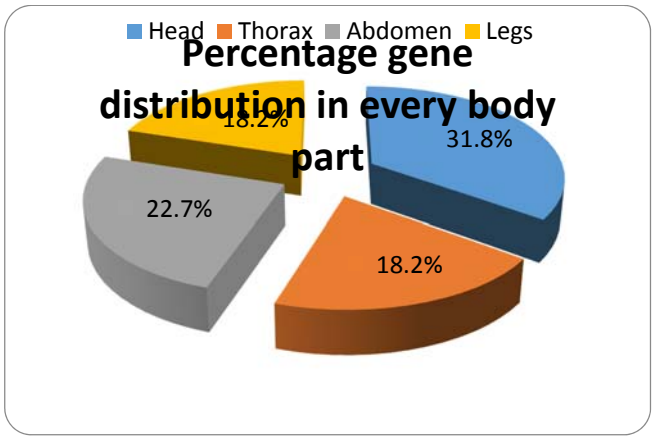

Figure 5: Percentage OBP distribution across different body parts in Glossina f fuscipes

Table 1: The genomic DNA of different tsetse fly body parts tissues depicting their quantity in µl/ml and purity. 260A= Absorbance at the wavelength of 260 and 280A= Optical density at 280 wavelength

Female tsetse flies tissues of *G. f fuscipes*				
Parameter	Head	Abdomen	Thorax	Legs
Quantity µM/ml	20	80	74	53
Purity 260A/280A	1.85	2.02	2.01	1.83

INCLUDE CONCENTRATION OF EACH SAMPLE

Table 2: Primers from G. m morsitans OBP genes as designed by primer3 plus version 0.4.0 that gave positive amplification.

Gene	Sequence (5'►3')	
	Forward primer	Reverse primer
GmmOBP1	GCATGGAAGCGAAGAAAGTC	CTTGTCTGCGCATTTTTCAA
GmmOBP2	TGAAAATTGGAAACGGCCTA	TTTACCGCGACAAGGATTTC
GmmOBP3	AGTGAGAAGGGCTGGTTTGA	TCATTTCGGCCTGTTTAAGG
GmmOBP4	GCTGGATCGGCTTAGAGTTG	CTTCATCCAGAAGCACACGA
GmmOBP5	TAAGCCTGCGGATAATTTCG	TAAGCCTGCGGATAATTTCG
GmmOBP6	TGCTAATGGCAAATTGGTGA	CCGTACGTATCGGCTTGTTT
GmmOBP8	GTGAATGTTGTCGACGATGC	GAAGCAAACATGCCACGAC
GmmOBP9	CGATGACAATGGTGACGTTC	CAGGGTCTGCTTTCTTCCAG
GmmOBP13	TGAAGTGCGTCATGGAGAAA	CGCAAGCACATGGATACCTT