SCREENING OF ODORANT BINDING PROTEINS GENES IN *GLOSSINA FUSCIPES FUSCIPES*

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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. Convectional PCR was used to screen for the presence of OBP genes previously identified in *Glossina morsitans morsitans.* Twenty two primers were designed for G*. m. morsitans* OBP genes and used to screen G*. f. fuscipes* head, thorax, abdomen and legs. 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 diversity between OBPs from riverine (*G. f. fuscipes*) and savanna (*G. m morsitans*) species which may be key in understanding 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 reproduction (mating and oviposition site finding) and in nutrition (detection of food sources) (Kaissling, 2001). Insect sense the volatile chemicals from the environment through the antennae (Shabhag *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, 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) OBPs 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, 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 of 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 on it. 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). Mosquitoes the OBPs are used to identify and discriminate hosts by temperature and chemical sensation (Dekker *et al*., 2002) and 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) in which it was observed that common animals, such as zebra and wildebeest, are not hosts and each glossina species has a specialist range of host animals; for example, the preferred host for *G. m. morsitans* is the warthog but also observed by Den Otter *et al.,* (1992) to feed on feeds on ox, buffalo, kudu and human.

2.0 Materials and Methods

2.1 Study Site

Samples were collected from Mbita in Homa bay 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) baited with acetone (Brightwell *et al.,* 1991), Cages were emptied after every 4 hours catch.

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

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. The insects were then preserved in absolute ethanol and RNA later before being stored in liquid nitrogen then transported to the laboratory. In the lab the tsetse flies were in 80^{O}C freezer until the time of use (FAO, 1982a).

2.4 DNA Extraction

Before DNA extraction, the insects were dissected under a light microscope to separate the different body part tissues in their respective labeled 1.5 ml eppendorf tubes. DNA was extracted from the antenna, 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, legs and antenae) were separated to different sterile eppendorf tubes. The tissues were then washed in 200 µl of T.E buffer of pH 8.5 and centrifuged at 16000 rpm for 5 min at room temperature. This was repeated twice to ensure that all the ethanol was washed off. This was then followed by crashing the tissues in the eppendorf into powder and then vortexed. 25 µl of 10% SDS and centrifuged at 16,000 rpm for 5 minutes room temperature, 15 µl of both proteinase k and RNase were added and mixed thoroughly. The contents were incubated at 38ºC water bath overnight while ensuring that the eppendorf tubes were well immersed in the water bath. Equal volume of phenol/chloroform was

added twice and centrifuged as above. Then, 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.

2.5 DNA Quantification

UV spectrophotometer assay was used to quantify extracted DNA. Briefly, 1 ml of Tris EDTA (T.E) buffer of 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 to 900 µl of T.E buffer and mixed well, T.E buffer was used to blank the spectrophotometer (Gaurav and Reject, 2012).

2.6 Genomic Gel Electrophoresis

0.8 g of Agarose was weighed and put in a bottle where 100 ml of 1X tris base EDTA (1XTBE) buffer was added and boiled in a microwave for 4 minutes. The contents were then removed from the microwave and cooled to 45 ºC then 3 µl of ethidium bromide was added then the cooled gel was poured in the casting plate making sure that the combs do not touch the bottom of the plate then left to solidify properly then the comb removed and the gel 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 power connected for genomic and PCR product. The gel was then let to run for 40 minutes after which the gel was removed, placed in the UV chamber and the DNA or the amplified gene presence viewed and recorded by photography.

2.9 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 identified from NCBI using Primer2 V0.4.0 (Liu *et al*., 2010) and are shown in table 4. 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, 0.5 µl of reverse and forward primer then 1.5 µl of the template. The amplification was carried out in a DNA thermal cycler (MiniCycler, MJ Research, and Watertown, MA). The reaction mixtures was initially heated at 95^0C for 1 min and then subjected to 33 cycles at 95^0C for 1 min, 55 ^{0}C for 1 min, and 72 ^{0}C for 1 min. Finally, an elongation step at 72^0C 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 – 80ul/mi while the spectrophotometer quantification had values ranging from 1.83 to 2.02 (Table 1).

Table 1: Shows DNA quantification and purity check results

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

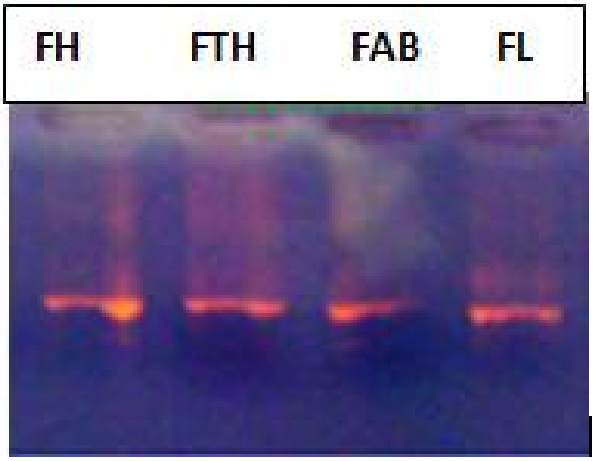

Figure 2: Genomic gel, DNA extracted from different tsetse body parts tissues; FH=Female head, FTH= Female thorax, FAB= Female abdomen and FL= Female legs

3.2 PCR Amplification of *G. F. Fuscipes* Body Parts

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 3). Amplification of the *G. f. fuscipes* head, thorax, legs and abdomen with internal control primer (GAPDH) identified a band size of 377bp (Figure 4). Out of the 22 *G. m. morsitans* OBP primers, only 10 amplified *G. f. fuscipes* body parts while the other 12 didn't pick any of the *G. f. fuscipes* body part (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, thorax and abdomen with molecular weight 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 (Table 2).

Table 2: Shows results for screening for the presence of OBP in G. f fuscipes using primers designed from G. m. morsitans in this table positive sign (+) signifies presence of a given OBP gene in that tissue while negative (-) shows the absence of that given give in the respective tissue

	Screening of OBP genes in *G. f fuscipes* using primers designed from *G. m morsitans* OBP genes																					**Percentage gene presence in respective body part**
Primers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Head	√	√	√	√	√	√	X	X	X	X	X	X	√	X	X	X	X	X	X	X	X	X	31.8%
Thorax	X	X	X	X	√	√	X	√	√	X	X	X	X	X	X	X	X	X	X	X	X	X	18.2%
Abdomen	X	X	√	√	√	√	X	X	X	X	X	X	√	X	X	X	X	X	X	X	X	X	22.7%
Legs	√	√	√	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	√	X	X	X	18.2%

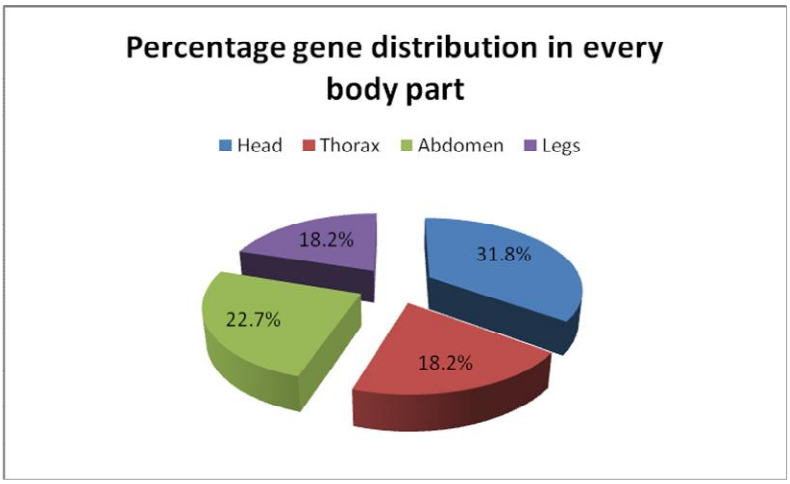

Figure 3: OBP distribution across different body parts in Glossina f fuscipes

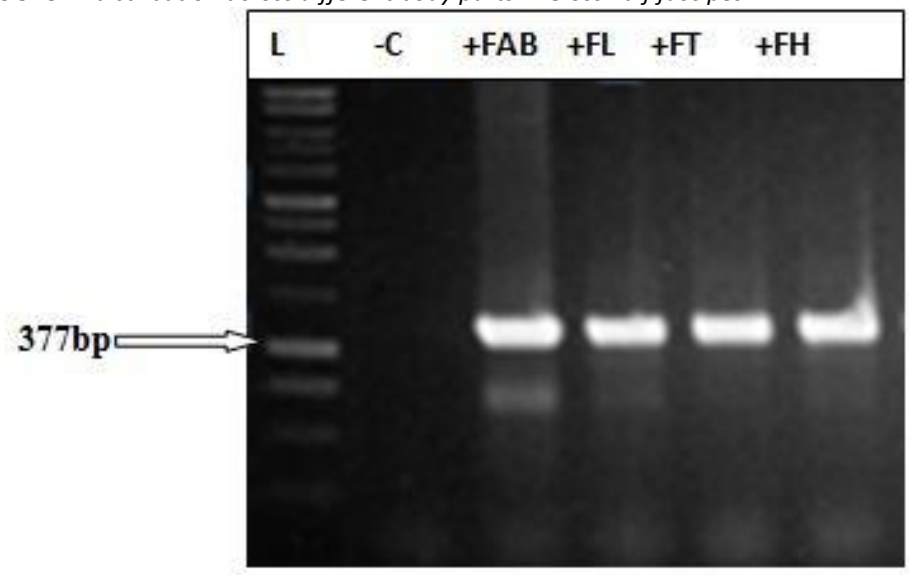

Figure 4: Control gel, G. f fuscipes body parts amplified with GAPDH primer (377bp). L = 1kb base pair; -C = negative control; +FAB = Female abdomen; +FL = female legs; +FT = female thorax; +FH = Female head

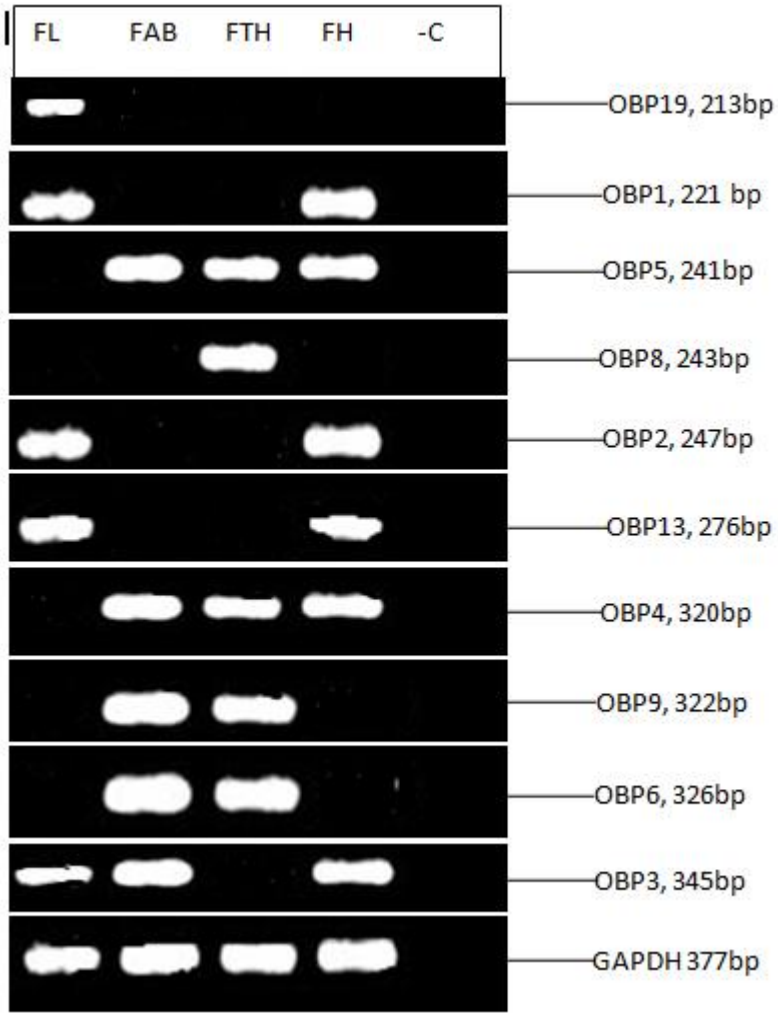

Figure 5: Distribution of OBP genes in different body parts, G. f fuscipes PCR products amplified with OBP primers. L = 1 kb ladder; FL = female legs; FAB = female abdomen; FTH = female thorax; –C = negative control

3.3 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 were similar proportion of the sample got from different Tsetse body parts, this could be an indication that despite the tissue 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 are 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; Shiraiwa, 2008; 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 & 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).

From this study we conclude that there exist different OBP genes among tsetse flies i.e. between *morsitans* and *palpalis* with the OBPs playing a critical role in identifying 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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