SPECIES COMPOSITION AND GENETIC STRUCTURES OF *GLOSSINA* POPULATIONS IN SELECTED NIGERIAN NATIONAL PARKS

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OCTOBER, 2018

DECLARATION

I declare that the work in this thesis entitled "Species Composition and Genetic Structures of *Glossina* Populations in Selected Nigerian National Parks" has been performed by me in the Department of Zoology. The information derived from literature have been duly acknowledged in text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Shaida, Stephen Saikiu _____ _____

Signature Date

CERTIFICATION

This thesis entitled SPECIES COMPOSITION AND GENETIC STRUCTURES OF *GLOSSINA* POPULATIONS IN SELECTED NIGERIA NATIONAL PARKS by Stephen Saikiu SHAIDA meets the regulations governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literally presentation.

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ABSTRACT

A study of population genetic structure of tsetse flies (*Glossina* spp), vectors of Human and Animal trypanosomiasis, was undertaken in order to increase our understanding on the distribution and dynamics of tsetse fly populations in Nigeria. Tsetse flies were collected in selected four Nigeria National Parks/Game Reserve namely, Kainji Lake National Park, Old Oyo National Park, Cross River National Park, Yankari Game Reserve and a highly tsetse infested agropastoral area within the Federal Capital Territory Abuja at ijah Gwari, and examined using Cytochrome Oxidase C SU1 (CO 1), Internal transcribed spacer-1(ITS-1) sequences and geometric wing morphometrics. Flies were sampled using standard biconical traps, while ambient temperature and relative humidity conditions were recorded with a whirling hygrometer and co-ordinates of sampled locations and trapping points were recorded with GPS device for geo-referencing. A total of 1891 tsetse flies were collected comprising, *Glossina tachinoides* (1050, 55.53%), *Glossina palpalis palpalis* (568, 30.04%), *Glossina morsitans submorsitans* (270, 14.23%) and *Glossina fusca* (3, 0.16%). Analysed data showed significant difference (χ^2 (4) = 1375.417, $p < 0.001$) in the relative abundance of *Glossina* spp. per sampled location, whereas pairwise comparison showed no significant difference ($p = 1.000$) in the relative abundance of *Glossina* spp. between Cross River and Ijah Gwari sampled locations. Apparent densities of *Glossina* populations were significantly different (χ^2 (4) = 20.500, $p < 0.001$) per sampled location. Pairwise comparison however, showed that Old Oyo, Yankari, and Kainji locations had comparatively high apparent densities of *Glossina* spp.

Male and female fly ratios were significantly different (χ^2 (1) = 4.000, p =0.046) with higher proportion of male flies caught than females in some but not all species in all regions sampled. The composition of *Glossina* species in the sampled locations showed no significant association (χ^2 (12) = 2134.106, $p > 0.001$), as well as no significant difference (t = -1.580, p 0.133 > α = 0.05)

in tsetse catches in relation to ambient temperature and relative humidity conditions was observed in Yankari Game Reserve. Significant difference ($t = -10.633$, $p = 0.000 < \alpha = 0.05$) in tsetse catches in relation to ambient temperature and relative humidity conditions was observed in Kainji Lake National Park, ($t = -36.290$, $p = 0.000 < \alpha = 0.05$) in Old Oyo National Park, ($t = -18.939$, $p = 0.000 < \alpha = 0.05$) in Cross River National Park and ($t = -4.613$, $p = 0.01 < \alpha = 0.05$) in Ijah Gwari. No significant correlations between tsetse catches and ambient temperature and relative humidity conditions were observed in most of the trapping locations visited, suggesting that the distribution of flies across the sampled locations were probably influenced by other environmental factors than climatic conditions alone.

Tenerity phenomenon showed significant difference ($\chi^2(4) = 20.500$, $p < 0.001$) in the proportion of teneral and non teneral flies amongst the four *Glossina* species caught, with the proportion of teneral flies in each *Glossina* species being significantly higher than the non-teneral flies, except in *G. fusca* in which there was no significant difference between the number of teneral and non-teneral flies caught. Pairwise comparison showed that Yankari Game Reserve, Old Oyo and Kainji Lake National Parks had comparable tenerity of *Glossina* species.

Five *Glossina* spp. for which no information on their COI sequences is available on the Genbank, could not be identified molecularly using phylogenetic analysis. However, the study demonstrated the splitting of *G. p. palpalis* populations into two major groups, a West African Clade (WAC) and a Central Africa Clade (CAC), with the convergence zone of the two major groups (WAC and CAC) found co-existing sypatrically in the region of Cross River National Park at the Nigerian-Cameroonian border area. While the ITS-1 regions of the *G. p. palpalis* subpopulations did not show any differences, the geometric wing morphometrics showed a sex dependent shape dimorphism in 41 flies examined from Dodeo region in Cameroon.

Since the COI sequences have clearly revealed the splitting of *Glossina palpalis palpalis* populations into two major groups, the question requiring answer is whether the two groups of *G. p. palpalis* identified in this study can interbreed and if so are the vectorial capacities of the two groups of flies the same, these need to be investigated. This is even so as the isolation status of flies in the National Parks need to be investigated especially as migration of other flies into cleared areas may hinder the success of tsetse control using SIT, whereas limited migration may lead to success.

It is concluded that information generated from this study, would aid the choice of effective anti-tsetse management approaches in the country's National Parks using a component of the sterile insect technique (SIT) as being advocated by the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC).

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LIST OF ABREVIATIONS/ACRONYMS

AAT	Animal African Trypanosomiasis
ACENTDFB	African Center of Excellence for Neglected Tropical Disease and Forensic Biotechnology
AFLP	Amplified fragment length polymorphism
AHG	African Heads of Government
AMOVA	Analysis of Molecular Variance
AU	African Union
BICOT	Biological Control of Tsetse
BLAST	Basic Local Alignment Search Tool
CATT	Card Agglutination Test for Trypanosomiasis
CIRDES	Centre Internationale de Recherche Development sur l'Elevage en zone Subhumide
CO1	Cytochrome Oxidase I
COII	Cytochrome Oxidase II
CytB	Cytochrome B
DDT	Dichlorodiphenyltrichloroethane

DFG	Deutsche Forschungsgemeinschaft
DNA	Deoxy-ribonucleic acid
DRC	Democratic Republic of Congo
F/T/D	Fly per Trap per Day
FAQ	Frequently asked questions
G	*Glossina*
GPS	Global Positioning System
HAT	Human African Trypanosomiasis
IAEA	International Atomic Energy Agency
ITS-1	Internal Transcribed Spacer-1
MtDNA	Mitochondrial deoxynucleic acid
MTR	Mid Term Review
NARP	National Agricultural Research Project
NITR	Nigerian Institute for Trypanosomiasis Research
NTD	Neglected Tropical Disease
PATTEC	Pan African Tsetse and Trypanosome Eradication Campaign
PBS	Phosphate buffered Saline

PCR	Polymerase Chain Reaction
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RH	Relative Humidity
RNA	Ribonucleic acid
SIT	Sterile Insect Technique
SNP	Single nucleotide polymorphism
SNPs	Single Nucleotide Polymorphisms
SOP	Standard Operational Procedure
Spp	Species
SPSS	Statistical Parkage for the Social Sciences
SSR	Simple sequence repeat
T.	*Trypanosoma*
UV	Ultra violet
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

African Trypanosomiasis is a debilitating tropical disease complex of humans and livestock caused by protozoan parasites of the Genus *Trypanosoma*. The disease is the major cause of extensive morbidity and mortality in affected individuals and livestock across 38 endemic countries of sub-Saharan Africa (Leak, 1999). It is the major impediment to profitable agriculture and livestock development in the poverty stricken sub-region (Swallow, 2000).

Animal African trypanosomiasis (AAT) otherwise called "nagana" in cattle, is a wasting disease responsible for the death of about three million cattle annually and other breeds of livestock in sub-Saharan Africa (Abenga *et al*., 2002; Mulumba, 2003). Losses due to AAT alone are estimated at approximately 4.5 billion United States dollars (Hursey, 2001). Large areas of fertile land have been abandoned, and therefore not utilized for agricultural purposes. Animal African trypanosomiasis (AAT) impacts negatively on the socio-economic and political wellbeing of teeming human populations in affected countries (Nash, 1958). The resultant effects of these on large scale is food insecurity, low protein intake, low manure for local organic food production, low income from sale of farm produce, lack of employment and national insecurity (Swallow, 1999).

In Nigeria, losses due to AAT are estimated at about 837.20 million Naira in six states of the Federation (NARP/NITR Mid Term Review, 1998), largely because tsetse and trypanosomiasis have altered the economic health and social status of inhabitants of affected areas in the country. Nigeria, despite its cattle population of over 16 million head (FAO 2004), is the largest importer of meat and dairy products in West Africa (Bonnet *et al*., 2015).

Human African trypanosomiasis (HAT) otherwise called "Sleeping sickness" occurs in an area approximately 1.55 million Km^2 with over 70 million people mainly living in rural areas at risk of the disease (Aksoy *et al.,* 2017). The World Health Organisation (WHO) estimates the disease burden at 2.05 million disability adjusted life years (WHO, 2006). Epidemics of sleeping sickness which ravaged sub-Saharan African countries affected by the disease in the twentieth century, caused massive human displacement and depopulation (Steverding, 2008, Simarro *et al.,* 2012). Though the situation was brought under control, in the 1960s, it resurged again in the 1980s (Curtain, 2015), due mainly to economic decline, civil disturbances, war, population movements and refugees (Smith, 1998). The gradual encroachment of people into tsetse infested areas as a result of war, civil disturbances may be responsible for the resurgence of sleeping sickness in areas where the disease had previously been eliminated as observed in the Republic of Congo, Southern Sudan and Rwanda (Lyons, 1992; Arbyn *et al.*, 1995; More and Richer, 2001; Stanghellini and Josenado, 2001; Aksoy *et al.,* 2003; Büscher *et al.,* 2017). However the number of new cases of HAT in 2009 declined to below 10,000 for the first time in 50 years as against 30,000 reported in 1999 and 2804 reported in 2015 (WHO, 2018) due mainly to the improvement of surveillance and treatment activities (Simarro *et al*., 2011). Even though, with concerted efforts through active surveillance and treatment of infected individuals, the epidemic situation has been brought under control, sleeping sickness still remains endemic in many countries in sub-Saharan Africa, including Nigeria (Franco *et al.,* 2014).

In Nigeria, the disease which was previously regarded as a northern problem and conquered in the 1960s is re-emerging, with a shift to the south of the country (Edeghere *et al*., 1989; Anere *et al.,* 2006; Curtain, 2008; Adamu *et al.,* 2011). Reports of active surveillance activities have shown that the disease is on the rise even though the WHO has grouped the country amongst those with

low sleeping sickness cases (Onyebiguwa *et al.,* 2010; Franco *et al.,* 2014; Karshima *et al.,* 2016; WHO, 2017). In February, 2010, out of 470 subjects screened in Delta State Nigeria, 4 (0.85%) were parasitologically positive and 44 (9.34%) were serologically positive for trypanosomes (NITR unpublished report). In 2011, out of two reported cases (North and South), one was confirmed CATT positive and died (NITR unpublished report).

Trypanosomes are the causative agents of trypanosomiasis. They are single celled protozoan parasites belonging to the genus *Trypanosoma*. They are transmitted from host to host via the bite of infected blood-sucking insect, tsetse fly (*Glossina* species). The most economically important trypanosome parasites transmitted in livestock include *Trypanosoma vivax, T. congolense, T. brucei, T. simiae,* and *T. evansi*. Two sub-species of *T. brucei* that are responsible for the disease in humans include *T. brucei gambiense,* which causes the chronic form of the disease in west and central Africa, and *T. brucei rhodesiense* that causes the acute form in east and southern Africa where it is a zoonosis. The *T. brucei* forms that cause the disease in humans and animals are morphologically identical except that the animal parasites are susceptible to a trypanocidal factor due to the presence of a high density lipoprotein associated with the human blood (Rickman and Robson, 1970; Jenni and Brun, 1982; Hager and Hajduk, 1997). The trypanosome parasites that cause trypanosomiasis in both humans and animals are transmitted cyclically by the bites of infected tsetse flies (*Glossina* species) during blood meals. Other biting flies including tabanus, stomoxys and haematopota have been incriminated in the non-cyclic transmission of the disease in animals (Bitome-Essono, 2015).

Tsetse flies (*Glossina* species) are obligate haematophagous insects of human and animal hosts occupying about 10 million Km^2 (78 %) of the total land mass of the region of sub-Saharan Africa traversing the continent between latitude 14^o North and 20^o South (Swallow *et al.,* 1998). There

are 34 extant species and subspecies of tsetse flies distributed across 38 sub- Saharan African countries (Davies, 1977). Within their confines, tsetse flies are not uniformly distributed, but are distributed discontinuously due to habitat fragmentation (Krafsur, 2009). Added to that is the influence of environmental factors such as temperature, relative humidity, elevation, rainfall and vegetation cover on the relative abundance of the fly populations (Rogers, 1977; Brightwell *et al.,* 1992; Robinson, *et al.,* 1997a; 1997b). The above listed environmental factors in addition to host availability (Rogers and Boreham, 1973; Turner, 1981), cause uneven distribution of tsetse flies between vegetation types. Environmental changes (i.e. land use due to increasing human population and deforestation) have been implicated in the changing pattern of the epidemiology of trypanosomiasis (Van den Bossche *et al.,* 2001; 2010). Tsetse flies exist in geographically isolated units or subpopulations in which flies are adapted to and may be responsible for the observed intraspecific geographical variation in behavior and host preferences (Torr *et al.,* 1988; Colvin and Gibson, 1992).

Out of the 34 extant species and subspecies of tsetse flies so far described, 11 occur in Nigeria covering an area approximately 75-80% of Nigeria's 928,300 square kilometers total land mass. They are distributed according to habitat preferences between Mangroove swamp vegetation from the coast, through rainforest, derived savanna to Northern guinea /Sahel savannah, between latitude 12-13^{o}north with extensions along Hadejia-Jama'are hydrographic networks (Davies, 1977; Onyiah *et al*., 1983). Exceptions include areas above 1,200 meters above sea level (Dede *et al*., 2005). However, it has recently been observed that tsetse infestations have extended into areas in which they were previously not recorded probably due to the phenomenon of global warming (Jordan, 1989; Kalu, 1996; Aksoy *et al.,* 2003; Dede *et al.,* 2005; Ogedegbe and Rotimi, 2006; Tongue *et al.,* 2015)

All the 11 species and subspecies of tsetse in Nigeria are capable of transmitting animal trypanosomiasis, the most important being *G. m. submorsitans*, *G. longipalpis*, *G. tachinoides* and *G. p. palpalis* (Davies, 1977). Two members of the riverine group, (*Glossina palpalis palpalis* and *Glossina tachinoides*), in addition to transmitting animal trypanosomiasis, are able to transmit human trypanosomiasis. It is estimated that if tsetse and trypanosomiasis are eradicated and the area presently occupied by tsetse flies is opened up and put into full utilization, it could support additional 2.5-3.2 times the country's current estimated livestock population (Onyiah, 1997). In addition, the country could save approximately US $1.3 billion from the importation of meat and dairy products annually, and ensure increased employment opportunities, food availability and national security.

In view of the negative impact of trypanosomiasis on the economy of most countries affected by the disease, the African Heads of State and Government during the 36th Conference of African Union (AU) at Lome, Togo, declared through resolution *AHG/Dec.156 (XXXVI),* of 12 July, 2000, that tsetse flies be eliminated from the African continent through the Pan Africa Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)

Control of trypanosomiasis relies on the deliberate actions against the trypanosome parasites, the vector and/or the host. The disease could be controlled either by chemotherapy or chemoprophylaxis. However in view of the lack of effective vaccine against the parasites (Nok, 2005), due to the phenomenon of variable surface glycoproteins in trypanosomes (Barry, 1997; Pays *et al.,* 2004), unavailability and high cost of drugs, resistance of parasites to available drugs, vector control is considered the most effective method of trypanosomiasis control (Ravel, 2007). The vector could be controlled by means of aerial or ground application of chemical insecticides, impregnated screens and targets, pour- on formulations used on animals and the use of Sterile

Insect Technique (SIT) for mop up operations (Allsop, 2001). However, despite concerted efforts to control tsetse flies, these efforts are hampered by the reinvasion of cleared areas by other tsetse flies (Aksoy, 2003; Krafsur *et al.,* 2008; Beadell *et al.,* 2010). The limiting factors have mainly been attributed to inadequate knowledge on the dynamics of fly populations. Invariably, adequate knowledge on the population dynamics of tsetse flies is key to successful control of tsetse flies using the Sterile Insect Technique (Feldmann *et al*., 2005; Hendrichs, *et al*., 2005; Torr *et al*., 2006).

Earlier laboratory studies had shown that tsetse populations could be differentiated (Van Etten, 1981; 1982a; 1982b; Torr *et al.,* 1988; Colvin and Gibson, 1992; Moloo, 1992, 1993), but genetic basis of the differences were never investigated probably due to limited availability of molecular tools. With the current availability of molecular tools, studies have shown that the genetic basis of differentiations in tsetse populations could be investigated. It is possible for instance to employ molecular tools to provide data on genetic differentiations of tsetse populations, using either mitochondrial or microsatellite DNA markers (Solano *et al.,* 1999, 2000; Krafsur 2003; Marqueze *et al.,* 2004). Cytochrome oxidase1 gene which is important in oxidative activities during metabolism is a maternally inherited gene found in Mitochondrial DNA that could be used to assess genetic drift and bottlenecks in population size (Avise, 1994). It is a conserved gene and relatively stable, whereas Microsatellites are short repetitive nucleotide sequences with conserved flanking regions that could be amplified by polymerase chain reactions (Krafsur and Endsley, 2002). These techniques rapidly identify the levels of epidemiologically important population sub-structuring in tsetse fly vectors.

Investigating genetic variations in natural populations of *Glossina* species is imperative for ascertaining the success or otherwise of tsetse control programme using SIT. Where there is limited

gene flow for instance, SIT may succeed because chances of reinvasion from neighbouring populations may be minimal, but where there is no restriction to gene flow, the success of control campaign using SIT cannot be guaranteed (Krafsur, 2003).

To our knowledge, information on the genetic structures of Nigeria tsetse fly populations that would enable planning of effective control strategies against the vectors in the country is limited. Therefore investigating whether Cytochrome oxidase I mtDNA marker could be used to unravel genetic variations in natural tsetse fly populations could improve our understanding of the dynamics of tsetse fly populations in Nigeria and provide some answers. The result from this study might provide information that could aid choice of effective control strategy against tsetse fly vectors in Nigeria, especially as the country is part of the PATTEC campaign of eradication of economically important *Glosssina* spp. using a component of SIT.

1.2 Statement of the Research Problem

Despite concerted efforts to control tsetse flies, reinvasion of cleared areas by other tsetse flies often occurs due primarily to inadequate knowledge on the population dynamics of target *Glosssina* species and their role in disease transmission Ecological data needs to be supported by molecular techniques in providing clearer understanding on the extent of dispersal, delineation of cryptic species boundry, isolation and disease transmission potentials of a given target tsetse population. These are currently limited in Nigeria.

1.3 Justification

Knowledge on the population structure of tsetse flies is of paramount importance as it is expected to allow for the assessment of the menace of tsetse and trypanosomiasis in the country. Information generated from this study may aid the choice of effective anti-tsetse management approaches in the country's National Parks using a component of the SIT.

1.4 Aim and Objectives

1.4.1 Aim

To evaluate and compare the population genetic structures of *Glossina* species in Nigeria National Parks and Ijah Gwari, with a view to deciphering underlying changes in species composition.

1.4.2 Objectives

i) To determine the species composition and relative abundance of *Glossina* populations in some Nigeria National Parks and Ijah Gwari.

ii) To determine the apparent densities and distribution patterns of *Glossina* populations in Nigeria National Parks in relation to vegetation types and climatic conditions

iii) To determine genetic structures of *Glossina* species in Nigeria National Parks and Ijah Gwari using Cytochrome Oxidase C SU1 (CO1).

iv) To determine the phylogenetic profile/structure of *Glossina* populations in some Nigeria National Parks and Ijah Gwari.

v) To determine how narrow or wide *Glossina* populations have drifted in Nigeria National Parks and Ijah Gwari

1.5 Research Questions

i) Is the species composition and relative abundance of *Glossina* populations in the selected Nigeria National Parks the same?

ii) Are there any variations in the apparent density and distribution patterns in the *Glossina* populations in relation to vegetation types and climatic conditions in the different National Parks and Ijah Gwari?

iii) Are there genetic variations in the different species of *Glossina* in the different National Parks

iv) Are there variations in the phylogenetic relationship within, among and between the different populations of *Glossina* spp. in the different National Parks

v) Are the *Glossina* populations homogenous in the sampled locations?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Economic Importance of Trypanosomiasis

African trypanosomiasis is a parasitic disease of man and livestock. Trypanosomiasis constitutes one of the major causes of poverty in affected countries of sub-Saharan Africa (Aksoy, 2003). The human disease is ranked 9th out of 25 infectious diseases recognized by the WHO.

Animal Trypanosomiasis is a major threat to food security in Nigeria and other parts of sub-Saharan Africa (Swallow, 2000; Abenga *et al.,* 2002). Livestock industry plays a very important role in the national economy of any nation. In Nigeria it consistently contributes 5-6% of gross domestic product (GDP) and between 15-20% of the total agriculctural GDP over the years. It is also an important source of protein in local diet and provides employment and income for a large portion of the rural populace (Shuaibu *et al.*, 1997; FMARD, 2003).

In Nigeria a number of factors including nutrition, diseases and pests continue to militate against the attainment of food security by impacting negatively on the agricultural sector. (Shuaibu *et al.,* 1997). Losses due to animal diseases including trypanosomiasis are estimated at between 30-40% (Majiyagbe and Lamorde, 1997). Where trypanosomiasis is present, the disease constantly impedes livestock productivity and drains the financial resources of livestock keepers. The disease consistently results in mortality, reduced fertility and overall production in livestock if not treated (Shaw, 2004).

The Food and Agriculture Organisation (FAO) recognizes that the panacea for a successful fight against poverty is the improvement of food security in developing countries. The need to promote agriculture, health and food security is a pre-requisite to poverty reduction as noted during the

World Food Summit held in Rome in 1996. The need to reduce poverty is particularly important in tsetse infested areas of sub-Saharan Africa where half of the population suffers from food insecurity. Approximately, 85% of the poor are located in rural areas and more than 80% of the populace depends on agriculture (PAAT, 2008).

It is estimated that 70 million people (Aksoy, 2017) and 48 million cattle (Kristjanson *et al*., 1999) are at risk of African trypanosomiasis. About three million livestock deaths due to trypanosomiasis are reported annually and the loss in livestock production and mixed farming alone is valued at approximately 4.5 billion U.S dollars yearly in Africa (Abenga *et al.*, 2002; Mulumba, 2003).

Direct impact of the disease results in losses in milk and meat production as well as mortality and morbidity (FAO, 2007). Reductions in milk intake by 10-26% in trypanotolerant breeds of animals, calving rates by 1-12 % in trypanotolerant breeds and 11-12% in susceptible breeds, and increase in calf mortality by 0-10% per tolerant breeds and 10-20% per susceptible breeds have been reported (Swallow, 2000). The overall cattle population is estimated to be reduced by 30-50%, due to the avoidance by farmers of areas with high tsetse challenge or trypanosomiasis risk, even if such areas would be good for grazing. This negatively affects livestock population and production (Altman, 1985; Gombe, 2003).

The indirect impact of trypanosomiasis contributes to food shortage through reduced crop production, land use, ecosystem structure and function, human welfare and availability of healthy animals that provide traction power. Trypanosomiasis acts as an important constraint to human settlement pattern and migration (Swallow, 2000) in sub-Saharan Africa including Nigeria (Takele and Abebe, 1988). The main factor leading to changes in land use and settlement is migration. Outbreaks of the disease usually lead to large losses in livestock numbers forcing people to leave tsetse infested areas to uninfested areas (Abenga *et al.,* 2004). Also the fear of contracting the

disease and stigmatization has led people to migrate from tsetse fly infested fertile farm lands into areas that are tsetse fly free but less fertile. This results in poor crop production, over population, over exploitation and devaluation of the land and environment (Kabayo, 2002). According to Swallow (2000), the rate of migration from tsetse fly infested to tsetse fly controlled areas doubled during a study in Zimbabwe.

The human form of the disease in Nigeria is chronic in nature (Swallow, 2000) resulting in reduction of overall efficiency of the affected individuals while animal trypanosomiasis reduces work efficiency of oxen used in cultivation, produce transportation and manure, thereby discouraging the introduction of drought animals into crop farming in tsetse fly infested areas (Omotainse *et al*., 2004). The cost of treatment and management further drains the resources of infected individuals and if diverted elsewhere could go a long way in improving their standard of living (Swallow, 2000). All of these hamper farming and livestock production thereby increasing poverty (Kabayo, 2002).

Presently trypanosomiasis impacts negetively on livelihood of rural dwellers resulting in reduction of agricultural output of affected communities, thus greatly influencing food production, natural resource utilization and the pattern of livestock and human distribution throughout much of sub-Saharan Africa. The infection in livestock robs man of valuable protein intake in form of milk and meat. The economic losses in six states in Nigeria, due to the disease in cattle alone, are estimated at ₦837.2 million annually (NARP/NITR, 1998). Tsetse infested areas of Nigeria could potentially support additional 1.5 million herds of cattle and twice as much as the number of sheep, goats and pigs if the vector is eliminated (Onyiah, 1997). The cost of vector control is estimated at approximately ₦357/km for ground application of insectides and ₦400/km for helicopter

application. The cost of reclaiming almost 400,000km^2 of the Sudan and Northern Guinea Savanna zones between 1955 and 1983 may be about ₦1.6 billion (Samdi *et al*., 2010)

2.2 The Causative Agent - *Trypanosoma* species

2.2.1 Description of trypanosome

The name *Trypanosoma* is derived from the Greek word trypano (meaning borer) and soma (meaning body) because of their corkscrew-like motion (https://en.wikipedia.org>wiki>Trypano...). *Trypanosomes* (Plate I) are small and heterotrophic; they share common characteristics with other members of the phylum Euglenozoa, particularly the stiffening paraxial rod in the flagellum, a characteristic common to the order Kinetoplastida, specifically a large clump of DNA located at one end of the unusually long mitochondrion known as the kinetoplast. Trypanosoma's structure plays a vital role in allowing the parasite to morph into three froms (trypomastigote, epimastigote, and amastigote) during its lifecycle, depending on where the parasite is located in the host's anatomy. The location of the kinetoplast in relation to the nucleus and the flagellum emergence dictates in which stage the trypanosome parasite is found. Key sources of the trypanosome's energy, for example acid calcisomes and reservosomes, are not known. Trypanosomes use several methods in order to penetrate the host's cell, including active penetration and active induction of receptor-mediated phagocytosis. Trypanosome's unique structure allows the parasite to invade the host cell usually with little difficulty, resulting eventually in cell rupture, release of trypomastigotes, and their subsequent multiplication. *Trypanosoma cruzi* differs from its African relatives (*Trypanosoma brucei*) in that no replication occurs in the bloodstream; rather, the forms are only able to replicate after another cell has been penetrated.

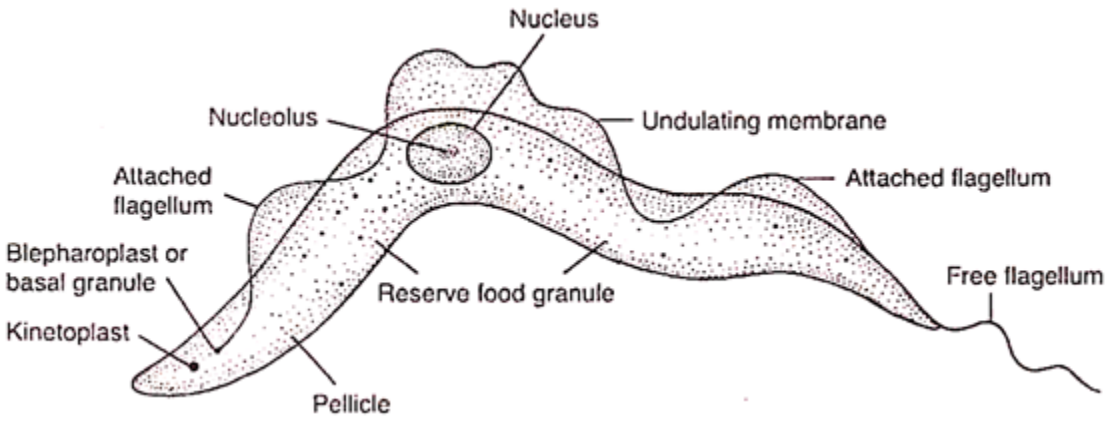


Plate I: Schema of *Trypanosoma brucei* (Source: https://www.google.com/biodiscusssion.com)

2.2.2 Classification of trypanosomes

Trypanosomes infect a variety of hosts and cause various diseases, including the fatal human disease, sleeping sickness, caused by *Trypanosoma brucei*, and Chagas disease caused by *Trypanosoma cruzi.* Trypanosomes belong to the Phylum Euglenozoa, Class Kinetoplastida*,* Family Trypanosomatidae and genus: *Trypanosoma* Gruby, 1843. The genus *Trypanosoma* is further split into two major groups based on some inherent morphological differences, the Stercorarian or non pathogenic and Salivarian or pathogenic groups (Hoffmann, *et al*., 1984). While the former are transmitted during blood meals by the insect vectors (Anterior station), the latter are transmitted via faeces of the insect vector (Posterior station). The pathogenic trypanosomes include Dutonnella (*T. vivax*)*,* Nannomonas (*T. congolense*), and Trypanozoon (*T. brucei*) groups. The stercorarian trypanosomes include amongst others *T. godfreyi*, *T. grayi* and *T. rangeli* which are trypanosomes infecting crocodiles and other reptiles. However, studies have shown that *T. grayi* infects *G. fuscipes fuscipes, G. palpalis palpalis* and *G. tachinoides* (Challier, 1982). The parasites (*T. grayi* and *T. theileri*) have recently been found in cattle and their pathogenicity is being investigated (Ngomtcho *et al,* 2017).

2.2.3 Life cycle of trypanosomes and disease distribution

2.2.3.1 Life cycle of trypanosomes

During a blood meal on the mammalian host, an infected tsetse fly (Genus *Glossina*) injects infective metacyclic trypomastigotes into the skin tissue (Figure 2.1). The parasites enter the lymphatic system and pass into the bloodstream. Inside the host, they transform into bloodstream trypomastigotes, are carried to other parts of the body and fluids (eg, lymph, spinal fluid), and continue the replication by binary fission. The entire life cycle of African trypanosomes is represented by extracellular stages. The tsetse fly becomes infected with bloodstream trypomastigotes when taking a blood meal on an infected mammalian host. In the fly's midgut, the

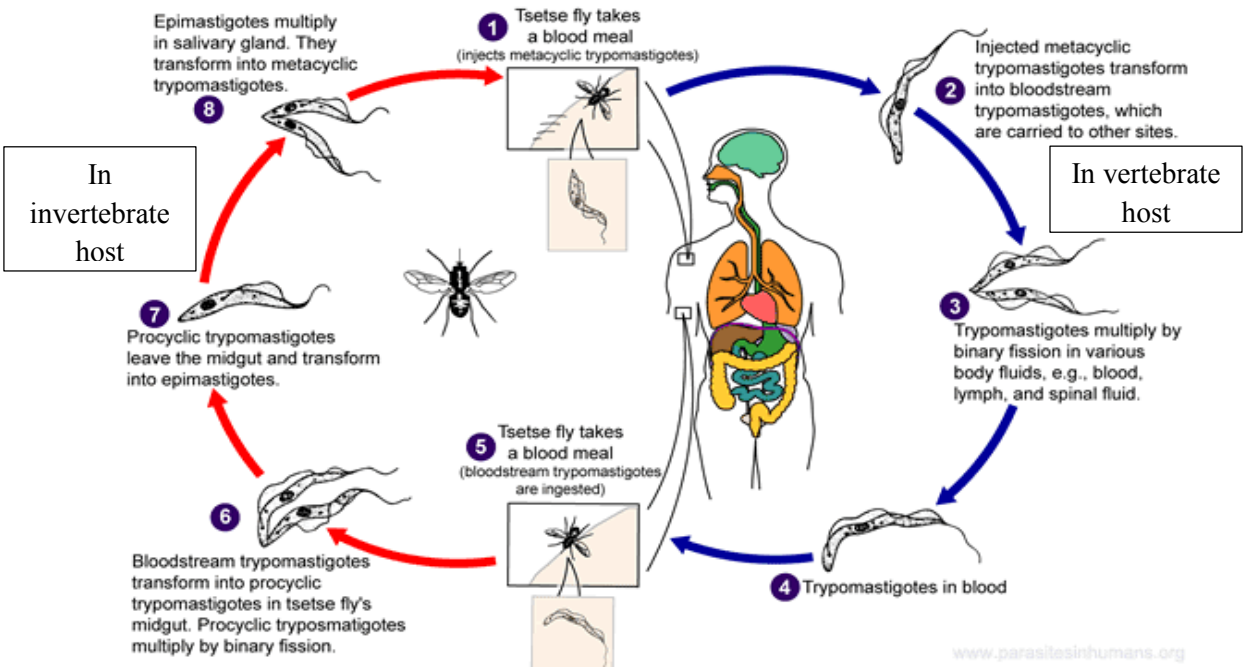


Figure 2. 1: Schema of the Life cycle of *trypanosoma brucei* within the insect vector and the vertebrate host (Figure: adapted from http//www.dpd.cdc.gov/dpdx)

parasites transform into procyclic trypomastigotes, multiply by binary fission, leave the midgut, and transform into epimastigotes. The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission. The cycle in the fly takes approximately 3weeks. Humans are the main reservoir for *Trypanosoma brucei gamgiense*, but this species could also be found in animals. Wild game animals are the main reservoir of *T. b. rhodesiense.*

The causative agents of both HAT and AAT are different species of trypanosomes. The economically important parasites include, for animal trypanosomiasis, *Trypanosoma congolense, T. vivax, T. evansi, T. suis, T. equiperdum,* and *T. brucei brucei*; Two sub-species of *T. brucei, T. b. gambiense* and *T. b. rhodesiense* are responsible for the chronic West Africa and acute East Africa forms of sleeping sickness respectively (Van den Bossche, 2001). The *brucei* forms that cause the disease in humans and animals are morphologically indistinguishable except that the animal parasites are susceptible to a trypanocidal factor due to the presence of a high density lipoprotein associated with the human blood (Rickman and Robson, 1970; Jenni and Brun, 1982; Hager and Hajduk, 1997; Thomson *et al.,* 2009). All the parasites however rely on tsetse flies for their cyclical development and transmission, except trypanosomes of equines (*T. equinum* and *T. equiperdum*) and of camels (*T. evansi*) which are not transmitted by tsetse flies but by direct blood contact during copulation (horses) or by other biting insects such as horse flies (tabanids) in the case of camels (Ravel *et al.,* 2007). Research has shown that some enzymes play important roles in the life clycle of trypanosomes. It is thought that some of the trans-sialidase enzymes are essential in the sustenance of trypanosomes inside of the vector tsetse fly and in the mammalian host and thus may be important in the pathophysiology of the disease (Gbem *et al*., 2013).

2.2.3.2 Disease distribution

The current distribution of trypanosomiasis in Africa corresponds to the range of distribution of tsetse flies which currently spans an area approximately 10 million km^2 between latitude 14°North and 20°South (Molyneux *et al.,* 1996). HAT is discontinuously spread over 9 million square kilometers and affects populations across 38 sub-Saharan countries (Figure 2.2). Animal trypanosomiasis (AAT), caused by a wider number of trypanosome species and carried with higher prevalence by a greater number of *Glossina* species, is invariably the greater epidemic across the African continent with dire economic consequences. In general, trypanosome infections that threaten livestock are over 100 to 150-fold higher in *G. morsitans* than the trypanosome infections that cause human trypanosomiasis (Jordan, 1976). Historically, the impact of animal trypanosomiasis were so profound that it influenced the migration routes of cattle-owning tribes into the continent who were forced to avoid the *G. morsitans* "fly-belts", as well as the movements of early European and Arab settlers into the continent who depended on horses and oxen (McKelvey, 1973 as cited by Steverding, 2008). Trypanosomiasis has severely hampered the socio-economic development of sub-Saharan Africa (Steverding, 2008).

Disease distribution in Nigeria

The origin of trypanosomiasis in Nigeria is not known. The disease seems to have been in Nigeria since time immemorial, precisely before the coming of the colonial masters. Evidences however, abound indicating that the natives were conversant with the disease and have had traditional methods of its treatment before the era of colonialism. Natives and pastoralists seemed to have developed some type of response in preventive and curative medicine as far as human and animal trypanosomiasis were concerned. Research on trypanosomiasis in different parts of Africa reveals that the people had practiced evacuation of tsetse infested areas, avoidance of certain riparian areas

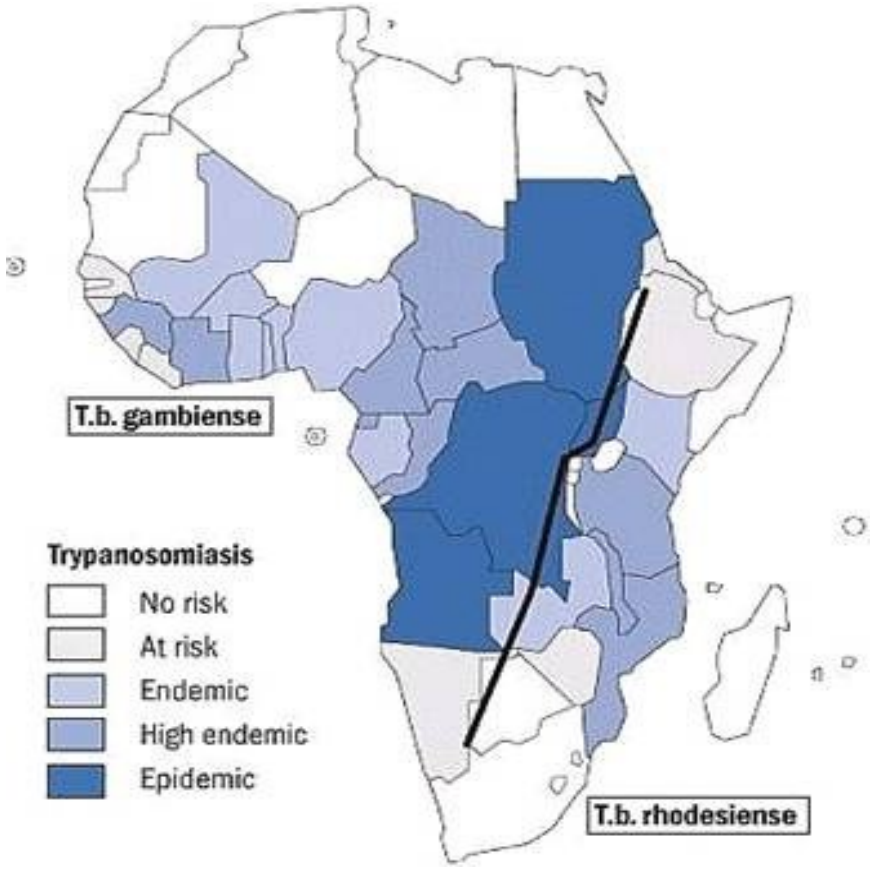


Figure 2. 2: East and West African Sleeping Sickness separated by the thin dark line

(Souce: Map provided by WHO, Report on Global Surveillance of Epidemic- prone Infectious Diseases- African Trypanosomiasis, 1999).

in the dry seasons, produced traditional concoctions to treat affected persons and animals, and practiced the isolation of persons affected by the disease, etc. Even though in some instances some of these movements were ignorantly thought to be caused by "spirits". In 1907, a colonial officer, Dr. McGahey, reported the case of a "native doctor" at Loko on the Benue River who had been treating sleeping sickness since the beginning of the 20 century. According to him, sleeping sickness patients were treated by this "doctor" for nineteen weeks. The treatment consisted of "incising the glands in the neck and dressing the wounds with a certain leaf which had previously been burned, powdered and made into a paste with water. A decoction of other leaves was made for internal administration." This native doctor claimed that he inherited the remedy from his father and that he had treated 83 cases in the previous two years and lost only two. Consciously or unconsciously, the practice of bush burning in the dry season also reduced the amount of space tsetse flies could use, thereby reducing them to vegetation and shades near water holes, streams and ponds.

The first reported case of sleeping sickness in Nigeria was by MacFie and Gallagher (1914) in Eket district in South-East Nigeria. It has been reported that the greater part of Northern Nigeria was virtually free from human trypanosomiasis until the beginning of the 20 century when the disease rose to an epidemic proportion probably through labour forces employed on the opening up of road and rail transport infrastructures. Infections introduced in this way were probably carried a stage further by travelers and traders traversing the newly developed lines of communication and eventually foci of infection became established along hundreds of kilometers of these newly opened routes from which, in due course, the disease filtered through to the remoter areas. Once a reservoir of infection had been introduced, the altered mode of life of the community

created ideal conditions for the spread of sleeping sickness. This was probably made possible by the increase in man-fly contact that resulted from increased pastoral and agricultural activities, and the practice on the tin mines of washing the mineral deposits in the hundreds of streams known to be the habitat of tsetse flies.

Recent reports however, show changes in the dynamics and epidemiology of human trypanosomiasis in the country. New endemic foci have been reported in the southern part of the country (Edeghere *et al.,* 1989). A disease which was previously confined to the north seems to be shifting to the south as revealed in Figure 2.3 (NITR records), whereas animal trypanosomiasis is presently endemic in all the 36 states of the federation including the Federal Capital Territory Abuja.

2.2.4 Historical trends

The historical trend of the evolution of trypanosomes and their role in the socio-economic development of the African continent has been succinctly captured by Steverding (2008). It is suggested that the origin of trypanosomiasis predates recorded history. However, evidence from phylogenetic reconstruction suggests that all Salivarian trypanosomes diverged from other trypanosomes approximately 300 million years ago (Haag *et al.,* 1998). It seems most probable that soon after their emergence, Salivarian trypanosomes became gut parasites of early insects which evolved around 380 million years ago. Tsetse flies are thought to have evolved about 35 million years ago and have been involved in the transmission of trypanosomes to mammals since then. The trypanotolerant status of African wild animals as a result, could reasonably be traced to the long co-existence of both tsetse flies and the game animals. In contrast, domestic animals have not yet fully developed resistance against trypanosomiasis, despite their breeding over 13 000 years now (Lambrecht, 1985 as cited by Steverding, 2008).

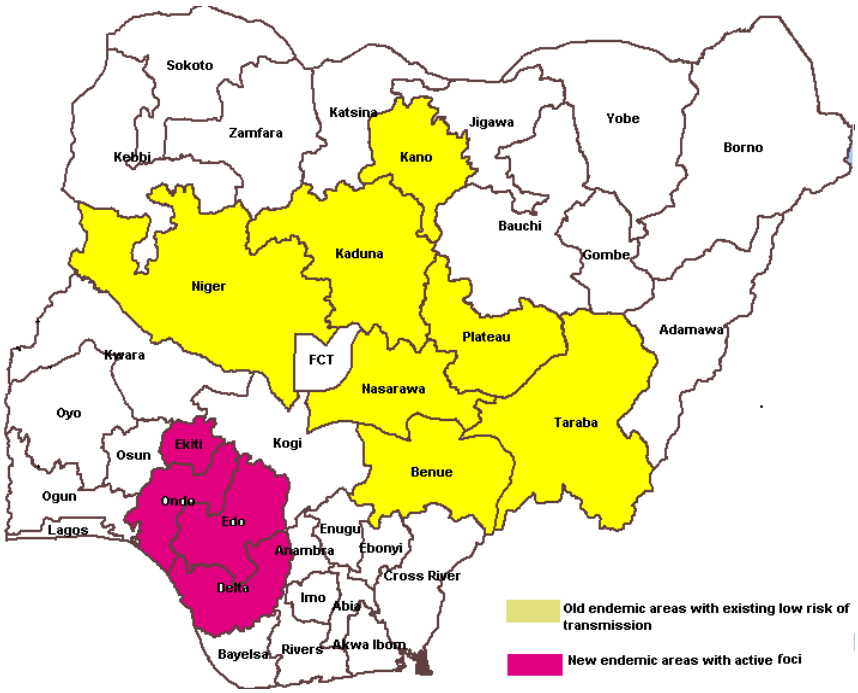


Figure 2. 3: HAT distribution in Nigeria (Soure: NITR unpublished Records)

From antiquity, it has been established that, the ancient time north coast of the African continent held more flush vegetation than what it is today (Jolly *et al.,* 1998). Tsetse flies distribution would probably have extended beyond the present limit much more northwards into the Nile delta. It seems most reasonable to mention that the native livestock keepers at that time must have been familiar with trypanosomiasis. Besides, further evidence of the presence of tsetse flies in ancient Egypt as provided by the Veterinary Papyrus of the Kahun Papyri dates back from second millinium, in which a disease resembling nagana was described (Griffith *et al.,* 1998 as cited by Steverding, 2008). Following the adjustment of the stream course of the Nile River during the course of the Middle Kingdom (2000BC-1300BC), the breeding sites of tsetse flies would probably have been largely destroyed. This would have led to the gradual eradication of tsetse flies from the Nile River and would probably have paved the way for raising pure breed zebu cattle and other domestic animals like horses (introduced in 16th Century) by the ancient Egyptians (Winkle *et al.,* 2005 as cited by Steverding, 2008).

In his review of the situation in the middle ages, Steverding (2008) mentioned that evidence of the occurrence of trypanosomiasis in Africa had been described following reports from the Arabs who kept close trade relations with the West African Kingdoms of Benin, Ghana, Mali and Songhai. He further stated that one of the earliest reported cases of human trypanosomiasis is credited to the famous Arabian geographer Abu Abdallah Yaqut who journeyed into Africa and found the "Country of Gold"(Wangara) (Kea, 2004), an underground village whose inhabitants and even their dogs were just skin and bones and asleep (Winkle, 2005 as cited by Steverding, 2008)). The report depicted the devastating sleeping sickness epidemic in Uganda at the turn of the 20 century. The review also gave account of the first sleeping sickness case that was reported by the Arabian historian Ibn Khaldun, in which he referred to a fellow country man who had earlier told him

about the death of the Sultan Mari Jata, Emperor of Mali from an illness whose description ("He told me that Jata had been smitten by the sleeping illness, a disease which frequently afflicts the inhabitants of that climate, especially the chieftains who are habitually affected by sleep") resembled that of human trypanosomiasis (Williams, 1996 as cited by Steverding, 2008).

Modern time account of the history of human trypanosomiasis is said to be closely related to slave trade. The first account of sleeping sickness would probably have come from ship doctors and medical officers who worked for slave-trade companies. As sleeping sickness caused increasing losses, ship-owners and slave traders would probably have insisted that their ship doctors had the cause investigated? It thus followed that in 1734, the first accurate medical report on African sleeping sickness was published by the English naval surgeon John Aktins, (COX, 2004 as cited by Steverding, 2008). It is also provided in the account that, whereas only the neurological symptoms of the late stage of sleeping sickness were described by Aktins, the English physician Thomas Winterbottom, published in 1803 a report referring to the characteristic sign of swollen lymph glands along the back of the neck in the early stage of the disease (COX, 2004). It was also mentioned that this symptom was known long ago by Arabian slave traders who refrained from buying slaves with this sign (Winkle, 2005). Although throughout the 19th century, reports on sleeping sickness increased and human trypanosomiasis became a well recognized disease, no one had any clue about the nature of the illness (COX, 2004 cited by Steverding, 2008).

2.3 Trypanosome –Tsetse Interaction

The review of Steverding (2008) gives account of the history of tsetse-trypanosome interactions, in which it is suggested that the Scottish missionary and explorer David Livingston was the first to suggest that nagana is caused by the bite of tsetse flies. It further states that following the death of all the cattle he carried along the valleys of the Limpopo and Zambezi rivers as well as at the banks of the lakes of Nyasa and Tanganyika, after they were bitten by tsetse flies, Livingston was prompted in 1852 to report the presence of the disease (Winkle, 2005). The report was viewed with scepticism until 40-50 years later that trypanosomes were identified as the causative agents of both animal and human trypanosomiasis. The Scottish pathologist and microscopist David Bruce, in 1895, discovered *T. brucei* as the cause of cattle trypanosomiasis (as cited by Steverding 2008). The presence of trypanosomes in human blood was first demonstration by the British Colonial surgeon Robert Michael Forde in 1901 following the examination of a steamboat captain in The Gambia by name Ford R.M (as cited by Steverding, 2008). The organisms were first thought to be worms, not until the English physician Joseph Everett Dutton, identified them as trypanosomes a few months later and proposed in 1902 the species name *Trypanosoma gambiense* (now *T. b. gambiense*). In yet another development, the Italian physician and pathologist Aldo Castellani in the same year, found trypanosomes in the cerebrospinal fluid of sleeping sickness patients and suggested that they cause sleeping sickness. However, Bruce one year later, provided conclusive evidence that sleeping sickness is transmitted by tsetse flies. But he believed that trypanosomes were transmitted mechanically by tsetse flies. This was countered by the German Military surgeon Friedrick Karl Kleine, who showed in 1909 the cyclical transmission of *T. brucei* in tsetse flies. Having been compelled to change his original opinion of mechanical transmission of trypanosomes, Bruce instead described the full developmental cycle of the parasites within their insect host. In his review, Steverding (2008), further gives account of the discoveries of two other

animal pathogenic trypanosome species *T. congolense* and *T. vivax* made in 1904 and 1905 by the Belgian physician Alphonse Broden and the German Naval Doctor Hans Ziemann respectively. In 1910 the second human pathogenic trypanosome species, *T. rhodesiense* (now *T. b. rhodesiense*), was eventually recovered by the parasitologists John Williiam Watson Stephens and Harold Benjamin Fantham as revealed (Steverding, 2008).

2.4 Evasion of Host Immune System by Trypanosomes

2.4.1 Variant Surface Glycoprotein (VSG)

One of the major characteristics of trypanosomes is the presence of variant surface glycoprotein (VSG) which covers nearly all the membrane of trypanosomes and is the predominant surface antigen of African trypanosomes. VSG constitutes an important molecular interface between the trypanosomes and the host immune system. Trypanosomes establish their presence in host animals by releasing numerous toxic substances and by mediating alternate expression of an extensive repertoire of variant surface glycoprotein, VSG, a process known as antigenic variation. The success of the establishment and the maturation of trypanosomes play a key role in the disease transmission cycle. In the fly's midgut, the trypanosomes survive digestive and trypanocidal environments (Welburn and Maudlin, 1999; Hao *et al.*, 2001 and Boulanger *et al*., 2002), proliferate and translocate into the salivary gland, where they become infectious to the next mammalian host (Vickerman *et al*., 1988). These survival mechanisms employed by the parasite are major factors that have made the chemotherapy and development of vaccine against trypanosomiasis difficult.

2.4.2 Anti-pathogenicity inducing "host" factors

Trypanosomes have evolved efficient immune escape mechanisms by sculpturing the host's innate/adaptive immune response in order to establish an environment suitable for parasite survival

and transmission. Usually this manipulation of the host's response undermines its ability to respond/recover following establishment of the parasites. Intervention strategies against African trypanosomiasis that would require the development of efficient anti-trypanosomal agents in combination with nuetralisation of anti-pathogenicity inducing "host" factors are currently being elucidated, with the view to reducing economic losses of affected countries (Stijlemans, *et al.,* 2016).

2.5 Tsetse Flies

According to Baldry and Riodan (1967), tsetse flies (Figure 2.4) could be described as robust, dark brown, blackish, yellowish-brown, or yellowish flies ranging in length (excluding the proboscis and wings) from about 6 or 8mm, in the case of *G. tachinoides*, to as long as 13 or 13-15mm, in the case of *G. brevipalpis* or *G. longipennis*. A prominent proboscis which has a distinct basal bulb and points forward when at rest is present in all species. The mouth parts consist of three parts (the upper lip or labium, the hypopharynx and the lower lip or labrum) surrounded by a pair of maxilla palps; the third segment of the antenna bears an arista with hairs which are themselves branched on the upper surface only. The compound eyes which are widely separated by the frons are important in short-range host location. The eyes (ocelli) may also distinguish between light and darkness, a useful attribute for seeking out shaded microclimates when ambient temperatures are at lethal levels. The wings at rest are folded like a pair of scissors, so that they are fully overlapping one another. The wing venation is characteristic with the discal cell (Figure 2.4), comprised between fourth and fifth longitudinal vein and the posterior cross-vein) being "hatchet" shape like a butcher's cleaver). The abdomen in the *G. morsitans* group and in *G. tachinoides* is of a paler colour and marked with sharply dark brown bands, interrupted in the middle line, but this part of the body is invisible when the insect is at rest, since it is concealed beneath the wings (FAO, 2000).

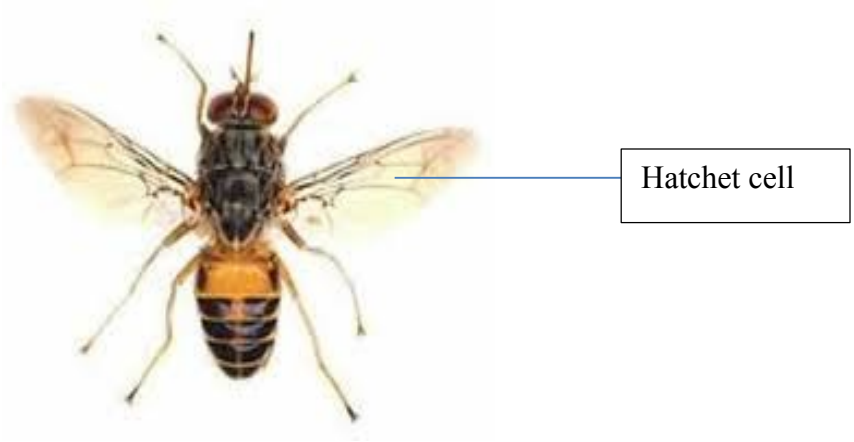


Figure 2. 4: Abdominal markings of adult tsetse.

(Adapted from: flyhttp://www.kznhealth.gov.za/environ/vector/tsetsefly.htm)

Tsetse flies detect odours by means of sensilla situated on the antennae. They may also be attracted by Kairomones produced in the host's breath (e.g. carbon dioxide, acetone, 1-octen-3-0l) and in the urine of African buffaloes and cattle (phenols) (Vale and Hall, 1985a and Vale *et al*., 1988). However some tsetse are repelled by the odours of humans and some animal species e.g. waterbuck (Gikonyo *et al*., 2002).

The internal anatomy of tsetse flies is characterized by two extremely long salivary glands which end in the anterior part of the abdomen, an alimentary tract subdivided in an oesophagus, a crop, which receives the major part of the blood meal before the meal is diverted to the midgut, the proventriculus which controls the passage of blood between the crop and the midgut and secretes the peritrophic membrane (a semi-permeable membrane protecting the midgut cells), a midgut responsible for water absorption, digestion of the meal and absorption of the nutrients and behind the insertion of the Malphigian tubules (with a function comparable to kidneys) the hindgut. The female reproductive system consists of paired ovaries each consisting of two ovarioles, the uterus and two spermathecae. The four ovarioles produce a single follicle in turn in a predicted order, which could be used to determine the age of an adult female tsetse (Saunders, 1960; Challier, 1965 as cited by Challier, 1982).

Importance of Tsetse flies

Tsetse flies have been extensively studied because of their role in transmitting diseases of humans and livestock. They have a prominent economic impact in sub-saharan Africa as the biological vectors of trypanosomes which cause human sleeping sickness and animal trypanosomiasis.

2.6 Male and Female Flies Differentiation

Tsetse flies of both sexes are obligate haematophagous insects. The sexes are readily differentiated when the external genitilia are examined. In the male the external genitalia forms a conspicuous knob-like protuberance (hypopygium) beneath the end of the abdomen, which is absent in the female (Figure 2.5). During copulation the hypopygium is deployed and reveals the superior claspers and penis. The claspers are used for withholding the female during copulation and their form is characteristic for each sub-genus.

During emergence, sex ratio in the field is always uniform but the ratio becomes distorted depending on the sampling method used. Hand net catches seem to have more representative segment of male flies, whereas sex ratio in trap catches is in favour of females (Pollock, 1982). In their attempt to unravel the riddle of sex ratio in tsetse flies, Randolph *et al.,* (1991) reported that trap catches of *Glossina longipenis* depended on movement of flies, timing of activity, nutritional status, trap efficiency, climatic conditions, host availability or a combination of some or all. Okiwelu, (1982) affirmed that different sampling methods often capture different sectors of *Glossina* populations. Study of sex ratio in tsetse populations is important while considering the control of target populations using SIT.

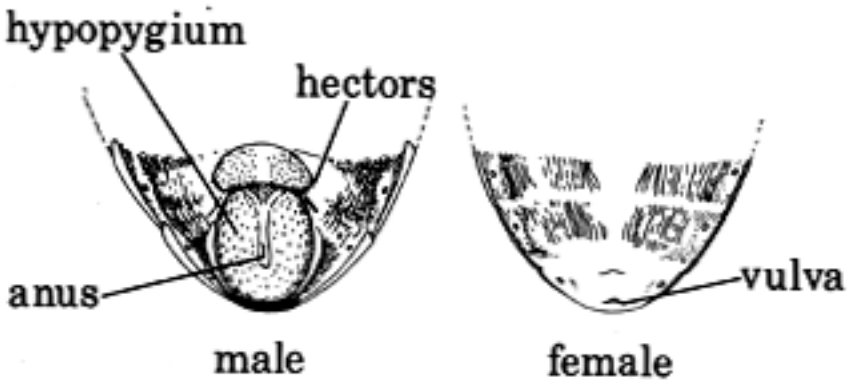


Figure 2. 5: Differences between male and female *Glossina* ventral view (Source: FAO, 1992)

2.7 *Glossina* Identification

Tsetse flies may be identified morphologically (Figures 2.6) using identification keys as previously described (Buxton, 1955; Potts, 1970; FAO, 1982). Other methods of species identification include length polymorphism, barcoding, cuticular hydrocarbons (Sutton and Carlson, 1997; Getahun *et al.,* 2014).

Very recently, wing geometric morphometrics have been shown to distinguish between species, subpopulations, ecotypes and even successive generations in forensic science (Patterson and Schofield, 2005). Besides it has the advantage of simplicity of data acquisition and low cost (Rohlf and Marcus, 1993). Phylogenetic analysis could also be used to differentiate between tsetse fly species, their subpopulations and their evolutionary relationships (Patterson and Schofield, 2005; Abila *et al*., 2008; Dyer *et al.,* 2008; Dyer *et al.,* 2011). Phylogenetic analysis is also important as a tool for species identification (Christopher *et al.,* 2015). Recently, Mauricio and Juan (2016) using Molecular phylogenetics were able to identify a new frog species group (*Pristimimantis lacrineosus* spp. (Anura: *Gaugaetoridae*) from Colombia.

Each of these methods however has its advantages and disadvantages. While, the phylogenetics method is expected to give greater accuracy when fully developed, a combination of identification methods is expected to give more accurate identification than the single methods. It was proposed that in control programs that involve Sterile Insect Technique (SIT) as suggested for a *G. f. fuscipes* SIT program in Ethiopia, morphological classification alone is not used to classify such populations (Dyer *et al*., 2011).

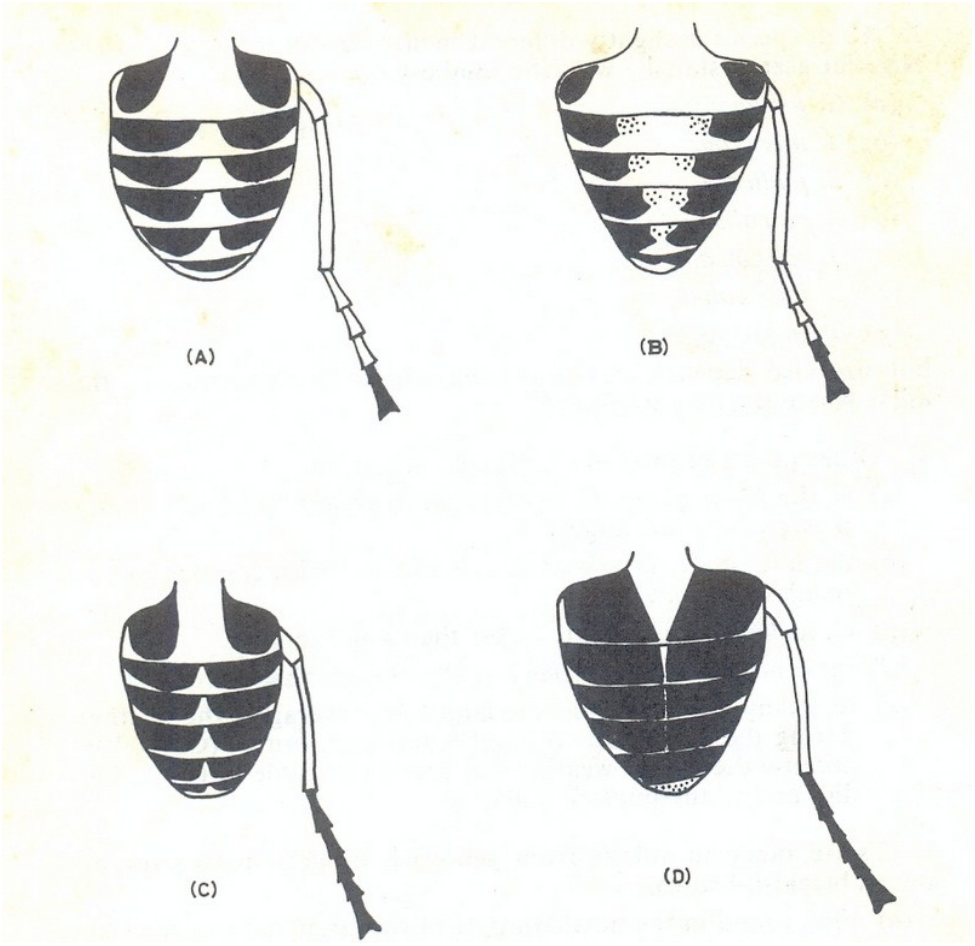


Figure 2. 6: Morphological differences among economically important *Glossina* spp. found in Nigeria

(A) *G. m. submorsitans* (B) *G. longipalpis* (C) *G. tachinoides* (D) *G. palpalis palpalis*

(Source: Davies, 1977)

2.8 Tsetse Life History and Biology

2.8.1 Mating

Tsetse reproduce by adenotrophic viviparity (Tobe and Langley, 1978). Tsetse flies mate during the days after emergence, when the females are teneral. One insermination is enough for the whole life of the female, but males mate several times during their life, and perhaps some females too. Non-teneral wild caught females are always inseminated. Homologous matings are the rule but sometimes mating occurs in nature between closely related species; such matings are generally not fertile and may be lethal to the female.

2.8.2 Ovulation

The two ovaries are composed of two ovarioles each, and each ovariole develops in turn (Saunders, 1962; Challier, 1963). The first ovulation occurs 3 to 11 days after the emergence of the female (Figure 2.7).

2.8.3 Egg stage

There is no free egg stage, and females retain each egg within the uterus. The fertilized egg hatches into a first stage larva that passes through three instars, nourished by milk glands that are highly modified accessory glands inside the uterus (Denlinger and Ma 1974). A female tsetse deposits its first mature larva when it is 16 to 17days old. Thereafter, progenies are produced optimally every 9 to 10 days. The larvae burrow into the soil and pupate 1 to 2hours after larviposition. Pupae remain in the ground for about 30 to 60 days before adults emerge depending on temperature (Gooding, 1998). The development rate of the various stages of the tsetse fly is directly proportional to the temperature. High temperatures could shorten the interlarval period, the pupal stage (20 days at 30^{o}C but 100 days at 16^{o}C), the life span of the adult and the period between successive feeds.

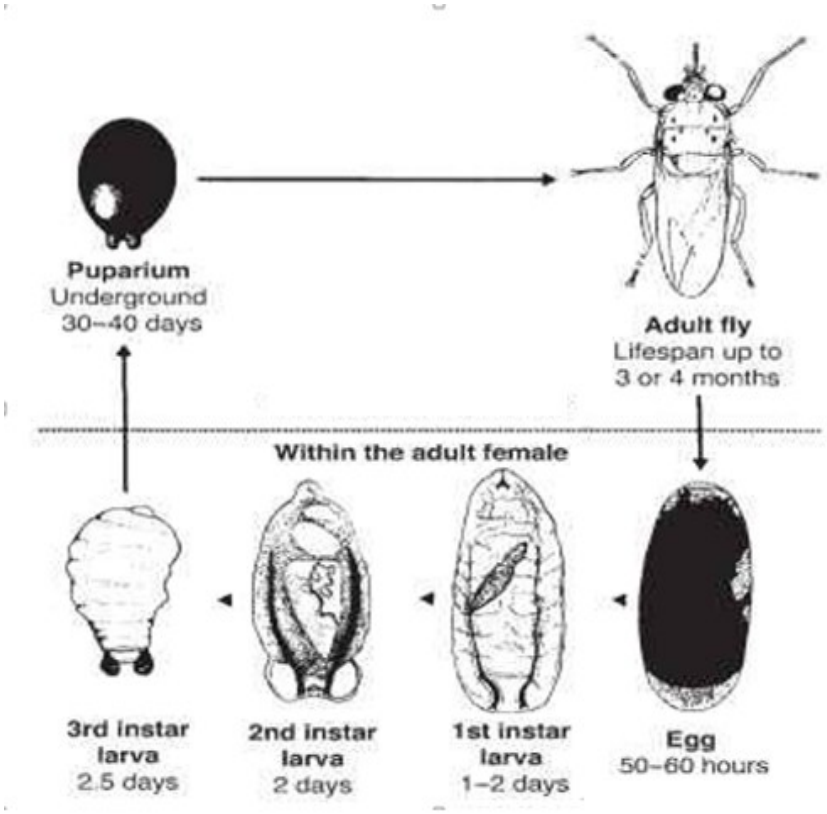


Figure 2. 7: Life cycle of tsetse fly

(Figure: adapted from: http://what-when-how.com/insects/tsetse-fly-insects/)

Consequently, tsetse flies have been reported to have a very low reproductive rate. The low fecundity and peculiar life cycle of tsetse qualifies them as ideal targets for control or eradication using genetic methods (Vreysen, 2001).

2.8.4 Larval and pupal stages

The depth to which the larvae enter the soil varies with environmental conditions; in the rainy season the larva could pupate almost on the ground or only one or two centimeters below. In the dry season the larva could enter five to eight centimeters below the ground before pupating. The pupae cannot move and so are susceptible to wide variations of temperature and humidity. They can withstand high relative humidities but neither submersion by water nor dryness (Bursell, 1963). Pupal duration is an important parameter in timing tsetse control operations. High temperatures reduce the length of pupal life, but too high temperatures may kill the pupae in some days and even some hours and pupal sites are usually situated in well shaded places, under logs, bushes, and so on. For some species, eg *G. tachinoides* and *G. p. palpalis*, breeding sites could be entirely man-made, such as mango and banana plantations

2.8.5 Adult Emergence

During emergence, a young fly emerges by breaking the puparium at the opposite end of the end which has the two respiratory lobes, with the aid of a swelling on top of the head of the fly called ptillinium. It is with the aid of this same structure that it forces itself out of the soil in which it was buried. Upon emergence, the wings of the fly flatten out into their normal shape within few minutes, the young fly then craws to a suitable place and rests waiting for its first blood meal.

2.9 Teneral and Non-Teneral Flies

From emergence to taking of its first blood meal, a young fly is referred to as a teneral fly. A teneral fly may be recognized by the whitish semi- transparent appearance of the underside of the abdomen, the ptilinium may sometimes be everted by squeezing both sides of the head capsule between two fingers and the fly has a soft soapy touch (Pollock, 1982). After the first blood meal has been taken, the fly ceases to be teneral, but rather referred to as non teneral fly. It could be recognized by the more creamy yellow, appearance of the underside of the abdomen and when held up to the light, the dark shape of the last meal could be seen. The thorax feels firmer and harder, because of the greater development of muscles in it. The ptilinium cannot easily be everted when the head is squeezed between two fingers (FAO, 2008).

2.10 Age Determination in Male and Female Flies

The assessment of the age structure of samples collected after insecticide spraying has been used as a means to evaluate the effectiveness of treatments in tsetse control trials (Chapman, 1976; Davies, 1978; Molyneux *et al.,* 1978). Age determination in male and female tsetse flies requires the use of different methods. In the males the wing fray method is used, whereas ovarian configuration is used in the case of female flies.

2.10.1 Wing fray analysis in males

Age estimation in male tsetse flies is done according to the degree of wear or fraying on the hind margin of the wing (Figure 2.8) According to the degree of wear, flies are assigned a number corresponding to one or other of the six categories as described by Jackson (1946) and Challier (1965). After giving the wing fray category (Table 2.1), the age is estimated using the guide for estimating the mean age of a sample of the tsetse flies (Table 2.2), and mean wing fray is calculated as the sum of each category divided by the sum of fly number for each category and finding the

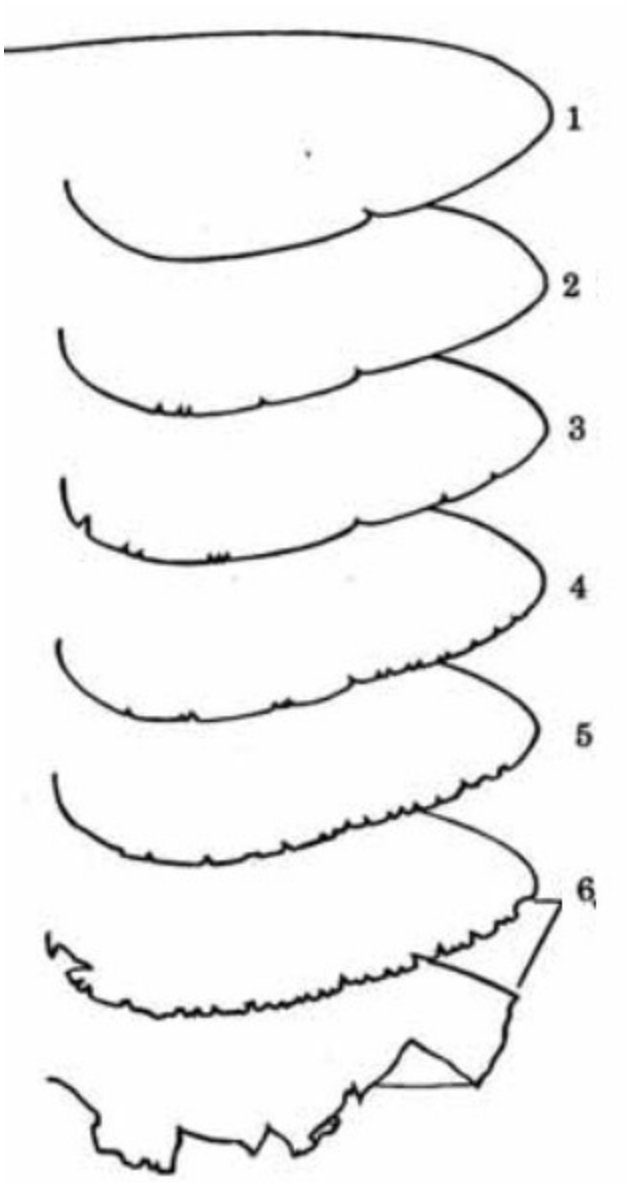


Figure 2. 8: Wing fray analysis for the estimation of the age of male flies. Numbers from 1-6 correspond to the degree of wear or fraying on the hind margin of the wing.

(Adapted from: FAO, 1982)

Table 2. 1: Analysis of wing fray data from 40 flies

Wing fray category	No. of flies in each category	Factor	Product
1	5	1	5
2	20	2	40
3	9	3	27
4	5	4.4	22
5	1	5.5	5.5
6	0	6.9	0
	40		99.5
	Total flies		Total (sum of products)

$$\frac{\text{sum of products}}{\text{Total flies}} = \frac{99.5}{40} \text{ or 2.7 days approx.}$$

(Source: FAO, 1982)

Table 2. 2: Correspondence between mean wing fray value (MWFV) and estimated mean age in days of flies in sample (Est. age)

MWFV	Est. age	MWFV	Est. age	MWFV	Est. age	MWFV	Est. age
1.6	11	2.8	21	3.9	31	5.1	41
1.8	12	2.9	22	4.0	32	5.2	42
1.9	13	3.0	23	4.2	33	5.3	43
2.0	14	3.1	24	4.3	34	5.4	44
2.1	15	3.3	25	4.4	35	5.5	45
2.2	16	3.4	26	4.5	36	5.6	46
2.3	17	3.5	27	4.6	37	5.8	47
2.4	18	3.6	28	4.7	38	5.9	48
2.6	19	3.7	29	4.8	39	6.0	49
2.7	20	3.8	30	5.0	40	-	-

(Source: FAO, 1982)

given value on the table as given in the Food and Agriculture Organisation (FAO) Training manual for tsetse control personnel (FAO, 2000).

2.10.2 Ovarian configuration in female flies

Age estimation in female flies is done using ovarian configuration (Figure 2.9). Flies are dissected to examine the contents of the uterus. Based on the relative sizes of the follicles in each of the two ovarioles and in each of the two ovules that constitute each ovary, the flies are assigned an age category following methods described by Saunders (1962) and as illustrated in Food and Agriculture Organisation (FAO) Training manual for tsetse control personnel (FAO, 2000).

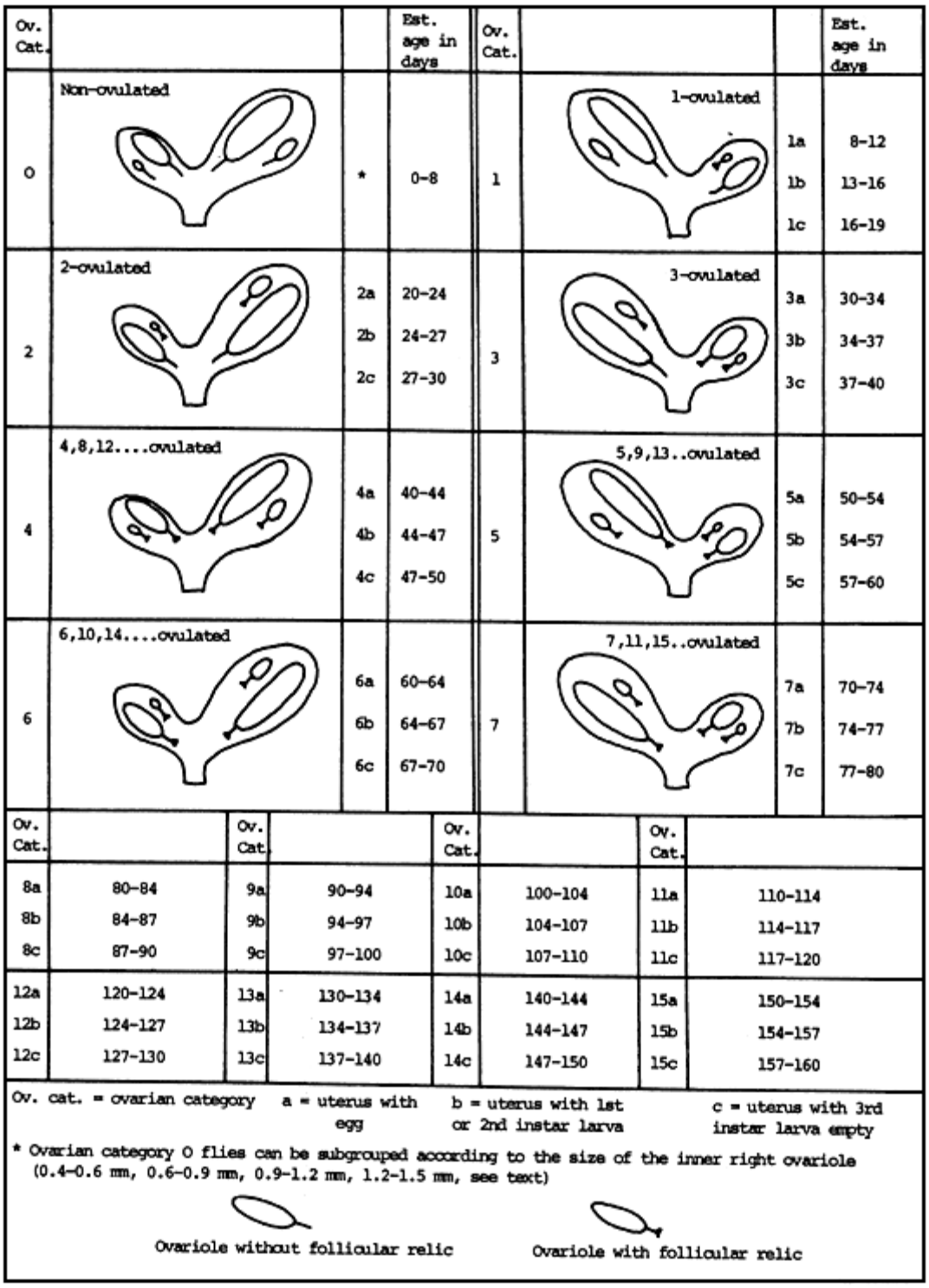


Figure 2. 9: Successive changes in ovaries with increasing age (Source: FAO, 1982)

2.11 Physiological States in Female Flies

It is very difficult to recognize a pregnant female fly when the larva is small unless it is dissected. Assessing pregnancy rates requires careful examination of the contents of the uterus whether the uterus is empty, has an egg, first, second or third instar larva (recognized by the size of the developing polypnuestic lobes). Based on the above observations, the female fly can be categorized as pregnant or non pregnant.

2.12 Hunger Staging

Hunger staging is done only on non-teneral male flies. It is always very difficult to hunger stage pregnant females due to the development of larva which obscures the manifestation of the hunger cycle. Some workers hunger stage flies into three categories i.e. stage 1 when a fly is engorged; stage 2, when a fly is non hungry and stage 3, when a fly is hungry (Davies, 1977). The classical method of assessing hunger stage however, is that of Jackson (1933) which gives the mean hunger stage (MHS). This parameter enables comparison to be made between various categories of flies or between different populations or different seasons. Male flies could be categorized into four hunger stages. Stage 1: Gorged when abdomen is distended with red or blue-black blood. Stage 2: Replete when abdomen is slightly convex with no red or black color visible and two- thirds opaque. Stage 3: Intermediate when abdomen is slightly concave and a quarter to two- thirds opaque. Stage 4: Hungry when abdomen is flat and underside wrinkled. The Mean Hunger Stage (MHS) could then be computed by multiplying the number of flies in stages 1, 2, 3 and 4 by 1, 2, 3, 4 respectively and dividing the sum of these numbers by the sum of the numbers of flies assigned to each of these stages. This gives, for the MHS, a figure between 2 and 4. A figure between 2 and 2.9 shows a non-hungry population while a figure between 3 and 3.9 shows a hungry population.

2.13 Tsetse Ecology

Vegetation is determined by climate and soil. Apart from grasslands, which do not support tsetse flies, all forms of woodland, from savannah to rain forest, could provide suitable habitat for some species of tsetse flies, but no single vegetation type is suitable for all species. Some species (e.g. *G. p. palpalis*) adjust easily to artificial biotopes, such as coffee, cocoa and palm oil plantations. Thickets (sometimes comprising chiefly *Lantana camara*), which develop on abandoned agricultural land, are also often good habitats for them. As the type of vegetation, its photosynthetic activity over the course of the year and the rate of fragmentation of the natural habitat could easily be monitored by satellite data; these are often used to predict the distribution and density of *Glossina* species in an area.

Each species of tsetse fly has a specific distribution area characterized by climate, vegetation and soil, this last element being important for larval deposit and pupal survival. In each distribution area, it is often possible, to distinguish three zones (Mouchet *et al*., 1961). The permanent breeding sites, where flies occur all year round, the temporary breeding sites, and where they congregate in unfavourable seasons, and usually where they lay larvae, and where they cannot survive in dry season. The breeding grounds are generally open areas, not very far from the breeding sites, where flies could easily detect their hosts, and feed upon them; feeding grounds could extend a few kilometers around breeding sites, but are to their immediate vicinity. In each zone resting places of the flies vary according to environmental conditions and physiological condition of the fly, but they are usually constituted by poles, small trunks and medium sized branches, up to some meters above the ground. But some species like the *G. swynnertoni* and *G. pallidipes* rest extensively on leaves by night and on the underside of branches by day (Harley, 1960; 1963). Some species of trees afford better resting-places than areas in woody savanna selected by the majority of flies.

2.14 Survival and Longevity of Tsetse Flies

Adults of *Glossina* have been reported to have a high survival rate, in the laboratory as well as in nature. *G. p. palpalis* has been observed to live for 154 days with males living for two or three months. Unfavourable environmental conditions, predators (birds, spiders, and insects), parasites and parasitoids (fungi and protozoa) could killed the fly. Apart from death from aging, flies could also die from starvation. Larvae and pupae are more proned to death from predators (insectivorous birds, insectivorous insects, ants etc). Insects which parasitise tsetse pupae include Diptera bombylidae, Hymenoptera chalcidae and Mutillidae. Pupae are also much more easily killed by unfavourable environmental changes than adult flies which could move away. In nature probably less than half of the laid larvae reach adult stage.

2.15 Activity Cycle

Tsetse flies have been observed to completely rely on blood meals for their nutritional requirements. It thus suggested that adult tsetse must regularly make flights to contact host animals to feed off them. In female tsetse, routine activities appear to consist mainly on a cycle of finding a blood meal, resting for digestion of blood meals, larviposition and searching for another bloodmeal. In that manner, flight could consume energy up to 100 times the resting rate (Bursell, 1973), and feeding, moreover carries its own risk. Optimal feeding behavior in females thus involves balancing the requirements of getting enough blood to produce a larva, whilst using as little energy as possible, and avoiding being killed while feeding.

In male flies, the optimal behavior consists entirely of mating with as many virgin females as possible. Rates of energy replenishment must nonetheless be balanced against the risks associated with feeding and the high costs of flight. This balance for both sexes is modified by climate. Thus, when temperatures rise, metabolic rates increase in tsetse flies like in other poikilotherms. If flies

are to maintain condition and, in the case of females, the size of the pupae they produce, they must feeed more frequently, and/or modify their behavior so as to reduce body temperature below ambient. Failure to do so could lead to increases in mortality and consequent decreases in population growth rate (Hargrove, 1994 as cited in www.tsetse.org>FAQ>Pdf>hargro04).

2.16 Feeding Behaviour and Host Selection

Tsetse flies are haematophagous insects i.e. they feed only on blood of their hosts. Host seeking and selection in tsetse flies is a complex phenomenon and is central to the transmission of trypanosomiasis. While some workers claim host selection is influenced by host abundance, others feel host selection in tsetse flies depends on host availability. Some still feel, learning influences host selection in tsetse flies (Bouyer *et al.,* 2007a)

Each species of tsetse fly exhibits definite feeding preferences, but the majority of *Glossina* are able to bite a great variety of vertebrates. *G. swynnertoni, G. austeni, G. fuscipleuris* and *G. tabaniformis* have been reported to feed mainly on suids, with bovids and other vertebrates (rhinoceros, hippopotamus, and porcupine) as secondary hosts (Weitz, 1963). The *Morsitans* group flies are catholic in feeding behaviuor deriving their blood meals mainly from large bovids and ungulates whereas the *Palpalis* group feed on any available hosts including reptiles.

G. morsitans s.*l*., equally feeds mainly on suids and bovids, warthog (*Phacochoerus* spp) being the preferred host. *G. pallidipes*, *G. fusca* and *G. longipalpis* feed extensively on bushbuck (*Tragelaphus scriptus*), and the first and second species bite also suids to some extent.

G. longipennis feeds mainly on rhinoceros, with giraffe, elephant, buffalo and ostrich as secondary hosts.

G. brevipalpis feeds mainly on hippopotami, suids, buffaloes and bushbuck. *G. palpalis, G. fuscipes* and *G. tachinoides* feed extensively on man, crocodiles, lizards and bovids, the last

species being particularly infeodated to man and cattle. The behavior of each species is characteristic and is not entirely dependent on the availability of different hosts; this view is supported by the fact that commonly occurring animals are not fed on by *Glossina,* the best example being Zebra (*Equus burchelli*) in *G. morsitans* areas of East Africa and water bucks (*Kobus* sp.).

Few data are available about the adaptability of tsetse flies to a varying host fauna. It is likely that flies like *G. morsitans s.l.,* the *G. palpalis* group, and possibly *G. swynnertoni* and *G. pallidipes* would quickly adapt to changes in fauna. The anthropophily of *G. palpalis* and *G. tachinoides* increases when the wild fauna and livestock decrease (Onyiah, 1980a). However some species such as *G. fuscipleuris*, *G. austeni* and *G. tabaniformis* (infeodated to suids), *G. longipalpis* and *G. fusca* (infeodated to bushbuck and buffalo), and *G. longipennis* (rhinoceros, elephant and buffalo), could be very rapidly affected by the removal of only a few species.

2.17 Host Detection

Mechanism of host location in tsetse flies has been studied extensively and observed to be influenced by visual and olfactory factors (Onyiah, 1980b). Both visual and odour detection are used by the tsetse fly to locate its host, vision is used in short-range recognition (about 15m) while odours are responsible for attracting flies from greater distances. In any case, odours play important role in attracting flies to hosts even in the open savanna where sight is predominantly used for locating host positions. Tsetse flies detect odours by means of sensilla situated on the antennae. They are also attracted by Kairomones produced in the host's breath (e.g. carbon dioxide, acetone, 1-octen-3-0l) and in the urine of African buffaloes and cattle (phenols) (Vale and Hall, 1985b and Vale *et al*., 1988). However some tsetse are repelled by the odours of humans and some animal species e.g. waterbuck (Gikonyo *et al*., 2002). In the savanna flies, host location is by visual means

though flies could move upwind in the direction of emanating odours even when the hosts are hidden (Vale, 1978 as cited by Onyiah, 1980b).

Tsetse flies respond differently to different odours emanating from animal hosts (Gikonyo *et al*., 2002). The choice of a host may depend primarily on the host preference of the fly but, as tsetse flies are opportunistic feeders, host availability could also play an important role (e.g. in the forest *G. p. palpalis* feeds mainly on reptiles). However in West and Central Africa the most important densities of this fly are met around villages where pigs are bred. For tsetse flies of the *morsitans* group such peridomestic habits are seldom observed.

It was shown that tsetse flies, which have fed on a specific host species, have the tendency to return to the same host species for their following meals (Bouyer *et al.,* 2007a). Repeated feeding on the same host species by a disease vector is likely to increase the within-species diseases-transmission risk, but to decrease it between species. In the laboratory, feeding sterile, warm defibrinated blood through a membrane has generally been used to maintain colonies of tsetse flies. While the behavior of some *Glossina* species is considered to be catholic, *G. palpalis* is able to adapt to environmental changes and could feed on any available animal hosts including humans. Madubunyi (1990) observed that the peridomestic populations of *G. palpalis* in south eastern Nigeria obtained most of its blood meal from domestic pigs. Also Ahmed (2004) inferred that a peridomestic population of *G. p. palpalis* in Kontagora, northern Guinea savanna zone Nigeria, obtained most of its blood meals by feeding on people who visited a public park for relaxation.

2.18 Resting Behaviour

Proper selective application of insecticides in tsetse control requires sound knowledge of the resting behavior of *Glossina* species in order to optimize the contact between flies and insecticide-

treated surfaces. Thus, efficient, economic and environmentally safe control methods could be devised.

The general feature of tsetse resting behaviour is that daytime resting sites differ from the night-time ones. Males and females behave in the same manner regarding resting substrates. Substrates utilized as night resting sites mainly consist of leaves, twigs and creepers, and, to a much smaller extent, of branches, stems, petioles, trunks, roots and fruits-all as live organs of plants and never when dead. Daytime resting sites mostly consist of branches, creepers, trunks, twigs and roots-and, to a small extent, of rocks, stems and sometimes burrows. Usually, flies rest with their body parallel to the length of the plant organ and mostly on the underside of it. The head-up position is commonest on vertical organs (Abdurrahim, 1971; Van Vegten, 1971a, b; Challier, 1973; Bois *et al.,* 1977; Laveissière *et al.,* 1978; Spielberger and Barwinek, 1978; Laveissière *et al.,* 1979; Turner, 1980) and the horizontal position on horizontal organs (Onyiah, 1981). According to Challier, (1982), the general pattern of vertical distribution of resting tsetse seems to be: (a) decreasing number of flies with increasing height, (b) resting sites lower in the daytime than at night, and (c) resting sites lower in the dry than in the wet season.

2.19 Classification of Tsetse Flies

Tsetse flies belong to the Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: *Glossinidae* and Genus: *Glossina*. The genus *Glossina* comprises 34 extant species and subspecies of tsetse flies distributed across 38 countries of sub-Saharan Africa (Leak, 1999). The genus is further split into three sub groups, *Glossina* (*Morsitans* group), *Nemorhina* (*Palpalis* group) and the *Austenina* (*Fusca* group) based on some minor inherent differences in their morphological characteristics such as differences in genitalia, wing size and shape and abdominal colouration, body hairs and habitat preferences (Buxton, 1955; FAO, 1982; Gouteux *et al*., 1987; Gooding, *et al*., 1991;

Gooding and Krafsur, 2005). Members of the *Austenina* (*Fusca* group) are predominantly inhabitants of the tropical forests and are considered to be of less economic importance because they seldom come in contact with man or domestic animals. Members of the *Glossina* (*Morsitans*) and *Nemorhina* (*Palpalis*) groups are the major vectors of Nagana or Animal African Trypanosomiasis (AAT) in livestock and sleeping sickness or Human African trypanosomiasis (HAT) in humans respectively, thus constituting a great public health hazard (Jordan, 1986, Abila *et al.,* 2008).

2.20 Distinctive Characteristics

Here attempt is made to describe some external characters of the different *Glossina* species by means of which they could most easily be distinguished as well as providing some information on where they could be found. The following description is according to the identification keys as edited by FAO: (ftp://ftp.fao.org/docrep/fao/011/i0535e/i0535e03.pdf)

2.20.1 *G. palpalis* group

Tsetse flies of the *palpalis* group are of small to average size (6.5 to 11mm). Their abdomen is uniformly brown or grey with some dark spots (except *G. tachinoides* of which the abdomen often resembles that of a *morsitans* fly). The male genital amature comprises divergent superior claspers joined by an intercalated membrane. The tarsi of the hind legs are uniformly dark (Figure 2.5c and d). Bristles on the pteropleuron are composed of rather small, uniform hairs, which are much shorter and weaker than those on the sternopleuron. Straight hairs cover the fringe of the thoracic squama. *Palpalis* flies are mainly species living in the forests or in riverine or lakeside habitats; they are diurnal; apart from domestic animals they often feed on reptiles and birds and are more or less anthropophagic. The *Palpalis* group includes five species and four sub-species and is restricted to West and Central Africa, with relics in Ethiopia and Arabia.

2.20.1.1 *G. tachinoides*

This species occurs in the Sudanese savanna and in the southern part of the Sahelian area of West Africa with residual foci in North-Eastern Africa and could withstand very dry weather conditions but congregates in the dry season around water holes, residual pools of temporary rivers etc.

2.20.1.2 *G. palpalis* and *G. fuscipes*

These species occur in the forest as well as in the Guinean and Sudanese savannahs, but in this last environment they are restricted to rivers and rivulets in the neighbourhood.

2.20.1.3 *G. pallicera* and *G. caliginea*

These species occur in the high forests but the first may be caught in the gallery forests of the Guinean savannah.

2.20.2 *G. morsitans* group

Tsetse flies of the *morsitans* group are small or medium sized (7.5 to 11mm) and the abdomen is usually coloured with dark spots on a yellow background. *Glossina austeni* has a small size compared to the other flies of the group. Male genital amature comprises convergent claspers with an intercalated membrane. Only the last two segments of the hind tarsi are darker than the more proximal segments (Figure 2.5a and b), except in *G. austeni* where the tarsi could be uniformly dark. Bristles on the pteropleuron are composed of rather small, uniform hairs, which are much shorter and weaker than those on the sternopleuron. Straight hairs cover the fringe of the thoracic squama. The *morsitans* species are primarily found in savannah woodland and evergreen thickets. They are diurnal, feed on wildlife or livestock and are more or less anthropophagic.

This group includes seven species and three sub-species, all inhabiting savannahs.

G. morsitans complex occurs mainly in Sudanese savannah with *G. m. submorsitans* in West and Central Africa and *G. morsitans* in East Africa. *Glossina longipalpis* is restricted to the Guinean

savannahs of West Africa. *G. austeni* occurs mainly in the coastal areas of East Africa. *G. swynnertoni* and *G. pallidipes* are highland species of East Africa, the first being restricted to Kenya and Tanzania and the second species occurring from Ethiopia to Mozambique and being present in some coastal areas. *G. pallidipes* has been eradicated from Natal and *G. borgesi* has been recently described from Mozambique.

2.20.3 *G. fusca* group

Tsetse flies of the *fusca* group are large (10.5 to 15.5mm) and the abdomen is generally of dark and uniform colour. The genital armature comprises divergent claspers not joined by an intercalated membrane. Either all or only two last segments of the hind tarsi are uniformly dark dorsally (FAO, 2008). On the pteropleuron (a plate on the side of the thorax under the insertion point of the wing) short, thin hairs are intermingled with longer, stronger hairs. Silks that cover the fringe of the thoracic squama are long and corrugated and appear muddled and disordered.

The *fusca* group includes 13 species and two sub-species which inhabit mainly densely forested areas (except *G. longipennis* and *G. brevipalpis*), are most active at dusk and mainly feeding on vertebrate animals. *Glossina nashi, G. schwetzi, G. tabaniformis, G. haningtoni, G. severini, G. vanhoofi* and *G. fuscipleuris* occur in the lower Guinean forests, sometimes in very restricted areas. *Glossina fusca* occurs in all forested areas from Sierraleone to Uganda, and *G. nigrofusca* is wide spread in the upper Guinean forests and in the northern part of the lower Guinean forest, from Guinea to Uganda. *G. medicorum* is restricted to the Upper Guinea, sanannah and forest *G. brevipalpis* is very widespread in gallery forests and savannahs of East Africa from Natal to Eastern Congo (Léopoldville) and to Southern Ethiopia and Somalia. *G. longipennis* is also an Eastern Africa savannah species but restricted to Kenya, Ethiopia, Southern Somalia, South-Eastern Sudan, North-Western Uganda and Northern Tanzania.

2.21 Tsetse Fly Distribution and Abundance

2.21.1 Historical trends

The origin of tsetse flies is not known; different sources give different accounts of the real origin of tsetse flies. The Biblical account in Isaiah Chapter 7 verses 18 - 19, in New King James Version of The Holy Bible (Thomas Nelson, 1982 edn), states "In that day the LORD will whistle for the fly that is in the remotest part of the rivers of Egypt and for the bee that is in the land of Assyria. They will all come and settle on the steep ravines, on the ledges of the cliffs, on all the thorn bushes and on all the watering places". From the evolutionary point, the origin of tsetse flies has been captured more succinctly by Krafsur (2009). The existence of tsetse flies predates recorded history as shown by the discovery of fossils of the flies in Florissant shale of Colorado, dating from 35million years ago (MYA) (Grimaldi, 1992) and from Oligocene strata in Germany (Grimaldi and Engel, 2005). It is assumed that tsetse had nearly a worldwide distribution some 30 to 40 million years ago. It seems, therefore that tsetse flies would probably have evolved predating continental separation in the Cretacious more than 100 million years ago (Krafsur, 2009).

2.21.2 Speciation in tsetse flies

Speciation is the evolutionary process by which reproductively isolated biological populations evolve to become distinct species. The term was first coined by F. Cook to describe the splitting lineages or cladogenesis as opposed to anagenesis or phyletic evolution occurring within lineages. The role of natural selection in speciation was first described by Charles Darwin. Four geographic modes of speciation occur in nature based on the extent to which speciating populations are isolated from one another, allopatric, peripatric, and parapatric and sympatric. Speciation could also be induced artificially through animal husbandry, agriculture or laboratory experiments.

2.21.3 Phylogeny of *Glossina*

According to Gooding and Krafsur (2004), 34 extant taxa have been described of 23 species that include five species complexes with well defined habitats in terms of satellite imagery derived variables. Evidence exists that the genus *Glossina* is ancient. Examples of fossil of four species have been traced from sedimentary Shales in Colarado USA (Cockerel, 1918)

There are speculations from a number of authorities on the relationship between the fossil and extant species of *Glossina*, and on the likely distribution of the genes in earlier geological periods. Current opinion on this subject analysed by Ford (1970), suggest that, the fossil species were closely related to the present day species of the *Fusca* group as postulated by Evens (1953). This was disputed by Machado (1959), submitting that the paleontological evidence reveals no more than that the genes have shown considerable variation for a long period of time and that is previously extended beyond its present range. Evens (1953) suggested that the living members of the *fusca* group show more primitive features than the species of the *Palpalis* and *Morsitans* groups. Newstead *et al.,* (1924) considered that the superior claspers of the male become progressively more complicated through the groups *fusca-palpalis-morsitans*. Bursell (1958) also considered the modern *fusca* group to be the most primitive. He postulated that the ancestral *Glossina* inhabited a humid forest environment as do most members of the *fusca* group of today. Bursell also suggested that divergence from this ancestral type was associated with increased water-proofing of the puparia, resulting in speciation and evolution of forms able to survive in drier environments. He saw evolution as progressing from a predominantly xerophyllic *morsitans* group. *Glossina longipennis* and *G. austeni*, were considered as examples of reversion to drier or wetter environments after the adoption of a basic habitat, by the group to which they belong.

Superior claspers appear to become progressively more complex for the *fusca* group to the *morsitans* group (Machado, 1959). The complicated shapes of many species of the former could be considered as possessing the most primitive features and those of the *fusca* group, the most specialized ones. It has been shown that species of the *morsitans* group are morphologically much closer to *gasterophilus* species of the *fusca* group. This could be based on the knowledge of the origin of the extant *gastrophilidae* and *Glossinidae* in a basic gastrophilid stock.

2.21.4 *Glossina* distribution

Abundant information is available on significant interactions between tsetse flies and their environment, especially regarding climate, vegetation and fauna. The biology and ecology of tsetse flies has been comprehensively reviewed by Leak (1999). Tsetse flies are distributed discontinuously spanning an area approximately 9-10 million Km^2 across 38 countries of sub-Saharan Africa. The discontinuous distribution of tsetse flies across the African continent is governed by climate particularly temperature, relative humidity and altitude (Nash, 1937). A humid atmosphere makes it possible for tsetse to move away from the protected habitats where it survives during harsh weather conditions. For instance, *G. tachinoides* and *G. palpalis gambiensis* in the rainy season have been observed to leave the gallery forests in the Sudanian savanna and *G. pallidipes* leaves the Lambwe Valley National Park in Kenya and reach the surrounding arable land. Temperatures above 38ºC and below 17ºC are detrimental to adult flies. They could seek shelter as soon as the temperatures reach 35ºC (negative phototactism). At these temperatures the fat reserve of the pupa becomes also easily exhausted.

The distribution area of the genus *Glossina* is restricted to the lowland rainforest and woody savannah regions of Africa south of the Sahara (Figure 2.10). Some species had also been observed in south-west Saudi-Arabia. To be precise, tsetse flies may be found between latitude 14 degrees

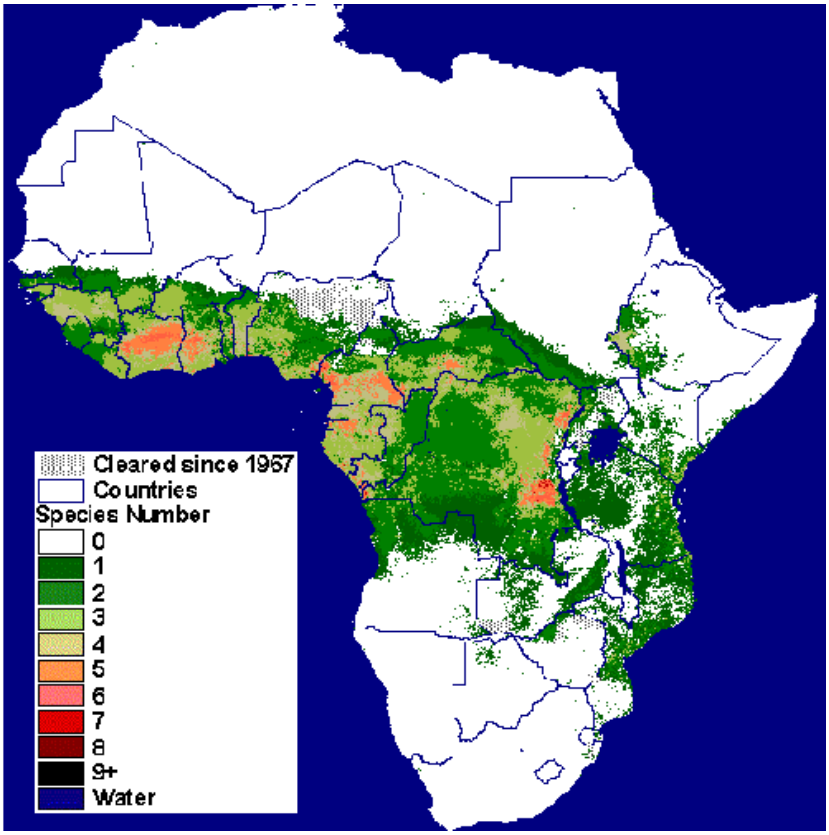


Figure 2. 10: Tsetse distribution in sub-Saharan Africa

(Source: http://www.tsetse.org/FAQ/Pics/allspec.gif)

North and latitude 29 degrees South (Buxton, 1955). The distribution is however not uniform but often patchy. Country lying below the 500mm isohyets does not support tsetse flies unless there are watercourses with some vegetation along them (forest galleries). Mountains exceeding 1600 meters of height generally form an insuperable obstacle for tsetse flies. However it has been established that it is not the altitude itself which hinders colonization by tsetse, but rather the low temperature prevailing at these heights. Recently, however, *G. m. submorsitans* has been observed in Ethiopia and Cameroon at a height of 2000 meters, corroborating the hypothesis of global warming of the earth. Likewise in Nigeria, Dede *et al.,* (2005) observed *Glossina palpalis palpalis* populations on the highlands of the Jos plateau, suggesting that the highland might have lost its previous status as a tsetse free zone. For the purposes of emphasis, within the confines of its distribution in much of sub-Saharan Africa, the distribution of the genus *Glossina* in nature is discontinuous and patchy due to factors involving climate and animal host distribution and abundance (Rogers, 1998). *G. morsitans* appears to have the widest distribution, extending from Senegal in West Africa, easterly to the lowlands in Ethiopia and southwards to Botswana, Zimbabwe and Mozambique (Krafsur and Endsley, 2002). Each sub-species is charaterised by a disjunctive distribution (Rogers, 1998). *G. morsitans s l* consists of three subspecies, *G. m. morsitans* Westwood, *G. m. centralis* Machado and *G. m. submorsitans* Newstead. The geographical distribution of subspecies is allopatric. *G. m. submorsitans* occurs in the north from below the Sahel from Senegal to Ethiopia. *G. m. morsitans* occurs in Tanzania to Mozambique and eastern Zambia and Zimbabwe. *G. m. centralis* occurs in Angola, northern Botswana, Uganda, Zaire and western Zambia (Jordan, 1993).

G. tachinoides is distributed from Mali, Ghana, Guinea, Ivory Coast, Burkina Faso, Togo, Benin, Nigeria, Cameroon, in the west, Southern Sudan, Chad, Niger in the north and Central African Republic to Ethiopia in the east (Krafsur and Endsley, 2002). *G. palpalis* complex is composed of two subspecies, *G. palpalis palpalis* and *G. palpalis gambiensis* which show high levels of mitochondrial sequence divergence and postzygotic isolation, with F1 males of intersubspecific crosses being sterile (Gooding, 1997). The subspecies are almost entirely allopatric, with *G. p. palpalis* having a more southerly distribution. Challier *et al.,* (1983) proposed that the subspecies diverged from an ancestral *palpalis* following the contraction of their riverine forest habitat during a dry period at the last glacial maximum around 19 000-13 000 years ago. Mating experiments between colonies of *G. p. palpalis* originating in Nigeria and the Democratic Republic of Congo (DRC) yielded sterile hybrid males, suggesting that *G. p. palpalis* itself may consist of more than one subspecies (Gooding *et al.,* 2004). A molecular phylogenetic study of the *palpalis* group produced results consistent with this hypothesis, as *G. p. palpalis* from DRC and West Africa had divergent Cytochrome Oxidase I(CO1) gene sequences and ribosomal Internal transcribed spacer1(ITS-1) lengths (Dyer *et al.,* 2008). Interestingly, *G. p. palpalis* from Equatorial Guinea had ITS-1 length polymorphism, containing copies of the same size as both West African and DRC flies, suggesting the possible presence of hybrids in this population (Dyer *et al.,* 2008). *G. palpalis s.l.* occurs in Angola, Benin, Cameroon, Central African Republic, Congo Peoples Republic, Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea Bissau, Ivory Coast, Liberia, Mali, Nigeria, Senegal Sierra Leon Togo and Burkina Faso.

The limits of tsetse distribution and abundance are influenced by environmental factors such as temperature, relative humidity, elevation, rainfall and vegetation cover (Rogers, 1977; Brightwell *et al.,* 1992; Robinson *et al.,* 1997a; 1997b). Host availability also plays a part in determining local

distribution and abundance of flies (Turner, 1981; Rogers and Boreham, 1973). Tsetse flies move randomly in their habitats (Bursell, 1970). Such random movement is able to cause an even distribution of tsetse flies and homogenize gene frequencies among subpopulations. However the above listed environmental factors could cause uneven distribution of tsetse flies between vegetation types. For example, such uneven distribution in Zimbabwe's Zambezi valley was attributed to extreme climatic conditions during the hot dry season, which made riverine woodland more suitable to tsetse (Hargrove and Vale, 1980).

Satellite data could be used to predict suitable habitat for *G. m. centralis, G. morsitans* and *G. pallidipes* and indeed they have been used to infer the environmental factors that shape the distribution of these species in Zimbabwe and Zambia (Robinson *et al.,* (1997a). Tsetse distribution cuts across various ecological zones and different models and variables are required to describe the distribution across these different zones. This may suggest that tsetse populations exist in geographically isolated units or subpopulations in which flies are adapted to the local environment. Indeed, genetic data show that tsetse populations are highly differentiated, consistent with a discontinuous distribution (Krafsur *et al.,* 1997a; Krafsur and Wohlford, 1999; Krafsur and Endsley, 2002). Whereas genetic data show pattern of restricted gene flow, ecological data show that tsetse of the *morsitans* group have great capacities for dispersal (Vale *et al*., 1984; Williams *et al.,* 1992).

2.21.5 Tsetse distribution in Nigeria

Tsetse flies are distributed discontinuously in Nigeria spaning an area approximately 75-80% of the country's 928,300 square Kilometers total land mass (Figure 2.11). They are found in areas from the coast to latitude 12-13^{o}North with extensions along Hadejia-Jama'are hydrographic networks (Davies, 1977; Onyiah *et al*., 1983). Due to the phenomenon of global warming, tsetse

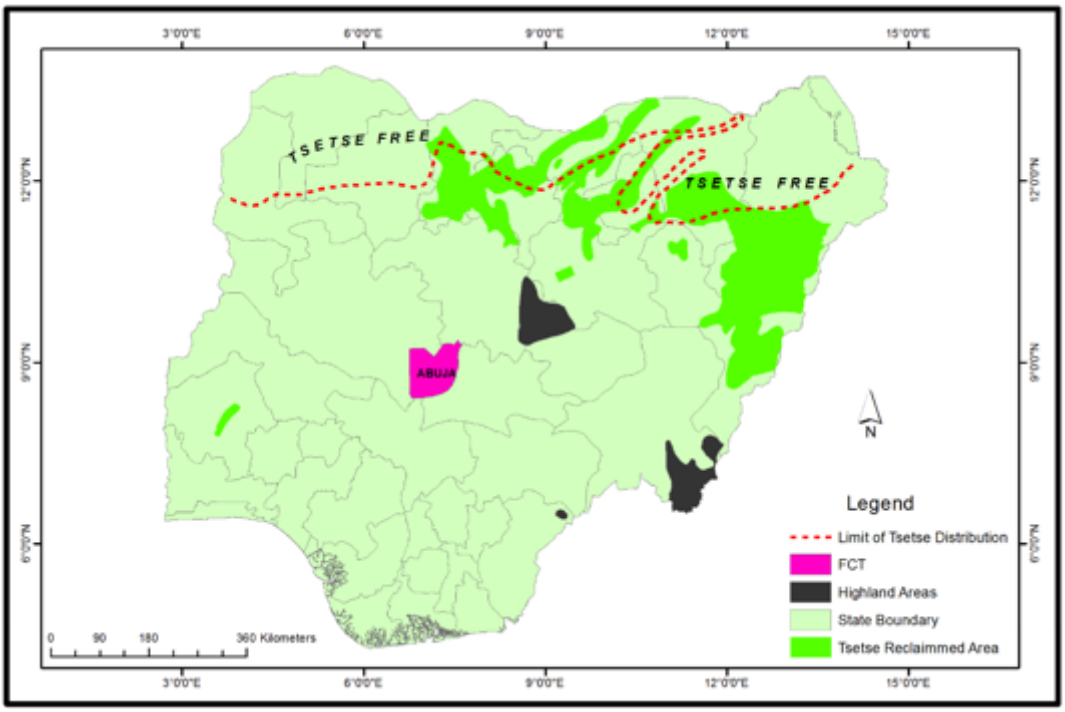


Figure 2. 11: Tsetse Distribution in Nigeria (Adapted from Ford 1970)

flies have recently been observed to exist on the Jos Plateau, previously regarded as tsetse free (Jordan, 1989; Kalu, 1996; Dede *et al.,* 2005). Climatic factors particularly relative humidity and temperature (Appendix XXXVII) and vegetation type (Appendix XXXVIII) play major roles in the distribution of tsetse in the country.

There are 11 different species and subspecies of tsetse flies in Nigeria which have further been grouped based on their habitat preferences to include the forest (*Fusca* group), riverine (*Palpalis* group) and the savanna (*Morsitans* group) flies (Table 2.3). In order to give an insight into the various types of habitats that these flies may be found, a brief description of the habitats of representative members of these groups is given as follows:

The *Fusca* group (forest flies) are predominantly forest dwellers occupying both rain forest and riverine forest in the savanna zones (Appendix XXXII). In many instances suitable habitats for the *Fusca* group flies are fast disappearing due to the influence of anthropogenic factors, such as logging and poaching of wild animal hosts. They seldom come in contact with humans or livestock and are considered of less economic importance. They derive most of their blood meals from red river- hug in the rain forest or bush buck in forest outliers of the savannah. The group is represented by *Glossina fusca* and *Glossina medicorum* as illustrated (Appendix XXXII).

The *Palpalis* group (Riverine flies) are found in riparian vegetations at the banks of rivers/stream systems, where precipitation is within 1000-2000 mm of rainfall, and mean temperatures of 25

Table 2.3: *Glossina* species known to be present in Nigeria according to their habitat preferences

Fusca group – Forest flies	*Palpalis* group – Riverine flies	*Morsitans* group – Savanna flies
Glossina tabaniformis Westwood (1850)	*Glossina palpalis palpalis* Robineau Desvoidy (1830)	*Glossina morsitans submorsitans* Newstead (1910)
Glossina tabaniformis Westwood (1850)	*Glossina tachinoides* Westwood (1830)	*Glossina longipalpis* Wiedmann (1830)
Glossina medicorum Austen (1911)	*Glossina pallicera pallicera* Bigot (1891)	
Glossina fusca congolensis Newstead and Evans (1921)	*Glossina caliginea* Austen (1911)	
Glossina haningtonii Newstead and Evans (1922)		

(Adapted from Baldry, 1967)

±2°C and 80-85% R.H. They are found in dense vegetation along watersides with closed canopy from both sides to prevent wind from penetrating. The shade that ensures as a result of the canopy allows temperature to remain lower in the afternoon and higher at night than the surrounding environmental conditions in the open savannah. At extremely dry conditions when temperatures are high, flies find resting places close to water bodies. In the dry season when most streams dry up flies leave and concentrate in areas where humidity is high. Other habitats where *Glossina palpalis* group flies may be found include sacred grooves where natives perform prayers to their gods. Such places are characterized by patches of vegetation consisting of large old trees, dense creepers and shrubs, often around water holes or flooded areas. The Mangrove swamps form the lower limit of *G. p. palpalis* distribution in Nigeria. Lake shores with gallery forests as found in the savannah. Other atypical habitats include coffee, cocoa and banana plantations and peridomestic situations (Madubunyi, 1990; Ahmed, 2004). The *Palpalis* group derive most of their blood meals from monitor lizard (Varanus), crocodile and man visiting the habitat for fishing, cutting wood, hunting or farming at the bank of rivers/streams. They are constantly in contact with humans and livestock and are of high economic importance. The group is represented by *Glossina tachinoides* (Appendix XXXIII) and *Glossina palpalis palpalis* (Appendix XXXIV) as illustrated.

The *Morsitans* group (Savanna flies) is found in the open woodland savannahs where the mean annual rainfall is between 800 and 1350mm (Rogers and Randolph 1986), where it is neither very humid nor very dry. Their distribution is patchy or more appropriately in broken belts where they are found. At extremely dry weather conditions, *Glossina morsitans* may behave as riverine flies by concentrating in cooler and moister part of the habitat to escape from the worst effect of the dry season. The typical habitat of *G. m. submorsitans* in Nigeria, is open woodland and woodland savannah in which the dominant tree is *Isoberlina doka* or its close relatives, with plenty of game

animals living in the area. In the more northernly zone, *G. m. submorsitans* is found in areas in which the dominant trees are *Anogeissus, Balanites, Lannea, Combretum* and certain *Acasia* species. In the southern Guinea and derived savannah zones, *G. m. submorsitans* is found in vegetations dominated by *Daniella, Hymenocardia, Detarium, Afzelia, Lophira,* and *Terminalia* trees. In the absence of game, *G. m. submorsitans* may derive most of its blood meals from livestock eg cattle. They are constantly in contact with humans and livestock and are equally of high economic importance as vectors of AAT. In Eastern and Central Africa, *G. morsitans* is an important vector of *Trypanosoma rhodesiense* causing sleeping sickness in man. The range of distribution of the group represented by *G. longipalpis* (Appendix XXXV) and *G. m. submorsitans* (Appendix XXXVI) in Nigeria is as illustrated. *G. m. submorsitans* is not found in very high rainfall areas such as rain forest and mangrove swamp

In Nigeria, AAT is cyclically transmitted by *G. m. submorsitans, Glossina longipalpis, G. p. palpalis* and *Glossina tachinoides.* AAT is also transmitted mechanically by biting flies eg tabanids like in the case of *T. evansi* in camels, or sexually in the case of *T. equiperdum* in horses (Isaac *et al*., 2016). While HAT is transmitted by *Glossina p. palpalis* and *G. tachinoides*.

2.21.6 Tsetse flies in Nigeria National Parks and Game Reserves

National parks are protected areas which can be defined as lands and waters with defined boundaries established primarily to permanently protect natural heritage features such as unique landforms, plant communities and animal life. In Nigeria, protected areas include; game/forest reserves, national parks, strict nature reserves, ramser sites, etc. They make a significant contribution to maintaining ecosystem health and diversity.

Protected areas, protect habitats for wildlife, including endangered species and diversify local and regional economies by supporting tourism, especially ecotourism. They support forest "certification" that helps companies make their practices sustainable and market their products. In addition, they preserve genetic materials that have potential for new foods, medicines and other products and therefore have important scientific and educational value.

The prominent protected areas are the National Parks, managed by the Nigeria National Park Service (NNPS). The eight National Parks namely, Chad Basin, Cross River, Gashaka Gumti, Kainji, Kamuku, Okomu, Old Oyo and Yankari Game Reserve, cover a total land area of approximately 20,156km^2, or about 3% of Nigeria's total land area (Fig 2.12., Table 2.4). The three major natural resources in the protected areas are biodiversity, forests and freshwater.

The Nigeria Government's policy and goal on the conservation of biodiversity are to ensure sustainable use of forest resources and preservation of the many benefits accruing from soil, water, and wildlife conservation for economic development. Among the current priority programmes are the extension of National Parks and Reserves and the compilation of the flora and fauna of Nigeria

The policies and activities of the Federal Government on the three major resources (Wildlife, forests, freshwater) of protected areas are vital in addressing the environmental considerations on *Glossina* control in these areas. Since *Glossina* control under the auspices of the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) is to be implemented based on the principle of the rolling carpet, emphasis has to be shifted to protected areas were tsetse populations have been reported to be highest.

In Nigeria, the expansion in human population has led to the reduction of areas previously occupied by tsetse flies, restricting their distributions to the National Parks/Game Reserves. Presently

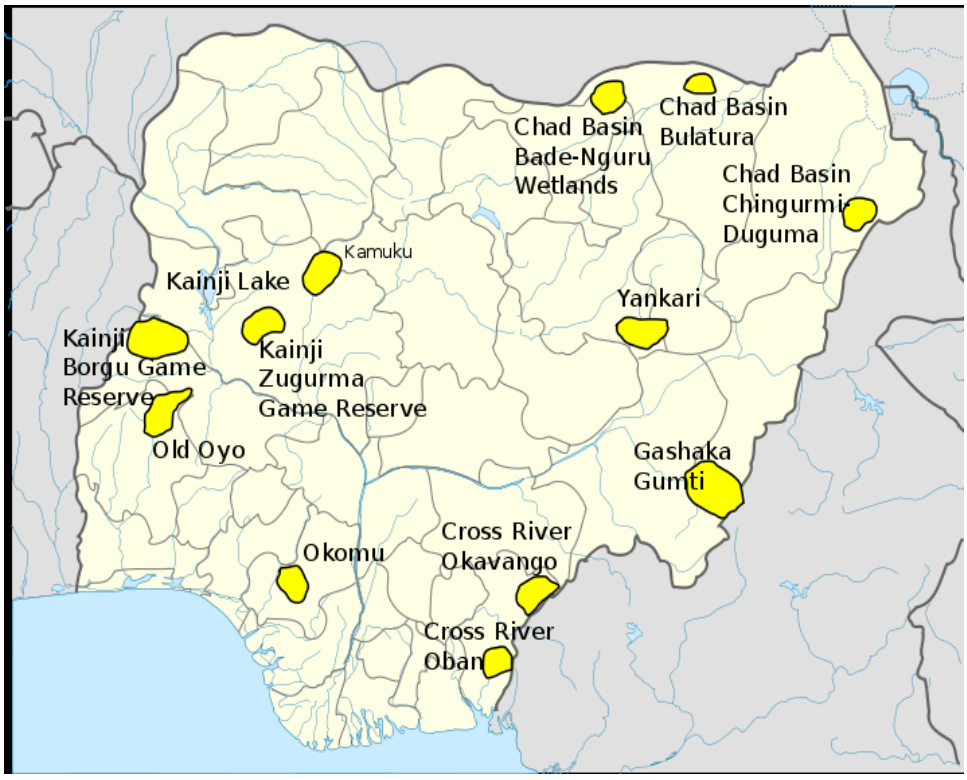


Figure 2. 12: Location of the National Parks of Nigeria

(Source: *http://en.wikipedia.org/wiki/st_of_national_parks_of_Nigeria,* 2017).

Table 2.4: Co-ordinates of locations and some characteristics of Nigeria National Parks and Game Reserve

S/N	National Park/Game Reserve	Year established	Area (Km^2)	Co-ordinates	State/ Ecological zone
1	Old Oyo	1999	2 512	Lat.8º10′and 9º05′N and Long. 3º00′ and 4º02′E.	Oyo Derived savanna
2	Kamuku	1999	1 121	Lat. 10º45′N and Long. 6º30′E	Kaduna/ Northern Guinea
3	Chad Basin	1991	2 258	Lat. 11º00′and13º00′N and Long. 13º00′ and 15.30′E	Borno/Yobe Sudan savanna
4	Cross River	1991	4 000	Long. 5º05′and 6º29′N and Lat. 8º15′and9º30′E.	Cross River/ Rain forest
5	Gashaka-Gumti	1991	6 731	Lat.6º55′ and 8º12′N and Long. 11.11′ and 12º.13′E.	Adamawa/Taraba Northern Guinea savanna
6	Okomu	1991	202	Lat. 6º.15′ and 6º25′N and Long. 5º9′ and 5º23′E	Edo/ Rain forest
7	Kainji Lake	1979	5 382	Lat.9º45′and10º23′N and Long. 3º41′ and 5º47′E	Niger/Kwara Southern Guinea
8	Yankari Game Reserve	1962	2 244	Lat.09º.50′N and Long.10º.30′E	Bauchi/ Northern Guinea

National Parks and Game Reserves, hold the highest density of *Glossina* populations in the country.

2.22 Dispersal in Tsetse Flies

Dispersal of tsetse flies simply means movement of flies from infested to unfested areas for the purposes of colonization. These movements are influenced by environmental factors such as temperature, relative humidity, elevation, rainfall and vegetation cover and host availability resulting in the uneven distribution and abundance of tsetse flies (Randolph *et al*., 1991).

Early estimates of rate of dispersal in tsetse flies depended on classical mark- release- recapture method of Jackson (1944, 1946). Jackson concluded from such experiments that tsetse dispersal involved to-and-fro ambit movement between resting sites and feeding grounds. This observation was however countered by Bursell (1970) who observed that catches from bait cattle gave different information on fly distribution and movement than previously provided by man-fly rounds. Further Bursell (1970), suggested that tsetse movement could be explained by a random walk model. The idea of random movement was upheld by Rogers (1977) who fitted a random walk model to Jackson's 1946 data and to his own mark-release-recapture data on *G. fuscipes fuscipes and G. morsitans morsitans.* Subsequent re-examination of Jackson's 1946 data showed they conformed well to a simple diffusion model (Hargrove, 1981; Hargrove and Lange, 1989).

It is predicted that the mean rate of frontal advance of *G. morsitans* group flies is of the order of 200 m day^{-1} or 7 km $year^{-1}$ (Williams *et al.,* (1992). In a theoretical study of re-invasion of cleared areas by *G. pallidipes,* Hargrove (2000) suggested that areas as large as 10,000Km^2 should be treated to minimize reinvasion. In summary, tsetse dispersal may be modeled as a random

movement or as a diffusion process. Additionally, savanna group tsetse flies are highly vagile (Ouma, 2004)

From the fore going it is pertinent to note that the distribution of tsetse in nature is homogenous, but the distribution becomes disjointed due to habitat fragmentation (Van den Bossche *et al.*, 2009). Apart from physical features like mountains, oceans and rivers, anthropogenic factors also have great influence on the distribution of *Glossina* species. Most of the great forests have been destroyed forcing the forest flies (*Fusca* group) to either go extinct or new species evolving as a means of adaptation to new conditions. Dispersal of tsetse flies is always for exploitation of new grounds during favourable conditions (immigration) or for escape from harsh weather conditions (emigration). Dispersal in *morsitans* group flies is said to be limited due to the patchy nature of its distribution. However, *G. palpalis* is considered to have the widest distribution since its movement is horizontal following the hydrographic networks. In the past the classical mark-release-recapture method of Jackson (1944, 1946) was used to estimate extent of dispersal in tsetse flies. But because of the challenges involved in the technique whereby not all individual flies released to the environment may be recovered, it was difficult to determine the extent of dispersal of a target species. Genetic methods can now be used in predicting fly movements; barriers to gene flow and exchanges, as well as assessing risks of possible recolonisation from neighbouring areas where control is not implemented (Echodu *et al.,* 2013). Of equal importance is the spatial delineation, temporal stability and size of the targeted population. All these attributes could be defined by genetic tools. This information could be used in defining the effectiveness and appropriate scales of control measures that have become a vital tool in guiding the implementation of tsetse control strategies.

2.23 Disease Transmission

2.23.1 Cyclic transmission

The transmission of trypanosomes by tsetse flies occurs cyclically. That means that the fly after taken a blood meal from an infected host, does not immediately become infectious but that the pathogen during "the extrinsic incubation period" has to pass through a developmental cycle inside the vector. During this period the trypanosome undergoes substantial morphological and metabolic changes. The developmental cycle of *T. vivax* is restricted to the mouthparts and lasts 5 to 14 days. *T. congolense* develops in the midgut and the proboscis and its development takes generally about 12-18 days. *T. brucei* has the most complicated developmental cycle with trypanosomes migrating from the midgut to the salivary glands of the tsetse fly. The cycle takes between 17 and 45 days.

The basic reproductive rate of an infection R_0 is the mean number of secondary cases caused by an infected individual into a population with no pre-existing immunity to the disease and in the absence of control interventions. Reproductive rate is obtained by a simple formula (Macdonald, (1957) and Smith *et al.,* (2007) $R_0 = \frac{\mathrm{Ma^2\ b\ c\ p^2}}{\mathrm{r\ g}}$

Where

m = rate of vectors to hosts (amongst others depending on the space utilization of the host and alternative hosts in relation to tsetse mobility and distribution).

a = feeding rate of the vector on the host (depends on the feeding rate "f" at that moment, and also on the feeding preferences "q" of tsetse flies for a particular host) so a = fq

b = probability that a host could become infected once bitten by an infected vector

c = the probability that the vector becomes infected from a bite on an infected host

p = daily survival rate of the vector, so p = e-g and the death rate g = -ln(p)

g = death rate of the vector

n = extrinsic incubation period (number of days before the vector becomes infectious)

r = the rate with which the host cures the infection.

Factors affecting m;

- The rate of vectors to hosts (m) may decrease as a result of vector control, disappearance or fragmentation of tsetse habitat, or a massive immigration of cattle in an area (e.g. temporarily available pasture land)
- The rate of vectors to hosts (m) may increase as a result of livestock movements (e.g. cattle moving into game reserves),
- Particularly beneficial climatic conditions for tsetse (at the end of the rainy season the tsetse fly population density is generally at its top because of an increased reproduction in the previous months and an increased longevity at that time).
- Tsetse flies seeking refuge in the forest galleries during the hot dry season resulting in a temporarily increased transmission risk for cattle at watering points. Notwithstanding the tsetse population is not at its highest density at that time high trypanosomiasis incidences could be observed.
- Sudden immigrations of tsetse flies (this may be particularly high in case of tsetse control operations when the control area is not isolated making the control effort often unsustainable).

Factors affecting a:

This important parameter depends on the number of feeds taken by the vector per unit of time, which could increase when the climate becomes dry and hot, but it depends also on the feeding prefences of the flies.

Furthermore it was demonstrated that upon colonization of the tsetse salivary glands with *T. brucei,* the anti-haemostatic activity of the saliva changes resulting in a prolonged probing/feeding time, which could result in an increase of interrupted feeding and thus contribute to an increased parasite transmission (Van den Abbeele *et al*., 2010). Increased probing activity have been observed in *T. congolense* infected *G. morsitans* flies, which could possibly be due to physical interference of the parasite with phagoreceptors in the proboscis in combination with a reduced diameter of the tsetse labrum due to the presence of parasite rosettes (Livesey *et al*., 1980).

With regard to b: This may depend on the "trypanotolerance" or "susceptibily" to trypanosome infection of the host. Trypanotolerant animals are more difficult to infect (smaller value of "b") and once infected they could clear the infection more rapidly, resulting in a larger value for the parameter "r". The parameter "b" could also be reduced when prophylactic drugs are used.

With regard to c: this may depend on the vectorial competence (ability to become infected) and vectorial capacity (the ease with which the development cycle in the vector is completed) of the vector. In most tsetse flies taking up trypanosomes, these protozoa could be killed by the combined action of proteases, lectins, immunopeptides and oxidative stress (Lehane, 2004; Macleod, 2007). Young flies infect themselves more easily with trypanosomes than old ones. However, it was shown that even older flies may show a similar vectorial capacity in case the vector undergoes a stress of some kind which affects the fly's immune gene expression (Akoda *et al*., 2009). The *morsitans* group flies have higher infection rates than those of the *palpalis* group (except for *T.*

vivax in West Africa). The *fusca* group is generally a poor vector for the *T. brucei* group of trypanosomes but experiments showed it to be a good vector for *T. vivax* and *T. congolense*.

With regard to p and g:

These depend on the longevity of the vector, which could be influenced by environmental conditions (climate, host availability, tsetse density dependent mortality) and possibly by local tsetse control operations.

With regard to n:

To become infectious the longevity of an infected tsetse fly must be superior to the extrinsic incubation period of the trypanosome. This period varies according to the trypanosome species and the environmental conditions (e.g when temperatures are rising, the pathogen could develop more rapidly in the vector).

2.23.2 Non-cyclic transmission (Mechanical transmission)

This mode of transmission refers to the direct transmission of trypanosomes from an infected host into an uninfected host without the parasite undergoing any form of development. It is similar to mechanical injection of trypanosome parasites into a host with a syringe. Mechanical transmission requires that tsetse feed on an infected host and acquire trypanosomes in the bloodmeal, and then, within a relatively short period, tsetse feed on an uninfected host and regurgitate some of the infected blood from the first blood meal into the tissue of the uninfected animal. This type of transmission occurs most frequently when tsetse are interrupted during a blood meal and attempt to satiate themselves with another meal. Other flies, such as horse flies also transmit trypanosomes mechanically (Cherenet *et al*., 2004).

2.24 Vectorial Capacity and Transmission Potential

The vectorial capacity of a target *Glossina* species is its ability to acquire infection, whereas the ability of a fly to transmit infection is its potential. Vectorial capacity depends on several factors including, the population dynamics of the fly, survivorship, species diversity, genotype, sex, age, hunger stage, seasonal changes, habitat/vegetation type, anthropogenic factors, availability of hosts, host seeking behaviour and selection and the presence of symbiotic organisms in the fly. These factors have been shown to have an impact on the vector competence of tsetse flies. Once established, parasites migrate to the mouthparts where they differentiate into epimastigote forms and, finally, into infectious metacyclic forms (maturation step), which could then be transmitted to mammals by the fly during its blood feeding (Vickerman *et al*., 1988; Kazadi *et al*., 1998). However, only a small part of tsetse flies showing midgut infection develop mature infection and are capable of transmitting the disease (Maudlin and Welburn, 1994). This ability to acquire the trypanosome, favor its maturation, and transmit it to a mammalian host is known as the vectorial competence. A large variability in vectorial competence has been recorded between different species of *Glossina.* It is known that the *Morsitans* group flies carry higher infection rates than the *Palpalis* group (Jordan, 1976).

2.25 Infection Rates in Tsetse

Infection rates in tsetse flies may be determined by examining dissected mouth parts, salivary glands and gut of tsetse flies for trypanosome parasites. The process involves removal of the wings of freshly killed flies and placing them with ventral side up under a dissecting microscope in 0.9% normal saline. A cover slip is placed on each part of the slide where the proboscis, salivary gland, or the midgut (including the proventriculus) are placed, and trypanosome infections in the tsetse are identified using a compound microscope at x400 magnification according to the methods of

Lloyd and Johnson (1924). Parasites are then identified based on their location in the dissected tissues. Parasites found in the mouth parts only belong to the *Duttonellla* group (*Trypanosome vivax*-type infection), those in the mouth parts and midguts *Nanomonas* (*Trypanosoma congolense*-type), and those found in the mouth parts, midgut, and salivary glands are regarded as belonging to the *Trypanozoon*, (*Trypanosoma brucei* – type), immature infections, when only the midgut is infected. The infection rate can then be calculated as:

$$\text{Infection rate (IR)} = \frac{\text{Number of tsetse flies infected}}{\text{Total number of tsetse flies dissected in a given period}} \text{ x } 100$$

The intrinsic vectorial capacity of a tsetse fly refers to the intrinsic capability of the fly to develop a metacyclic infection (Le Ray, 1989). According to Jordan (1974), generally, infection rates in the three salivarian subgenera (Duttonella, Nannomonas and Trypanozoon) are usually low in populations of tsetse flies, with infections determined by the host, the vector, the parasite and the environment. *Trypanosoma* infections in tsetse flies varies between species with *T. vivax* ranking the highest and *T. brucei* the lowest. Factors influencing trypanosome infection rates in tsetse flies include, endogenous factors (vector), parasite factors, ecological factors (environment) and host factors. Endogenous factors include tsetse species, sex, longevity, age at taking infective blood meal, age structure of tsetse populations, genetic differences (variations within species), concurrent infections (virus, bacteria, fungi), interaction between lectins and rickettsia-like organisms, physiological and biochemical states. Parasites factors include, parasite numbers available to tsetse, parasite species and its infectivity to tsetse, subspecies/strain of parasites. Ecological factors include, climatic factors, availability of infected hosts and hosts available for subsequent feeds. Host factors include susceptibility, immune state of host and host behavior and attractiveness to tsetse.

Tsetse flies differ in their ability to develop infections as stated above. Female tsetse flies usually have higher infection rates than males (Kubi *et al*., 2007; Moloo *et al*., 1992; Welburn and Maudlin, 1992; Desta *et al*., 2013; Isaac *et al*., 2016), probably due to a higher longevity of female flies than males and therefore have a higher probability of infections. However, it is not yet known whether infection rates in tsetse flies is determined by sex of the fly (Kazadi, 1991; Mihok, 1992). Within species, it has been found that infection rates vary greatly depending on individual host factors. It has also been shown that susceptibility of flies to *T. brucei* infection is also due to maternally inherited characteristic, associated with presence of intracellular rickettsia-like organisms (RLOs). Tsetse flies carrying RLOs in their midgut were found to be six times more likely to be infected with trypanosomes than those without (Maudlin *et al*., 1990). Within any given species, indivuals and sexes of the same species respond differently to infection suggesting the involvement of some genetic differences (Jordan, 1974).

2.26 Tsetse Immunity

The immune system in tsetse is made up of both cellular and non-cellular defence mechanisms to act against a wide range of micro-organisms (Brehelin, 1986; Dunn, 1986). Naturally occurring non-specific and in some cases inducible 'immune' molecules which act as agglutinins, lysins, precipitins, opsonins, complement-like and microbicides, are present in insects haemolymph, (Komano *et al.,* 1980; Karp, 1985; De Azambuja *et al.,* 1986; Ka'aya *et al.*, 1986). Lectins are sugar binding proteins or glycoproteins of non-immune origin which may selectively agglutinate cells or precipitate glycoproteins and which have specific receptors for certain carbohadrates configurations (Franz *et al*., 1982). They are unlike anti-bodies in that they are not synthesized during an immune response and do not have the typical immunoglobulin structure, nor do they show enzymatic activities toward the carbohydrate to which they bind (Kocourek and Horejsi,

1981). Endogenous lectins of insect hosts of trypanosomatid parasites could mediate susceptibility and vectorial capacity (Pereira *et al*., 1981; Wallbanks *et al*., 1986; Molyneux and Killick-Kendrick, 1987; Maudlin and Welburn, 1987, 1988; Ingram and Molyneux, 1988).

Such insect derived lectins may bind to the surface carbohaydrates which, like lectins show inter and intraspecific variation. This variation could be demonstrated by the use of commercial lectins which agglutinate parasites carrying, on their surface, the sugars for which the lectins are specific (Wallbanks *et al*., 1986).

Insect haemolymph agglutinins or lectins are thought to mediate fly susceptibility to and maturation of trypanosome infections (Maudlin *et al*., 1987; Welburn and Maudlin, 1989). The interaction between tsetse lectins and parasite surface coats may be responsible for trypanosome transmissibility and partly distribution of trypanosomiasis in Africa (Okolo *et al.,* 1990; Welburn and Maudlin, 1990). However inter and intraspecific variations occur in *Glossina* spp. based on levels (titres) of haemolymph and midgut lectins (Welbun and Maudlin, 1990). Flies with high midgut and haemolymph lectin titres (e.g. *G. morsitans*) may have high transmission indices for *T. congolense* and *T. b. rhodesiense* whereas those with low titres of haemolymph lectin (e.g. *G. p. palpalis*), may mature few infections of those trypanosomes, even in the presence of high midgut lectin titres.

Inter and intraspecific variation in exposed carbohydrate residues in six *Glossina* spp. have been investigated using Flourescene-conjugate as marker to determine the presence of surface carbohydrate on the peritrophic membrane of *Glossina* spp. (Okolo *et al*., 1988). Similarly, Ingram *et al*., (1988) detected the presence of relatively heat-labile, human ABO (H) blood group non-specific lectins or lectin-like agglutinins, titre range 2^{-9}-2^{-16} in *G. m. morsitans, G. p. gambiensis* and *G. tachinoides* haemolymph. The haemagglutinins exhibited wide specificities for

carbohydrate residues on the surface of human erythrocytes, indicative of heterogeneity, which varied according to the *Glossina* spp. examined and the type of erythrocyte used. It has further been reported that midgut haemagglutinins, titres 2^{-6} or 2^{-7} were only found in *G. m. morsitans* exclusively against 'AB' erythrocyte types. A minimum concentration of lectins (μg/ml) is required for agglutination of different species of Herpetosoma trypanosome, in other words the degree of trypanosome agglutination is dependent on lectin concentration (Maraghi *et al*., 1989). The longer exposure to midgut lectin the higher the frequency of *T. congolense* maturation, suggesting that there is clonal variation in response to lectin stimulation within trypanosome stocks (Welburn and Maudlin, 1989). It is most probable that this variation corresponds to differences in lectin binding sites on the trypanosome surface. Midgut trypanosomes retain their ability to mature throughout their life in the fly. When lectin activity in the midgut is inhibited, the trypanosomes remain as procyclic forms but when this inhibition is removed maturation is able to proceed (Welburn and Maudlin, 1989).

2.27 Tsetse Sampling

In tsetse fly population studies, flies are most often sampled in order to assess their availability and apparent densities in a given area. During sampling, different segments of the population are collected depending on sampling method used (Okiwelu, 1982). For instance, fly rounds are generally considered to give bias representatives of the population in favour of males, whereas the inverse is the case in trap catches.

Availability of flies is also affected by seasonal changes, river systems, vegetation type, land fragmentation, anthropogenic factors, availability of hosts, fly species, sex, age, hunger stage (nutritional status), physiological state (Davies, 1977).

2.27.1 Fly rounds

One of the earliest methods of tsetse survey is fly rounds. This was based on fundamental knowledge on behaviors characteristic of certain species of tsetse flies. On the basis of that Nash, in 1934, recommended different mechanisms for finding the insects and catching them. In his words: "When looking for *G. palpalis* the river banks should be followed, and constant stops made under shade so as to detect the insect, which usually settles fairly high up on the body. Any glades in the dense forest should be inspected, and near settlements mango trees should be investigated, as they are often a favourite haunt of this tsetse. *G. tachinoides* is often a very secretive species, and to find it in its true home, one must enter a Kurumi, find an open glade with high overhead shade and stand motionless watching one's ankles and those of one's (fly) boys. It usually attacks very low down and is exceedingly restive; it approaches silently and alights so gently that nothing is felt. *G. morsitans* is not of a secretive disposition, but attacks openly alighting most frequently on the back, and could often be heard before it is seen; when numerous they frequently swarm around the investigator's head and shoulders". This method was later improved to include a black piece of cloth carried by two fly catchers during fly rounds along a definite path in the fly habitat. The screen is often baited with tsetse fly attractant (butanone or octenol) to increase its attractiveness to tsetse flies. The fly catchers move along the path, stopping at defined intervals to catch flies attracted to them using hand nets. This method gives idea of the true fly density but it makes it possible to observe fluctuations in the density of the flies over time. This method captures much more males than females (not withstanding female tsetse flies live longer than males and thus a population could comprise more females than males). With this method teneral males are often under represented and teneral females relatively over represented. Instead of a black cloth to attract the flies an ox could be used as a bait to increase the catch.

2.27.2 Traps

Tsetses respond differently to different objects. *G. morsitans morsitans* is attracted by different shapes of stationary objects (Vale, 1977). This might explain why the design of the first set of traps which were made to resemble ungulates (Harris, 1930). But because most animal traps were bulky and could not be moved easily from one place to another, this gave rise to the design of the geometric traps, which are considered lighter, easily collapsible and could be moved from place to place (Kuzoe and Schofield, 2005). Traps have the added advantage of working round the clock which indirectly eliminates the human factor in fly sampling that is associated with fly round technique. Because different species of tsetse flies behave differently to different traps the proliferation of traps has become inevitable. It is envisaged that this may continue until a universal trap is produced that could act at least against all species of flies in one region or against all species in all regions. The prototype NITSE trap produced by Omoogun *et al*., (1994), upon experimental trial at the Yankari Game Reserve, performed well against *G. tachinoides* in Nigeria. The modified version showed no significant difference in performance against standard biconical trap, at the Kainji Lake National Park (Shaida *et al.*, (2000) unpublished report). Furthermore, in that same experiment, NGU trap and F4 trap from east Africa performed poorly against the West Africa *Glossina* species when compared together (Shaida *et al.*, (2000) unpublished report).

Traps attract and capture tsetse flies but colour is key in fly's orientated behavior. Blue attracts tsetse flies while black encourages them to land. The efficiency may vary according to the tsetse fly species. The biconical trap Challier and Laveissière (1973) is often considard as the gold standard. The biconical trap performance depends on its location and visibility against the surrounding vegetation. Chemical odours (CO_2, acetone, octenol and phenols) or natural odours (cow urine) enhance trap catches, especially those of the savanna group. Teneral flies (especially

nulliparous females) are very often under represented in trap samples, but traps give more representative of sex ratio than fly rounds. Large proportions of flies captured by traps are hungry and have low fat Reserves.

2.27.3 Electric nets

Electric screens are made of a metal frame and parallel electric wires between which a high voltage is passed through. Flies colliding with the screen are electrocuted and fall into the collecting device. Electric screens are used to evaluate the effectiveness of traps, target screens, odours etc.

At equilibrium, in the absence of immigration or emigration, the real density of a tsetse population may be estimated using mark / release / recapture technique. Here a random sample of the population is marked, released and recaptured at a latter date. The population density (X) is then calculated as: $\frac{X}{Y} = \frac{\mathrm{M}}{\mathrm{R}}$ where Y= the number of all recaptured flies (marked and unmarked), M = 3221number of all marked flies, R= number of marked flies recaptured.

2.27.4 Visual and olfactory attractants of tsetse flies

Tsetse flies use both visual and olfactory detection to locate their hosts. These visual and olfactory stimuli attract tsetse flies respectively at short (<15m) and long range (<100m for the odour of a single ox). The different sampling methods make use of this principle.

2.27.5 Screens, targets and traps

Screens and targets are simple cloth materials designed to attract tsetse flies or to catch them in the case of traps. The number of flies that are attracted to their vicinity and those that actually land on them or, in the case of traps are caught by them, depends on the shape and size of these objects.

Studies have shown that the visual acquity in tsetse spans from ultra violet (UV) to infra red (IFR) on the optical spectrum. Jordan and Green (1984) described the performances of targets made of different coloured materials of known spectral reflectance and concluded that the most attractive

colour was blue (400-500nm) and the unattractive was green-yellow (500-600nm). Optimising the visual attractiveness of targets is particularly important if odour is not employed to effect the initial attraction of tsetse to their vicinity (Lindh *et al.,* 2012). Further studies have shown that tsetse flies respond differently to different colours, for instance the *Palpalis* group responds only to the blue colour, the *Morsitans* to both blue and black, except *G. longipennis* which only responds to blue. Black is to be preferred as it induces a landing response in both *G. pallidipes* and *G. moristans. G. medicorum*, and *Fusca* group fly, is attracted to blue from the additional mosquito netting to induce landing (FAO, 2017). Not all flies are attracted to traps, and not all flies attracted end up landing on traps. Vale (1977) using electric grids estimated that the proportion of flies that come near traps but avoid being caught are more in number than those that end up being caught by traps. In order to increase landing rates, a number of studies have been undertaken to identify techniques that could encourage landing of flies. Green (1988) reported that flies respond differently to different colours based on the reflectivity of the ultra violet (UV) ray on the wave length band and concluded that the blue (pthalogen blue) colour is for attraction while the black colour is for landing. This was later to be accepted for use in the construction of blue black targets for the control of vectors of sleeping sickness as described (Cuisance *et al.,* 1990). Recently a study on the tsetse genome led to the identification of a gene responsible for colour selection in tsetse flies (Aksoy *et al*., 2014; Frazer, 2014).

2.27.6 Odour attractants

Acetone, 1-Octen-3-Ol and CO_2 found in breath odour and Butanone and 'TF 86/85'(a mixture of phenols) found in cattle urine are known to be potent olfactory attractants for tsetse flies particularly the *Morsitans* group flies. Vale *et al*., (1988) reported 99.99% reduction of *G. m. morsitans* and *G. pallidipes* populations in 600 Km^2 area of Zambezi valley, using targets baited

with acetone and octenol and coated with deltamethrin. Mèrot *et al*., (1986) while using charcoal filter observed that CO_2 was not the only attractant of *G. palpalis* and *G. tachinoides* found in breath odour. However, Cheke *et al*., (1988) had reported that twice as many *G. p. palpalis* were caught in traps baited with acetone or 1-octen-3-0l as against unbaited traps. No evidence for attraction neither by 'TF 86/05' nor for a combination of all the three chemical baits for *G. p. palpalis*. All species of tsetse so far tested have been shown to be attracted to carbon dioxide, acetone, butanone, octenol and phenols (Vale *et al*., 1988). The *Fusca* group is attracted by the phenol components from bushbuck urine while the *Morsitans* group is attracted by the component from cattle or buffalo urine. However, in Somalia *G. pallidipes* does not seem to respond to phenols from these components. It is believed that other odour components remain to be discovered.

2.28 Population Dynamics

Tsetse fly populations are affected by climatic conditions (seasonal changes) and population growth rate (Hargrove, 1988). Growth rates are determined by the interplay of rates of dispersal, birth and death, as summerised by the equation:

Growth rate = (Birth rate + Immigration rate) – (Death rate and Immigration rate).

Studies by Rogers and Randolph (1986) suggest that it is possible to predict the behavior of tsetse populations under a wide variety of circumstances. It is possible for instance to predict how quickly fly populations grow under different levels of imposed mortality, how quickly they could re-invade cleared areas and what needs to be done in order to prevent re- invasion. And with the present advances in the knowledge on population dynamics, it is possible to predict the outcome of various approaches to tsetse and trypanosomiasis control (Hargrove, 2003).

2.29 Population Analysis

Population genetics tracks patterns of inheritance from individual parents to their offspring. These patterns could relatively be simply determined statistically. Genetic traits are distributed in breeding populations following particular patterns. The interrelated patterns of phenotypic, genotypic and allelic frequencies within populations could be studied and expressed in relatively formal statistical notations. At the core of all of the genotypic and phenotypic variation are, of course, alleles. And the frequencies of those alleles are at the core of the calculations of population genetics. The total genetic bank of a population is its gene pool. Calculating these frequencies, and tracking changes in those frequencies are important due to influences they have in selection and chance. (Http: www2. /e. ac.uk/project/Vgec/schoolsccolleges/…)

Presently, with the knowledge of genotypic frequencies, it is possible to calculate the allelic frequencies for any population.

Basic priciples of population genetics are founded on the assumption of an ideal population described by the Hardy-Weinberg equation given by $p^2 + q^2 + (p+q)^2 = 1$. A population which simulates this ideal population is often described as being "in equilibrium." The relationship between the allelic and genotypic frequencies in this ideal population must meet the following five criteria:

1. There must be completely non-random mating.
2. There must be no selection, natural or artificial.
3. There must be no mutation. Noting that this is impossible, as mutation rates can't be reduced to zero.
4. There must be no migration of species members from outside populations (as they could come from a population with different allelic frequencies).

5. There must be no genetic drift. Genetic drift results from chance alterations in allelic frequencies, and again cannot realistically be reduced to zero. Genetic drift could be minimized if the population is very large. The smaller the population, the more influential genetic drift could be.

The H-W equation shows the relationship between allelic and genotypic frequencies in a population which meets these exacting standards. Exceptions to the H-W equation occur when there is

1. Sexual selection as a result of random mating,

2. Genetic drift is any changes to allelic frequencies which are simply the result of chance. It comprises of sampling error, founder effect and bottlenecking

3. Mutation also changes allelic frequencies, though this effect is generally very small

4. Natural selection or artificial selections

5. Migration occurs when two populations of the same species are combined together to form a single new population. This is generally viewed as a smaller population joining ("migrating into") a larger population, but the equation works no matter the respective sizes of the populations.

2.30 Genetic Variation in Tsetse Flies

2.30.1 Laboratory studies

Evidence exists of intraspecific geographical variation in behavior and host selection in tsetse flies (Torr, 1988; Colvin and Gibson, 1992). Earlier empirical data on variation predominantly came from studies with laboratory colonies of tsetse flies. A comparative study on the performance of

G. pallidipes originating from two allopatric Kenya populations, one from the Shimba Hills and the other from the Nguruman, showed that there was no difference in the bionomic parameters (insemination rates, female survival rates, puparial weights and survival rates) between the two colonies (Moloo, 1992). However, fecundity of the Shimba Hills culture flies was significantly greater than that of the Nguruman culture. In addition, Nguruman culture flies were significantly susceptible to infection with *Trypanosoma congolense*, and were more likely to develop mature infection (Moloo, 1993).

In another experiment, Van Etten (1982a; 1982b) reported differences in feeding frequencies, activity patterns and responses to temperature in his examination of female *G. pallidipes* from allopatric Kenyan populations. In yet another study, it was demonstrated that *G. pallidipes* cultures established from the Lambwe and from Kibwezi Forest populations differed significantly in the duration of copulation (Van Etten, 1981). The above studies show that the laboratory colonies of *Glossina* species differed genetically. However, the genetic basis of these differences, were never investigated probably due to limited availability of appropriate genetic markers.

2.30.2 Variation in natural populations

Genetic variation among natural populations of *G. pallidipes* has been studied by using gel electrophoresis of allozymes (Van Etten, 1982c; Gooding, 1990; Nesbitt *et al.,* 1990). Results from these studies show that genetic variations exist within and among *Glossina* populations and these variations may be responsible for the differences in infection rates as well as the spatial distribution and epidemiology of trypanosomiasis (Lanzaro and Warburg, 1995; Gooding, 1996; Ouma *et al.,* 2003*;* Awono-Ambene *et al*., 2004). For instance studies carried out in Cote d'Ivoire have revealed

that *Glossina palpalis palpalis s.l* is a species complex consisting of genetically distinct siblings /subspecies (Ravel *et al*., 2007).

2.31 Genetic Markers in Tsetse Flies

Studies on population genetics in tsetse flies can now be carried out by the use of a range of molecular markers following the discovery of markers which have revolutionised studies in tsetse population genetics. Genetic markers are biological features that are determined by allelic forms of genes or genetic loci and can be transmitted from one generation to another, and thus they can be used as experimental probes or tags to keep track of an individual, a tissue, a cell, a nucleus, a chromosome or a gene. DNA markers have developed into many systems based on different polymorphism-detecting techniques or methods (southern blotting – nuclear acid hybridization, PCR – polymerase chain reaction, and DNA sequencing) such as restricted fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), single nucleotide polymorphism (SNP), etc. (Collard *et al*., 2005).

2.32 Biochemical / Protein Markers

2.32.1 Allozymes

Allozymes can be defined as codominant protein variants (alleles) that can be visualized by appropriate staining and starch-gel electrophoresis. Allozymes are the most cost effective protein markers for investigating genetic variation at the population level, as such are the most widely used (Murphy *et al*., 1990). Enzymes are being widely used in genetic variation studies of laboratory and field populations of tsetse flies. Isozymes are alternative forms or structural variants of an enzyme that have different molecular weights and electrophoretic mobility but have the same catalytic activity or function. Isozymes reflect the products of different alleles rather than different

genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010).Variants of the same enzyme (Isozymes) for instance, were used for interspecific comparisons of genetic variation, and for inferring phylogenetic relatedness among *Glossina* species (Gooding *et al.,* 1991). Also isozymes have been used for designation of linkage groups and for mapping in *Glossina palpalis palpalis* (Gooding and Rolseth, 1995). The use of these markers has provided information on the genetic structure of natural *Glossina* populations (Nesbitt *et al*., 1990; Krafsur *et al*., 1997b; Krafsur and Griffiths, 1997; Krafsur and Endsley, 2002). The disadvantage of the use of isozyme markers for genetic studies of population is that they reveal little or no variation and a low frequency of polymorphic loci (Gooding, 1981; Gooding, 1993). They also have the disadvantage of low detectable variability of many enzymes, and most importantly, fresh or deep frozen biological material is required. Obtaining liquid nitrogen for sample preservation for electrophoretic studies in developing countries could be quiet challenging because of unavailability and high cost. Alternatively, DNA markers could be used for assessing genetic variation within and among populations, since they could provide more characters per gene and less sample and storage costs.

2.33 Molecular / DNA Markers

2.33.1 Microsatellites

Genetic variation in tsetse flies could also be investigated by the use of Microsatellite markers. Microsatellites are short repetitive nucleotide sequences with conserved flanking regions that could be amplified by polymerase chain reactions (Krafsur and Endsley, 2002). They occur throughout the genomes of many eukaryotic organisms, including insects (Queller *et al*., 1993; Haymer, 1994). Microsatellite loci tend to be highly polymorphic. Variation at these loci is reflected by length polymorphism, which is likely due to slippage during DNA replication

(Schlôtterer and Tautz, 1992). Due to their codominance, supposed selective neutrality and Mendelian inheritance, microsatellites are very useful for population studies (Ouma, 2004). Microsatellites, have the disadvantage of high mutation rates which could lead to underestimation of genetic differentiation by using F- statistics particularly when migration is low (Hedrick, 1999). Underestimation of genetic differentiation among population variants could lead to overestimation of gene flow (Krafsur, 2003). However the benefits of the use of microsatellites far outweigh the shortcomings. A limited number of microsatellite markers have been developed in tsetse flies. For instance, nuclear DNA markers such as microsatellites provide information that could be used to document colonization by both males and females. It is also possible to examine behavioral differences between sexes by comparing sex-specific data. An autosomal and two X-linked microsatellite loci from *G. palpalis gambiensis* have been isolated (Solano *et al.,* 1997). From *G. p. palpalis* group, 13 polymorphic microsatellite markers have been identified (Luna *et al.,* 2001). And amongst the *G. morsitans* group flies, microsatellites have been isolated from *G. morsitans sl.* (Baker and Krafsur, 2001) and *G. pallidipes* (Ouma *et al*., 2003).

2.33.2 Mitochondrial DNA (mtDNA)

Mitochondrial DNA has been widely used in taxonomic and population studies (Simon *et al.,* 1994). It is maternally inherited and could be used to assess genetic drift and bottlenecks in population studies (Avise, 1994). Insect mtDNA could be rapidly isolated for mitochondrial DNA studies (Martinez *et al.,* 1992), and there are available universal primers that can amplify across the entire insect mtDNA (Simon *et al.,* 1994). Analysis of variation in mitochondrial DNA could be done using the Single Strand Conformation Polymorphism (SSCP) method (Black and Du Teau, 1997). The application of SSCP to infer gene flow and gene diversity in house flies (Marquez and Krafsur, 2002; 2003), and in several species of tsetse flies (Krafsur and Wohlford *et al.,* 1999;

Wohlford *et al*., 1999; Krafsur *et al.,* 2000a; 2001) has been described. Despite the usefulness of mtDNA for population studies, its matrilineal inheritance tends to lead to erroneous interpretation of colonizing insects relative to their source population (Roderick, 1996). For instance, if mtDNA is used to study gene flow, the direct consequences of dispersal by males is missed (Palumbi and Baker, 1994). This is because the colonizing ability of male and female insects obtained from mtDNA markers frequently differ (Birky *et al*., 1989; Slatkin and Maddison, 1989; Roderick and Caldwell, 1992).

The above studies show that *Glossina* populations could be differentiated using molecular tools.

2.34 Geometry Wing Morphometrics

Studies on tsetse population structures have become increasingly important as a guide for planning effective control interventions against *Glossina* species as envisaged by AU- PATTEC initiative. Such studies are necessary for post control monitoring and analyzing the possibility of recolonisation of controlled areas. The use of molecular techniques for analyzing DNA sequences in such studies has proven to be expensive. Other alternative methods that could minimize the cost of DNA sequence analysis are being explored. Geometric wing morphometrics seems most promising as an inexpensive alternative technique for genetic analysis of tsetse populations. The method involves removal of wings and dry-mounting them between microscope slides. Each of the wings so mounted are photographed using a digital camera after land marks have been assigned and analysis carried out according to methods previously described (Patterson and Schofield, 2005).

2.35 Tsetse and Trypanosomiasis Control

Control of trypanosomiasis relies on actions against the parasites and the vectors.

2.35.1 Control of trypanosomiasis using Chemotherapy and Chemoprophylaxis

Through sustained surveillance of human and animal populations at risk, the disease could be controlled by chemotherapy and/or chemoprophylaxis. Lack of effective vaccine against the disease due to the phenomenon of variable surface glycoprotein (VSGs), the high cost and unavailability of drugs are limiting factors against the control of the disease by the use of prophylactic and therapeutic means. A wide range of drugs are available for the treatment of trypanosomiasis (Table 2.5), however, the development of resistance by trypanosome parasites to drugs in current use has been reported in several countries including Nigeria (Randolph, *et al.,* 2011). Because of the limitations associated with control actions against the parasites, vector control is considered the most efficacious method of trypanosomiasis control (Ravel *et al*., 2007).

Table 2.5: Chemotherapy of AAT (Calous *et al*., 2013) and HAT (Nok, 2003)

AAT	Year of discovery	HAT	Year of discovery
Homidium	1950s	Nifurtimox	2009
Diminazene aceturate (Berenil)	1955	α-Difloromethylonithine DFMO (Eflonithine)	1990
Isometamidium Chloride (Samorin)	1945	Melarsoprol	1949
		Pentamidine	1940
		Suramin	1920

2.35.2 Use of trypanosome resistant (Trypanotolerant) breeds

Trypanotolerant breeds are breeds of animals that have the ability to withstand high tsetse challenge when compared to others not found in the regions. Several species of African wildlife are known to tolerate trypanosomiasis infections without showing clinical symptoms and with no apparent detriment to their health. Certain indigenous breeds of cattle such as N'Dama and small ruminants in West Africa also tolerate trypanosomiasis and in some cases recovery from the disease without chemotherapy has been reported. Potential exists to replace susceptible herds with those that are trypanotolerant and this possibility is currently being investigated in several West African countries, in collaboration with FAO and ILRI. It has been established that only 200,000 out of the 10-14 million cattle in Nigeria are trypanotolerant (Adeniji, 1993).

2.36 Tsetse Control

Methods for tsetse fly control just before and after the Second World War as captured by Hamon *et al.,* (1965), which were essentially crude methods have recently been replaced by methods that are cheaper, more direct, and ecologically friendly. Presently tsetse control involves use of selective application of insecticides, impregnated traps and targets, pour-on formulations and the use of Sterile Insect Technique for mop-up operations (Allsopp, 2001).

In many instances tsetse control has proven to be a very efficient way to stop human trypanosomiasis transmission in restricted areas as a complement to chemotherapeutic measures, and tsetse control or eradication is often the cheapest method for preventing livestock trypanosomiasis transmission in cattle grazing areas of Sudanese and Guinean savannah (Hocking *et al*., 1963).

2.36.1 Direct destruction of tsetse flies

Hand–catching using nets and sticky panels has been used as a method of tsetse control since the early twentieth century against *G. palpalis* in Principe Island (Austen and Hegh, 1922) and in Riamugasire on Lake Victoria (Kock, 1914). Also extermination of *G. f. fuscipes* from an experimental site through hand-catching has been described (Glasgow and Duffy, 1947). Successes have been recorded in some areas whereas others have resulted in failures (Glasgow and Potts, 1970). Destruction by trapping (Harris, 1938) has been successful in controlling *G. pallidipes* in a restricted breeding area of Zululand, South Africa. In Nigeria, Offor and Nlumanze, (1986) reported the catching out of a tsetse population in an area near Abuja, Niger state. Also Onyiah and Riordan (1978), reported the fluctuation in numbers and eventual collapse of a wild population of *Glossina palpalis* (R-D) population in Anara forest Reserve, Nigeria, following intense anthropogenic activities. On the contrary failures to exterminate tsetse populations from certain areas have been reported to be entirely due to non-isolation and the use of smaller number of catchers in the study areas (Symes and Vane, 1937; Symes and Southby, 1938; Glasgow and Duffy, 1951). Undoubtedly, the most important advantage of hand-catching as a control method of tsetse is that it is absolutely species specific making sure that only tsetse are caught as the target species. The advent of modern tools makes hand catching unpopular as a tsetse control method.

2.36.2 Tsetse control by game elimination

One of the earliest methods of tsetse fly control is game elimination. Tsetse and game have a long history of association, this is evidenced by strong reduction or even disappearance of *Glossina* populations following game elimination, during rinderpest epidemics. Game destruction has been used as a routine method of tsetse fly control in Eastern and South- Eastern Africa, and has

succeeded in clearing many thousands of square kilometers of flies belonging to the *morsitans* group, the best known experiment being that of Shinyanga, in Tanganyika (Glasgow, 1960). However, Weitz (1963) and Glover (1964), stressed that the *palpalis* group flies cannot be starved except if reptiles and humans are also eradicated, and the control by starvation of many *Glossina* species (such as *swynnertoni*, *austeni*, *fuscipleuris*, *tabaniformis*, *morsitans* and *submorsitans*) supposed total elimination of suids, which constitute a group more difficult to locate and kill than antelopes and big game. Besides, Dasmann (1962) and Hocking *et al*., (1963), have underscored the importance of game farming as a more profitable venture in some marginal areas than cattle raising as a source of meat, hides and skins and also for the tourist industry, so tsetse control by game destruction is almost entirely abandoned now.

2.36.3 Tsetse fly control by bush clearing

Bushes and trees are widely used by tsetse flies as resting places, but they are vital as shelters for pupae. Pupae can only survive if the soil is sufficiently shaded, protected from direct insulation and preserved from rapid radiations, humidity and temperature. The clearing of all woody vegetation in a fly infested area has rarely been used for tsetse fly eradication, but has extensively been employed to provide barrier areas or to reduce the contact between flies and humans, around villages and water holes, at river crossings, and along the main routes of communication of humans and cattle (Le Rouzic, 1948). Such clearings called “agronomical prophylaxis” in French- speaking countries could be used for cultivation to be kept clear of trees and bushes. They cannot be extended on large areas in sparsely inhabited zones because they require larger manpower supply than available, and are very costly if carried out by administrative agencies. However, for special purposes, clearings and protective barriers have been done up to 100 kilometers long, and three kilimeters wide as in Southern Zimbabwe.

Selective clearing has been much more extensively used for tsetse fly control. It is based on the tendency of flies, in unfavourable seasons, to concentrate in permanent breeding or resting sites that comprise identifiable plant communities and a comparatively small proportion of the bush or woodland as a whole.

The requirements of the common tsetse fly vectors of trypanosomiasis have been described and the types of vegetation most favourable as refuges are known. They must be located before tsetse fly control measures are undertaken. Selective clearance could be done directly or by use of chemicals; it suffers from the same deficiency as total clearing; it is a costly measure, the regrowth of bush must be kept down and it is much more efficient in dry areas than in humid ones. In Guinea savannah zones, temporary control of riverine tsetse could be done by use of obstructive clearing, by felling of trees forming the overhead canopy and blocking the stream bed to obstruct the tsetse flight line. The flies cannot move freely under shade and either are starved or forced into the open where the climate in the dry season is intolerable (Nash and Steiner, 1957). With the discovery of modern insecticides it appears that it is very often more efficient to spray a residual toxicant on the tsetse permanent resting sites than to destroy them, because tsetse could change resting sites when the preferred ones are destroyed, but they do not abandone the sites after spraying (Hocking, 1964).

2.36.4 Tsetse control by population resettlement

Tsetse clearance by concentration of population involves massive movements or the removal of many thousands of families and placing them in new areas. In many cases entire new villages had to be built, and the sites for these had to be carefully selected. These new villages were situated sufficiently close together to produce something of a land hunger so that inhabitants were to go in

for intensive farming. Under such conditions it was probably thought that all the tsetse bush would be cleared as all the land available would be wanted by the farmers and the timber and scrub needed for domestic use. A very good example of this project was the Anchau resettlement scheme in northern Nigeria. The major benefits of population concentration included, the facts that the villages so moved were well planned and laid out before they were built; the inhabitants were theoretically all to be eventually protected from tsetse flies and other diseases (like malaria and guinea worm) were to be more easily controlled because there were to be provided in these villages good sources of water from wells.

2.36.5 Tsetse control by aerial spraying of insecticides

Insecticides could be used as aerosols of temporary efficacy on huge areas or as residual sprays on resting places, in traps and on baits. Insecticide residues have very short residual effect and kill tsetse flies during some hours only after their application. They could control or eradicate the flies only if they are applied on large areas at convenient intervals to kill all flies before their reproduction and emergence from pupae since the previous application. A convenient rhythm seems to be about eight applications at intervals of two to four weeks (Burnett, 1962a). Aircraft application could be advantageous for covering large areas within a short time and could be efficient against savannah inhabiting species like *G. morsitans, G. swynnertoni* and *G. pallidipes.* It is less adequate than ground application against riverine tsetse flies like *palpalis* (Burnett, 1962a), and is almost imposible to carry out against *Glossina* species living in high forests.

Ground application could be done either with lorry-carried generators, or with light generators, like Swingfog (Challier *et al*., 1964). Insecticide aerosols can only be applied some hours a day, just after dawn and before dusk and sometimes by night, when air currents are downwards; during

other day-time periods the insecticide cloud dispenses very rapidly and if applied by aircraft, does not reach ground level. Only a very small amount of insecticide reaches each individual fly, and gravid females, less susceptible to insecticides than males and females of other physiological conditions (Burnett, 1962b), are not easily killed by chlorinated insecticides, some organophosphate compounds like fenthion could perhaps be more efficient (Hocking *et al*., 1963). For eradication programmes dieldrin and 3HC aerosols have been used mainly in East Africa, with variable results including fair success (Cockbill *et al*., 1963).

2.36.6 Tsetse control by ground spraying of insecticides

Residual treatment of tsetse fly resting places must be lethal for the fly on short contact for a longer period than the maximum duration of the pupal life. In such conditions only one spraying could be sufficient to control the species, and perhaps eradicate it in an isolated area. The first residual applications have been done against riverine species, like *palpalis* and *fuscipes*, with habitats restricted to water edge. In larger gallery forests, it is sometimes possible to open paths in the forest, which could be extensively used by moving flies, and to treat them for controlling flies. Dichlorodiphenyltrichloroethane (DDT) suspensions and emulsions, which have been used in the first experiments, have usually been replaced by dieldrin emulsions, which assumed to be efficient almost one year and sometimes more than one year if applied at 47% (Kernaghan, 1962). According to Baldry (1963; 1964), DDT emulsions have a longer residual effect than dieldrin and telodrin (isobenzan) when applied at the concentration of 5% and even, in some conditions at lower concentrations.

Such control procedures are 5 to 10 times cheaper than bush clearing and of a more permanent effect (Hocking, 1963; Maclennan and Aitchison, 1963; Challier, 1965). Improvement on the knowledge of tsetse fly resting places has permitted the use of residual insecticides against savannah species of tsetse like *G. morsitans* and *G. swynnertoni*, by selective spraying of the lower side of the branches of acacia trees in fly concentration areas (Hocking, 1961; Chadwick, 1964; Chadwick *et al*., 1964). The cost of control by selective spraying, is similar to or lower than by aircraft fogging but the work is more difficult to supervise. Residual insecticides have been used also against *G. tachinoides* in Northern Nigeria, along Tchad-Cameroons border, but that fly is not as concentrated as *G. palpalis* along places and trees and bushes must be treated on a large width on both sides of the rivers and water holes (Mouchet *et al*., 1961). They have also been used with fair success against *G. fusca* and *G. fuscipes* in large gallery forests of the Central African Republic (Finelle *et al*., 1962; 1963; Yvore *et al*., 1962). Tsetse fly control by residual insecticides has not been carried out against high forest species and is only promising when the fly habitats are restricted.

2.36.7 Trapping

The history of tsetse trapping began when a farmer in Principe Island had his workers wear on their backs a black piece of cloth covered with glue, and with partial clearance of vegetation and elimination of wild animal hosts, the island was rid of the tsetse fly *Glossina p. palpalis* (Da Costa *et al.,* 1916). Trapping techniques are considered very important by entomologists because they could be used to estimate tsetse populations (Harris, 1930; 1938; Morris and Morris, 1949). Some authors, stress that traps give the best way for catching representative samples of fly populations (Morris, 1960;1961), whereas others consider traps as useless (Abedi, 1963). Traps by their nature attract tsetse flies by virture of their shapes and colours (Green and Flint, 1986). The attraction of

tsetse flies to traps varies from species to species and from region to region (Kuzoe and Schofield, 2005). The responses of male and female flies to colours also differ (Okello-Onen *et al.,* (2004).

Traps however have remained the most acceptabl tool for tsetse sampling since their discovery by Harris in 1930 (Kuzoe and Schofield, 2005). Traps by their nature are environmentally friendly since they act only against the target species. The performance of the biconical trap (Challier and Laveisseir, 1973) against *Glossina* species in West Africa had attracted a lot of interest the world over leading to the proliferation of traps in many parts of sub-Saharan Africa aimed at tsetse control. Such traps include the alpha, beta and gamma traps, the F1, F2 and F3 traps (Flint, 1985), the S trap, the vauvou trap and the NGU traps (Kuzoe and Schofield, 2005), and the NITSE trap (Omoogun *et al.,* 1994). But because different species of *Glossina* respond differently to different traps, their use is limited especially in epidemic situations as traps are slow in action. However their action could be enhanced by impregnating them with chemical insecticides and the use of odour baits to increase the attractiveness of traps to flies. But because of the limitations associated with the use of traps for control, e.g the avoidance of landing by some categories of tsetse as outlined by Olet, (2001), their effectiveness on a large scale has been put to question.

2.36.8 Insecticide treated traps and targets

Before the advent of insecticides, the control of animal trypanosomiasis had relied solely on the physical elimination of the vectors of the disease, as it was logically thought that absence of the vectors suggests absence of the disease in the animals. To accomplish this task, ruthless clearance of vegetations serving as resting and breeding sites of the tsetse vectors and selective game destruction were carried out (Duggan, 1962). With the advent of insecticides however, the use of which began in 1955 to control tsetse in Nigeria, the vector had been eradicated from large areas of the country (Maclennan and Kirby, 1958; Davies, 1964; Spielberger *et al*., 1977). But the

method becomes less effective with increase in humidity as one moves from the northern sahelian region to the southern part of the country. Unfortunately the application of persistent insecticides had undesirable side effects on non target organisms in the ecosystem. Severe mortalities in insectivorous vertebrates were reported in Nigeria following Dieldrin applications (Koeman *et al*., 1978). High fish mortality and acute mortalities in mammals, birds, reptiles and amphibians were similarly reported following Endosulfan spraying in Nigeria (Koeman, *et al*., 1978; Nagel, 1995). However, from the beginning of the 1980s other non-insecticidal method of control and methods requiring selective or less use of insecticides received prominence such as the Sterile Insect Technique (SIT) and the use of traps and screens/targets to control tsetse flies. Insecticide impregnated traps for instance have been shown to reduce tsetse populations almost to the point of extinction (Laveissiere and Couret, 1980). A 91% reduction of *Glossina palpalis palpalis* population within three months was achieved in the forest zone of Ivory Coast (Gouteux *et al*., 1982). Similarly, a 90% reduction in the *G. palpalis* density in seven weeks and 95% in twenty weeks was achieved using Deltamehtrin-impregnated screens at a concentration of 0.104% during the rainy season, in the sub-humid savanna zone of Nigeria (Oladunmade *et al*., 1985). Continuous trapping with biconical traps in the Tsakuwa and Maisamari forests of the Akunni catchment zone of the BICOT project area, for two weeks and seven weeks each reduced the average female fly population density by 80% and 95% in each of the locations respectively (Oladunmade *et al*., 1988). However, the major constraint to the use of traps for control purposes is that traps lack efficiency in capturing all species of tsetse in a given region or the same species in other regions, presumably because of changes in their behavior occasioned by changes in their ecological requirements. Thus as observed by Jordan (1986), there are still prospects for the design of new tsetse traps. This factor is attributed to the observation that traps could be used to reduce tsetse

populations and incidence of trypanosomiasis. They are ecologically friendly, and are accessible to the African rural communities afflicted by the disease (Brightwell *et al*., 1987; Vale *et al*., 1988).

2.36.9 Pour-on formulations

Insecticide treatment of cattle and other livestock is considered one of the important methods of tsetse control and reduction of incidence of trypanosomiasis. The principle involves use of cattle as mobile targets (or baits). Tsetse are attracted via visual and olfactory means, and as they land on their target, they pick up insectide deposites enough to kill them off. Cattle constitute natural attractants and both systematic and residual conventional insecticides applied to cattle are being used to kill tsetse flies in restricted areas where wild game is scarce or absent. It has been shown that a single insecticide treated cow potentially kills about as many tsetse as a single odour-baited target. Therefore, if there is a reasonably large population of cattle in an area, the use of insecticide treated cattle offers several advantages over using targets. Treating an animal is cheaper than constructing and deploying an insecticide –treated target. In addition, cattle owners are strongly inclined to treat their cattle with insecticide, to maintain them and protect them from theft. As a result, several problems associated with using targets are overcome by using insecticide-treated cattle, including the loss of targets due to general wear and tear, theft, animal damage. However, in practice a combined approach using both traps and insecticide treated cattle could represent the best option. Pour-ons are formulations of a number of different synthetic pyrethroid (SP) insecticides that could be applied to livestock. These products have been shown to be very effective against tsetse flies as well as ticks. Pour-on treatment involves the application of an insecticide along the backline of livestock at a prescribed dosage (http://www.tsetse.org/http://www.nri.org/tsetse/FAO/itc.html.

2.37 Biological control of tsetse

Tsetse flies can be controlled by the release of natural enemies (Jenkins, 1964). Biological control techniques employing nematodes, fungi, viruses and rickettsia-like organisms have been successful in laboratory experimental trials only (Laird, 1977). Cross-mating of closely related species to produce a high degree of sterility in a tsetse fly population have largely been unsuccessful (Glasgow, 1960). Sterilization of of tsetse flies is possible, either by radiation or with chemosterilants (Knippling, 1963; Smith and Dame, 1963).

2.37.1 Predators

Lizards, snakes, birds, bats, mongoose, rodents, insects, spiders, and ants). Okiwelu, (1976) recorded predators of *G. tachinoides, G. pallidipes and G. m. submorsitans* to include Asilids and spiders.

2.37.2 Parasites

Tsetse fly pupae have been found to be parasitized where eggs of some parasites are deposited in tsetse fly pupae. Percentage of pupae parasitism could however fluctuate strongly depending on seasonal changes.

2.37.3 Parasitoids

Parasitoids belong to the Diptera. Twelve different species of *Exhyalanthrax* (*Thyridanthrax*) were found to parasitise tsetse flies as well as other flies, 10 in eastern Africa and two in Western Africa. The cycle from egg to adult generally lasts 20 to 35 days, but sometimes there could be a kind of hibernation of the larvae so that the adults hatch only after several months (up to 14 months). The breeding of these dipteral could not be achieved. Parasitoids that have been found to infect pupae of various species of *Glossina* include, *Chrestoinutilla* (*Mutilla auct.*), *Exhyalaiithrax* (*Thyridanthrax auct.*) and Acari in pupae of *G. morsitans* (van der Vloedt, 1970). *E. beckerianus*

have been found to parasitise the pupae of *G. tachinoides* and *G. Pallidipes*. *Nesolynx* spp. (*Syntomospherium* spp) do not attack the pupae of tsetse flies exclusively (Onyiah and Riordan, 1978; Baldry, 1981). Establishment of breeding colonies of this parasite is possible, but significant releases could be challenging, while the insect is not able to dig the ground to search for the pupae of tsetse flies. *Chrestomutilla* spp. (*Mutilla* spp) are able to reproduce by parthenogenesis, the unfertilized eggs giving rise to males. The female lays 2 to 3 eggs in the same pupa, but only one adult parasitoid could emerge. The development of the immature stages takes approximately a month and half. Breeding of this insect in the laboratory has not been successful.

2.37.4 Pathogens

Pathogens include microbial agents (e.g. *Bacterium mathisi*), several fungi and *Baculovirus* (salivary gland hypertrophy virus) are pathogenic to tsetse flies but none of these pathogens can be used as biological control agent yet.

2.37.5 Nematodes

Mermithid larvae have been found to parasitise tsetse flies though at low levels. They penetrate newly emerged flies through thin places in their body walls. *Hexamermis glossinae species* found in *G. palpalis, G. pallicera* and *G. n. nigrofusca* in Ivory Coast have been described (Poinar *et al.,* 1981).

2.38 Genetic Control of Tsetse Flies

2.38.1 Transgenesis

Transgenesis involves the manipulation of the vector genetic make-up through genetic engineering so as to render it sterile or refractory to infection (Aksoy, 2003)**.** Genetic control of tsetse flies by transgenesis involves modulating vector competence of the flies, which could eliminate their ability to transmit pathogens by introducing and expressing foreign genes with anti-pathogenic

properties that interfere with pathogen viability, development or transmission. These genetically engineered refractory insects could then be driven into natural populations to replace their susceptible counterparts. The endosymbionts of the tsetse fly (*Sodalis glossinidius*, *Wigglesworthia glossinidium* and a strain of *Wolbachia*) are all maternally transmitted to progeny. Using recombinant DNA (rDNA) technology, it is possible to alter the genome of the endosymbiont, *Sodalis glossinidius*, so that the symbiont expresses trypanolytic substances into the fly. *Wolbachia* is able to introduce cytoplasmic incompatibility in natural tsetse populations. Cytoplasmic incompatibility is achieved when wild type females are mated with males infected by a *Wolbachian* strain that is non-existent in the female. The intracellular *Wolbachia* then causes embryonic mortality. Using cytoplasmic incompatibility (CI), it is also possible to sterilize male flies and release them to mate with their wild female counterparts, as a vector control technique which at the long run, could increase their efficacy and reduce cost as against the use of gamma irradiation for the SIT programme (Aksoy and Rio, 2005). When both methods are combined it is expected that the wild – type, trypanosome-susceptible tsetse population could be replaced by the engineered, trypanosome refractory lines.

2.38.2 Sterile Insect Technique (SIT)

Sterile insect technique is a form of birth control in tsetse flies. The concept was first developed by Knipling (Knipling, 1955). It involves the sterilization of male tsetse flies using gamma radiation and releasing them to compete with their wild counterparts taking advantage of the reproductive biology of the female flies which mate only once in their life time. SIT was first used for the control of the fruit flies (Allsopp *et al.,* 2000), and the new world Screw worm *Cochliomyia*

hominovorax (Coquerel) from the Island of Curacao, Netherlands Antilles in 1954 (Baumhover *et al*., 1955).

Attempts have been made to control *Glossina* species using SIT in Zimbabwe, Burkina Faso, Nigeria, and Tanzania and most recently in Unguja Island, Zanzibar. In Nigeria SIT was achieved by the successful elimination of *G. palpalis palpalis* populations from 1,500Km^2 area in the BICOT operational area (Oladunmade *et al*., 1990), and in Zanzibar, *Glossina austeni* (*Glossinidae*) was eradicated from the Unguja island using SIT (Vreysen *et al*., 2000). Failures have been recorded in some instances (Oluwafemi *et al.,* 2008) due to non isolation of controlled areas thus making it possible for reinvasion of other tsetse flies from neighbouring uncontrolled areas into areas that have been controlled. The ability of remnants of tsetse populations to recover quickly has also been identified as a factor responsible for failures in some tsetse control operations (Hendrichs *et al*., 2005; Torr *et al*., 2006).

But basically failures have been attributed to a large extent on inadequate knowledge on the population dynamics of the target species. This is primarily attributed to the limitations of conventional methods for population studies using morphological means. For instance it is not possible to use Mark-release-recapture method of Jackson (1944, 1946) to infer the exact extent of dispersal of species, spatial delineation, assessing risk of re-colonisation of controlled areas, size of target population. Molecular tools have overcome the limitations of the use of Mark - release-recapture method for tsetse population studies. Gene flow could be used to infer dispersal indirectly in tsetse populations (Krafsur, 2003). They also provide the platform for which informed choice by authorities (e.g. PATTEC) of effective and appropriate control strategies against tsetse and trypanosomiasis using SIT could be made.

The sterile insect technique via irradiation considers primarily the eradication of isolated populations of tsetse flies. It involves the breeding of millions of tsetse flies of each subspecies present in the area and the male tsetse are then sterilized using gamma (or X-) radiation and releasing at regular intervals, thus swarming the population with males that are unable to fertilise females successfully. *Glossina austeni* has been successfully eradicated from Zanzibar using this method (Vreysen *et al*., 2000). During this campaign 5.5 million sterile males were released in total. Before flooding the area with the sterile males the local wild tsetse population must be largely reduced by other local control methods such as the use of arial or ground spraying of insecticides, use of traps, impregnated screens and targets and pour-on formulations. It is estimated that a proportion of 10 to 30 sterile males per fertile female is needed in order to ensure that most couplings with the few remaining wild females could occur with sterile males and, since most female tsetse flies do not copulate more than once with a male, the population could be eradicated. Sterilization by irradiation is possible for adult male and female tsetse as well as for pupae. Before being released the sterile males are nourished with blood from animals treated with trypanocides in order to decrease as much as possible the chances of transmission by the sterile flies. For riverine tsetse fly species it seems easier for the sterilized, released male to find wild flies of the opposite sex than for the savannah species.

2.38.3 The Sterile Insect Technique via chemical sterilisation:

In this method of control, the reproductive potential of the vector or its vectorial competence is altered. Males and / or female flies could be autosterilized in the field using a system of traps or screens by allowing sufficient contact between the insect and a sterilizing chemical product (e.g. bisazir, tepa, hempa). Mass breeding followed by gamma ray sterilization of males could possibly be replaced by this direct method of sterilization. These products are relatively dangerous and toxic

to mammals, but could be substituted by analogues of the juvenile hormone (e.g. pyriproxyfen: (Langley, 1994). An amount of 0.02 µg of pyriproxyfen applied on a tsetse fly female could inhibit pupariation by the female. Pyriproxyfen is relatively stable under tropical conditions, screens treated with a mixture of oil (Cerechlor 545) and of pyriproxyfen 2mg/cm^2 could be effective for a period of nine months. This juvenile hormone mimics the chitin synthesis inhibitor, triflurmuron, and could provide a safe way to effectively autosterilise female tsetse flies. These methods could eliminate the need for the costly artifially maintained membrane feeding tsetse fly colonies.

2.39 Benefits of Tsetse and Trypanosomiasis Control

The benefits of tsetse and trypanosomiasis control are enormous. Elimination of HAT and AAT could bring about increased livestock and agricultural production, increased revenue accruing to the Federal Government, improved standard of living, increased employment opportunities especially in the agricultural sector and National security

In general terms, the defeat of sleeping sickness and nagana would be of immense benefit to rural development and contribute to poverty alleviation and improved food security in affected countries of sub-Saharan Africa.

2.40 Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC)

Despite several decades of conserted efforts to control tsetse and trypanosomiasis, the disease still persists. Following the successful elimination of *G. austeni* from the Unguja Island, Zanzibar (Vreysen *et al*., 2000) using SIT and in view of the apparent devastating effects of trypanosomiasis on the economy of most affected sub-Sahara African countries, the African Heads of State and Government during the 36 conference of AU at Lome, declared via a resolution (Resolution *AHG/Dec.156 (XXXVI*), of 12 July, 2000) to eliminate tsetse from the African continent through the Pan Africa Tsetse and Trypanosomiasis Eradication Campaign (PATTEC). This is because

SIT is the most environmentally friendly tsetse control technique. It makes sure that only the target insects are eliminated. This is to be done using a combination of insecticide impregnated screens and targets, pour on formulations to suppress tsetse populations and the use of sterile insect for mop up operations.

2.41 Criteria for Tsetse Control Using SIT

The criteria for a successful area-wide tsetse control using SIT adopted by PATTTEC for its operations is comprehensively given by Vreysen (2001). Study components comprising pre-control baseline data generation, include a full comprehensive species diversity and composition, distribution and infection rates, dispersal, population density, genetic structure for determining isolation status of target populations, wing morphometrics, establishment of tsetse colonies, monitoring and evaluation of control programmes etc. All these attributes could be defined by genetic tools. Information obtained in the study would be used in defining the effectiveness and appropriate scales of control measures that have become a vital tool in guiding the implementation of tsetse control strategies.

CHAPTER THREE
3.0 MATERIALS AND METHODS

3.1 Description of Study Areas

Location of study areas is as shown on Figure 3.1. The study was carried out in Yankari Game Reserve (Figure 3.2), Kainji Lake (Figure 3.3), Old Oyo (Figure 3.4), Cross River (Figure 3.5) National Parks, and a highly tsetse infested agropastoral zone in Ijah Gwari (Figure 3.6). All, except Ijah Gwari, are national parks (Table 2.4). National parks are protected areas, where hunting, fishing, lumbering, firewood gathering, livestock grazing, farming activities etc are prohibited. Ijah Gwari was chosen to compare tsetse populations outside the national parks with those in the national parks.

3.1.1 Yankari Game Reserve

Yankari Game Reserve (Figure 3.2) is located in the south-eastern part of Bauchi State, North eastern Nigeria between latitude 09^{o}.50'N and 10^{o}.30'E and lies within the Northern guinea/Sudan-savannah vegetation zone. It is an ecotourism and tourism destination. The reserve covers an area of 2,244km^2 and is home to several natural warm water springs as well as a wide variety of flora and fauna. Yankari is rich in wildlife resources; the reserve is an important refuge for over 50 species of mammals, including *Loxodonta Africana* (Elephants), *Papio papio,* (Olive Baboons), *Makaka fascicularis* (Patas Monkeys), *Chlorocebus tantalus* (Tantalus Monkeys), *Hippotragus equinus* (Roan Antelopes), *Alcelaphus buselaphus* (Western Hartebeest), *Panthera leo* (Lion), *Syncerus caffer* (African Buffalo), *Kobus ellipsiprymnus* (Waterbuck), *Tragelaphus sylvaticus* (Bushbuck) and *Hippopotamus amphibious* (Hippopotamus) (National parks Hand bills, 2004). It has a large and diverse freshwater ecosystem around its freshwater springs. Grasses with scattered

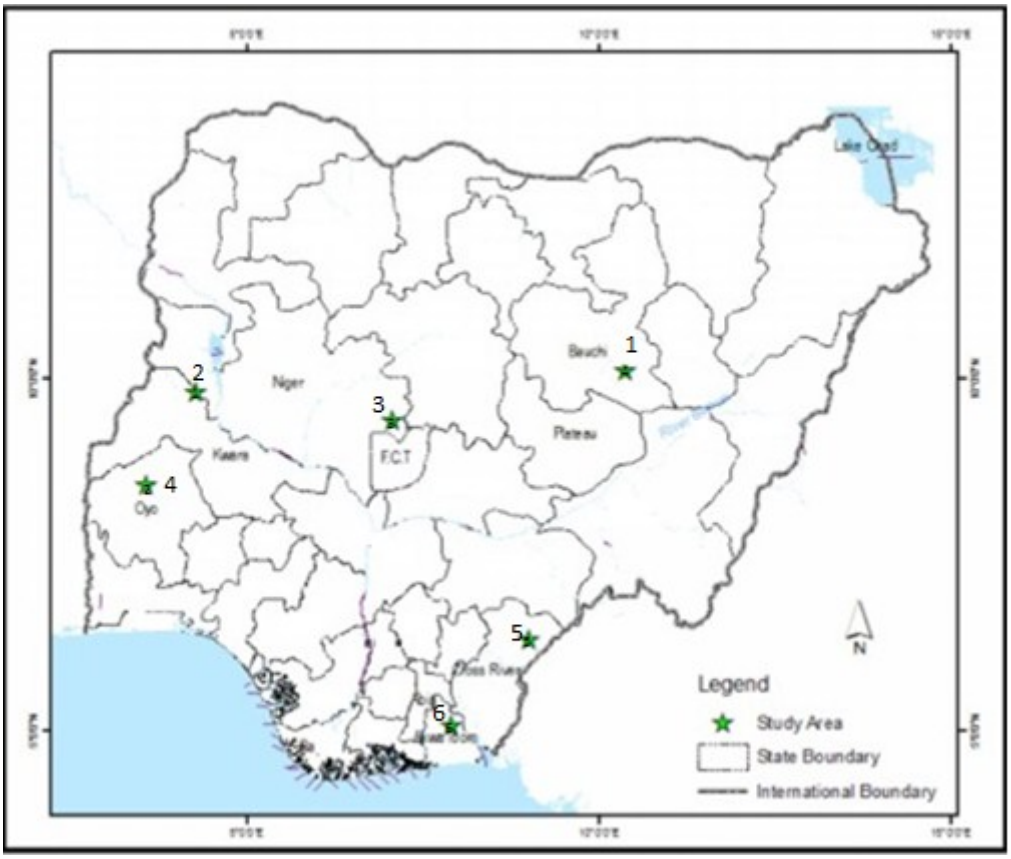


Figure 3. 1: Study areas in Nigeria

1. Yankari Game Reserve, 2. Kainji Lake National Park, 3. Ijah Gwari, 4. Old Oyo National Park, 5. Cross River National Park (Okwango Division), 6. Cross River National Park (Bama Division)

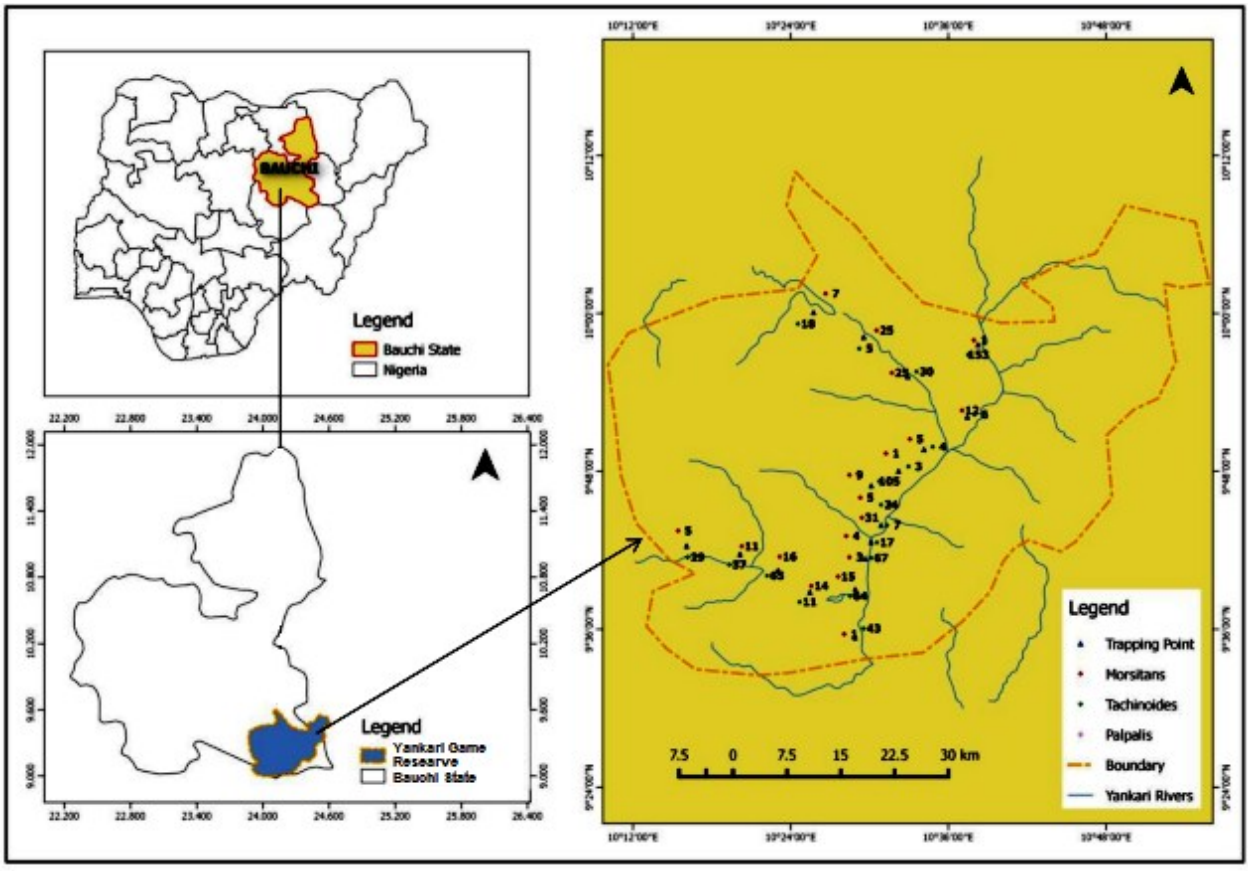


Figure 3. 2: Yankari Game Reserve and tsetse trapping points.

trees and shrubs dominate the vegetation. Trees are shorter and thorny and are fewer in numbers than in the southern guinea savanna. Tree species found in the Northern guinea savanna include *Isorberlina Doka, Vapola, Somona* and *Monotest kertingii*. The park consists of two vegetation types, a savannah woodland at the hinterland, and a riverine vegetation at the bank of river courses.

The climate of Yankari Game Reserve is tropical in nature and is characterized by wet and dry seasons. The temperature ranges between 31 and 35.5^{o}C, while the mean annual rainfall ranges between 510 and 1,140mm. The wet season is associated with the Southwest monsoon wind from the Atlantic Ocean while the dry season is associated with the northeast trade winds from the Sahara desert. The dry season lasts between 5 – 7months (October- April). There are 350 species of birds; of these, 130 are residents, 50 Paleantic migrants and the rest are intra-African migrants that move locally within Nigeria. These birds include the *Ephippiorhynchus senegalensis* (saddle-billed stork), *Gyps bengalensis* (white-rumped vulture), *Numididae* (Guinea fowl), *Ocyceros birostris* (Grey hornbill) and the *Bubulcus ibis* (cattle Egret). The park has some of the largest populations of *Loxodonta* spp. (elephants) in West Africa, estimated at more than 300 in 2005 (National Parks of Nigeria Hand bills, 2004).

3.1.2 Kainji Lake National Park

Kainji Lake National Park (Figure 3.3) is located in the northwest part of the country, between latitude 9^{o}45′ and 10^{o}23′N and longitudes 3^{o}4′ and 5^{o}47′E in Niger and Kwara States within the Southern Guinea vegetation zone. The mean annual rainfall is above 500mm. Dry season is shorter and less intense than in the northern guinea savanna. The vegetation is an open savanna wood land type, with tall grasses up to 3m high. The trees have short, large and broad leaves. Major characteristics of the zone are the common occurance of wild fires in the dry season. Several of

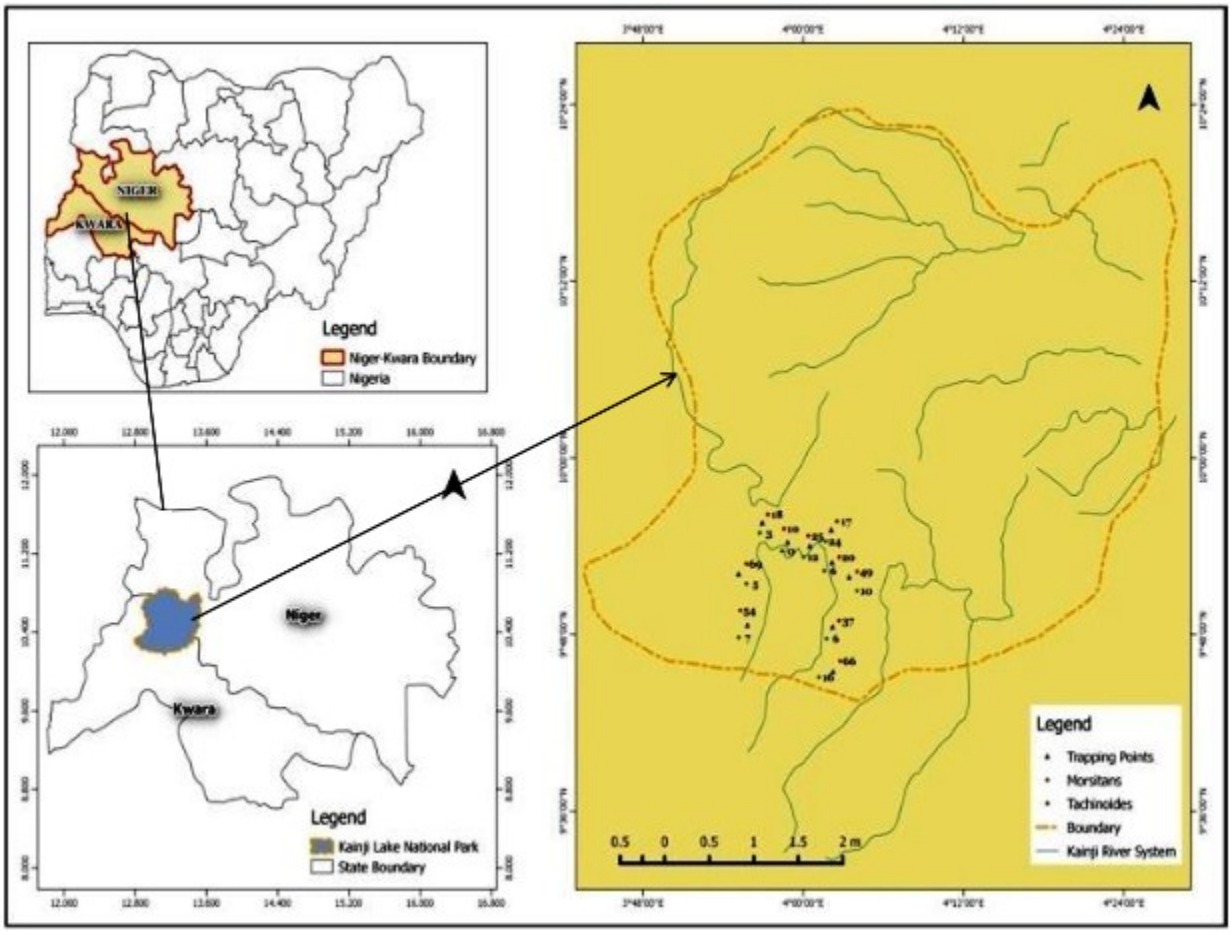


Figure 3. 3: Kainji Lake National Park and tsese trapping points.

the trees are adapted to fires by the possession of thick, fire resistant barks. Trees include *Doniellia oliveri: Hypharrenia* and *pennisetum*. The park covers an area of about 5382km^2 separated into two distinct non-contiguous sectors, namely Borgu sector with an area of 3970.02km^2 and Zugurma sector covering an area of 1370.80km^2 (National Parks of Nigeria Hand bills, 2004).

3.1.3 Old Oyo National Park

Old Oyo National Park (Figure 3.4) is located in the northern part of Oyo State, South Western Nigeria. It has a total land area of 2,512km^2. Dry season is usually three months with mean annual rainfall of 1,440-1,780mm. The park lies between latitudes 8º10′ and 9º05′N and longitudes 3º00′ and 4º02′E within the derived Savannah vegetation zone. The park is rich in fauna and flora resources, significant among which are *Hippotragus equinus* (Roan antelope), *Kobus kob kob* (Buffon Kob), *Syncerus caffer* (Buffalo), *Tragelaphus sylvaticus* (Bushbuck) and a wide variety of Birdlife (National Parks of Nigeria Hand bills, 2004).

3.1.4 Cross River National Park

Cross River National Park (Figure 3.5) is located in the rainforest ecological zone in the extreme southeastern corner of Nigeria on the border with Republic of Cameroun between longitudes 5º05′ and 6º29′N and latitudes 8º15′and 9º30′E.. The park occupies a total land area of about 4000km^2 of tropical rain forest ecosystem which thins out progressively into montane savanna vegetation at the edge of the Obudu Plateau in Okwangwo area. It exists as two non-contiguous divisions, Oban and Okwangwo. The Southern Oban sector has an area of 3,000km^2 while the Northern Okwango division near Obudu covers an area of 1,000km^2. The park habours many species of plants and animals some of which are *Gorilla gorilla* (Gorilla), *Mandrillus leucophaeus* (Drill), *Pan troglodytes* (Chimpanzee), *Arctocebus aureus* (Angwantibo or Golden Potto), *Loxodonta cyclotis*

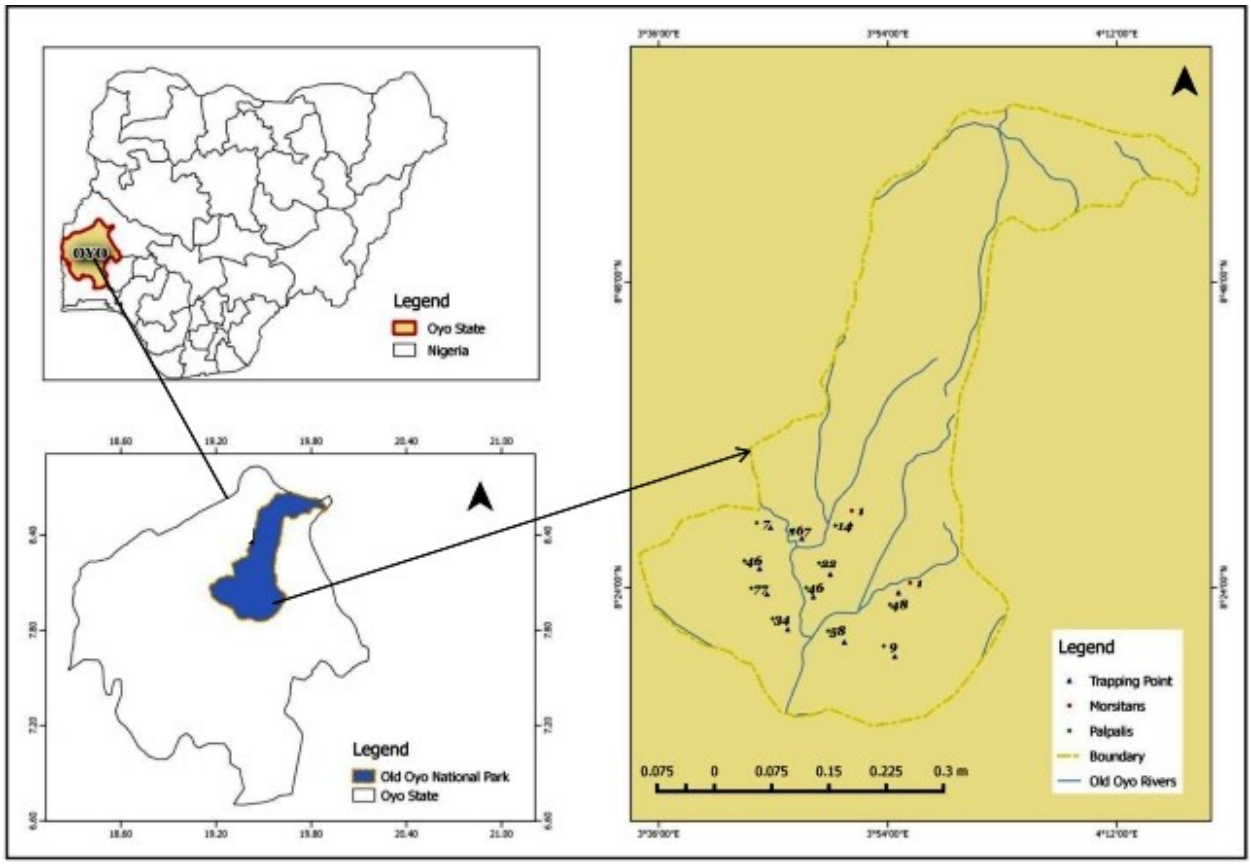


Figure 3. 4: Old Oyo National Park and tsetse trapping points.

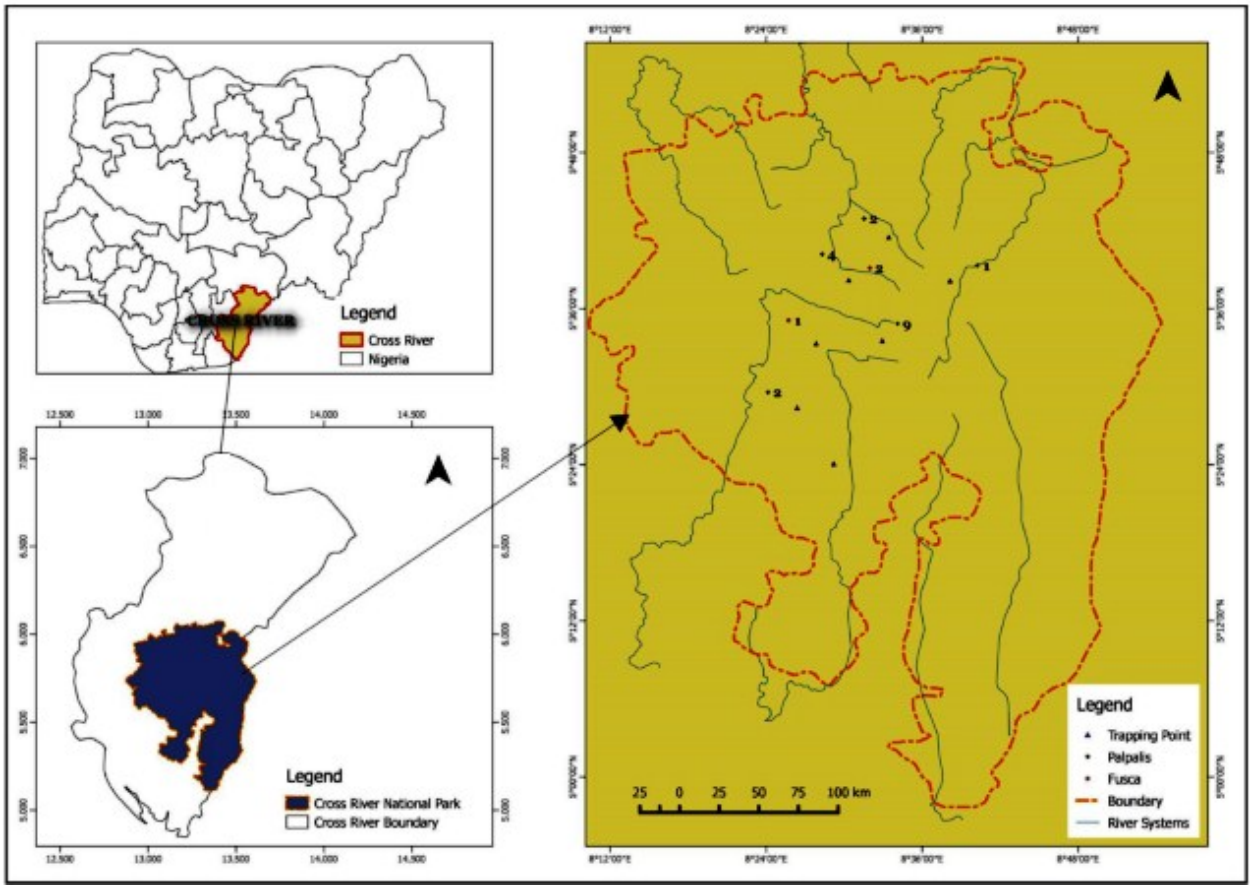


Figure 3. 5: Cross River National Park and tsetse trapping points.

the trees are adapted to fires by the possession of thick, fire resistant barks. Trees include *Doniellia oliveri: Hypharrenia* and *pennisetum*. The park covers an area of about 5382km^2 separated into The park has two entry points, Erokut entry point, at Akamkpa and Butatong Base Camp in Okwaango division (National Parks of Nigeria Hand bills, 2004).

3.1.5 Ijah Gwari

Ijah Gwari (Figure 3.6) lies between latititude 9º12′0″N and 9º24′N and longitude 7º12′E and 7º 20″E in Tarfa Local Government Area of Niger State, within the Southern guinea savannah vegetation zone. The area is traversed by several small streams which empty into the major River Muye that flows through Abuja Federal Capital Territory (FCT). The vegetation is riverine forest forming a dense two storey canopy. The area is a refuge to large populations of terrestrial and aboreal animals including, reptiles, squirrels and primates. The lanscape is characterized by rocky outcrops of small and medium sizes. The major settlement in the area is 6-10kilometers to the collection site. Fulani households are about 4kilometers to the study site. Anthropogenic activities observed include logging, firewood collection, charcoal making, palm wine tapping, rearing of domestic animals and crop cultivation at the hinterland. Ijah Gwari is a field experimental site under the supervision of the Suleja outstation of the Nigerian Institute for Trypanosomiasis Research.

3.2 Entry Permit into National Parks

Entry permit into the National Parks for tsetse collections was sought and obtained from the Office of the Conservator-General of National Parks (Appendix 1). Entry permit into Ijah Gwari was obtained from traditional leaders and local administrative authorities according to Nigerian laws.

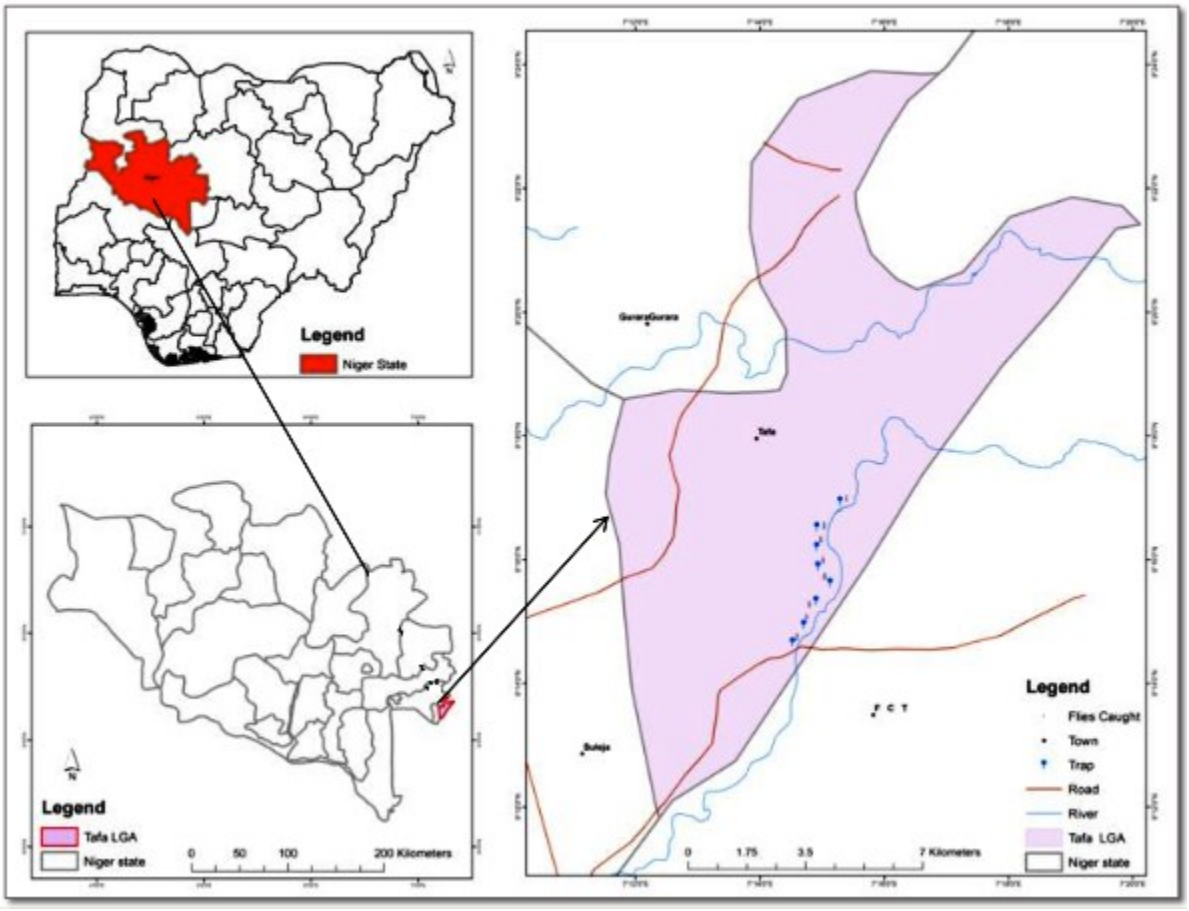


Figure 3. 6: Ijah Gwari and tsetse trapping points.

3.3 Methodology

3.3.1 Sample collection, handling and documentation:

Tsetse flies were collected using standard biconical traps (Plate II), between February and March 2014. A total of fifty one traps were deployed to tsetse collection sites during the sampling comprising, 18 in Yankari Game Reserve, 10 Kainji, 11 Old Oyo, 7 Cross river and 5 in Ijah Gwari. Trapping was carried out over a period of 2 days each in Yankari and Cross River, 3days each in Kainji and Old Oyo, while only 1 day's collection was obtained from Ijah Gwari, making a total of 118 trap days. Traps were deployed in riparian vegetations at the banks of river/stream system, at water points where animals water, at animal tracts, and areas where animal-man/fly contact were suspected to be highest. Traps were deployed at distances of 100 meters apart and were harvested after a minimum of 24 hours and a maximum of 48 hours before removing and redeploying to new positions. Trap catches were conveyed to camp in cool boxes for further entomological evaluations. Flies were sorted out according to species and sexes using morphological identification keys as previously described (FAO, 1982). Data entry and documentation was done according to standard operational procedure (SOP) developed by the African Center of Excellence on Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB, 2014).

3.3.2 Mapping of *Glossina* species distribution using the Global Positioning System (GPS)

Positions of traps were captured using GPS device (GPSMAP® 60CSx Garmin) for imputing on the map for georeferencing the spatial distribution of the flies. The captured points in the GPS were downloaded on to the computer using the DNR Garmin/Easy GPS software. The downloaded data from the GPS were further transferred to the MAPSOURCE and processed using EXCEL program and Arc GIS/ MapInfo/ Arc Map/ Quantum GIS. Spatial patterns of tsetse distribution were



Plate II: The biconical trap (Challier and Lavessiere, 1973) in a tsetse collection site (Source: From field work collections)

determined using the average nearest neighbour analysis feature in ESRI Arc GIS 9.0 and geo-referenced distribution maps drawn to scale (Figures 3.3-3.6).

3.3.3 Micro climatic conditions in National Parks

The ambient temperature and relative humidity were taken with whirling hygrometer during positioning and harvesting of traps. Whereas ambient temperature readings where obtained with the dry bulb thermometer, the wet bulb thermometer readings where converted by the slide rule to obtain relative humidity values.

3.3.4 Species composition, relative abundance and distribution

In each study area, trap catches were sorted out and examined for species composition, relative abundance and distribution. Sum total of each parameter was documented. Flies were identified morphologically using identification keys as previously described (FAO, 1982).

3.3.5 Apparent density of *Glossina* species in sampled locations

Apparent density is measured as the relative number of fly species in a locality. It is calculated by summing the number of flies collected divided by number of traps multiplied by number of trapping days. And the density is in the scale of 1-20 fly/trap/day, where values betweens1-5 are regarded as low density, 5-20 medium density and above 20 high density.

3.3.6 Sex ratio

Sex ratio is the proportion of male and female flies in a population expressed as a ratio. It was determined by simply sorting out male and female flies' component of the catches and dividing the total number of female flies captured by the total number of males in the catch. Male and female flies were distinguished by simply examining the external genitalia of the adult flies. A fly that possessed an external genitalia forming a conspicuous knob-like protuberance (hypopygium)

beneath the end of the abdomen, was identified as a male, whereas a fly without this structure was regarded as a female (Figure 2.5).

3.3.7 Teneral and non-teneral flies

Teneral and non-teneral flies were sorted out and counted as a percentile proportion of the overall catch and according to species. Teneral flies are flies that have never taken a blood meal since their emergence, whereas non-tenerals are those that have taken blood meals. The two categories of flies were distinguished by looking closely at the ventral side of the abdomen. Teneral flies were identified by a soft soapy touch, by a whitish semi-transparent appearance of the underside of the abdomen, and the evertion of the ptilinium upon squeezing of both sides of the head capsule between two fingers as earlier described (Pollock, 1982). Whereas the non-teneral flies were identified by the more creamy yellow, appearance of the underside of the abdomen and when held up to the light, the dark shape of the last meal could be seen. The thorax in the non-teneral flies when felt, was firmer and harder, because of the greater development of muscles. The ptilinium could not be easily everted when the head was squeezed between two fingers as described in FAO (2008).

3.3.8 Hunger staging

The classical method for estimating hunger stage in tsetse flies is according to the method earlier described in Jackson (1933). However, due to logistical problems, this method could not be used to hunger stage flies in this study. Rather the method described by Madubunyi (1989) which involves dissection of both male and female flies and assessing blood meal contents in the guts of dissected flies was used. Flies were hunger staged by simply examining the blood contents in the guts of dissected flies. Where traces of blood were detected in the guts of dissected flies, they were regarded as having fed and where no traces of blood meal were detected, flies were regarded as

not having fed. The proportion of hungry and non-hungry flies were determined by counting and the sum totals in each category documented.

3.3.9 Collection and preservation of tsetse fly tissues for genetic analysis

Live flies collected by trapping according to the standard operational procedures (SOPs) were dissected to acquire the following tissues, wings, legs, proboscis, salivary glands, and midguts. Wings were collected into 2ml tubes for morphometric analysis, 2 to 3 legs, in tubes containing 200µL RNA*later* for genetic analysis, while proboscis, salivary glands and whole/midguts in tubes containing 200µL RNA*later* for trypanosome infection rate studies. RNA*later* (25 mM sodium citrate, 10 mM ethylene diamine tetraacetic acid (EDTA), 70 g ammonium sulfate/100 mL solution, pH 7.5) was dispensed to each of the tubes for preservation. Samples containing RNA*later* were kept at room temperature for 24hours and later at -20^{o}C or -80^{o}C until needed for further analysis. In order to avoid contermination, after each dissection, dissection pins were dipped in decontamination solution containing 70% alcohol or 50:50% distilled water/ sodium hypochloride (bleach) for 30minutes and then rinsed in double distilled water.

3.4 Molecular Studies

Molecular aspect of this work was carried out at the Center for Biomolecular Interactions, University of Bremen, Germany during the period 2014 to 2015.

3.4.1 DNA Extraction

DNA was extracted using Dneasy DNA kit (Qiagen, Germany) following the manufacturer's instructions. 2µL tubes containing 2-3 legs in 250µL RNA*later* earlier collected, were centrifuged to obtain the supernatant. RNA*later* was decanted and the legs were transferred into new 2µL tubes and quashed using metal beads. 180µL Buffer ATL and 200µl Proteinase K was added and

mixed thoroughly by vortexing. The mixture was then incubated at 56ºC until completely lysed, with vortexing at intervals for 15 minutes. 700µL Buffer ATL was added and mixed thoroughly by vortexing and incubated at 56ºC for 10 minutes. 100µL absolute ethanol was added and mixed thoroughly by vortexing. The mixture was pippetted into a DNeasy minispin column placed in 2ml collection tubes and centrifuged at ≥6000g (8000rpm) for 1minute. The flow through was discarded and the spin column was then placed in a new 2µL collection tube, to which 500µl Buffer AW2 (wash buffer) was added and centrifuged for 3minutes at 20000xg (14,000rpm).

The flow through was again discarded with the collection tube. The spin column was transferred to a new 1.5ml micro centrifuge tube, where the DNA was eluded by adding 200µl Buffer AE (elusion buffer) to the center of the spin column membrane and incubated for 1minute at room temperature (15-25ºC) and finally centrifuged for 1minute at ≥6000g and stored.

3.4.2 DNA Quantification

DNA was quantified by Spectrophotometry (Nanodrop ND1000, Thermo Scientific, Germany) at a wavelength of 260nm. The process involved three stages of initialization, calibration and measurement. The machine was set to Nucleic Acid programme. The sample loading peddestral of the Nanodrop was wiped with clean white tissue paper before and after every measurement. Then 1.5µL double distilled water (ddH_20) was applied to the loading peddestral of the Nanodrop for initialisation, followed by 1.5µL elusion buffer for blank test followed by measurement of the concentration by applying 1.5µL of DNA sample on to the loading peddestral of the Nanodrop and readings were taken.

3.4.3 Preparation of Master Mix

Reaction mixtures were carried out in 25 µL final volume containing 2.5 µL 10x Dream*Taq* Green Buffer, 0.5µL dNTPs), 0.5µL Dream*Taq* Polymerase (all supplied by Thermo Scientific, Dreieich, Germany), 0.5µL 100 µM Primers (forward and reverse), 13.5µL ddH_2O and 5.0 µL DNA template (Dyer *et al*., 2008).

3.4.4 PCR Amplifications

Partial gene fragments of Cytochrome C Oxidase SUI and Internal transcribed spacer I (ITS-1) were amplified as described in Dyer *et al*., (2008). Tubes containing 25 µL final volume Master Mix were placed in the thermocycler (2720 Thermal Cycler Applied Biosystems). Partial Cytochrome C Oxidase SU1 (CO1) was amplified with Primers (forward 5′ TTG ATT TTT TGG TCA TCC AGA AGT-3′ and reverse 5′ -TGA AGC TTA AAT TCA TTG CAC TAA TC-3′) at initial denaturation step of 95 °C for 5 minutes, followed by 35 cycles of 1 minute at 94°C, 1 minute annealing temperature at 55°C and 2 minutes elongation at 72 °C. The final elongation was 10 minutes at 72 °C and 4°C final hold.

The ITS-1 region was amplified with ITS-1-forward (5′-GTG ATC CAC CGC TTA GAG TGA-3’) and ITS-1-reverse (5′-GCA AAA GTT GAC CGA ACT TGA-3′) primers, with an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 1 minute at 94 °C, 1 minute at 60 °C and 1.5 minutes at 72 °C. The final elongation was 7 minutes at 72 °C.

3.4.5 Preparation of Agarose Gel

A 1.5 - 2% (w/v) Agarose gel was constituted by weighing 1.5 -2g of agarose gel in a conical flask and 100ml of TAE buffer (40 mM Tris, 10mM Na-Acetate, 1mM EDTA, pH 8.0) was added to the powder and mixed. Heat was applied to the mixture for 3-5 minutes in a microwave to dissolve

the power and allowed to cool to 56ºC, after which 1.5µL G- stain was added. The gel solution was then cast in a gel mould in which the gel comb had been appropriately inserted. The agarose gel was allowed to solidify for 45 minutes at room temperature.

3.4.6 Gel Electrophoresis

10µL of 100bp molecular marker was loaded with micropipette into the first well followed by the loading of 10µL test sample plus 2µL loading dye (bromophenol blue) in other wells of agarose. The loading dye helps to increase the viscosity of the sample (DNA) ensuring that samples drop evenly into the wells of agarose to prevent diffusion into the TAE, in addition to adding colour to the sample thereby simplifying the loading process. The sample was run on 100 volts for 45 minutes for migration after which the gel was removed and transferred to the transilluminator and for documentation.

3.4.7 Purification of DNA samples for sequencing

To obtain high grade DNA for sequencing, PCR amplicons were subjected to another round of amplifications. 20µL of PCR products were loaded on to the wells made with preparatory combs of a 2% (w/v) solidified gel. Excised PCR products were gel purified using GeneJet DNA purification kit (Thermo Scientific, Dreieich, Germany) following the manufacturer's instruction. DNA concenrations were determined at a wavelength of 260nm on a Nanodrop 1000 device (Thermo Scientific, Dreiech, Germany). Purified PCR products were cloned into linearised pJET 1.2/blunt plasmid using the CloneJET PCR (Thermo Scientific, Dreiech, Germany) according to the manufacturer's instructions. Positive clones were identified by colony PCR and selected single colonies were cultured in LB plus ampicillin (100 µg/mL) with shaking overnight at 37 °C. Bacteria were collected by centrifugation (4,500 × g, 15 minutes at 4 °C) and the plasmid DNA

was purified using the GeneJET Plasmid Mini Prep Kit (Thermo Fischer Scientific, Dreiech, Germany) according to the manufacturer's instructions.

3.4.8 Sequensing reactions

Purified DNA samples obtained were subjected to sequencing reactions in final volumes of 11µL consisting of 2.0µL Big Dye, 1.0µL using 20 - 50ng template Cytochrome Oxidase C SU1 insect generic primer (forward 5′-TTG ATT TTT TGG TCA TCC AGA AGT-3′ and reverse 5′ -TGA AGC TTA AAT TCA TTG CAC TAA TC-3′), (Dyer *et al*., 2008). Sequencing reactions were left over night after which they were subjected to PCR (Thermocycler 2720 Applied Biosystems) for 60cycles regime at 95ºC for 2 minutes, 95ºC for 15 seconds, and 56 ºC for 15 seconds, 60 ºC for 4 minutes and 60ºC for 7 minutes. Reactions were then purified using Sephadex 50 in 96 well plates previously equilibrated with distilled water at 4ºC and sent for sequencing at the Max Planck Institute for Marine Microbiology-Bremen.

3.4.9 Sequencing

Sequencing was carried out at the Max plank Institute, Bremen Germany, according to Sanger termination method (Sanger *et al.,* 1977), using a Genetic Analyser (Applied Biosystems, Germany).

3.5 Data Analyses

3.5.1 Differential Statistics

The Student's t-test was used to determine if there was any statistical relationship between ambient temperature and relative humidity conditions in relation to fly catches for each sampling site using the IBM SPSS Statistical parkage version 20. Correletion statistics was also used to determine if there was any correlation between tsetse catches and climatic conditions of temperature and relative humidity. Analysis of variance (ANOVA) was used to compare if there was any significant

difference between tsetse catches and sampled locations. Where there was similarity in means, the Ducan's Multiple Range test was used to separate the means.

3.6 Genetic Analyses

3.6.1 Sequence alignments

For each PCR products sequenced, obtained sequences forward and reverse were aligned using the Geneious software version 5.5.9 (Drummond *et al.,* 2001). Alignments were trimmed and analysed using the ClustalW algorithm in MEGA version 6.0 with gap opening penalty 15, gap extension penalty 7.5, IUB weight matrix, transition weight 0.5 and delay divergent cut-off 30. MEGA was also used to calculate the proportion of sites differing between pairs of sequences, which are quoted in the text as percentage differences. ITS-1 sequences were analysed as described above, using pairwise gap deletion.

3.6.2 Molecular species identification

Species identification was done by sequence similarity searches using the Basic Local Alignment Search Tool (BLAST) based on alignments of closely related sequences on the Genbank database of the National Centre for Biotechnology Information (NCBI:http//www.ncbi.nlm.nih.gov/ BLAST). Sequences from the database that best matched that of the samples were retrieved for phylogenetic analysis using the programme encapsulated in Genius Version 5.5.9 (Drummond 2001). The sample and GenBank database sequences of the species were aligned in MEGA 4.1 to generate neighbour joining tree separately for the two genes. The MEGA 4.1 Kimura-2 parameter model simultaneously calculates the genetic distances between pairs of sequences and constructs a neighbour joining tree (Saitou and Nei, 1987). The bootstrap test of phylogeny (Hillis and Bull, 1993) option of MEGA 4.1 was selected and used. DnaSP version 5 was used to generate the

haplotypes, an index of DNA polymorphism (Librado and Rozas, 2009). ITS-1 sequences were analysed as described above, using pairwise gap deletion.

3.6.3 Haplotype diversity

Haplotype diversity is defined as the probability that two randomly chosen haplotypes are different (Nei, 1987). The unbiased estimate of haplotype diversity is given by $he = n\ (1 - \Sigma xk2)/(n - 1)$, where x is the frequency of haplotype k and n is the number of individuals (Nei, 1987). For the parsimony analysis, haplotype trees for CO1 data from *G. tachinoides*, *G. m. submorsitans* and *G. p. palpalis* were generated using the algorithm of Templeton *et al.,* (1992). The TCS 1.21 programme was used to estimate the haplotype tree, with the connection limit (probability of parsimony) at 95% (Clement *et al.,* 2000). The software Bottleneck ver. 1.2 (Piry *et al.*, 1999) was used to test hypotheses that populations were in mutation-drift equilibrium at selectively neutral loci (Cornuet and Luikart, 1996). Departures from equilibrium could be caused by recent bottlenecks in population size or recent increases in population size (Maruyama and Fuerst, 1985). A test of neutrality, the McDonald–Kreitman test (Mcdonald and Kreitman, 1991), was implemented in DnaSP for the CO1 dataset (Rozas and Rozas, 1995; 1997; 1999; Rozas *et al*., 2003). DnaSP was also used to calculate the average number of nucleotide differences between the clades defined by the TCS analysis.

3.6.4 Nucleotide diversity (Genetic variation)

Nucleotide diversity is defined as the average number of nucleotide differences per site between two randomly chosen DNA sequences (Nei and Li, 1979). It is the variation in composite nucleotides between a pair of randomly selected sequences. Analyses of nucleotide diversity were

carried out using FSTAT version 2.9.3.2 (Goudet, 1995). The level of similarity or variation is estimated in terms of the number of neocleotides the sequences have in common.

3.6.5 Genetic differentiation (Average Evolutionary Divergence)

Differentiations were estimated from the number of base substitutions per site from averaging over all sequence pairs, using the Maximum Composite Likelihood model (Tamura *et al*., 2004). The analysis involved all nucleotide sequences per *Glossina* species per site. All positions containing gaps and missing data were eliminated to get a total number of positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013). Tests for Hardy-Weinberg equilibrium of the substitution frequencies were carried out with Arlequin version 6.0 (Schneider *et al.,* 2000). Differentiation between regions, blocks and trapping sites was assessed by exact tests of homogeneity of nucleotide distribution among samples (Raymond and Rousset, 1995). Significance in pairwise comparisons was evaluated after a Bonferroni correction of critical probabilities. The extent of population differentiation was quantified by computing Weir and Cockerham's (1984) estimators of Wright's (Wright, 1951) F-statistics (F_{ST}, F_{IS} and F_G) by using Genepop. The genetic structure was analyzed using an analysis of variance method framework (Weir and Cockerham, 1984; Excoffier *et al.,* 1992; Weir 1996) as encapsulated in Arlequin 2.0 (Schneider *et al.,* 2000). Arlequin was also used to determine statistical significance of pairwise F_{ST} based on the permutation test of Manly (1985) with 1000 iterations. The relationship between genetic distance and geographic distance was tested according to methods of Hutchinson and Templeton (1999). Hierarchical analysis of diversity were computed according to methods of Nei (1987). Single locus statistics and genetic differentiation within and among natural populations were estimated using Analysis of Molecular Variance (AMOVA) with Alequin version 6.0

(Excoffier *et al.,* 1992). Statistical significance of deviation of single locus F_{Sj} estimates from zero was tested by using chi-square as outlined by Workman and Niswander (1970).

3.6.6 Phylogenetic relationships

Distance based, maximum likelihood and Bayesian phylogenies were calculated for CO1, and ITS1 separately, and also for the concatenated mtDNA dataset consisting of CO1 and ITS1. Phylogenetic analysis was performed using MEGA 6.0. Alignments were calculated with Muscle alignment tool (Gap open penalty -400, Gap extension 0, Clustering methods (all iterations) UPGMB). Neighbor joining trees were calculated using complete gap deletion and 900 bootstrap replications. Maximum likelihood trees were calculated using the model finder tool of MEGA. Tamura 3-parameter model was picked, assuming Gamma distribution with invariant sites with the default setting for number of discrete gamma categories (5). For all the phylogenetic inferences, positions containing gaps or missing data were not used. Phylogenetic reconstructions were carried out using neighbour joining trees in MEGA 6 (Tamura *et al.,* 2013).

3.7 Geometric Wing Morphometrics

Geometric morphometrics are considered a cheap and fast technique for investigating population structure. The technique was used to investigate population structure of *Glossina palpalis palpalis* populations for comparative purposes with CO 1 sequences. To achieve this, 41 individual flies collected at the border region with Cameroon had wings in a state suitable for morphometric analysis. Wings were secured on a microscope slide using a drop of Faure'sche solution on the wing root. They were covered with a cover glass and dried overnight. Pictures were taken using an Olympus SZX16 Binocular (Olympus, Hamburg, Germany) with upward light (Olympus KL1500 LCD, Olympus, Hamburg, Germany) employing 10x magnification and scale bar added using Cell^F program (Olympus, Hamburg, Germany). 9 landmarks were selected for analysis as

described previously (Achukwi *et al.,* 2013). The CLIC package (http://mome-clic.com) was used for landmarks analysis. COO was used to digitalize the landmarks. Procrustes superimposition (centering of configuration of landmarks, scaling, and rotation) and comparison of centroid sizes was done using MOG. Samples were segregated according to their genetic group or sex. Data obtained were compared with the COI sequences.

CHAPTER FOUR

4.0 RESULTS

4.1 Ecological Studies

4.1.1 *Glossina* species composition and relative abundance

Table 4.1 shows pattern of catches of *Glossina* species in the national parks and Ijah Gwari. A total of 1891 tsetse flies was captured during the sampling. Flies were morphologically identified to include *Glossina tachinoides* 1050*, Glossina palpalis palpalis* 568*, Glossina morsitans submorsitans* 270 and *Glossina fusca* 3. Figure 4.1 shows total *Glossina* species caught in the national parks and Ijah Gwari. Catches were higher in Yankari Game Reserve 863 (45.64%), than Old Oyo 530 (28.027%), Kainji Lake 457 (24.16%), and Cross river 21 (1%) National Parks respectively, while in Ijah Gwari 20 (1.05%) flies were caught. Figure 4.2 shows abundance of *Glossina* species caught in the national parks and Ijah Gwari. The predominant *Glossina* spp. in the catches was *G. tachinoides* 1050 (55%), followed by *G. p. palpalis* 568 (30%), *G. m. submorsitans* 270 (14%) and the least *G. fusca* (1%). In terms of composition, Yankari Game Reserve was composed of *Glossina tachinoides* 682 (78.02%), followed by *G. m. submorsitans* 179 (20.74%) and *G. p. palpalis* 2 (0.23%) respectively. Kainji Lake National Park was composed of *G. tachinoides* 368 (80.52%), and *G. m. submorsitans* 89 (19.47%). In Old Oyo, the catch composition of *Glossina* spp. was *G. p. palpalis* 528 (99.63%), followed by *G. m. submorsitans* 2 (0.37%). A total of 18 (85.71%) *G. p. palpalis* was recorded in Cross River National Park followed by 3 (14.28%) *G. fusca*, while 20 (100%) *G. p. palpalis* were caught in Ijah Gwari. Statistical analysis shows significant difference (χ^2 (4) = 1375.417, $p < 0.001$) in the relative abundance of *Glossina* spp. per sampled location. However, pairwise comparison shows no significant association (χ^2 (12) = 2134.106, $p > 0.001$). Whereas no significant difference (t = -1.580, p 0.133

Table 4. 1: Pattern of *Glossina* species caught in the National Parks and Ijah Gwari

Location	*Glossina* species caught								Total	%
	G. tachinoides		*G. m. submorsitans*		*G. p. palpalis*		*G. fusca*			
	♂	♀	♂	♀	♂	♀	♂	♀		
Yankari	476	206	80	99	1	1			863[a]	46
Old Oyo			1	1	276	252			530[b]	28
Kainji	250	118	39	50					457[c]	24
Cross River					6	12		3	21[d]	1
Ijah Gwari*					10	10			20[d]	1
Total by sex	726	324	120	150	293	275		3	1891	100
Total	1050		270		568		3		1891	100

*NITR Suleja Outstation collection site.

Values in column total with different superscripts have significantly different ($p<0.05$) relative abundance of *Glossina* spp. per site.

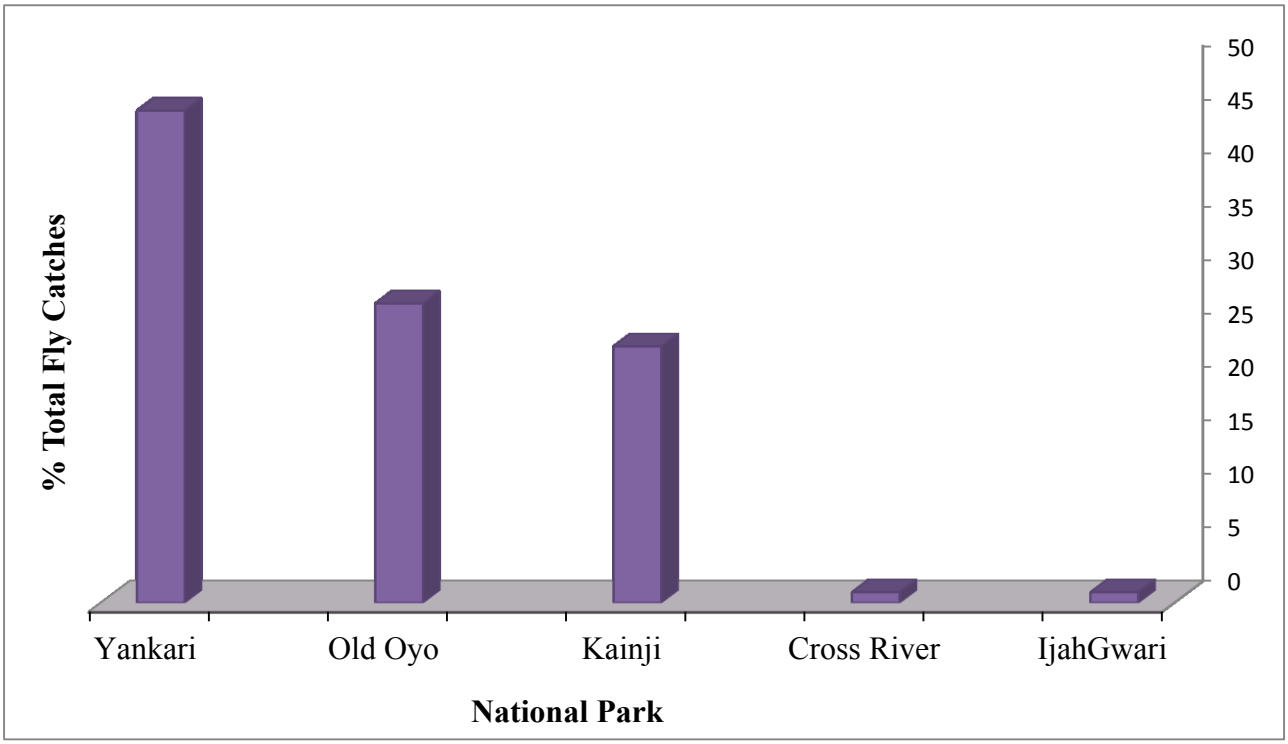


Figure 4. 1: Total *Glossina* species caught in the National Parks and Ijah Gwari

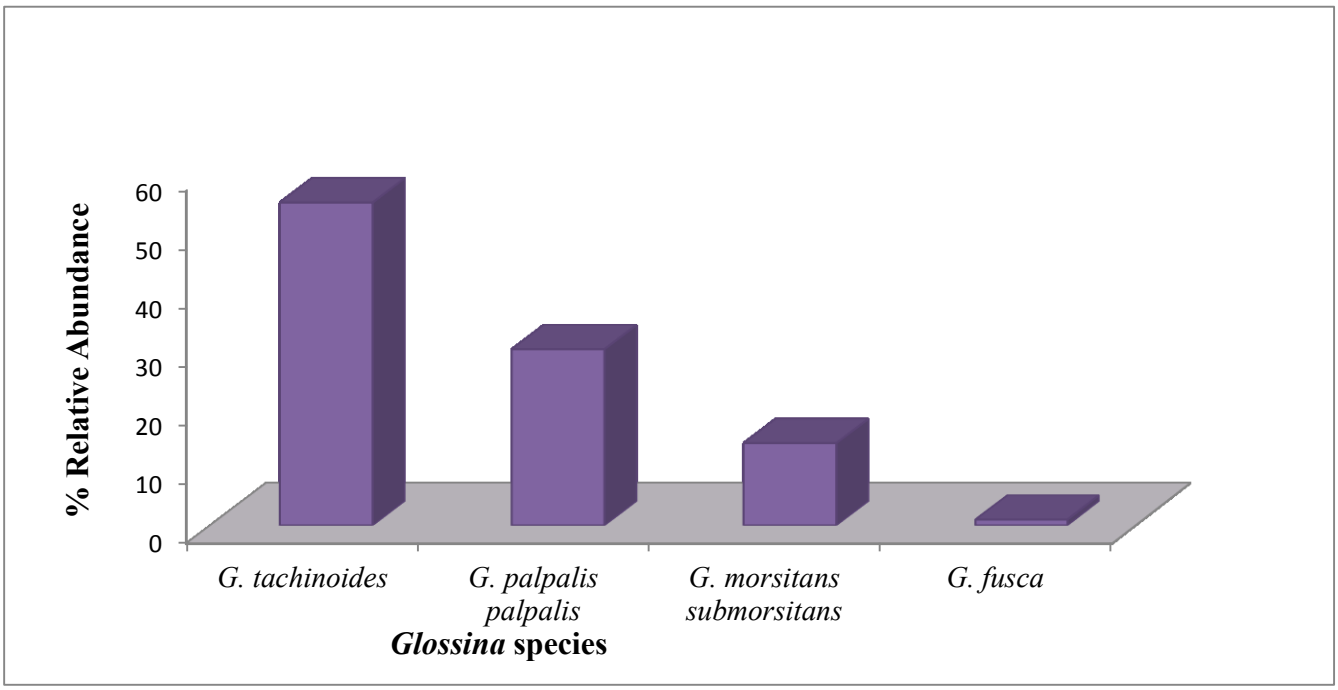


Figure 4. 2: Abundance of *Glossina* species across sampled National Parks and Ijah Gwari

Significant difference (p = 1.000) in the relative abundance of *Glossina* spp. between Cross River and Ijah Gwari sampled locations.

> α = 0.05) in tsetse catches in relation to ambient temperature and relative humidity conditions was recorded in Yankari Game Reserve, significant difference (t = -10.633, p =0.000 < α =0.05) was recorded in Kainji Lake National Park, (t = -36.290, p = 0.000 < α = 0.05), Old Oyo National Park (t = -18.939, p = 0.000 < α = 0.05), Cross River National Park (t = -18.939), p = 0.000 < α = 0.05) and Ijah Gwari (t = -4.613, p = 0.01 < α = 0.05) respectively.

4.1.2 *Glossina* spp. distribution in the National Parks and Ijah Gwari

Results (Figure 4.3) show uneven distribution of *Glossina* species across the national parks and Ijah Gwari. In Yankari Game Reserve, the most frequently caught *Glossina* spp. was *Glossina tachinoides* 682 (78.02%), followed by *G. m. submorsitans* 179 (20.74%) and *G. p. palpalis* 2 (0.23%) respectively. In Kainji Lake National Park, the most frequently caught *Glossina* spp. was *G. tachinoides* 368 (80.52%), followed by *G. m. submorsitans* 89 (19.47%). In Old Oyo, the most frequent *Glossina* spp. caught was *G. p. palpalis* 528 (99.63%), followed by *G. m. submorsitans* 2 (0.37%). A total of 18 (85.71%) *G. p. palpalis* was recorded in Cross River National Park followed by 3 (14.28%) *G. fusca*, while 20 (100%) *G. p. palpalis* were caught in Ijah Gwari. The distribution of fly catches shows that out of a total of 1050 *G. tachinoides* caught, 682 (65%) were captured in Yankari Game Reserve and 368 (35%) in Kainji Lake National Park. Out of a total of 270 *G. m. submorsitans* caught, 179 (66.3%) were captured in Yankari Game Reserve, 89 (33%) in Kainji Lake and 2 (0.74%) in Old Oyo National Parks. Of the 568 *G. p. palpalis* caught, 528 (93 %) were captured in Old Oyo National Park, 20 (3.5%) in Ijah Gwari, 18 (3%) in Cross River National Park and 2 (0.35%) in Yankari Game Reserve respectively. All the 3 *Glossina fusca* caught in the study, were from Cross River National Park. Statistical analysis showed no significant difference (χ^2 (12) = 2134.106, $p > 0.001$) in *Glossina* species distribution between the sampled locations.

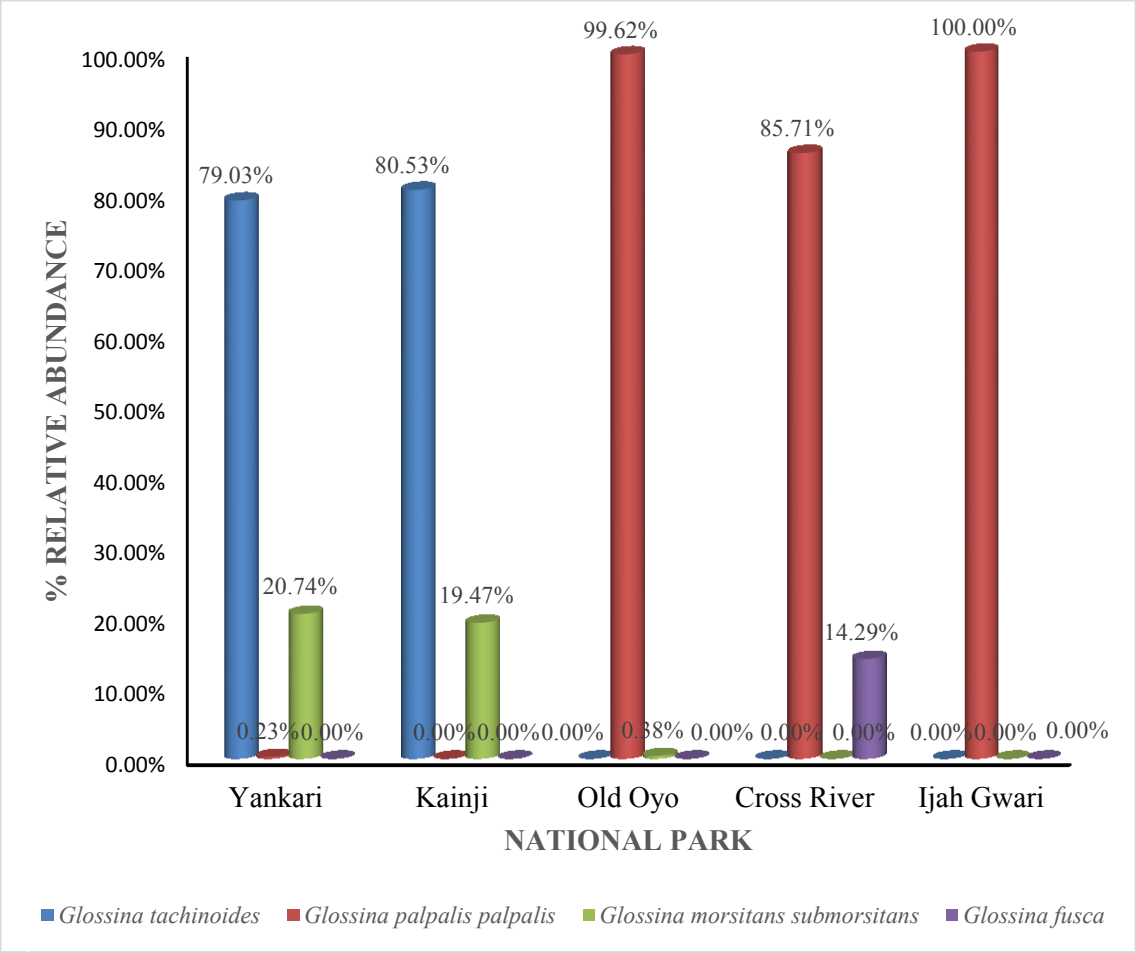


Figure 4. 3: *Glossina* species distribution in the National Parks and Ijah Gwari.

4.1.3 *Glossina* spp. distribution in relation to ambient temperature and relative humidity conditions

Figure 4.4 showed the distribution in catches of *G. m. submorsitans*, *G. tachinoides* and *G. p. palpalis* in relation to ambient temperature and relative humidity conditions in Yankari Game Reserve. Flies were collected under prevailing average ambient temperature range of 31 - 35.5ºC and relative humidity range of 28.0 - 46.0%. In Kainji Lake National Park (Figure 4.5), only two tsetse species were caught (*G. tachinoides* and *G. m. submorsitans*) under prevailing ambient temperatures range of 26.5 - 36ºC and relative humidity range of 43.5 - 57.5%. In Old Oyo National Park (Figure 4.6), *G. p. palpalis* and *G. m. submorsitans* were caught under prevailing ambient temperatures range of 29 - 31.8ºC and relative humidity range of 58.5- 69%. In Cross River National Park (Figure 4.7), *G. fusca* and *G. p. palpalis* were caught under prevailing ambient temperature range of 24.5 - 29ºC and relative humidity range of 76.5 - 100%. In Ijah Gwari (Figure 4.8), *G. p. palpalis* was caught under prevailing temperatures range of 27 - 34ºC and relative humidity range of 50 - 84.5%. A decreasing pattern in prevailing ambient temperature range of 35.5 - 29ºC and increasing relative humidity range of 46 - 100% from the Northern Guinea savannah to the Rain forest region was detected during the sampling. So also a decreasing pattern in fluctuations in tsetse catches in relation to ambient temperature and relative humidity conditions from the Northern Guinea savanna (Figure 4.4) to the Southern Guinea savanna through the rain forest (Figure 4.7) vegetation zones was observed during the study.

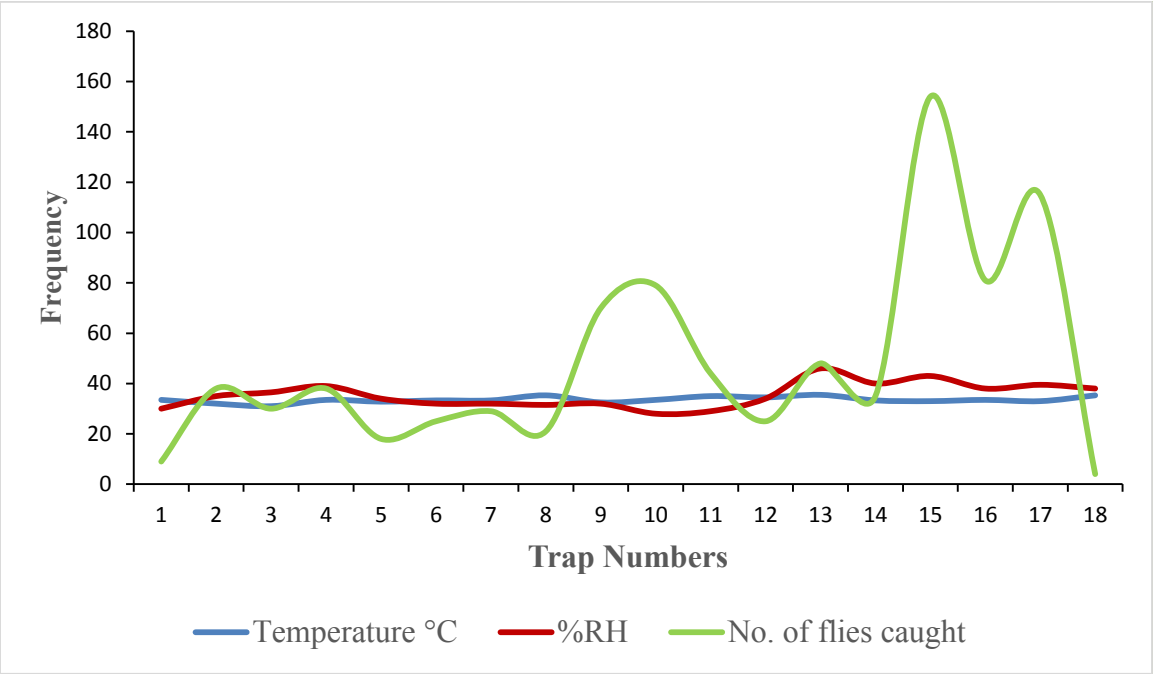


Figure 4. 4: Catch distribution of *Glossina* spp. in relation to ambient temperature and relative humidity conditions in Yankari Game Reserve.

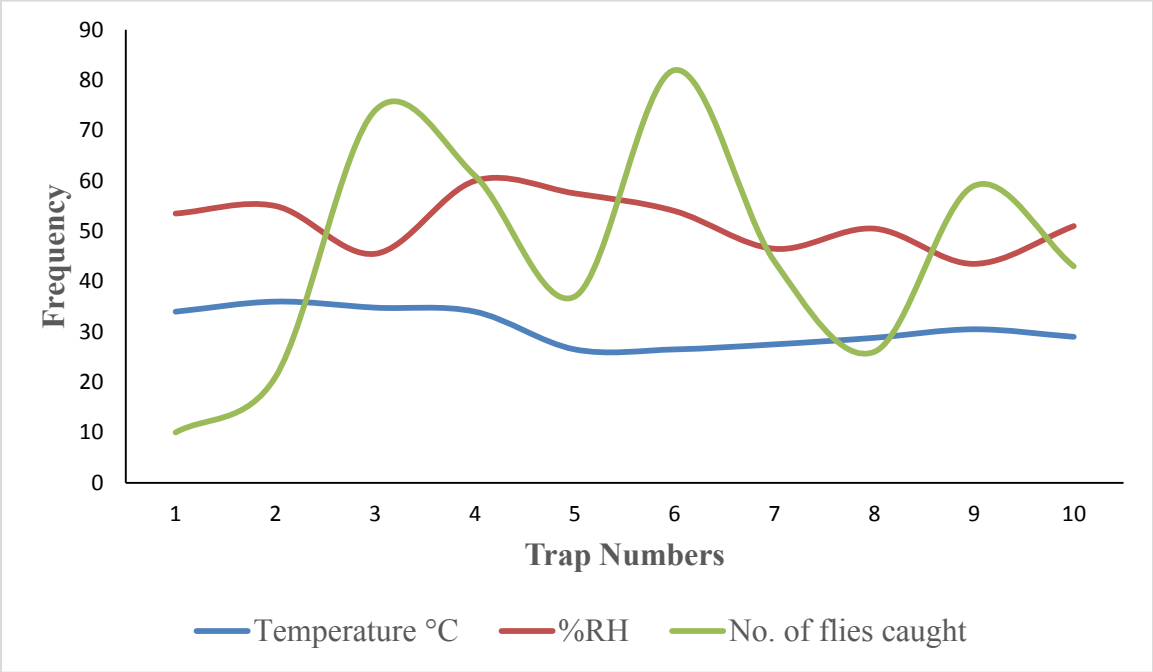


Figure 4. 5: Catch distribution of *Glossina* spp. in relation to ambient temperature and relative humidity conditions in Kainji Lake National Park.

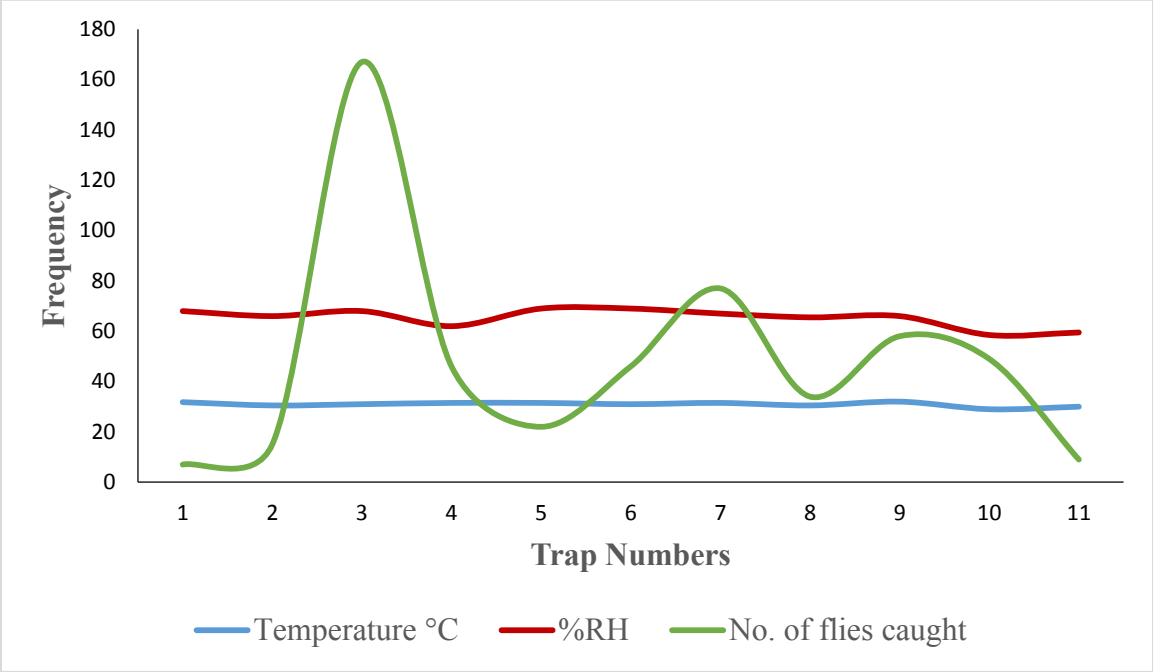


Figure 4. 6: Catch distribution of *Glossina* spp. in relation to ambient temperature and relative humidity conditions in Old Oyo National Park.

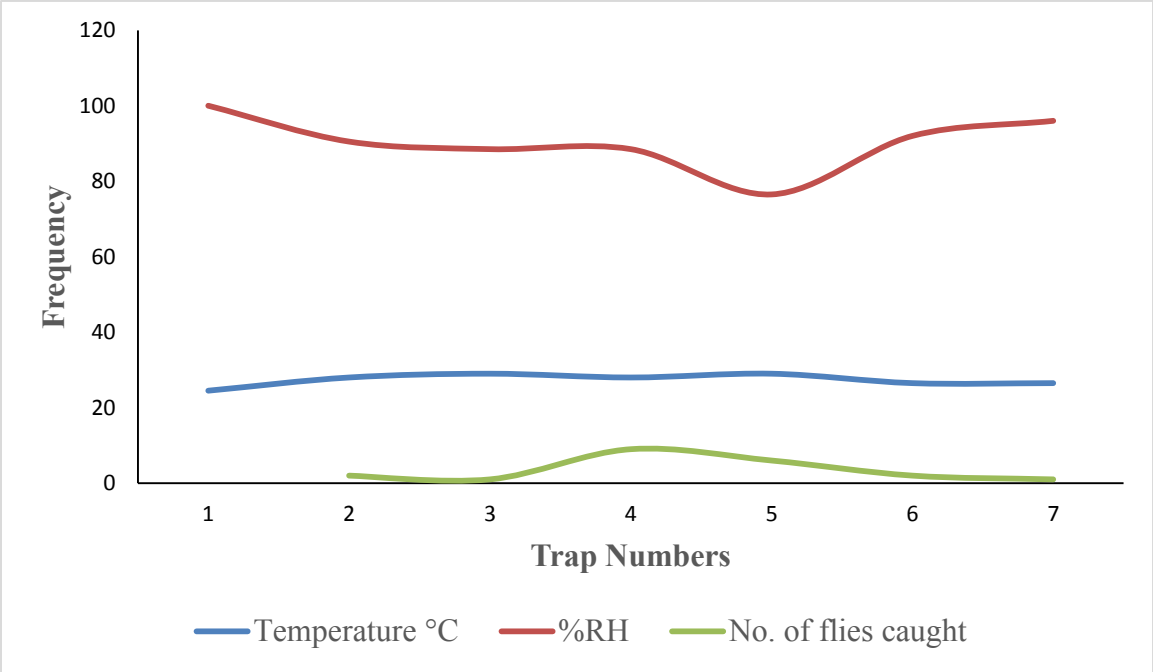


Figure 4. 7: Catch distribution of *Glossina* spp. in relation to ambient temperature and relative humidity conditions in Cross River National Park.

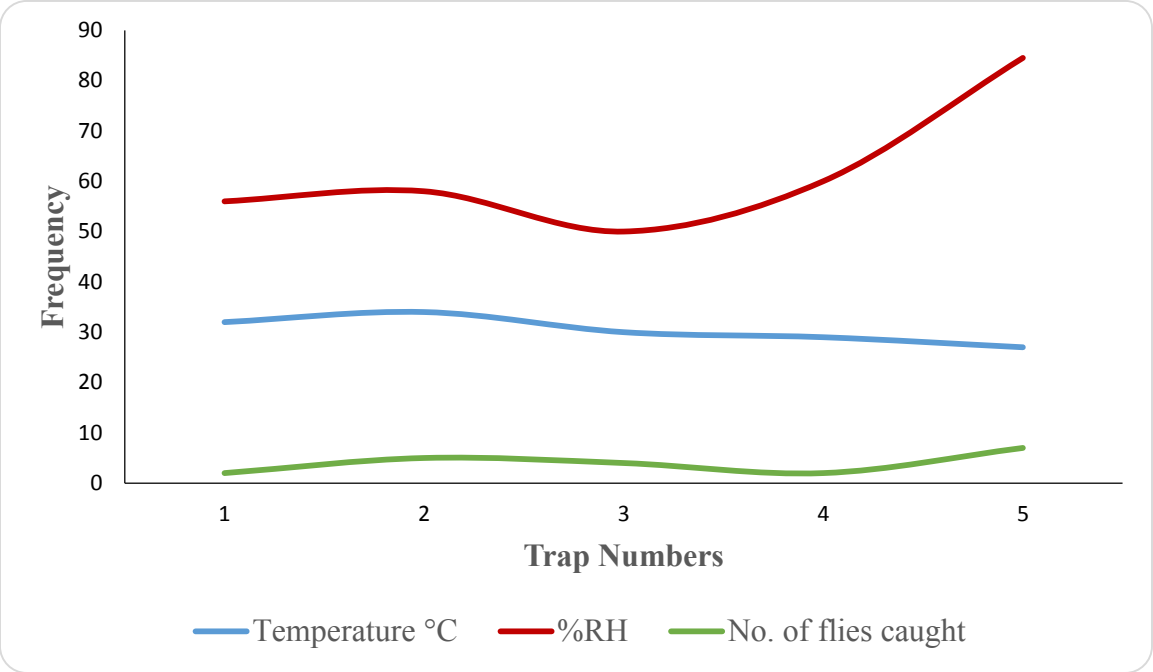


Figure 4. 8: Catch distribution of *Glossina* spp. in relation to ambient temperature and relative humidity conditions in Ijah Gwari

Statistical analysis showed no significant difference ($t = -1.580$, $p\ 0.133 > \alpha = 0.05$) in tsetse catches in relation to ambient temperature and relative humidity conditions in Yankari Game Reserve. In Kainji Lake National Park, result showed significant difference ($t = -10.633$, $p = 0.000 < \alpha = 0.05$) in tsetse catches in relation to ambient temperature and relative humidity conditions. Significant difference, was obtained in tsetse catches in relation to ambient temperature and relative humidity conditions in Old Oyo ($t = -36.290$, $p = 0.000 < \alpha = 0.05$), Cross River ($t = -18.939$), $p = 0.000 < \alpha = 0.05$) National Parks and Ijah Gwari ($t = -4.613$, $p = 0.01 < \alpha = 0.05$), respectively. However, correlation statistics showed no significant difference ($p > 0.05$) between tsetse catches and ambient temperature and relative humidity conditions in all the parks visited. Figure 4.9a showed that tsetse catches negatively correlated with ambient temperature, but correlated positively with relative humidity conditions (Figure 4.9b) in Yankari Game Reserve. In Kainji Lake National Park, tsetse catches correlated negatively with both temperature (Figure 4.10a) and relative humidity conditions (Figure 4.10b). However when the species were considered separately, *Glossina tachinoides* marginally correlated positively with ambient temperature (Figure 4.10c). In Old Oyo National Park however, *G. p. palpalis* correlated positively with both temperature (Figure 4.11a) and relative humidity conditions (Figure 4.11b). In Cross River National Park, correlation statistics showed that tsetse catches correlated positively with ambient temperature (Figure 4.12a) and negatively with relative humidity (Figure 4.12b). This result is a direct inverse of the result obtained in Yankari Game Reserve described above. Furthermore, in Ijah Gwari, catches of *G. p. palpalis* correlated negatively with ambient temperature (Figure 4.13a) and positively with relative humidity conditions (Figure 4.13b).

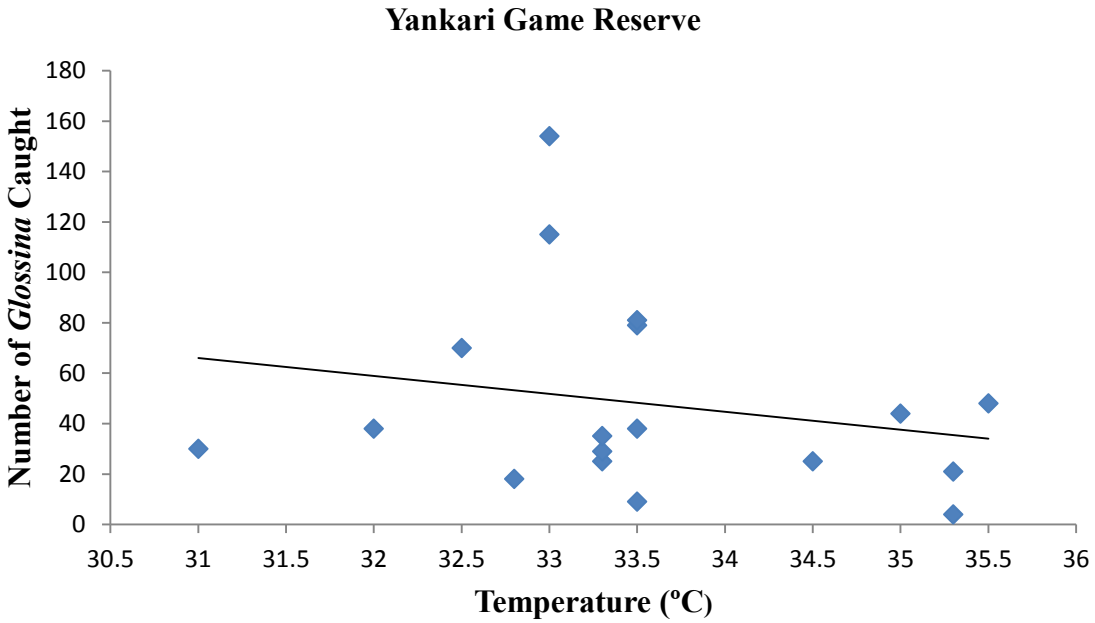


Figure 4.9a: Negative correlation (r (16) = - 0.220, p = 0.380 > α = 0.05) between ambient temperature and number of *Glossina* caught in Yankari Game Reserve

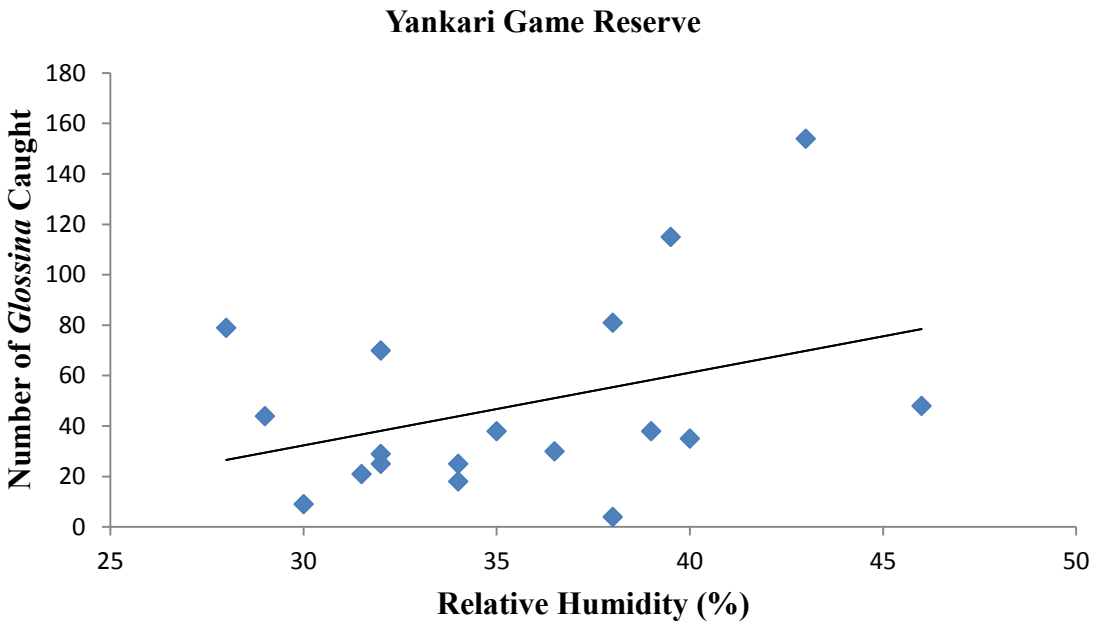


Figure 4.9b: Positive correlation (r (16) = 0.368, p = 0.134 > α = 0.05) between relative humidity and number of *Glossina* caught in Yankari Game Reserve.

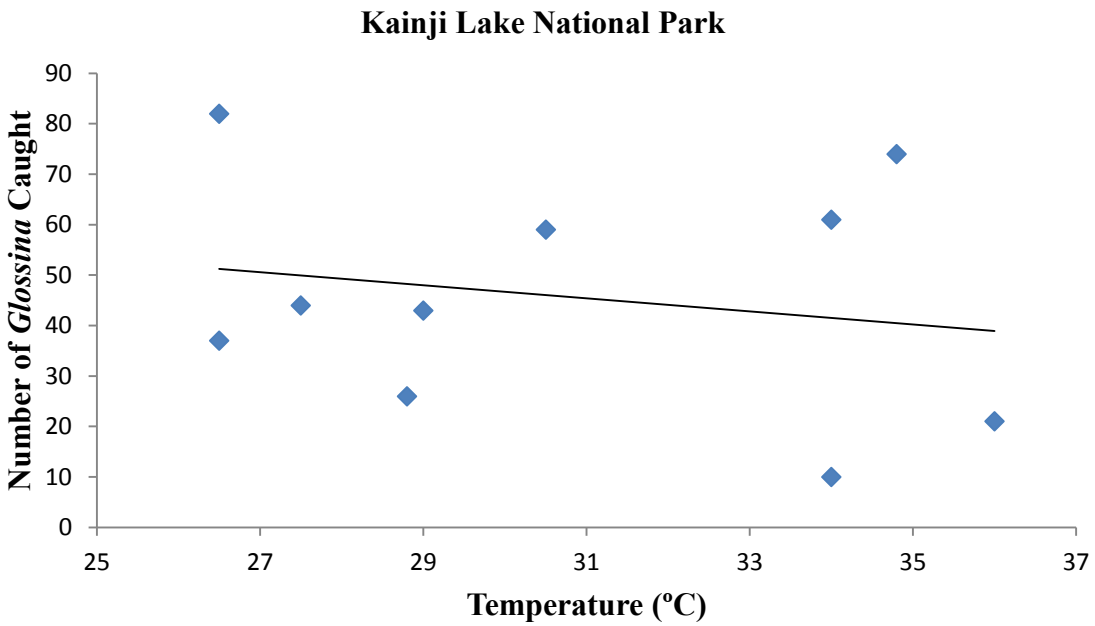


Figure 4.10a: Negative correlation (r (8) = -0.201, p = 0.577 > a = 0.05) between ambient temperature and number of *Glossina* caught in Kainji Lake National Park.

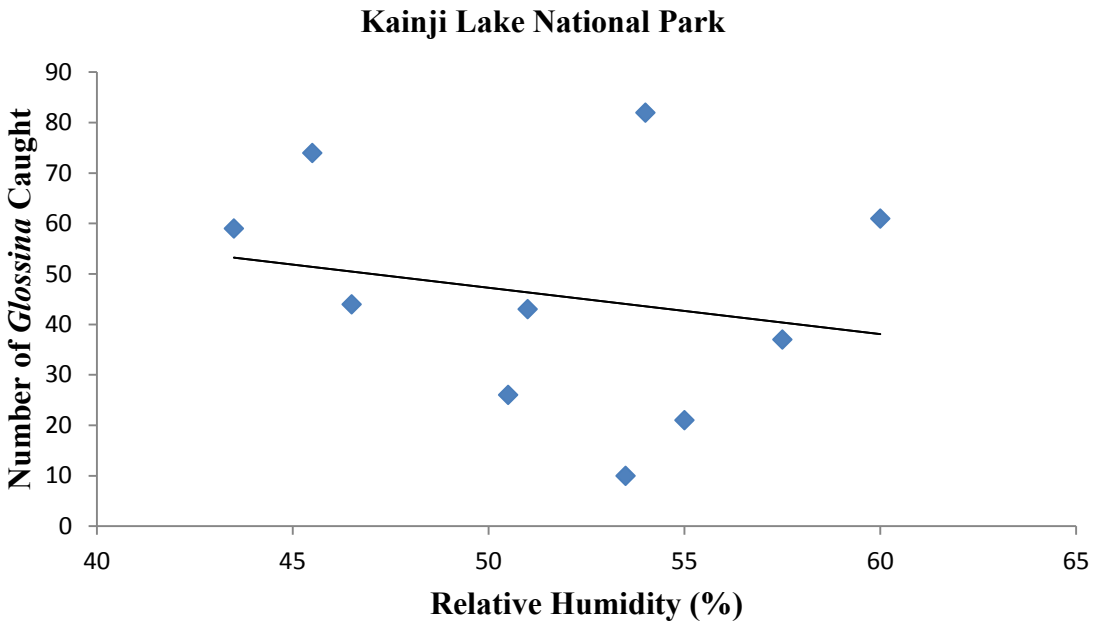


Figure 4.10b: Negative correlation (r (8) = -0.211, p = 0.559> α = 0.05) between relative humidity and number of *G. m. submorsitans* and *G. tachinoides* caught in Kainji Lake National Park.

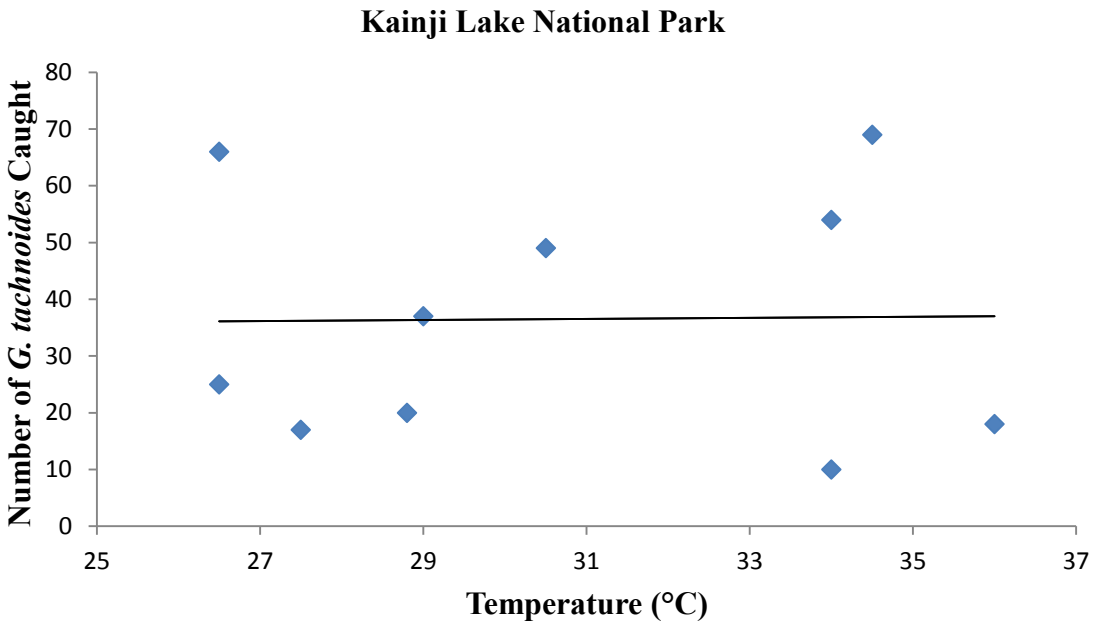


Figure 4.10c: Marginal positive correlation (r (8) = 0.029, p = 0.937>α=0.05) between ambient temperature and number of *G. tachinoides* caught in Kainji Lake National Park.

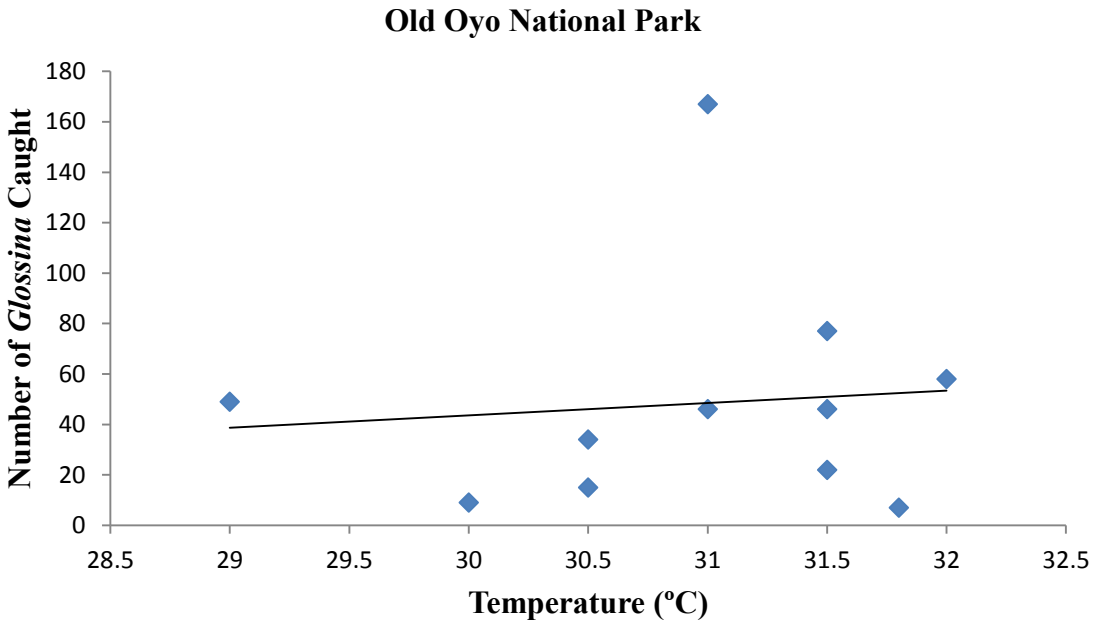


Figure 4.11a: Positive correlation (r (9) = 0.096, p = 0.779 > α = 0.05) between ambient temperature and number of *Glossina* caught in Old Oyo National Park.

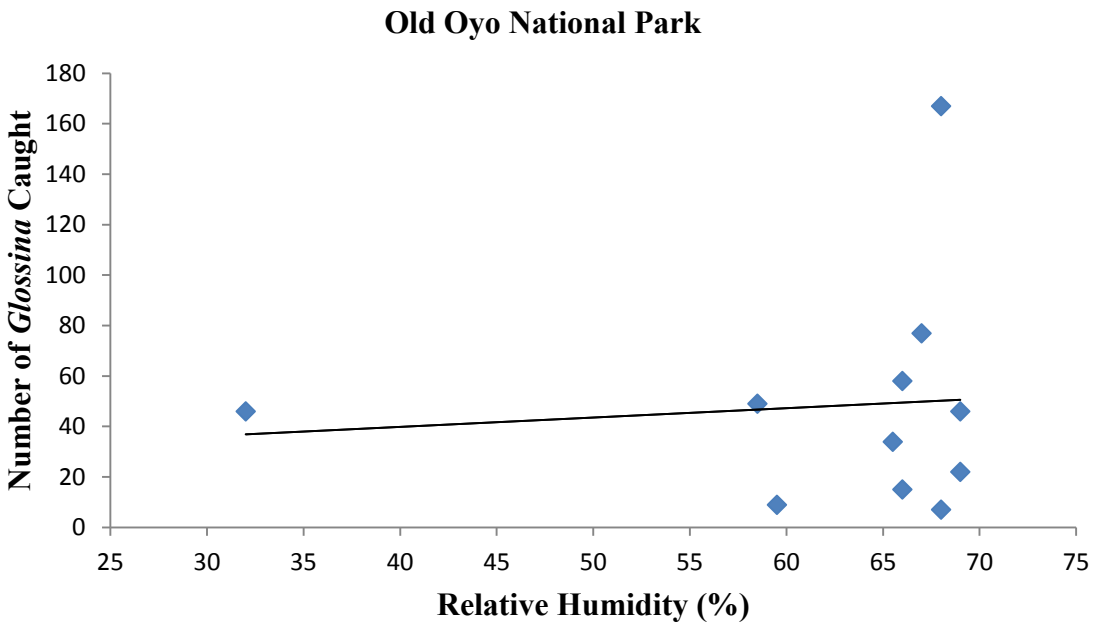


Figure 4.11b: Positve correlation (r (9) = 0.089, p = 0.795 > α = 0.05) between relative humidity and number of *Glossina* caught in Old Oyo National Park

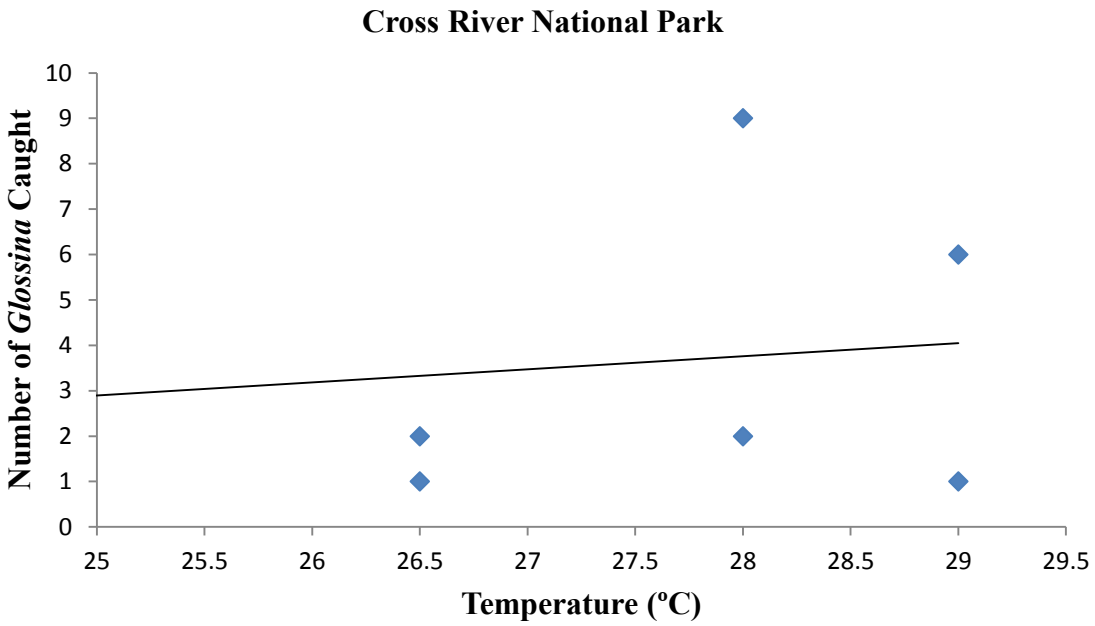


Figure 4.12a: Positive correlation (r (4) = 0.326, p = 0.528 > α = 0.05) between ambient temperature and number of *Glossina* caught in Cross River National Park.

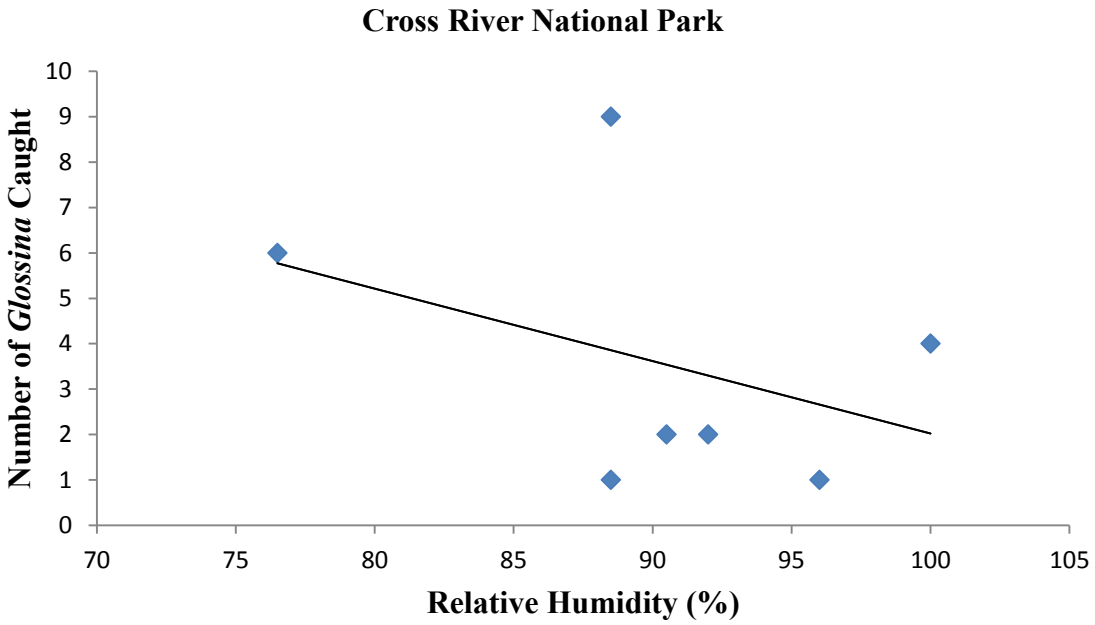


Figure 4.12b: Negative correlation (r (4) = -0.530, p = 0.280 > α = 0.05) between relative humidity and number of *Glossina* caught in Cross River National Park.

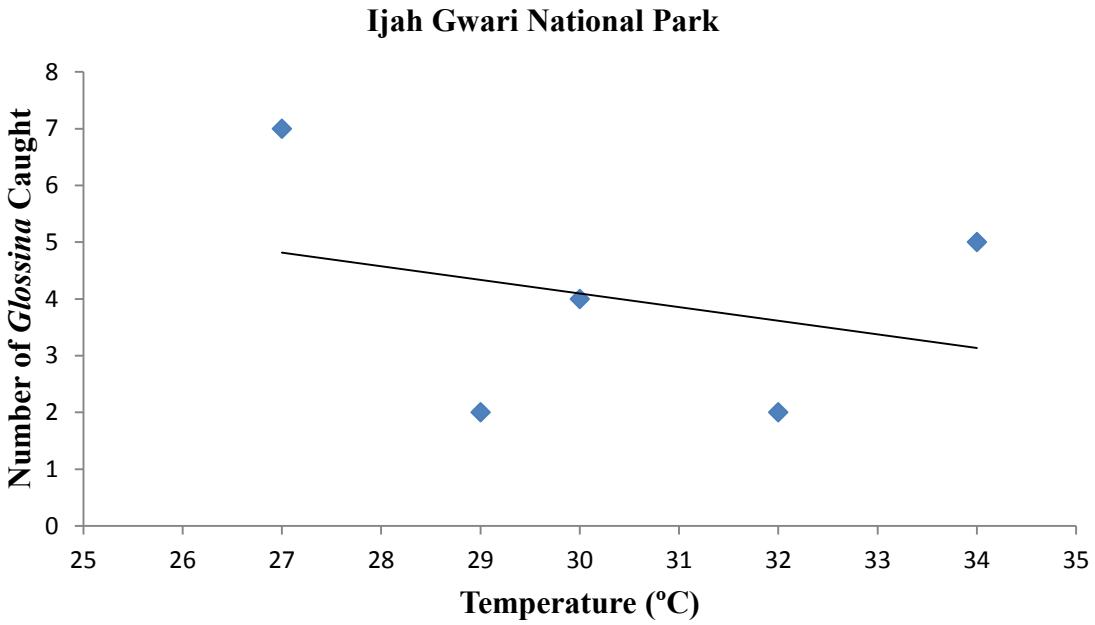


Figure 4.13a: Negative correlation (r (4) = - 0.305, p = 0.617 > α = 0.05) between ambient temperature and number of *G. p. palpalis* caught in Ijah Gwari.

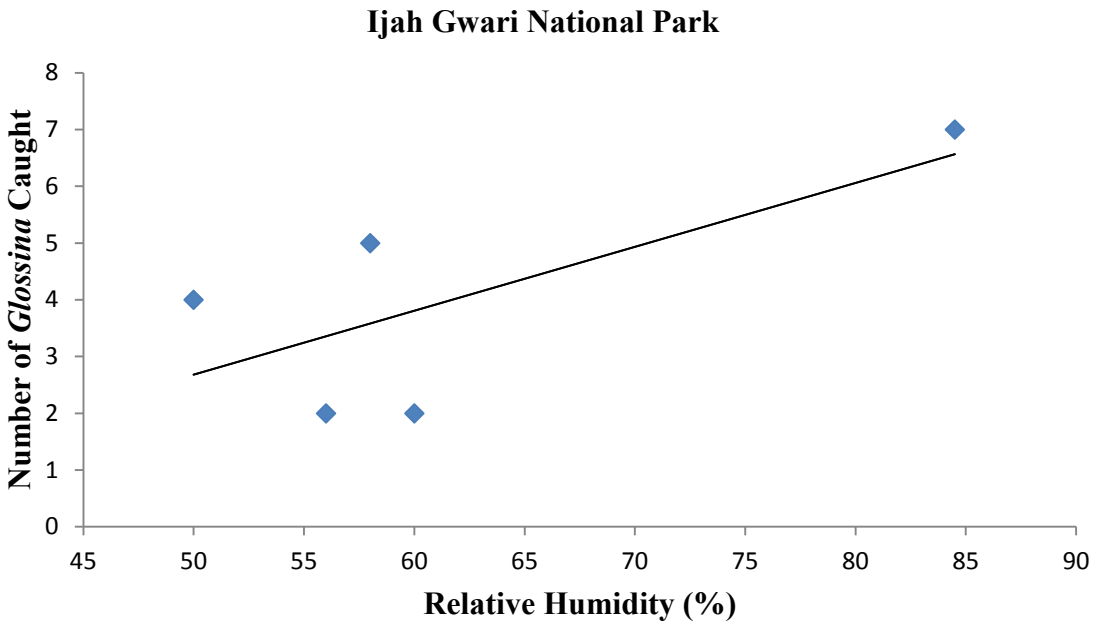


Figure 4.13b: Positive correlation (r (4) = 0.705, p = 0.183 > α = 0.05) between relative humidity and number of *G. p. palpalis* caught in Ijah Gwari.

4.1.4 Apparent density of tsetse fly populations in the National Parks and Ijah Gwari

Figure 4.14 shows apparent density of fly populations caught in the National Parks and Ijah Gwari. Fly apparent density of 16.06 fly per trap per day (F/T/D), was recorded in Old Oyo National Park, followed by 15.98 (F/T/D) in Yankari Game Reserve, 15.23F/T/D in Kainji Lake National Park, 4.0 F/T/D in Ijah Gwari and 1.0 F/T/D in Cross River National Park respectively. Further results (Appendix XII and XIII) show that in Old Oyo National Park, apparent density of *G. p. palpalis* populations was 24F/T/D and 0.09F/T/D in *G. m. submorsitans*. In Yankari Game Reserve, apparent density was 18.94F/T/D in *G. tachinoides*, 4.97 F/T/D in *G. m. submorsitans* and 0.05F/T/D in *G. p. palpalis* respectively. In Kainji, apparent density of *G. tachinoides* was 12.26 F/T/D and 2.96F/T/D in *G. m. submorsitans*, while in Ijah Gwari, apparent density of *G. p. palpalis* was 4.0F/T/D. In Cross River National Park, apparent density of *G. p. palpalis* was 1.28F/T/D and 0.21F/T/D in *G. fusca* respectively.

Statistical analysis showed significant difference $\chi^2(4) = 20.500$, $p < 0.001$ in the apparent density of *Glossina* spp. per sampled location. Pairwise comparison showed that Old Oyo, Yankari, and Kainji sampled locations had comparable apparent densities of *Glossina* spp.

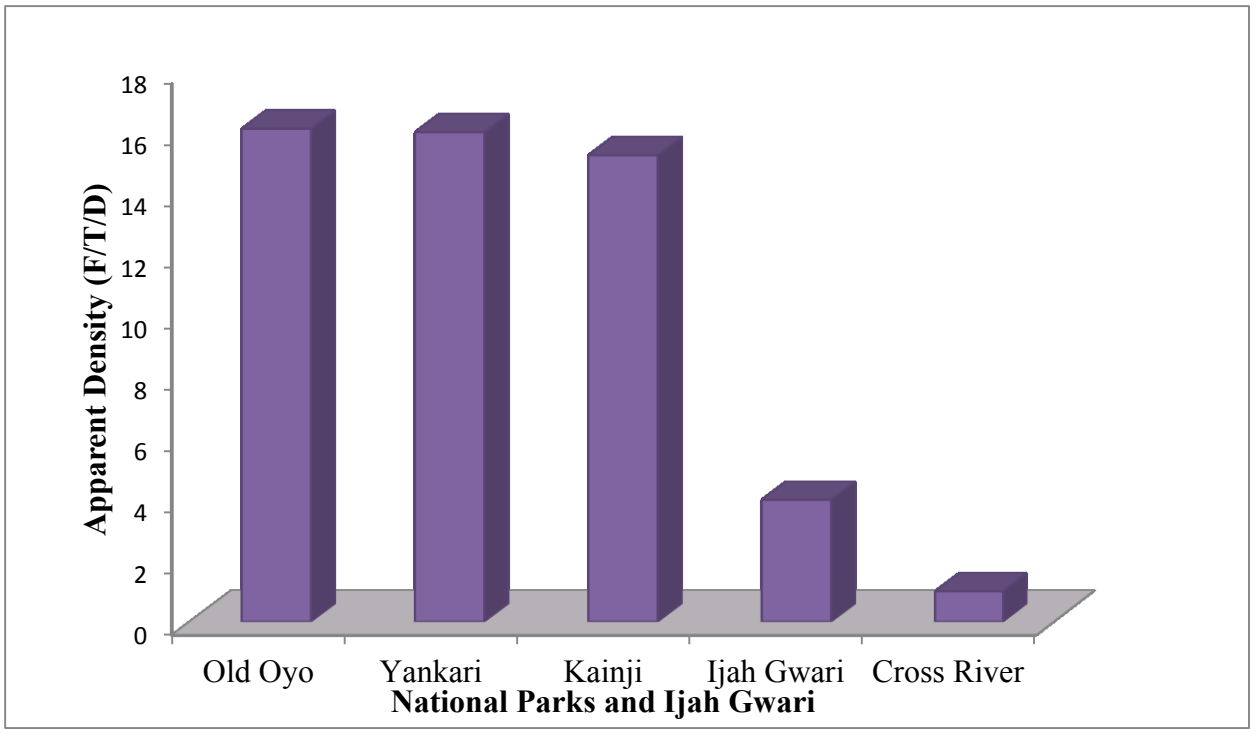


Figure 4. 14: Apparent density of *Glossina* species caught in sampled National Parks and Ijah Gwari.

4.1.5 Sex ratio

Figure 4.15 showed abundance of male and female *Glossina* species caught in sampled locations. In Yankari Game Reserve, more male 476 (70%) than female 206 (30%) *G. tachinoides* were caught giving a sex ratio of 2:1 males to females. In *G. p. palpalis* however, only 2 flies (1male and 1 female) were caught with a ratio 1:1 a piece. Whereas 80 (45%) male and 99 (55%) female *G. m. submorsitans* were caught with sex ratio in sampled flies being 1: 1.5 in favour of females. In Kainji Lake National Park, 250 (68%) male and 118 (32%) female *G. tachinoides* were caught, sex ratio was 2: 1 in favour of males, whereas in *G. m. submorsitans,* it was 1:1.5 in favour of females in 30 (37%) male and 50 (43%) female flies caught. In Old Oyo National Park, whereas 276 (52%) male and 252 (48%) female *G. p. palpalis* were caught with a sex ratio of 1.5:1 in favour of males, in *G. m. submorsitans* only 2 flies (1male and 1female) were caught with sex ratio of 1:1 a piece. In Cross River National Park, whereas 6 (33%) male and 12 (67%) female *G. p. palpalis* were caught with sex ratio of 1.5:1 in favour of males, in 3 female *G. fusca* caught, ratio was 0:1 all in favour of females. In Ijah Gwari, 10 (50%) male and 10 (50%) female *G. p. palpalis* were caught, sex ratio was 1:1 a piece. Overall fly catches (Appendix XV) show that more males (60%) were caught during the sampling than females (40%) given a sex ratio of 1.5:1 females to males. Statistical analysis revealed statistically significant difference (χ^2 (1) = 4.000, p =0.046) in sex ratio between male and female flies in sampled locations with a higher proportion of male than female flies caught.

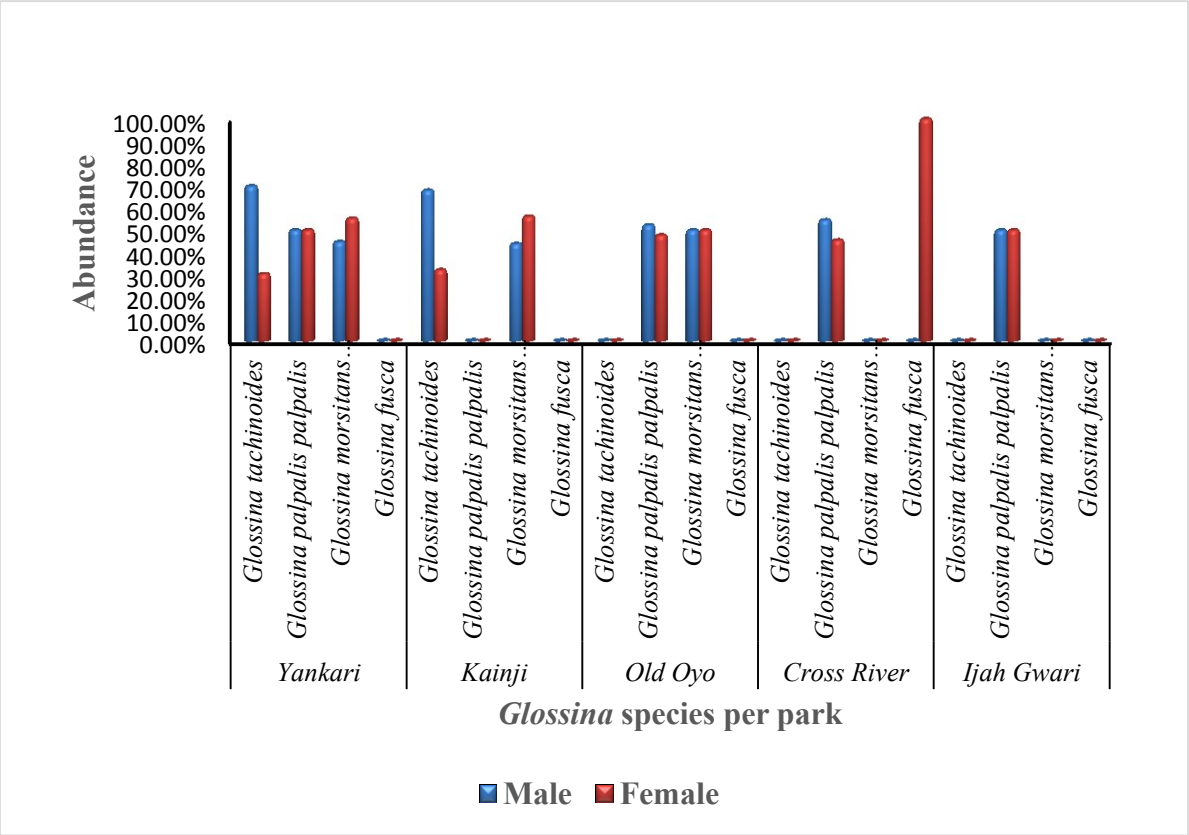


Figure 4. 15: Abundance of male and female flies in *Glossina* species per sampled location.

4.1.6 Teneral and non-teneral flies

Table 4.2 shows the proportion of teneral and non teneral flies in trap catches. Out of 1891tsetse flies collected, 1500 (79%) were teneral and 391 (21%) non-teneral. A total of 824 (55.53%) *G. tachinoides*, 451(30%) *G. p. palpalis*, 225 (15%) *G. m. submorsitans* and 0 (0.0%) *G. fusca* teneral flies were caught, while the non-teneral flies constituted 226 (21.52%) in *G. tachinoides*, 117 (20.6%) *G. p. palpalis*, 45 (16.67%) *G. m. submorsitans* and 3 (0.77%) *G. fusca* of the catches.

Chi-square analysis revealed significant difference (χ^2 (4) = 20.500, $p < 0.001$) in the proportion of teneral and non-teneral flies among the four *Glossina* species caught. Within each *Glossina* species, the result showed significantly higher proportion of teneral flies than the non- teneral flies, except in *G. m. submorsitans* and *G. fusca* in which no significant difference was found between teneral and non teneral flies caught. Pairwise comparison showed comparable tenerity of *Glossina* species in Old Oyo, Yankari, and Kainji National Parks.

4.1.7 Hunger staging

Hunger stage estimated by dissecting and examining blood meal contents in both male and female flies was carried out following the method described by Madubunyi (1989). Results (Table 4.3) showed that of the total of 391 flies dissected, 38(9.7 %) had traces of blood meal detected in their guts. Of these, more females 28 (74%) had blood meals in their guts than 10 (26%) males.

Table 4. 2: Teneral and non-teneral flies

Glossina species	Total collected	Teneral (%)	Non-teneral (%)
Glossina tachinoides	1050(55.53)	824(78.47)(55)	226(21.52)(57.8)
Glossina palpalis palpalis	568(30.04)	451(79.40)(30)	117(20.60)(29.9)
Glossina morsitans submorsitans	270(14.28)	225(83.33)(15)	45(16.67)(11.51)
Glossina fusca	3(0.16)	0(0.0)(0)	3(100)(0.76)
Total	1891	1500(79)(100)	391(21)(100)

( ) ( ): First bracket ( ) shows percentage tenerality within individual *Glossina* spp; Second bracket ( ) shows percentage tenerality in overall fly catches.

Table 4. 3: Hungry and non-hungry *Glossina* species collected from the National Parks

Glossina species	Non- hungry (%)		Total non-hungry	Hungry (%)		Total hungry
	♂	♀		♂	♀	
Glossina morsitans submorsitans	1	2	3	9	33	42
Glossina palpalis palpalis	6	19	25	84	117	201
Glossina tachinoides	3	6	9	62	46	108
Glossina fusca	0	1	1	0	2	2
Total	10(26)	28(74)	38	155	198	353

4.2 Molecular Studies

4.2.1 Amplifications

Results (Table 4.4) showed that, 391 *Glossina* species out of the total of 1891 flies caught, were subjected to polymerase chain reactions. Amplifications were obtained from 26 *G. m. submorsitans*, 12 *G. tachinoides*, 29 *G. p. palpalis* and 2 *G. fusca*. Amplifications obtained showed expected product size of about 900bp for all the four species of tsetse subjected to PCR targeting CO1 gene (Figure 4.16a). Electrophoresis analysis obtained showed single amplicons indicating that the fly populations were homogeneous. Similarly, Figure 4.16b shows results of PCR on *G. m. submorsitans* only, with expected product size of about 900bp.

4.2.2 Molecular species identification

Sequences of *Glossina* species obtained in conjunction with sequence similarity searches in the Genbank, were used to build phylogenetic trees for species identification. Result (Figure 4.17) showed that whereas 60 (91%) out of 66 flies were accurately identified using phylogenetic analysis, six flies (nos. 17, 74, 162, 273, 352 and 353) could not be identified beyond generic level. Closely related *Glossina* species clustered together and separately from those of distantly related siblings. Flies of the same species clustered together, whereas different species clustered separately.

Table 4. 4: Amplified samples of *Glossina* species obtained by PCR targeting Cytochrome Oxidase I gene.

Glossina species	Location	No. of flies Dissected	No. Positive	% positive
G. m. submorsitans (45)	Yankari Game Reserve	26	13	50.0
	Kainji Lake National Park	17	12	70.58
	Old Oyo	2	1	50.0
G. tachinoides (117)	Yankari Game Reserve	23	5	22.0
	Kainji Lake National Park	94	7	7.40
G. p. palpalis (226)	Old Oyo	188	14	7.44
	Cross River	18	10	55.55
	Ijah Gwari	20	5	25.0
G. fusca (3)	Cross River	3	2	66.66

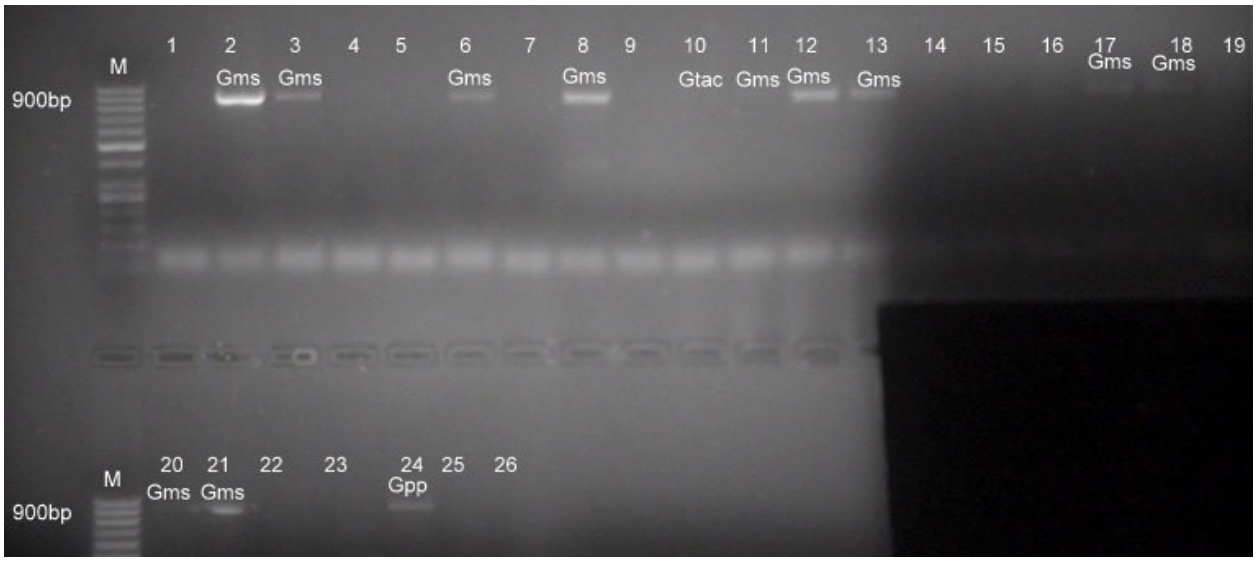


Figure 4.16a: Gel Electropherographs of amplified Cytochrome oxidase I (C01) gene in *G. m. submorsitans* (Gms), *G. p. palpalis* (Gpp) and *G. tachinoides* (Gtac) collected in sampled locations.

Amplifications were obtained from samples in wells 2, 3, 6, 8, 10, 11, 12, 13, 17, 18, 20, 21 and 24; whereas numbers 1, 4, 5, 7, 9,14, 15, 16, 19, 22, 23, 25 and 26 did not amplify within the expected band size region of 900 bp.

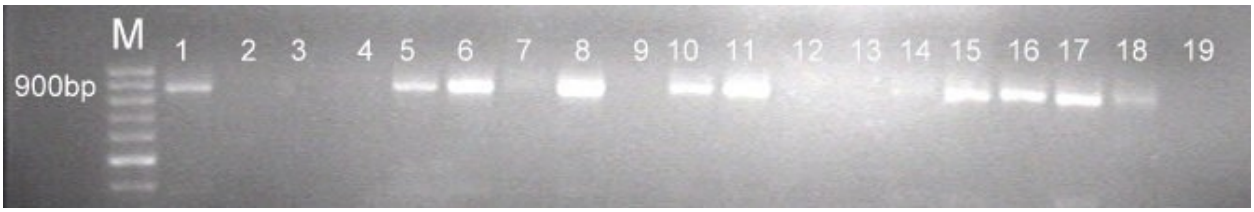


Figure 4.16b: Gel Electropherographs of amplified Cytochrome Oxidase I (CO1) gene in *G. m. submorsitans* collected in Yankari and Kainji Lake National Parks.

Amplifications were obtained from well numbers 1, 5, 6, 7, 8, 10, 11, 14, 15, 16, 17 and18; whereas numbers 2, 3, 4, 9, 12, 13, and 19 did not amplify within the expected region of 900 base pairs PCR product size.

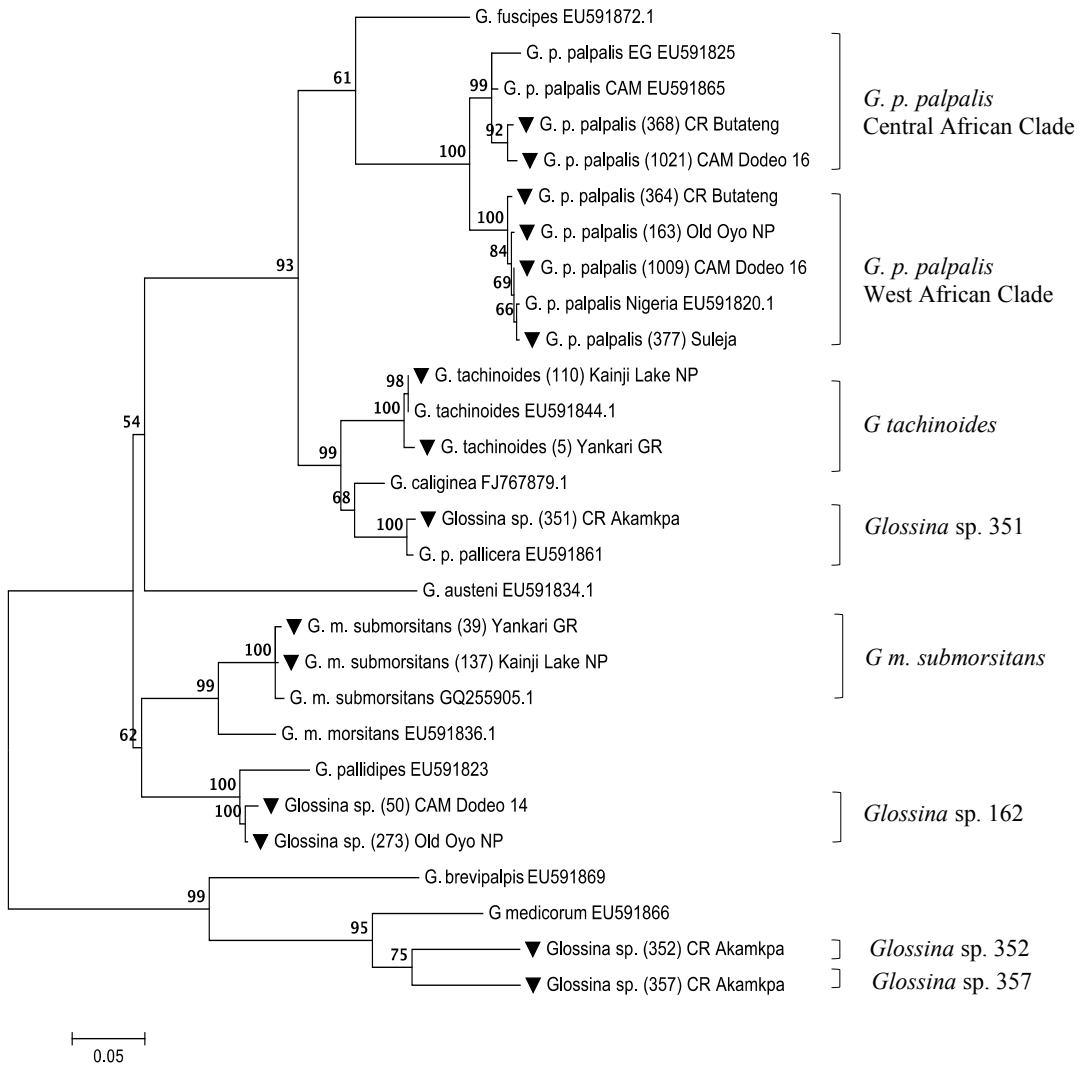


Figure 4.17: Molecular identification of *Glossina* sp. collected in Nigeria National Parks and Ijah Gwari

4.2.3 Characteristics of CO1 sequences of *Glossina* spp. in sampled locations

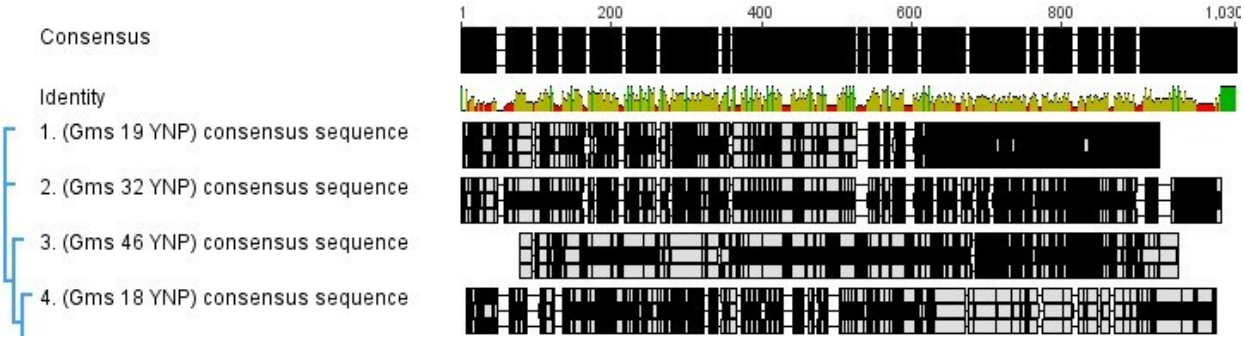
Results in Table 4.5 showed variation in nucleotide frequencies of sequences of *Glossina* species in sampled locations. Partial CO1 fragment lengths of mtDNA gene obtained from sequence alignments of individuals of *Glossina* from the five different sampled locations, show that the average nucleotide frequencies of A, C, G, T in all sequences were 31.7%, 13.3%, 13.2% and 31.2% respectively. The AT content (29.7%) was higher than the GC content (21.3%). In Yankari Game Reserve, average nucleotide frequencies of A, C, G, T in *Glossina tachinoides* population were A=27.1%, C=11.0%, G=11.0%, T=31.9%. The AT content was higher (19.2%) than GC content (12.9%), while in *G. m. submorsitans* populations, the average nucleotide frequencies of A, C, G, T in all 5 sequences were 27.8%, 10.6%, 10.9%, 28.9% with the GC content higher (19.5%) than AT 9.0% content. In Kainji Lake National Park, the average nucleotide frequencies of A, C, G, T in 5 sequences of *G. tachinoides* populations were 31.4%, 11.4%, 12.1%, 30.6%. The AT content (27.8%) was higher than GC content (20.8%), while in *G. m. submorsitans* populations, the average nucleotide frequencies of A, C, G, T in all 5 sequences were 33.4%, 13.1%, 13.1%, 34.0% with the GC content being higher (21.4%), than (18.1%) AT content. In Old Oyo National Park, the average nucleotide frequencies of A, C, G, T in 6 *G. p. palpalis* sequences were 33.4%, 12.5%, 12.7%, 27.7%, with the AT content higher (20.7%) than (17.9%) the GC content. In Cross River National Park, the average nucleotide frequencies of A, C, G, T in 6 *G. p. palpalis* sequences were 33.3%, 14.2%, 14.1%, 32.0% respectively. The AT content was higher (29.7%) than GC (24.9%) content. In Ijah Gwari, the average nucleotide frequencies of A, C, G, T, in 5 *G. p. palpalis* sequences were 36.0%, 14.7%, 14.7%, 34.5%, with a higher (29.7%) AT content than GC (23.6%) content. Molecular based statistical analysis of *G. tachinoides* and *G. m. submorsitans,* the two dominant *Glossina* species identified in Yankari Game Reserve

Table 4. 5: Characteristics of sequences of *Glossina* spp. in the different sampled sites

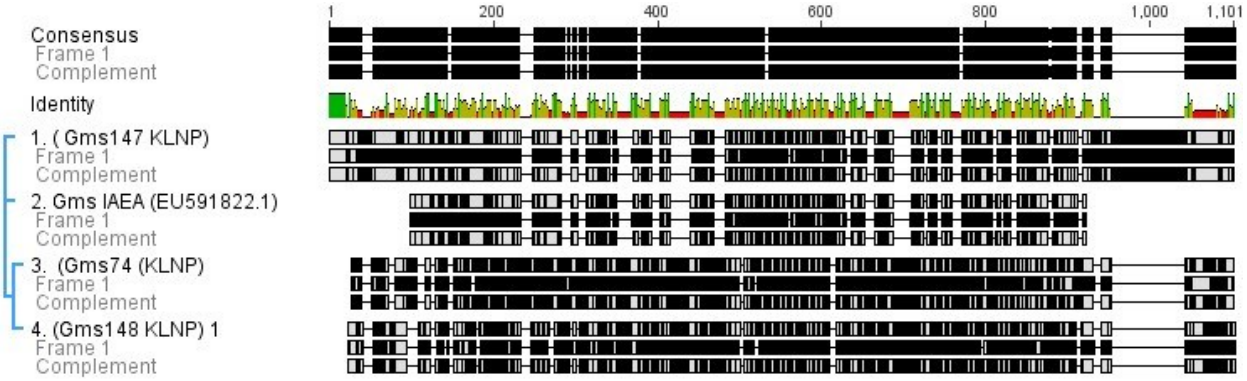
Sampled site	*Glossina* species	No. of sequences	% Average nucleotide frequencies				% AT content	% GC content
			A	C	G	T		
Yankari Game Reserve	*G. tachinoides*	5	27.1	11.0	11.0	31.9	19.2	12.9
	G. m. submorsitans	5	27.8	10.6	10.9	28.9	9.0	19.5
Kainji Lake National Park	*G. tachinoides*	5	31.4	11.4	12.1	30.6	27.8	20.8
	G. m. submorsitans	4	33.3	13.0	13.1	34.0	18.1	21.4
Old Oyo National Park	*G. p. palpalis*	6	33.4	12.5	12.7	27.7	29.5	20.9
Cross River National Park	*G. p. palpalis*	6	33.3	14.2	14.1	32.0	29.7	24.9
Ijah Gwari	*G. p. palpalis*	5	36.0	14.7	14.7	34.5	29.7	23.6

revealed that, there was no significant difference (χ^2 (1) = 1.125, p = 0.2289) in the AT and GC content in *G. tachinoides*. However, there was significant difference (χ^2 (1) = 0.172, p = 0.041) in the AT and GC content in *G. m. submorsitans* with a higher proportion of GC to AT content.

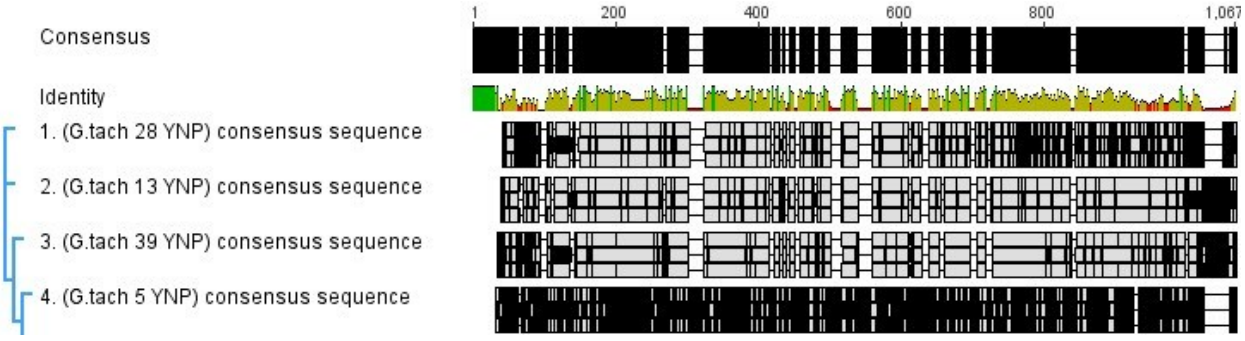
In Kainji Lake National Park, statistical analyses showed that there was no significant difference in AT and GC content in both *G. tachinoides* (χ^2 (1) = 2.455, p = 0.117) and *G. m. submorsitans* (χ^2 (1) = 0.231, p = 0.631) populations. Similarly, in Old Oyo National Park, whereas, there was no statistically significant difference *(*χ^2 (1) = 0.231, p = 0.631) in AT and GC content in *G. p. palpalis*, in Cross River National Park, the difference in AT and GC content in *G. p. palpalis* was not statistically significant (χ^2 (1) = 0.610, p = 0.435). And in Ijah Gwari, the difference in AT and GC content in *G. p. palpalis* was not statistically significant (χ^2 (1) = 2.632, p = 0.105). (Figure 4.18a-g) shows banding patterns in sequences of *Glossina* species caught in the national parks and Ijah Gwari.



(a)

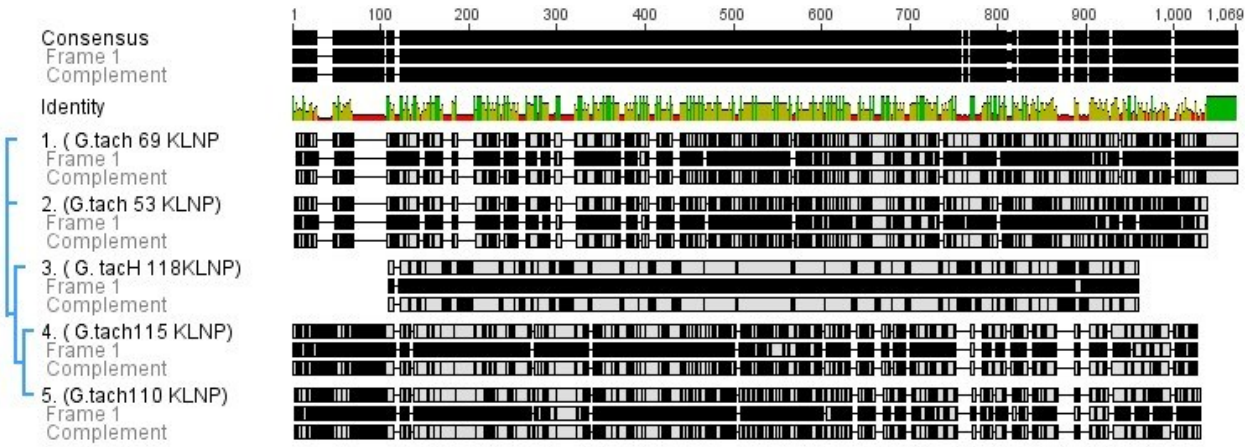


(b)

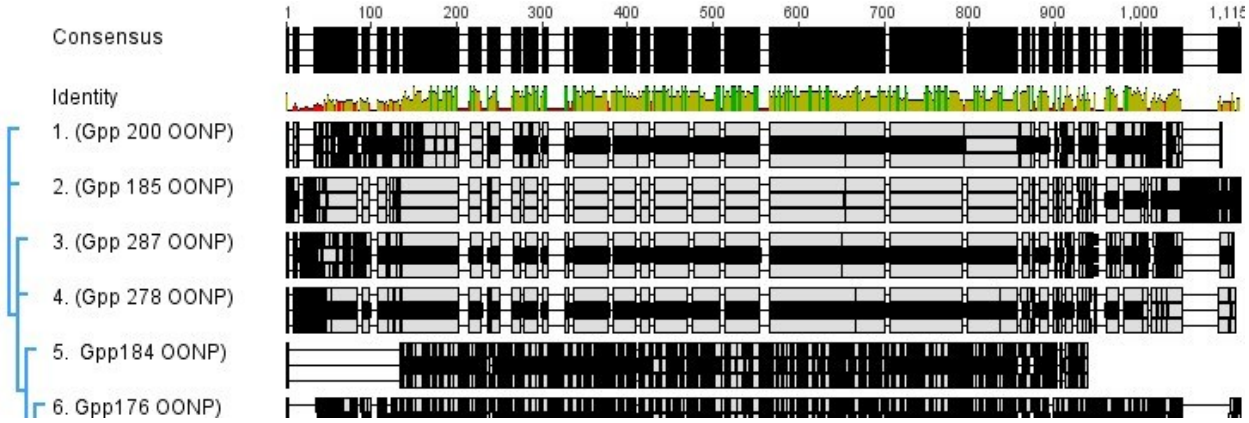


(c)

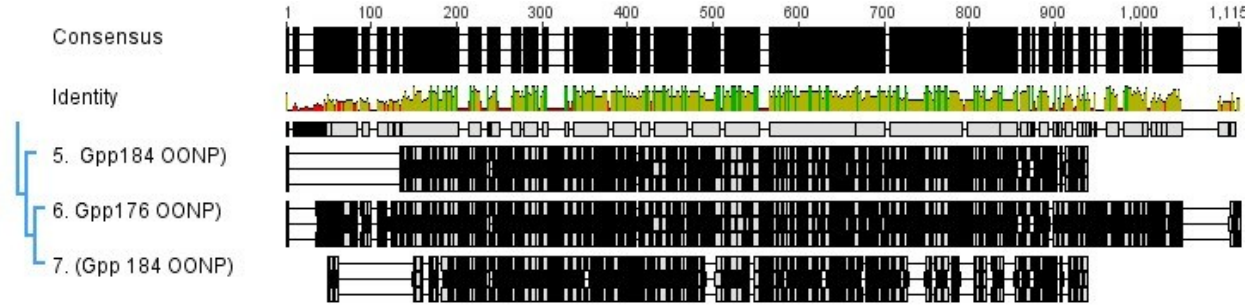
Figure 4. 18(a-c): Banding patterns of Cytochrome Oxidase I sequences in (a) *G. m. submorsitans* (Gms) populations in Yankari Game Reserve (b) *Glossina tachinoides* (G.tach) populations inYankari Game Reserve (c) *G. m. submorsitans* (Gms) populations in Kainji Lake National



(d)

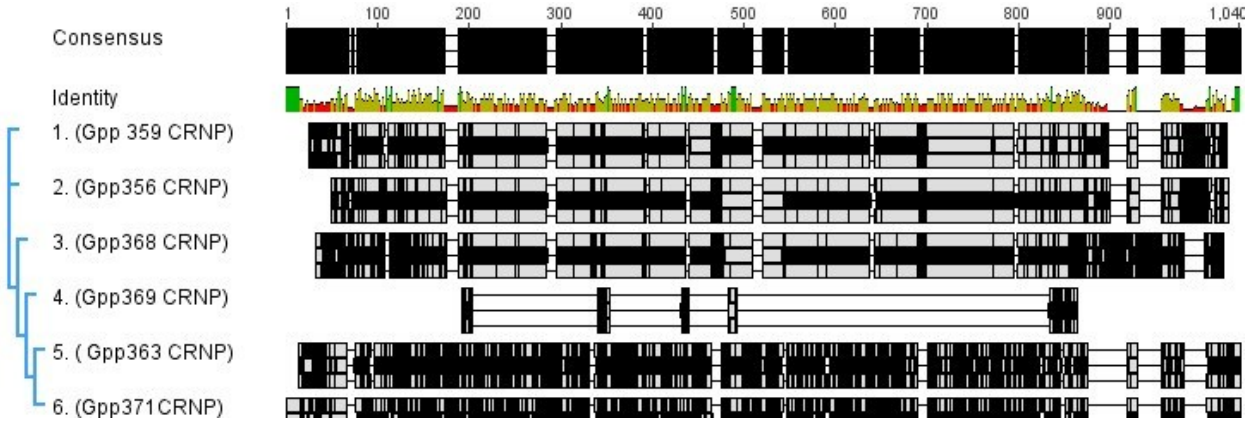


(e)

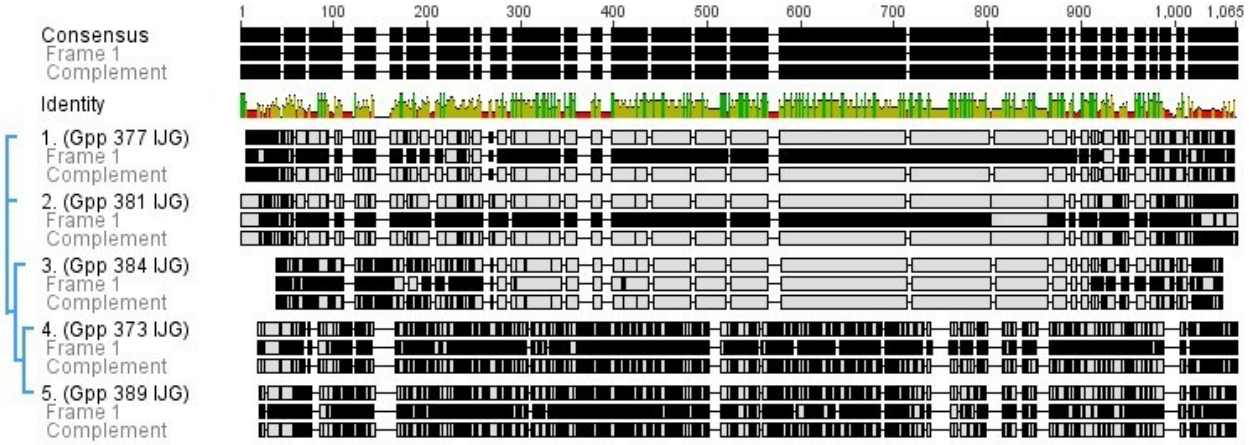


(e) Cont'd

Figure 4.18(d & e): Banding pattern of Cytochrome Oxidase I sequences in (d) *Glossina tachinoides* (G.tach) populations in Kainji Lake National Park (e) *G. p. palpalis* (Gpp) populations in Old Oyo National Park (f) *G. p. palpalis* (Gpp) populations in Old Oyo National Park continued. Sequences with similar banding patterns clustered together.



(f)



(g)

Figure 4.18(f & g): Banding pattern of Cytochrome Oxidase I sequences in **(f)** *G. p. palpalis* (Gpp) populations in Cross River National Park and in **(g)** *G. p. palpalis* (Gpp) populations in Ijah Gwari. Sequences with similar banding pattern clustered together.

4.2.4 Haplotype diversity

Result of analysis of haplotype diversity (Table 4.6), shows the number of composite haplotypes per *Glossina* species caught per sampled location. In *G. m. submorsitans* population in Yankari Game Reserve (Figure 4.19a), number of detected haplotypes was 4 with 2 of them being singletons or private haplotypes, while 2 were shared. The population of *G. m. submorsitans* in Kainji Lake National Park (Figure 4.19b), showed 2 shared haplotypes, and none private. *G. tachinoides* population in Yankari *(*Figure 4.19c), showed 3 detected haplotypes, 1 private, and 2 shared, while the population in Kainji Lake National Park (Figure 4.19d) showed 1 shared haplotype and 2 private. In *G. p. palpalis* in Old Oyo National Park (Figure 4.19e), 2 private and 2 shared haplotypes were detected. *Glossina. p. palpalis* population in Cross River National Park (Figure 4.19f), consisted of 2 shared haplotypes and 1 private, while the *G. p. palpalis* population in Ijah Gwari (Figure 4.19g), showed that out of 3 haplotypes detected, 1 was private and 2 were shared. Overall results showed no significant difference in haplotype diversity ($p > 0.05$) in *G. tachinoides*, *G. m. submorsitans* and *G. p. palpalis* populations.

4.2.5 Nucleotide diversity (Genetic variations)

Result (Table 4.7) shows composite nucleotide diversity in *Glossina* populations sampled. A 53.2% variation in composite nucleotides was recorded in *G. m. submorsitans* in Yankari Game Reserve and 47.2% variation was recorded in the same species in Kainji Lake National Park. In *G. tachinoides*, variations were 44.3% in Yankari Game Reserve and 43.3% in Kainji Lake National Park. In *G. p. palpalis* populations, Old Oyo had 36.8% variation, Cross River National Park had 50.6% and Ijah Gwari had 40.8%. The three *Glossina fusca* group flies captured in this study were very negligible to be subjected to sequence analysis, so genetic variation in them was not determined.

Table 4. 6: Haplotype diversity in *Glossina* populations sampled in National Parks and Ijah Gwari

Glossina species	Sampled Location	Sequence Length	No. of Haplotypes	No. of shared Haplotypes	No. of private haplotypes (singletons)	Confidence Mean	% Pairwise Variation
G. tachinoides	Yankari	1067	3	2	1	88.2	44.3
G. m. submorsitans	Yankari	1075	4	2	2	81.5	53.2
*G. p. palpalis**	Yankari	-	-	-	-	-	-
G. tachinoides	Kainji Lake	1067	3	1	2	88.2	44.3
G. m. submorsitans	Kainji Lake	1071	2	2	0	84.0	47.2
G. p. palpalis	Old Oyo	1100	4	2	2	84.4	36.8
G. p. palpalis	Cross River	999	3	2	1	85.7	50.6
G. p. palpalis	Ijah Gwari	1065	3	2	1	87.5	40.8

*Could not be computed due to low sample size

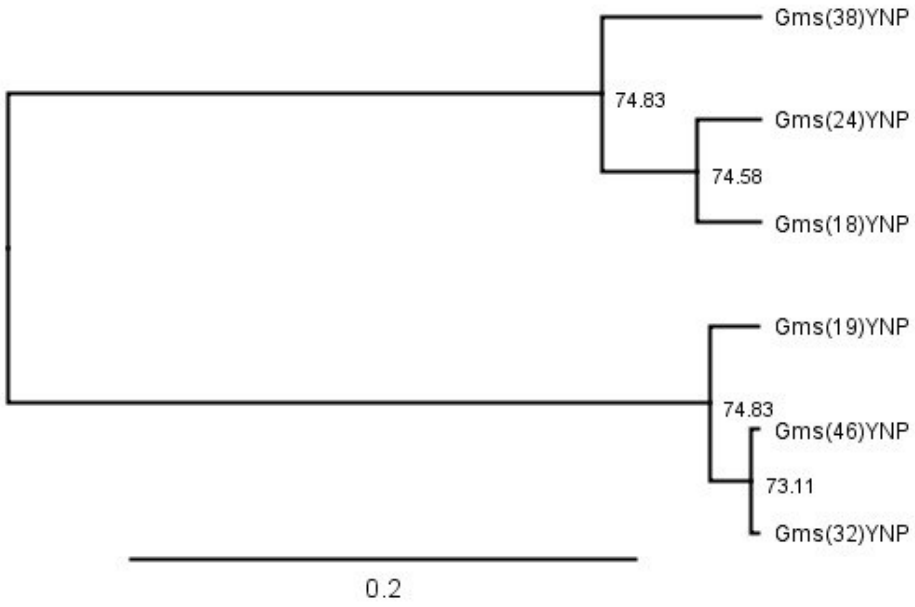


Figure 4.19a: Composite haplotype diversity in *Glossina morsitans submorsitans* populations in Yankari Game Reserve.

Branches with Gms 19 and 38 represent single/private haplotypes, while branches with Gms18, 24, 32 and 46 represent 2 shared haplotypes.

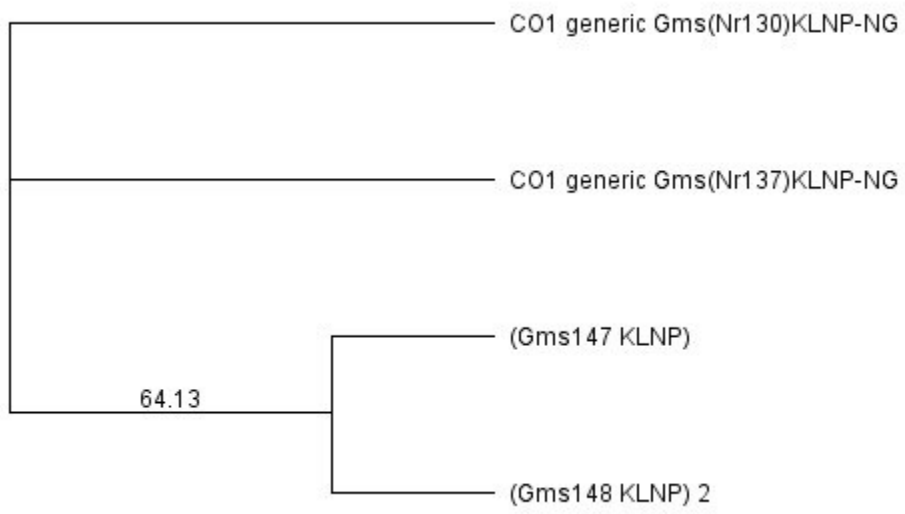


Figure 4.19b: Composite haplotype diversity in *Glossina morsitans submorsitans* populations in Kainji Lake National Park.

Branches with Gms 130, 137, 147, and 148 represent 2 shared haplotypes. No private haplotypes/singletons were detected here.

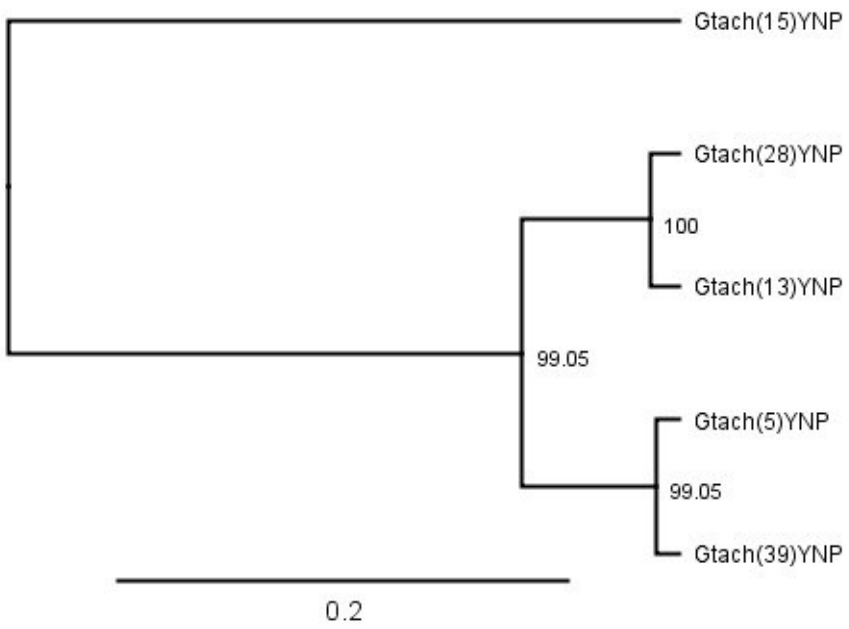


Figure 4.19c: Composite haplotype diversity in *Glossina tachinoides* populations at Yankari Game Reserve.

Branches with Gtach15 represent single/private haplotype, while branches with Gtach 5, 13, 28 and 39 represent 2 shared haplotypes

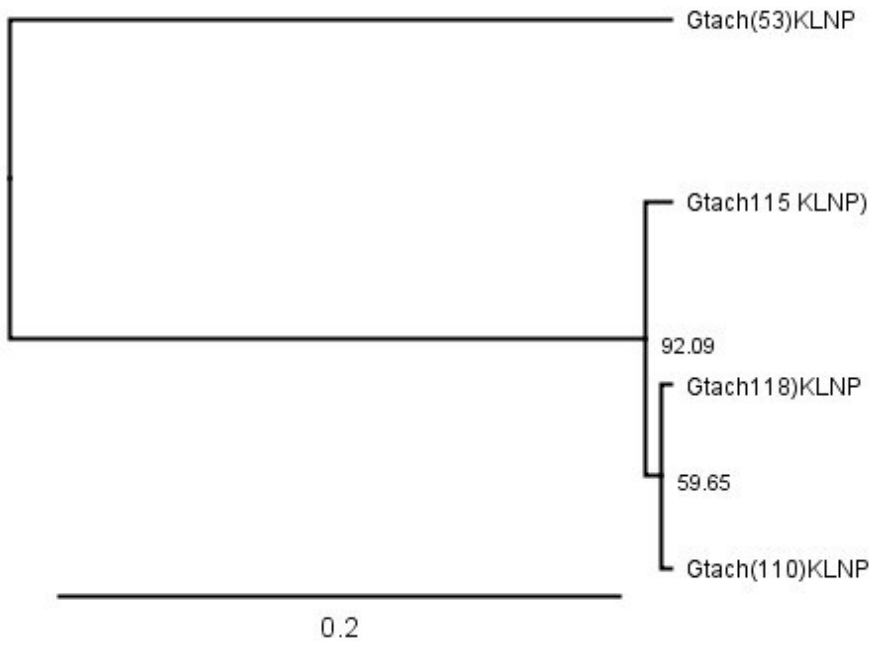


Figure 4.19d: Composite haplotype diversity in *Glossina tachinoides* populations at Kainji Lake National Park.

Branches with Gtach 53 and 115 represent single/private haplotypes, while branches with Gtach110 and 118 represent 1 shared haplotype

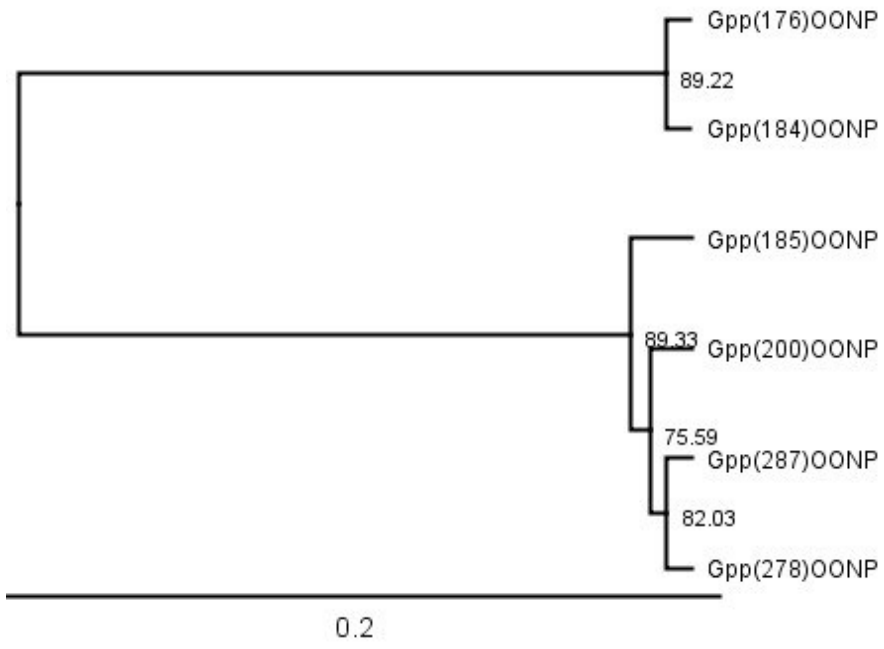


Figure 4.19e: Composite haplotype diversity in *Glossina palpalis palpalis* populations at Old Oyo National Park.

Branches with Gpp 185 and 200 represent single/private haplotypes, while branches with Gpp176, 184, 278 and 287 represent 2 shared haplotypes

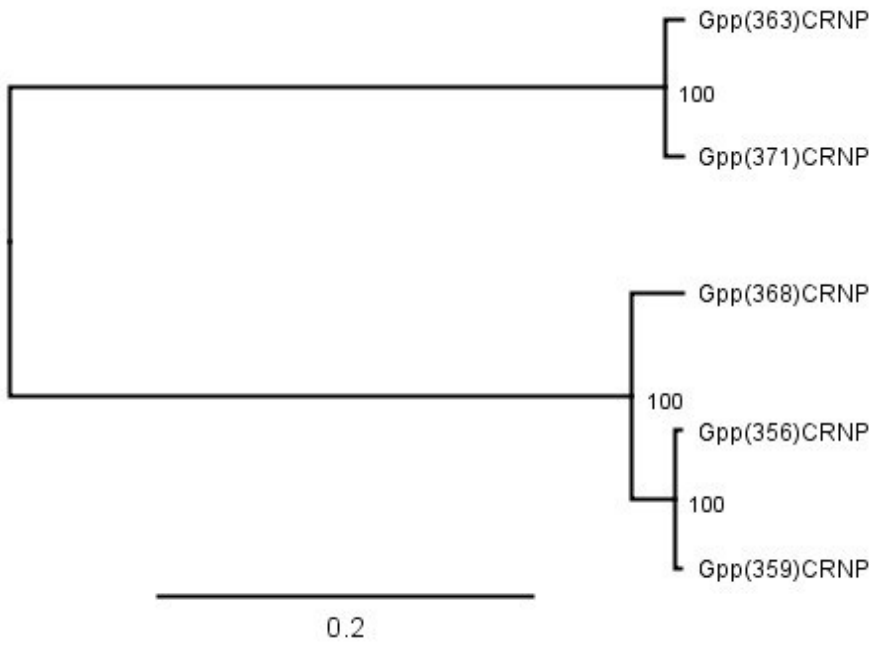


Figure 4.19f: Composite haplotypes in *Glossina palpalis palpalis* populations at Cross River National Park.

Branches with Gpp 368 represent single/private haplotype, while branches with Gpp356, 359, 363 and 371 represent 2 shared haplotypes

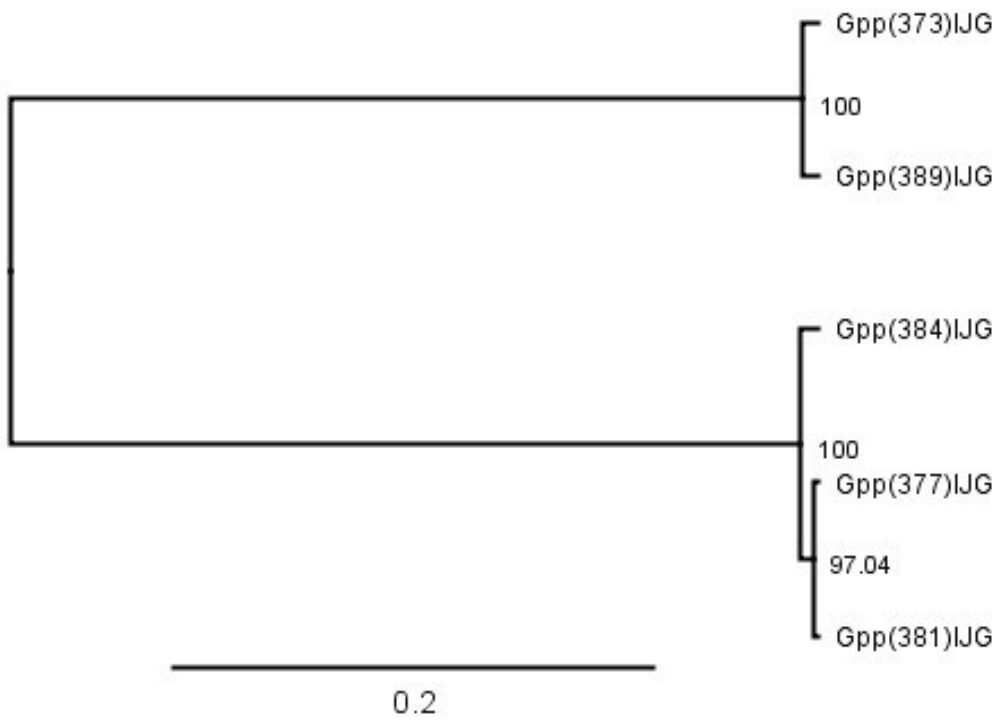


Figure 4.19g: Composite haplotype diversity in *Glossina palpalis palpalis* populations in Ijah Gwari sampled site.

Branches with Gpp 384 represent single/private haplotype, while branches with Gpp373, 377, 381 and 389 represent 2 shared haplotypes

Table 4. 7: Nucleotide diversity in *Glossina* populations sampled in National Parks and Ijah Gwari

Location/ National Park	*Glossina* species	No. of Sequences	Sequence Length	Confidence Mean	% Pairwise Similarity
Yankari	*G. tachinoides*	4	1067	88.2	55.7
	G. m. submorsitans	4	1075	81.5	46.8
	*G. p. palpalis**	-	-	-	-
Kainji Lake	*G. tachinoides*	5	1067	88.2	55.7
	G. m. submorsitans	4	1071	84.0	52.8
Old Oyo	*G. p. palpalis*	4	1100	84.4	63.2
Cross river	*G. p. palpalis*	6	999	85.7	49.4
Ijah Gwari	*G. p. palpalis*	5	1065	87.5	59.2

*Could not be computed due to low sample size.

4.3 Genetic Differentiation (Average Evolutionary Divergence) in Sampled *Glossina* spp

4.3.1 *G. m. submorsitans* populations in Yankari Game Reserve

Genetic differentiation was measured as frequency of nucleotide substitutions in sequences of 5 *Glossina m. submorsitans* flies. Results of analysed data (Table 4.8) revealed high (F_{ST}= 0.7643) levels genetic differentiations in aligned sequences within the 1075 fragment size in *G. m. submorsitans* populations collected in Yankari Game Reserve. Figure 4.20a, shows nucleotide substitution at the 560 base pair region of the sequence fragment of fly number 38. The dominant nucleotide Thymine (T) was substituted with Adenine (A) as a single nucleotide substitution. Whereas, at the 569 base pair region, the dominant nucleotide, Adenine (A), was substituted with Thymine (T).

4.3.2 *G. m. submorsitans* populations in Kainji Lake National Park

Differentiation was determined by examining the frequency of nucleotide substitutions using sequences of 4 *G. m. submorsitans*. Results (Table 4.8) showed high (F_{ST}= 0.6508) level differentiation in aligned sequences within the 1071 fragment size. Figure 4.20b shows nucleotide substitution of the dominant nucleotide Adenine (A) with Thymine (T), expressed in the 536 bp region of nucleotide sequence of fly No.147.

4.3.3 *G. tachinoides* populations in Yankari Game Reserve

Genetic differentiation within *G. tachinoides* populations in Yankari Game Reserve was examined using sequences of 5 *G. tachinoides*. Results (Table 4.8) showed that differentiation based on frequency of nucleotide substitutions was (F_{ST}=0.3427). Figure 4.20c shows differentiation expressed within a portion of the 1067 base pair fragment length of the sequences, indicating nucleotide substitutions at the 579 and 580bp regions of fly no. 15. The dominant nucleotide Thymine (T) was substituted with Adenine (A) in a dinucleotide substitutions. It thus appear from

Table 4. 8. Genetic differentiations based on frequency of nucleotide substitutions in *Glossina* populations in sampled locations.

Location	*Glossina* spp	Number of sequences	Mean diversity	Standard deviation	P – value
Yankari Game	*G. tachinoides*	5	0.3427	45.0	0.175
Reserve	*G. m. submorsitans*	5	0.7643	13.0	0.174
Kainji Lake	*G. tachinoides*	5	0.3025	24.3	0.268
	G. m. submorsitans	4	0.6508	122.5	0.315
Old Oyo	*G. p. palpalis*	6	0.2805	96.6	0.403
Cross river	*G. p. palpalis*	6	0.4033	306.7	0.116
Ijah Gwari	*G. p. palpalis*	5	0.3856	17.3	0.472

Standard range = 0.0-1.0 based on Hardy-Weinberg equilibrium.

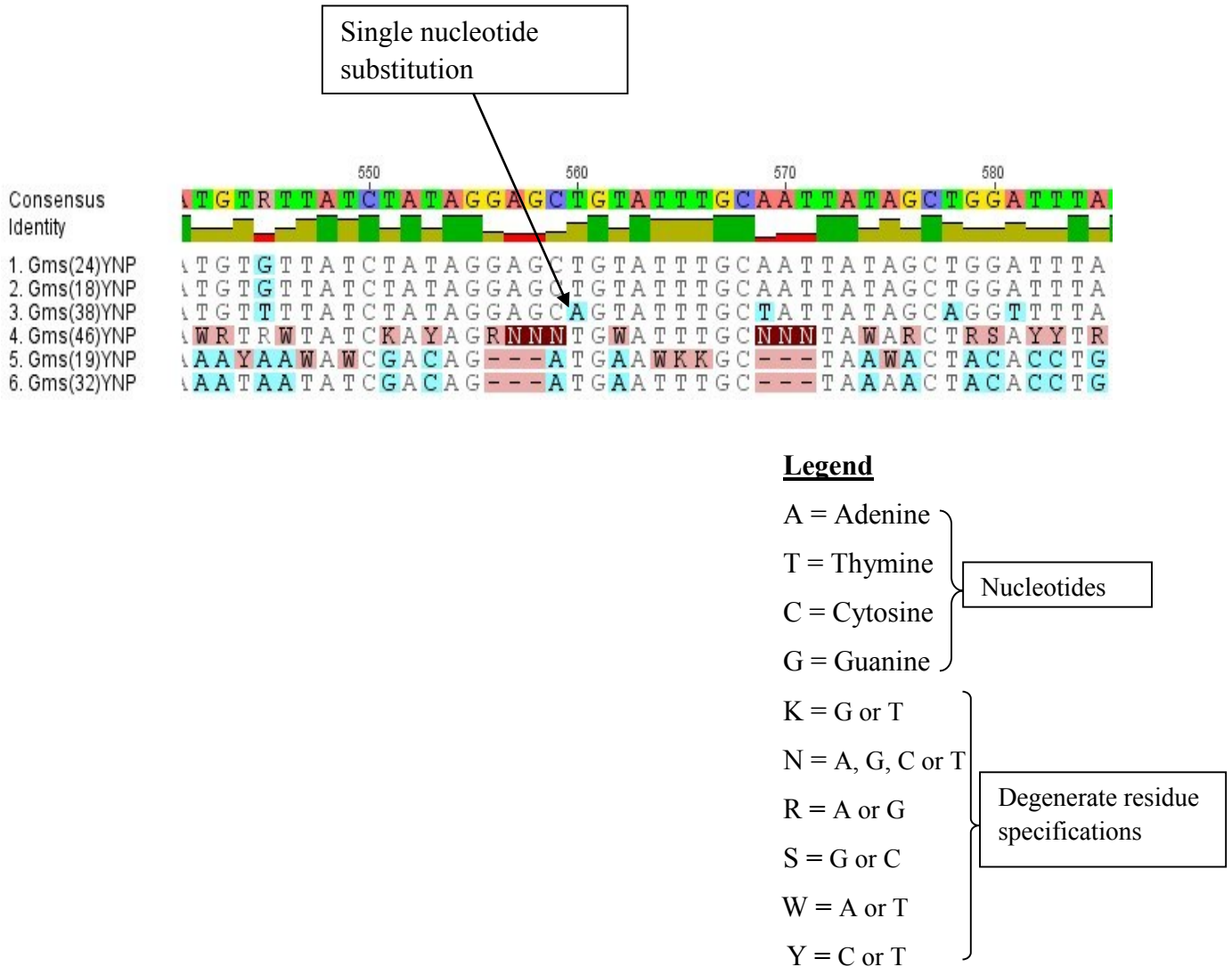


Figure 4.20a. Some nucleotide substitutions in CO I sequences in *G. m. submorsitans* populations in Yankari Game Reserve (YGR).

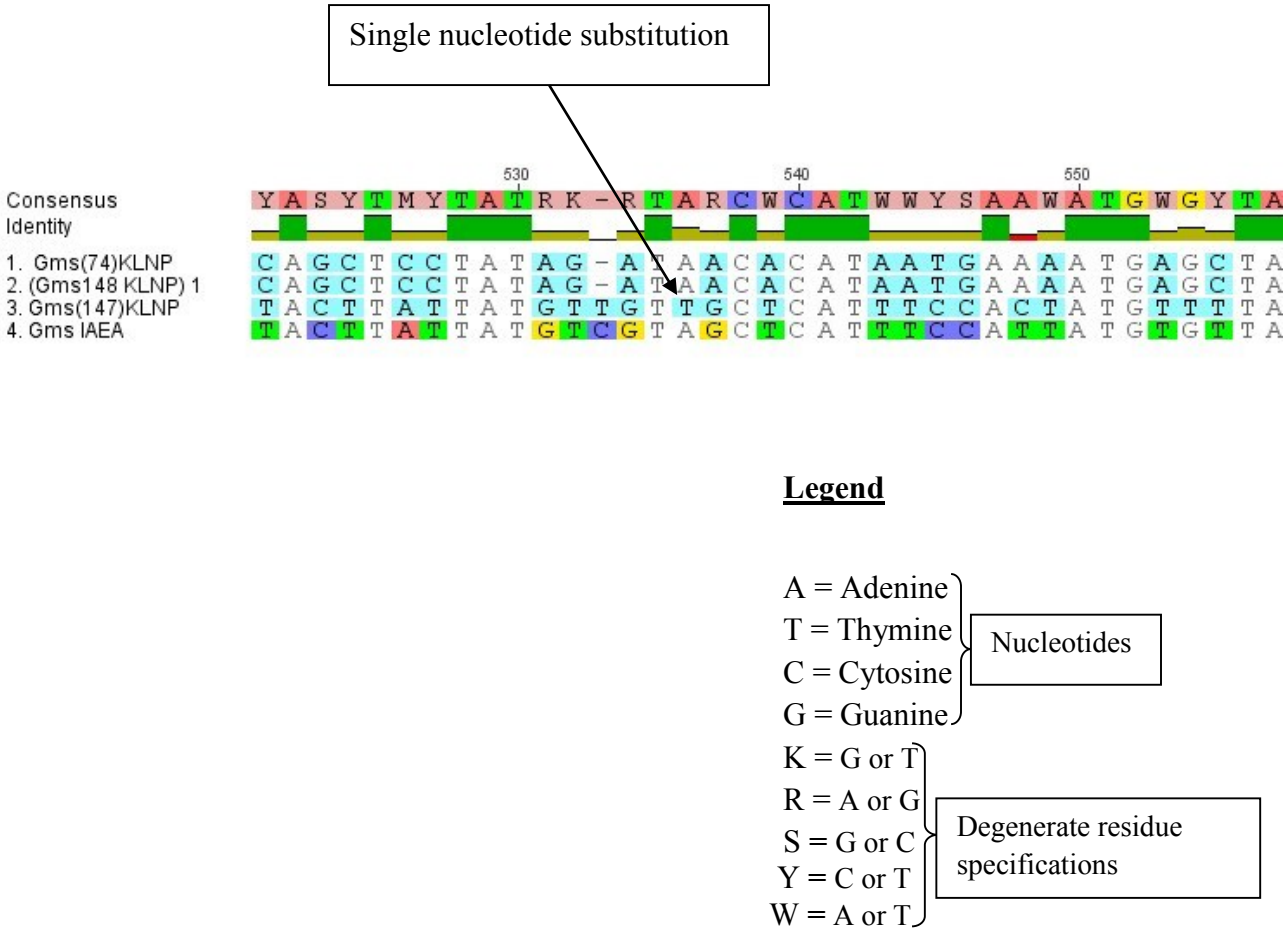


Figure 4.20b. A nucleotide substitution in CO I sequences in *G. m. submorsitans* populations in Kainji Lake National Park (KLNP).

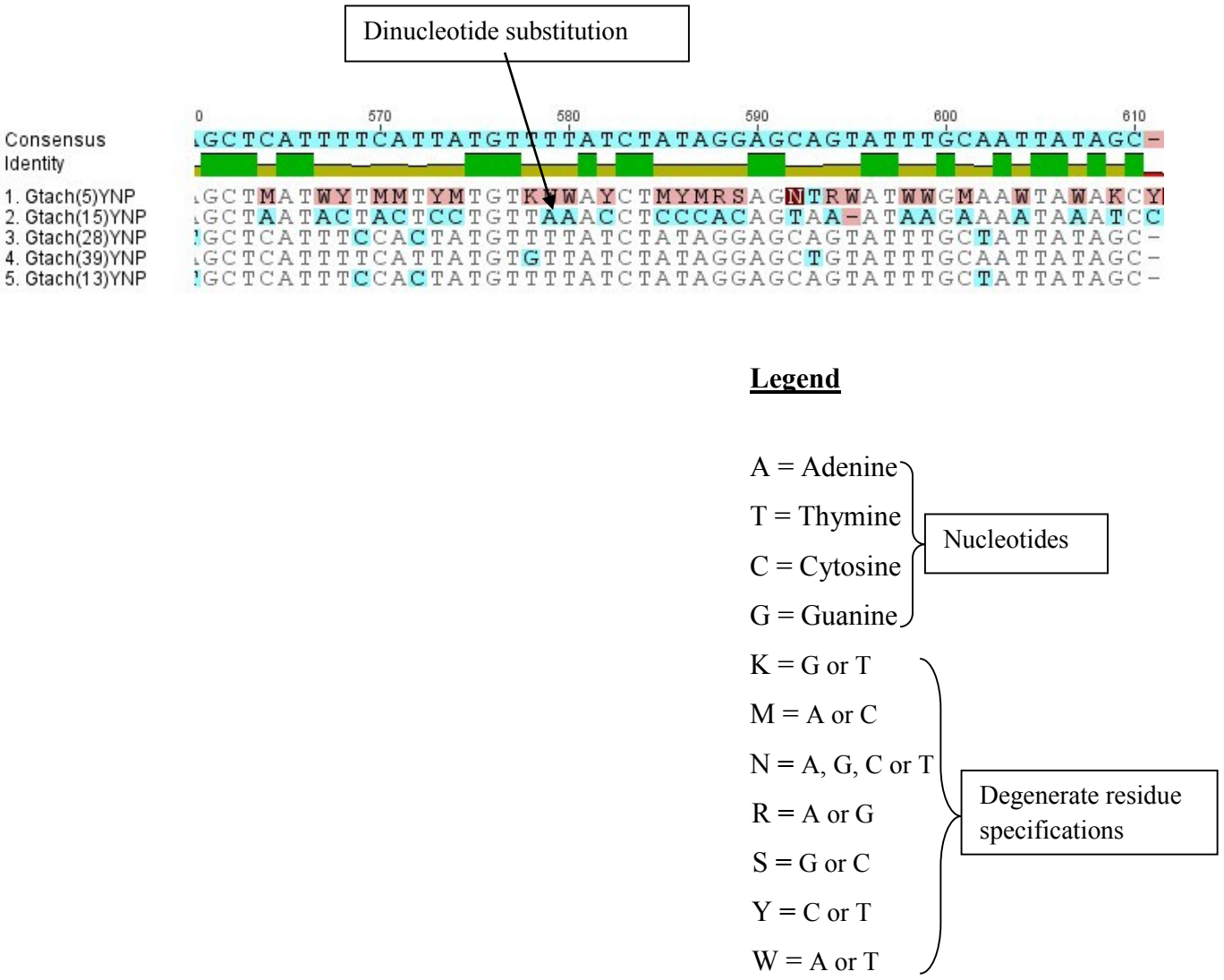


Figure 4.20c. Some nucleotide substitutions in CO I sequences in *G. tachinoides* populations in Yankari Game Reserve (YGR).

the analysis that the *G. tachinoides* populations were more homogeneous compared to *G. m. submorsitans* populations.

4.3.4 *G. tachinoides* populations in Kainji Lake National Park

Results (Table 4.8) show low level differentiation (F_{ST}= 0.3025) in 5 examined sequences of *G. tachinoides* in Kainji Lake National Park. Figure 4.20d shows substitution of the dominant nucleotide Guanine (G) with Cytosine (C) expressed at the region of 460 bp in fly no.53 within the fragment length of 1067 base pairs.

4.3.5 *G. p. palpalis* populations in Old Oyo National Park

Result (Table 4.8) shows low level (F_{ST}= 0.2805) genetic differentiation within *G. p. palpalis* population in Old Oyo National Park. Analysis of sequences (Figure 4.20e) shows substitutions within the 1100 bp fragment length portion of sequences of 6 *G. p. palpalis*. Substitution of the dominant nucleotide Adenine (A) with Guanine (G), at the 749 bp sequence region of fly no.278 is shown.

4.3.6 *G. p. palpalis* populations in Cross River National Park

Result (Table 4.8) shows low level genetic differentiation (F_{ST}= 0.4033) in *G. p. palpalis* in Cross River National Park. Figure 4.20f shows nucleotide substitutions in sequences of 6 *G. p. palpalis* flies with dominant nucleotide Guanine (G) being substituted with Adenine (A) in 834bp sequence region of fly no363. Also in the sequence region of 860 bp, the dominant nucleotide Thymine (T) was substituted with Guanine (G) in fly no.369, within the fragment length of 999bp.

4.3.7 *G. p. palpalis* populations in Ijah Gwari

Result obtained (Table 4.8) shows low level differentiation (F_{ST}= 0.3856), in 5 *G. p. palpalis* populations in Ijah Gwari. Figure 4.20g shows substitutions of the dominant nucleotide Adenine (A) with Guanine (G) occurring at the 450bp sequence region of 1067bp fragment lenght of fly no

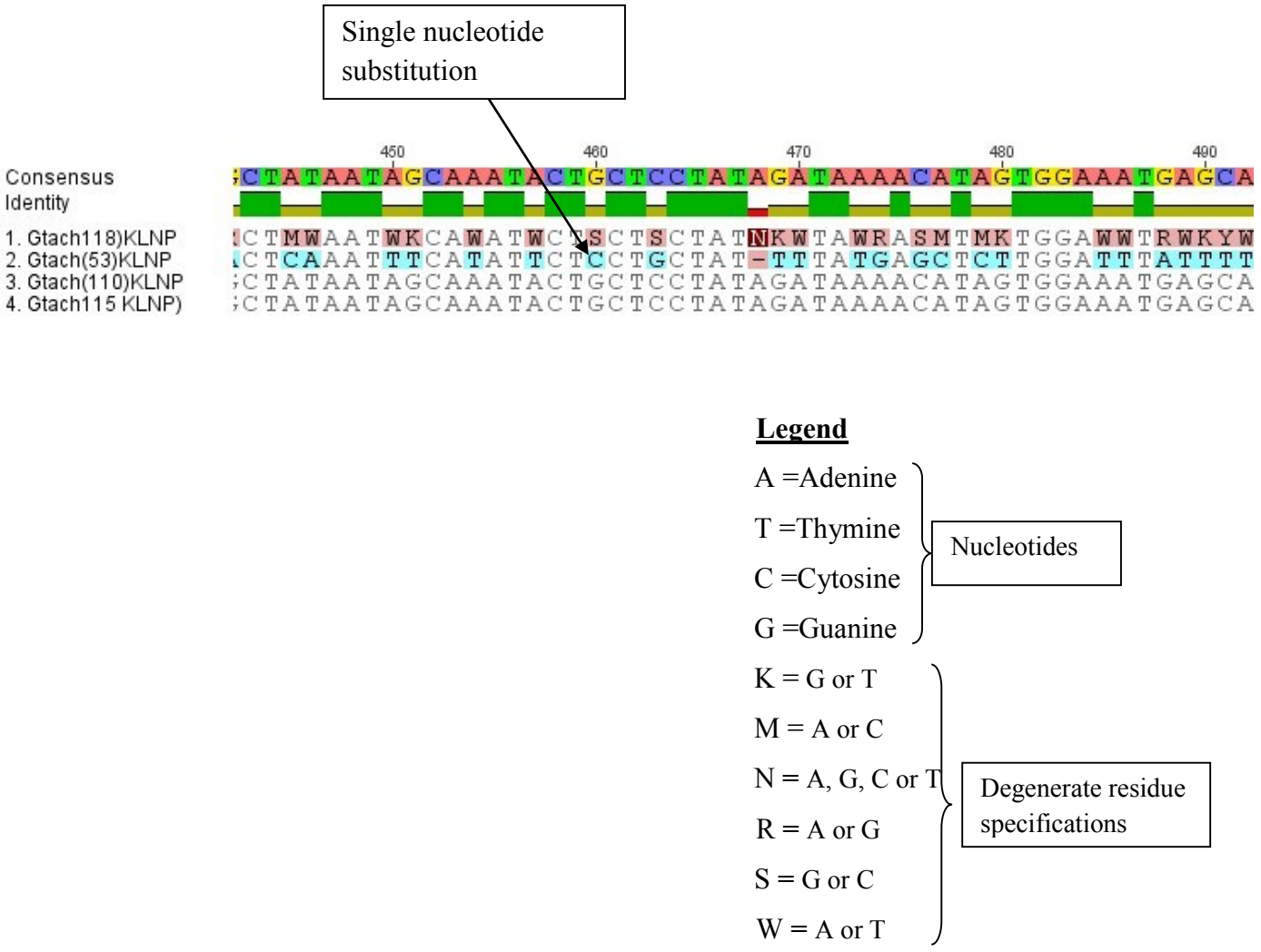


Figure 4.20d. Genetic differentiations based on some nucleotide substitutions in CO I sequences in *G. tachinoides* populations in Kainji Lake National Park (KLNP).

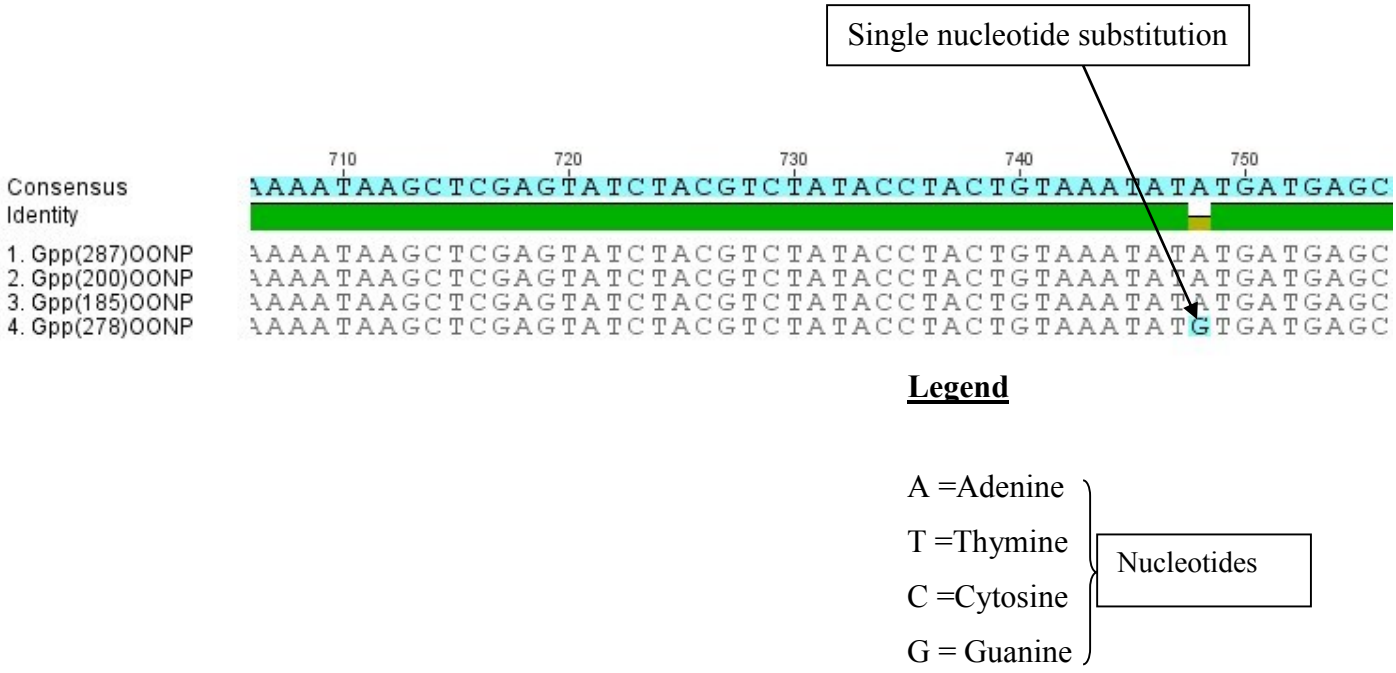


Figure 4.20e. Genetic differentiations based on some nucleotide substitutions in CO I sequences in *G. p. palpalis* populations in Old Oyo National Park (OONP)

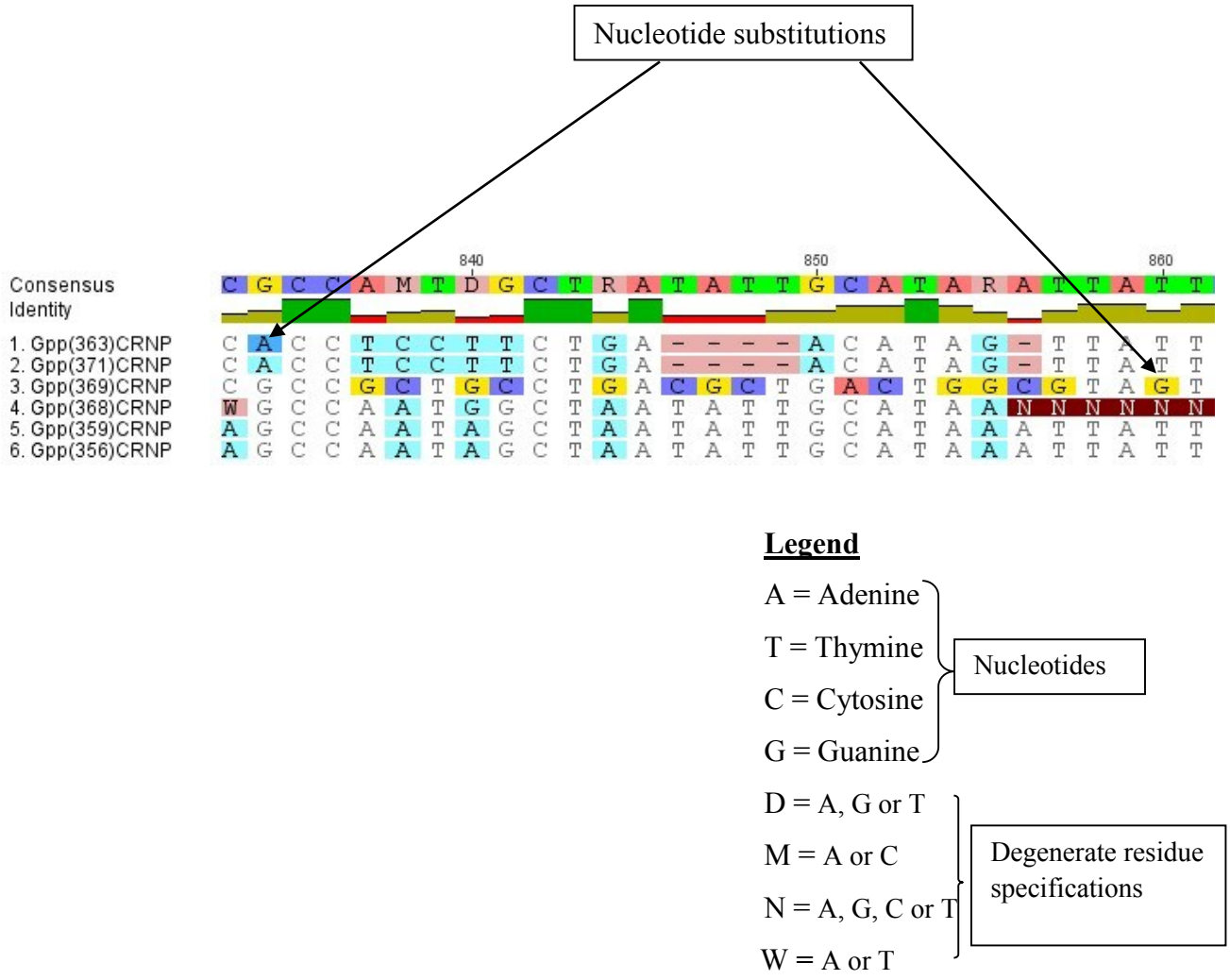


Figure 4.20f. Genetic differentiations based on some nucleotide substitutions in CO I sequences in *G. p. palpalis* populations in Cross River National Park (CRNP)

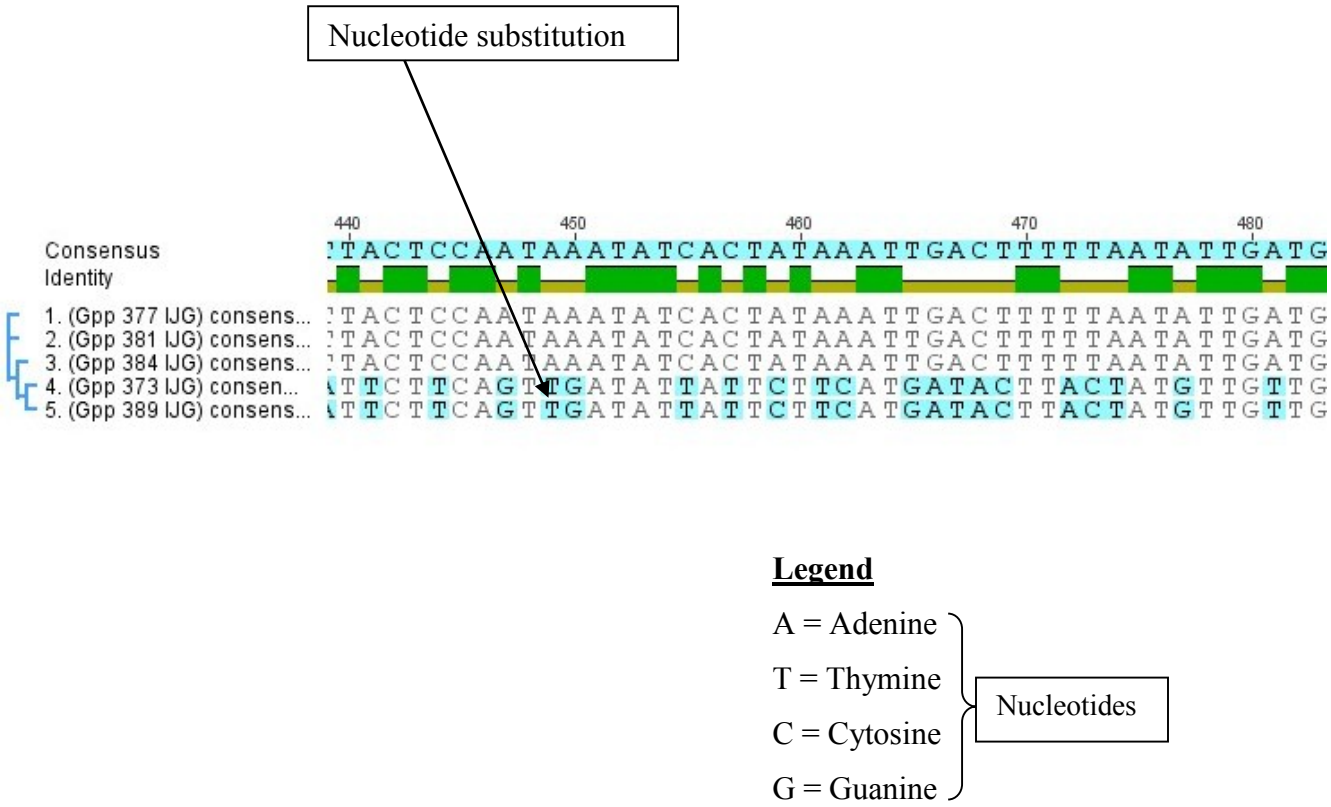


Figure 4.20g. Some nucleotide substitutions in CO I sequences in G. p. palpalis populations in Ijah Gwari (IJG)

373. Substitution of dominant nucleotide Adenine (A) with Thymine (T) at the 481bp sequence region of fly no.389 is also shown.

Overall result in Table 4.8 shows that genetic differentiation in fly populations in the sampled national parks and Ijah Gwari, based on frequency of nucleotide substitutions averaged across all sequences, varied between species and between parks. Differentiation was higher (F_{ST}=0.7643) in *G. m. submorsitans* population in Yankari Game Reserve than in Kainji Lake National Park (F_{ST}= 0.6508). Differentiations in *G. tachinoides* populations in Yankari Game Reserve was higher (F_{ST}=0.3427) than in Kainji Lake National Park (F_{ST}=0.3025). Differentiation in *G. p. palpalis* was higher in Cross River National Park (F_{ST}= 0.4033), followed by low level genetic differentiation in Ijah Gwari (F_{ST}= 0.3856) and in Old Oyo National Park (F_{ST}= 0.2805). Overall results show high level genetic differentiations in the savanna tsetse fly, *G. m. submorsitans* while low level differentiations were recorded in the riverine flies *G. p. palpalis* and *G. tachinoides* in the parks sampled.

4.4 Phylogenetic Relationships of *Glossina* Species in National Parks and Ijah Gwari

Results obtained from phylogenetic analysis of *G. m. submorsitans*, *G. tachinoides*, *G. p. palpalis* and *G. fusca* caught in this study (Figure 4.21) shows that *Glossina* spp. evolved from a single ancestor. Through evolutionary relationships, the neighbor joining tree reconstruction showed clear patterns of clustering of closely related species within one clade, and distantly related species forming another clade. Within the clades, closely related siblings clustered together and separately from their distant relations suggesting that tsetse populations at the National Parks exist in smaller population subunits that are genetically similar. Areas of conflict identified were in four instances where specimen numbers 17 and 74 morphologically identified as *Glossina tachinoides* clustered with *G. m. submorsitans* instead of their closely related siblings *G. tachinoides*. Also specimen numbers 162 and 273 caught in Old Oyo morphologically identified as *G. m. submorsitans*, did not cluster with its sister group, but rather clustered with *G. pallidipes* sequences obtained from the GenBank.

4.5 Phylogenetic Relationships with Particular Reference to *G. p. palpalis* Populations

Results obtained from phylogenetic analysis of *G. p. palpalis* (Figure 4.22) showed relationships of *G. p. palpalis* populations in sampled locations. The neighbor joining tree reconstruction showed clear patterns of clustering of closely related siblings separately from their distant cousins. It is interesting to note the splitting of *G. p. palpalis* populations into two major groups, a West African Clade (WAC) and a Central Africa Clade (CAC), with the groups co-existing sympatrically in Cross River National Park at the Nigerian-Cameroonian border area (Figure 4. 16). *G. p. palpalis* populations in Old Oyo National Park clustered with populations in Republics of Benin, Togo, Cote d'voire and Liberia, forming the West African clade, whereas *G. p. palpalis* populations in Cross River National Park clustered with those populations in Cameroon, Congo,

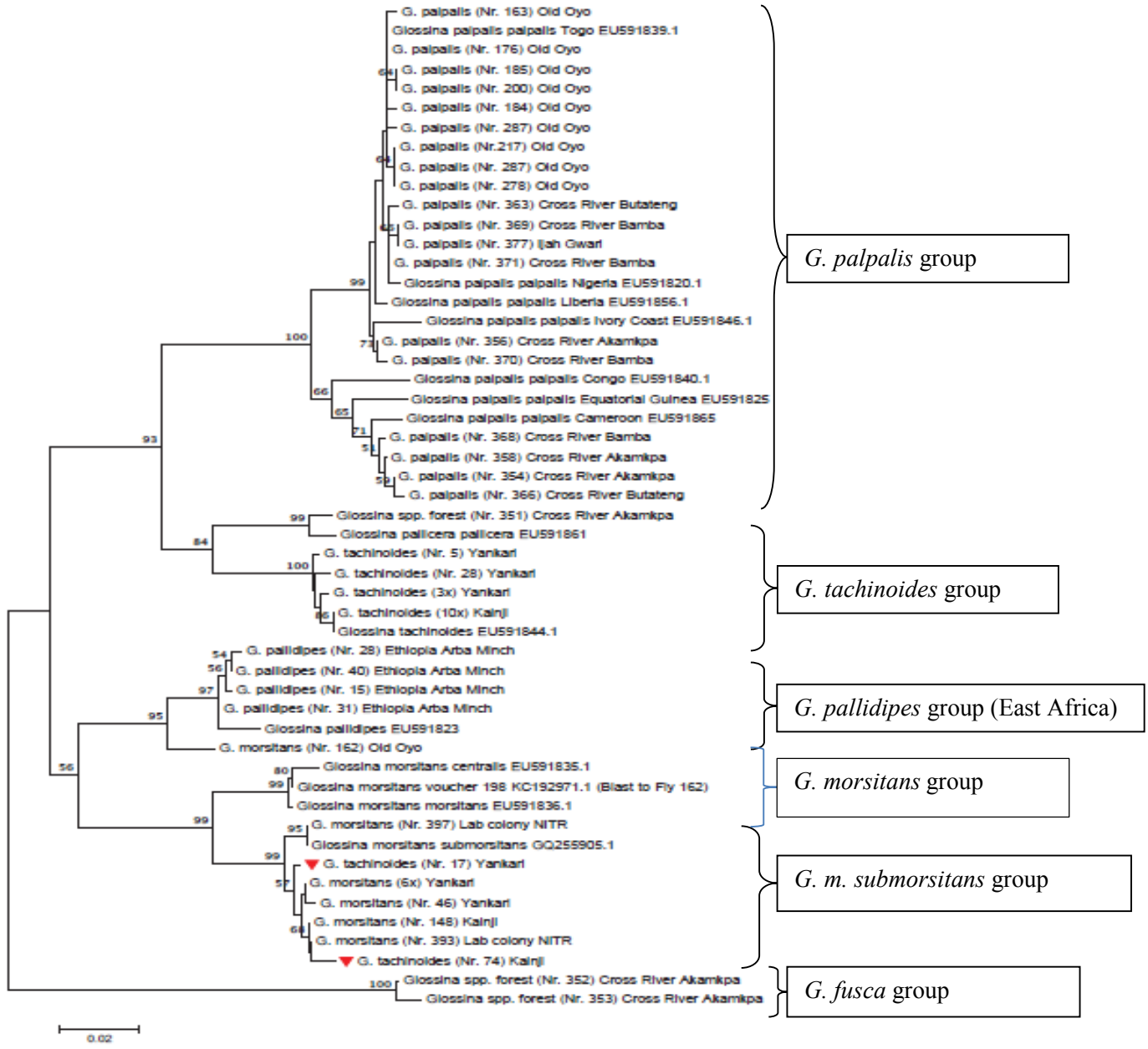


Figure 4.21. Phylogenetic relationships within *Glossina* spp. populations in Nigeria National Parks based on CO1 sequences.

Samples marked in red are morphologically indistinguishable from *G. tachinoides*, but phylogenetically related to *G. m. submorsitans*. Similarly, fly numbers 162 and 273 are morphologically indistinguishable from *G. m. submorsitans,* but phylogenetically related to *G. pallidipes* found in Central and Eastern Africa.

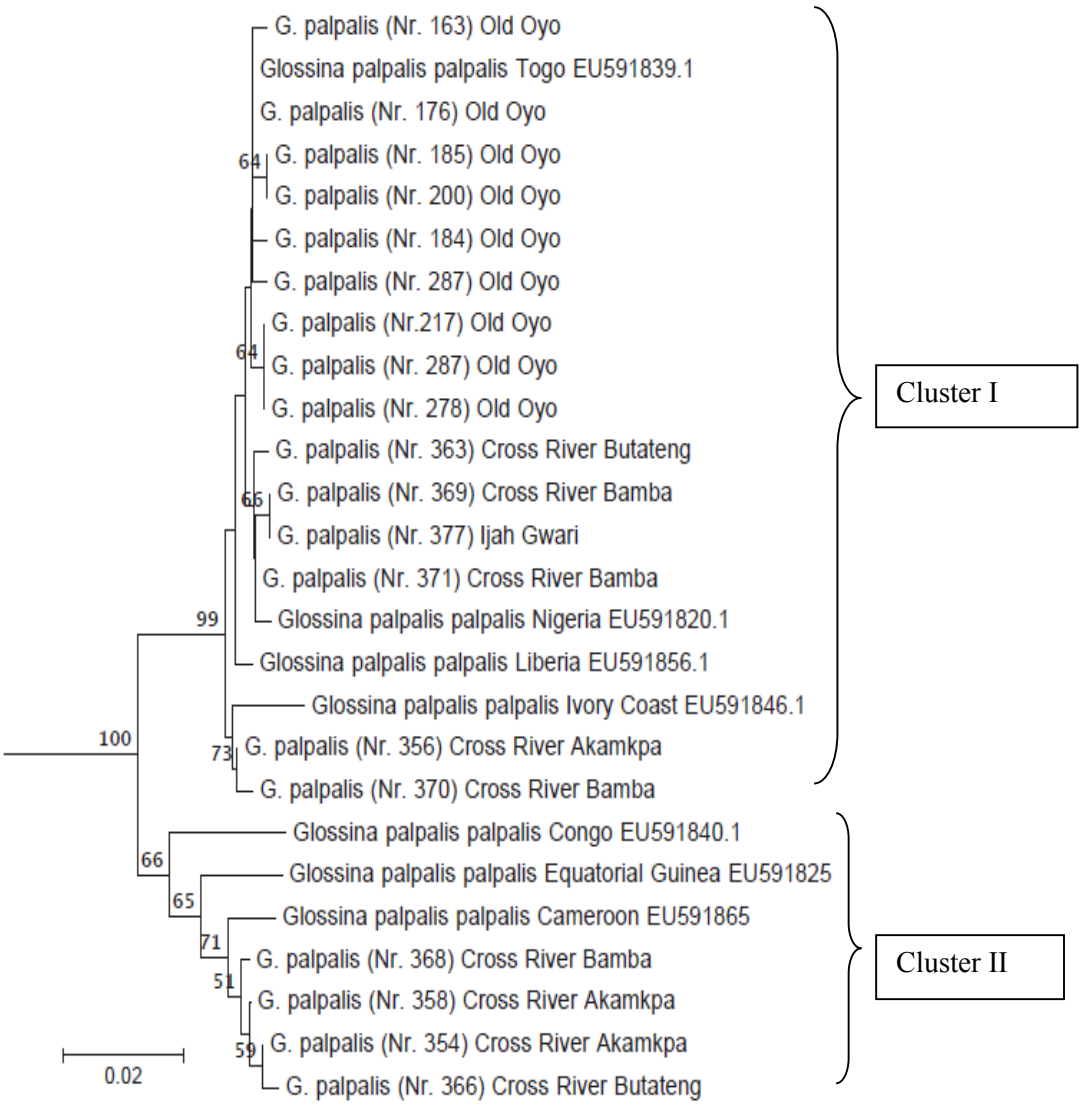


Figure 4.22. Phylogenetic relationships among *Glossina palpalis palpalis* populations based on CO I sequences in Nigeria and other sub-Sahara African countries of Liberia, Cote d'Ivoire, Togo, Cameroon, DRC, and Equatorial Guinea whose sequences were obtained from the Genbank.

Central African Republic to form the Central African clade. In Cross River National Park at the Nigerian-Camerounian boarder, it was observed that the two groups of *G. p. palpalis* detected in this study exist in sympatry, based on the capture of flies (Nos 356 and 370) representing the two groups (WAC and CAC) in the same location. The overlap in the ecologies of the two *G. p. palpalis* groups identified may suggest that the contact zone of the two groups identified exists around the Nigerian-Cameroonian border area. However the extent of admixture of the two groups of *G. p. palpalis* populations identified in this work remains unknown.

4.6 ITS-1 Analysis

The two *G. p. palpalis* clusters and various unidentified *Glossina* sp. were further analysed regarding changes in their ITS-1 region, while the ITS-1 regions of the *G. p. palpalis* subpopulations did not show any differences, the marker could be used for looking more closely into *Glossina* species. In contrast to the homogeneous population shown by the CO1 analysis, ITS-1 comparison, grouped the sequences into two branches (data not shown). The observed differences may be due to very distinct nucleotide deletions as well as several substitutions.

4.7 Geometric Wing Morphometrics

Analysis of 9 landmarks for morphometry of the wing (Figure 4.23a) was performed as described (Achukwi *et al*., 2013) on 41 tsetse fly wings from *G. p. palpalis* of the two genetic clusters (WAC and CAC) obtained by CO1 analysis. However, due to the low number of samples collected in Nigeria's Cross River National Park, samples collected in Toungo in Adamawa State at the Nigeria - Cameroonian boarder, were used for this analysis. CO1 sequences from all these flies were analysed and grouped in the two observed clusters. 20 flies belonged to the Central African cluster, while 21 samples grouped in the West African cluster.

The centroid size was used as a measure of geometric size (Rohlf *et al*., 1996) and the distance showed a difference in the two genetic clusters in the total sample, with the Central African group showing a smaller median than the West African group. However, when comparing the principal component analysis (PCA) of the partial warps, no differences were seen between the centroid sizes of the two populations (data not shown).

When the sex distribution of the subgroups was taken into account and the samples were split accordingly, the samples showed a sex dependent shape dimorphism (Figure 4.23b). The centroid distance was larger in female flies. Interestingly, PCA on the partial warps showed that the female subgroup of the Central African cluster segregated from the others (Figure 4.23c)



Figure 4.23a. Landmarks for geometric wing morphometric analysis

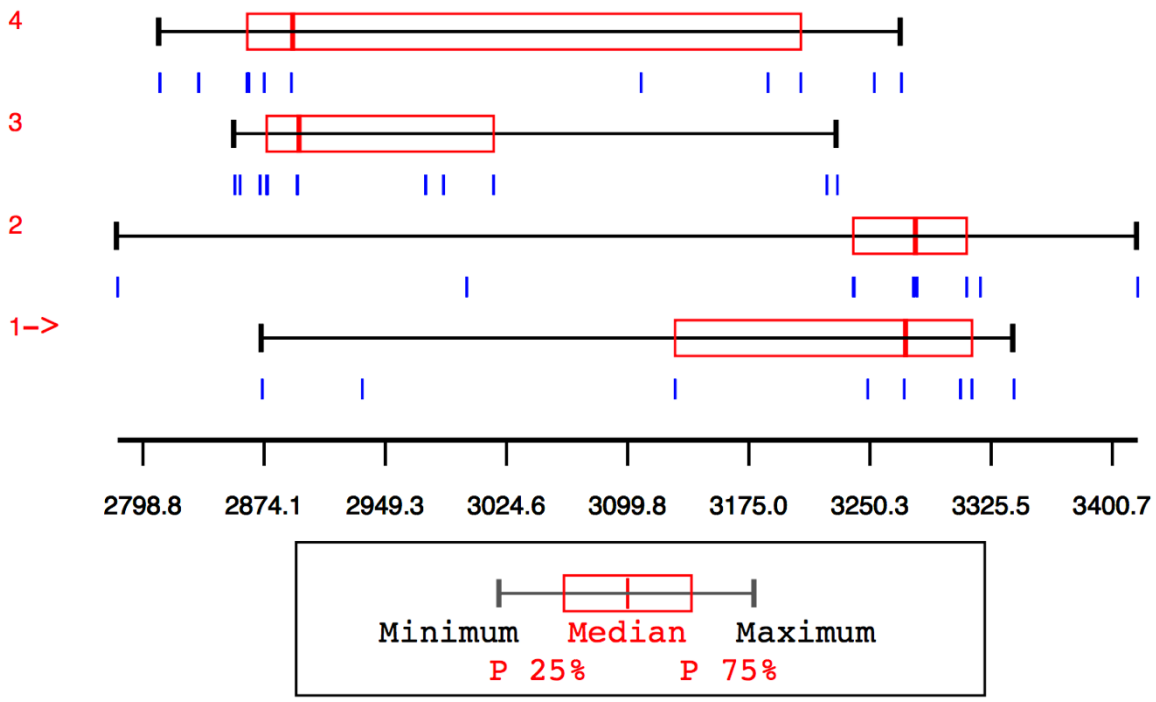


Figure 4.23b. Centroid distance.1=Female Central African Clade, 2=Female West African Clade, 3=Male Central African Clade, 4=Male West African Clade.

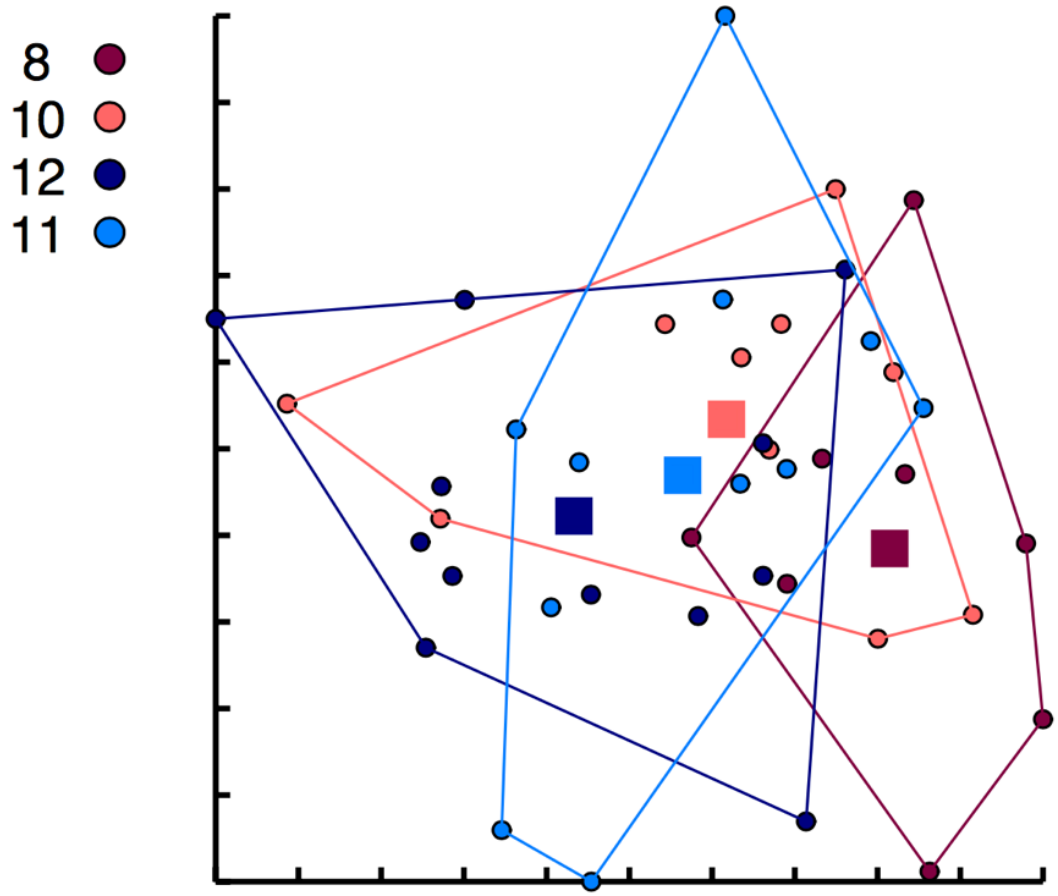


Figure 4.23c. Principal Component analysis on Partial warps. 11= Female Central African Clade, 12 = Female West African Clade, 10 = Male Central African Clade, 8 = Male West African Clade.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Ecological Studies

5.1.1 *Glossina* species composition and relative abundance in National Parks and Ijah Gwari

5.1.2 Yankari Game Reserve

From the results obtained (Table 4.1), Yankari Game Reserve is infested with *G. m. submorsitans, G. tachinoides* and *G. p. palpalis* populations, with *G. tachinoides* being more abundant than *G. m. submorsitans* followed by *G. p. palpalis*. This agrees with Molynuex (1973), in which presence of large numbers of *G. tachinoides* and *G. submorsitans* as well as a small *G. p. palpalis* populations had been described. The result shows that less anthropogenic activities that could threaten the existence of tsetse flies within the park may have taken place over the years. In contrast, Isaac *et al.,* (2016) observed that only *G. tachinoides* and *G. m. submorsitans* were present at the Yankari Game Reserve. This discrepancy in the composition and abundance in catches of *Glossina* spp. in Yankari Game Reserve might be attributed to variation in environmental conditions and possibly due to differences in timing of sampling activities. In Yankari Game Reserve, *G. m. submorsitans* were caught in areas with high ambient temperatures and low relative humidity conditions while *G. tachinoides* and *G. p. palpalis* were mostly caught near water bodies with lower ambient temperature and high relative humidity conditions. Results of statistical analysis in this study shows that tsetse catches correlated negatively with mean ambient temperature and positively with relative humidity conditions. Mean ambient temperatures between 31 and 35.5^{o}C and relative humidity between 28 and 46% obtained in this study are consistent with data previously described (Nash, 1937; Davies, 1977), suggesting that tsetse distribution in nature is governed by climatic factors.

The differences in the observed fly composition may also have been influenced by differences in vegetation types. *Glossina m. submorsitans* was captured in the open woodland areas of Yankari Game Reserve, while *G. tachinoides* and *G. p. palpalis* were caught along the banks of rivers/ stream courses. Whereas in the same vegetation zone mentioned above, *G. tachinoides* and *G. m. submorsitans* were found in sympatry in some instances in open woodland*, G. p. palpalis* (caught in Yankari) was however found in gallery forests at the bank of river courses. The low population of *G. p. palpalis* observed in Yankari may be due to its less tolerance to high temperatures, as this species has been reported to thrive best under prevailing temperature range of 25 ±2^{o}C and 80-85% relative humidity, with 1000-2000 mm of rainfall (Davies, 1977). This is lower than temperature range of 31-35.3^{o}C and relative humidity range of 28-46% recorded in Yankari during this study (Appendix IX).

The overlap in the ecologies of *G. m. submorsitans* a savannah species and *G. tachinoides* a riverine species in Yankari Game Reserve is in consonance with earlier works suggesting the co-existence of *G. tachinoides* and *G. m. submorsitans* at certain times of the year (Jordan, 1986). The extremely dry weather conditions might have made *G. m. submorsitans* become less tolerant to high temperatures and low relative humidity conditions and behaved like a riverine species by retreating from open woodland and stayed close to the banks of river systems to avoid desiccation, while *G. tachinoides* though a riverine species but because of its broad tolerance to high temperature and low relative humidity conditions might have dispersed widely into areas with dryer conditions. Those conditions as described above, might have brought both species of flies to co-exist at that period of the year as earlier described (Rogers and Randolph, 1986).

Results (Figure 4. 1) further showed that tsetse flies were most abundant in Yankari than any other location sampled. This might be due to the high concentration of game in the park, as host

availability has been described to play important part in determining local distribution and abundance of flies (Turner, 1981; Rogers and Boreham, 1973). In addition, it was observed that *G. tachinoides* population was more abundant 682 (79.03%) than *G. m. submorsitans* 179 (20.74%) in the reserve. The dominance of *G. tachinoides* in our trap catches in Yankari is consistent with the works of Omoogun *et al.,* (1994), who described the dominance of *G. tachinoides* over *G. m. submorsitans* in Yankari during trap trials. This observation further buttresses the view expressed on the interplay of hosts' availability and their selection on tsetse fly abundance (Onyiah, 1980b). *Glossina tachinoides* has a wide host range compared to *G. m. submorsitans* which is reported to have a limited choice of hosts because of its catholic feeding behaviour (Madubunyi, 1990). *Glossina. m. submorsitans* derives most of its blood meals from large bovids and ungulates which graze in the open woodland and move to riverine areas only when they are thirsty, suggesting that fluctuations in numbers of host animals may have direct impact on the abundance of flies. It has been established that elimination of large game (as occurred during the rinderpest pandemic) could lead to extermination of *morsitans* populations, but less so for the *palpalis* group which are able to switch to any available hosts including humans for their blood meals (Onyiah, 1980a).

Glosssina tachinoides abundance may be associated with the extensive hydrographic network in Yankari Game Reserve. The reserve is traversed by several tributaries which discharge their contents into the major perennial river Gaji in the area. Several breeding sites become available from the exposed sandy beds as the smaller tributaries dry up during dry season. Being a riverine species, it is most probable that *G. tachinoides* would have taken advantage of the breeding grounds associated with the extensive hydrographic network in Yankari Game Reserve for its breeding activity.

The dominance of *G. tachinoides* populations in Yankari (Figure 4.2) in addition, may be due to the occurrence of riparian vegetation, which may be providing shelter to the large populations of small animal hosts, reptiles, birds and primates which abound the park from which *tachinoides* possibly obtains its blood meals. The preponderance of young teneral flies in the catch composition (Table 4.2) shows that the flies were probably at the peak of their breeding activities.

From the overall outlook, it seems most reasonable to conclude that the dominance of *G. tachinoides* at Yankari over *G. m. submorsitans* and *G. p. palpalis* might possibly be attributed to its ability to explore more niches and to adapt to changing environmental conditions better than *G. m. submorsitans.* Environmental conditions such as ambient temperature and relative humidity, breeding sites, foraging or feeding grounds, resting sites and wide choice of animal hosts might be responsible for the dominance of *G. tachinoides* in the game reserve.

The dominance of *G. tachinoides* in our catches may in addition be attributed to sampling biase of the biconical trap. It has been reported that tsetse respond differently to different traps (Kuzoe and Schofield, 2005). The biconical trap is reported to be more effective at catching the riverine species of flies than it does for the *morsitans* group (Omoogun *et al.,* 1994; Ajibade and Agbede, 2008). This may be attributed to the vertical shape of biconical trap, which *G. tachinoides* seems to prefer. In contrast *G. morsitans* preference for horizontally cylindrical shapes (drum shape), endears it to large bovids and ungulates, except the Zebra which tsetse do not feed on because of its strips (Gibson, 1992).

5.1.3 Kainji Lake National Park

Tsetse catches in Kainji Lake National Park (Table 4.1) showed variations in species composition and relative abundance during the study. The most abundant *Glossina* species caught was *G.*

tachinoides 368 (80.53%), followed by *G. m. submorsitans* with 89 (19.47%) of the catches. The predominance of *G. tachinoides* in the catches was similar to the observation in Yankari Game Reserve described earlier in this study. Although *G. p. palpalis* was recorded in Kainji during an earlier study, (Ahmed *et al.*, 2000), it was not caught during this study probably due to differences in timing of sampling activities. Similar reports by Ajibade and Agbede (2008), showed that *G. m. submorsitans, G. tachinoides* and *G. p. palpalis* exist in different habitats in Kainji Lake National Park. Whereas *G. m. submorsitans* inhabited open woodland savannah in the two sectors of the park (Zugurma and Borgu sectors), the riverine tsetse *G. p. palpalis and G. tachinoides* were found along the course of River Oli. These discrepances in species composition may be due to differences in trapping points and other environmental factors. Earlier studies did not provide co-ordinates of trapping positions. In an earlier study (Shaida *et al.,* 2002, unpublished report) in Kainji Lake National Park, the co-existence of *G. tachinoides* and *G. m. submorsitans* in the same habitat was described, suggesting an over lap in the ecologies of those flies. This is consistent with works of Rogers and Randolph (1986) in which the co-existence of *Glossina* species in studies of ecodistribution and abundance of the vectors had been described.

5.1.4 Old Oyo National Park

In Old Oyo National Park (Table 4.1), *G. p. palpalis* and *G. m. submorsitans* were caught at varying levels of relative abundance. However *G. p. palpalis* was the dominant *Glossina* spp. caught here. The dominance of *G. p. palpalis* 528 (99.62%) populations over *G. m. submorsitans* 2(0.37%) might be attributed to the hydrographic network ensuring a lush riverine vegetation, which created unsuitable environmental condition for *G. m. submorsitans.* Ambient temperature conditions of 29 to 31.8^{o}C and relative humidity conditions of 58.5 to 69% recorded here (Figure 4.8) were lower compared with conditions obtained in Kainji Lake National Park and Yankari

Game Reserve (where *G. tachinoides* and *G. m. submorsitans* codominate) located in the guinea savannah northwards of Old Oyo. However, in a recent separate study between 2014 and 2015 (unpublished report), a total of 41 *G. p. palpalis* and 30 *G. tachinoides* were collected during the wet season and 486 *G. p. palpalis* and 2 *G. tachinoides* collected during the dry season in some localities in Old Oyo at the proximity of the Old Oyo National Park, indicating the presence of low population of *G. tachinoides* within the zone.

Old Oyo National Park falls within the derived savannah zone, where the vegetation is a transition from the forest to the guinea savannah vegetation zone. The vegetation is typical of the derived savannah type as earlier described (Keay, 1953). The low catches of *G. m. submorsitans* may be due to unsuitable microclimatic conditions. Although according to Omoogun, *et al*., (1989), anthropogenic activities such as lumbering, farming, charcoal stacking and hunting, were responsible for the collapse of *morsitans* belts in Egbe in Kwara state, Nigeria and indeed have been shown to be responsible for the decline and collapse of tsetse belts and disappearance of tsetse flies from various regions of sub-Saharan Africa, these activities were not pronounced in Old Oyo National Park being a protected area.

It is thought that a lot of illegal game poaching activities may be taking place in the park which may be detrimental to fly survival. This may be due to weak legislation and lack of observance or enforcement of extant rules and regulations of protected areas (Faleyimu *et al.,* 2012). Wildlife elimination results in tsetse starvation and death. One of the earliest methods of tsetse control was wild life extermination (de Raadt, 2005). However the method had to be suspended due to the negative effect on the ecosystem. Rather game farming was encouraged due to the economic value derivable from the sale of game meat, hides and skins, and ecotourism (Hamon, 1965). Anthropogenic activities were however observed to be existing outside the national park which

could threaten the survival of tsetse populations at the hinterland. The buffer zone separating the game park from the communities was not protected making it easy to be encroached upon for farming activities or hunting within the perimeter of the park. Of course vegetation clearance for farming activities otherwise referred to in some palance as "agricultural prophylaxis" had been advocated as a means of tsetse control (Hamon, 1965).

5.1.5 Cross River National Park

In Cross River National Park (Table 4.1), only the forest species of tsetse flies consisting of the *fusca* group and some *G. p. palpalis* were caught, constituting 1% of the total flies caught (Figure 4.1). This may be due to the low availability of wild animal hosts as compared with what is obtainable in the savanna region. According to Rogers and Randolph (1986), low availability of hosts in the high rain forest areas may limit *G. palpalis* abundance in areas which are bioclimatically optimal. The *fusca* group flies were less abundant compared with the *palpalis* group probably due to environmental conditions or sampling method used. Tsetse species have been reported to behave differently to different traps (Kuzoe and Schofield, 2005). In Nigeria, there have been very little information on the *fusca* group flies in the recent past probably due to their less economic value. Their disappearance owing to destruction of the great forests, through massive human activies as earlier predicted (Jordan, 1989), remains speculative, due literally to the absence of anthropogenic activities within the National Park. Results of this study showing presence of the *fusca* group flies is fundamental and requires a review of their economic importance owing to the present environmental changes. Similar studies showing the presence of the *fusca* species in the Niger delta area of Nigeria has also been described (Ogedegbe *et al*., 2006). Average ambient temperatures recorded in this area were relatively low, and relative humidity conditions were high reaching precipitation levels at some instances (Figure 4.7). Tsetse

populations caught were generally low in all sites visited probably due to wide dispersal of flies. Similar low catches (5%) of *Glossina* species among other bitting flies were recorded by Oku *et al*., (2011), in Rhoko forest in AKamkpa Local Government Area, Cross River State. It has been established that the rain forest region maintains relatively steady temperature and relative humidity conditions through out the year (Wama *et al*., 2018). This characteristic of the rain forest prevents localization of tsetse populations, resulting in wide dispersal of tsetse flies through out the region as indicated by the low tsetse catches during the study period. In addition, low tsetse catches made may be due to effect of local rains. Whenever it rained, tsetses were not caught probably due to high humidity conditions, which possibly depressed fly catches. Similar studies showing that rains depress tsetse catches have earlier been described (Muhigwa *et al*., 1998).

In this study, the use of traps for tsetse sampling in the rain forest region was not very effective probably due to less attractive properties of traps to the forest flies. Forest flies seemed to be less attracted by visual objects probably due to the high canopy associated with the region. It appears, less light penetration was allowed to enable traps to be sighted by flies. Even though tsetse flies have been reported to be positively phototactic below about 30°C (Popham and Vickers, 1979), very few of such flies came to traps, prompting the use of acetone vapour to remedy the situation, since odours enhance trap catches (Cheke *et al*., 1988). Host location in riverine and forest flies is mainly by olfactory means (Cheke *et al*., 1988). In the savanna flies however, host location is by visual means though flies can move upwind in the direction of odours even when the hosts are hidden (Vale, 1978 as cited by Onyiah, 1980b).

5.1.6 Ijah Gwari

Results from Ijah Gwari (Table 4.1) showed that the tsetse fly *G. p. palpalis* was the only *Glossina* species caught during the sampling constituting 1% of the total flies caught in all the locations

sampled (Figure 4.1). Similar reports of the composition of catches of *G. p. palpalis* solely as the dominant species in this locality have been described (Offor and Nlumanze, 1986; Christopher *et al.*, 2015). This may be due to the massive encroachments into the fly's habitat due to extensive anthropogenic activities, going on in the sampled location owing to its proximity to the Federal Capital Territory (FCT). Previous studies (Falana, 1974; Riordan, 1974) showed very high populations of *Glossina* species present within the FCT. For instance, Falana, (1974) captured a total of 7,850 *G. p. palpalis* at Gimi between 1971 and 1973. He also captured 93,700 and 2,260,000 *G. longipalpis* at Chizako and Gwacipi, all within the FCT. Furthermore, between 1973 and 1983, Offor and Nlumanze (1986) recorded a total of 67,708 females, 101,266 males and 280,902 puparia of *G. p. palpalis* and 1,023 females, 3,243 males and 200 puparia of *G. longipalpis* in 1974, 1975 and 1978. The situation is no longer the same due to environmental changes that have taken place in the FCT over the years. It seems most likely that the *Morsitans* group flies represented by *G. longipalpis* which used to be present in this locality might no longer be available for capture and the relative abundance of *G. p. palpalis* presently existing has declined tremendously. The *Morsitans* group flies appear to be most sensitive to environmental changes which impact negatively on their populations. Nash (1948), had reported that *Glossina morsitans* would not be found where human population densities exceed 40 per square kilometer. The current census figure for FCT is put at approximately 1.4million people (NPC, 2006). With the high influx of people into FCT, this figure far exceeds the limit for the existence of *G. morsitans* flies. While increase in anthropogenic activities such as farming, lumbering, hunting, and industrialization due to increase in human population in the FCT, may have had a negative impact on *G. morsitans* flies, *G. p. palpalis* has been known to be able to adjust quickly to environmental changes and have been found to exist in peridomestic situations (Ahmed, 2004). Tsetse distribution in the FCT which is

now restricted to the relic forest following the destruction of savanna vegetation, previously inhabited by the *Morsitans* group flies, probably still maintains small populations of diverse species of small wild animal hosts that are preferred by the riverine group flies from which they derive their means of livelihood through their opportunistic feeding habits as earlier described (Clausen *et al*., 1998). Farmers that cultivate on farm lands at the bank of the river may as well be sources of blood meal to the flies. It was observed in this study that nomadic pastoralists that are permanently settled in the proximity of the sampled location often graze their cattle and other livestock close to the banks of the rivers inhabited by the *Glossina* species. These flies could take advantage of the presence of these domestic animals to obtain blood meals to meet their nutritional needs in Ijah Gwari. The lush vegetation which probably used to persist through out the year possibly being sustained by the permanent river, may no longer be providing adequate shelter to the wild animal hosts both terrestrial and aborreal and unable to maintain the internal meliu typical of the forest vegetation as done previously. The present *G. p. palpalis* population would probably have had to adjust its breeding, resting and feeding habits in order to survive in the changing environment. Presently, capture of *G. p. palpalis* as the only *Glossina* species existing within Ijah Gwari following the decline of the other *Morsitans* group flies previously recorded in the FCT has been corroborated by Ajuwan *et al*., (2017). This is even the case in the study by Bourn (1988), where the impact of environmental changes on tserse distribution in Nigeria had been described.

From the foregoing, Chi - square (χ^2) analysis showed significant difference χ^2 (4) = 1375.417, $p < 0.001$ in the relative abundance of *Glossina* spp. per sampled location. Pairwise comparison of the composition and relative abundance of *Glossina* populations in sampled locations, shows that whereas significant difference exists in catches in Yankari Game Reserve, Kainji and Old Oyo National Parks, there was no significant difference (p = 1.000) in the relative abundance of

Glossina spp. caught between Cross River National Park and Ijah Gwari locations. This may be due to similarity in environmental conditions that prevailed during the study period between Cross River and Ijah Gwari sampled locations.

5.1.7 *Glossina* spp. distribution in the National Parks and Ijah Gwari

Results (Figure 4.3) showed that populations of *Glossina* species were not uniformly distributed, with their distribution varying between vegetations zones. The most widely distributed *Glossina* species were the riverine species, *G. p. palpalis* and *G. tachinoides*. Similar uneven distribution of *Glossina* species in Gashaka Gumti National Park Nigeria has been described (Wama, *et al*., 2018). *Glossina p. palpalis* was caught in Yankari Game Reserve, Old Oyo, Cross River National Parks and Ijah Gwari, while *G. tachinoides* was caught in Yankari Game Reserve and Kainji lake National park only. Although *G. tachinoides* was recorded in some communities outside the Old Oyo National Park in a separate study (unpublished report), it is an indication of the low presence of the species within the zone. The distribution of *G. palpalis* was confined to the hydrographic networks. This may be due to less tolerance to high temperature conditions as found in the northern and southern Guinea savannah vegetation zones. Although some riverine species may occasionally be found in drier conditions as was the case with *G. tachinoides* which were caught in sympatry with *G. submorsitans* in Yankari and Kainji, they have also been reported to exhibit peridomestic behaviours by maintaining close association with urban and suburban settings and agricultural plantations (Ahmed, 2004). The savannah species, *G. m. submorsitans* was restricted to the northern and southern Guinea savanna vegetation zones (where it thrives best under drier conditions) dominated by *isoberlinea doka* and other xerophytic plants, where it is discretely distributed within the *morsitans* belts, while the forest species, *Glossina fusca* was restricted to the forest vegetation zone. Similar pattern of distribution of *Glossina* species found in Nigeria has

long been described (Davies, 1977). Statistical analysis shows significant difference ($p<0.05$) in relative abundance of *Glossina* spp. caught per location except in Cross River National Park and Ijah Gwari locations where catches were not significantly different probably due to similarity in ecological conditions.

5.1.8 *Glossina* spp. distribution in relation to ambient temperature and relative humidity conditions.

Results of tsetse sampling obtained in this study (Figure 4.4-4.8), showed that tsetse flies were unevenly distributed across the sampled locations based on variations in ambient temperature and relative humidity conditions. Whereas *G. m. submorsitans* populations had a more discrete distribution and probably confined to the *G. morsitans* belts where they were caught, the riverine species (*G. p. palpalis* and *G. tachinoides*) had more extensive distributions along the hydrographic networks. *Glossina fusca* populations were however restricted to the forest region only. Even though only four *Glosssina* species (*G. tachinoides, G. m. submorsitans, G. p. palpalis and G. fusca*) were caught during the sampling, in contrast to the 11 species and subspecies described earlier (Baldry 1967), this may be due to the inability to visit all ecological niches during this study. Ogedegbe and Rotimi (2006) for instance recorded *Glossina fusca congolensis, G. nigrofusca nigrofusca, G. caligenia*, *G. pallicera pallicera* and *G. p. palpalis* in the Niger Delta area of the country. It is interesting though to note that despite the low number of *Glossina* species caught, all flies caught represent the three groups which the genus *Glossina* comprises (i.e the *Austenina* or Fusca group, the *Nemorhina* or Palpalis group and the *Glossina* or Morsitans group). This finding is thus in conformity with earlier classification of *Glossina* species present in Nigeria into the three major groups of forest, riverine and savanna flies. It is also interesting that three (*G. tachinoides, G. m. submorsitans* and *G. p. palpalis*) out of the four economically important

Glossina species in Nigeria were caught during this study. Davies (1977) had reported that *G. m. submorsitans, G. longipalpis, G. tachinoides,* and *G. p. palpalis* are the most economically important *Glossina* species in Nigeria due to their ability to transmit HAT and AAT. The distributions of the various *Glossina* species caught in the sampled locations were not uniform as shown by fluctuations in number of catches between trapping points and between sampled locations (Figure 4.4 - 4.8). This supports the observation by Challier (1982), that tsetse species exhibit various patterns of population fluctuations related to local climates and vegetation. Fluctuation in tsetse catches in relation to ambient temperature and relative humidity conditions that prevailed, appeared more frequent in Yankari Game Reserve (Figure 4.4) than in Kainji Lake National Park (Figure 4.5). Similarly, fluctuations were more frequent in Old Oyo (Figure 4.6) than in Cross River National Parks (Figure 4.7). Fly catches did not show apparent fluctuations in Cross River National Park probably due to relatively steady conditions that prevailed through out the period of trapping. Slight fluctions were however observed in Ijah Gwari gallery forest relic (Figure 4.8), possibly due to the infiltration of Guinea savanna conditions due to increasing anthropogenic activities within the sampled location. Onyiah (1978) had observed that a decrease in atmospheric humidity and increase in human activities led to a decline and eventual collapse of a *G. palpalis* population in a forest reserve in Nigeria. All the variations in *Glossina* species distribution observed above may be attributed to several factors chief amongst which might possibly be variations in microclimatic conditions (Appendix VIII), vegetation types, availability of wild animal hosts and possibly other environmental conditions. Results of statistical analysis obtained in this study show that, at the two extreme ends of our study areas (North and South) there was complete inverse relationships between the numbers of *Glossina* spp. captured in relation to climatic conditions. Results showed that in Yankari Game Reserve, there was negative

correlation between the number of *G. m. submorsitans* and *G. tachinoides* caught (Figure 4.9a) and ambient temperature and significant positive correlation (Figure 4.9b) between the number of flies caught and relative humidity. In Cross River National Park, tsetse catches correlated positively with ambient temperature (Figure 4.12a) and negatively with relative humidity (Figure 4.12b). In Yankari Game Reserve at the Northern Guinea savanna vegetation zone, the number of tsetse flies caught in relation to ambient temperature and relative humidity conditions did not show any significant difference, there was significant difference in Cross River National Park located at the Rain forest vegetation zone in relation to tsetse catches. Further results showed that the number of catches of tsetse flies at Yankari was almost twice as many as all the other locations put together. This is in line with earlier observation that at extremely dry weather conditions as found in the Northern Guinea savanna, the distribution of tsetse flies becomes concentrated along river courses where humidity is high to avoid desiccation and subsequent death from harsh weather conditions at the hinterland. In Kainji Lake National Park, number of tsetse flies caught correlated negatively (Figure 4.10a) with ambient temperature, however when species were considered separately, number of *G. tachinoides* marginally correlated positively with ambient temperature (Figure 4.10b). This may be due to a broader tolerance to higher temperature ranges by *G. tachinoides* as opined by Rogers and Randolph (1986*).* Catches of *G. m. submorsitans* and *G. tachinoides* however correlated negatively (Figure 4.10c) with relative humidity in Kainji Lake National Park. This result is not unexpected due to the location of Kainji Lake National Park which is situated in the southern sub-humid Guinea savanna vegetation zone, as a result tsetse distribution would be less concentrated along river courses as compared to Yankari Game Reserve. The low number of tsetse catches in Kainji may therefore be reflective of the microclimatic conditions recorded. In Old Oyo National Park there was significant difference between the number of *G. p. palpalis*

caught and the climatic conditions, with *G. p. palpalis* catches correlating positively with both ambient temperature (Figure 4.11a) and relative humidity (Figure 4.11b). It is interesting to note that both temperature and relative humidity were favourable to *G. p. palpalis* and perhaps that may be responsible for the higher apparent density in fly populations than all other sampled locations. In Cross River National Park however, fly catches correlated positively (Figure 4.12a) with ambient temperature and negatively (Figure 4.12b) with relative humidity. This may be responsible for the low fly catches recorded here as a result of wide dispersal of flies within the zone. In Ijah Gwari, tsetse catches correlated negatively (Figure 4.13a) with temperature and positively (Figure 4.13b) with relative humidity. Even though Ijah Gwari is a forest relic, it is located within the northern guinea savanna vegetation zone, with climatic conditions peculiar with the northern guinea savanna infiltrating. The low number of flies recorded here may not necessarilly be due to high dispersal of tsetse flies as it were, in a typical forest region, but might be due to low population size brought about by anthropogenic factors.

From the foregoing, it is pertinent to note that variations in microclimatic conditions that prevailed in the study areas, may be responsible in part for the uneven distribution and the occurence of some *Glossina* species in some parks and their absence in others. For instance, whereas *G. m. submorsitans* was caught only in the savanna vegetations of Yankari and Kainji Lake National Parks, it was not found in the forest zone. Similarly, *G. tachinoides*, though a riverine species like other members of the *palpalis* group tsetse, exists in much drier habitats than *G. palpalis* and does not occur in the forest zone (Rogers and Randolph, 1986). In contrast, *G. fusca* being a forest fly caught in the forests of Cross River National Park in the rain forest vegetation zone, was not found in the northern Guinea savanna vegetation zones. It was observed during the survey that there was a steady decrease in ambient temperature from a range of 31- 35.5^{o}C in Yankari Game Reserve,

in northern guinea savanna geo-ecological zone to a range of 24.5 - 29^{o}C in Cross River National Park, in rain forest geo-ecological zone and a corresponding increase in relative humidity conditions from a range of 28 - 46.0% in Yankari Game Reserve to a range of 76.5 - 100% in Cross River National Park (Appendix VIII). This may be due to altitudinal change between sampling locations as shown in Appendix III – VII. While high altitudes between 230 and 530 meters above sea level obtained in the northern Guinea savanna would probably had ensured low relative humidity due to the low level of the water table, low altitudes between 123 and 151meters above sea level obtained in the rainforest region would probably had ensured high humidity conditions due to high level of water table in addition to the region's proximity to the coast (Atlantic Ocean). These variations in microclimatic conditions might possibly be responsible in part for uneven distribution of *Glossina* species in the sampled locations. Besides, the change in vegetation type from a sparsely dry woody savannah in the north to luxuriant forest in the south as earlier described (Keay, 1953), might be a factor in the flies' distribution. Statistical analyses revealed no significant correlations between tsetse catches and ambient temperature and relative humidity conditions in most of the trapping locations visited. This might possibly suggest that the distribution of flies across the sampled locations were probably influenced by other environmental factors than climatic conditions alone as described above. Gondwe *et al.,* (2009), observed that the uneven distribution of *G. m. morsitans* inside Nkhotakota Game Reserve in Malawi is attributed to the uneven distribution of wildlife the main hosts of tsetse flies. It would be most reasonable therefore to conclude that tsetse abundance and distribution in the sampled locations were most possibly influenced by the relative abundance and distribution of wild animal hosts, vegetation types and wildlife conservation management practices in addition to ambient temperature and relative humidity conditions. It is interesting however, to note the intra and inter species variations

in behaviour of *Glossina* species caught in this study in relation to these biotic and abiotic environmental factors, a crucial information for area-wide tsetse population management with particular reference to the sterile insect technique (SIT).

5.2 Apparent Density of Tsetse Fly Populations in the National Parks and Ijah Gwari

Results in Figure 4.14 showed variations in apparent density between fly populations in national parks and Ijah Gwari sampled locations. From the results obtained, it could be deduced that the highest apparent density of fly populations was recorded in Old Oyo National Park, followed by Yankari Game Reserve, Kainji Lake, Cross river National Parks, and the least was Ijah Gwari. Old Oyo National Park spans an area 2,512Km^2 just slightly bigger than Yankari Game Reserve. The park is ranked the first in terms of apparent density of tsetse populations. This may be due to the presence of gallery forests and dense vegetation of tall shade trees along the banks of rivers which provide conducive climatic conditions for the flies as well as shelter for their wild animal hosts. The gallery forests are kept lush throughout the year being watered by the perenial River Ogun. The small size and plenty of grazing for the wild animals may be responsible for the high population of the game throughout the year. This high population of game possibly ensures sustainance of the high density of tsetse populations in the Park. The extremely low number of *morsitans* group flies caught in Old Oyo may be attributed to unsuitable climatic conditions for its existence and possibly low population of large bovids, the preferred hosts of the *morsitans* flies. *Glossina m. submorsitans* is found in open woodland and woodland savannah with mean annual rainfall between 800-1350mm and in which the dominant tree is *Isoberlinea doka* or its close relatives, with plenty of game animals living in the area (Rogers and Randolph, 2006). This is contrary to the mean annual rainfall of 1440-1780mm earlier described for the derived savanna zone (Davies, 1977). Anthropogenic activities such as lumbering, vegetation clearance for farming

activites and hunting, creat unfavourable conditions for the survival of tsetse flies. These anthropogenic activities were absent in Old Oyo National Park, therefore the low catch of *G. m. submorsitans* during this study may not be attributed to anthropogenic activities. Although illegal hunting activities might possibly be going on unabated due to poor legislation and enforcement, it may lead to gradual decimation of the wild animal hosts which are main sources of blood meals for the *Morsitans* group flies. The riverine group flies have been reported to be less affected by such activities. It thus appear that the park may presently be sustaining populations of small game which are preferred hosts of *G. p. palpalis* populations in the lush riparian vegetation as well as providing conducive resting, hunting and breeding sites for the *G. p. palpalis* populations which abound the park.

In Yankari Game Reserve (Appendix XII), apparent density in fly populations was 15.98 F/T/D. This is contrary to apparent density of 128 F/T/D and 101 F/T/D obtaind in 2012 as reported by Isaac *et al.,* (2016) during a dry and wet season's tsetse survey in Yankari Game Reserve reflecting a seasonal trend. This discrepancy in results obtained may be due to differences in trapping points and environmental conditions during tsetse samplings. Other factors which were probably responsible for the high apparent density in fly populations observed in Yankari, might be local concentration of fly populations, recruitment of young flies into existing populations, timing of tsetse survey, pupal duration, vegetation type, *Glossina* species, ambient temperature, relative humidity conditions, and availability of wild animal hosts and activity patterns of flies. Local concentration of flies observed around riverine vegetations might be responsible for the high apparent density of tsetse populations in Yankari Game Reserve at the time of sampling. The high number of teneral flies in the fly catches (Table 4.2) might be due to the recruitment of young flies into the population from proximal breeding sites during the peak breeding activity. This finding is

in consonance with observations of Turner (1987), suggesting that preponderance of teneral flies indicates an active population. It is interesting that the timing of tsetse survey in this study (February to March), probably coincided with the peak of pupal emergence which has been reported to be around the middle of dry season (Challier, 1982). It has been established that pupal duration decreases with increase in temperature (Jaenson, 1981). This suggests that a decrease in pupal duration might have ensured a faster turnover in fly populations resulting in increase in apparent density. Apparent density was however low within the forest vegetation zone as compared to what was obtained at the Northern Guinea savanna region probably due to differences in vegetation type and micro climatic conditions (Appendix XII). Whereas apparent density was 4.0 fly/trap/day in Ijah Gwari gallery forest relic, it was 1.0 fly/trap/day in Cross River National Park. This result is consistent with the low apparent density obtained in 1999 by Ogedegbe and Rotimi (2006). The forest region is known to be characterised by high relative humidity and constant temperature conditions almost throughout the year. This might be responsible for the low tsetse catches during our survey as a result of wide dispersal of tsetse flies within the zone. Climate is considered a very important factor determining the abundance of *Glossina* species particularly in the northern guinea savanna regions (Rogers and Randolph, 1986). It has been established that during extremely dry weather conditions e.g during the dry season, most fodder from which the game obtain their feed at the hinterland dries up forcing the animals to retreat from the hinterland and move closer to river courses for fresh grazing and for their water requirements. The distribution of flies at this time also becomes localized around water bodies. Both tsetse and wild animal hosts are brought into intimate contact, as flies obtain their blood meals with minimal efforts. This might be responsible for the high apparent density of flies recorded at Yankari during the survey (Figure

4.2). It is possible that during rainy season, relative humidity might increase impacting negatively on the apparent density of flies due to extensive dispersal of fly populations.

Activities of host animals could influence tsetse behaviour and hence their availability. Yankari Game Reserve with an area 2,244Km^2 is ranked fifth in terms of land mass. However, the Game Reserve has a high concentration of game, and is considered to be the richest in fauna resources than any other national parks in Nigeria. High apparent density of tsetse populations recorded may not be unconnected with the high concentration and activities of game at the park during the survey.

Tsetse fly activities have been known to be diurnal, crepurscular or nocturnal in nature, and this could be influenced by activities of their animal hosts. Members of the family *Hippopotamidae* (*Hippopotamus amphibious*) for instance, feed only at night and would probably be visited by flies that have nocturnal feeding behaviour. Tenabe (1983), studied the diurnal activities of *G. p. palpalis* within the Guinea savannah vegetation zone, in Niger State, Northern Nigeria, and concluded that the fly activities were both diurnal and crepurscular suggesting some strain differences in the *G. p. palpalis* populations caught.

Further results in this study showed that the apparent density of tsetse caught did not depend on the number of traps used for sampling (Appendix XI). This might be due to lack of uniformity in the number of traps deployed at the parks due to differences in terrain. Trap performance depends on its siting and visibility against background vegetation. Tsetse flies are attracted to traps from distances on short and long term basis. It has been suggested that for effective performance, traps should be sitted in such a manner that their action within their ambits would be within the radius

of at least 50 meters (Laird, 1977). This underscores the importance of trap positioning during tsetse samplings.

Kainji Lake National Park, with an area 5,382Km^2, is ranked second out of all the National Parks in terms of land mass. Results (Figure 4.14) showed that the park has the third highest apparent density (15.23fly/trap/day) of tsetse populations (Appendix XIII). Though, about twice the size of Yankari, Kainji is a tourist destination in terms of number of game present. The high tsetse density recorded may be partly due to abundant game and localization of tsetse populations along riparian vegetations banking the river system and its tributaries. Since both Kainji and Yankari fall within the same Guinea savannah zone, the high density of *G. tachinoides* and *G. m. submorsitans* caught in the park is not expected to be different except for the larger land mass Kainji enjoys.

Cross River National Park spans an area about 4,000Km^2 and is ranked third in terms of land mass. *G. p. palpalis* and *G. fusca* caught here (Figure 4.14), have a combined apparent density of 1.0 fly/trap/day. The rain forest region is noted for uniformity of climatic conditions throughout the year. Low temperature and high humidity conditions associated with this region prevents any localization of tsetse populations. This may be responsible for the wide dispersal and the low apparent density of *Glossina* populations caught in the region during the study. Seven out of the 11 *Glossina* species recorded in Nigeria occur in this region probably because the forest areas are noted for high biodiversity, but the prevailing environmental conditions during tsetse survey may be responsible for the low fly density recorded during this study due to wide dispersal of flies.

In Ija Gwari, results (Figure 4.14) showed that the apparent density of *G. p. palpalis* population (4.0fly/trap/day) was comparatively low. This may be attributed to a relatively wide dispersal of the flies within the sampled location. Ijah Gwari is a gallery forest relic within a Guinea savannah

zone, with a lot of farming activisties at the bank of the river. Partial localization of flies may be due to relatively low ambient temperatures, and the presence of small wild animal hosts including primates which abound the forest. The large vertebrate mammalian hosts might have been decimated due to illegal pouching. The massive encroachment into the flies' habitat may be due to natural or anthropogenic factors since the area is contiguous with the Federal Capital Territory (FTC) Abuja. The absence of the *morsitans* flies is to be expected due to a combination of factors including vegetation clearance, unfavourable ambient temperature and relative humidity conditions, increase in anthropogenic activities, lack of large game, illegal encroachment of the forest for farming activities, lumbering and hunting.

5.3 Sex Ratio

Results (Figure 4.15) showed differences in the proportion of male and female tsetse flies caught during the survey. Variations in male and female ratio occured between species and between species and vegetation type from which they were caught. In Yankari Game Reserve, whereas 70% males and 30% females constituted the catch in *G. tachinoides* giving a sex ratio of 2:1 in favour of males, ratio was 1: 1.5 in favour of females in *G. m. submorsitans* with 55% of the catches. In *G. p. palpalis* however, sex ratio was 1:1 a piece. This is contrary to 20% male and 80% female *G. tachinoides* and 19% male and 79% female *G. m. submorsitans* with sex ratio consistently in favour of females recorded in 2012 by Isaac *et al*., (2016). These discrepancies may be due to differences in environmental conditions and or sampling techniques at the time of tsetse survey. Earlier records show that sex ratio in trap catches were always in favour of females (FAO, 1982). This was attributed to a claim that female flies live longer than males (Challier and Gouteur, 1980; Jaenson, 1981). However, with time this concept seems no longer tenable in view of the opinions expressed by several workers who have observed distortions to sex ratio in tsetse flies. According

to Okiwelu (1982), at emergence, sex ratio in field populations is always thought to be unity but the ratio becomes distorted depending on the sampling method used. The preponderance of male flies in *G. tachinoides* samples in Yankari may be attributed to the search for mating partners. It has been established that cuticular hydrocarbons (pheromones), play a major role in the reproductive behavior of male and female tsetse flies. Pheromones are expressed in female flies by virtue of which male flies are attracted for mating expedition (Offor *et al*., 1981). Recent studies have however, shown that male and female flies respond differently to different colours. The dominance of male over female flies in *G. tachinoides* catches may be attributed to their strong affinity to blue colour in biconical traps. This view corroborates observations by Aksoy *et al*., (2014) suggesting that attraction of male flies to blue colour is governed by a gene. It has also been suggested that the preponderance of males in trap catches may be due to trap bias (Rogers and Randolph, 1986). Female flies may have visited traps probably because traps were mistaken for larviposition sites as opined by Saunders (1964). A good proportion of females may have also visited traps in search of blood meals probably because they were hungry in conformity with FAO, (1982). That notwithstanding, sex ratio in *G. p. palpalis* at Yankari gives representative ratio of 1:1 males to females which closely resembles natural field situations.

In Kainji Lake National Park, sex ratio in *G. tachinoides* was in favour of males, while in *G. m. submorsitans*, ratio was in favour of females (Figure 4.15). This is similar to results obtained in Yankari in the Northern Guinea savannah. This result is expected since both Yankari and Kainji Lake National Parks are located within the Guinea savannah vegetation zone. In Old Oyo National Park, sex ratio in *G. p. palpalis* was in favour of males, whereas in *G. m. submorsitans* sex ratio was unity (Figure 4.15). Ordinarily more female flies in a population could show a hungry population, or a population in search of larviposition sites since females need more blood meal for

the maturation of their follicles. This is not the case here, but rather more males seem to signify a population in search of mating partners.

In Cross River National Park, sex ratio in *G. p. palpalis* was 1.5:1 in favour of males, whereas in *G. fusca* ratio was 0:1 in favour of females (Figure 4.15). This may be due to the reason adduced above, where *G. p. palpalis* males probably visited traps in search of mating partners In Ijah Gwari, sex ratio in *G. p. palpalis* caught was unity (Figure 4.15). Ijah Gwari is within the Northern Guinea savanna vegetation zone. The sex ratio obtained in *G. p. palpalis* seems to agree with the ratio in other locations (Yankari) within the zone.

It seems therefore that the general believe that hand net catches seem to have more representative segment of male flies, while trap catches give ratio in favour of females (Pollock, 1982), may no longer be tenable. Results in this study showing variation in sex ratio from one *Glossina* species to another (Figure 4.15) and from one vegetation zone to another are therefore consistent with works of earlier workers suggesting that, trap catches in tsetse flies depend on movement of flies, timing of activity, nutritional status, trap efficiency, climatic conditions, host availability or a combination of some or all (Randolph *et al.,* 1991). The above foregoings describe sex ratio in natural conditions, which however differ from what is obtainable in the laboratory. Sex ratio in laboratory colonies of *G. m. submorsitans* for instance gives ratio in excess of females suggesting the occurrence of genetic aberration in laboratory colonies (Rawlings and Maudlin, 1984). The biased sex ratio in favour of females in *G. m. submorsitans* may be due to some heritable factors that have to do with presence of supernumerary sex chromosomes (Gooding and Krafsur, 2005). Such biased sex ratios are adverse and not acceptable in breeding stocks meant for SIT. The possibility of raising breeding stocks which would yield male biased sex ratios needs to be explored to enhance the efficacy of the SIT program.

5.4 Teneral and Non-Teneral Flies

Results obtained (Table 4.2), showed very high levels of tenerity 79% (1500) in the total fly collection. This may be due to the recruitment of young emergents into the population at the period of fly collection which seemed to have coincided with the peak of flies breeding activities. The tenerity phenomenon implies that flies which have not taken a single blood meal since emergence, have higher chances of being infected and transmitting trypanosomes upon taking an infected blood meal than older non-teneral ones (Haines, 2013). This previledge however reduces as the fly becomes older leading to refractoriness in older flies (Oteino *et al*., 1983).

5.5 Hunger Staging

Results (Table 4.3) obtained showed that, most of the flies captured were hungry. The high preponderance of hungry flies captured in Yankari Game Reserve as compared to other locations may be due to prevailing high ambient temperature conditions in the game reserve at the time of sampling. It has been established that rate of blood meal digestion increases with increase in temperature (Terblanche *et al.,* 2008). It thus appear that increase in temperature would have led to a shortened hunger cycle resulting in high number of hungry flies in our catches. This is consistent with observation of other workers (Terblanche *et al.,* 2009). Besides, it was also observed that more female flies were found to be hungry than the males. Similar observations in *Glossina tachinoides* have been described by Madubunyi (1989). The higher proportion of hungry females in our catches may be due to the attraction of this category of flies to traps which are often being mistaken for animal hosts, to meet the need of more blood meals by the female flies for the maturation of their follicules.

5.6 Molecular Studies

5.6.1 Amplifications

From the results obtained (Table 4.4), out of 391 surviving flies subjected to polymerase chain reactions, amplifications were obtained from only 66 (17%) flies while others did not amplify (Figures 16a and b). Reasons for the lack of amplifications from the other flies subjected to PCR is not known but sequencing error during hybridation has been observed to cause failure (Dyer *et al*., 2008). Different methods of DNA extraction could also give different yields of PCR products as the quality of PCR products some times depends on the extraction method used. Polymerase chain reaction is known to be highly sensitive and specific and its application to population studies has increased our understanding on population dynamics of tsetse flies and other disease vectors (Dyer *et al.,* 2009; Christopher *et al*., 2015). The sensitivity of the method may reveal the extent to which morphological techniques could provide under estimates of ecological data as described in this study.

5.6.2 Molecular species identification

Results (Figure 4.17) of sequence similarity searches on the Genbank database and phylogenic reconstruction of sequences, showed that closely related species of flies clustered together, whereas distant relations clustered separately giving a total of 60 (94%) flies with matching identities out of 66 flies analysed. Results showed that accurate identification of natural populations of *Glossina* species in Nigeria national parks using CO1 mtDNA gene was successfully achieved. Similar combination of sample DNA and database sequences were used to generate neighbour joining tree for the identification of blow flies (*Cordylobia anthropophaga*), another insect of medical, veterinary and forensic importance (Ogo *et al*., 2012). Several reports have shown that this tool can be used for species identification with accuracy (Dyer *et al.,* 2008;

2011; Christopher *et al.,* 2015; Mauricio and Juan, 2016). The 6 unidentified flies due to mismatch of their sequencies in the database suggest that CO1 mtDNA marker could be used for dual purposes, namely, species identification and for molecular evolutionary genetic analyses. The unidentified *Glossina* species for which no information on their COI sequences is available in the Genbank may require other markers in additional to microsatellites for possible accurate identification.

5.6.3 Characteristics of CO1 sequences of *Glossina* spp. in sampled locations

Results obtained (Table 4.5) showed that the GC content was significantly higher than the AT content in *G. m. submorsitans* populations in Yankari Game Reserve. In *G. tachinoides* however, even though the AT content was higher than the GC, the difference was not statistically significant. This discrepancy may be due to differences in the distribution patterns of *G. m. submorsitans* and *tachinoides* populations in the game reserve. In Yankari Game Reserve, *G. m. submorsitans* seems to have a more restricted distribution confined to *morsitans* belt 29, while *G. tachinoides* distribution appears less restricted due probably to the extensive hydrographic network which forms the major habitat of *G. tachinoides* within the game reserve. This may have resulted in extensive migration of flies resulting in random mating forming a more diffused population as indicated by the GC/AT content. The GC/AT content did not show any significant difference in *G. m. submorsitans*, *G. tachinoides,* and *G. p. palpalis* populations found in Kainji Lake, Old Oyo, and Cross River National Parks. Similarly no significant difference was found between GC and AT content in *G. p. palpalis* in Ijah Gwari, even though AT content was higher than GC content. All these may be due to homogenisation of the populations due to random mating within the southernmost populations as a result of extensive migrations within their respective vegetation zones. It is thought that the detected variation in banding patterns (Figure 4.18a-g) in sequences,

shows that *Glossina* species could be differentiated based on differences in sequence banding patterns. Similar use of banding patterns for identification of tsetse flies based on randomly amplified polymorphic DNA (RAPD) approach has earlier been described (Hide *et al*., 1991).

5.6.4 Haplotype diversity (Genetic variability)

Results (Table 4.6) of analysis of composite haplotypes showed that, tsetse populations in the various sampled locations were genetically structured and that structuring in the fly populations varied between sampled locations and between species. The frequency of composite haplotypes was from one private haplotype to as many as 4 shared haplotypes as shown in Figures 4.19a-g. In *G. m. submorsitans* population in Yankari Game Reserve (Figure 4.19a), two private haplotypes were detected. Haplotype diversity measures the rate of evolutionary change in a population. From the results obtained, it is only most reasonable to state that the rate of evolutionary change in *G. m. submorsitans* population in Yankari was higher than other *Glossina* populations sampled in other locations in this study based on the number of private haplotypes detected in this study. Private haplotypes detected may be indication of new adaptations the fly populations have had to undergo in order to survive in their environments. Similar observation of private haplotypes being new adaptations flies have to undergo in order to survive in their environments has long been described (Sjöstrand *et al*., 2014). Long term environmental changes such as the long period of draught experienced in Yankari Game Reserve and the extensive anthropogenic activities going on in Ijah Gwari within the Nigeria's Federal Capital territory may lead to disappearance of native species and possibly introduction of alien ones.

5.6.5 Nucleotide diversity (Genetic variation)

Results (Table 4.7) of analysis of nucleotide diversity which is measured as frequency of composite nucleotides sequences have in common, showed that variation in *G. m. submorsitans*

population in Yankari Game Reserve was higher (53.2%) than in *G. m. submorsitans* (47.2%) populations in Kainji Lake National Park. The high level variation in composite nucleotides in *G. m. submorsitans* populations in Yankari Game Reserve is probably not unconnected with the more restricted nature of its distribution in the park. Unlike *G. m. submorsitans* population in Yankari, the population in Kainji Lake appeared to be more extensively distributed probably due to the size of the *morsitans* belt in Kainji Lake National Park which is twice as large as the size of the belt in Yankari, where the flies are more restricted in their distribution. Likewise in *G. tachinoides* populations, genetic variation was higher in Yankari Game Reserve (44.3%) than in Kainji Lake National Park (43.3%). This low level variation in composite nucleotide frequency obtained in *G. tachinoides* populations in Kainji may be due to extensive migrations of flies forming more homogeneous populations, the result describes the wider dispersal and distribution of *G. tachinoides* populations relative to the dense hydrographic network in the park. Despite the higher apparent density recorded in *G. tachinoides* populations, low level variation in composite nucleotides was detected in this fly population as compared with high level variations obtained in *G. m. submorsitans* population with low apparent density.

These differences in frequency of composite nucleotides may be the underlying factors responsible for observed differences in behavior and indeed distribution patterns of *G. m. submorsitans* and *G. tachinoides* caught in both Yankari Game Reserve and Kainji Lake National Park, given that the two parks have differences in microclimatic and other environmental conditions. This study has revealed sub-structuring in tsetse fly populations caught in Yankari Game Reserve and Kainji Lake National Park and corroborates earlier observation by Krafsur, (2009) suggesting that tsetse populations are genetically structured and that variations in structuring exist within and between species.

In *G. p. palpalis* populations in Old Oyo National Park (Table 4.7), low genetic variation (36.8%) in composite nucleotide frequency was recorded. This suggests that *G. p. palpalis* populations caught in Old Oyo National Park may be extensively dispersed possibly due to a much denser hydrographic network resulting in more homogenisation of the populations throughout the range of its distribution bringing about the low level of diversity. Similar low level genetic variation in *G. p. gambiensis*, a major vector of sleeping sickness in West Africa, has been described in populations in continental locations due to wide dispersal than Island populations in Coastal Guinea (Solano *et al.*, 2000; Bouyer *et al*., 2007b). *Glossina p. palpalis* populations caught in Cross River National Park (Table 4.7), however show marginally high level (50.6%) genetic variation. This is not unexpected due to the number of locations where flies were collected from during the sampling. Samples were collected from three different locations ostensibly to beef up the number of tsetse catches due to poor performance of the biconical trap in the forest region. The apparent density in the fly population was 1fly/trap/day (Appendix XII). Flies were collected from Akamkpa, Bamba, and Butateng all at various distances apart and pooled together for ease of analysis. This may have brought about the marginally high level composite nucleotide frequency obtained in Cross River National Park. In Ijah Gwari (Table 4.7), nucleotide diversity was 40.8% showing relatively low level variation in composite nucleotide frequency as compared to what was obtained in Cross River. This result is not unexpected in view of the low apparent density of *G. p. palpalis* population in Ijah Gwari. The apparent density of *G. p. palpalis* population recorded in Ijah Gwari was 4flies /trap /day (Appendix XIII). This low apparent density in the fly population may be attributed to demographic pressure as this *Glossina* species is known to be capable of extensive dispersal especially during wet seasons. The low apparent density of tsetse populations

in Ijah Gwari may in addition be due to tremendous encroachment on the fly's habitat by anthropogenic activities owing to its proximity to Abuja, the Nigeria Federal Capital.

From the foregoing, it would appear that there is a more restricted distribution of the savanna fly *G. m. submorsitans* population in Yankari Game Reserve than it is obtained in *G. m. submorsitans* population in Kainji Lake National Park. In view of the above, control of *G. m. submorsitans* population using SIT may be less challenging in Yankari Game Reserve than controlling *G. m. submorsitans* population in Kainji Lake National Park which appears to have less restricted migration. Further studies are however required using microsatellites to determine the level of isolation (gene flow), population size and delineation of these different fly populations, that could aid the decision on whether control would be implemented selectively or simultaneously in the context of Area-wide tsetse control following the principle of the 'rolling carpet' being advocated by PATTEC. The riverine group flies, *G. p. palpalis* and *G. tachinoides* populations analysed in this study that showed low nucleotide diversity appear to have less restricted migrations compared to the savanna fly *G. m. submorsitans* in Yankari and may therefore pose some challenges for control using SIT. This also needs to be confirmed using other genetic markers including microsatellites.

5.7 Genetic Differentiation in *Glossina* spp. in Sampled Locations

5.7.1 *G. m. submorsitans* populations in Yankari Game Reserve

Results of analysis of genetic differentiations measured as the frequency of nucleotide substitutions showed high level genetic differentiation (F_{ST}=0.7643) within *G. m. submorsitans* populations in Yankari Game Reserve (Table 4.8). A similar observation in East, West and Southern Africa, suggest that *G. morsitans sl* may be associated with high levels genetic

differentiation at mitochondrial and microsatellite loci (Wohlford, *et al*., 1999; Krafsur *et al*., 2000b; Krafsur *et al*., 2001; Krafsur and Endsley, 2002). The high level genetic differentiation in *G. m. submorsitans* populations in Yankari Game Reserve supports result of ecological work in this study suggesting that the restricted distribution of *G. m. submorsitans* population within the park may be responsible for the high level genetic differentiation. Besides, the long period of dry season lasting longer than rainy season, may have modified the fly's behavior in Yankari.

5.7.2 *G. m. submorsitans* populations in Kainji Lake National Park

Results of analysis showed high level (F_{ST}=0.6508) genetic differentiation in *G. m. submorsitans* populations in Kainji Lake National Park (Table 4.8). This shows a lower level of differentiation compared to the *G. m. submorsitans* population in Yankari Game Reserve. This is not unexpected bearing in mind that the two populations are siblings of the same *Glossina* spp. seperated through habitat fragmentation. Lower levels of genetic differentiation displayed by *G. m. submorsitans* in Kainji Lake National Park, may be due to more extensive distribution than their counterparts in Yankari Game Reserve.

5.7.3 *G. tachinoides* populations in Yankari Game Reserve

Results of analysis showed low level genetic differentiation (F_{ST}=0.3427) in *G. tachinoides* populations in Yankari Game Reserve (Table 4.8). Similar studies showing low and infact no genetic differentiation in *G. tachinoides* have been reported by other workers (Kone, 2010; Adam *et al*., 2014). It thus appear from the analysis that the *G. tachinoides* populations in Yankari Game Reserve were more homogeneous compared with *G. m. submorsitans* populations, suggesting that *G. tachinoides* populations possibly have more wider distribution than *G. m. submorsitans* populations which are rather more restricted in their distribution.

5.7.4 *G. tachinoides* populations in Kainji Lake National Park

In Kainji Lake National Park (Table 4.8) results of analysis of genetic differentiation showed low levels genetic differentiation (F_{ST}=0.3025) in *G. tachinoides* populations. Low genetic differentiation in *G. tachinoides* in Kainji Lake National Park as compared to what was obtained in *G. tachinoides* populations in Yankari Game Reserve at the northern Guinea savanna vegetation zone, was not unexpected due to differences in environmental conditions between Yankari Game Reserve and Kainji Lake National Park. This low genetic differentiation in *G. tachinoides* in Kainji Lake National Park may be due to a more homogeneous population brought about possibly by random mating.

5.7.5 *G. p. palpalis* populations in Old Oyo National Park

Results of analysis showed low (F_{ST}=0.2805) genetic differentiation in *G. p. palpalis* populations in Old Oyo National Park (Table 4.8). The extensive migrations of members of *palpalis* populations during dispersal may have resulted in more homogeneous populations resulting in the observed low level differentiation. Low level genetic differentiation has been observed to be associated with the *palpalis* group flies (Adam *et al*., 2014). This may perhaps explain why despite high relative abundance and apparent density of *G. p. palpalis* obtained in this study in Old Oyo National Park, yet low level genetic differentiation was recorded. Similarly, low genetic differentiation (F_{ST}=0.016) in *G. p. gambiensis* populations inhabiting two adjacent river basins in Burkina Faso has been described (Bouyer *et al*., 2010).

5.7.6 *G. p. palpalis* populations in Cross River National Park

Table 4.8 showed low differentiation (F_{ST}=0.4033) in *G. p. palpalis* population in Cross River National Park. The low level of differentiation may be due to low tsetse population size. It has

been established that low population size could possibly bring about reduced differentiation and increased variation especially in southern populations (Krafsur and Endsley 2002). This is in consonance with observation that the forest zone has the highest reservoir of biodiversity (Oku *et al*., 2011). Perhaps this may explain why out of the 11 extant *Glossina* species identified in Nigeria, 7 occur in the rain forest region alone (Davies, 1977).

5.7.7 *G. p. palpalis* populations in Ijah Gwari

Results (Table 4.8) showed low differentiation (F_{ST}=0.3856) in *G. p. palpalis* populations in Ijah Gwari. This low differentiation may be due to low population size brought about by anthropogenic factors. Low apparent density of 4 flies / trap / day was recorded during this study at Ijah Gwari.

From the foregoing, results of analysis of genetic differentiations, shows that *Glossina* populations in the locations surveyed are genetically differentiated in line with discontinuous distributions of tsetse as observed earlier in the ecological work in this study. Whereas high genetic differentiation was recorded in *G. m. submorsitans* populations probably due to the restricted and patchy nature of its distribution, low differentiations detected in the *Palpalis* group flies may be due to their extensive distributions along the hydrographic networks. These differentiations observed may be due to drift, inbreeding or restricted gene flow as a result of several nucleotide substitutions, insertions and / or deletions as shown (Figures 4.20a – g). Genetic drift have been known to cause gene variants to disappear completely and thereby reduce genetic variation. When there are a few copies of an allele, the effect of genetic drift would be larger, and when there are many copies the effect would be smaller (Fisher, 1999). When populations become small and discontinuous, drift will cause gene frequencies to diverge in proportion to numbers and time since divergence (Krafsur *et al*., 2000a). Abundant variation occurring at mitochondrial loci in *morsitans* group flies have been reported by several workers (Krafsur *et al*., 2001; Bouyer *et al*., 2010). A high degree of

genetic differentiation (F_{ST}=0.866) in six natural populations of *G. m. centralis* from, Botswana, the Caprivi Strip (Namibia), Zambia, and in a laboratory culture derived from Singida, Tanzania have been described (Krafsur *et al*., 2001). Similarly, low genetic differentiation (F_{ST}=0.016) in *G. p. gambiensis* populations inhabiting two adjacent river basins in Burkina Faso have also been described (Bouyer *et al*., 2010). This is in conformity with results of ecological data obtained in this study showing significant differences in population parameters (relative abundance, apparent density, distribution, altitude, ambient temperature, relative humidity, etc.) measured in all the fly populations sampled across the various vegetation zones. Similarly, analyses of sequences, showed significant differences in genetic variations (GC/AT content, haplotype diversity and nucleotide diversity), and these differences appeared to be in line with differences in the distribution patterns of the flies across the sampled locations. These differences in tsetse populations across the various national parks may be due to differences in genetic make-up of the flies, which in turn may be responsible for differences in flies' behavior especially to climatic conditions as observed in this study. It has been established that differences in flies' behavior could possibly be responsible for differences in infection rates as well as the spatial distribution and epidemiology of trypanosomiasis (Lanzaro and Warburg, 1995; Gooding, 1996; Ouma *et al.,* 2003; Awono-Ambene *et al*., 2004).

Tsetse populations in Nigeria are discontinuously distributed possibly due to habitat fragmentation by physical features such as mountains, rivers, and roads. Similar observation had earlier been made suggesting that, differences in tsetse distribution, appear to be consistent with a discontinuous distribution in line with habitat fragmentation (Krafsur *et al.,* 1997a; Krafsur and Wohlford, 1999; Krafsur, 2002; Bouyer *et al.,* 2007b). Habitat fragmentation could lead to loss of genetic variability and increased differentiation through random genetic drift, inbreeding and

reduced gene flow between fragmented populations (Templeton *et al.,* 1990; Young *et al.,* 1996). In Nigeria, increase in human population has been known to bring about increase in anthropogenic activities, such as bush burning, clearance of vegetation for agricultural purposes, wildlife elimination for "bush meat," human habitation, and road construction. All these activities could threaten tsetse existence leading to fragmentation of tsetse populations. Such anthropogenic activities are restricted in the National Parks but prevalent in Ijah Gwari which is contiguous with Nigeria's Federal Capital Territory. Earlier records show that the Federal Capital Territory was infested with high populations of *Glossina* species (*G. longipalpis,* and *G. p. palpalis*) but extensive anthropogenic activities in the area, has led to the decline of other *Glossina* species inhabiting the area with the exception of *G. p. palpalis* which presently exists in low numbers.

5.8 Phylogenetic Relationships of *Glossina* species in National Parks and Ijah Gwari

Tsetse flies caught in this study comprised *Glossina* species derived from the forest (*Fusca*), riverine (*Palpalis*) and savannah (*Morsitans*) groups. The neighbor joining tree (Figure 4.21) revealed that closely related *Glossina* species clustered together, whereas distant relations clustered separately. The evolutionary link between the *Morsitans*, *Palpalis* and *Fusca* groups, as revealed in this work, suggests that the flies have a common ancestry from where they all evolved from. A similar observation had been made by Dyer *et al.,* (2009) showing that the three groups of *Glossina* species belong to a single phylum. Further analyses in this study have revealed that natural tsetse fly populations in Nigeria national parks are genetically differentiated (Table 4.8). Variations in number of clusters of closlely related siblings observed per *Glossina* species per national park may be due to the level of differentions existing in the fly populations as shown by the number of haplotypes. The clustering together of closely related species may be due to the number of identical nucleotides in their sequences. It appers the higher the number of identical

nucleotides, the more closely related and the lower the number of identical nucleotides, the less closely related and the less chances of clustering together.

Figure 4.21, also revealed that intraspecific genetic variation exists among flies of the same species in different vegetation zones as shown by the dendogrames. Tsetse flies of the same species from the same park clustered together but separately from flies of the same species in other parks. For instance, *G. m. submorsitans* from Yankari Game Reserve clustered together but separately from *G. m. submorsitans* in Kainji Lake National Park. So also, *G. tachinoides* from Yankari were found to cluster separately from *G. tachinoides* from Kainji Lake National Park. The only exceptions occurred in clustering together of two flies previously identified morphologically as *G. tachinoides* with *G. m. submorsitans* in Yankari (Fig 4.21). It is interesting that, phylogenetic analysis in this study revealed that the *Glossina* spp. from Cross River National Park are clustering in different groups, which are not described in the database. Firstly, *Glossina* spp. 351 position within the *Glossina* species is not clearly understood in respect of sequence similarity searches. It clustered with the *G. palpalis* group, closely together with *G. pallicera*. Similarly, *Glossina* spp. 352 clustered away from the *Palpalis* and *Morsitans* groups and appeared to be closely related to *G. medicorum* a *Fusca* group. Also, *Glossina* spp. 357 clustered closely with *G. medicorum*, probably belonging to the *Fusca* group.

Secondly, in the course of this study, two flies (Nr 162 and 273) found in Old Oyo National Park (Figure 4.21), identified as *G. m. submorsitans* (morphologically indistinguishable) and subjected to phylogenetic analysis were found to cluster closely with *G. pallidipes,* an East African savannah species. From the phylogenetic analysis, fly nos 162 and 273 may not be *G. m. submorsitans*. Krafsur, (2009) described both *G. longipalpis* exclusively found in West Africa and *G. pallidipes* found in East Africa, as morphologically and ecologically similar, and suggested investigation of

their phylogenetic relationship. But no information on CO1sequences of *G. longipalpis* is available in the Genbank that would allow for conclusive inference. In addition, Dyer *et al*., (2008), observed that *G. f. fuscipes* specimens originally derived from the Central African Republic, clustered together with *G. f. quanzensis* from the Democratic Republic of Congo (DRC), and not with *G. f. fuscipes* from southern Uganda and Kenya. In another experiment, Dyer *et al*., (2008), observed clustering of *G. fuscipes* sequence obtained from Petersen *et al*., (2007), with *G. p. gambiensis* and attributed this to either misidentification of this specimen, or to mitochondrial introgression. This is because, very often field workers are confronted with challenges of accurate identification of closely related species subjected to morphological means of identification and this results in misplacement of taxa (Dyer *et al.*, 2009).

Phylogenetic analysis has become important in resolving issues of species identification making it possible to identify species which could not be resolved morphologically. Closely related species will normally cluster together in a neighbor joining tree, while distantly related species will cluster differently. In view of the foregoing, it will be very necessary to carry out further investigations on the relative abundance and extent of distribution of these unidentified *Glossina* species caught in this study especially as their distribution extends beyond Dedeo at the Nigerian-Cameroonian border. Recent studies have shown a changing pattern in the epidemiology of trypanosomiasis in sub-Saharan Africa (Gooding and Kafsur, 2005). In Nigeria, recent localized studies have shown that the pattern of the distribution of trypanosomiasis and its vector tsetse flies is changing (Majekodunmi *et al*., 2013). HAT which was thought to be confined to the northern region seems to have shifted to the south (Figue 2.3). Recently, the presence of some *Glossina* species in areas which they were previously not recorded have been reported. For instance, *G. p. palpalis* have been reported in Niger Delta area, Nigeria (Ogedegbe and Rotimi, 2006), a hygrophytic riverine

species, *G. fuscipes fuscipes* in Gashaka - Gumti National Park Nigeria, (Wama *et al*., 2018), *G. fusca congolensis* and *G. fuscipes* in Northern Cameroon (Tongue *et al*., 2015). All these *Glossina* species have recently been recorded in areas previously not recorded, suggesting that environmental changes may be impacting on the distribution of tsetse flies in sub-Saharan Africa. It seems most reasonable therefore to conclude that in view of the changing pattern in the distribution and epidemiology of trypanosomiasis across 38 countries of sub-Saharan Africa, the existing tsetse distribution maps earlier produced by Ford and Katondo (1971) more than 5 decades ago may pose some challenges in interpreting current tsetse ecological data and therefore may need updating. This is because long term environmental changes could lead to disappearance of indigenous species or emergence of newer ones.

5.9 Phylogenetic Relationships with Particular Reference to *Glossina palpalis palpalis* Populations.

As regards *G. p. palpalis* populations caught in this study, phylogenetic analysis revealed evidence sufficiently suggesting that *G. p. palpalis* population in Nigeria is composed of genetically distinct subpopulations (Figure 4.22). The neighbor joining tree showed that *G. p. palpalis* populations in Ijah Gwari clustered together but separately from *G. p. palpalis* in Old Oyo National Park. Likewise populations in Old Oyo National park clustered separately from populations in Cross River National Park. It is interesting to note that *G. p. palpalis* from Old Oyo clustered together with *G. p. palpalis* from Togo suggesting that the distribution of *G. p. palpalis* sub-population in Old Oyo National park extends beyond Republic of Benin (which shares border with Nigeria) up to Togo. This sub-population of *palpalis* is genetically distinct from the population in Cross River National Park, suggesting that *G. p. palpalis* may be existing as a complex in Nigeria. This finding supports similar observation in Cote d'Ivoire which has suggested that *G. palpalis s.l* exists as a

species complex consisting of genetically distinct siblings /subspecies (Ravel *et al*., 2007). In Burkina Faso, discrete populations of *G. p. gambiensis* with limited gene flow among populations have also been reported (Solano *et al.*, 2000).

In this study (Figure 4.22), two clearly distinct groups of *G. p. palpalis* populations have been revealed, a group comprising of flies that cluster with those from Cameroun, Democratic Republic of Congo (DRC), Central African Republic and Equatorial Guinea. The second group comprises of flies that cluster with those from Togo, Cote d'Ivoire and Liberia. The two major groups exist sympatrically in the same location in Cross River National Park at the Nigerian-Cameroonian border area, suggesting that there is an overlap in the distribution of the two groups identified above. It is however not known whether the two groups of *G. p. palpalis* (Clade I and CladeII) can interbreed. However, this finding is consistent with results of earlier studies by Carlos, (2014) revealling that *G. p. palpalis* populations from Equatorial Guinea phylogenetically belong to two distinct allopatric clades, the West African Clade (WAC) and the Central African Clade (CAC). Similarly, Dyer *et al.,* (2009) also revealed through phylogenetic analysis that *G. p. palpalis* population in Equatorial Guinea exists as a complex. A further molecular phylogenetic study of the *palpalis* group tsetse by Dyer *et al.,* (2008) produced results consistent with this hypothesis, as *G. p. palpalis* from DRC and West Africa had divergent Cytochrome Oxidase I (CO1) gene sequences and ribosomal internal transcribed spacer1 (ITS-1) lengths. From the studies refered to above, it is suggested that the *G. p. palpalis* populations in Nigeria is genetically structured into two major groups, a West African Clade (WAC) and a Central Africa Clade (CAC). This finding is consistent with earlier observations that *Glossina* populations can be genetically differentiated using molecular tools (Gooding and Krafsur, 2005). These differences in the flies appear to be consistent with discontinuous distribution of the species due to habitat fragmentation as earlier

described (van den Bossche *et al.*, 2009). In Nigeria, the presence of physical features like mountains, rivers, vegetation and increasing anthropogenic activities may have a great impact on tsetse distribution leading to loss of gene flow. Added to these, is the role of the famous Niger River with its tributary Benue River. The Niger River is the principal river of West Africa (Gleick, 2000). It takes its source in the Guinean highlands in southeastern Guinea. It runs in a crescent through Mali, Niger, on the border with Benin and then through Nigeria, discharging through a massive delta, known as the Niger Delta or the Oil Rivers, into the Gulf of Guinea in the Atlantic Ocean. The Niger is the third-longest river in Africa, exceeded only by the Nile and the Congo River. Its main tributary is the Benue River which merges with the river at Lokoja forming a larger river which runs through the middle of Nigeria discharging its content into the coast (FAO, 1997). Benue River in West Africa, is the longest tributary of the Niger. It rises in northern Cameroon, joined by the Gongola, and then flows east and south. The Niger River in Nigeria and Sanaga River in Cameroon have similarly been identified as barriers to gene flow between genetically distinct populations of Chimpanzees, *Pan troglodytes verus* and *Pan troglodytes troglodytes* (Gonder, 2000). In another study, Niger and Benue Rivers have been identified as partial barriers to gene flow in *Mastomys natalensis* Rodents, vectors of Lassa fever in Nigeria (Olayemi *et al.,* 2016).

In the light of the above, it is only most reasonable to conclude that the Niger River might have had a great impact on tsetse distribution and differentiation in Nigeria tsetse flies and may be a physical barrier to gene flow between the two major groups of *G. p. palpalis* populations identified in this work. This finding is the first on tsetse flies in Nigeria and corroborates the River Barrier to gene flow hypothesis (RBH), which posists that tropical rivers can be effective barriers to gene flow (Wallace 1852 as cited by Voelker *et al*., 2013). The origin of tsetse flies in Nigeria is not known, however it is thought that tsetse would probably have existed in Nigeria preceding

continental separation over 100 Million years ago. It thus appear very likely that before the formation of Niger River (probably through tectonic forces) about 23million years ago in the Miocene (Bonne, 2014), tsetse flies would probably have had their distribution in West Africa. With the formation of the Niger River, the modern population structure of many *Glossina* species with particular reference to *G. p. palpalis*, would probably have been shaped into two major clades, one to the west and the other to the east, currently referred to as West and Central African Clades. Their uniqueness indicates that these populations have been isolated for significant amounts of time. Movements of flies between western and regions to the east of the Niger River may have not occurred for hundreds of thousands of years. Though some members belonging to the Central African Clade share similarity with some members in the West African Clade by clustering together, this may have been induced by some evolutionary forces of Natural selection, drift, mutation, migration and/or random mating. Speciation could also occur artificially through animal husbandry, agriculture or laboratory investigations. In Nigeria, the transhumant nature of animal husbandary is highly incriminated in this case. It is very unlikely that tsetse flies would disperse actively across Niger River which has a large drainage basin covering several thousand kilometers. Flight in tsetse flies consists of short bursts. Bursell and Taylor (1980) estimated that the daily flight duration is about 15 and 30 min for males in the hot and cold seasons respectively; for females, the flight duration is limited to a few minutes. Since it has been predicted that the mean rate of frontal advance of *G. morsitans* group flies is about 200 m day^{-1} or 7 km $year^{-1}$ (Williams *et al.,* (1992), it seems most inconceivable to think that flies would disperse actively across Niger River, except conveyed passively by cattle during seasonal movements as opined by Lambrecht (1972).

All the above factors could separate tsetse populations in the country leading to restricted gene flow in tsetse and may bring about differentiation within species. This observation lends credence to the works of Ouma, (2004) who observed differentiation in *G. pallidepes* populations in Kenya and attributed that to presence of physical features like mountains and valleys in the study area. Recently, Carlos *et al.,* (2014) observed genetic structuring and differentiation in two *G. p. palpalis* populations in Equatorial Guinea, from the costal and the continental regions conforming to the hypothesis of habitat fragmentation. Similarly, Lake Kyoga in Uganda has been described as a barrier to gene flow between two genetically distinct populations of *G. fuscipes fuscipes* a riverine species (Echodu *et al*., 2013).

Genetic differentiation, if associated with differences in physiology, behavior, and /or symbiont composition affecting vector competence, could have implications for the epidemiology of trypanosomiasis, as well as vector control (Beadell *et al.,* 2010). Moloo (1992) compared the performance of *G. pallidipes* originating from allopatric Kenya populations, one from the Shimba Hills and the other from the Nguruman. He observed that bionomic parameters (insemination rates, female survivorship, puparial weights and survival rates) did not differ between the two populations. However, fecundity of the Shimba Hills culture females was significantly greater than that of the Nguruman culture. In addition, Nguruman culture flies were more likely to develop mature infections (Moloo, 1993). Also van Etten (1982a) demonstrated different feeding frequencies of female *G. pallidipes* from allopatric Kenya populations. Differences were also recorded in activity patterns and responses to temperature (van Etten, 1982b). In a separate study, it was shown that *G. pallidipes* cultures established from the Lambwe and from Kibwezi forest populations differed significantly in the duration of copulation (van Etten, 1981). Gooding *et al.,* (2004) revealed through mating experiments between *G. p. palpalis* originating from Nigeria and

Democratic Republic of Congo (DRC) yielding sterile F1 hybrid males, suggesting that *G. p. palpalis* itself may consist of more than one subspecies.

At present, available data indicate that the actual zone of contact between West Africa and Central Africa Clades of *G. p. palpalis* populations in Nigeria, is in the Nigerian-Cameroonian border area where the two groups meet, however, the level of admixture between these two population groups in locations where they co-exist and empirical data on the extent of dispersal of *Glossina* species from the National Parks surveyed is not known. Of equal importance is the need to investigate the status of cryptic (unusual) species of tsetse flies in Nigeria in view of the presence together in the same place (Nigerian-Cameroonian border area) of closely related sibling species of *G. p. palpalis* populations which are geographically distributed distinctly in the country. Using flies from colonies originating from Nigeria and Bras – Zaire (the present day Democratic Republic of Congo DRC), in X-chromosome mapping experiments, Gooding *et al.,* (2004), revealed the occurrence of cryptic species in the tsetse fly *G. p. palpalis*. The finding showed that the populations were reproductively isolated and also showed variation in vectorial capacity between the two groups.
A clear understanding of the structure of tsetse populations and their role in disease transmission in Nigeria could define the scale in which vector control is likely to be more effective. Further breeding experiments are required to determine if the two distinct groups of *G. p. palpalis* revealed in this study are reproductively compatible. It is also important to determine if there is any differences in the vectorial capacities of these two groups of *G. p. palpalis* identified phylogenetically. The out come of the study could reveal whether breeding stock of flies for use in the SIT programme would be done selectively or not. Further studies are also required on gene flow using microsatellites to determine the isolation status of the parks to guide against reinvassion should control be implemented. Results obtained from these analyses would define whether control

would be implemented simultaneously or progressively following the principle of the "rolling carpet" (Vreysen *et al.,* 2007).

5.10 Internal Transcribed Spacer (ITS)-1 Analysis

ITS-1 region is characterized by high variability and could thus show more recent changes within species and even sufficiently isolated populations. However, in the case of Nigerian *G. p. palpalis* populations, differences could not be identified. This inability of ITS-1 to distinguish between *G. p. palpalis* at the population level has also been observed by others (Dyer *et al*., 2008). The phylogenetic analysis revealed that the population was homogenous. This could possibly imply that the segregation in two groups as observed by looking at CO1 resembles two different maternal lineages, but not clades segregating into distinct species.

5.11 Geometric Wing Morphometrics

Landmarks of wings for morphormetrics (Figure 4.23a) were chosen according to methods described by Achukwi *et al*., (2013), in a study of subpopulations of *G. m. submorsitans* for segregation. The sexual dimorphism (Figure 4.23b) observed here has been described previously for *G. p. gambiensis* (Camara *et al.,* 2006). Principal component analysis of the partial warps showed a segregation of the female population belonging to the central African group (Figure 4.23c). However, due to the low sample size available for analysis, no final conclusion could be drawn and it remains to be seen if this can be reproduced with a larger sample size. It is interesting that the observed difference is not detected if data from male and female flies are pooled together.

As this divergence of *G. p. palpalis* is strongly supported by CO1 analysis, it is important to acknowledge this factor in breeding and vector competence, as it reflects the maternal lineage. It was shown in breeding experiments with *G. m. centralis* that susceptibility to trypanosomal infection is maternally inherited (Moloo *et al*., 1998). A sex dependent breeding preference has

been observed and sterility in tsetse fly males has been linked to sex-chromosomal factors (Gooding, 1997; Gooding *et al.,* 2004). It has been proposed that this may be due to chromosomal incompatibility (Gooding *et al.*, 2004). Interestingly, in that same study a breeding barrier has been observed between *G. p. palpalis* colonies originating from Nigeria and Zaire, respectively, while they also had different vector capacities. Similarly, in a study comparing the performance of *G. pallidipes* originating from two allopatric Kenyan populations, one from the Shimba Hills and the other from the Nguruman, it was observed that bionomic parameters did not differ between the two (Moloo, 1992). However, fecundity of the Shimba Hills culture females was significantly greater than that of the Nguruman culture. In addition, Nguruman culture flies were more likely to develop mature infections (Moloo, 1993). Furthermore, different feeding frequencies of female *G. pallidipes* from allopatric Kenya populations was demonstrated in an earlier study (van Etten, 1982a). Differences were also recorded in activity patterns and responses to temperature (van Etten, 1982b). In a separate study, it was shown that *G. pallidipes* cultures established from the Lambwe and from Kibwezi Forest populations differed significantly in the duration of copulation (Jaenson, 1978).

This together brings up the questions, if the coexisting clusters observed in this study are actually representing subspeciation or even subspecies in the classical sense in *G. p. palpalis*, and if so: Can these flies interbreed or do they exhibit different breeding preferences or breeding incompatibility as observed by Gooding *et al.,* (2004) and is there a difference in vector competence regarding trypanosomal infections between these clusters. These questions have to be investigated closely to see whether gene flow occurs between these clusters, as this will provide vital information for the application of SIT or paratransgenesis approaches in tsetse control.

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Tsetse flies (*Glossina* species) are the main vectors of trypanosomes, protozoan parasites that cause human African trypanosomiasis (HAT) in humans and animal African trypanosomiasis (AAT) in livestock across 38 countries of sub-Saharan Africa. Adequate knowledge on *Glossina* species present in an area and population dynamics is required for planning effective control measures against the target species in the target area. In order to achieve this on tsetse populations in Nigeria, tsetse flies were collected from five different locations in Nigeria, from the rain forest, through derived savanna, southern Guinea, northern Guinea to Sudan savannah vegetation zones. These locations included, Yankari Game Reserve, Kanji Lake National Park, Old Oyo National Park, Cross River National Park and Ijah Gwari, a highly tsetse infested area within the Federal Capital Territory Abuja for purposes of determining the population structure of the *Glossina* species as well as to have increased understanding on their population dynamics. Flies collected with standard biconical traps were identified morphologically and later confirmed molecularly by phylogenetic analysis using Cytochrome C Oxidase (SU1) and Internal Transcribed Spacer region-1 (ITS-1) sequences as genetic markers for population structure as well as Geometric wing morphometric analysis of the wing land marks. Results obtained in the study showed that of 1891 tsetse flies caught and identified morphologically included, *Glossina tachinoides* 1050*, G. p. palpalis* 568*, G. m. submorsitans* 270 and *Glossina fusca* 3. Three out of the four *Glossina* species caught namely, *G. tachinoides, G. p. palpalis,* and *G. m. submorsitans,* are of high economic importance in Nigeria as vectors of both human and animal African trypanosomiases. *Glossina tachinoides* was the dominant species caught in Yankari Game Reserve and Kainji Lake National

Park, followed by *G. m. submorsitans*. Whereas *G. p. palpalis* dominated catches in Old Oyo and Cross River National Parks, it also constituted the sole species caught in Ijah Gwari.

The discontinuous distribution of *Glossina* species caught in this study could possibly be due to habitat fragmentation, variation in vegetation types, relative humidity, ambient temperature conditions, altitude and distribution of wild animal hosts across the sampled locations. Sex ratio was in favour of female *G. m. submorsitans* in Yankari Game Reserve and Kainji Lake National Park, while catches of *G. tachinoides* gave sex ratio in favour of males. In Old Oyo and Cross River National Parks, sex ratio in *G. p. palpalis* was in favour of males. These observations suggest that sex ratio in tsetse trap catches depends on fly species, vegetation type, temperature and relative humidity conditions among other factors, which is a crucial information for area-wide tsetse population management with particular reference to the sterile insect technique (SIT).

Results of statictical analysis showed no significant difference ($t = -1.580$, $p\ 0.133 > \alpha\ 0.05$) between ambient temperature and mean relative humidity conditions in relation to fly catches in Yankari Game Reserve. However, in Kainji Lake National Park, there was significant difference ($t = -10.633$, $p = -0.000 < 0.05$) between mean ambient temperature and mean relative humidity in relation to fly catches.

In Old Oyo National Park, fly catches were significantly different ($t = -36.290$, $p = 0.000 < 0.05$) in relation to mean ambient temperature and mean relative humidity conditions. In Cross River National Park, significant difference ($t = -18.939$, $p = 0.000 < 0.05$) between mean ambient temperature and mean relative humidity conditions in relation to tsetse catches was recorded.

Analysis of Cytochrome C Oxidase SU1, sequences revealed that *Glossina* populations in Nigeria are diverse and could be differentiated genetically. High level genetic differentiations were

recorded in *G. m. submorsitans* populations, whereas the *palpalis* group exhibited low genetic differentiations. The underlying causes of differentiations in *Glossina* species caught may be due to nucleotide deletions, insertions and various substitutions. It was possible to use CO 1 sequences in conjunction with sequence similarity searches on the Genbank to accurately identify 91% *Glossina* species subjected to phylogenetic analysis. The six unidentified *Glossina* spp. to our knowledge, have not their CO1 sequences deposited on the Genbank database. The study revealed that two of the unidentified flies closely resemble *Glossina pallidipes*, an East African species, while some other flies aligned closely with *G. medicorum* ostensibly belonging to the *Fusca* group. There is the need to employ some other markers including microsatellites to look more closely at the unidentified *Glossina* spp. for possible new country records. It has been shown in this study that *G. p. palpalis* population in Nigeria is composed of two genetically distinct groups, a West African Clade (WAC) and a Central African Clade (CAC). It is not known whether the two distinct groups are reproductively compatible or there is a mating barrier between them. It is also not known whether there is variation in vectorial capacities between the two groups.

It is interesting that wing morphometric analysis could not distinquish between the two groups of *Glossina palpalis palpalis* revealed by CO 1 sequences in this study, but rather grouped the flies based on sexual dimorphism. Other geometric morphometric tools other than the 'Clic' software used in this study, could be employed to look more closely into the possibility of distinguishing between the two identified groups of *G. p. palpalis* as revealed by CO1.

From the findings in this study, it is possible to control *Glossina* populations in the National Parks, by the use of SIT since it is species specific, in view of the existing genetically diverse *Glossina* populations. However the level of isolation of *Glossina* species in the national parks in addition to suggestions made above need to be investigated.

6.2 Conclusion

From the findings in this study, we most reasonably conclude that:

i. The ecological data obtained in this study have shown evidence of variations in species composition and relative abundance of *G. m. submorsitans, G. tachinoides*, *G. p. palpalis* and *G. fusca* during the study, and that the variations may be due to variation in environmental factors across sampled locations.

ii. The apparent density and spatial distribution of *Glossina* populations in the National Parks and Ijah Gwari vary from species to species and from sampled location to location, in relation to variations in vegetation types and micro-climatic conditions.

iii. To our knowledge, the population genetic structure of *Glossina* populations in the National Parks and Ijah Gwari has been revealed for the first time and that the tsetse flies exist in small clusters or subpopulations/units of closely related sibling species based on phylogenetic profiles.

iv. The genetic data obtained in this study have shown that tsetse populations in Nigeria National Parks and Ijah Gwari are genetically structured and are even more diverse than ecological data could reveal. The study has sufficiently demonstrated that, *Glossina palpalis palpalis* population in Nigeria is composed of two major genetically distinct groups, a West African Clade (WAC) and Central African Clade (CAC), with the two groups co-existing sympatrically in the region of Cross river National Park, at the Nigerian-Cameroonian border area.

v. The study has shown that *Glossina* populations in the National Parks and Ijah Gwari may be drifting as shown by single nucleotide polymorphisms (SNPs).

vi. Finally, the ecological data supported by molecular data obtained in this study have given a clearer understanding on the population dynamics of tsetse flies in the selected National Parks and Ijah Gwari, which may be a vital information that would aid the choice of effective control strategies against tsetse flies and trypanosomiasis management in Nigeria.

6.3 Recommendations

From the results obtained in this study, it is recommended that:

1. For effective control of tsetse flies in Nigeria using SIT, the dominant economically important tsetse flies present in each National Park should be targeted for elimination.
2. Suppression of tsetse flies using density dependent methods (impregnated traps and screens, pour-on formulations) should precede the use of SIT in the selected National Parks /Game Reserve studied.
3. Tsetse control in the National Parks using SIT is feasible in view of species specificity of the method. However, it is necessary to find out if those closely related species could interbreed, as this may pose some challenges to the successful implementation of the control campaign.
4. The isolation status of each *Glossina* species in each sampled location should be determined in addition to their host range through blood meal analysis. This is because where there is migration, SIT may not succeed, but where there is limited migration, SIT may succeed.
5. The genotypes of flies across sampled locations should be determined in view of the perceived population drift as indicated by SNPs.

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APPENDICES

Appendix I: Entry Permit

Office of the
Director-General/CEO
NITR Kaduna
RECEIVED
Sign ..... Date 11.4.14

NATIONAL PARK SERVICE

Nnamdi Azikiwe International Airport Expressway, P.M.B. 0258, Garki - Abuja.
Tel: 09-6714926. E-mail: consgenparks@hotmail.com

NPH/GEN/501/I/90 — 4th March, 2014

Our Ref. ---------- Date: ----------

Director-General/CEO,
Nigerian Institute for Trypanosomiasis Research,
Federal Ministry of Science & Technology,
NO. 1 Surame Road,
Ungwan Rimi G.R.A,
P.M.B 2077,
Kaduna.

Attention: Director Research/Project Officer,
PATTEC-Nigeria.

RE: REQUEST FOR ENTRY PERMIT AND PERMISSION TO CARRY OUT RESEARCH ACTIVITIES WITHIN THE PARKS

Your letter dated 27th February, 2014, vide S/TR/2003/VII/154 on the above subject matter refers please.

2. I am directed to convey approval for your team of researchers from your Institute to visit the National Parks and collect tsetse flies for research purposes as indicated;

i. Kainji Lake National Park (4th-9th March, 2014),

ii. Old Oyo National Park (10th-15th March, 2014),

iii. Cross River National Park (16th-20th March, 2014).

3. Thank you.

Jonah C. Moses
For: Conservator-General

Appendix II: Tsetse trapping using biconical trap



Appendix III: Co-ordinates of trapping points, environmental conditions and *Glossina* spp. caught in Yankari Game Reserve

Location	Trap No.	Co-ordinate			Ave Temp °C	Ave R.H %	*Glossina* species caught								Total
		Longitude	Latitude	Elev			*G.p.palpalis*		*G.tachinoides*		*G.m.submorsitans*		*G.fusca*		
		E°	N°	M			♂	♀	♂	♀	♂	♀	♂	♀	
Yankari	1	09 44.252′	10 30.721′	227	33.5	30			1	3	2	3			9
	2	09 43.906′	10 30.886′	232	32	35			7		17	14			38
	3	09 43.873′	10 30.879′	232	31	36.5			5		15	10			30
	4	09 43.763′	10 30.901′	232	33.5	39			13		11	14			38
	5	09 43.625′	10 30.837′	233	32.8	34			6		6	6			18
	6	09 43.577′	10 30.815′	234	33.3	32			17	1		7			25
	7	09 43.504′	10 30.790′	234	33.3	32			17	7	1	4			29
	8	09 43.443′	10 30.721′	235	35.3	31.5			11	6		4			21
	9	09 43.395′	10 30.717′	237	32.5	32			46	21		3			70
	10	09 43.385′	10 30.396′	232	33.5	28			49	15	4	11			79
	11	09 43.365′	10 30.369′	232	35	29			30	13	1				44
	12	09 43.306′	10 30.329′	232	34.5	34			7	4	8	6			25
	13	09 43.258′	10 30.280′	233	35.5	46			23	14	7	4			48
	14	09 43.009′	10 30.061′	234	33.3	40		1	21	8		5			35
	15	09 43.641′	10 29.994′	233	33	43			93	60		1			154
	16	09 43.644′	10 29.972′	234	33.5	38			44	21	6	10			81
	17	09 43.662′	10 29.955′	234	33	39.5	1		76	29	2	7			115
	18	09 43.679′	10 29.962′	235	35.3	38				3	1				4
Total															863

Appendix IV: Co-ordinates of trapping points, environmental conditions and *Glossina* spp. caught in Kainji Lake National Park

Location	Trap No	Co-ordinate			Ave Temp °C	Ave R.H %	*Glossina* species caught								Total
		Longitude	Latitude	Elev			*G.p.palpalis*		*G.tachinoides*		*G.m.submorsitans*		*G.fusca*		
		E°	N°	M			♂	♀	♂	♀	♂	♀	♂	♀	
Kainji	1	0954.722′	03 57.180′	245	34	53.5			7	3					10
	2	0954.704′	03 57.115′	245	36	55			13	5	1	2			21
	3	0954.658′	03 57.054′	243	34.8	45.5			50	19	3	2			74
	4	0954.678′	03 57.266′	239	34	60			34	20	2	5			61
	5	0954.295′	03 59.184′	227	26.5	57.5			15	10	7	5			37
	6	0954.945′	03 59.566′	220	26.5	54			46	20	8	8			82
	7	0954.097′	03 59.681′	226	27.5	46.5			9	8	11	13			44
	8	0954.050′	03 59.710′	224	28.8	50.5			14	6	2	4			26
	9	0954.621′	03 56.919′	238	30.5	43.5			36	13	3	7			59
	10	0954.378′	03 56.811′	236	29	51			23	14	2	4			43
Total															457

Appendix V: Co-ordinates of trapping points, environmental conditions and *Glossina* spp. caught in Old Oyo National Park

Location	Trap No	Co-ordinate			Ave Temp °C	Ave R.H %	*Glossina* species caught								Total
		Longitude	Latitude	Elev			*G.p.palpalis*		*G.tachinoides*		*G.m.submorsitans*		*G.fusca*		
		E°	N°	M			♂	♀	♂	♀	♂	♀	♂	♀	
Old Oyo	1	0827.281′	0346.591′	307	31.8	68	2	5							7
	2	0827.223′	0346.581′	309	30.5	66	5	9				1			15
	3	0827.157′	0346.589′	306	31	68	81	86							167
	4	0827.127′	0346.574′	305	31.5	62	30	16							46
	5	0827.124′	0346.541′	305	31.5	69	5	17							22
	6	0827.128′	0346.398′	300	31	69	24	22							46
	7	0827.118′	0346.377′	300	31.5	67	39	38							77
	8	0827.066′	0346.383′	301	30.5	65.5	16	18							34
	9	0827.027′	0346.368′	301	32	66	35	23							58
	10	0826.995′	0346.374′	303	29	58.5	34	14			1				49
	11	0825.051′	03 6.726′	298	30	59.5	5	4							9
Total															530

Appendix VI: Co-ordinates of trapping points, environmental conditions and *Glossina* spp. caught in Cross River National Park

Location	Trap No	Co-ordinate			Ave Temp ℃	Ave R.H %	*Glossina* species caught								Total
		Longitude	Latitude	Elev			*G.p.palpalis*		*G.tachinoides*		*G.m.submorsitans*		*G.fusca*		
		E°	N°	M			♂	♀	♂	♀	♂	♀	♂	♀	
Cross River	1	0521.779′	0827.479′	123	24.5	100									
	2	0623.031′	0910.916′	151	28	90.5		2							2
	3	0623.063′	0910.903′	155	29	88.5							1		1
	4	0623.049′	0910.903′	152	28	88.5	1	8							9
	5	0623.433′	0910.901′	130	29	76.5	4						2		6
	6	0521.781′	0826.394′	155	26.5	92	1	1							2
	7	0521.829′	0826.180′	151	26.5	96		1							1
Total															21

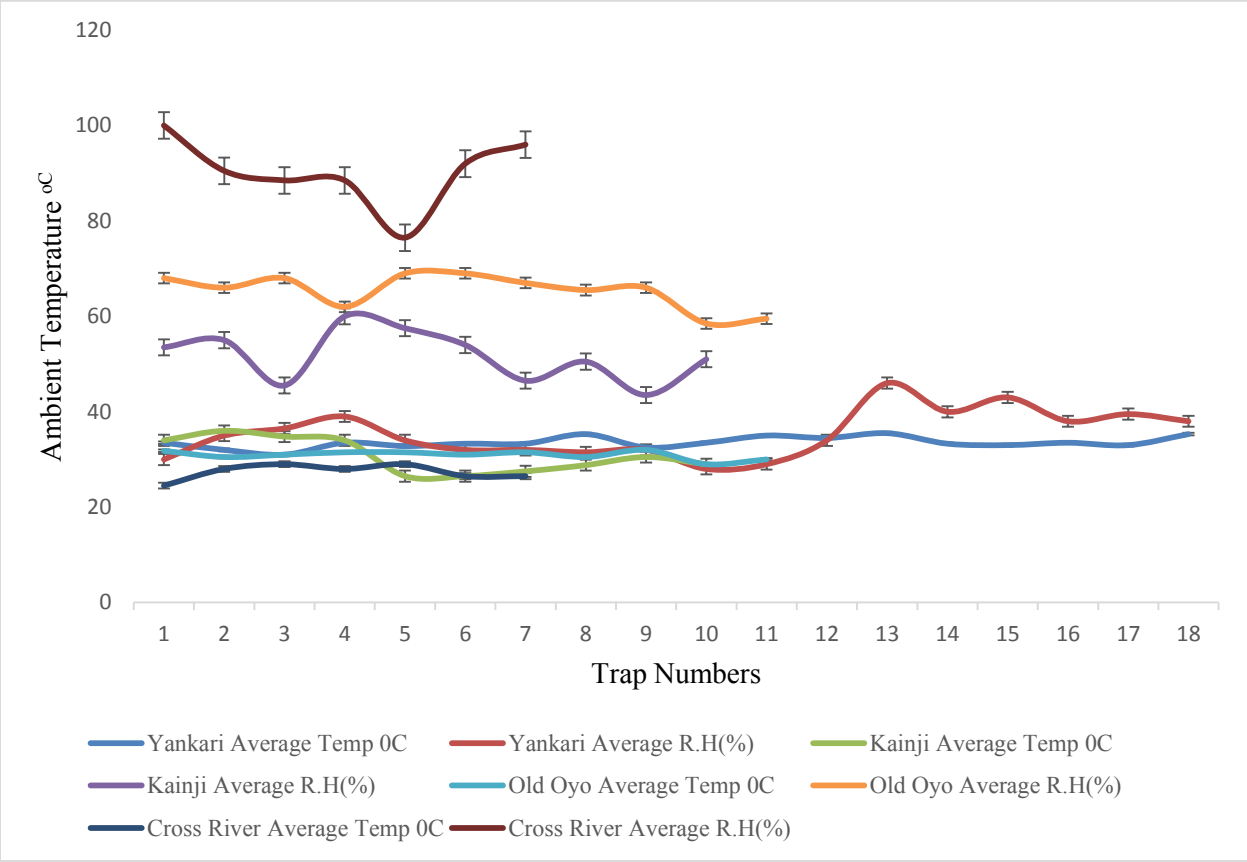
Appendix VII: Co-ordinates of trapping points, environmental conditions and *Glossina* spp. caught in Ijah Gwari

Location	trap no.	Co-ordinates			Ave temp	Ave RH	*Glossina* species caught								Total
		Longitude	Latitude	Elev	°c	%	*G.p.palpalis*		*G.tachinoides*		*G.m.submorsitans*		*G.fusca*		
		N°	E°				♂	♀	♂	♀	♂	♀	♂	♀	
Ijah Gwari	1	09 18.860′	07 26.814′	524	32	56		2							2
	2	09 18.734′	07 26.823′	508	34	58	2	3							5
	3	09 17.858′	07 26.768′	474	30	50	2	2							4
	4	09 17.874′	07 26.608′	446	29	60	1	1							2
	5	09 17.798′	07 26.609′	446	27	84.5	5	2							7
Total							10	10							20

Appendix VIII: *Glossina* spp. distribution in relation to climatic conditions and vegetation zones

National Park	*Glossina* species	Relative abundance	Ambient temperature (°C)	Relative Humidity (%RH)	Vegetation type
Yankari	*G. tachinoides*	682	31-35.5	28.0-46.0	Sudan savannah /Gallery forests
	G. m. submorsitans	179			
	G. p. palpalis	2			
Kainji	*G. tachinoides*	368	26.5-36	43.5-57.5	Southern Guinea
	G. m. submorsitans	89			
Old Oyo	*G. p. palpalis*	528	29-31.8	58.5-69.0	Derived Savannah
	G. m. submorsitans	2			
Cross river	*G. p. palpalis*	18	24.5-29	76.5-100	Rain forest
	G. fusca	3			
Ijah Gwari	*G. p. palpalis*	20	27-34	50-84.5	Northern Guinea/Fringing forest

Appendix IX: Environmental conditions of sampled locations (mean temperature and relative humidity)

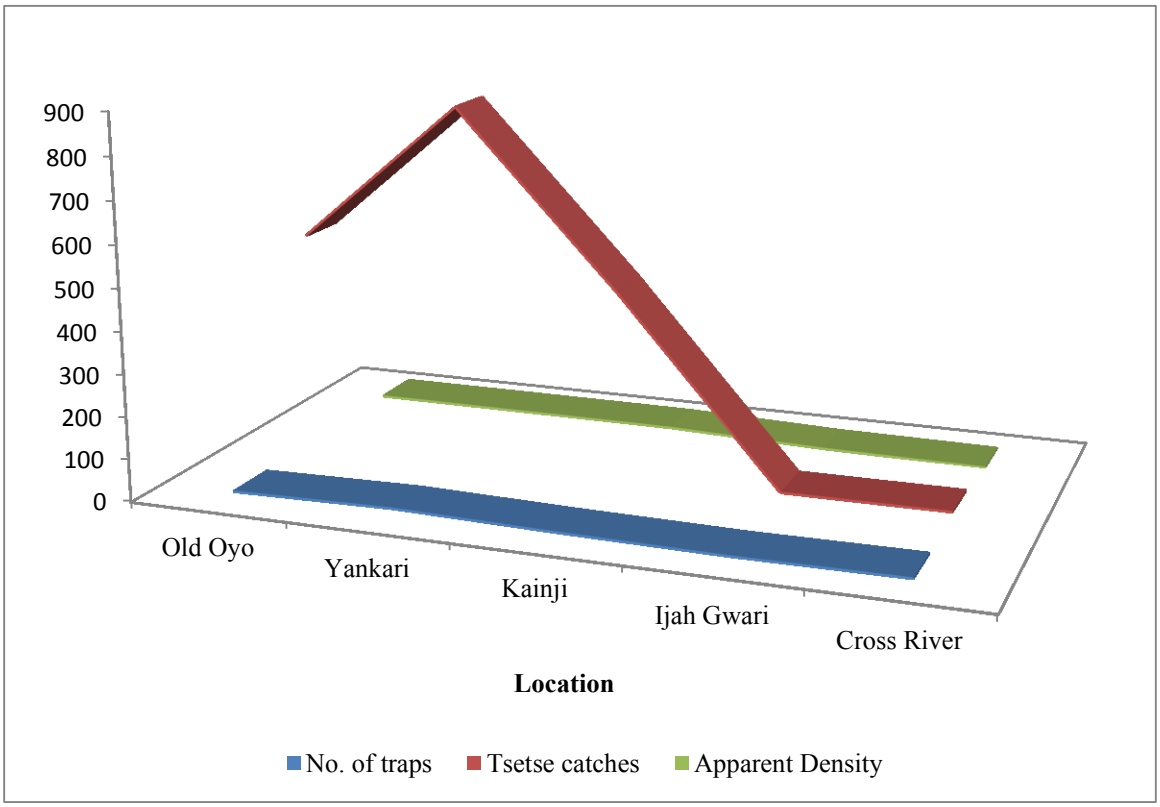


Appendix X: Total number of *Glossina* spp. caught per location

Location	*Glossina* species caught								Total	%
	G. p. palpalis		*G. tachinoides*		*G. m. submorsitans*		*G. fusca*			
	♂	♀	♂	♀	♂	♀	♂	♀		
Yankari	1	1	476	206	80	99	-	-	863	46
Old Oyo	276	252	-	-	1	1	-	-	530	28
Kainji	-	-	250	118	30	50	-	-	457	24
Cross River	6	12	-	-	-	-	-	3	21	1
Ijah Gwari*	10	10	-	-	-	-	-	-	20	1
Total by sex	293	275	726	324	120	150		3	1891	100
Total	568		1050		270		3		1891	

*NITR Suleja Outstation collection site

Appendix XI: Tsetse catches in relation to location and number of traps used



Appendix XII: Apparent density of *Glossina* spp. in relation to location and vegetation zone type

Location	No. of Traps	No. of Tsetse flies caught	Apparent Density (F/T/D)	Type of vegetation
Old Oyo	11	530	16.09	Derived savannah and riverine
Yankari	18	863	15.98	Northern guinea savannah and riverine
Kainji	10	457	15.23	Riverine and Southern guinea
Ijah Gwari*	5	20	4.00	Riverine and forest relic
Cross River	7	21	1.00	Tropical rain forest

*NITR Suleja outstation collection site

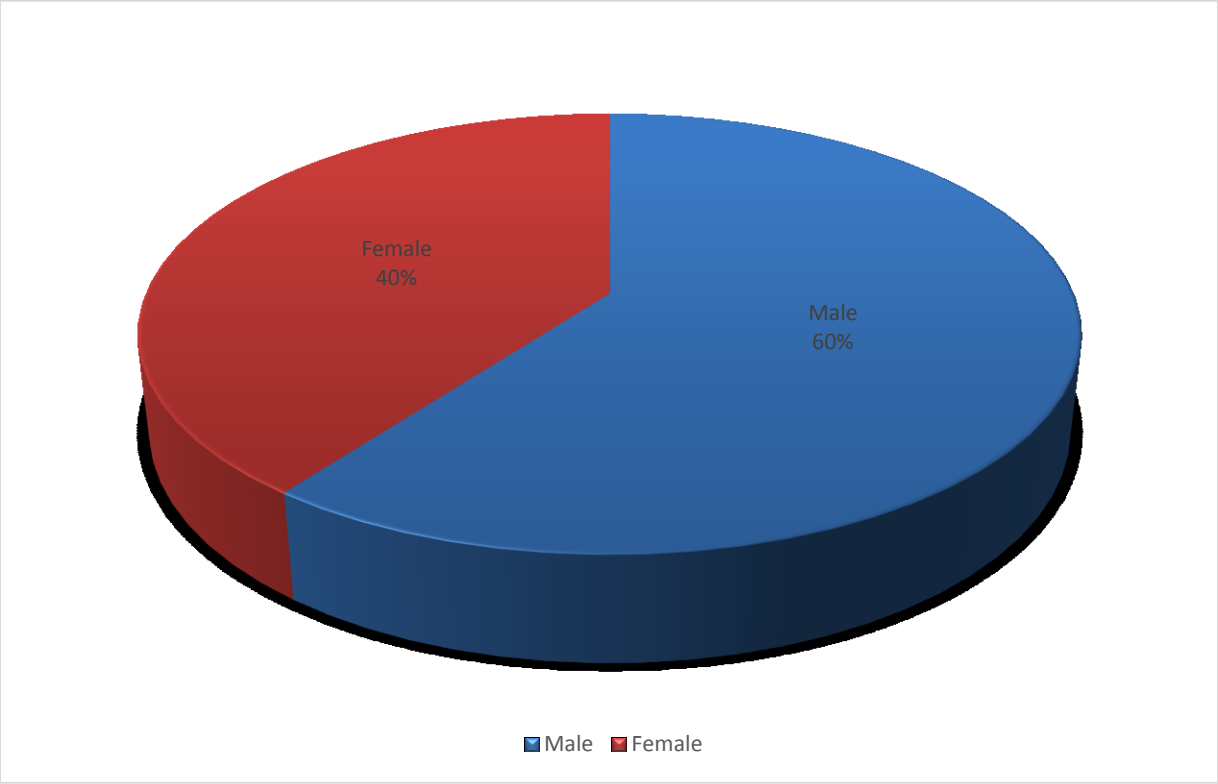
Appendix XIII Apparent density of *Glossina* per species per sampling site

Location	*Glossina* spp.	Total No. caught	Total No. of traps used	Trapping days	Trap days	Apparent Density (F/T/D)
Old Oyo National Park	*G. p. palpalis*	528	11	3	33	16
	G. m. submorsitans	2	-	-	-	0.09
Yankari Game Reserve	*G. tachinoides*	682	18	2	36	18.94
	G. m. submorsitans	179				4.97
	G. p. palpalis	2	-	-	-	0.05
Kainji Lake National Park	*G. tachinoides*	368	10	3	30	12.26
	G. m. submorsitans	89	-	-	-	2.96
Ijah Gwari	*G. p. palpalis*	20	5	1	5	4.0
Cross River National Park	*G. p. palpalis*	18	7	2	14	1.28
	G. fusca	3	-	-	-	0.21

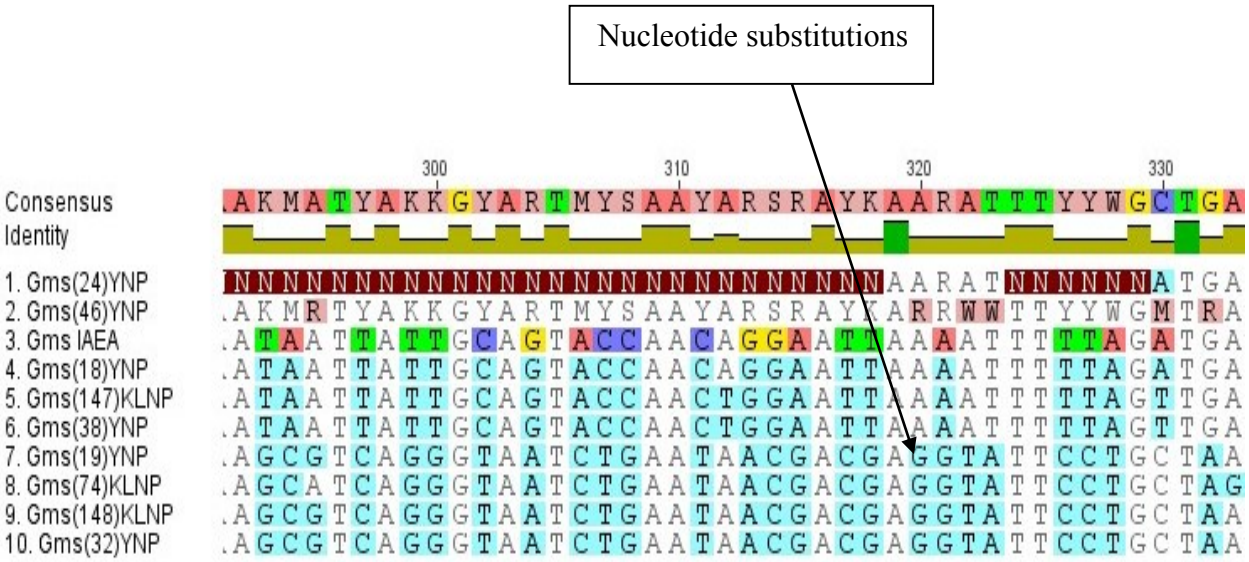
Appendix XIV: Pattern of tsetse fly catches according to species per location

Glossina spp.	Locations										Total	%
	Yankari		Kainji		Old Oyo		Cross River		Ijah Gwari			
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀		
G. tachinoides	476	206	250	118							1050	55
G. p. palpalis	1	1			276	252	6	12	10	10	568	30
G. m. submorsitans	80	99	39	50	1	1					270	14
G. fusca								3			3	1
Total by sex	557	306	289	168	277	253	6	15	10	10	1891	100
Total	863		455		530		21		20		1891	

Appendix XV: Catch composition of *Glossina* species according to sex in sampled Nigeria National Parks



Appendix XVI: Comparative nucleotide substitutions based on CO1 sequences among *G. m. submorsitans* populations in Yankari and Kainji Lake National Parks (KLNP)



Legend

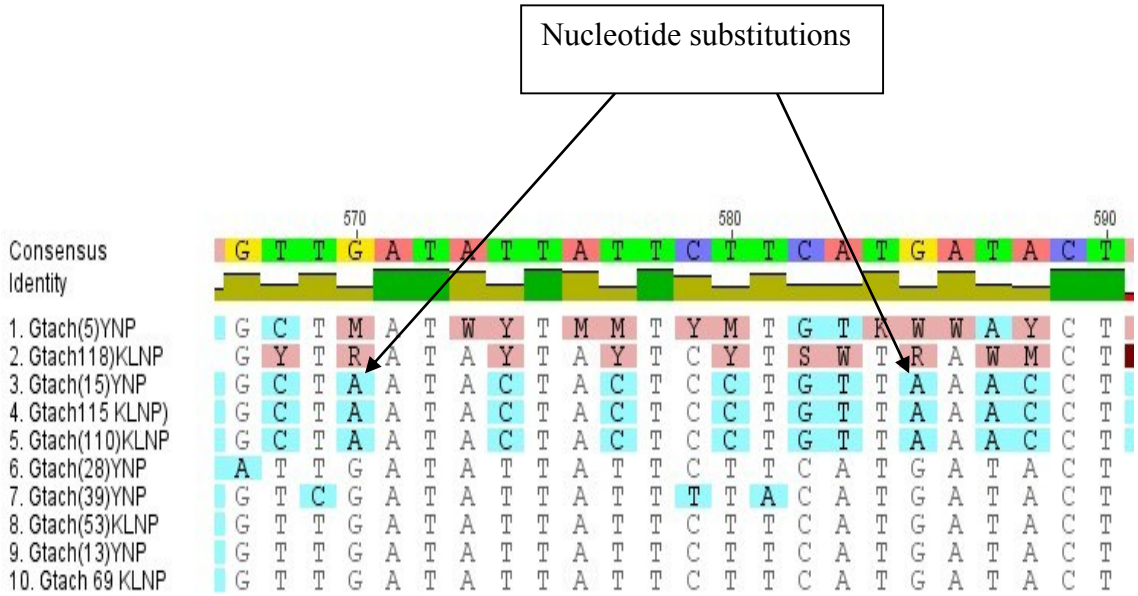
A = Adenine
T = Thymine
C = Cytosine
G = Guanine

Nucleotides

K = G or T
M = A or C
N = A, G, C or T
R = A or G
S = G or C
W = A or T
Y = C or T

Degenerate residue specification

Appendix XVII: Comparative nucleotide substitutions based on CO1 sequences in *G. tachinoides* populations at Yankari and Kainji National Parks (KLNP)



Legend

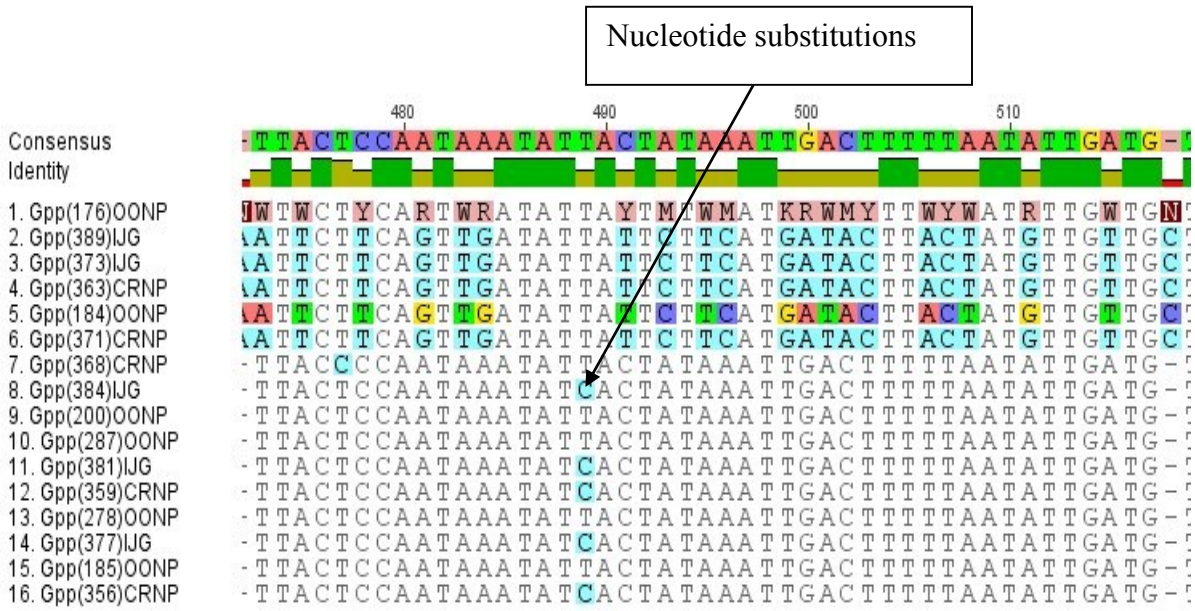
A = Adenine
T = Thymine
C = Cytosine
G = Guanine

Nucleotides

M =
R =
S =
W =
Y =

Degenerate residue specification

Appendix XVIII: Comparative nucleotide substitutions based on CO1 sequences in *G. p. palpalis* populations in Old Oyo, Cross River National Parks (CRNP) and Ijah Gwari (IJG)



Legend

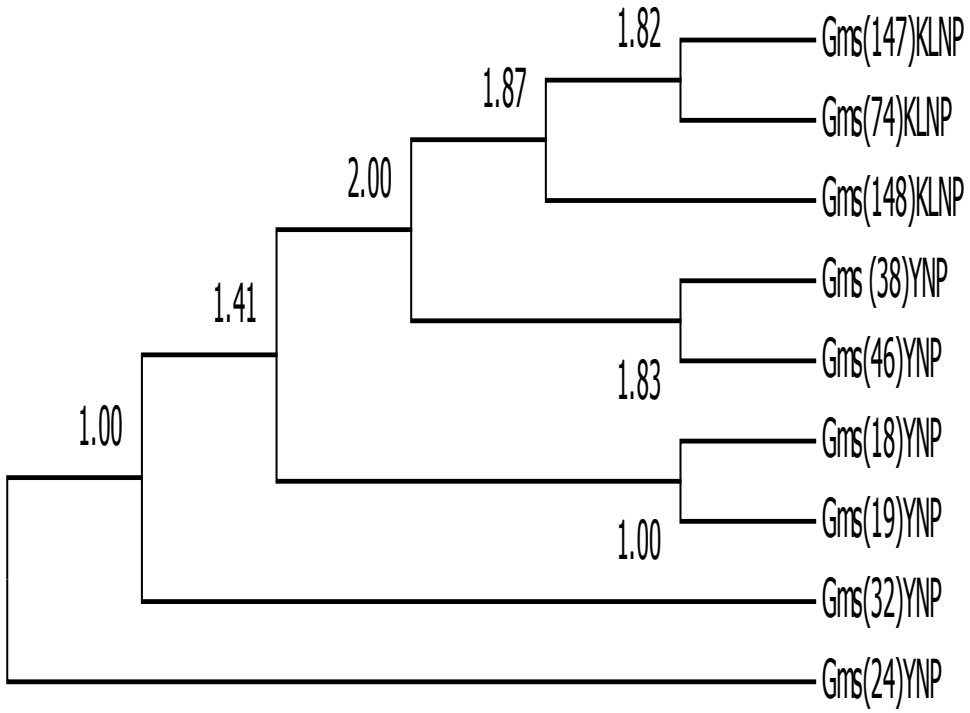
A = Adenine
T = Thymine
C = Cytosine
G = Guanine

Nucleotides

K = G or T
M = G or T
N = A, G, C or T
R = A or G
W = A or T
Y = C or T

Degenerate residue specification

Appendix XIX: Divergence time (2MYA) of *G. m. submorsitans* populations in Yankari from same population in Kainji Lake National Park.



Appendix XX: Sample of *Glossina* spp. used for DNA extraction

Location	*Glossina* species								Total
	G. m. submorsitans		*G. tachinoides*		*G. p. palpalis*		*G. fusca*		
	♂	♀	♂	♀	♂	♀	♂	♀	
Yankari	4	22	10	13	-	-	-	-	49
Kainji	5	12	55	39	-	-	-	-	111
Old Oyo	1	1	-	-	74	114	-	-	190
Cross river 1	-	-	-	-	3	1	-	3	7
Cross river 2	-	-	-	-	3	11	-	-	14
Ijah Gwari	-	-	-	-	10	10	-	-	20
Total by sex	10	35	65	52	90	136	-	3	391
Total	45		117		226		3		391

Appendix XXI: Cytochrome Oxidase I ampligfied samples

Glossina species	Location	No. Dissected	No. Amplified	% Amplification
G. m. submorsitans (45)	Yankari Game Reserve	26	13	50.0
	Kainji	17	12	70.58
	Old Oyo	2	1	50
G. tachinoides (117)	Yankari Game Reserve	23	5	22.0
	Kainji	94	7	7.40
G. p. palpalis (226)	Old Oyo	188	14	7.44
	Cross River	18	10	55.5
	Ijah Gwari	20	5	20.0
G. fusca (3)	Cross River	3	2	66.66

Appendix XXII: Student's t-test on environmental conditions in Yankari Game Reserve

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
1	Mean temperature	33.544	18	1.1957	.2818
	Mean R.H	35.417	18	4.9386	1.1640

Paired Samples Test

		independent Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Mean temperature - Mean R.H	-1.8722	5.0270	1.1849	-4.3721	.6276	-1.580	17	.133

Appendix XXIII: Student's t-test analysis of environmental conditions in Kainji Lake National Park

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
1	Mean temperature	30.760	10	3.6317	1.1484
	Mean R.H	51.700	10	5.3396	1.6885

Paired Samples Test

		Independent Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
paired 1	Mean temperature - Mean R.H	-20.9400	6.2276	1.9693	-25.3949	-16.4851	-10.633	9	.000

Appendix XXIV: Student's t-test on environmental conditions in Old Oyo National Park

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
1	Mean temperature	30.936	11	.8857	.2671
	Mean R.H	65.318	11	3.6964	1.1145

Paired Samples Test

		Independent Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Independent 1	Mean temperature - Mean R.H	-34.3818	3.1422	.9474	-36.4928	-32.2708	-36.290	10	.000

Appendix XXV: Student's t- test analysis of environmental conditions in Cross River National Park

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Mean temperature	27.357	7	1.6257	.6145
	Mean R.H	90.286	7	7.3760	2.7879

Paired Samples Test

		Independent Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
paire 1	Mean temperature - Mean R.H	-62.9286	8.7912	3.3228	-71.0591	-54.7980	-18.939	6	.000

Appendix XXVI: Student's t-test on environmental conditions in Ijah Gwari National Park

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Temperature	30.40	5	2.702	1.208
	Relative humidity	61.70	5	13.283	5.941

Paired Samples Test

		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Temperature - Relative humidity	-31.300	15.172	6.785	-50.139	-12.461	-4.613	4	.010

Appendix XXVII: Correlation between tsetse catches, ambient temperature and relative humidity in Yankari Game Reserve

Correlations[a,b]

Temperature vs number of tsetse caught		Total population per point of collection	Temperature (ºC) at catch site
Total population per point of collection	Pearson Correlation	1	**-.220**
	Sig. (2-tailed)		**.380**
	N	18	18
Temperature (ºC) at catch site	Pearson Correlation	**-.220**	1
	Sig. (2-tailed)	**.380**	
	N	18	18

a. Catch site = Yankari National Game Reserves.

b. Footnote: there is a negative correlation ($r_{(16)} = -0.220$) between number tsetse flies and temperature; however, the relationship is not statistically significant ($p = 0.380$).

Correlations[a,b]

Humidity vs number of tsetse caught		Total population per point of collection	Relative humidity at catch site
Total population per point of collection	Pearson Correlation	1	**.368**
	Sig. (2-tailed)		**.134**
	N	18	18
Relative humidity at catch site	Pearson Correlation	**.368**	1
	Sig. (2-tailed)	**.134**	
	N	18	18

a. Catch site = Yankari Game Reserve

b. Footnote: there is a positive correlation ($r_{(16)} = 0.368$) between number tsetse flies and relative humidity; however, the relationship is not statistically significant ($p = 0.134$)

Kainji Lake National Park

Correlations[a,b]

Temperature vs number of tsetse caught		Total population per point of collection	Temperature (ºC) at catch site
Total population per point of collection	Pearson Correlation	1	**-.201**
	Sig. (2-tailed)		**.577**
	N	10[b]	10
Temperature (ºC) at catch site	Pearson Correlation	**-.201**	1
	Sig. (2-tailed)	**.577**	
	N	10	10

a. Catch site = Kainji Lake National Park

b. Footnote: there is a negative correlation ($r_{(8)} = -0.201$) between number tsetse flies and temperature; however, the relationship is not statistically significant ($p = 0.577$)

Correlations[a,b]

Humidity vs number of tsetse caught		Total population per point of collection	Relative humidity at catch site
Total population per point of collection	Pearson Correlation	1	**-.211**
	Sig. (2-tailed)		**.559**
	N	10	10
Relative humidity at catch site	Pearson Correlation	**-.211**	1
	Sig. (2-tailed)	**.559**	
	N	10	10

a. Catch site = Kainji Lake National Park

b. Footnote: there is a negative correlation ($r_{(8)} = -0.211$) between number tsetse flies and relative humidity; however, the relationship is not statistically significant ($p = 0.559$)

Old Oyo National Park

Correlation a

Temperature vs number of tsetse caught		Total population per point of collection	Temperature (ºC) at catch site
Total population per point of collection	Pearson Correlation	1	**.096**
	Sig. (2-tailed)		**.779**
	N	11	11
Temperature (ºC) at catch site	Pearson Correlation	**.096**	1
	Sig. (2-tailed)	**.779**	
	N	11	11

a Catch site = Old Oyo National Park

b. Footnote: there is a positive correlation (r (9) = 0.096) between number tsetse flies and temperature; however, the relationship is not statistically significant (p = 0.779)

Correlations b

Humidity vs number of tsetse caught		Total population per point of collection	Relative humidity at catch site	
Total population per point of collection	pearson correlation	1	**.089**	
	sig. (2-tailed)		**.795**	
	n	11	11	
Relative humidity at catch site	pearson correlation	**.089**	1	
	sig. (2-tailed)	**.795**		
	n	11	11	

a. Catch site = Old Oyo National Park

b. footnote: there is a positive correlation ($r_{(9)}$ = 0.089) between number tsetse flies and relative humidity; however, the relationship is not statistically significant (p = 0.795).

Cross River National Park

Correlationsa,b

Temperature vs number of tsetse		Total population per point of collection	Temperature (ºC) at catch site	
Total population per point of collection	Pearson Correlation	1	.326	
	Sig. (2-tailed)		.528	
	N	6	6	
Temperature (ºC) at catch site	Pearson Correlation	.326[b]	1	
	Sig. (2-tailed)	.528		
	N	6	6	

a. Catch Site = Cross River National Park

b. Footnote: there was a positive correlation ($r_{(4)} = 0.326$) between number of tsetse and temperature; however, the relation is no statistically significant ($p = 0.528$).

Correlations a,b

Humidity vs number of tsetse caught		Total population per point of collection		relative humidity at catch site
Total population per point of collection	Pearson Correlation	1		**-.530**
	Sig. (2-tailed)			**.280**
	N	6		6[b]
Relative humidity at catch site	Pearson Correlation	**-.530**		1
	Sig. (2-tailed)	**.280**		
	N	6		7

a. Catch site = Cross river

b. Footnote: there is a negative correlation ($r_{(4)} = -0.530$) between number tsetse flies and relative humidity; however, the relationship is not statistically significant ($p = 0.280$)

Ijah Gwari

Correlationsa

Temperature vs number of tsetse caught		Total population per point of collection	Temperature (ºC) at catch site
Total population per point of collection	Pearson Correlation	1	-.305
	Sig. (2-tailed)		.617
	N	5	5
Temperature (ºC) at catch site	Pearson Correlation	-.305	1
	Sig. (2-tailed)	.617	
	N	5	5

a. Catch site = Ijah Gwari

b. Footnote: there is a negative correlation ($r_{(4)} = -0.305$) between number tsetse flies and relative humidity; however, the relationship is not statistically significant ($p = 0.617$)

Correlations[a,b]

Humidity vs number of tsetse caught		Total population per point of collection	Relative humidity at catch site
Total population per point of collection	Pearson Correlation	1	0.705
	Sig. (2-tailed)		0.183
	N	5	5
Relative humidity at catch site	Pearson Correlation	0.705	1
	Sig. (2-tailed)	0.183	
	N	5	5

a. Catch site = Ijah Gwari

b. Footnote: there is a positive correlation ($r_{(4)} = 0.705$) between number tsetse flies and relative humidity; however, the relationship is not statistically significant ($p = 0.183$)

Appendix XXVIII: Protocol for DNA extraction using Chelex 100

- Add 1ml of 5% Chelex into microtubes containing tsetse fly mid-gut
- Incubate mixture in water bath for 1hr at 56^{o}C, then for 30minutes at 100^{o}C
- Centrifuge the tubes and their contents for 10minutes at 14,000rpm.
- Carefully collect supernatant into new tubes and discard former with debris
- Use supernatant as DNA template for your PCR reactions.

(Source: Walsh *et al*., 1991)

Appendix XXIX: PCR protocols for *Glossina* species identification/ population genetics

1. Cytochrome oxidase 1

a. *Glossina morsitans* specific

Sequence

Forward: TTACCTGGATTTGGT

Reverse: TGGAAATGAGCTACG

PCR-Reaction mix:

OSK2335	100 pmol/µL	0.5 µL
OSK2336	100 pmol/ µL	0.5 µL
Taq-Buffer (+KCl/-$MgCl_2$)	10x	2.5 µL
$MgCl_2$	25 mM	2.5 µL
DNTPs	10 mM	0.5 µL
Taq-Polymerase	5 U/µL	0.5 µL
ddH_2O		13.0 µL
Template	1 ng/µL	5 µL

PCR-Programm:

Initial denaturation	95 °C	5 min	
Denaturation	95 °C	30 s	
Annealing	51 °C	30 s	30 x
Elongation	72 °C	30 s	
Final Elongation	72 °C	6 min	
Store	4 °C	∞	

Notes:

Amplifies to *G. morsitans* only

Fragment size:

b. Insect generic primer

Sequence

Forward:
TTGATTTTTTGGTCATCCAGAAGT

Reverse:
TGAAGCTTAAATTCATTGCACTAATC

PCR-Reaction mix:

OSK2499	100 pmol/µL	0.5 µL
OSK2500	100 pmol/ µL	0.5 µL
Dream *Taq*-Buffer	10x	2.5 µL
BSA	2 mg/mL	2.5 µL
DNTPs	10 mM	0.5 µL
Dream *Taq*-Polymerase	5 U/µL	0.5 µL
ddH_2O		Fill up to 25 µL
Template	1 ng/µL	2.5 µL – 5 µL

PCR-Programm:

Initial denaturation	95 °C	5 min	
Denaturation	94 °C	1 min	
Annealing	55 °C	1 min	35 x
Elongation	72 °C	2 min	
Final Elongation	72 °C	10 min	
Store	4 °C	∞	

Notes:

Fragment size?! Prominent signal at ≈900 bp

Cycle number reduced to 30?

Appendix XXX: Hypothetical example of the principle of the Sterile Insect Technique

Generation .	No. of Insects	No. of insects released	The sterile to wild insects ratio	Percent sterility	No. of insects remaining fertile	Growth rate
P	1 000 000	9 000 000	91	90.0	100 000	Five fold
FI	500 000	9 000 000	18:1	94.7	26 316	Five fold
F2	131 579	9 000 000	68:1	98.6	1896	Five fold
F3	9 480	9 000 000	949:1	99.9	10	Five fold
F4	50	9 000 000	180 000:1	99.9	1	Five fold

(Adapted from Knippling 1955)

Appendix XXXI: List of consumable and non consumable items

Non - consumables

Item	**Supplier/Specification**
Agaroose- gel chamber	BioRad- SubCell gt
-80^{0}C Ultra Freezer	Eppendorf High efficiency
-20 Freezer	Vestfrost SZ 247C
Heat block	Eppendorf thermomixer comfort (1.5mL)
High speed Centrifuge	Eppendorf 5810R/5804R
Swing out rotor (plates)	Eppendorf A-2-DWP
Micropipettes	
100-1000µL	AB MATE
20-200µL	ABI MED ht
2-20Ml	ABIMED ht
0.1-2.5µL	Eppendorf research
Spectrophotometer	Nanodrop® Spectrophotometer ND1000
Table centrifuge	Eppendorf table centrifuge FAS 12-11
Thermocycler	
Thermocycler gradient	Eppendorf
2720 Thermo cycler	Applied biosystems
UV-chamber/camera	
Transilluminator	Herolab
Programme	EasyWin 32
Binocular Dissecting-	
Microscope	Leica S6E

Biconical traps	Vestergaard Fransen
High speed centrifuge	
Centrifuge 5417R	Eppendorf
Rotor FA45-24-1	Eppendorf
Microscope slides	VWR
Vortex mixer	CHINCAN XH-C vortex

Consumables

Microscope Slides	VXR
Microtitre plates	
96-well transparent	Sarsdedt
Parafilm	Bemis® Parafilm 'M'
Pipet tips micro pipets	Sarsdedt

Reaction tubes

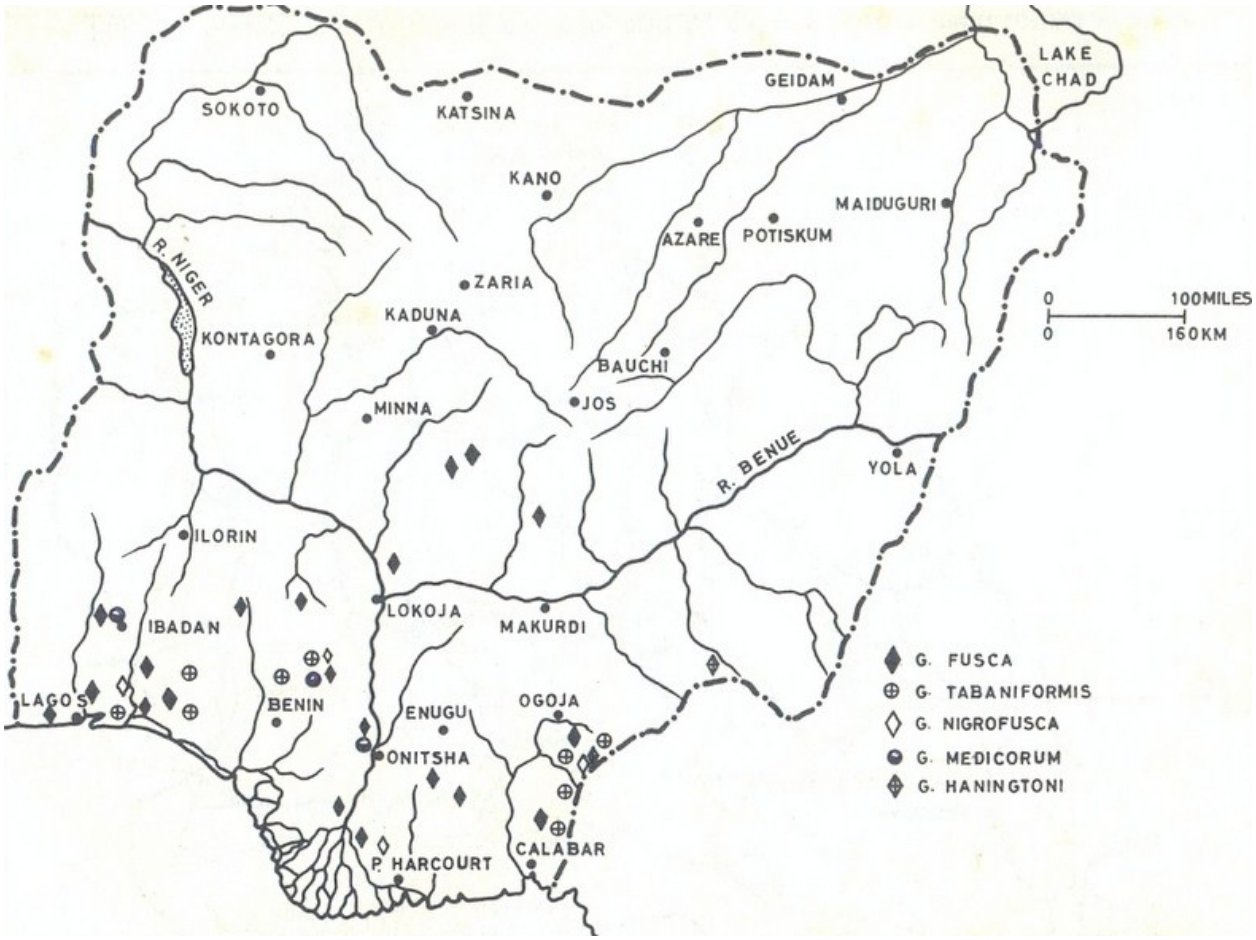
0.5mL tubes	Eppendorf
1.5mL tubes	Eppendorf
2.0mL tubes	Eppendorf
Acetone	Sigma-Aldrich
Agarose	Biozyme LE
EDTA	AnalaR Normapur
G-Stain	SERVA Electrophoresis

PCR Reagents

Dream-*Taq* Polymerase	Thermoscientific
X10 Green buffer	Thermoscientific
Taq Polymerase	Thermoscientific
X10 *Taq* buffer +KCl	Thermoscientific

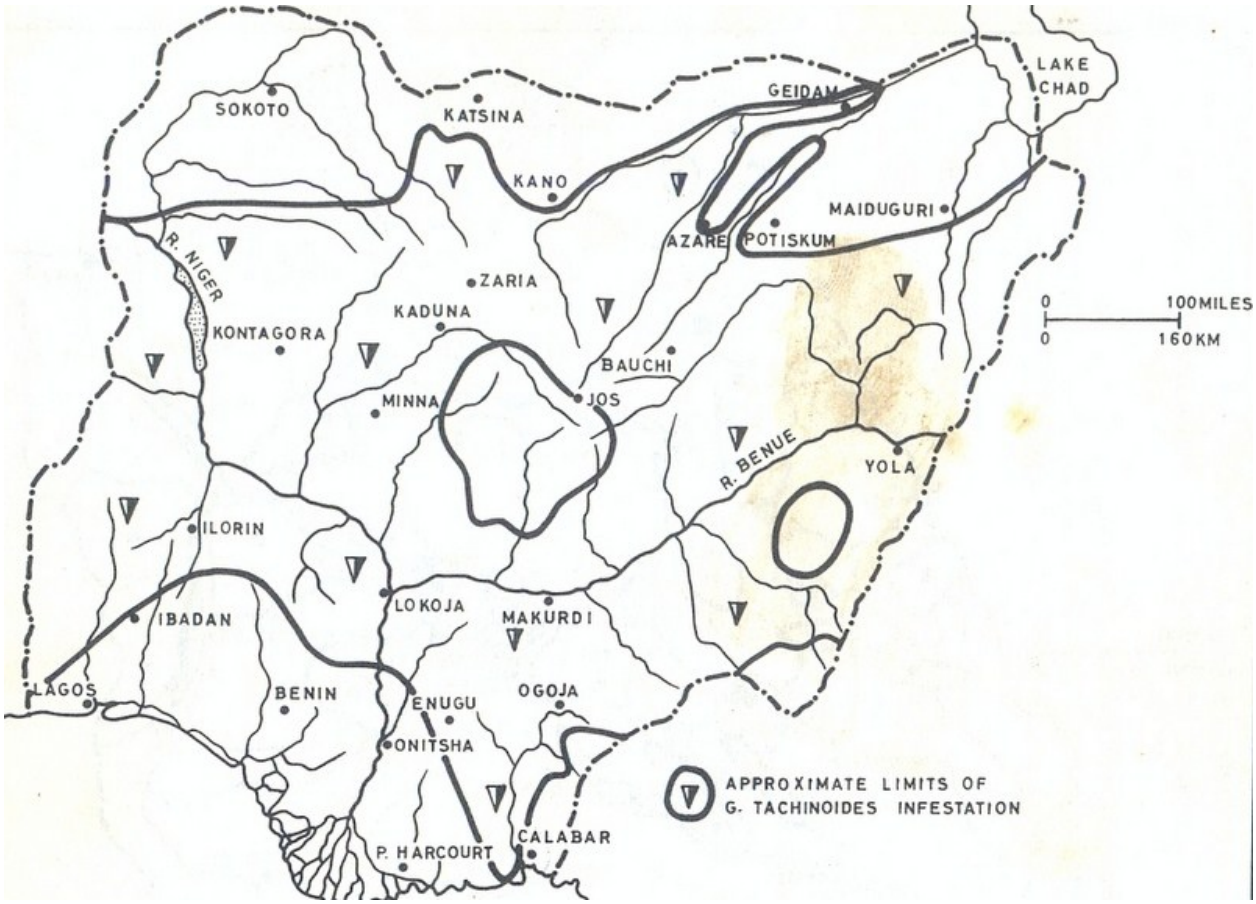
MgCl	Thermoscientific
dNTPs - mix (50mM)	Thermoscientific
Primers	Eurofins
RNA*later*	

Appendix XXXII: Distribution of the *fusca* group in Nigeria



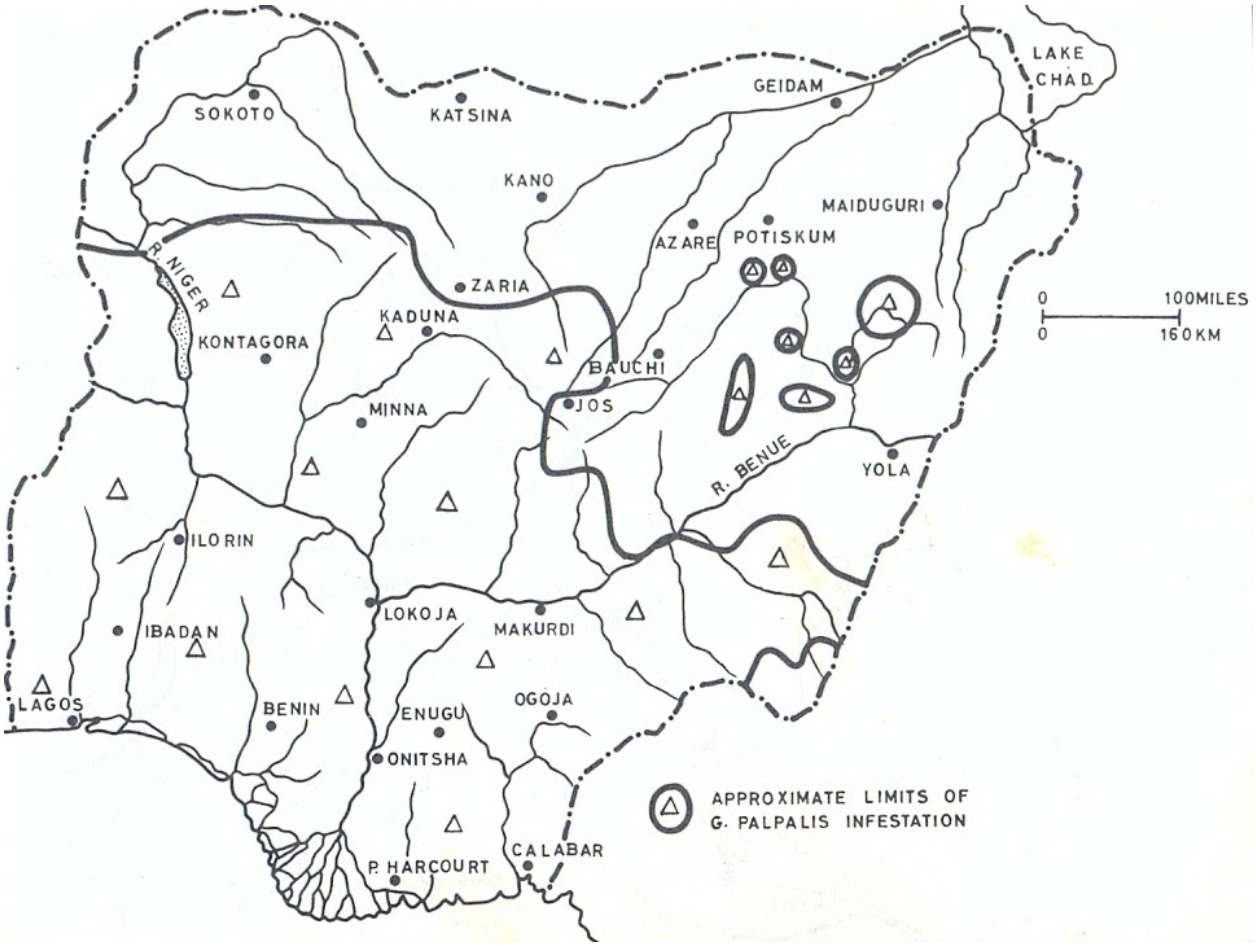
(Source: Davies 1977)

Appendix XXXIII: Distribution of *Glossina tachinoides* in Nigeria



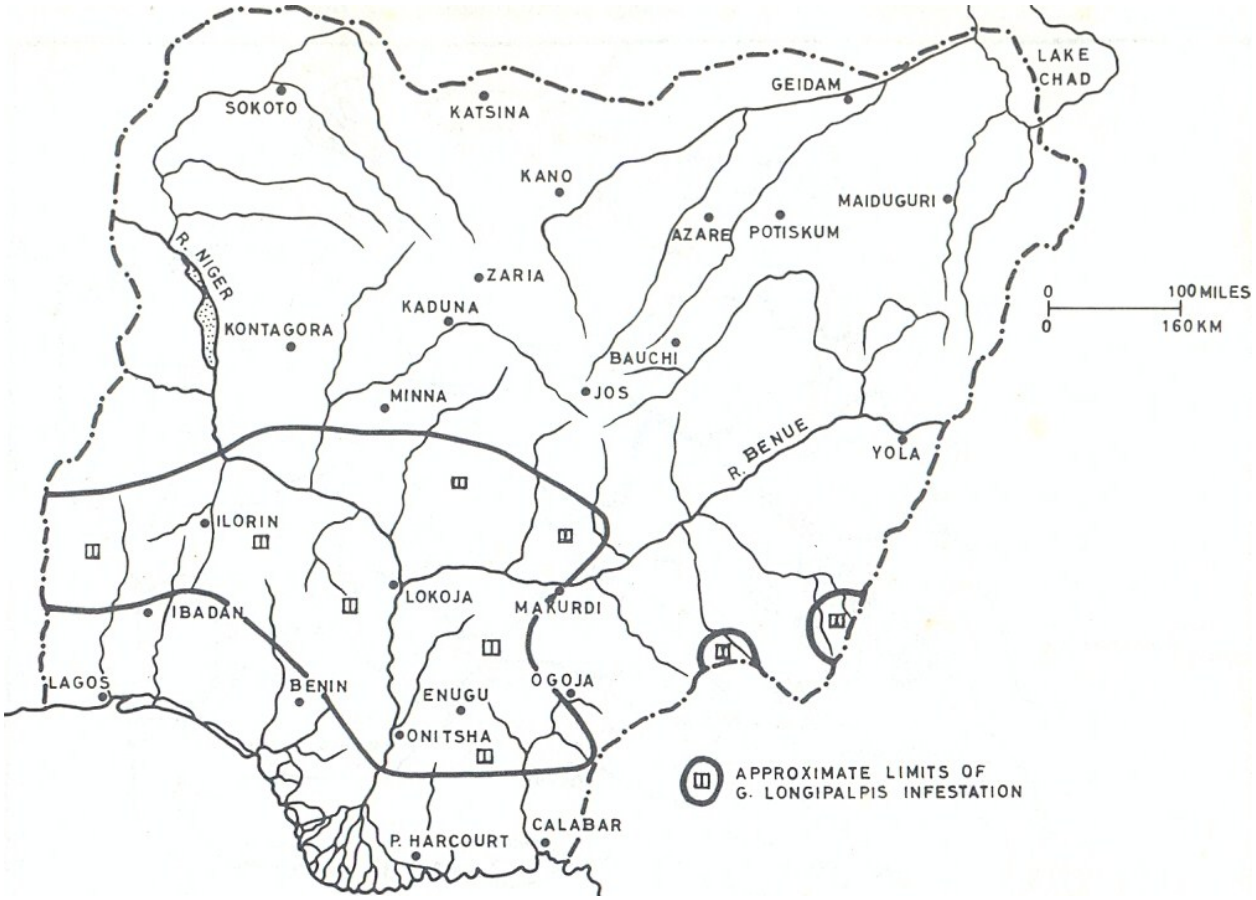
(Source: Davies 1977)

Appendix XXXIV: Distribution of *Glossina palpalis* in Nigeria



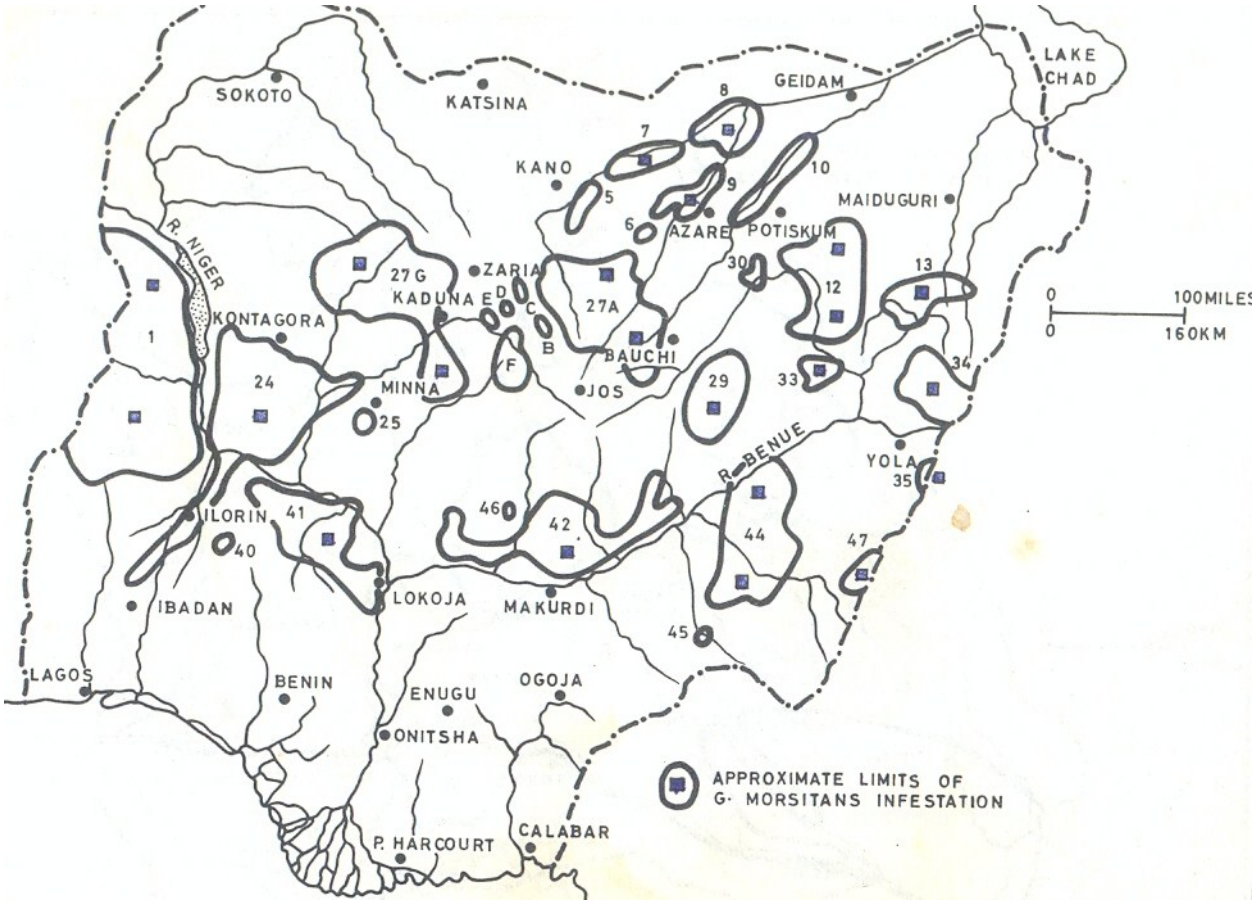
(Source: Davies 1977)

Appendix XXXV: Distribution of *Glossina longipalpis* in Nigeria



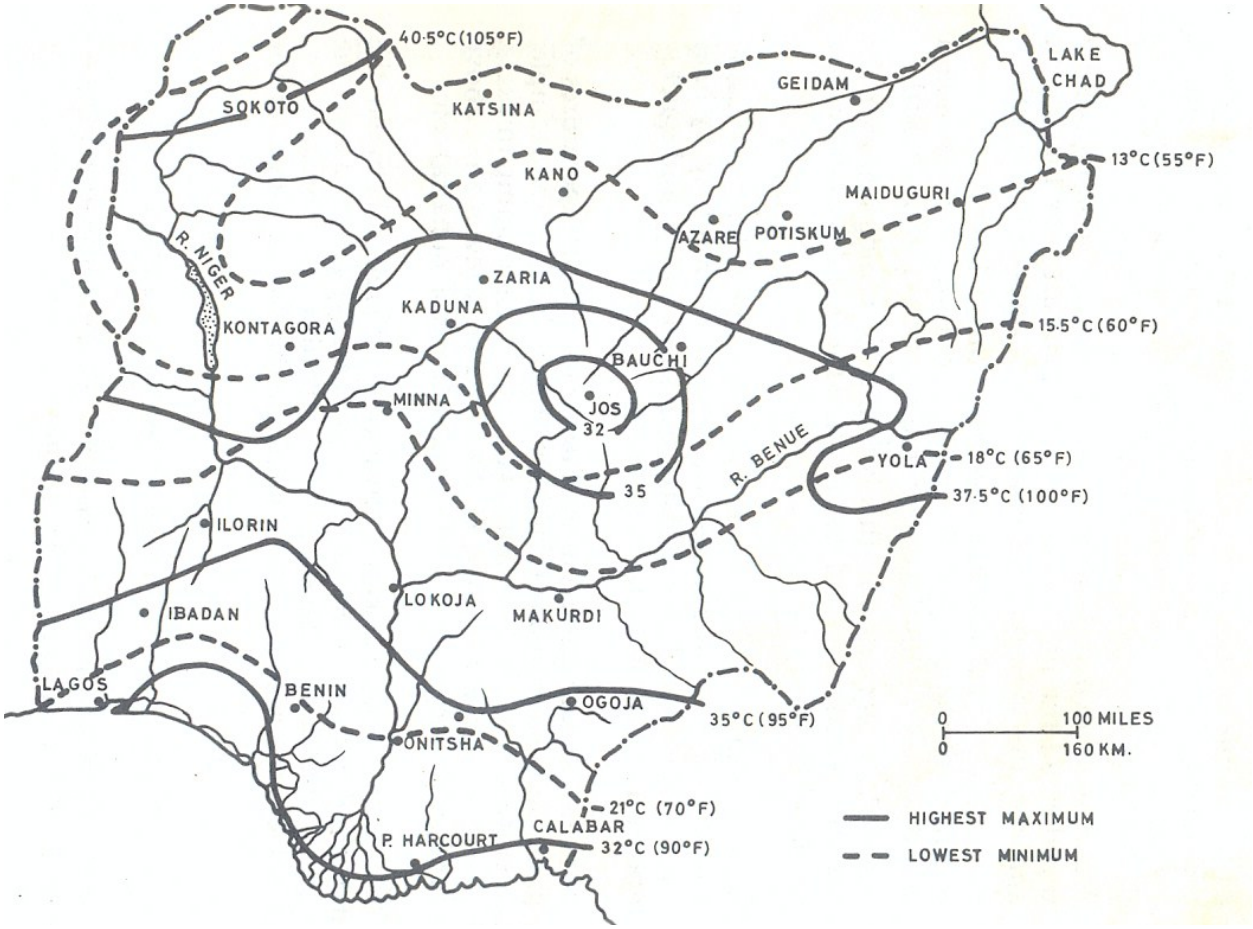
(Source: Davies 1977)

Appendix XXVIIVI: Distribution of *Glossina morsitans submorsitans* in Nigeria



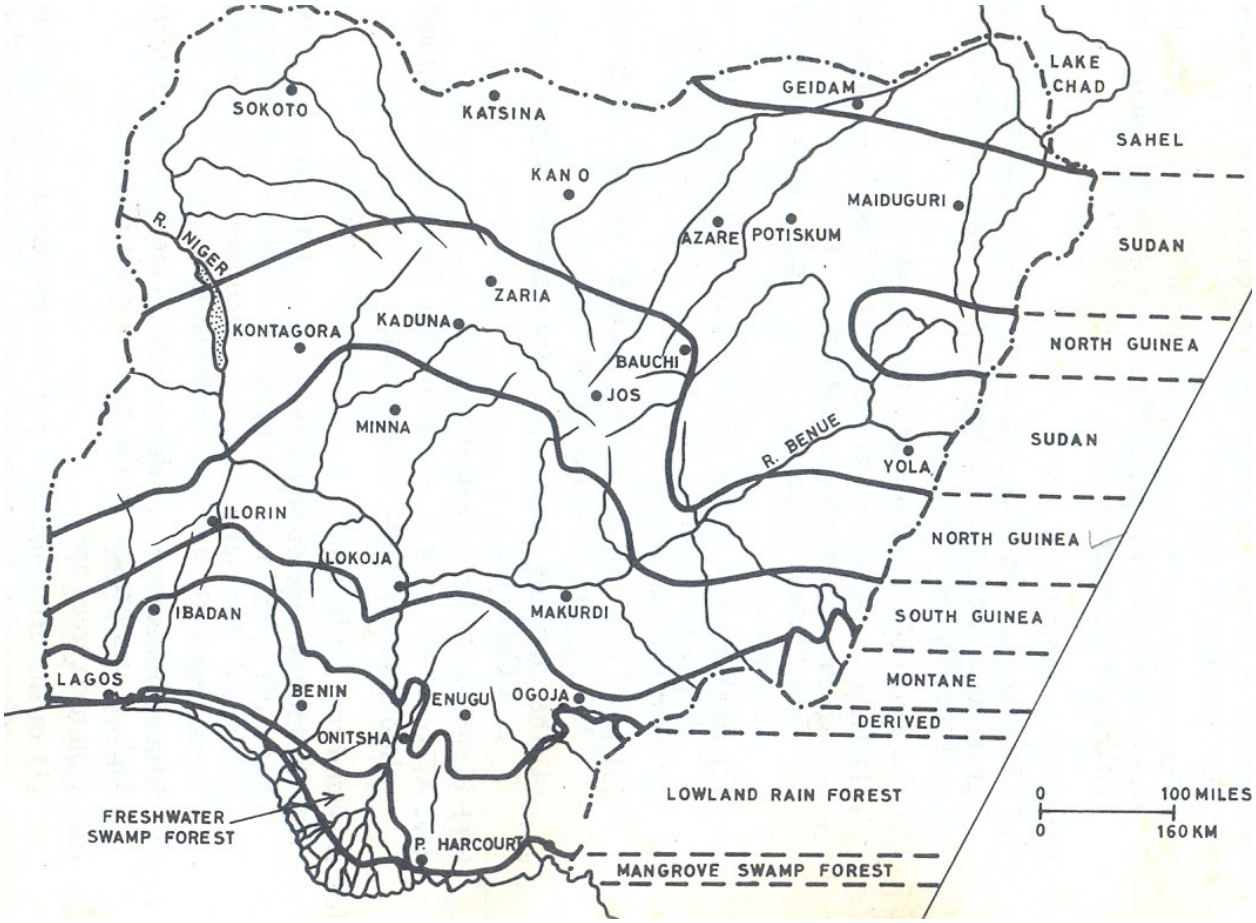
(Source: Davies 1977)

Appendix XXXVII: Minimum and maximum temperatures in Nigeria



(Source: Davies 1977)

Appendix XXXVIIIXXVIII: Vegetation zones of Nigeria



(Source: Davies 1977)

Appendix XXXIX: Sub-sample of tsetse flies used for DNA extraction

location	*Glossina* species								Total
	G. m. submorsitans		*G. tachinoides*		*G. p. palpalis*		*G. fusca*		
	♂	♀	♂	♀	♂	♀	♂	♀	
Yankari	4	22	10	13	-	-	-	-	49
Kainji	5	12	55	39	-	-	-	-	111
Old oyo	1	1	-	-	74	114	-	-	190
Cross river 1	-	-	-	-	3	1	-	3	7
Cross river 2	-	-	-	-	3	11	-	-	14
Ijah Gwari	-	-	-	-	10	10	-	-	20
Total by sex	10	35	65	52	90	136	-	3	391
Total	45		117		226		3		391

Appendix XL: Cytochrome Oxidase I Generic primer PCR positive samples

Glossina species	Location	No. Dissected	No. Amplified	% Amplification
G. m. submorsitans (45)	Yankari Game Reserve	26	13	50.0
	Kainji	17	12	70.58
	Old Oyo	2	1	50
G. tachinoides (117)	Yankari Game Reserve	23	5	22.0
	Kainji	94	7	7.40
G. p. palpalis (226)	Old Oyo	188	14	7.44
	Cross River	18	10	55.5
	Ijah Gwari	20	5	20.0
G. fusca (3)	Cross River	3	2	66.66

Appendix XLI: A female roan antelop killed by illegal poachers in one of the Game Reserves in Nigeria during the study.



Appendix XLII: A forest fly *Glossina fusca* caught during the survey being examined by one of the technical staff

