LOCALIZATION AND EXPRESSION PROFILES OF ODORANT RECEPTOR GENES IN NON-OLFACTORY TISSUES OF MALE AND FEMALE TSETSE FLY *Glossina morsitans morsitans*

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2021

Localization and Expression Profiles of Odorant Receptor Genes in Non-olfactory Tissues of Male and Female Tsetse Fly *Glossina morsitans morsitans*

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Molecular Biology and Bioinformatics of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

Signature……..Date……

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This research thesis has been submitted for examination with our approval as the university supervisors

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DEDICATION

I dedicate this work to my parents Chrispinus and Jean Musundi, siblings Christine, Beryl, Reagan Musundi, and nephews, Braville, Christian and Leris.

ACKNOWLEDGEMENTS

Throughout carrying out this research and writing this thesis, I have received a great deal of assistance and support from various quarters. I would first like to thank my supervisors, Dr. Steven Ger Nyanjom and Professor Fred Wamunyokoli, for their support, direction, insights, valuable comments, and immense knowledge, which were key in completing the above thesis. I would also like to acknowledge my laboratory colleagues, Josephat Bungei and Kerubo Rebecca, for their encouragement while undertaking the above study. I am also indebted to my family members and friends for their encouragement and patience throughout this entire study

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LIST OF ABBREVIATIONS AND ACRONYMS

AAT	Animal African Trypanosomiasis
cDNA	Complementary DNA
CNS	Central Nervous System
CP	Chemosensory Proteins
GR	Gustatory Receptors
HAT	Human African Trypanosomiasis
IRs	Ionotropic Receptors
NECT	Nifurtimox–Eflornithine Combination Treatment
ODE	Odorant Degrading Enzyme
OBP	Odorant Binding Proteins
OR	Odorant Receptors
ORN	Odorant Receptor Neuron
ORCO	Odorant Co-receptor
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction.
SIT	Sterile Insect Technique
SNMP	Sensory Neuron Membrane Proteins

ABSTRACT

Glossina morsitans morsitans, a savannah species of tsetse fly, are vectors of trypanosome which cause African trypanosomiasis. Tsetse flies rely on olfaction in identifying hosts, mates, larviposition site, and escape from predators. Olfactory proteins located in the tsetse antennae are involved in perceiving odors from the external environment, processing and signal transduction. Odorant receptors (ORs) primarily located in the antennae's olfactory receptor neuron have a vital role in the signal transduction process. Recent evidence in the mosquito, *Anopheles gambiae*, and fruit fly *Drosophila melanogaster* suggests expression of ORs in insect's non-olfactory tissues implying other physiological functions that could aid in vector control. The aim was this study was therefore to study the expression of Ors in non-olfactory tissues in males and females *G. m. morsitans.* Herein, ORs expression in the brain, gut, and reproductive tissues of male and female *G. m. morsitans* was determined. Total RNA was extracted from brain, gut, and reproductive tissues (testis, and accessory, spermathecae and ovary) and used to synthesize cDNA. 46 OR genes identified in *G. m. morsitans* were screened in individual tissues using conventional PCR. Amplicons were sequenced and searched against VectorBase and their protein sequences used to identify homologs in *D. melanogaster* and other *Glossina* spp genomes in FlyBase and VectorBase, respectively. ORs domains and the number of transmembrane helices were confirmed using the Conserved Domain Database and TMHMM, respectively. Gene ontology functions of the identified *D. melanogaster* homologs were inferred from FlyBase. Tissue expression of homologs in *D. melanogaster* was checked in Fly Atlas 2. Real-time PCR was used to determine the expression profile of 46 ORs in male and female *G. m. morsitans* brain, gut and reproductive tissues. Results revealed the localization of Ors in the male brain (41), gut (38) and reproductive tissue (39) and similarly, female brain (44), gut (23) and reproductive tissues (40) respectively. Sequence identity between *G. m. morsitans* and other *Glossina* genomes ranged from 21.7% to 99.4% while for *D. melanogaster* it ranged from 22.4 to 77.5%. All sequenced ORs belonged to the seven transmembrane odorant receptors (7tm_6) family and varied in number of transmembrane helices. Gene ontology identified signal transduction and response to stimuli as the main biological processes. The membrane was identified as the main cellular component, while odorant receptor activity and odorant binding were the main molecular function. The presence of transmembrane helices and biological process such as signal transduction suggests ORs act as receptors for endogenous ligands. Fly Atlas results revealed expression of *G. m. morsitans* orthologs in *D. melanogaster* including DmelOR85f (GmmOR28) and DmelOR67d (GmmOR42) in the male brain, DmelOR94a (GmmOR12) in the male gut, and DmelOR67c (GmmOR27) and DmelOR13a (GmmOR32) in the male testis. DmelOR67c (GmmOR27) was also present in female brain and DmelOR49b (GmmOR33) in female gut. Quantitative real time PCR revealed four significantly highly expressed OR genes in male *G. m. morsitans* brain (GmmOR19, GmmOR28, GmmOR30, GmmOR31),six in the gut (GmmOR7, GmmOR12, GmmOR32, GmmOR34, GmmOR42, GmmOR44) and three ORs in the testis and accessory glands (GmmOR32, GmmOR44, GmmOR46). In females, two ORs were highly expressed the

brain (GmmOR25, GmmOR27), three in the gut (GmmORr17, GmmOR33, GmmOR35) and three in the reproductive tissues (GmmOR9, GmmOR22, GmmOR27). OR expression in non-olfactory tissues suggests unique physiological roles in the brain, gut and reproductive tissues in male and female *G. m. morsitans*. Thus, GmmOR28 expression in the male and female brain may suggest a role in brain related functions while presence of GmmOR32 and GmmOR44 in the male gut and reproductive tissue and GmmOR27 in the female brain and reproductive tissue suggests conserved non-olfactory functions across different tissues. The conserved odorant receptor, Orco was expressed in male reproductive tissue and may suggest male reproductive roles such as spermatozoa activation. The findings provide insight on the localization and expression profile of OR genes in non-olfactory organs (brain, gut and reproductive tissues) in male and female *G. m. morsitans* and open avenues to investigate functional roles of significantly expressed ORs for the control of *Glossina* and trypanosomiasis.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

African trypanosomiasis is a neglected tropical disease that affects both livestock and human beings resulting in devastating economic and health effects (Brun et al., 2010; Hotez & Kamath, 2009). In humans, the disease is referred to as human African trypanosomiasis (HAT), while in animals, animal African trypanosomiasis (AAT) (Steverding, 2008). HAT exists in two forms; (i) the chronic form caused by the protozoa *Trypanosoma brucei gambiense* found in Central and West Africa and, (ii) the acute form caused by the protozoa *Trypanosoma brucei rhodesiense* present in East and Southern Africa (Brun *e*t al., 2010). The rhodesiense form of the disease is zoonotic with occasional infections reported in human beings, while the gambiense form is anthroponotic and responsible for approximately 98% of HAT cases (World Health Organization, 2013). *Trypanosoma brucei*, *Trypanosoma simiae*, *Trypanosoma congolense*, *Trypanosoma evansi*, *Trypanosoma equiperdum*, and *Trypanosoma vivax* cause AAT (WHO, 2015).

Tsetse flies are vectors of African trypanosome, the protozoan parasite which cause HAT and AAT (WHO, 2018). The members of the genus *Glossina* are classified into subgroups depending on the environment they inhabit: thus, savannah (morsitans), forest-dwelling tsetse (fusca), and riverine tsetse (palpalis). 33 species and subspecies of *Glossina* are present in sub-Saharan Africa, with two species, *G. morsitans submorsitans*, and *G. fuscipes fuscipes*, being reported in the southwestern corner of the Arabian Peninsula (Elsen et al., 1990). *Glossina morsitans morsitans* is restricted to savanna grasslands, preferentially feeding on livestock and, thus, is an important vector for AAT.

Tsetse flies show unique feeding preferences when selecting hosts (Clausen et al., 1998). Blood meal analysis from the gut of *G. fuscipleuris* and *Glossina austeni* revealed a feeding preference for bushpigs while *Glossina morsitans* mainly fed on warthogs, as well as ruminants or hippopotamuses (Clausen et al., 1998). Bloodmeal analysis from the gut of *G. swynnertoni* revealed preference for *w*arthog and giraffe but not Thompson gazelle, zebra and wildebeest despite being tsetse flies abundant areas (Auty et al., 2016). The differential preference to specific hosts is attributed to odor compounds released from the vertebrates which act as repellents or attractants. This olfactory uniqueness has been exploited in developing artificial bait technologies that mimic behaviors of odors produced by hosts and non-hosts (Nagagi et al., 2017). Naturally occurring repellents from a non-preferred host body, such as the waterbuck, *Kobus defassa,* have employed to develop an innovative cattle neck dispenser which reduced trypanosome infection by 80%, and enhanced traction power among smallholder cattle in Kenya (Saini et al., 2017). The combination of 4-methyl guaiacol and the waterbuck repellant compounds also reduced catches of *Glossina fuscipes fuscipes* from the palpalis group suggesting such repellants act across a broad spectrum (Mbewe et al., 2019). These odor-based targets directly target the olfaction system of insects. However, there remains limited information on which specific olfaction proteins are affected by various attractant and repellant molecules across various tsetse species.

Soluble proteins such as chemosensory proteins (CSPs) odorant degrading enzymes (ODEs), and odorant-binding proteins (OBPs) (Liu et al., 2010: Liu et al., 2012) and receptor proteins like gustatory receptors (GRs), ionotropic receptors (IRs), sensory neuron membrane proteins (SNMPS) and odorant receptors (ORs) mediate olfaction in tsetse fly (Macharia et al., 2016; Obiero et al., 2014). Genome-wide comparative studies revealed *G. m. morsitans* contains 46 OR genes, 14 GR genes, 30 ionotropic & ionotropic glutamate receptors genes (IR/IGR), 30 OBP genes, and 5 CSPs (Macharia et al., 2016; Obiero et al., 2014). ORs are heteromeric proteins with seven transmembrane domains (Lundin et al., 2007; Smart et al., 2008), which operate as ligand-gated ion channels (Sato et al., 2008; Wicher et al., 2008). Amongst insects, odorant co-

receptor(Orco) is a highly conserved canonical co-receptor and functions as a heteromer with an odor-specific OrX (Larsson et al., 2004; Neuhaus et al., 2005). In the absence of a tuning OR, Orco fails to confer odorant sensitivity (Elmore et al., 2003).

The major olfactory organs in insects is the antennae that houses Olfactory Receptor Neurons (ORN) containing olfactory sensilla, which contains pores allowing for entry of various odors (Mucignat-Caretta, 2014). High expression of GmmOR33 and GmmOR45 genes in the male and female antennae of *G. m. morsitans* implies their role in olfaction (Nyanjom et al., 2018). Electrophysiological recordings of GmmOR9 in *G. m. morsitans* antennae's sensory pit also showed a strong positive response to acetone, 2-propanol, and 2-butanone (Chahda et al., 2019). Butanone and acetone are well-known attractants used for luring tsetse flies into traps.

ORs are not exclusively localized in the primary olfactory organs (Li et al., 2017; Xu et al., 2019). Orco's expression in adult male and female leg tissues, OR20 in larvae and OR28 pupae of *G. m. morsitans* demonstrates presence of ORs in non-olfactory tissues (Moindi *e*t al., 2018). Recent studies reported the expression of odorant receptors in non-olfactory tissues across various insect orders and implicated ORs in mediating endogenous ligand recognition, fertilization, sperm chemotaxis, and spermatozoa activation (Hansen *e*t al., 2014; Pitts et al., 2014; Wang et al., 2015). Other olfactory proteins such as OBPs and CSPs are implicated in various non-olfactory functions such as pheromone production and release from pheromone glands (Dani et al., 2011; Gu et al., 2013; Li et al., 2008; Zhang et al., 2013) and development of eggs and ovaries (Costa-Da-Silva et al., 2013; Marinotti et al., 2014). There is limited information on the expression of ORs in non-olfactory organs in *Glossina*. Identification of ORs expressed in non-olfactory tissues could provide possible insights on their physiological functions which could be exploited to manage trypanosomiasis.

1.2 Statement of the Problem

Tsetse flies inhabit approximately 9 million km^2 which translates to much of the tropical Africa landmass (Cecchi & Mattioli, 2009). This places about 50 million herds of cattle and 70 million people within 36 sub-Saharan Africa countries at risk of exposure to AAT and HAT respectively. In Kenya, tsetse flies inhabit about 138,000 Km^2 or 23% of the total land mass which includes 38 out of 47 counties, placing about 11 million people at the risk of disease exposure (Shaw et al., 2014) The cases of AAT in Kenya range from 1.39% to 4.19% in cattle (Adungo et al., 2020; Ngari et al., 2020). However, large areas still remain at risk of AAT due to movement of infected animals, and mechanical transmission of trypanosomes by other biting flies such as *Stomoxys* and tabanids (Ahmed et al., 2016). Communities affected by AAT report poor quality meat and reduced milk yield, livestock mortality, reduced land for grazing and cattle sales (Food and Agriculture Organization, 2019). In addition, 36 million doses of trypanocidal drugs are annually used in managing AAT increasing incidences of drug resistance (FAO, 2019). The total agricultural losses within sub-Saharan Africa associated with cattle production annually exceeds US$ 1 billion while the total agricultural losses amount to US$ 4.5 billion annually (FAO, 2019). AAT therefore increases poverty levels among pastoral communities which are solely dependent on livestock farming. Models on the effect of climate and anthropogenic changes in Kenya suggests an expansion of tsetse flies into areas such as Bomet and Narok with the infestation rate estimated to be 83% and 67% respectively by 2051-2059 (Messina et al., 2012). This could threaten dairy agricultural practices and places large populations in these areas at risk of AAT and HAT.

Apart from agriculture, trypanosomiasis infection also impacts human health and tourism sector. Previous accounts of patients affected by sleeping sickness revealed a socioeconomic burden to families. Treatment regimen such as NECT(nifurtimox–eflornithine combination treatment) is expensive with a single dose cost ranging from about £ 288- 366 (Simarro et al., 2012). The cost of medical services places a strain in

family resources resulting in either the leasing or sale of family assets, use of personal saving or seeking credit which serve to maintain a cycle of poverty among neglected communities (Bukachi et al., 2017). Although Kenya last reported local cases of HAT in 2009, two cases were reported among tourists who visited Maasai Mara Reserve in 2012 (Clerinx et al., 2012; Wolf et al., 2012). The risk of infection in parks and animals remains low but serve as an important source of exposure to tourists and travellers. Also, pastoralist communities residing close to game reserves are at risk of AAT infection considering wild animals are reservoirs of trypanosomes. The total cost saving in the next 20 years associated with the elimination of trypanosomiasis in Kenya and the remaining East African region amounts to US$590 million and US$ 2.4 billion respectively (Shaw et al., 2014). Targeting of tsetse flies remains a viable approach in the eradication of African Trypanosomiasis.

Management of trypanosomiasis focuses on either targeting the trypanosome parasite or vector, *Glossina spp.* Targeting the trypanosome parasite is limited by the lack of vaccines due to antigenic variation, lack of specific diagnostic tests, drug toxicity and drug resistance (Bottieau & Clerinx, 2019; Field et al., 2017). Although vector control approaches successfully reduced tsetse populations, some methods are prone to different limitations. For example, use of traps and targets in capturing tsetse flies only covers a small geographical region while the Sterile Insect Technique (SIT) is limited by the high cost and lack of irradiation facilities within the Sub-Saharan region (Aksoy et al., 2017). Moreover, the use of insecticides in reducing tsetse populations results in the development of insecticide resistance (WHO, 2004). The baiting of traps with odorants that mimic the behavior of natural hosts or non-bovine hosts has been successfully used to eliminate *Glossina* populations and development of the cattle neck repellant dispenser (Saini et al., 2017). The repellant is detected by olfactory proteins, including ORs that play a role in signal transduction in the brain, ultimately resulting in behavior change in tsetse flies. The ORs are expressed as dimers with odorant co-receptor, Orco, which facilitates signal transduction processes resulting in behavior change (Sato et al., 2008; Wicher et al., 2008). The antennae and maxillary palps are the main olfactory organs and

therefore OR's expression in these sites is directly linked to olfaction functions (Mucignat-Caretta, 2014). However, ORs are not exclusively expressed in the antennae and therefore their expression in non-olfactory tissues could signal non-chemoreception roles that could serve as future potential targets for tsetse control.

1.3 Justification

The antennae and maxillary palps are the primary olfactory organs in insects (Mucignat-Caretta, 2014). Expression of olfactory proteins such as ORs in these tissues is directly associated with olfaction functions such as searching for mates, hosts, or larviposition sites (Hansson & Stensmyr, 2011). ORs are of particular interest because of their important role in signal transduction where they act independently to open ligand-gated ion channels or as metabotropic G-protein coupled receptors activating secondary messengers which alter intracellular calcium levels, and gene transcription (Sato et al., 2008; Wicher et al., 2008). Although ORs are primarily expressed in the antennae, their expression in other non-olfactory tissues has been reported suggesting possible non-chemosensory functions. For instance, ORs expressed in the male reproductive system of *An. gambiae* and *D. melanogaster* are linked to spermatozoa activation and maintaining the actin cap respectively (Dubey et al., 2016; Pitts et al., 2014). There is limited information about the functions of these ORs *G.m. morsitans* despite belonging to the same insect order as *An. gambiae* and *D. melanogaster*. Understanding the role of these ORs in non-olfactory tissue will provide basic knowledge that future studies may leverage on to develop control strategies of the tsetse fly vector, which will contribute to disease control.

1.4 Research Questions

1). Which ORs are localized in the brain, gut, and reproductive tissues of *G. m. morsitans*?

2). Which OR homologs are found in the brain, gut and reproductive tissues of *G. m. morsitans?*

3.) How do gene expression levels of ORs compare in the brain, gut, and reproductive tissues of male and female *G. m. morsitans*?

1.5 Objectives

1.5.1 Overall Objective

To determine OR genes localized and expressed in the brain, gut and reproductive tissues of male and female *G. m. morsitans*

1.5.2 Specific Objectives

1. To identify ORs localized in the brain, gut and reproductive tissues of *G. m. morsitans.*

2. To identify homologs of ORs in the brain, gut and reproductive tissues of *G. m. morsitans*

3. To determine the relative gene expression levels of ORs in the brain, gut and reproductive tissues of *G. m. morsitans*

CHAPTER TWO

LITERATURE REVIEW

2.1 Tsetse Distribution in Africa

Thirty-one species of *Glossina* currently exist. These species fall under three subgenera based on their habitat; *Austenina* Townsend, *Glossina* Wiedemann and *Nemorhina* Robineau-Desvoidy which correspond to the fusca (forest), morsitans *(*savannah) and palpalis (riverine) group (Krafsur, 2009). The fusca group is largely present in forest belts, coastal forests and savannah regions although increasing human activity in forests is associated with the disappearance of the subgenus. Fusca group members such as *Glossina longipennis* are present in the southern border of Ethiopia, north eastern Kenya, Somalia and Tanzania as well as south east Sudan while *Glossina brevipalpis* is found across eastern parts of Africa from Mozambique to South Africa, to Ethiopia, around Lake Tanganyika and Somalia (Pollock, 1982). The remainder of the fusca species is largely limited to Africa's thickly forested areas (Pollock, 1982).

Members of the subgenus *Glossina* are largely present in the woodland Savannah. These species represent main vectors of trypanosomes in wild animals and cattle (Bekele et al., 2015). *G. pallidipes*, *G. swynnertoni* and *G. morsitans* are present in East Africa and are involved in *T. b. rhodesiense* transmission (Franco et al., 2014). The palpalis group dominates western and central Africa and are found in the forest, lakes and riverbanks, cocoa or coffee plantations, mangroves and swamps and can adapt to different environmental conditions surviving even in towns (Brun et al., 2010). The palpalis group are known vectors of HAT. *G. p. gambiensis* and *G. palpalis palpalis* are vectors of *T. b. gambiense,* while *G. fuscipes* transmits *T. b. rhodesiense* and *T. b. gambiense* (Franco et al., 2014). *G. fuscipes* is present from Cameroon to Congo, while *G. palpalis* is found from Senegal to Angola alongside the Atlantic coast. *Glossina fuscipes fuscipes* occupies the inland areas of Africa centered around the Democratic Republic of Congo but covers some regions in Gabon and Cameroon to Uganda and western parts of Kenya

(Pollock, 1982). *Glossina fuscipes fuscipes* has been documented in Lake Victoria, Buvuma and islands of Kalangala (Albert et al., 2015; Okoth, 1991). *Glossina tachinoides* are distributed around the belt of Guinea and Central African Republic while *Glossina caliginea* and *G. pallicera* are present in the thick forest areas and mangrove forest of West Africa (Pollock, 1982) (Figure 2.1).

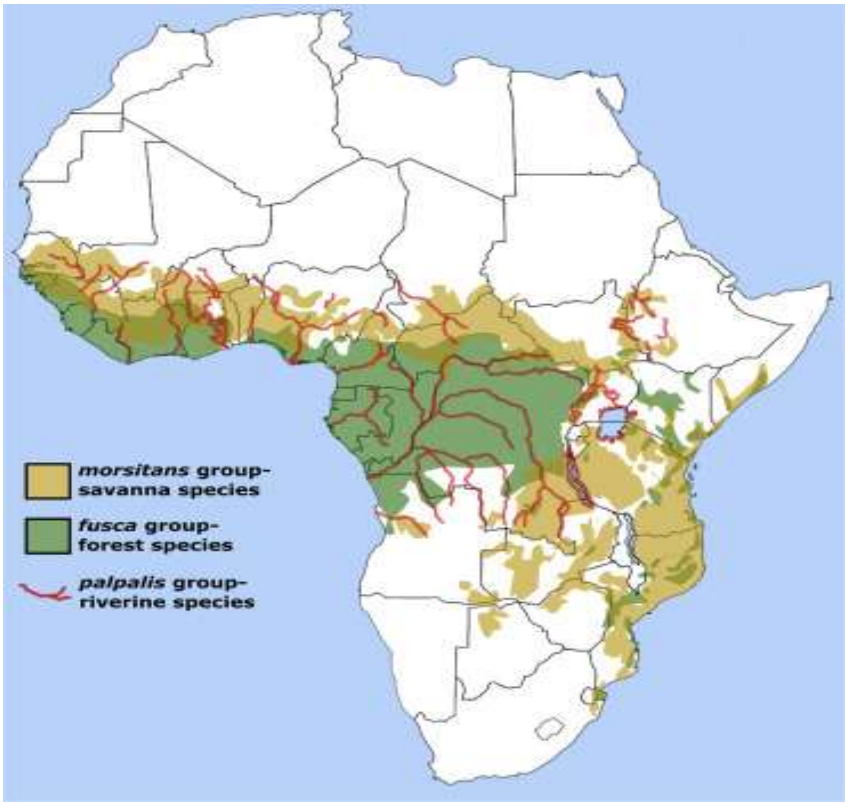


Figure 2.1: Distribution of morsitans, palpalis and fusca groups of tsetse flies

(Source: Krinsky, 2019).

2.2 Tsetse Biology

Adult tsetse flies of both sexes feed exclusively on blood (Benoit et al, 2015). Each *Glossina* species has one to three prokaryotic symbionts. *Wigglesworthia glossinidia* is the most important maternally transmitted endosymbiont found in the anterior part of the midgut and plays several roles including nutrient provisioning, vitamin B synthesis that is absent in blood and influencing host immunological response (Michalkova et al.,

2014; Weiss et al., 2012). *Wolbachia* is another maternal inherited symbiont found in the gonadal tissues which is associated with cytoplasmic incompatibility, a phenomenon which eggs and sperms are unable to form viable offspring (Wang et al., 2013). The effect occurs due to changes made by *Wolbachia.* Mating of a fly infected with Wolbachia with an uninfected or infected female of a different strain causes cytoplasmic incompatibility (Schneider et al., 2013). *Sodalis glossinidius*, is another secondary symbiont that is non-essential to any *Glossina* present in the midgut and other tissues including fat bodies, hemolymph, reproductive system and fat bodies. *Sodalis glossinidius* transmission occurs horizontally during mating, vertically in the larvae or through the hemolymph (De Vooght et al., 2015). The presence of *Sodalis glossinidius* in tsetse increases trypanosome vector competence in *Glossina* (Channumsin et al., 2018).

Tsetse flies fall in the genus *Glossina*, suborder Brachycera, infraorder Cyclorrhapha, superfamily Hippoboscoidea/Pupiparia, and Family Glossinidae (Grimaldi et al., 2005). A key characteristic of all members in this superfamily pupiparia is reproduction via adenotrophic viviparity where zygote development occurs in the female reproduction system. The larva feeds on nutrients provided by the female reproductive system until it develops and a single offspring is produced one at a time (Benoit et al., 2015). When the larvae reach the third instar, it develops within the uterus and is larviposited into humid soil for maturity in shady places. The larva buries in sandy or clay soil to pupate within 1-2 hours and the pupa lives within the soil utilizing soil reserves and adults emerge 30 days after larviposition based on humidity and temperature. In laboratory conditions, females are normally mated after emergence and only one insemination is sufficient since spermatozoid are stored in the spermathecae and stay for approximately 200 days. The female then deposits a larva every 10 days. Ovulation occurs after 10 days after adult emergence and is followed by intrauterine embryogenesis where the first instar develops and takes approximately 3-4 days. Larval development takes approximately 4-5 days and therefore females give birth 20 days post-eclosion. The second oocyte development begins within the left ovary and matures before larval development is

complete (Figure 2.2). Fully developed oocyte is ovulated 20-35 minutes after larviposition and the developmental cycle allows for the deposition of second larva 9-10 days after the first birth (Benoit et al., 2015: World Health Organization, 2013)

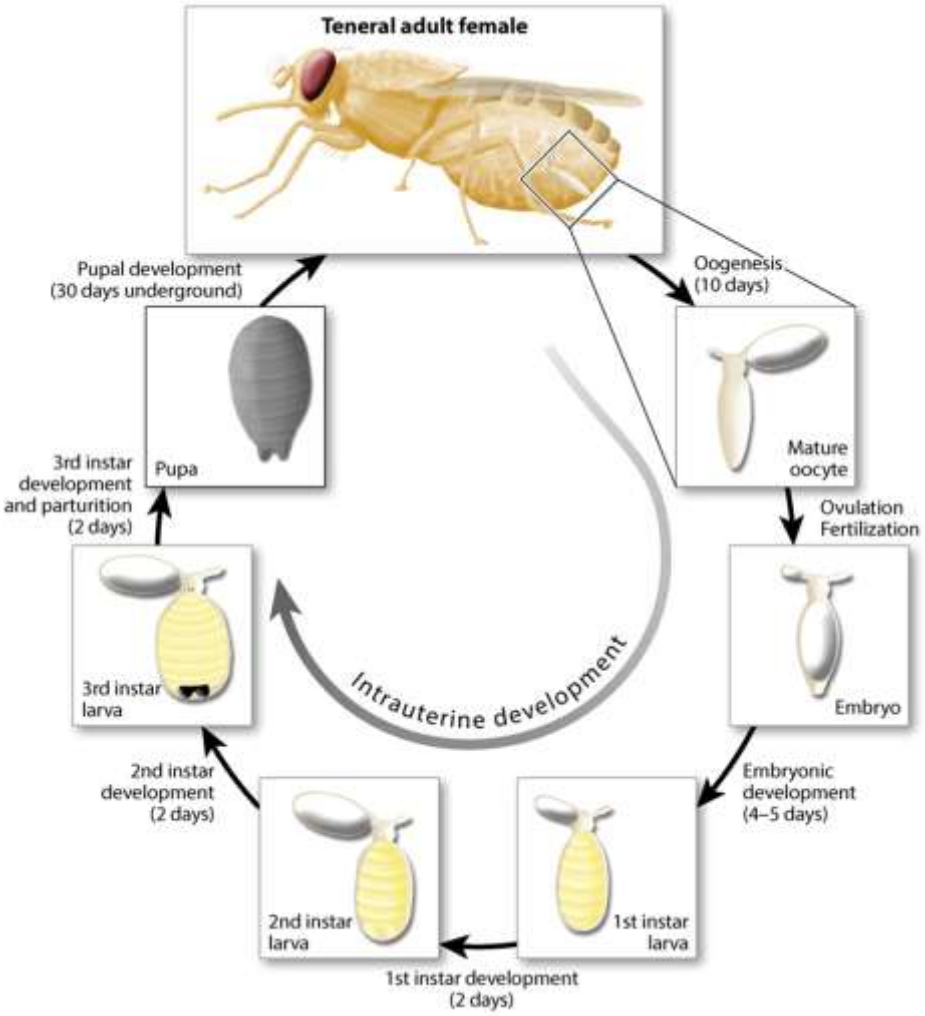


Figure 2.2: Life cycle of Tsetse fly.

(Source: Beno*it et* al., 2015)

2.3 Economic Significance of Tsetse Flies

HAT impedes economic development of poor rural communities in Sub-Saharan African. It causes approximately 1.3 million DALYs or disability-adjusted life year, which estimates years lost to disability or premature death (Fèvre et al., 2008). The disease has a negative impact on health systems and households with direct medical costs of US$25.50, indirect medical costs $68.40 for living expenses and transport and US$83.80 cost for local health services per patient (Matemba et al., 2010). In the Democratic Republic of Congo (DRC), the total treatment of gambiense HAT equates to the 5 months household income for families with the value rising to 17 months the total income in case of any complications (Houweling et al., 2016). Models reveal the total cost for control and elimination measures for Trypanosomiasis from 2013 to 2020 incurred a cost of US$630.6 million. The introduction of elimination programs which recommends switching to new technologies for treatment such as the use of fexinidazole, use of diagnostic kits and implementing vector control in high-risk areas alone and moderate and high-risk areas averts US$700 and US$1500 per DALY respectively (Sutherland & Tediosi, 2019).

2.4 Trypanosomiasis Control

Reducing the tsetse population and targeting the trypanosome remains the two key strategies for control of African trypanosomiasis. Human beings are a hosts for *Trypanosoma brucei gambiense* (Franco et al., 2014). Active and passive screening of HAT followed by treatment of detected cases is the main method of controlling gambiense HAT (WHO, 2013). However, persistent reinfections of gambiense HAT in rainforests by asymptomatic carriers (Jamonneau et al., 2012), additional latent infections in domestic animals (Karshima et al., 2016: N'Djet*chi et* al., 2017), lack of sensitivity of serological (Ngoyi et al., 2014) and limited coverage of active case detection to approximately 75% of the population leaving residual levels of community infection (Checchi et al., 2012; Robays et al., 2004). Unlike gambiense HAT, where the

transmission cycle is from human-fly-human, the transmission cycle in rhodesiense HAT is animal-tsetse-animal/human with reservoirs being domestic and wild animals (Wamwiri et al., 2007). Control of rhodesiense HAT focuses on reducing the tsetse vector population, which aids in transmitting the parasite. The combined use of medical and vector strategies such as small insecticide-impregnated targets has proven effective in reducing *T. b. gambiense* transmission (Courtin et al., 2015). The presence of asymptomatic carriers after years of infection of *T. b. gambiense* has been reported (Jamonneau et al., 2012; Sudarshi et al., 2014). The existence of asymptomatic careers of *T. b. gambiense* supports the need to incorporate vector-based strategies in interrupting transmission of gambiense HAT (Bucheton et al., 2011).

The major obstacle towards HAT control is the lack of a vaccine against various parasites due to antigenic variation of the variant surface glycoprotein. This allows for continued proliferation and differentiation of the parasite and evasion of the immune system (Bangs, 2018). Apart from antigenic variation, trypanosomes manipulate the trypanosome lytic factor 1 and 2 (TLF1) and TLF2 which are components of the innate immune system with anti-parasitic ability by releasing a serum resistance associated (SRA) gene. SRA binds to apolipoprotein L1, a component of a TLF1, resulting in its inactivation and degradation by proteases (Zoll et al., 2018). Other methods of immune evasion used by trypanosomes include inducing non-specific activation of B-cells, decoy shedding of VSGs resulting in hypocomplementemia and suppressing the proliferation of lymphocytes (Onyilagha & Uzonna, 2019). Thus, the available viable measure of the controlling the parasite is the use of drugs. Chemotherapy treatment is dependent on disease stage and species. Pentamidine which accumulates in the trypanosome and disrupts mitochondrial functioning is effective against peripheral blood stream *Trypanosoma brucei gambiense* (Sanderson et al., 2009). Suramin which disrupts glycolysis by binding to glycosome enzymes is also effective against peripheral bloodstream *T. b. rhodesiense* (de Koning, 2020) . However, neither Suramin nor Pentamidine cross the blood brain barrier. Therefore the two drugs are ineffective against the second stage which passes the Central Nervous System (CNS) and settles in

the cerebrospinal fluid (Sanderson et al., 2009). Melarsoprol which disrupts trypanosomal glycolysis and redox metabolism is effective against bloodstream and cerebrospinal fluid parasites. However, Melarsoprol is toxic and incidences of trypanosomal resistance have been reported (Fairlamb & Horn, 2018). Eflornithine inhibits ornithine decarboxylase activity and is effective against *T. b. gambiense* parasites in the cerebrospinal fluid. However, Eflornithine treatment is time consuming due to the treatment duration and its ineffective against *T. b. rhodesiense* (Babokhov et al., 2013). NECT (nifurtimox–eflornithine combination treatment), the latest treatment regimen bases its action on the inhibition of ornithine decarboxylase activity by Eflornithine and oxidative attacks by Nifurtimox with the treatment showing high cure rates for blood and cerebrospinal fluid while reporting no death cases. However, NECT treatment remains expensive with a single dose ranging from about £288-366 in 2010 (Simarro et al., 2012).

Trypanocides such as isometamidium chloride and diminazene aceturate are the two largely used drugs in controlling AAT and are effective against *T. vivax, T. congolense,* and *T. b. brucei* (Giordani et al., 2016). Administration of 35 million doses of isometamidium, diminazene and homidium occurs annually on livestock although the unsupervised and indiscriminate application has increased levels of drug resistance (Delespaux *e*t al., 2008). The lack of a vaccine, high treatment costs, toxicity and increased resistance to trypanocidal drugs reduces control of the trypanosomes in reservoir, thus, the need of an alternative strategy which largely focuses on vector control.

Vector control approaches provide an effective method of controlling Trypanosomiasis by reducing contact between the vector and host Previous efforts such as game eradication by shooting infected animals to deprive flies their primary food source and bush clearing via creation of barrier zones to prevent fly migration from one area to another have been abandoned due to the adverse environmental effects. Vector control measures such as use of insecticides has adverse environmental effect by increasing the

risk of developing resistance and targeting non-intended insects. Effective environmental measures include sterile insect technique (SIT) and use of traps and targets.

2.4.1 Insecticides

Spraying of *Glossina* resting sites with residual insecticides such as Benzene hexachloride (BHC) and dichlorodiphenyltrichloroethane (DDT) was initially used in vector control method but later discouraged after targeting other non-intended organisms (WHO, 2004). The use of persistent insecticides also provided room for insects to develop resistance to the insecticide, new pests outbreak after eliminating predators, killing beneficial non-targeted insects, and general pollution due to insecticide bioaccumulation (Vreysen et al., 2013). Application of persistent pesticides was replaced with aerial spraying or ultra-low concentrations of pesticides through fixed wings airplanes. Utilizing the global positioning system (GPS), sequential aerial technique (SAT) was used to spray pyrethroids like deltamethrin at low doses and suppressed riverine *Glossina* species in Ghana. (Adam et al., 2013). SAT effectively clears areas infested with *Glossina* across a short duration, although the approach remains expensive (Pere P. Simarro et al., 2008).

2.4.2 Sterile Insect Technique (SIT)

SIT involves systemic mass production and sterilization of male adult flies by radiation. The sterilized males are continually released to the environment to outcompete wild male flies for mating opportunities with virgin females. When sterilized male flies mates with wild female, it renders the female flies infertile. Over time, no fertile mating occurs, and the insect population is eliminated. SIT was successfully applied in Unguja Island to eliminate *Glossina austeni* (Vreysen et al., 2014). SIT was used in combination insecticide-impregnated targets and traps to suppress *G. p. palpalis* in Nigeria (Takken et al., 1986), and *G. tachinoides* and *G. palpalis gambiensis* populations in Burkina Faso (Van der Vloedt et al., 1980). The approach faces several limitations related to mass

rearing and establishment of a successful colony, irradiation effects on tsetse beneficial microbiota (Lauzon & Potter, 2012), pathogenic infections which reduce colony fitness (Abd-Alla et al., 2013), and compromised performance of released male flies (Terblanche & Chown, 2007). Feasibility of SIT in sub-Saharan Africa is limited by the high number of diverse species, and high cost of infrastructure facilities such as airplane, irradiation facilities and large insectaries (Aksoy et al., 2017; Kabayo, 2002).

2.4.3 Traps and Targets

As exclusive hematophagous insects, tsetse flies rely on the use of olfactory and visual signals to identify their hosts. As a result, targets and traps which detect visual and olfactory signals have been designed as a control measure. Tsetse fly traps are made of blue and black material with blue material used for attraction and black material for landing purposes. Visual stimulus in insects depends on the reflected light wavelength. Tsetse attraction correlates positively with reflecting in the blue region (460nm). Tsetse attraction correlates negatively with light reflection in the green and ultraviolet region at 360nm and 520nm respectively (Lindh et al., 2012). Tsetse landing responses tend to be stimulated by low luminance although strong landing responses occur at high ultraviolet reflectivity indicating that ultraviolet wavelengths negatively contribute to attraction from far but positively to landing at very close distances (Santer, 2015). Advances in developing traps for trapping various *Glossina* species have been made. For example, Ngu trap was developed for *Glossina pallidipes* (Brightwell et al., 1987), biconical trap for sampling *Glossina morsitans,* F3 and Epilson for *Glossina pallidipes* (Green & Flint, 1986), pyramidal for *Glossina tachinoides* (Gouteux & Lancien, 1986) and S3 for *Glossina swynnertoni* (Ndegwa & Mihok, 1999). Comparative analysis of various traps including NGU, biconical, pyramidal, S3 and NZI revealed that NGU was the most effective trap in tsetse control programs (Malele et al., 2016). Traps rely with visual stimuli which is hindered by vegetation. In such instances, attraction of files can be greatly improved using odor-baited traps. Kairomones such as 1-octen-3-ol (Hall et al., 1984), acetone and carbon dioxide (Vale, 1980) have been identified from cattle breath,

3-propylphenol and 4-methylphenol from cattle urine (Vale et al., 1988) and 3-n-propylphenol and 4-cresol were identified from buffalos urine (Owaga et al., 1988). Odor baited traps laced with cow urine and acetone were used in controlling *G. pallidipes Austen* and *G. longipennis* in Kenya (Brightwell et al., 1991). Allomones such as guaiacol, pentanoic acid, geranylacetone, and δ-octalactone were identified from waterbucks (Gikonyo, et al., 2002; Gikonyo et al., 2003) while acetophenone, geranylacetone, and 6-methyl-5-hepten-2-one from the zebras body surfaces (Olaide et al., 2019). Crude zebra skin extracts and electrophysiologically-active components such as geranylacetone, acetophenone, 6-methyl-5-hepten-2-one, nonanal, octanal, heptanal and decanal reduced *G. pallidipes* catches by 66.7% and 48.9% respectively (Olaide et al., 2019). 4-methylguiacol was shown to reduce catches in unbaited and baited traps by 70% and 80% respectively in savanna tsetse (Saini & Hassanali, 2007), while δ-octalactone, guaiacol, geranylacetone, hexanoic and pentanoic acid, allomones produced on the body surface of the waterbuck also reduced *G. pallidipes* catches by 84% (Bett et al., 2015). The identification of repellants has resulted in the development of repellant dispenser collars containing δ-octalactone, geranylacetone, guaiacol, and pentanoic acids which act to repel tsetse flies (push) towards targets laced with attractants such as acetone and cow urine (pull) and a fly killing agent such as deltamethrin. The approach significantly increased traction power, reduced trypanosomiasis by more than 80%, reduced trypanocide use and significantly increased cattle weight (Saini et al., 2017).

2.5 Olfaction in Insects

Use of odor baited traps as a method of controlling tsetse fly is based on chemoreception or the olfaction system. Insects utilize the olfaction system in search of food, mating partners, escaping predators, locating oviposition sites, escaping predators and discriminating toxic food (Hansson & Stensmyr, 2011). Olfaction proteins include soluble proteins such as OBPs, CSPs and ODEs (Liu et al., 2010; Macharia et al., 2016) and receptor proteins including ORs, IRs and GRs (Macharia et al., 2016;Obiero et al., 2014). The recent annotation of chemosensory genes in the *Glossina* genomes revealed

the presence of 118 genes in *Glossina austeni*, 115 in *G. brevipalpis* 115, 124 in *G. f. fuscipes*, 123 in *G. pallidipes* and (127) in *G. m. morsitans* (127) (International Glossina Genome Initiative, 2014; Macharia et al., 2016; Obiero et al., 2014).

The antennae and maxillary palps are olfactory organ in insects (Shiraiwa, 2008; Su et al., 2009). Sensory hairs-like projections or sensilla which house dendrites of Odorant Receptor Neurons (ORN) cover olfactory organs. Sensilla are classified morphologically into three types: trichoid which detect pheromones (Wang & Anderson, 2010),coeloconic which detect food odors (Silbering et al., 2011) and basiconic which detects amines and organic acids (Hallem & Carlson, 2006).*G. m. morsitans* trichoid, coeloconic and basiconic sensilla resemble those in *Drosophila* in morphology but differ functionally based on odor recognition (Chahda et al., 2019: Soni et al., 2019). Each ORN contains olfactory receptor proteins which after binding of odorants induce signal transduction processes which pass from the antennal body to mushroom bodies for processing in higher brain centers. (Figure 2.3)

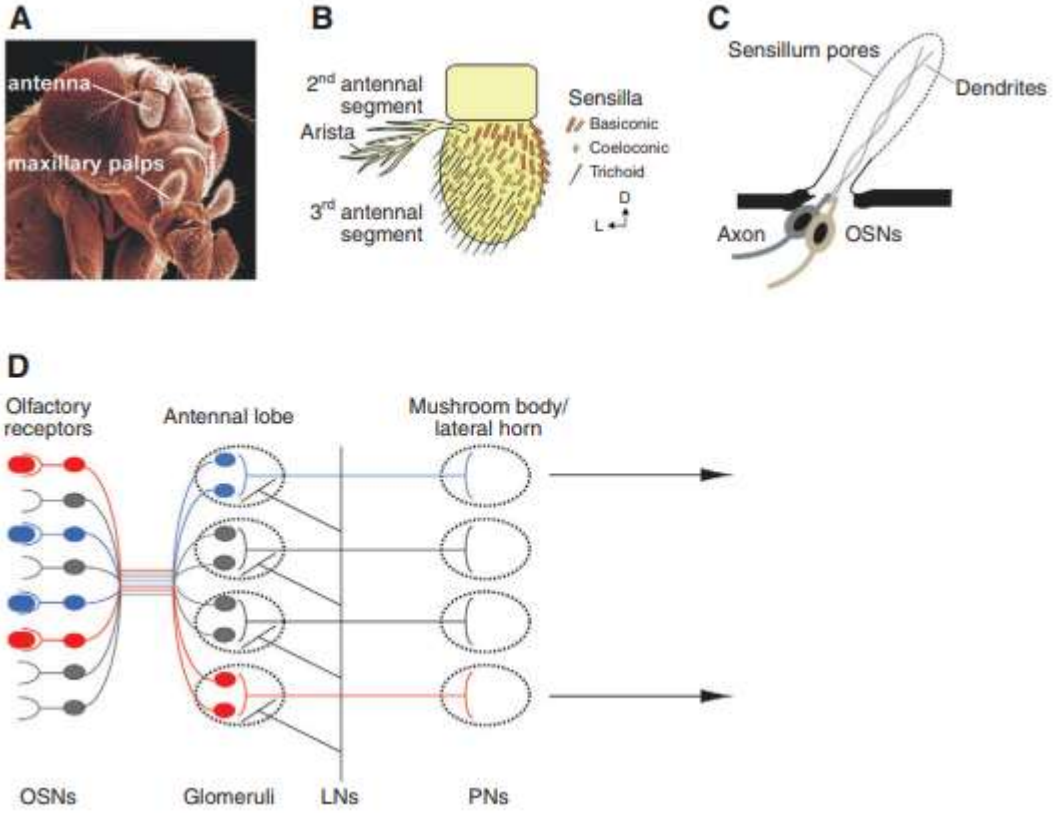


Figure 2.3:The olfactory system of the model fly *D. melanogaster*

(Source: Pellegrino & Nakagawa, 2009)

A) *D. melanogaster* head showing the antennae and maxillary palps. **B**) Classes of sensilla; trichoid, coelonic and basiconic **C**) The anatomy of the sensillum containing 2-4 ORN with the dendrites projecting to sensillum lumen and axon projecting to antennal lobe. Sensillum contains pores which allow odorant reach neurons**. D**) *Drosophila* olfactory system organization. Odorants from the surrounding environment bind to the ORN which dens information to the glomeruli. Information travels to higher brain centers, lateral horns and mushroom body through projection neurons

OBPs bind and solubilize odorants which move though sensilla pores into the sensillar lymph and finally in the dendrites of ORN. ORs play a role in conferring odor specificity of an ORN for general odorants while OBPs play roles in odor recognition (Guo & Smith, 2017). A recent study in *Anopheles gambiae* Odorant Binding Protein 1 (AgaOB1) reported that an indole, an odor which binds to AgaOB1 produced no electrophysiological response after RNAi-mediated gene silencing (Biessmann et al., 2010) while another study where *Culex quinquefasciatus* OBP1 (*CquiOBP1)* which is sensitive to mosquito oviposition pheromone showed lower electrophysiological responses to known mosquito attractants after RNA interference (RNAi) (Pelletier et al., 2010). Functional studies conducted in *D. melanogaster* OR repertoire *in vivo* expression system referred to as an empty neuron provides insights on odor coding. The deletion of OR22a creates an empty neuron that fails to express any functional receptor. Expression of OR genes in the OR22a mutant empty neuron is done through the GAL4/UAS system. The results revealed that ORs confer odor response similar to those observed in individual ORN in wild-type *D. melanogaster* which can be measured physiologically using single unit recordings. Olfactory coding of the OR repertoire also revealed that a subset of receptors may activate individual odorant while some receptors are activated by general odorants (Figure 2.4) (Hallem et al., 2004; Kreher et al., 2005).

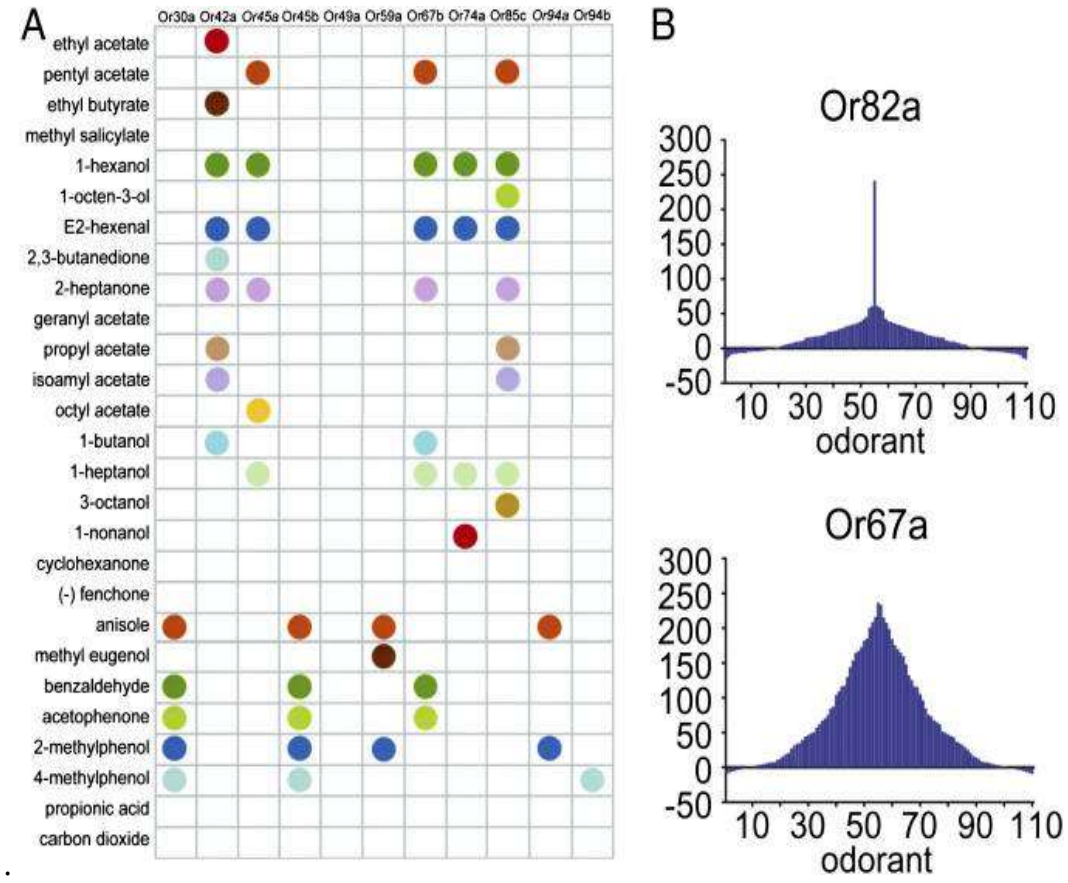


Figure 2.4: Odorant receptor odor coding

A) Combinational coding of odorant receptors in larvae with colored dots indicating a strong odor response (Source: Kreher et al. 2005). **(B)** Example of individual tuned odorant receptor OR82a and general tuned odorant receptor OR67a in an empty neuron (Source: Hallem & Carlson, 2006)

2.5.1 Chemosensory Proteins

Chemosensory proteins (CSPs) are small soluble, globular acidic proteins with molecular mass of 13 kilodaltons and length of between 100-120 amino acid residues. CSPs contain four conserved cysteine residues which form disulphide bridges (Pelosi et al., 2006; McKenna *e*t al., 1994). CSPs contain six alpha-helices which make up the

hydrophobic binding cavity and a highly conserved N-terminal motif (Forêt et al., 2007; Tegoni et al., 2004). CSPs are categorized as a class of binding protein because of three reasons. First, CSPs are abundant in the antennae of insects such as locust (Jin et al., 2005). Secondly, CSPs bind various semiochemicals with the same micromolar dissociation constant as OBPs (Briand et al., 2002). Lastly, some CSPs are exclusively abundant in some insect species' antennae like ants (McKenzie et al., 2014). CSPs are present in non-olfactory tissues including abdomen, truncus, cuticle (Li et al., 2013), legs (Li et al., 2016), tarsus (Ozaki et al., 2008), and pheromone glands (Jacquin-Joly et al., 2001). CSPs expression in non-olfactory tissues is associated with a wide range of functions. In *Locusta migratoria manilensis* LmigCSP91 was identified in the male reproductive organ and lacked in the female reproductive organs. LmigCSP91 was detected after mating suggesting its possible role during mating. At least other 16CSPs are present in ovaries and accessory glands of *Locusta migratoria manilensis* (Zhang et al., 2013).6 CSPs identified in the pheromone gland of silkmoth Bombyx mori act as pheromone carriers (Dani et al., 2011) while CSP p10 in cockroach *Periplaneta Americana* has a role in limb regeneration (Nomura Kitabayashi et al., 1998) CSP5 in honeybee eggs is involved in embryo development (Maleszka et al., 2007) and expression of CSP-1 is transitions locusts from the solitary to gregarious phase (Guo et al., 2011). Genome annotation of five tsetse fly species revealed CSPs are highly conserved than OBPs (Macharia et al., 2016). Expression of chemosensory proteins *GmmCSP2* relates to female host-seeking behavior in *G. m morsitans* (Liu et al., 2012).

2.5.2 Odorant Binding Proteins

OBPs features include a conserved six-cysteine signature, alpha-helix pattern, signal peptide, and size ranging from 15-20kDA (Pelosi et al., 2006). Insect OBPs are diverse and are classified based on conservation of cysteine residues. As a result, OBPs fall in six classes. Classic OBPs contain six conserved cysteine, dimer OBPs , two-six-cysteine signature, and Plus-C contains an extra conserved cysteine (Kang et al., 2018) and one proline group (Zhou et al., 2004). Minus-C OBPs has four conserved cysteines after two

residues while atypical OBPs have 9-10 residues with an extended long C-terminus (Jiang et al., 2017). OBPs mediate the interaction between odorant in the external environment and transportation of specific hydrophobic ligands to ORs (Fan et al., 2011).The antennae contains high concentrations of OBPs (Calvello et al., 2005; Shanbhag et al., 2001). OBPs functions in odorant recognition. *Drosophila* OBP LUSH is required for sex pheromone detection since deleting the encoding gene significantly reduces behavioral and electrophysiological responses to pheromone 11-vaccenyl acetate (Xu et al., 2005). Binding of LUSH to pheromone 11-vaccenyl acetate results in firing of pheromone sensitive neurons (Laughlin et al., 2008). OBPs have been reported across non-olfactory tissues of various insects. Transfer of OBP22 from sperms of *Aedes Aegypti* to females occurs during mating. Other OBPs present in the seminal fluid transferred to females during mating include OBP9 in honey bee A*pis mellifera* (Baer et al., 2012), OBP44a, OBP99a, OBP51a, OBP56f, OBP56g in *D. melanogaster* (Findlay et al., 2008; Takemori & Yamamoto, 2009) and OBP10 in *Helicoverpa armigera* (Sun et al., 2012). The expression of three OBPs together with OR47a in *D. melanogaster* suggests a role in sperm-egg communication (Prokupek et al., 2010).Overexpression of RproOBP1, RproOBP11, RproOBP14, and RproOBP24 in the gut of bloodsucking Bug, *Rhodnius prolixus* is implicated in transportation of nutrients and gut function (Ribeiro et al., 2014). GmmOBP4 and GmmOBP17 were reported in the male reproductive tissues in *G. m .morsitans* (Scolari et al., 2016). GmmOBP8, GmmOBP9, GmmOBP14 showed high expression in *G. m. morsitans* antennae and probably play a role in olfaction. Four OBP genes including GmmOBP4, GmmOBP15, GmmOBP17, and GmmOBP19 were expressed in the adult bodies while three OBP including GmmOBP3, GmmOBP5, and GmmOBP7were expressed in larvae stages (Liu et al., 2010). *Glossina brevipalpis* GbrOBP2 was highly expressed in pupal and larval stages while Gbr*OBP7* was only expressed in the pupal stage (Murithi et al., 2018). OBPs expression in non-olfactory tissues suggest non-canonical chemoreception roles.

2.5.3 Odorant Degrading Enzymes

Odorant Degrading Enzymes (ODEs) are found in the insect antennae sensilla lymph ODEs play a role in signal inactivation step that functions to terminate the action of the odorant molecule (Ishida & Leal, 2005). Various enzymes described as candidate ODEs include carboxyesterases, aldehyde oxidases, epoxide hydrolyses and cytochrome P450 (Jacquin-joly & Maïbèche-Coisne, 2009). Some enzymes like carboxyesterase and esterase-6 are involved in cVA degradation in *D. melanogaster* (Mane et al., 1983). Esterases known as ApolPDE are expressed on the antenna pheromone detecting sensilla of *Antheraea polyphemus* and they rapidly degrade components of sex pheromone E6Z11-16Oac (Ishida & Leal, 2005). In *Manduca sexta*, an antenna-specific aldehyde oxidase (MsexAOX) was identified in both males and females and it was shown to function in degrading bombykal, a pheromone released to attract males (Blomquist & Vogt, 2003).

2.5.4 Sensory Neuron Membrane Proteins (SNMPs)

Sensory Neuron Membrane Proteins (SNMPs) receives pheromone in insects (Benton et al., 2007). SNMPs are similar to mammalian CD36 protein which detect aggregation pheromone in *Drosophila* (Benton et al., 2007). Expression of SNMP1 and SNMP2 in various coleopteran insects occurs in the pheromone sensitive hairs present in different location (Gu et al., 2015). Expression of SNMP1 takes place in olfactory sensillium dendritic membrane while SNMP2 is present in the antenatal sensillar lymph. SNMP1 and SNMP2 orthologs previously reported in *Ae. aegypti, D. melanogaster* and other Lepidoptera species were also reported in all *Glossina* species (Macharia et al., 2016). 15 CD36 like genes annotated as SNMP1 and SNMP2 were also identified in *G. m. morsitans* (Obiero, 2014)

2.5.5 Gustatory Receptors

Gustatory Receptors distinguish between contact pheromones and odor tastes (Amrein & Thorne, 2005; Matsunami & Amrein, 2003). Expression of GRs occurs in the Gustatory Receptor Neuron and antennal dendrites where response to carbon dioxide changes occurs (Montell, 2009). GRs are more conserved in their structure and sequence as compared to ORs (McBride & Arguello, 2007). In *G. m. morsitans* 14 Grs were identified through *in silico* computing. Sweet taster GRs present in *D. melanogaster* were absent in *G. m. morsitans* due to the blood feeding nature (Obiero *et al*., 2014). High levels of AgGR22 which detects carbon dioxide in the antennae in *An. gambiae* is also present in the testis (Pitts et al., 2014). Among the sequenced *Glossina* species, GR32 which responds to pheromones, was present in all five tsetse fly species and could be conserved in function (Macharia et al., 2016).

2.5.6 Ionotropic Receptors

Ionotropic Receptors were evolutionary derived from the ionotropic glutamate receptors (iGluRs), a family of ligand gated ion channels (Rytz et al., 2013). Ionotropic receptors are present in the *Drosophila* coeloconic sensilla. Ionotropic receptor respond to acid based and amine odorants largely ignored by odorant receptors (Rytz et al., 2013). Other odorants commonly detected by IRs include ketones and aldehydes (Abuin et al., 2011). Ionotropic receptors are divided into antennal and divergent receptors. Antennal receptors occurs are involved in odor recognition while divergent receptors have a role in taste (Rimal & Lee, 2018). IRs differ from iGluRs by lacking the conserved glutamate residue (Rytz et al., 2013). IRs can also be co-expressed in the neurons and require between two to three heterodimer for odor perception (Abuin et al., 2011; Rytz et al., 2013).Genome annotation of *Glossina spp* revealed that IRs/iGLURs are conserved in copy number (Macharia et al., 2016). Ir84a implicated in supporting male courtship is also conserved across all *Glossina* species (Macharia et al., 2016). 19 IRs were annotated and identified in *Glossina morsitans morsitans* (Obiero et al., 2014).

2.5.7 Odorant Receptors

Odorant receptors (ORs) possess an inverted seven transmembrane domain (Smart et al., 2008). ORs function as odor gated ion channels with a role in signal transduction processes (Sato et al., 2008). The first ORs in *D. melanogaster* antennae were discovered over a decade ago (Cl*yne e*t al., 1999). *Tribolium castaneum*, the red flour beetle is predicted to contain 341 ORs while *Drosophila melanogaster* has 60 OR genes encoding 62 different genes with some undergoing alternative splicing (Touhara & Vosshall, 2009). A total of 79 ORs are present in *An. Gambiae* (Fox et al., 2002). Computational genome analysis revealed 60 OR genes in *D. melanogaster* and 341 ORs in the red flour bettle, *Tribolium castaneum* (Touhara & Vosshall, 2009), and 131 ORs in *Ae. aegypti* (Bohbot et al., 2007).

An exceptional receptor formerly known as OR83b, currently referred to as Orco is co-expressed in most ORN of adults and larvae (Gao & Chess, 1999; Larsson et al., 2004). Orthologs of OR83b are present in moths and mosquitoes as OR2 and OR7 respectively (Tsitoura et al., 2010). ORs form heterodimers with Orco before acting together as odorant gated ion channel. Studies indicate that Orco can function independently of the canonical ORs when stimulated by cAMP (Wicher et al., 2008). Also, Orco can signal through G proteins though at slower time scales (Tsitoura et al., 2010; Wicher et al., 2008). OR/Orco heteromer forms ligand gated non-selective cation channels (Gao & Chess, 1999). Orco is a highly conserved gene with the seven transmembrane domain sharing a high sequence with other insects orthologs (Abdel-Latief, 2007; Melo et al., 2004; Pitts et al., 2004; Xia & Zwiebel, 2006). The disruption of Orco affects the entire insect transduction system (Zhou et al., 2014; Degennaro et al., 2013; Zhao et al., 2011). Silencing of Orco in red weevils *Rhynchophorus ferrugineus* showed a decrease in pheromone response (Soffan et al., 2016) while in *Rhodnius prolixus* it resulted in loss of activity to find a host in time, decreased ingested blood volume, decreased egg laying and increased mortality rate (Franco et al., 2016).

Orco is expressed in the spermatozoa of *An. gambiae* in the puncta along the flagella of mature spermatozoa (Pitts et al., 2014). Orco protein expression in the sperm has also been elucidated in the Asian tiger mosquito, *Ae. albopictus,* the jewel wasp, *Nasonia vitripennis,* bedbug *Cimex lectularius* and the fruit fly, *D. melanogaster* (Hansen et al., 2014). Orco is also expressed in the testis of *Locusta migratoria* and is implicated in endogenous ligand recognition, fertilization, sperm chemotaxis, and spermatozoa activation (Wang et al., 2015). In *An. gambiae* Orco is implicated in sperm activation and maintenance of sperm fidelity (Pitts et al., 2014). Expression of Orco also occurs in *D. melanogaster* testis somatic cysts cells (Dubey et al., 2016). Disruption of Orco results in loss of activity to the actin cap which prevents spermatid release from the enclosed cyst (Dubey et al., 2016). Although ORs are expressed in non-head appendages, studies investigating expression of ORs in the brain, gut and reproductive tissues in *G. m. morsitans* have not been conducted.

2.6 Gene Expression by Quantitative Real-time PCR

Real-time PCR is a method used to determine gene expression. Real-time PCR has been used in validating olfaction genes identified via RNA-sequencing across various insect species such as longhorn beetle, *Apriona germari* and the green plant bug *Apolygus lucorum* (An et al., 2016; Qian et al., 2020). Real-time PCR is a relatively fast and easy process of perform compared in situ hybridization and northern blotting. Furthermore, qPCR is a more sensitive and specific tests and allows mRNA quantification over a wide range (Kuang et al., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site, and Sample Collection

This study was carried out at the JKUAT Biochemistry Department Laboratory. Ten (10) day-old adult male and female *G. m. morsitans* flies were sourced from Biotechnology Research Centre of Kenya Agricultural Livestock Research organization (BRC-KALRO) in October 2018, from Muguga Kenya. Rearing of flies followed the standard laboratory conditions of relative humidity 75 ± 10%, temperature of 25 ± 1°C, and feeding on sterilized blood using an artificial membrane after every 24 hours in the BRC-KALRO insectary. The study utilized the following formula to calculate the sample size

$$\text{Sample size (n)} = \frac{S}{1 + \frac{(S-1)}{N}}$$

$$S = \frac{Z^2PQ}{ME^2} = \frac{1.96^2 \times 0.4 \times 0.6}{0.01^2} = 9219.84$$

N= Target population of 100 10-day year old tsetse flies

Z= Confidence interval at 95%

P= Proportion of 10-day year old flies (0.4)

Q= Proportion of tsetse flies not in the target group (0.6)

ME = Margin of error (1%)

$$\text{Therefore the total sample size n} = \frac{9219.84}{1 + \frac{(9218.84)}{100}} = \frac{9219.84}{93} = 99.1 \text{ rounded off to at least } 100$$

Thus, the total number of flies collected was 100 (50 males and 50 females).

3.2 Localization of Odorant Receptors Genes in *G. m. morsitans* Tissues.

To localize the 46 odorant receptor genes, brain, gut and reproductive tissues (testis and accessory glands for male) and (spermathecae and ovary for female) were first dissected (Macharia et al., 2016). Total RNA was extracted, quantified and its integrity determined. cDNA was then synthesized through reverse transcription and the resulting product amplified using 46 OR primers via polymerase chain reaction as indicated herein.

3.2.1 Tissue Dissection

Tissue dissection was carried out 15 hours after the collection of flies. Ten (10) day old flies (50 males and 50 females) were immobilized at 20^{o}C for one minute and placed in petri dish on ice. The brain, gut and reproductive tissues (testis and accessory glands for male) and (spermathecae and ovary for female) were then dissected using a X40 Leica microsystem microscope (Wetzlar, Germany) and immediately stored in a 1.5ml Eppendorf tube containing 250µl TRIzol (Invitrogen, Carlsbad CA, USA) at -80^{o}C awaiting RNA extraction.

3.2.2 Total RNA Extraction

The frozen tissues containing TRIzol (Invitrogen, Carlsbad CA, USA) were thawed and the samples homogenized to fine paste using micropestles. The contents were then vortexed for 1 minutes before the addition of 750 µl of TRIzol LS reagent (Chomczynski & Mackey, 1995). The content stood at room temperature for 10 minutes to permit dissociation of nucleoproteins. 200 µl of chloroform was added and a further ten minutes of incubation followed. The content were mixed and centrifuged at 12000 rpm for 10 minutes at 4^{o}C resulting in three layers; a colorless upper aqueous phase, a phenol-chloroform interphase and a lower red phase. The colorless upper aqueous phase which contained RNA was pipetted carefully into a clean RNAse free Eppendorf tube. 1 µl of glycogen (20 mg/mL) and 500 µl of absolute ice-cold isopropanol was added for

RNA to precipitate. The contents were incubated overnight at -20^{0}C (Sambrook and Russel, 2001). After the overnight incubation, samples were centrifuged at 12,000 rpm for 10 minutes at 4^{0}C. The supernatant was discarded with caution with care to ensure the pellet was not lost or dislodged. Afterwards, the RNA pellet was washed gently by 50 µl of 75% ethanol. The tubes were then centrifuged at 12,000 rpm for 10 minutes at 4^{0}C. A further 3 seconds centrifugation of the samples at 12000 rpm at 4^{0}C was done to remove the pellet. Afterwards the pellet was resuspended in 12 µl of RNase free water, gently tapped and allowed to dissolve. The sample was then treated with 2 µl 10X reaction buffer and 2 µl of DNase I (1 U/µL) to remove any genomic DNA and incubated at 37°C for 30 min. 1 µL 50 mM EDTA was then added and incubated at 65°C for 10 minutes.

3.2.3 Determination of RNA Yield and Quality

The extracted brain, gut and reproductive tissues (testis and accessory glands for male) and (spermathecae and ovary for female) RNA samples were quantified using a NanoDrop 2000 spectrophotometer (Biospec-mini, Shimadzu Corporation, Tokyo). 1 µl of nuclease free water blanked the machine and 1µl of the sample was placed to measure the yield and quality of DNA. RNA yield was determined in µg/ml while the RNA quality was determined at the ratio wavelength 260/280nm

3.2.4 Determination of RNA Integrity

1.2% formaldehyde agarose gel electrophoresis was prepared and used to determine the integrity of brain, gut and reproductive tissues extracted RNA samples. 1X formaldehyde gel running buffer was prepared by adding 100ml of 10X MOPS buffer (3-(N-morpholinopropanesulfonic acid (209.3 gmol^{-1}), sodium acetate (136.1 gmol^{-1}) EDTA (372.24 gmol^{-1}), 20 ml of 37% formaldehyde and 800ml of 1% Diethyl pyrocarbonate (DEPC-treated water). The gel was prepared by dissolving 1.2 g of agarose in 100ml of 1% DEPC treated water. 10 ml of 10X MOPS buffer and 1.8 ml of 37% formaldehyde was added. The contents were heated for 1 minute in the microwave

and left to cool before the addition of 0.5 µl of ethidium bromide. 6 µl of RNA sample was mixed with 2 µl of the loading dye and heated at 65ºC for 5 minutes and immediately placed on ice before being loaded on the wells alongside the ladder. The samples were then run at 70V 80mA and 50W for 40 minutes. The gel images were then viewed within a gel imaging system and the images captured for analysis.

3.2.5 First Strand cDNA Synthesis

First strand cDNA templates were prepared using RevertAid First Strand cDNA synthesis kit (Fermentas, Thermoscientific, UK) according to the manufacturer's protocol. 20ng/µl template RNA were placed in a microcentrifuge tube followed by the addition of 1µ of 0.25 µM Oligo $(dT)_{18}$ and 7µl of nuclease free water bringing the total volume to 12µl. The contents were gently mixed, spun down and incubated at 65ºC for 5 minutes. The tubes were cooled on ice for 1 minute and final concentrations of the following reagents were added; 4µl of 1 × reaction buffer (250mM Tris-HCL pH 8.3, 250mM KCl, 20mM $MgCl_2$, 50mM Dithiothreitol), 1µl of 1U/µl Ribolock RNAse inhibitor, 2µl of 1mM dNTP Mix and 1ul of 10U/µl RevertAid M-MULV Reverse Transcriptase. The reaction mixture was spun briefly and incubated at 42ºC for 60 minutes, followed by a termination step at 70ºC for 5 minutes and finally chilled on ice. The negative control was composed of all reagents and processes minus Reverse Transcriptase.

3.2.6 Amplification of Synthetized cDNA

Primers for designing the 46 *G. m. morsitans* OR genes were downloaded from Vectorbase (VB-2014-04) (Giraldo-Calde*ron e*t al., 2015) and made using Primer3 plus (v0.4.0) (Untergas*ser e*t al., 2012). The set primer sequence length was between 18-20 base pairs and melting temperature between 55-58ºC. The GC content ranged from 20-80% and product size range of 150 to 200bp. The designed primers were synthesized by Inqaba Biotec, South Africa (Appendix I). The integrity of *G. m. morsitans* cDNA was validated through PCR amplification with glyceraldehyde-3-phosphate (GAPDH), beta-

actin and beta-tubulin as reference genes in an ABI GeneAMP 9700 (Applied Biosystem). The male and female brain, gut and reproductive tissues cDNA were used as template for the PCR amplification in a 50µl reaction containing: 32 µl nuclease free water, 10µl ×5x MyTaq reaction buffer, 1µl MyTaq DNA Polymerase Bioline (Bioline, London, UK) and 2.5 µl (10mM) each forward and reverse primers of 46 GmmOR genes and 2µl (20ng/µl) of cDNA sample. The cycling conditions were initial denaturation at 95ºC for 1 minutes, 35 cycles of subsequent denaturation at 95ºC for 30 seconds, annealing at 55ºC for 30 seconds, extension at 72ºC for 20 seconds followed by a final extension at 72ºC for 7 minutes.

3.2.7 Gel Electrophoresis of PCR Products

The PCR amplicons were analyzed using 2% agarose gel electrophoresis. 1.2g of agarose gel electrophoresis was mixed with 50ml of TAE buffer and 0.5 µl of RedSafe added before heating in a microwave for two minutes. 3 µl of the amplified products was mixed with 2 µl of the loading dye and loaded in the well alongside a 100 base pair DNA ladder (Bioline). The gel was run at 70Volts 80mA and 50W for one and a half hours. The images were viewed using a UV trans-illuminator.

3.2.8 Purification of Amplified Products

Purification was carried out using Qiagen PCR purification kit. 100µl of Buffer BP was added to 20 µl of the PCR sample and mixed. The mixture was added to the QIAquick column and was followed by a round of centrifugation for 60 seconds at 13,000 rpm. After centrifugation the flow through was discarded. 750 µl of Buffer PE was added to the QIAquick column and centrifuged for 30 to 60 seconds at 13,000 rpm and was followed by a round of centrifugation for 60 seconds at 13,000 rpm. The flow through was discarded and the QIAquick column centrifuged for an additional 1 minute at 13,000 rpm to eliminate any residual buffers. 35 µl of Buffer EB was added at the center of the QIAquick membrane and centrifuge for 1 min at 13,000 rpm. 5 µl of eluted DNA was run on 1% agarose gel with RedSafe to confirm the presence of the recovered DNA.

Sequencing was outsourced at Macrogen Inc. Netherlands and the sequenced samples analyzed by BLAST suite on Vector Base to confirm the identity of the amplicons.

3.3 Identification of OR Homologs in *D. melanogaster* and Gene Ontology

Odorant receptor protein sequences of the amplified genes of *G. m. morsitans* were retrieved from Vector Base by carrying out a protein name search (Giraldo-Calde*ron et* al., 2015). The *G. m. morsitans* OR sequences were screened against conserved domain database (Marchler-Ba*uer e*t al., 2011) and checked for the seven transmembrane domains using TMHMM (Kr*ogh e*t al., 2001). The obtained sequences were queried against the other *Glossina* genomes (*G. palpalis*, *G. austeni, G. fuscipes fuscipes*, and *G. pallidipes*) in VectorBase using a cut off e-value of $1.0e^{-05}$(Giraldo-Calde*ron et* al., 2015). The *G. m. morsitans* protein sequences were also queried against FlyBase to identify homologs in *D. melanogaster* (St. Pi*erre et* al., 2014). Since genome annotation in *G. m. morsitans* is incomplete, the identified homologs were used to infer gene ontology functions from the gene ontology database and Flybase (St. Pi*erre et* al., 2014). Expression of odorant tissues in the homologs in *D. melanogaster* was searched across Fly Atlas 2 to identify specific tissues in which such genes were expressed (Lea*der et* al., 2018).

3.4 Quantitative Real-Time Polymerase Chain Reaction

Expression levels of amplified *G. m. morsitans* odorant receptor genes were determined by qPCR using the Light cycler 96 (Roche, Indianapolis, IN). Reaction volumes of 10µl containing 2x SensiFAST SYBR® No-ROX Mix (Bioline, London, UK) 2ng/µl of cDNA template, 0.4mM of each primer and nuclease free water were prepared. The thermocycling conditions were set at one cycle of 95ºC for 2 minutes, followed by 40 cycles at 95ºC for 5 seconds, 55ºC for 20 seconds and 72ºC for 10 seconds. Data acquisition was performed during the extension step. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization of the template cDNA. Tests were carried out in three replicates. Melting curve analysis was carried out to check for

specificity of amplicons. The expression level of odorant receptor genes in male and female brain, gut and reproductive tissues of *G. m. morsitans* was calculated using the delta delta Ct ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen, 2001).

CHAPTER FOUR

RESULTS

4.1 Tissue Dissection

The brain, gut, and reproductive tissues (testis and accessory glands for male) and (spermathecae and ovary for female) were identified after being dissected under the 40 Leica microsystem microscope (Wetzlar, Germany) (Figure 4.1 A-D).

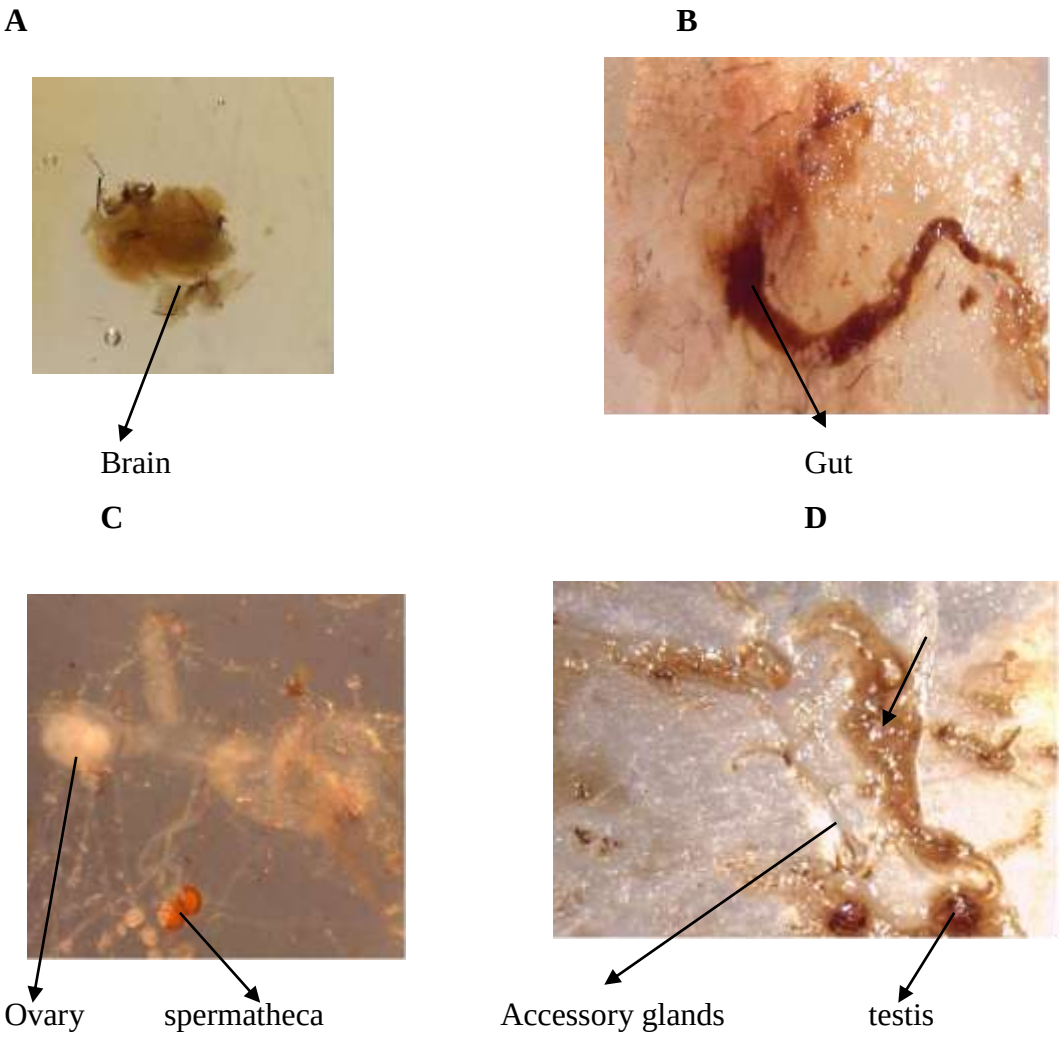


Figure 4.1: Microscopic image of *G. m. morsitans* tissues.

A. Brain **B.** Gut **C.** Ovary and spermatheca, **D** male testis and accessory gland (Magnification x40)

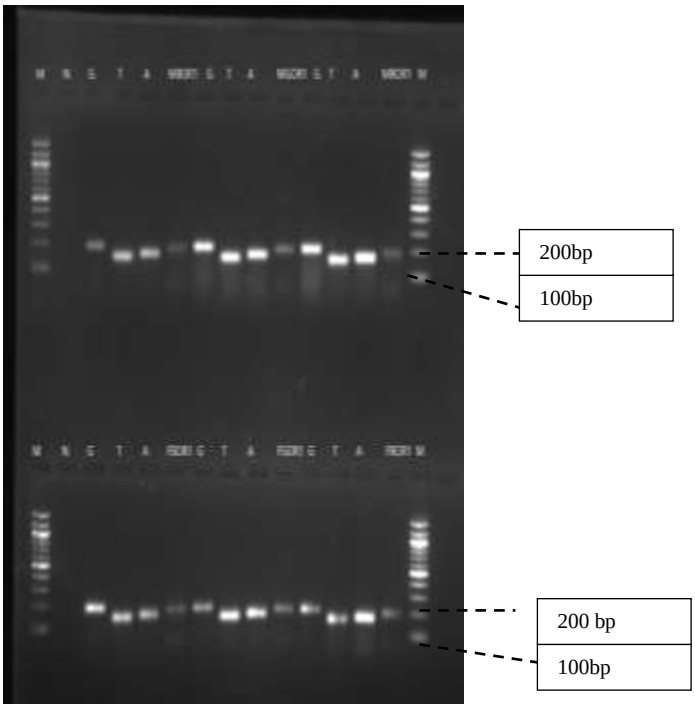
4.2 RNA Yield and Quality

RNA yield and concentration from the male and female brain, gut and reproductive tissues were analyzed using a NanoDrop 2000 spectrophotometer (Biospec-mini, Shimadzu Corporation, Tokyo). The concentration of all samples was above 350 µg/ml. The highest concentration among the samples was recorded in the male gut (1627 µg/ml) while the lowest concentration was reported for male brain (419.89 µg/ml). All the RNA samples gave an $A_{260/280}$ absorbance within the range of 1.944 to 2.188 (Appendix II). RNA integrity was assessed using 1.2 % formaldehyde agarose gel electrophoresis which showed one band of 18S of approximately 1.5 kb (Appendix III)

4.3 Screening of OR genes in Male and Female Non-Olfactory Tissues

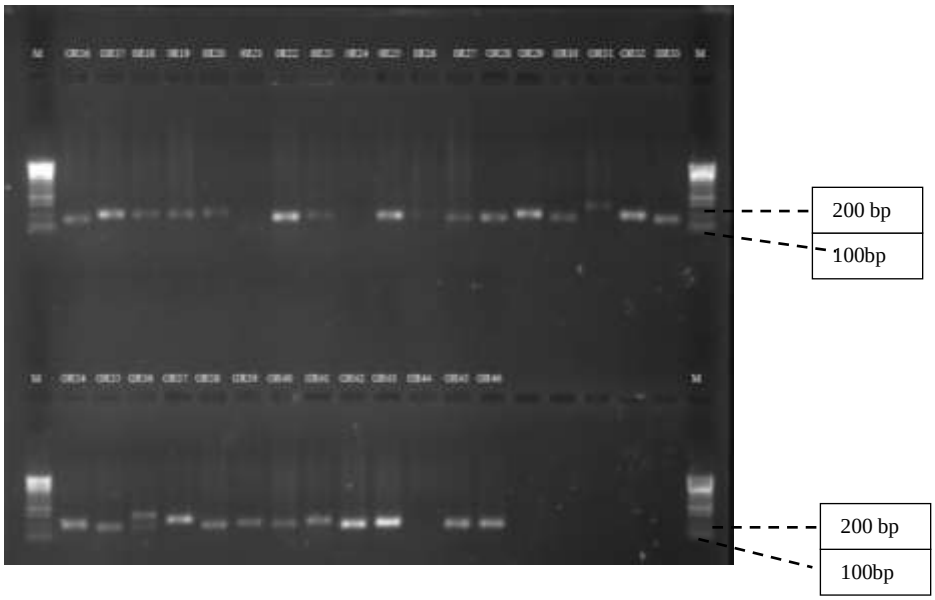
mRNA was converted to cDNA via reverse transcription. The 46 OR genes were then screened in the brain, gut and reproductive tissues of male and female *G. m. morsitans* using PCR. OR1 (Orco) was amplified and detected in all the male and female *G. m. morsitans* brain, gut and reproductive tissues. The band size of GAPDH, beta-tubulin and beta actin were 193bp, 149bp, 164bp respectively while the band size for OR1 was 190bp (Figure 4.2 A). 41 ORs amplified in the male brain (Figure 4.2 B-C), 38 ORs in the male gut (Figure 4.2 D-E) and 38 ORs in the male reproductive system (Figure 4.2 F-G) (Table 4.1). The internal controls GAPDH, beta-tubulin and beta actin amplified in all three tissues while the negative control lacking cDNA did not amplify in all male tissues. 44 ORs amplified in the female brain (Figure 4.2 H-I), 23 ORs amplified in the female gut (Figure 4.2 J-K) and 40 ORs amplified in the female reproductive tissues (Figure 4.2 L-M) (Table 4.1) The internal controls GAPDH, and beta-tubulin, beta actin amplified in the female brain and gut tissues alone while the negative control lacking cDNA did not amplify in any tissue

A



B

C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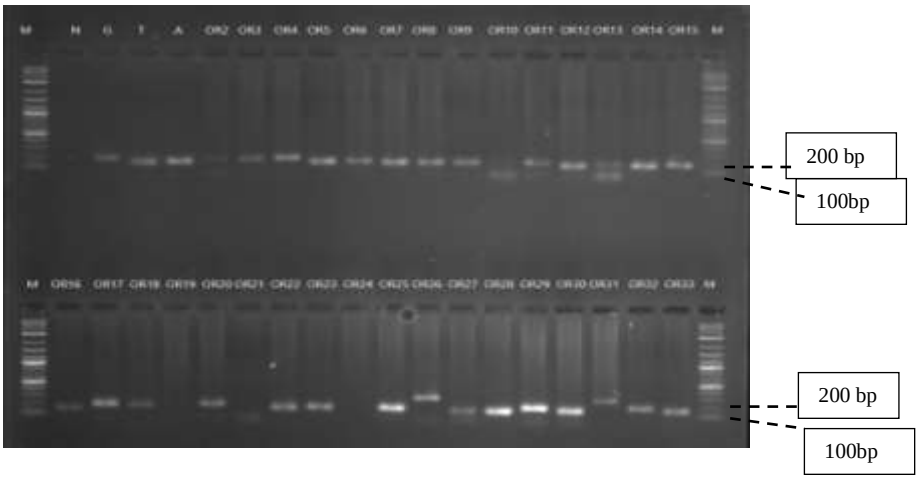
200 bp
100bp

D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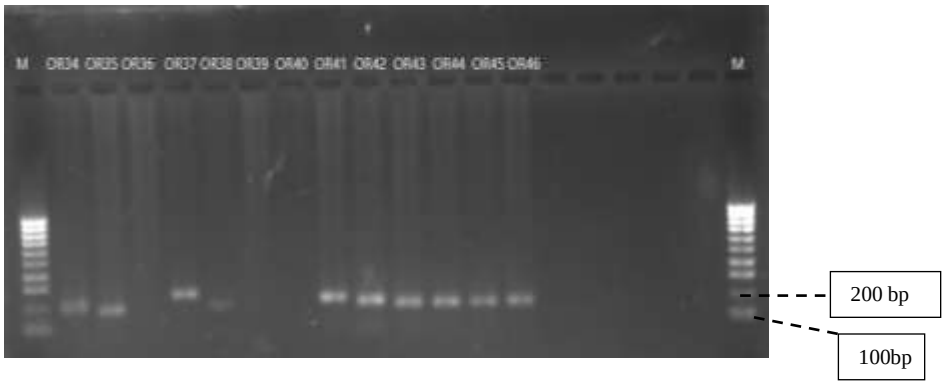
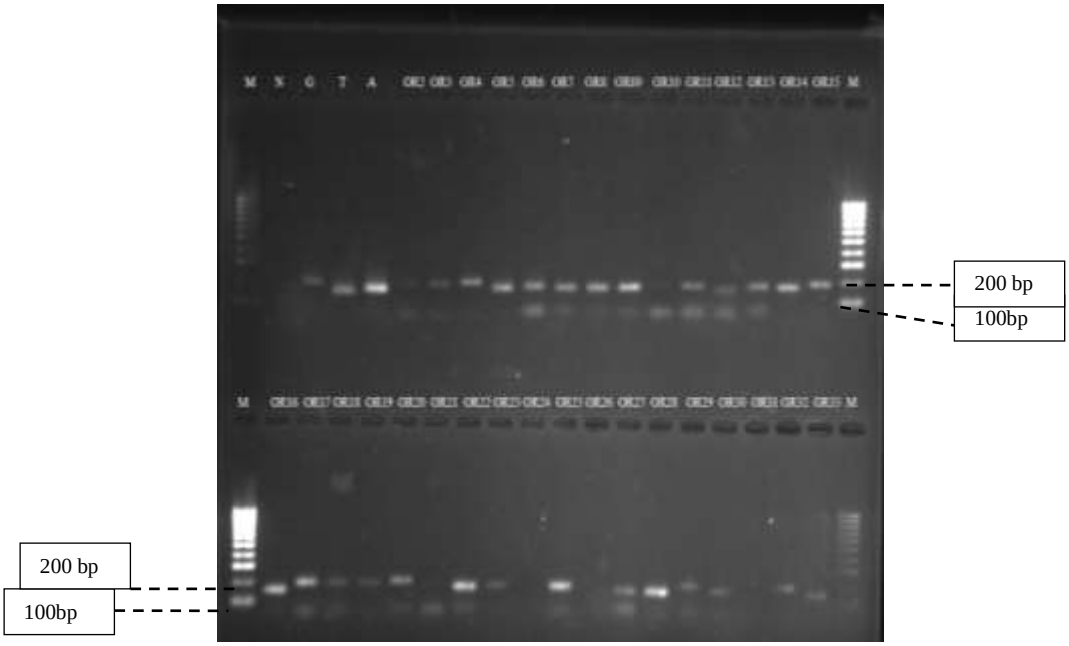
100bp

E

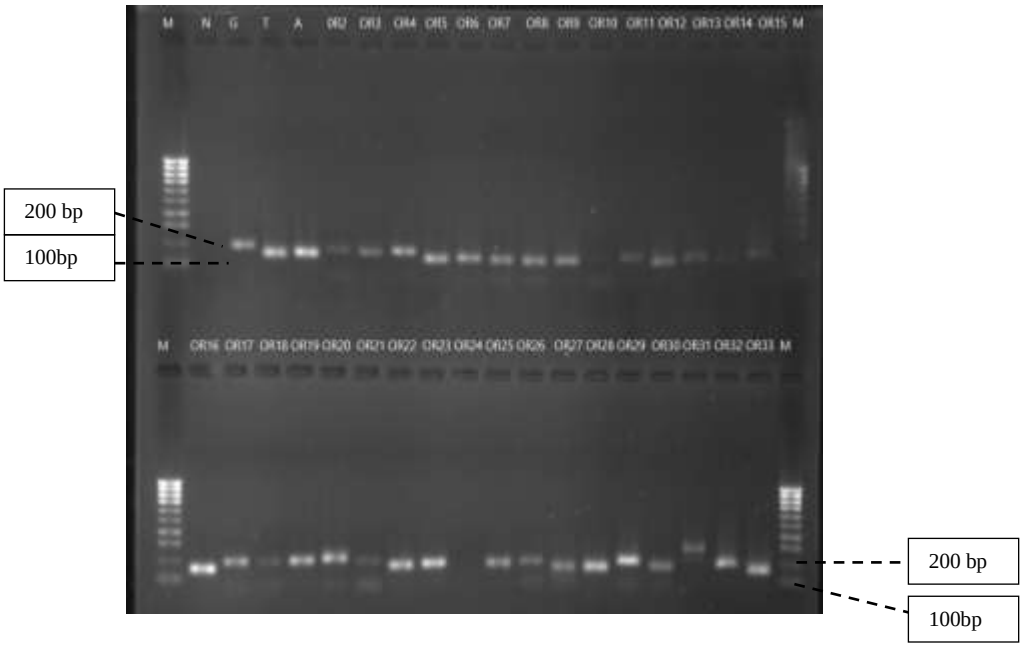

M OR34 OR35 OR36 OR37 OR38 OR39 OR40 OR41 OR42 OR43 OR44 OR45 OR46 M
200 bp
100bp

F

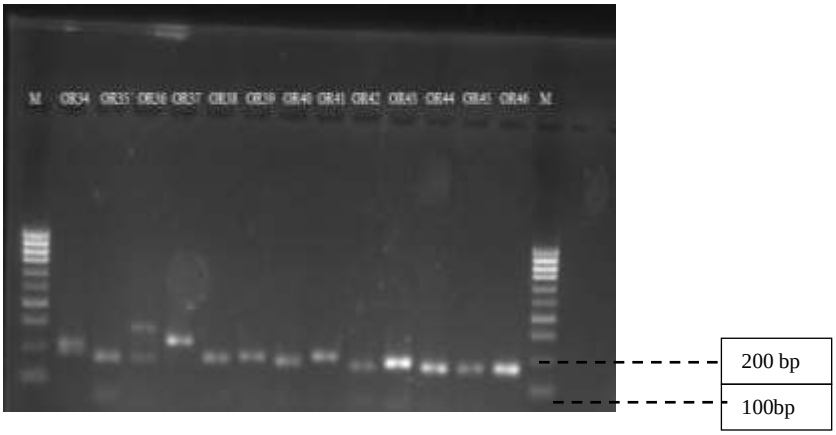


G

H



I



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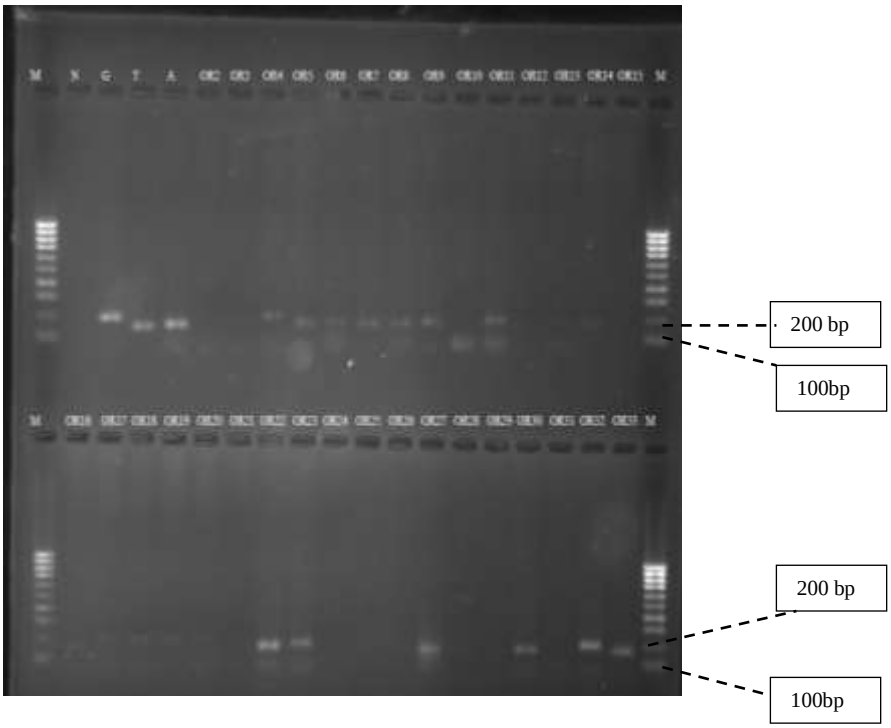

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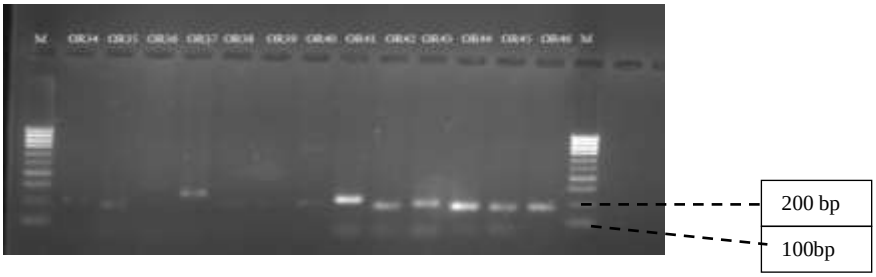

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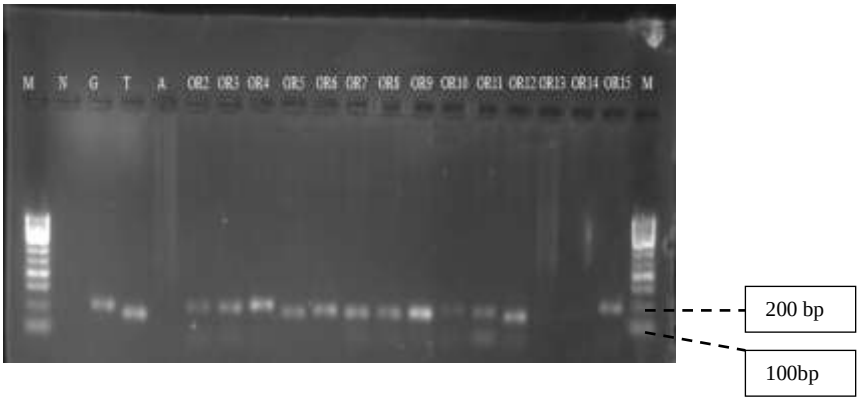


M

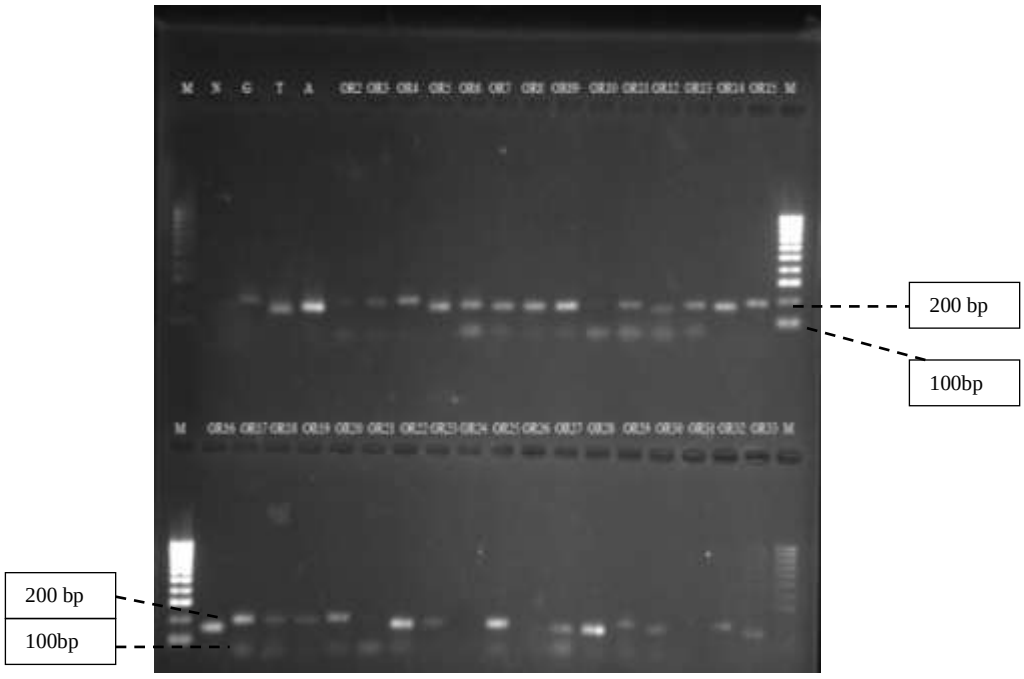
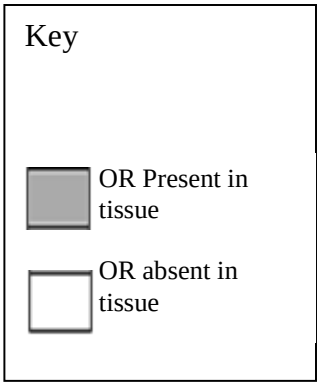


Figure 4.2: Amplified *G. m. morsitans* OR genes in non-olfactory tissues.

Gel image A represents OR1 amplification in the male and female brain gut and reproductive tissues. Image B and C amplification of ORs in male brain, D and E in the male gut, Fand G male reproductive tissues, H and I female brain, J and K female gut and L and M female reproductive tissues. The following letters represents individuals wells within the gel: **M.** 100bp molecular ladder, **N:** Negative control, **G** GAPDH (Glyceraldehyde-3-phosphate dehydrogenase), **T** β-Tubulin (Beta-Tubulin), **A** β-actin (Beta-actin), **MBOR1** OR1 male brain, **MGOR1** male gut, **MROR1** male reproductive tissue, **FBOR1** female brain, **FGOR2** female gut, **FROR1** female reproductive tissue and **ORX,** an odorant receptor gene where X is the number of the gene.

Table 4.1: Localized ORs in the non-olfactory tissues in *G.m. morsitans*

	Male			Female		
	Tissue type			Tissue type		
OR	B	G	R	B	G	R
01.						
02.						
03.						
04.						
05.						
06.						
07.						
08.						
09.						
10.						
11.						
12.						
13.						
14.						
15.						
16.						
17.						
18.						
19.						
20.						
21.						
22.						
23.						
24.						
25.						
26.						
27.						
28.						
29.						
30.						
31.						
32.						
33.						
34.						
35.						
36.						
37.						
38.						
39.						
40.						
41.						
42.						
43.						
44.						
45.						
46.						
Total present	41	38	39	44	23	40
Total absent	5	8	7	2	23	6



4.4 PCR Purification and Sequence Identification

38 amplicons from previously screened tissues were purified and the results viewed in 1% agarose gel before gene sequencing was carried out (Figure 4.3). The 38 amplicons consisted of different OR genes localized in the non-olfactory tissues. The genes BLAST similarity results of all the sequenced amplicons ranged from 84.8% to 98.1% and returned *G. m. morsitans* genes as best hits (Table 4.2). The sequences are presented in Appendix IV.

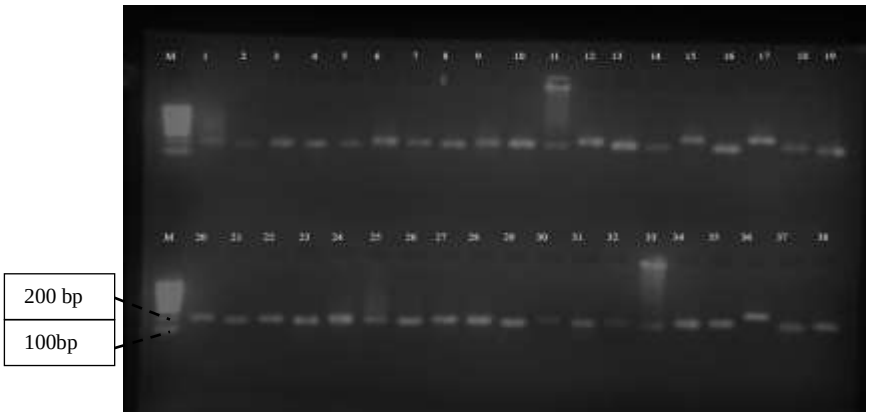


Figure 4.3: Agarose gel for purified amplified OR genes

M represents the molecular ladder: **1:** MBOR1 **2** MBOR5 **3**: MBOR6 **4**: MBOR7 **5**: MBOR8 **6:** MBOR9 **7**: MBOR14 **8**: FBOR11 **9:**FBOR12 **10** FBOR13 **11**:MGOR1 **12:** MGOR7 **13:** MGOR12 **14** FGOR33 **15** MGOR26 **16** FBOR28 **17** MBOR31 **18** MGOR32 **19** MGOR33 **20** MGOR34 **21** MGOR42 **22** MGOR44 **23** MGOR46 **24** FGOR43 **25** FGOR1 **26** FR0R9 **27** FR0R10 **28** FR0R11 **29** FROR15 **30** FROR22 **31** FROR25 **32** FROR27 **33** MROR1 **34** MROR42 **35** MROR44 **36** MROR37 **37** MROR45 **38** MROR46. **MB** represents male brain, **MG** male gut, **MR**-male reproductive, **FB** male brain, **FG** female gut, **FR**-female reproductive tissue

Table 4.2: Blast sequence results for amplified OR genes

Tissue	Gene	Accession number	E-value	Percentage identity	Query length	Gene name
Male Brain	GmmOR1	GMOY005610-RA	4e-76	97.1%	170	Orco
	GmmOR5	GMOY012018-RA	1e-68	98.1%	154	OR5
	GmmOR6	GMOY009475-RA	1e-49	93.9%	141	OR6
	GmmOR7	GMOY013231-RA	9e-45	92.6%	143	OR7
	GmmOR8	GMOY012193-RA	9e-58	94.5%	145	OR8
	GmmOR9	GMOY012276-RA	1e-6	97.4%	149	OR9
	GmmOR14	GMOY001365-RA	2e-53	91.8%	146	OR14
	GmmOR31	GMOY012239-RA	1e-49	93%	138	OR31
Female Brain	GmmOR11	GMOY010761-RA	3e-45	86.4%	144	OR11
	GmmOR12	GMOY009271-RA	5e-55	91.8%	148	OR12
	GmmOR13	GMOY003312-RA	6e-67	93.9%	171	OR13
	GmmOR28	GMOY012199-RA	1e-55	95.6%	129	OR28
Male Gut	GmmOR1	GMOY005610-RA	2e-22	89.2%	180	Orco
	GmmOR7	GMOY013231-RA	1e-61	94.8%	145	OR7
	GmmOR12	GMOY009271-RA	2e-59	92.9%	145	OR12
	GmmOR32	GMOY005084-RA	3e-57	96.3%	131	OR32
	GmmOR33	GMOY013237-RA	3e-57	93.3%	144	Or33
	GmmOR34	GMOY012295-RA	8e-58	95.8%	136	OR34
	GmmOR42	GMOY006479-RA	6e-67	95.1%	157	OR42
	GmmOR44	GMOY006265-RA	3e-70	97.4%	152	OR44
	GmmOR46	GMOY003305-RA	2e-85	95.4%	144	OR46
Female Gut	GmmOR33	GMOY013237-RA	5e-54	92.4%	140	OR33
	GmmOR43	GMOY006480-RA	1e-62	95.9%	148	0R43
Female reproductive tissue	GmmOR1	GMOY005610-RA	3e-71	96%	167	OR1
	GmmOR9	GMOY012276-RA	2e-60	94.1%	144	OR9
	GmmOR10	GMOY012214-RA	4e-56	93.3%	149	OR10
	GmmOR11	GMOY010761-RA	1e-62	96.2%	152	OR11
	GmmOR15	GMOY012287-RA	2e-65	94.4%	153	OR15
	GmmOR22	GMOY012287-RA	2e-59	95.7%	140	OR22
	GmmOR25	GMOY012357-RA	3e-64	97.3%	147	OR25
	GmmOR27	GMOY008038-RA	1e-55	97.0%	129	OR27
Male reproductive tissues	GmmOR1	GMOY005610-RA	3e-64	92.4%	170	Orco
	GmmOR42	GMOY006479-RA	1e-62	97.9%	142	OR42
	GmmOR44	GMOY006265-RA	5e-54	97.6%	123	OR44
	GmmOR37	GMOY012218-RA	8e-72	94%	172	OR37
	GmmOR45	GMOY007896-RA	1e-61	96.5%	137	OR45
	GmmOR46	GMOY003305-RA	2e-48	84.8%	153	OR46

4.5 Bioinformatics Analysis

4.5.1 Conserved Domains and Transmembrane Helices

All of the selected proteins belonged to the seven transmembrane odorant receptors (7tm_6) family which is composed of seven transmembrane odorant receptors. Seven transmembrane helices were identified in GmmOR32, GmmOR19, GmmOR15, GmmOR31, GmmOR33, and GmmOR35, while six transmembrane helices were identified in GmmOR7, GmmOR9, GmmOR10, GmmOR11, GmmOR13, GmmOR27, GmmOR30 GmmOR42, GmmOR43, GmmOR44. Three transmembrane helices were present in GmmOR12, and GmmOR28, two in GmmOR34, four in GmmOR37, five in GmmOR1, GmmOR5, GmmOR46 and GmmOR25, eight in GmmOR17 and none in GmmOR22 (Appendix V)

4.5.2 Identification of Homologs

Blast search against *G. palpalis* ranged between 24.1 % to 98%, *G. austeni* 29.2 %-98.8%, *G. fuscipes fuscipes* 23.1 to 97.6%, *G. pallidipes* 21.7% to 99.4% and *G. brevipalpis* 25.5% to 92% (Appendices 3). Orco similarity between *G. m morsitans* and the other *Glossina* species ranged 92% to 99.4% *D. melanogaster* homologs showed sequence similarity ranging from 22.4 to 77.5%. The highest sequence similarity homolog from *D. melanogaster* was Dmel\Orco (77.5%) while the lowest sequence similarity homolog was from *D. melanogaster* was Dmel\OR49a (23%) (Appendix VI).

4.5.3 Gene Ontology and Homolog Expression in *D. melanogaster* Tissues

Gene ontology was divided into three sections; molecular functions, biological processes and cellular component. Gene ontology functions retrieved from the various *Glossina* genomes (*G. palpalis, G. austeni, G. fuscipes fuscipes, G. pallidipes and G. brevipalpis*) homologs from Vector Base revealed that the molecular function was primarily odorant receptor activity and odorant function. The biological process of all of the identified homologs was signal transduction, response to stimuli and sensory perception of smell

while the cellular component was the plasma membrane, membrane and forming an integral part of a membrane. Similar molecular functions and biological processes were reported for *D. melanogaster* homologs. The cellular component of all homologs specified the dendrite membrane, plasma membrane and forming an integral part of the membrane (Appendix VI).

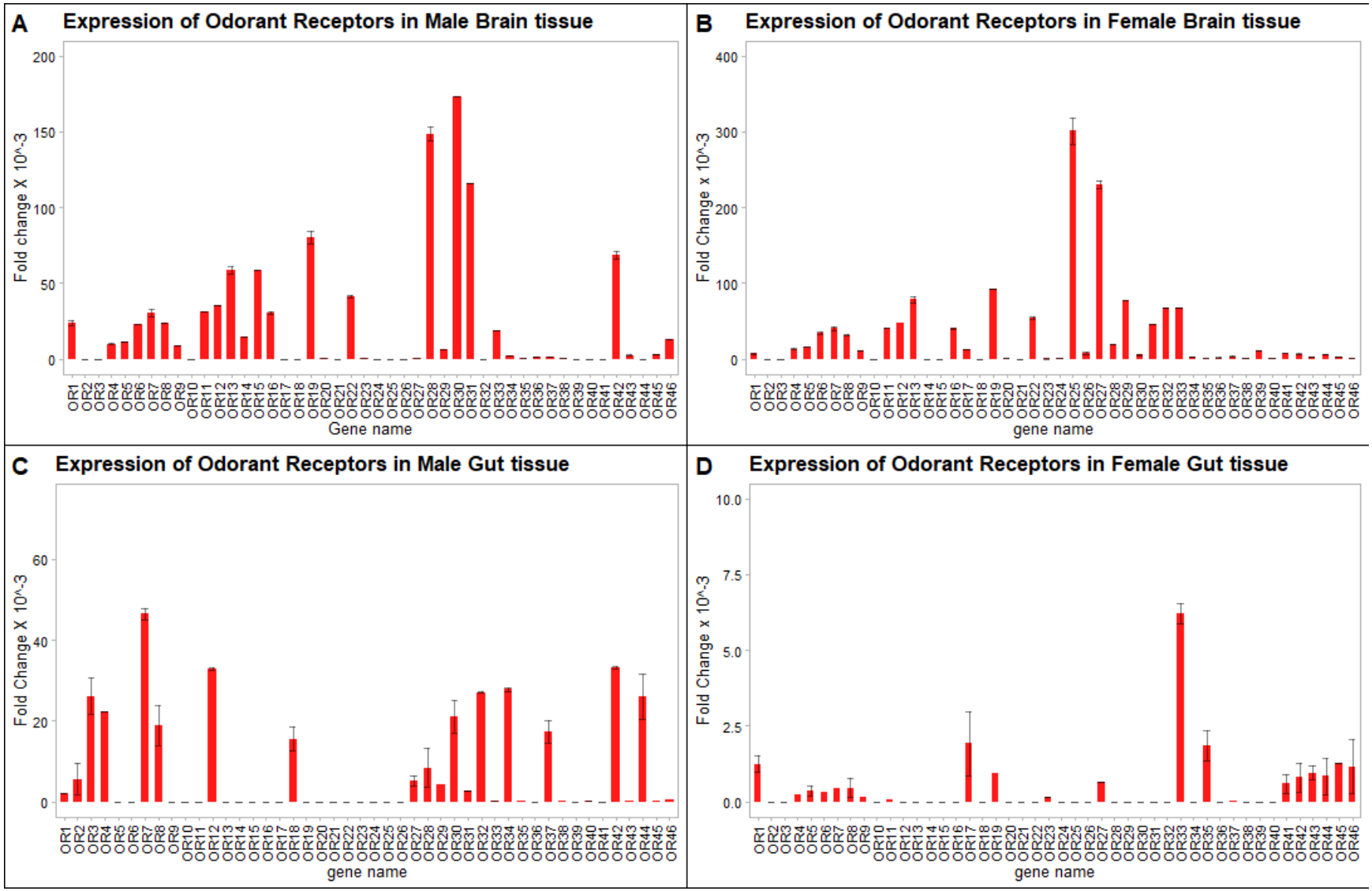
Fly Atlas 2 was used to illustrate the expression of *G. m. morsitans* homologs in *D. melanogaster* across different tissues. Most *G. m. morsitans* homologs were expressed in the head of *D. melanogaster* which houses the antennae, the main olfactory organ. *G. m. morsitans* homologs in *D. melanogaster* were also expressed or localized in non-olfactory tissues. The homologs identified in the male brain included Dmel\Orco (GmmOR1), Dmel\Or7a (GmmOR6), Dmel\Or42b (GmmOR7), Dmel\Or82a(GmmOR13), Dmel\Or45a (GmmOR15), Dmel\OR67c (GmmOR27), Dmel\Or85f (GmmOR28), Dmel\Or13a (GmmOR32), Dmel/OR49b (GmmOR33), and Dmel\Or67d, a homolog of GmmOR42, GmmOR44, GmmOR45 and GmmOR46. All of the above identified homologs were present in the female *D. melanogaster* with the exception of Dmel\Or13a (GmmOR32). Dmel\OR7a (GmmOR8) was present in the female gut, Dmel\OR42a (GmmOR9), Dmel\Or67b (GmmOR19) in the male gut, DmelOR94a (GmmOR12), Dmel\OR45a (GmmOR15), Dmel\Or85f (GmmOR28) Dmel\Or49b (GmmOR33) in both male and female gut. The identified homologs in the male testis included Dmel\Orco (GmmOR1), Dmel\Or7a (GmmOR6), Dmel\OR46a (GmmOR10), Dmel\Or46a (GmmOR11) Dmel\Or85d (GmmOR17), DmelOR67b (GmmOR19), Dmel\OR67c (GmmOR27) Dmel\Or85f (GmmOR28) Dmel\Or13a (GmmOR32), while Dmel\Or42b (GmmOR7), Dmel\OR7a (GmmOR8), Dmel\Or82a (GmmOR13) were present in the accessory glands.in the female reproductive system Dmel\Or24 (GmmOR31) and Dmel\OR82a (GmmOR13) were located in the ovary and virgin and mated spermatheca respectively (Appendix VI).

The results revealed presence of ORs in similar tissues in *G. m. morsitans* and *D. melanogaster*. For instance, GmmOR28 and GmmOR42 screened in the male brain *G.*

m. morsitans were identified as *D. melanogaster* homolog DmelOR85f and DmelOR67d in the *D. melanogaster* brain while GmmOR12 screened in the male gut was present in *D. melanogaster* hindgut as DmelOR94a. GmmOR27 and GmmOR32 localized in *G. m. morsitans* male reproductive system was expressed in *D. melanogaster* testis as DmelOR67c and DmelOR13a respectively. GmmOR27 localized in the female brain was identified in *D. melanogaster* as DmelOR67c in the female brain while GmmOR33 localized in female gut was identified in *D. melanogaster* as DmelOR49b (Appendix VI)

4.6. Relative Expression of *G. m. morsitans* ORs in Brain, Gut and Reproductive Tissues

The expression pattern of ORs was determined by qRT-PCR on the brain, gut and reproductive tissues in male and female *G. m. morsitans.* qRT-PCR results revealed ORs are abundantly expressed in male and female brain gut and reproductive tissues.Quantitative real time PCR revealed four significantly highly expressed OR genes in male *G. m. morsitans* brain (GmmOR19, GmmOR28, GmmOR30, GmmOR31) (Figure 4.10A), six in the gut (GmmOR7, GmmOR12, GmmOR32, GmmOR34, GmmOR42, GmmOR44) (Figure 4.10C) and three ORs in the testis and accessory glands (GmmOR32, GmmOR44, GmmOR46) (Figure 4.10E). In the female *G. m. morsitans*, two ORs were significantly expressed the brain (GmmOR25, GmmOR27) (Figure 4.10B), three in the gut (GmmOR17, GmmOR33, GmmOR35) (Figure 4.10D) and three in the reproductive tissues (GmmOR9, GmmOR22, GmmOR27) (Figure 4.10F).GmmOR1 (Orco) which is highly conserved was lowly expressed in in all male and female non-olfactory tissues. In the male tissues only GmmOR28, GmmOR30 and GmmOR31 in the male brain, and GmmOR32, GmmOR44, GmmOR45 and GmmOR46 in the male reproductive tissues had a relative expression value greater than 0.1 in reference to GAPDH. In the female tissues, only GmmOR25 and GmmOR27 in the female brain had relative expression values of 0.3 and 0.23 respectively in reference to GAPDH (Appendix VII)


A
Expression of Odorant Receptors in Male Brain tissue
Fold change X 10^-3
Gene name
B
Expression of Odorant Receptors in Female Brain tissue
Fold Change x 10^-3
gene name
C
Expression of Odorant Receptors in Male Gut tissue
Fold Change X 10^-3
gene name
D
Expression of Odorant Receptors in Female Gut tissue
Fold Change x 10^-3
gene name

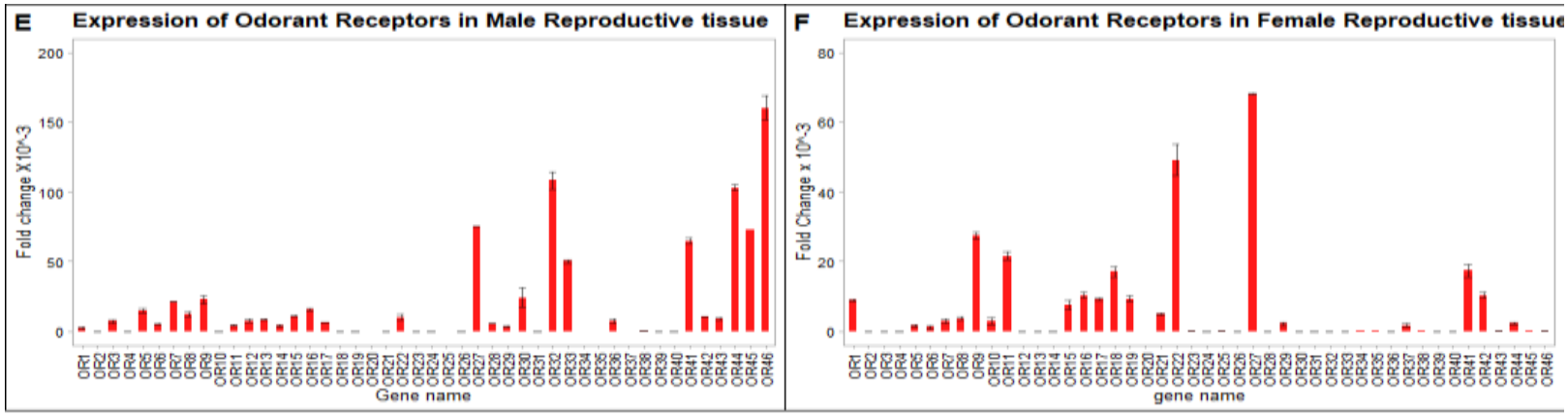


Figure 4.4: *G. m. morsitans* OR genes relative expression to GAPDH

(A) Male Brain, (**B**) female brain (**C**) male gut **(D)** female gut **(E)** male reproductive and **(F)** female reproductive tissues. The expression level of odorant receptor genes in male and female brain, gut and reproductive tissues of *G. m. morsitans* was calculated by delta delta Ct (ddCt) with GAPDH as the reference gene (Livak and Schmittgen, 2001)

CHAPTER FIVE

DISCUSSION

This study utilized 46 OR genes previously identified in *G. m. morsitans*. The results revealed the detection of 41, 38 and 39 OR genes in male, gut and reproductive tissues respectively. In the female, 44, 23 and 40 ORs were localized in the female brain, gut and reproductive tissues respectively. These results are consistent with previous studies which reported the presence of OR genes in non-olfactory tissues including GmmOR20 in larvae and GmmOR28 in pupae of *G. m.* morsitans (Moindi et al., 2018), GmmOR26 in female legs and GmmOR34 in male legs of *G. m.* morsitans (Nyanjom et al., 2018), LmigOrco and LmigOR95 in *Locusta migratoria* (Wang et al., 2015) and Orco in the head, reproductive tissue, legs and gut of the bed bug *Cimex lectularis* (Hansen et al., 2014). Expression of Orco has also been reported in the reproductive tissues of mosquito *Aedes albopictus* and parasitic wasp, *Nasonia vitripennis* (Pitts et al., 2014). Moreover, expression of odorant receptors AcerOR1 and Acer OR2 (ortholog to Orco) also occurred in the sperm and testes of male honeybee drones *Apis cerana cerana* with the expression of AcerOR2 being higher than AcerOR1 in the testis and sperm (Guo et al., 2018). Based on the above findings, the localization of ORs occurs in non-olfactory tissues in *G. m. morsitans* and other insects.

Expression of odorant receptors in non-olfactory tissues in the brain, gut and reproductive tissues in *G. m. morsitans* was also observed in *D. melanogaster* suggesting conservation of similar non-olfactory functions. For example, GmmOR12 expression in male gut and its presence as *D. melanogaster* hindgut as DmelOR94a and GmmOR33 in the female gut and its homolog in *D. melanogaster* as DmelOR49b may probably suggest gut related functions. Similarly, low expression of GmmOR28 and GmmOR42 in male brain and homologs DmelOR85f and DmelOR67d in *D. melanogaster* male brain as well as GmmOR27 in the female brain and its homolog DmelOR67c suggests either brain related functions or role in development of the olfactory system. The brain

houses the antennal lobe, which processes olfactory information and contains ORN projections in spherical modules referred to as glomeruli (Couto et al., 2005). GmmOR28 previously identified in the *G. m. morsitans* pupae was also identified in the male and female brain and its role could be associated with brain development (Moindi et al., 2018). Low expression of GmmOR27 and GmmOR32 expressed in the male reproductive system and homologs DmelOR67c and DmelOR13a respectively in *D. melanogaster* testis suggests reproductive related functions that were maintained after diversion of *D. melanogaster* and *G. m morsitans* over 75 million years ago (Wiegmann et al., 2011). The existing sequence similarities between *G. m. morsitans* tissues expressed in non-olfactory tissues and their *D. melanogaster* and other *Glossina* orthologs may suggests conservation in function and location in non-olfactory tissues.

Insects rely on the olfactory system in detecting and analyzing environmental cues. The antennae and maxillary palps are considered as the primary olfactory organs in insects and are covered by sensilla which house a small number ORN(Guo & Smith, 2017). The sensilla contains pores which allows compounds to contact the ORN in the lymph. ORNs express odorant receptors allow passage of ion current upon binding of odorants to its projections in the antenatal lobe to mushroom bodies and finally lateral horns for processing and subsequently change in behavior (Sato et al., 2008). Expression of odorant receptors within the primary olfactory organs is associated with olfaction functions. High expression of OR genes such as GmmOR33 in male antennae and GmmOR45 in female antennae, implied their role in olfaction (Nyanjom et al., 2018). The brain, gut and reproductive tissues have different roles as compared to the antennae. Therefore, ORs expression in non-olfactory tissues could be linked to non-olfactory roles. Existing evidence across other insect orders already links ORs in non-olfactory tissues to other non-olfactory functions. For instance, expression of AgOR7 or Orco in the testes of *An. gambiae* was linked to spermatozoa activation while the expression of HoblOR22 in *Hexachaeta oblita* non-olfactory tissues was consider as a ligand for other molecules rather than odorants. Also, the co-expression of *Locusta migratoria* LmigOrco and LmigOR95 in a non-olfactory tissue monitored levels of internal

metabolites levels by binding endogenous and exogenous ligands (Wang et al., 2015). Thus, the expression of OR1 or highly conserved Orco and other odorant receptors such as GmmOR32, GmmOR44 and GmmOR46 in *G. m. morsitans* male reproductive system may imply male reproduction roles.

Low expression patterns of Orco in the male gut and reproductive organs in the *G. m. morsitans* closely resembled expression patterns of Orco in similar tissues in *Cimex lectularius* (Hansen et al., 2014). In *Anopheles gambiae*, AgOrco transcripts in testis were not detected by RNA sequence analysis but later detected via Multidimensional Protein Identification Technology (MudPIT) with the apparent absence being associated with expression of Orco earlier in life stages perhaps during larval gonad development. However, the translated protein was highly stable and active in adult life (Pitts et al., 2014). Expression of Orco has also been reported in the reproductive tissues of mosquito *Aedes albopictus* and parasitic wasp, *Nasonia vitripennis* (Pitts et al., 2014). Odorant receptors AcerOR1 and Acer OR2 (ortholog to Orco) were also expressed in the sperm and testes of male honeybee drones *Apis cerana cerana* with the expression of AcerOR2 being higher than AcerOR1 in the testis and sperm (Guo et al., 2018). The presence of Orco and other odorant receptors such as GmmOR32, GmmOR44 and GmmOR46 similarly to expression of AgOR11 in the male testes in *Anopheles gambiae* suggested the formation of a heteromer complex with AgOrco (Pitts et al., 2014). Orco can form heterodimers with other odorant receptors to form ligated ion channels (Sato et al., 2008) or act independently as a functional ion homomeric protein (Jones et al., 2011). Considering that Orco is mostly conserved across most insect species (Jones et al., 2005) and is expressed with other odorant receptors in the male reproductive tissues of *G. m. morsitans*, it may play similar reproductive roles as those observed in *Anopheles gambiae* including fertilization, sperm chemotaxis, or spermatozoa activation (Pitts et al., 2014) or maintaining the actin cap and in the process preventing abnormal spermatids release from the cyst enclosure in *D. melanogaster* (Dubey et al., 2016).

The mechanism of ORs signal transduction processes may be similar in both olfactory and non-olfactory tissues. Odorant biding proteins normally bind to pheromones and odor molecules and act as carriers in delivering odorant to odorant receptors. It has been noted that other olfaction proteins such as OBPs are expressed in non-olfactory tissues and may be carriers of specific ligands other than odorant molecules (Bigiani et al., 2005). High expression of *LmigOBP8* in the brain of *Locusta migratoria* is suggested to carry ligands for signal transmission for neural development to the brain (Kang et al., 2018) while GmmOBP4 and GmmOBP17 have been reported in the male reproductive tissues of *G. m. morsitans* (Scolari et al., 2016). Proteome analysis in *D. melanogaster* revealed the presence of OBP44a, and OBP99a in the seminal vesicles and testis, OBP51 in the accessory glands, OBP56f in the ejaculatory duct and OBP56g in the ejaculatory bulb (Takemori & Yamamoto, 2009). Differential expressions of OBPs in *Drosophila's* reproductive organs suggest an organ-specific signaling function in reproduction. The presence of ORs in the male reproductive system of *G. m. morsitans* such as GmmOR32 and its homolog in *Drosophila melanogaster* DmelOR13a could be involved as signaling receptors for downstream process for chemoattractant OBP molecules identified in the reproductive system. Biological processes under gene ontology suggested that ORs are involved in signal transduction and response to stimuli. For genes expressed in the gut and reproductive tissues, signal transduction processes may likely be applicable. Exposure of a second messenger of cyclic adenosine monophosphate (8-Br-cAMP) to Orco in *A. gambiae* significantly increased flagellar beating in the sperm and was also unaffected by an Orco antagonist VUANT suggesting the highly conserved Orco either acts independently as an ion channel or utilizes secondary messengers (Pitts et al., 2014). Male honeybee drones *Apis cerana cerana* AcerOR2 expression in sperms was similar to Anopheles *gambiae* and both insects activates sperm motility via calcium ions/calmodulinCaM)/CaM-dependent protein kinase II (CaMKII) signaling (Guo et al., 2018). The presence of transmembrane helices identified in the expressed tissues in *G. m. morsitans* may further indicate signal transduction processes through ligand gated ions channels or metabotropically as G

protein-coupled receptors (GPCRs) channels (Sato et al., 2008;Wicher et al., 2008). Whether these OBPs transport ligands to odorant receptors identified in the reproductive tissues and other non-olfactory tissues such as the brain and gut warrants further investigation.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From the above study, it is evident that different ORs localize in the male and female *G. m. morsitans.* brain, gut and reproductive tissues. The study also showed that these *G. m. morsitans* ORs homologs are present in other non-olfactory tissues of *D. melanogaster*. The above findings suggest conservation of these genes across insect orders and possible roles outside the olfaction system. Lastly, different ORs were expressed in the male and female tissues non-olfactory tissues and provide basic knowledge that future studies could rely on develop vector control strategies.

6.2 Recommendations

1. Functional studies on the role of significantly expressed genes such as GmmOR28, GmmOR30 and GmmOR31 in the male brain, GmmOR32, GmmOR44, GmmOR45 and GmmOR46 in the male reproductive tissues and GmmOR25 and GmmOR27 in the female brain to be investigated.
2. Functional and imaging studies on Orco/OR1 in the male reproductive tissues (testis) to check whether it has similar functions related to spermatozoa activation and possible target for vector control in the reproduction system of *G. m. morsitans.*

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APPENDICES

Appendix I: Primers for amplification of Odorant Receptor

Receptor	Product Size	Forward Sequence	Melting temperature forward sequence	Reverse Sequence	Melting temperature reverse sequence
Or1	190	CTGTTCGCTCTATCTCATGC	57.2	ATTCGGCCTTGGAATGTG	60.4
Or2	191	TCCCGCTTGGTATGGTTTAG	60	AGAGACCCACAGAACGCACT	59.9
Or3	191	GCTTGGAAGCTGAAGCAAGT	59.8	GATCATCATTCGGACCAAAA	58.3
Or4	191	CTTAGTCGCAACCATGTGGA	59.7	TGATGAGTGCCGTGTAAAGG	59.7
Or5	163	TACACGGGTCTGACCACATT	58.9	ACAGGTCCCGTTGTAATTCG	59.9
Or6	175	CTGCAACCATCATCACAACC	60	GTCCGCAGATGATACGATGA	59.6
Or7	160	ACGATGTAACCGATGTGCTG	59.6	CAGATTAGCTGCTCGTTTGC	58.8
Or8	159	TCTATGGAGGCATGTCCTTG	58.7	GGCCAAAACCCAGATACTCA	59.9
Or9	164	CTGGGCATAGAACCTTACGC	59.7	GTGGCCTCCAGGAAATTGT	59.9
OR10	185	TACAGCTCTCGCAGTTCTGG	59	AGCGAAAGAACGCAGTAAGC	60.3
Or11	176	TCACCGGAGCGTATCCTAAT	59.6	ATATGGCCGATCAAGACGAC	59.9
Or12	157	CAGGCGGCCATCAATATC	60	CGGTAATTGCACAGTGACG	58.7
Or13	174	CGCGAATGTAGCGATGATAG	59.4	GTGAAGGAAATAGCCCATCG	59.5
Or14	168	GCCGATCGTTTAAGTGAAGC	59.9	TCGCATACGTCGCTGTAAGT	59.5
Or15	184	GACGTAGGCTGGAATGCAC	59.3	TGCCGCAAGTGTAGGTAATG	59.8
Or16	101	AAAAGAATTGGCGACGCTAT	58.9	AATTGAGTCGATACCCTCCT	55.2

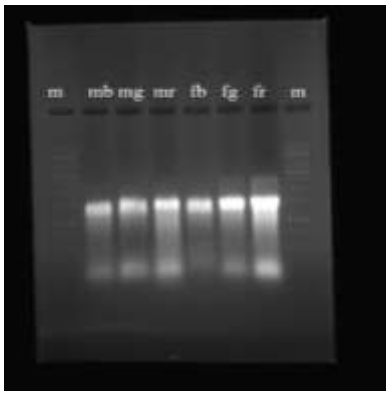
Or17	194	CGATCCCAATACCCATACG	58.7	CGCTTTCATTGGTCTCCTC	58.4
Or18	190	TTACGATGGCAAATTGTTGG	59.4	TTCCGATTCGGTCAATATCC	59.7
Or19	166	CACGATACGAATACGCCTTG	59.2	CCCGGTATCAACGCAGTTA	59.6
Or20	150	AGGATAGAGGCGCACGATAC	59.3	CCCGTTTGCCTGTTATTAGC	59.6
OR21	189	GCCCGATAGAACGTTGGTT	59.9	ACATCCAATAAGCCGAGGAG	59.1
OR22	162	CAAACGTGCCGCTTTTCT	58.2	GGTATGAATGTGGCTCTTCG	50
Or23	155	GCGTTGTACGTATGGGATTC	58	TTACCGCCGTTTTCTTGG	59.7
Or24	189	GCCCGATAGAACGTTGGTT	59.9	ACATCCAATAAGCCGAGGAG	59.1
OR25	180	TTACGATGGCAAATTGTTGG	59.7	TTCCGATTCGGTCAATATCC	60.3
Or26	184	CTCGTTGGCTGTACGCATTA	59.9	GCCAATCATCACAAGTACCG	59
Or27	150	CTGCATATGGCACGGAAA	59.2	GTATGCTGCCGTCATACCAT	58.5
Or28	151	AGAGCTCAAAGGCCAGCTC	59.8	CGTTGTCAGTGGCTACAGGA	59.9
Or29	192	TGACGAGCATCCTTGTGAAC	59.8	ATAATGGTGCCAGGCTGAAC	60
Or30	159	GTTCAGCCTGGCACCATTAT	60	GCAGCAAAGGCTTGAGTTG	59.7
Or31	151	TCAACACAAGCACCGCTTAC	59.9	CCATCAGCACCAACAAACC	60
OR32	184	GACGTAGGCTGGAATGCAC	59.3	TGCCGCAAGTGTAGGTAATG	59.8
OR33	101	AAAAGAATTGGCGACGCTAT	58.9	AATTGAGTCGATACCCTCCT	55.2
Or34	175	CGAAGAGCCACATTCATACC	58.2	TGGATGGTCAAAGCTGTACG	59.7
Or35	164	GTTGCCGGTCTGTGTAATGA	59.6	GGAATGCCTCGAACTTATCG	59.7
Or36	173	TACCGTTATGCTGTGCGTTC	59.8	TACGGTAAGCAGCATCAGGA	59.4
Or37	179	CGCAGAATCCTTTGGGTAGT	59.2	ATTTTCGCAGGAGTCTGAGG	59.4
Or38	177	CCACCGCTGTTAACTCATCA	59.7	GAAGATTCCGCAAGCAAGAC	60
Or40	166	GTGGCGCTGTGTTTACGATA	59.8	CTCACCACTCTTGCTCGTTG	59.6
OR41	150	AGGATAGAGGCGCACGATAC	59.3	CCCGTTTGCCTGTTATTAGC	59.6

OR42	166	CACGATACGAATACGCCTTG	59.2	CCCGGTATCAACGCAGTTA	59.6
Or43	179	GGCACACAATACGAATACGC	59.1	GGATCAATTCCCGGTATCAG	59.2
OR44	194	CGATCCCAATACCCATACG	58.7	CGCTTTCATTGGTCTCCTC	58.4
Or45	166	CAGTGATCGTATGGCCGTTA	59.6	GCTGCAAAGTTTCCATAGGC	59.9
Or46	164	GCGAGTGATACGCTGTTACG	59.5	AATTGTAGGGCGCTGCTTAG	59.5
GAPDH	180	GTGAACGCGATCCCACTAAT	60.4	GGGGAGTAAGCCTCCAAGTT	62.45
Actin	164	GGGTATGGAATCTTGCGGTA	60.4	GAGCCAAAGCAGTGATTTCC	60.4
Tubulin	149	CCATTCCCACGTCTTCACTT	60.4	GACCATGACGTGGATCACAG	62.5

Appendix II: Total RNA readings from *G. m. morsitans* tissues

Sample type	**Tissue**	**Concentration (µg/ml)**	**Absorbance 260/280nm**
Male	Brain	419.89	2.188
	Gut	1627.5	2.166
	Reproductive tissue	978.74	1.944
Female	Brain	611.23	2.138
	Gut	935.21	2.081
	Reproductive tissue	604.54	2.154

Appendix III: Total RNA Formaldehyde gel image for *G. m. morsitans* tissues



Appendix IV: Odorant Receptor Amplicons

Male Brain OR1

CTCATGCAAATTTATGCGATGTATTATTCTGTNCATGGTAATATCGCTTGTGA
GCAATTGCAACATTTAAAGAGTATTATGAAACCATTAATGGAATTATCCGCC
TCATTGGATACATTTCGTCCCGATTCCGGAGCTCTATCGTTCATTGTCAGCAC
ATTCCAAGGCCG

Male Brain OR5

TGGGTGCTATCGTCGACCAACGTTTAATGGTCCTGCTTGGTTTCCATTTGATT
GGAAATCGTCATCTACGTTATACTGGTCGGCTTTGTATATCAATTTATTGGCC
TTAATATGTTAATTATGCAGAATTTGGTTAATGACACCGTAGTCCAAA

Male Brain OR6

ATTGGTTGCAGCCATAAGTCCAATGTTTCGTTAACCATATTTGTACAATTTGC
CATTACTGAGTATGTGGCTACGAAATGTTAATATTTCGTTCTTTTCCAATGTC
GTTGGTCGTATGCTTCGAATTTTAATTATATTGTG

Male Brain OR7

AATTTACAATACGTTTATCTATAATTTCATAACAATATTTTGCAATTTAAATA
ACTCCACCATATTGGTACGCTCTTATAATATCCTACTGTTTGCGACCAACTTC
GCTTCTATAGTTCATCATGTTTTACGTATTGTGGTCG

Male Brain OR8

CTTATTCGAGACTATTGCTCTTATTTCGACCACGGTATTCGTTCAATTTATGA
TCACTGCAGCCATAATTGCTCTCACGCTTATCAATATGTTATTTTTCACTACC
AATATATCGTCGCAAATTCATGCGTTTAGTATTAGAAAT

Male Brain OR9

CTTTATAATCTATTTAGCCCAGTTATCTCTGGCACCATGTTTGTGCAGTTTTT
GATCATCGGTATTATTTTGGGTGTTACAACAATCCAGTTTTGTTTGCAAATTT
TTTTGCGATTGTAGCATCCTTGTTTTATGTGGCTTCTATTTTA

Male brain OR14

TGAGCAGTACCCGAGGAACTGACTATAATTCTGAAGCGAATCATCGAGATT
ATTGTTTAGTCTGACGTTTGTATCAACGTTATAACAACAGGAGTTTTGGCGTT
AGCGGCCCTTTTAGCCACCTTTTATACGTGCAAGGTGAGAAA

Male Brain OR31

CCTATAACCTTTGACATGTAGCATATCGCATTGGCAGCTATATAACAGTTGC
GGGGTTGTTGTGCTGATGGTTTCTTCTTAGGATTCTGTTTTTACTTTGCCACA
CTTTTTAAAAGCTGAACAGGATTTGAGTGACGC

Female Brain OR11

GATGTTCATTGAGAAGGTCATATCCTACATATTGGGTCAAATTCTGGTGTTG
TGCTTAGCATTTATCGTTTGCAAGCCATAAGTTTAGAGACACCATTTGATTTT
TTGTCTGTATTTCAAATATGTGGGTTATGGCCGCGCAAT

Female Brain OR12

TTACATGACCAGCGAAAAGCTTTCATTAGCTATTTATAGAGCTGGCTGGGTC
GACTGGAATGAGAAAAACGAAAACTCGCCCTCAAATGATGGGTCGTTCGAC
TTACCTATTCGCATTAAAACTATAAACAGATGTTACTCTTTCAATTTGGCGCT
TTTACATCC

Female Brain OR13

ATGATTGACTTATTGACGCAAAGGTTTTGTTTGCGTGTAATGGGGCATAATT
TTGTACGTGATAGATCAAACAAGCGGCGGTGGCACAATACAAGATGTACAT
ACAAATACATACTATGTCTGTTCTGGCGTTCGGCCCAAGTGCCAATGATAAA
TTACACAATTTATTAT

Female Brain OR28

TGGCCGGCAACAGCATGCTCGATATAACTTATCTTTTTATATCGTCCAATCTA
TTGATAATGTATTACTTGTCTGTGCATATGGCACGGAAATGATTAGATTGGT
AAATGGAAATGAAATTGAAATGAA

Male Gut OR1

AGTGGATTATCCGCCTCATTGGAACATTTCGTCCCGAGAGCTATTTCGTTCAT
TGTCAGCACATTCCAAGGCCGAATAGAAGCTCTATTTCGTTCATTGTCAGCA

CATTCCAAGGCGAATAACAGGTGTTACTTTGTCGGCACCCTCCCCGGCCGAA
TACATGAATTAGTCCTTCGGTAT

Male Gut OR7

TTCATCTATAATTTATAACAATATTTGTGCAATTTACAATAACTGCCACCATA
TTGGGTACGACTCTTATTAATATCCTACTGTTTGCGACCAACTTCGCTTCTAT
AGATCATGCTTTTACGTATTGGCTGTTCGTGGAAATCTT

Male Gut OR12

GATGTCGCGTCATTGGAGAAGGTCATATCCTATCCCATATTGGGTCAAATTT
TGTTTAGCTCTATGGTGTTGTGCTTTAGCATTTACGTTTGCAAGCCATAAGTT
TCATAGAGACACCATTTGAGTGTATTTCAATATATGTGGG

Male Gut OR32

ATTTTCAAATCGTTTGGAGCACTTTCAATCCGATCTTATTAGTAAATTTTCTA
ATATCGTCGGTATTAATTTGCATGGTGGGTCAATTGGTTACTGGTCAGGAAA
TGTTTATTGGCGATTATGTCAAATTC

Male Gut OR33

TGTTACCTTTGGCAGCATTATACCCGGAATCAATGAGTACAGTTCGCCGTTTT
ATGAGCTTTGGTATTTGTGATGTGCATCACACCGATAGGATGCTGTATGTAC
ATACCCTATACTAGTCTCATAGAGTATTTCGTTTATACT

Male Gut OR34

CGACTGATTATTTTGGTAATTGCTACAATCTCGACGACTTATTATTTTAAATT
TTTTTTCCAGAGTAAATATCGGCAATTCTGCTTATTCATCGTTGGTACATAGC
TTCATCTTCTTACAAGAAATCGATTTTGAT

Male Gut OR42

TTCATGGAATACATTGAAGAACTTTTTTCTGGCATTATTTTTGTACACATCAC
TACAAGCTGTGTATCCATTTGCTCTCTCTATTTTGTATAGTACTTAAGGTCTG
GCCGTTGCGCCACTTTTGTTGATCTCGAACCTTCAG

Male Gut OR44

ATTTATAGCAGGTGTCGATAAAATTTATCGAACGATTATATTCATTGAAATA
ACAACCTGCGGTCTTAGCATTTGCTGTACAATACGATTGTCTTAGACGTATG

GCCAGCAGCGTACGGTTACATAATTTACCTAATCTTTTGCTTATATTCCTGCA
TTATGGGTACATTGATAGAAATATCG

Male Gut OR46

ATTTATAGCAGGTGTCGATAAAATTTATCGAACGATTATATTCATTGAAATA
ACAACCTGCGGTCTTAGCATTTGCTGTACAATACGATTGTCTTAGACGTATG
GCCAGCAGCGTACGGTTACATAATTTACCTAATCTTTTGCTTATATTCCT

Female Gut OR33

TGTTACCTTTGGCAGCATTATACCCGGAATCAATGAGTACAGTTCGCCGTTTT
ATGAGCTTTGGTATTTGTGATGTGCATCACACCGATAGGATGCTGTATGTAC
ATACCCTATACTAGTCTCATAGAGTATTTCATACT

Female Gut OR43

AACGATGACGTCACCAGAATGATTTATGTGAAGGTTTGCATCTGGTACAGAC
TGGAAGTGAAGGAACAGAAAATGATTCTTCTCATGCATGTGCGTAAATCGC
AATATGCCGTCGAACTAACTGTTGGTAAAATAATGCCTTTGAACT

Female reproductive OR1

CTCATGCAAATTTATGCGATGTATTATTCTGTCATGGTAATATCGCTTGTGAG
CAATTGCAACATTTAAAGAGTATTATGAAACCATTAATGGAATTATCCGCCT
CATTGGATACATTTCGTCCCGATTCCAGCTCTATCGTTCATTGTCAGCACATT
CCAAGGCCG

Female reproductive OR9

CTTTATAATCTATTTAGCCCAGTTATCTCTGCACCATGTTTGTGCAGTTTTTG
ATCATCGGTATTATTTTGGGTGTTACAACAATCCAGTTTTGTTTGCAAATTTT
TTTGCGATTGTAGCATCCTTGATGTGGCTTCTATTTTA

Female reproductive OR10

CTCAACTAAAGAATAAACATTTCACTACCGTCAATGGTGCGAGATAGTTGAA
AGTATACAACGTCTCCGTCCTAATAATGAGCGCGAAATTCAATTGTTTCAAC
AGTCCGAGAGATTAGCACGTTTAATACGTAACGCTTACTGCGTTCTTTCGCT

Female reproductive OR11

TGTTACCTTTGGCAGCATTATACCGGAATCAATGAGTACAGTTCGCCGTTTT
ATGAGCTTTGGTATAATTTGAGATGTGCATACACCGATAGGATGCTGTATGT
ACATACCCTATACTAGTCTCAAGTTTCGTTTATACTATTGGTGTTGTGATGAG
CAAAACCTTACAACATCGTTTACGTATCTACA

Female reproductive OR15

ATGCTTTTGCATACTCTACAACTTAAACGTTATGGGTAACGCGACGTTCATTC
AATCTTATTGGTATCGATATAACTGCTCTCGATCATGATATTGTAAAATATCC
GATGCGTTGCTTCCTGTTTACTGCATTCGCGGCCGTGTGCTTGGGCT

Female reproductive OR22

CGACTGATTATTTTGGTAATTGCTACAATCTCTTGACGACTTATTATTTTAAA
TTTTTTTTCCAGAGTAAAAATATCGGCAATTGTACTTCTGCTTATAATCATCG
TTGGTACATAGCTTCATCTTCTTACAAGAAATCGGTCAA

Female reproductive OR25

GGCGGGCGGCGCTTGGGGATAAACTGGTGCTTGGATTGCCATCATTATTGCC
GCAGCTGATTTATATTTGCTTGGTTGCGTTTTGCAGTTGTGTTTGCATTTCGA
ACTATTGACCAAGCAGTTAATGCAATTAGATACAAGGATATTGACCGAATCG
GA

Female reproductive OR27

CATGATTGATGAAGTGAACGATGTTTTGCTTTTCAGTCTTATTGAGTTTTTTC
GGCTTTGGTGGATTATTATGTTTAGTAGCTTTCATGCAGTGGTCGGCAGCAG
CATGCTCGATATTTTTAGTCAAAC

Male reproductive OR1

CATGTCTGTTCGCTCTATCTCATGCAAATTTATGCACGTATTATTCTGTTCAT
GGNTTAATATTCGCTTGTGAGCAATTGCAACNATTTAAAGAGTATTATGAAA
CCATTAATGGAATTATCCGCCTCATTGGATATATGCATACATTTCGTCCCGAT
TCTGGAGCTCTATTTCG

Male reproductive OR42

ATGCCACCTCTAGATCGCTTCCGCTTATCAAGCATTTACGCGTTGTGGTCCGT
TTCTGTGCTTGCGATATGTTCGACGACTTATACTATGTTTAATCCATTCTTTA
TGGTTTTACTACTGTGCTTAGCGATATACCGTATGCTTTATAAATACCA

Male reproductive OR44

ATTTATAGCAGGTGTCGATAAAATTTATCGAACGATTATATTCATTGAAATA
ACAACCTGCGGTCTTAGCATTGCTGTACAATATTCGATTGTCTTAGACGTAT
GGCCAGCAGCGTACGGTTACATAATTACCTAATCTTGCTTATATCTTTCTGCA
TTATGGGTACATTGATAGAAATATCG

Male reproductive OR37

ACCGATGAAGTCAGCACCATGTATTACAATCGAATTGGGAGTCTGTTATAGC
ACGTTCCTCAGACTCCTAAAATGTACGACTAATGACTGTTAACTATGGCAAT
CGCTTTGAATCGGAAACTCTATTACTGGCTTAAATTTCTTTACAGTTTCTCTA
AATACGGTAATTAAG

Male reproductive OR45

ATTCATTGAGACCGCTAGTTCCATTTACTTCTGGGTTATATTGGTGCAAATAA
GTACCGCTGTCACAGGTATTGTGTTTGGTATGTCAGTTCTTGGGCATTTATCC
TACTGCTGTGGGTTACTTAATTTATTGCGCT

Male reproductive OR46

ATGATTGTGGCTTGTGTTGACCATTTTCGTAAATTGGTTCGCATTTTTCGGTC
AGGTATATGCGGCGCCGATTTTGGGGACAGAATAACCTGTCCTTGGTTGTGA
CTTTATCATCAATAGTGTTTCTATGCACCGTTTACACCATATATGCCGT

Appendix V: *G. m. morsitans* homologs in other Tsetse species

G. m. morsitans			*G. austeni*			*G. brevipalpis*		
Gene Name	transmembrane proteins	Accession number	Accession number	e-value	identity (%)	Accession number	e-value	identity
OR1	5	GMOY005610	GAUT034813-PA	0	98.80%	GBRI045585-PA	6.00E-166	92%
OR5	5	GMOY012018-PA	GAUT028888-PA	0	73.70%	GBRI036342-PA	4.00E-156	75.20%
OR7	6	GMOY013231-PA	GAUT052946-PA	0	89.2	GBRI044639-PA	0	69.2
OR8	4	TMP_Or8-RA	GAUT052947-PA	0	92.90%	GBRI044639-PA	2.00E-97	40.4
OR9	6	GMOY012276-PA	GAUT022268-PA	0	96.2	GBRI034666-PA	1.00E-173	85
OR10	6	TMP_Or10	GAUT011101-PA	0	93.40%	GBRI028428-PA	6.00E-94	41.40%
OR11	6	GMOY0110761	GAUT011101-PA	4.00e-67	36.30%	GBRI028428-PA	0	72.40%
OR12	3	GMOY009271-PA	GAUT005363-PA	5.00e-59	94.6	GBRI012762-PA	0	76.8
OR13	6	GMOY003312	GAUT003281-PA	0	83.5	GBRI018811-PA	2.00E-116	66.80%
OR15	7	TMP_Or15-RA	GAUT022034-PA	0	86.5	GBRI026647-PA	6.00E-179	82.30%
OR17	8	GMOY005386-PA	GAUT006649-PA	0	93.1	GBRI030235-PA	0	76.2
OR19	7	GMOY012322-PA	GAUT003629-PA	2.00e-49	82.8	GBRI031534-PA	4.00E-135	96.9
OR22	3	GMOY012295-PA	GAUT005460-PA	2e-04	37.9	GBRI027004-PA	0.000005	26.3
OR25	5	GMOY012357-PA	GAUT042360-PA	0	93.3	GBRI011904-PA	1.00E-168	63.2
OR27	6	GMOY008038-PA	GAUT032244-PA	4.00e-178	71.78	GBRI026158-PA	0	73
OR28	3	GMOY012199-PA	GAUT032244-PA	5e-46	53.7	GBRI026158-PA	1.00E-39	46.3
OR30	6	GMOY012282-PA	GAUT038273-PA	8.00e-139	67.34	GBRI002179-PA	2.00E-59	35.5
OR31	7	GMOY012239-PA	GAUT004311-PA	0	76.1	GBRI036522-PA	3.00E-156	72.7
OR32	7	GMOY005084-PA	GAUT014395-PA	0	88.8	GBRI045111-PA	6.00E-175	86.2
OR33	7	GMOY013237-PA	GAUT005608-PA	6.00e-136	97	GBRI015995-PA	0	88.7
OR34	2	GMOY012295-PA	GAUT000836-PA	3.00e-05	29.2	GBRI027004-PA	3.00E-07	25.5

OR35	7	GMOY012253-PA	GAUT021583-PA	0	79.4	GBRI002464-PA	1.00E-179	67.8
OR37	4	TMP_Or37-RA	GAUT035779-PA	0	90.7	GBRI045694-PA	0	76
OR42	6	GMOY006479-PA	GAUT021320-PA	0	87.3	GBRI017432-PA	4.00E-91	67.9
OR43	6	TMP_Or43-RA	GAUT021320-PA	0	79.8	GBRI017432-PA	3e-76	64.4
OR44	6	GMOY006265-PA	GAUT021320-PA	6.00e-68	35.3	GBRI037236-PA	8.00E-82	78.7
OR46	5	GMOY003305-PA	GAUT021320-PA	2.00e-57	34.2	GBRI040021-PA	1.00E-117	52.9

G. m. morsitans			*G.pallidipes*			*G. fuscipes*		
Gene Name	Transmembrane helices	Accession number	Accession number	e-value	identity	Accession number	e-value	identity
OR1	5	GMOY005610	GPAI035133-PB	2.00E-134	99.4	GFUI035140-PB	0	97.6
OR5	5	GMOY012018-PA	GPAI034198-PA	1.00E-92	90.8	GFUI007794-PA	5.00E-152	88.1
OR7	6	GMOY013231-PA	GPAI049191-PA	0	93.4	GFUI003499-PA	0	87.3
OR8	4	TMP_Or8-RA	GPAI049191-PA	2.00E-99	41	GFUI003499-PA	4.00E-101	41.8
OR9	6	GMOY012276-PA	GPAI029610-PA	0	98	GFUI028213-PA	0	94.6
OR10	6	TMP_Or10	GPAI009882-PA	0	89.8	GFUI037305-PA	0	86.7
OR11	6	GMOY0110761	GPAI009200-PA	2.00E-177	98.1	GFUI034469-PA	4.00E-179	94
OR12	3	GMOY009271-PA	GPAI004010-PA	1.00E-18	21.7	GFUI012941-PA	2.00E-80	92.6
OR13	6	GMOY003312	GPAI024118-PA	1.00E-70	95.4	GFUI053522-PA	8.00E-108	89
OR15	7	TMP_Or15-RA	GPAI026906-PA	0	87.7	GFUI032116-PA	2.00E-100	78.2
OR17	8	GMOY005386-PA	GPAI002024-PA	0	93.3	GFUI049134-PA	1.00E-60	88.6
OR19	7	GMOY012322-PA	GPAI017649-PA	0	98.8	GFUI027050-PA	1.00E-86	96.2
OR22	3	GMOY012295-PA	GPAI032515-PA	1.00E-77	76.1	GFUI016705-PA	1.00E-02	23.1
OR25	5	GMOY012357-PA	GPAI045426-PA	0	97.1	GFUI038147-PA	0	93.5
OR27	6	GMOY008038-PA	GPAI033169-PA	3.00E-180	93.8	GFUI022126-PA	7.00E-110	79.1
OR28	3	GMOY012199-PA	GPAI033171-PA	3.00E-22	55.2	GFUI022126-PA	8.00E-13	62
OR30	6	GMOY012282-PA	GPAI041241-PA	1.00E-157	69.6	GFUI051694-PA	2.00E-139	64.6
OR31	7	GMOY012239-PA	GPAI015219-PA	0	85.9	GFUI032492-PA	4.00E-112	96.3
OR32	7	GMOY005084-PA	GPAI034871-PA	0	96	GFUI014938-PA	4.00E-85	90.6
OR33	7	GMOY013237-PA	GPAI004557-PA	1.00E-120	68	GFUI009255-PA	1.00E-37	95.7
OR34	2	GMOY012295-PA	GPAI032515-PA	6.00E-145	61.3	GFUI045476-PA	3.00E-04	31.5

OR35	7	GMOY012253-PA	GPAI039623-PA	1.00E-72	94.8	GFUI003104-PA	5.00E-72	93.7
OR37	4	TMP_Or37-RA	GPPI006826-PA	0	86.5	GFUI022472-PA	0	87.2
OR42	6	GMOY006479-PA	GPAI012945-PA	3.00E-85	83	GFUI007388-PA	6.00E-135	84.8
OR43	6	TMP_Or43-RA	GPAI012945-PA	8E-93	84.8	GFUI007388-PA	9e-120	75.4
OR44	6	GMOY006265-PA	GPAI012945-PA	5.00E-33	44.2	GFUI043789-PA	4.00E-163	91.6
OR46	5	GMOY003305-PA	GPAI042230-PA	1.00E-56	91.4	GFUI022534-PA	6.00E-104	53.9

G. m. morsitans	Transmembrane helices		G. palpalis		
Gene Name		Accession number	Accession number	e-value	identity
OR1	5	GMOY005610	GPPI049421-PA	0	98
OR5	5	GMOY012018-PA	GPPI013405-PA	2.00E-165	81.6
OR7	6	GMOY013231-PA	GPPI052405-PA	0	87.3
OR8	4	TMP_Or8-RA	GPPI052404-PA	0	90.4
OR9	6	GMOY012276-PA	GPPI041284-PA	0	93.5
OR10	6	TMP_Or10	GPPI003043-PA	0	86.2
OR11	6	GMOY0110761	GPPI027250-PA	0	88
OR12	3	GMOY009271-PA	GPPI034884-PA	0	87.6
OR13	6	GMOY003312	GPPI019134-PA	0	83.1
OR15	7	TMP_Or15-RA	GPPI050189-PA	0	85.2
OR17	8	GMOY005386-PA	GPPI049642-PA	0	89.6
OR19	7	GMOY012322-PA	GPPI043178-PA	0	95.3
OR22	3	GMOY012295-PA	GPPI039956-PA	0.0001	37.9
OR25	5	GMOY012357-PA	GPPI039956-PA	5.00E-22	24.1
OR27	6	GMOY008038-PA	GPPI004969-PA	0	74.8
OR28	3	GMOY012199-PA	GPPI004969-PA	4.00E-41	45.3
OR30	6	GMOY012282-PA	GPPI026824-PA	9.00E-136	65.2
OR31	7	GMOY012239-PA	GPPI009646-PA	0	85.9
OR32	7	GMOY005084-PA	GPPI020103-PA	0	91.4
OR33	7	GMOY013237-PA	GPPI029110-PA	2.00E-118	92.5
OR34	2	GMOY012295-PA	GPPI039956-PA	1.00E-06	34.2
OR35	7	GMOY012253-PA	GPPI048802-PA	2.00E-176	97.6

OR37	4	TMP_Or37-RA	GPPI006826-PA	0.00E+00	86.5
OR42	6	GMOY006479-PA	GPPI008074-PA	0	81.2
OR43	6	TMP_Or43-RA	GPPI008074-PA	0	76.4
OR44	6	GMOY006265-PA	GPPI011878-PA	0	91.3
OR46	5	GMOY003305-PA	GPPI011878-PA	2.00E-84	37.6

Appendix VI: OR gene expression in non-olfactory tissues in *D. melanogaster*

***G. m. morsitans* gene**	***D. melanogaster* gene**	**Sequence identity and e-value**	**Accession number**	**Expression in male tissues**	**Expression in female tissues**	**Gene Ontology**		
						Molecular function	**Biological process**	**Cellular component**
GmmOR1	Dmel\Orco	77.5%, 0.0	FBgn0037324	Head, eye, brain, testis, crop	Head, eye, brain, Thoracic abdominal ganglion, crop	calcium-release channel activity, calmodulin binding, odorant binding, olfactory receptor, protein dimerization	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR5	Dmel\Or33b	38.4%, 1.2e-49	FBgn0026391	Head	Head	Odorant receptor activity, odorant binding and other	signal transduction, transport/localization and	plasma membrane, membrane and integral

						biological function	response to stimuli	part of membrane
GmmOR6	Dmel\Or7a	37.9%1e-65	FBgn0030016	Head*, eye, brain, thoracic-abdominal ganglion , crop, salivary gland, testis	Head, eye, brain, thoracic-abdominal ganglion , crop	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR7	DmelOR42b	32.3%, 1.13e-56	FBgn0033043	Head, eye, brain and accessory glands	Head, eye, and brain	Odorant receptor activity, odorant binding and other biological function	signal transduction, nervous system process and response to stimuli	plasma membrane, membrane and integral part of membrane

GmmOR8	Dmel\Or7a	34.5%8.1e-66	FBgn0030016	Head, eye, Malpighian tubules, salivary gland and accessory gland.	Head, eye, crop, midgut, hindgut, fat body, salivary gland and carcass	Odorant receptor activity, odorant binding and other biological function	signal transduction, nervous system process and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR9	DmelOR42a	39.7%, 5e-91	FBgn0033041	Head, eye and midgut	Head and eye	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane

GmmOR10	Dmel\Or46a	33.8%, 4e-56	FBgn0026388	Head, eye, fat body, thoracic-abdominal ganglion , crop, salivary gland, testis	Head , eye, Malpighian tubules, fat body, and salivary gland	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR11	Dmel\Or46a	31%, 1.3e-44	FBgn0026388	Head, eye, fat body, thoracic-abdominal ganglion , crop, salivary gland, testis	Head , eye, Malpighian tubules, fat body, and salivary gland	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR12	DmelOR94a	39%, 8.2e-69	FBgn0039033	Hindgut (FPKM =10)**. Head, eye, crop midgut, Malpighian tubule, fat body	Hindgut (FPKM=10)**. Head, eye, Malpighian tubule, fat body and salivary	Odorant receptor activity, odorant binding and other	signal transduction, transport/localization and	plasma membrane, membrane and integral

				and salivary glands	glands	biological function	response to stimuli	part of membrane
GmmOR13	Dmel\Or82a	45.2%, 2.3e-76	FBgn0041621	Head, eye, brain, thoracic abdominal ganglion, fat body, accessory gland, carcass	Head, eye, brain, thoracic abdominal ganglion, fat body, salivary, gland, virgin spermatheca, mated spermatheca and carcass	Odorant receptor function, and other biological functions	Reproduction, response to stimuli, nervous system process	Extracellular region, cell membrane, cell periphery, and cell junction
GmmOR15	Dmel\Or45a	25.3%, 9.4e-33	FBgn0033404	Head, eye, brain, thoracic abdominal ganglion, crop, hindgut, salivary gland	Head, eye, brain, thoracic abdominal ganglion, crop, hindgut and fat body	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR17	Dmel\OR85d	33.6%, 2.4e-17	FBgn0037594	Head and testis	Head.	Odorant receptor	signal transductio	plasma membra

						activity, odorant binding and other biological function	n, transport/localization and response to stimuli	ne, membrane and integral part of membrane
GmmOR19	Dmel\OR67b	28.1%, 5.6e-49	FBgn0036019	Head , eye, salivary glands, midgut and testis	None	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR22	Dmel\OR49a	36.6%(5.9e-24	FBgn0033727	None	Head	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membra

								ne
GmmOR25	Dmel\OR92a	28.4%, 1.3e-27	FBgn0038798	Head, eye	Head eye and Malpighian tubules.	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR27	Dmel\OR67c	28.3%, 6.3e-25	FBgn0036078	Head, eye brain testis	Head and brain	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane

GmmOR28	Dmel\OR85f	26.2%, 0.0	FBgn0037685	Head, eye, brain , crop, midgut, hindgut, Malpighian tubule, salivary gland, testis, carcass and rectal pad	Head, eye, brain, crop, midgut, hindgut , Malpighian tubule, fat body, salivary gland, ovary, carcass, rectal pad	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR31	Dmel\OR24a	44.1%, 45e-10	FBgn0026394	None	ovary	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR32	Dmel\OR13a	57.9%, 1.4e-143	FBgn0030715	Head, eye, brain, salivary gland, testis	None	Odorant receptor activity, odorant binding	signal transduction, transport/localization	plasma membrane, membrane and

						and other biological function	and response to stimuli	integral part of membrane
GmmOR33	Dmel\OR49b	(54.9%, 6.9e-45	FBgn0028963	Head, brain, thoracic abdominal ganglion and midgut	Head, brain, thoracic abdominal ganglion and midgut	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR34	Dmel\OR49a	23%, 3.9e-96	FBgn0033727	None	Head	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR35	DmelOR43a	47.5%., 3e-96	FBgn0026389	Head	head	Odorant receptor	signal transductio	plasma membra

						activity, odorant binding and other biological function	n, transport/localization and response to stimuli	ne, membrane and integral part of membrane
GmmOR37	Dmel\Or74a	39%, 7.1e-93	FBgn0036709	Testis	Head and thoracic abdominal ganglion	Odorant receptor activity, odorant binding and other biological function	signal transduction, transport/localization and response to stimuli	plasma membrane, membrane and integral part of membrane
GmmOR42,	DmelOR67d	36.6%, 3e-68	FBgn0036080	Head, eye, brain, crop, carcass	Head, eye, brain , thoracic abdominal ganglion	Odorant receptor function, and other biological functions	Reproduction, response to stimuli, nervous system process and	Cell membrane, cell periphery, and cell junction

							behavior	
GmmOR43	DmelOR67d	36.6%, 3e-68	FBgn0036080	Head, eye, brain, crop, carcass	Head, eye, brain , thoracic abdominal ganglion	Odorant receptor function, and other biological functions	Reproduction, response to stimuli, nervous system process and behavior	Cell membrane, cell periphery, and cell junction
GmmOR44	DmelOR67d	36.2%, 2e-74	FBgn0036080	Head, eye, brain, crop, carcass	Head, eye, brain , thoracic abdominal ganglion	Odorant receptor function, and other biological functions	Reproduction, response to stimuli, nervous system process and behavior	Cell membrane, cell periphery, and cell junction
GmmOR45	DmelOR67d	38.5%, 9.3e-42	FBgn0036080	Head, eye, brain, crop, carcass	Head, eye, brain , thoracic abdominal ganglion	Odorant receptor function, and other biological	Reproduction, response to stimuli, nervous	Cell membrane, cell periphery, and

						functions	system process and behavior	cell junction
GmmOR46	DmelOR67d	37%, 5e-67	FBgn0036080	Head, eye, brain, crop, carcass	Head, eye, brain , thoracic abdominal ganglion	Odorant receptor function, and other biological functions	Reproduction, response to stimuli, nervous system process and behavior	Cell membrane, cell periphery, and cell junction

Appendix VII: Relative expression values for male and female OR genes

Gene name	Fold Expression x $10^{\wedge -3}$ male brain	p-value x $10^{\wedge -3}$ male brain	Fold Expression x $10^{\wedge -3}$ male gut	p-value x $10^{\wedge -3}$ male gut	Fold expression x $10^{\wedge -3}$ male reproductive tissue	p-value x $10^{\wedge -3}$ male reproductive tissue
OR1	23.63	1.79	1.99	0.01	2.13	1.031
OR2	0	0	5.55	3.917	0	0
OR3	0	0	26.16†	4.594	6.86	1.053
OR4	9.87	0.61	22.25†	0.006	0	0
OR5	11.65	0	0	0	14.41	1.509
OR6	22.82	0	0	0	4.99	0.464
OR7	30.12†	2.45	46.50†	1.382	21.64†	0
OR8	23.52	0.02	18.75	5.014	11.95	1.502
OR9	8.55	0	0	0	22.56	2.995
OR10	0	0	0	0	0	0
OR11	31.11	0	0	0	4.08	0.522
OR12	35.32†	0.003	32.96†	0.345	7.29	1.411
OR13	58.86†	2.49	0	0	8.13	0.511
OR14	14.95	0.01	0	0	3.61	0.733
OR15	58.72†	0.001	0	0	10.77	0.739
OR16	30.12†	0.68	0	0	15.41	1.158
OR17	0	0	0	0	6.48	0
OR18	0	0	15.59	2.945	0	0

OR19	80.03†	4.25	0	0	0	0
OR20	0.228	0.001	0	0	0.016	0.236
OR21	0	0	0	0	0	0
OR22	40.95†	0.85	0	0	9.78	2.034
OR23	0.68	0	0	0	0.034	0.001
OR24	0	0	0	0	0	0
OR25	0	0	0	0	0.047	0.083
OR26	0	0	0	0	0	0
OR27	0.75	0.002	5.19	1.354	75.02†	0.075
OR28	148.65†	4.75	8.35	4.805	5.39	0.301
OR29	6.33	0.011	4.21	8.675	3.18	0.311
OR30	173.14†	0.19	20.91	4.054	24.07†	7.185
OR31	116.09†	0.05	2.59	0.013	0	0
OR32	0	0	27.08†	0.257	108.07†	6.133
OR33	19.02	0.012	0.034	0.001	49.95†	1.192
OR34	2.05	0	27.84†	0.44	0.05	0.147
OR35	0.53	0	0.071	2.739	0.06	0.601
OR36	1.199	0.022	0	0	7.19	1.526
OR37	1.63	0.005	0.017	2.739	0.03	0.079
OR38	0.49	0	0.061	0.276	0.81	0
OR39	0	0	0	0	0	0
OR40	0	0	0.058	0	0	0
OR41	0	0	0	0	64.70†	2.067
OR42	0.07	2.57	33.11†	0.276	10.6	0.011

OR43	2.32	0.27	0.14	0.3	8.81	0.796
OR44	0	0	25.98†	5.618	102.95†	2.278
OR45	3.19	0.005	0.17	0.492	75.31†	1.82
OR46	12.66	0.048	0.37	0.702	160.06†	8.689

Gene name	**Fold Expression x 10^-3 Female brain**	**p-value x 10^-3 Female brain**	**Fold Expression x 10^-3 female gut**	**p-value x 10^-3 Female gut**	**Fold expression x 10^-3 Female reproductive tissue (rpkm)**	**p-value x 10^-3 Female reproductive tissue**
OR1	6.85	0.39	1.25†	0.267	8.79†	0.384
OR2	0	0	0	0	0	0
OR3	0	0	0	0	0	0
OR4	13.11	0.84	0.23	1.087	0	0
OR5	15.48	0.05	0.36	0.168	1.518	0.223
OR6	34.04	1.04	0.33	0.938	1.156	0.476
OR7	40.01†	3.16	0.43	2.888	2.926	0.653
OR8	31.25	0.98	0.45	0.303	3.645	0.405
OR9	11.1	0.001	0.15	0.493	27.46†	1.079
OR10	0	0	0	0	2.846	0.928
OR11	41.33†	0.03	0.69	0.209	21.59†	1.093
OR12	46.93	186.49	0	0	0	0
OR13	78.20†	4.64	0	0	0	0
OR14	0	0	0	0	0	0

OR15	0	0	0	0	7.563	1.281
OR16	40.01†	1.12	0	0	10.31†	0.787
OR17	13.05	0.001	1.92†	1.049	9.21†	0.411
OR18	0	0	0	0	16.98†	1.413
OR19	92.14†	0.1	0.93	1.318	9.25†	0.823
OR20	0.3.0	0.016	0	0	1.701	0.233
OR21	0	0	0	0	4.798	0.316
OR22	54.41†	1.46	0	0	49.15†	4.621
OR23	0.69	0.62	0.14	0	0.391	0
OR24	1.02	0.21	0	0	0	0
OR25	300.76†	17.53	0	0	0.47	0.001
OR26	7.14	2.012	0	0	0	0
OR27	230.05†	4.791	0.66	0.001	68.08†	0.006
OR28	19.87	0.08	0	0	0	0
OR29	78.02†	0.04	0	0	2.094	0.531
OR30	5.29	0.34	0	0	0	0
OR31	45.96	0	0	0	0	0
OR32	67.76†	0.002	0	0	0	0
OR33	67.29†	0.017	6.21†	0.324	0	0
OR34	2.69	0	0	0	0.677	0.211
OR35	1.07	0.451	1.84†	0.508	0.699	0.189
OR36	1.51	0.472	0	0	0	0
OR37	3.15	0.417	0.36	0.356	1.661	0.531
OR38	1.29	0.351	0	0	4.121	0.278

OR39	10.33	0	0	0	0	0
OR40	0.54	0	0	0	0	0
OR41	7.92	0.353	0.58	0.3	17.30†	1.732
OR42	6.66	1.016	0.81	0.477	10.261	0.861
OR43	2.2	0	0.95	0.228	6.496	0
OR44	6.52	0	0.85	0.597	2.221	0.431
OR45	2.25	0	1.27†	0	0.203	0.353
OR46	0.79	0.2924	1.16†	0.907	0.285	0.005

Appendix VIIII: Publication

Scientific African 10 (2020) e00591

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

Expression profile of odorant receptors in brain, gut and reproductive tissues in male and female *Glossina morsitans morsitans*

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ARTICLE INFO

Article history:
Received 1 March 2020
Revised 8 September 2020
Accepted 11 September 2020

Key words:
Glossina morsitans morsitans
Odorant receptors
Brain
Gut
Reproductive tissues

ABSTRACT

Glossina morsitans morsitans are vectors of Animal African Trypanosomiasis (nagana) and Human African Trypanosomiasis (sleeping sickness). *Glossina* uses odorant receptors (Ors) expressed within the olfactory receptor neurons to identify chemical cues within the environment, locate hosts and find larviposition sites and mating partners. Most Ors have been identified in insects' olfactory tissues but little is known about the potential functions of Ors in non-olfactory organs. In the present study, we determine the expression profile of 46 Ors in male and female *G. m. morsitans* brain, gut and reproductive tissues using quantitative polymerase chain reaction. Our results indicate that 38 Or genes were detected in the male brain and gut, 37 in reproductive tissues while the number identified in the female included 44 in brain, 22 in gut and 40 in reproductive tissue. The Or genes were abundantly distributed in both male and female brain, gut and reproductive tissues at different fold expression levels. Quantitative real time PCR revealed that 4 Or genes (*GmmOr19, GmmOr28, GmmOr30, GmmOr31*) were significantly highly expressed in the male *G. m. morsitans* brain while the gut had 6 Ors (*GmmOr7, GmmOr12, GmmOr32, GmmOr34, GmmOr42, GmmOr44*) and the testis and accessory glands had 3 Or genes (*GmmOr32, GmmOr44, GmmOr46*) significantly highly expressed. In the female *G. m. morsitans*, the Ors that were significantly expressed were 2 in the brain (*GmmOr25, GmmOr27*), 3 both in the gut (*GmmOr17, GmmOr33, GmmOr35*) and reproductive tissues (*GmmOr9, GmmOr22, GmmOr27*).

This results provides insight on the expression profile of Or genes in non-olfactory organs (brain, gut and reproductive tissues) in male and female *G. m. morsitans* and open avenues to investigate the possible role of the Or genes that were significantly expressed, which could provide targets that could be explored to control *Glossina* and the devastating trypanosomiasis disease.