GENETIC DIVERSITY IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER* MEIGEN, 1830 FROM SAVANNA ZONE OF NIGERIA USING MICROSATELLITE MARKERS

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERISTY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER DEGREE IN BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
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DECLARATION

I hereby declare that this dissertation entitled "Genetic Diversity in Natural Populations of *Drosophila melanogaster* Miegen 1830 from Savanna Zone of Nigeria Using Microsatellite Markers" has been written by me and that it is a record of my own research work under the supervision of Professor J. Auta and Dr. D. M. Shehu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at any University.

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Rashidatu Abdulazeez Date

CERTIFICATION

This dissertation entitled "**GENETIC DIVERSITY IN NATURAL POPULATIONS OF *DROSOPHILA MELANOGASTER* MEIGEN 1830 FROM SAVANNA ZONE OF NIGERIA USING MICROSATELLITE MARKERS**" by Rashidatu, Abdulazeez meets the requirements governing the award of the Master of Science Degree in Biology, Faculty of Science, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to my parents, Mr. and Mrs. Abdulazeez, S. Akhadelor; may Almighty Allah (S.W.T) sustain you in good health to enjoy the fruit of your labour.

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ABSTRACT

A study was carried out to evaluate the genetic diversity in natural populations of *Drosophila melanogaster* using seven microsatellite loci. Fermented banana in hand-made bottle traps was used as bait with 10 replicate traps per site of collection. Samples were collected from November, 2015 to March 2016. A total of 42 male flies from Northern Guinea, Sudan and Sahel Savanna were used for the analysis. Genomic DNA was extracted using the phenol chloroform method; PCR products were amplified on 1.5% Agarose gel by Electrophoresis and scored using Molecular Imager® Gel Doc™ XR+ system with image Lab™ Software of BIO-RAD. GenAlex version 6.501 and MEGA 6 softwares were used to determine the genetic diversity and population structure. The results revealed that the markers were highly polymorphic (PIC>0.5) in all the sampled populations. The mean observed heterozygosity for all populations (1.000) was greater than the mean expected heterozygosity (0.500), although the populations were in Hardy-Weinberg Equilibrium (P>0.05). The highest genetic distance was observed in Northern Guinea vs. Sahel (2.639) while no distance was observed in Northern Guinea vs. Sudan (0.000) and Sudan vs Sahel Savanna (0.000). The lowest genetic identity of 0.000 was observed in Sudan vs. Sahel Savanna, followed by identity of 0.071 in Northern Guinea vs. Sahel Savanna and the highest (1.000) was observed in Northern Guinea vs. Sudan Savanna. The values of genetic distance and genetic identity showed that Northern Guinea and Sudan Savanna *D. melanogaster* are closely related, while Northern Guinea and Sahel *D. melanogaster* are divergent species. The AMOVA showed an estimated variation of 2163.35 with variation of 9% among vegetation zones, 0.00 among populations within zone and 22320.139 estimated variation with 91% variation within populations. The F_{IS} value was -1.000 which was lower than the F_{IT} (-0.675) indicating random mating and excess heterozygosity and the F_{ST} was 0.162. The pairwise F_{ST} and gene flow was 0.333and 0.545 respectively for both Northern Guinea vs. Sudan Savanna and Sudan vs. Sahel Savanna, whereas in Northern Guinea vs. Sahel Savanna, a lower F_{ST} (0.314) and higher gene flow (0.545) indicating a high population sub-structure and genetic isolation in natural populations of *D. melanogaster*. Northern Guinea and Sudan Savanna presented a close cluster in the first quadrant while Sahel Savanna presented a distinct quadrant. The Global Spatial Autocorrelation showed that there was no correlation between genetic distance and geographic distance (r = 0.212, P>0.05). It can be concluded that the used markers are highly polymorphic (PIC>0.5). The populations of *D. melanogaster* are highly genetically diversified; outbreeding; excess of heterozygotes, and are highly sub-structured with consistent clusters which quantify the degree of relationships between the zones.

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
AZC	Azare City (Kasuwan Azare)
AZV	Azare Village (Kuzuru)
D	Nei's Genetic Distance
DNA	Deoxyribonucleic Acids
F_{ST}	Genetic Differentiation for total population
F_{IS}	Genetic Differentiation for sub-populations
F_{IT}	Coefficient of Inbreeding
GSA	Global Spatial Autocorrelation
Ho	Observed Heterozygosity
He	Expected Heterozygosity
HWE	Hardy-Weinberg Equilibrium
I	Nei's Genetic Identity
KDC	Kaduna City (Anguwan-Dosa)
KDV	Kaduna Village (Danhono)
KNC	Kano City (Hotoro)
KNV	Kano Village (Gunduwawa)
LS	Level of Significance
NJ	Neighbor- Joining
Nm	Gene flow
Ne	Effective Number of Alleles
PIC	Polymorphism Information Content
PCR	Polymerase Chain Reaction
SSR	Simple Sequence Repeats
Vs	Versus

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Evolution is the dual process of genetic change and diversification of organisms through time resulting in populations diverging from one another in their genetic characteristics thus giving rise to new species. The leading evolutionary forces such as mutation, natural selection, and genetic drift have created a vast diversity of sub populations which led to the formation of many well defined species with different levels of performance (Mahmut, 2012).

*Drosophila melanogaster (*Diptera: Drosophilidae) is generally known as fruit fly or vinegar fly. The fly is said to have probably originated from sub-Sahara Africa (Capy *et al.,* 2004), but is also able to proliferate under temperate climate which could be as a result of the spread of beneficial mutations in non- Africa populations (Kirby and Stephan 1996; Kauer *et al.,* 2003) and selection pressure imposed by man such as the species resistance to insecticides (Daborn *et al.,* 2001). *Drosophila melanogaster* is probably considered the most differentiated into geographic subpopulations (David *et al.,* 2007).Wild type fruit flies are yellow-brown, with brick red eyes and transverse black rings across the abdomen. They, exhibit sexual dimorphism, females are about 2.5 millimeters (0.098 in) long; males are slightly smaller with a distinct black patch on the abdomen, and a cluster of spiky hairs (claspers) surrounding the reproducing parts used for attachment to the female during mating (Flybase, 2009). The "Drosophila season" as stated by Pavkovic and Kekic (2014) is usually between March to October due to abundance of fruits and vegetables.

Although there are obvious differences between humans and *D. melanogaster*, there are many molecular and cellular processes that are common between humans and the fruit fly such as aggression, sleep, learning, memory, circadian rhythm and mating which makes the fruit fly

an important model in the investigation on functions of specific genes, diseases and effectiveness of various promising therapeutic drugs (Valente *et al.,* 2004). The short life cycle, large number of offsprings and small genome which was fully sequenced in year 2000 makes genetic manipulation of the fruit flies easy (Adams *et al.*, 2000).

D. melanogaster serves as a multiple model organism as its embryo, larva, pupa and adult can be used as models in different toxicological settings. For instance, the embryo and the pupa can be used as models in developmental toxicological studies; the larva can be used as a model for physiological and behavioural studies, while the adult fly posses structures that can mimic the equivalent functions of mammalian reproductive tract, heart, kidney, gut and lung. (Nichols *et al*., 2002; Wolf and Heberlein, 2003; Andretic *et al*., 2008). It has been estimated that about 75% of known human disease genes have a recognizable match in the genome of fruit flies (Reiter *et al*., 2001). *Drosophila* is nowadays often used as a "test tube" to screen for genetic components of disease-relevant processes or pathways, or to unravel their cellular and molecular mechanisms, covering a wide range of disease mechanisms including neurodegenerative (Parkinson's, Huntington's, spinocerebellar ataxia and Alzheimer's disease), neurotoxicology (Bier, 2005; Rand, 2010; Hu *et al.*, 2011; Jaiswal *et al*., 2012) and is also being used to study mechanisms underlying aging and oxidative stress, epilepsy, immunity, diabetes, and cancer, as well as drug abuse (Chien *et al*., 2002). This is to say that the fly has basic biological, biochemical, neurological, and physiological similarities with mammals (Abolaji *et al*., 2013).

The fruit fly may have ten or more generations per year, oviposit in a wide variety of substrates and considered a generalist feeder (Markow and Grady, 2008). *D. melanogaster* is commonly considered a pest due to its tendency to invade and establish populations where fruit crops are grown. The flies are seen in homes, restaurants, stores, and even in dump sites.

Reduction of an infestation on a fruit farm can be difficult, as larvae may continue to hatch in nearby fruit even as the adult population is eliminated (James, 2009).

The use of fruit flies as an invertebrate model organism in the field of classical genetics was introduced more than a century ago due to the fact that it is an omnipresent follower of human culture, easily obtainable and easily maintained in laboratories (Kohler, 1994). Its genetics have been systematically applied to the study of development, physiology and behaviour, generating new understanding of the principal genetic and molecular mechanisms underpinning biology, many being conserved with higher animals and humans (Ashburner, 1993; Keller, 1996; Martinez, 2008; Bellen *et al.*, 2010). Therefore, diversity analysis and identification of genotypes are vital to the *D. melanogaster* conservation, control and breeding programmes.

Genetic diversity which is the total number of genetic characteristics in the genetic makeup of a species is a combination of both variety and variability and a requirement for populations to evolve and cope with environmental changes, new diseases, and pest epidemics (Mahmut, 2012) and which also significantly influences the long-term viability and persistence of local populations (Sushila and Jaya, 2013). Genetic variation is one of the three levels of biodiversity that the World Conservation Union (IUCN) has recommended for conservation, as it is a very important requirement for evolution and a direct linkage to population fitness (Reed and Frankham, 2003). Genetic variation exists within and among members of populations which is brought about by mutation: which is a change in the chemical structure of a gene, random mating, and recombination between homologous chromosomes (Lars *et al*., 2006).This provides a huge source of information about the biology of an individual species, their history and spatial relationships between populations. The amount and nature of genetic variation in a population allows for the estimation of effective population size,

structure, how selection acts on genes and location of diseases on genes (QTL mapping), (David *et al.*, 2005).

The assessment of genetic diversity may be done at molecular level by using different markers based techniques such as allozymes (Biochemical marker), Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RADP-PCR), Restriction Fragment Length Polymorphism (RFLP), Microsatellite (Molecular markers) (Penzes *et al*., 2002). The molecular markers are more accepted because they overcome many of the limitations morphological and biochemical techniques poise since they are not affected by the environment or developmental stages and can detect a variation at the DNA level.

Microsatellite marker is among the most recently developed molecular marker which gives a much higher estimate resolution even at small spatial scales when compared with other markers such as allozymes, RAPD (Turlure *et al.*, 2014). It is currently the marker of choice for molecular genetic studies such as reconstruction of phylogenetics and relationships among populations (MacHugh *et al*., 1997), determination of paternity and kinship analyses, forensic studies, linkage analysis and population structures (Arora and Bhatia, 2004; Schlotterer, 2004) because they are highly polymorphic, highly abundant, co-dominantly inherited, easy to analyze and score. However, null alleles, or size homoplasy could be seen in using the marker (Schlotterer, 2004).

Microsatellite also known as Simple Sequence Repeats (SSR) is a class of repetitive DNA elements, which according to Kahl (2001) is any one of a series of very short (2-10 base pairs), middle repetitive, tandemly arranged, highly variable DNA sequences which are dispersed throughout living organisms genomes. They are generally found in nuclear genome, usually in the introns of genome. Microsatellites are "junk" DNA, and are selectively neutral (Li *et al*., 2002). Microsatellite alleles when amplified are of variable lengths which can be separated by gel electrophoresis and visualised by silver-staining,

autoradiography (if primers are radioactively labelled) or via automation (if primers are fluorescently labelled) (FAO/IAEA, 2002).

1.2 Statement of the Research Problem

Every organism in its natural habitat is faced with constantly changing pressure from natural forces, such as temperature, light, competition, predation, or from human impacts such as pollution, habitat destructions which result in a highly variable environment (Sofija and Vladimir, 2014). In order for a species to survive, part of the population of that species must exhibit sufficient genetic variability to adapt to the changing environment; this forms the basis of natural selection (Bader, 1998).

Genetic variations among *D. melanogaster* population have been analyzed using different genetic markers in different parts of the world (Kaurer *et al.,* 2003; Scholotter *et al.,* 2005). No reported studies have been conducted in the Savanna zone of Nigeria thereby leaving the genetic structure and genetic relationship of this species unexplored in this geographic area.

1.3 Justification

The level of genetic variation among populations has received considerable attention, because it is indicative of overall species fitness and potential for evolutionary responses to environmental changes (Mateus and Sene, 2003).

The recent ethical issues on the use of Mice, Bacteria, Nematodes and Zebra fish have led scientists to seek for a cost effective research organisms that can be studied for many, if not all perspective with little ethical concerns (Koushik and Krishna, 2013). *Drosophila* especially *D. melanogaster* may be such an organism whose genetics have revealed it to be a powerhouse for unraveling concepts and fundamental understanding of basic biology.

Knowledge on the genetic diversity in natural populations of *D. melanogaster* would provide relevant information for developing strategies to conserve its genetic resources, for genetic

control, developing new strains for neuroscience, for breeding programmes, and would also provide insights regarding the natural history and evolutionary relationship of this insect.

1.4 Aim of the Study

The aim of this study is to investigate the genetic diversity in natural populations of *D. melanogaster* from Savanna zones of Nigeria using Microsatellite markers.

1.5 Objectives of the Study

i. To determine the degree of polymorphism in markers from *D. melanogaster* populations.
ii. To determine the genetic diversity within and among natural populations of *D. melanogaster* from Savanna zone of Nigeria.
iii. To determine the genetic relationships within and among *D. melanogaster* populations from Savanna zone of Nigeria.

1.6 Hypotheses

i. There is no polymorphism in markers from *D. melanogaster* populations.
ii. There is no genetic diversity within and among natural populations of *D. melanogaster* from Savanna zone of Nigeria.
iii. *D. melanogaster* populations from Savanna zone of Nigeria are not genetically related.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Drosophila melanogaster*

Drosophila melanogaster belongs to the Kingdom: Animalia, Phylum:Arthropoda, Class: Insecta, Order: Diptera, Family: Drosophilidae, Genus: Drosophila, Subgenus: Sophophora, Species: melanogaster. The species is known generally as the common fruit fly or vinegar fly (Meigen, 1830).

Ashburner *et al*. (2005), in their various investigations about Drosophilids, discovered that *Drosophila melanogaster* undergoes complete metamorphosis (egg to larva to pupa and to adult), with a developmental period varying with temperature, as with many ectothermic species: its shortest developmental time (7 days), is achieved at 28˚C (82˚F), which increases to 11 days at higher temperature of 30˚C (86˚F) due to heat stress. Its lifespan is about 30 days at 29˚C (84˚F) and with a record of an increased lifespan of 3 months. Hirth (2010) also observed that diet such as cornmeal prolongs the lifespan of the fly, while diets with high quantities saccharides and cholesterol can reduce life expectancy. According to Meeta *et al.* (2008); Acurio *et al.* (2010); Penariol and Madi- Ravazzi (2013), Drosophilids especially *D. melanogaster, D. simulans, D. malerkotliana* are primarily found in environments disturbed by man, in open areas, or in degraded and urbanized environments which are characterized by a pronounced degree of environmental stress.

The genome of *D. melanogaster* consists of 139.5 million base pairs with approximately 15,682 genes NCBI, (2011) and it is known to have only one or very few genes coding for members of the same protein class (low redundancy) unlike the genome of higher organisms which have several paralogous genes coding for closely related proteins that tend to display functional redundancy and complicate loss-of-function analyses (Giacomotto and Segalat 2010). Reverse genetics through a wide range of available genetic strategies and tools can be

performed on virtually every gene of *Drosophila* (Ni *et al.*, 2009: Venkan, *et al.*, 2009). Bassett *et al.* (2013) adapted the CRISPR/Cas9 system to mutagenize genes in *Drosophila melanogaster* by injecting a mix of Cas9 mRNA and sgRNA intosyncytial blastoderm-stage embryos. They discovered that injection of RNA into the *Drosophila* embryo can induce highly efficient mutagenesis of desired target genes which can be transmitted efficiently through the germline to make stable lines.

The brain of the fly possesses more than 100,000 neurons that are important in flight navigation, circadian rhythms, memory, feeding, courtship and aggression (Abolaji *et al.*, 2013). *D. melanogaster* possesses circulating hormones, plasma membrane receptors (PMRs) and nuclear receptors (NRs) governed by the same chemical and biological mechanisms found in the hormonal system of vertebrates (Riddiford, 1993). The gene expression of *D. melanogaster* is controlled by more than 60% functional non- protein –coding DNA, (Halligan and Keightley, 2006). The fruit fly has four pairs of chromosomes: an X/Y pair and three autosomes labeled 2, 3, and 4 with the 4^{th} being so tiny that it is often been ignored, it is responsible for the *eyeless* gene (Adams *et al*., 2000). *Drosophila melanogaster* is an interesting model system for toxicologists due to the similarities of molecular processes involved in the control of lifespan and aging, coupled with good degree of genetic homology between *D. melanogaster* and human (Pandey and Nichols, 2011; Poddighe *et al*., 2013).

According to Wiemerslage *et al.* (2013), *D. melanogaster* have only innate immunity which is divided into two responses: humoral and cell-mediated, it lacks an adaptive immune response. A comparison study on the genome- wide variation in the human and *Drosophila melanogaster* was carried out by (Aquadro *et al.*, 2001). The result revealed that average levels of nucleotide diversity are ten-fold lower in humans than in *D. melanogaster*. Both species also show lower levels of variation on average in non-African compared to African

populations, reflecting a similar evolutionary history and perhaps occurrence of both natural selection and founder effects in new environments.

Drosophila melanogaster populations in Panama along a transect of 40 latitudinal degrees on the west coast of South America showed a negative correlations with latitude for Adh^{s} and $_{\alpha}Gpdh^{F}$ allele frequencies and for the frequency of the cosmopolitan inversion *In(2L)t* in $Adh^{s}\alpha pdh^{F}$ chromosomes while a positive correlation existed between wing length and latitude. Significant correlations were found between these traits and climatic variables like temperature and rainfall which was found to be considerably similar with those of other continents (Jan Van *et al.*, 2007).

Effect of low stressful temperature (13^{o}C) was investigated on genetic variation for five quantitative traits in *D. melanogaster* (thorax length, wing length, sternopleural bristle number, developmental time and larva-to-adult viability) using the half sib analysis. Both phenotypic and environmental variation showed a significant increase under stressful conditions in all traits, no statistically significant differences were found between the two environments for estimates of genetic variation, and the data suggested that the effect of stressful temperature may be trait-specific (Bubliy and Loeschcke, 2009). The genetic basis of adaptive pigmentation variation in *Drosophila melanogaster* was investigsted. A strong positive correlation was found between dark pigmentation and high altitude, suggesting adaptation to specific ecological conditions (Pool and Aquadro, 2006). The 'thermal budget hypothesis' that pigmentation differences help flies to maintain optimal body temperature in varying environments has been suggested to account for geographical variation of other pigmentation traits in *D. melanogaster* (David *et al*. 1985; Gibert *et al*. 2004).

Abundance of *Drosophila species* in Botanical garden and selected fruit stands in Zaria was investigated. The study revealed *Zaprionus tuberculatus* (69.38%), an invasive species was

more abundant than *Drosophila melanogaster* (30.63%) (Olajide *et al.,* 2016). Goji, *et al.* (2016), investigated the amelioration of *D. melanogaster* mutants with aqueous extract of *Commiphora Africana,* revealed that *C. africana* extract significantly ameliorated the body weight, total lipid and carbohydrate content which means *C. africana* may have some ameliorating some complications that come with diabetes mellitus.

2.2 DNA Markers and Genotyping

DNA which stands for DeoxyriboNucleic Acid is the genetic material whose genetic information for hereditary are encoded in the nucleotides Guanine (G), Adenine (A), Thymine (T) and Cytosine (C) which make sequences that encode for amino acids. DNA is a double helix structure whose backbone is made of deoxyribose sugar and phosphate groups. Nucleotides are attached to the backbone with hydrogen bindings. DNA is tightly arranged in chromosomes in the nucleus of a cell. A functional sequence of DNA is called a gene whose sequences of nucleotides vary in repeats, deletions/insertions, and transition/ transversions from one individual to another. These variations in DNA form the basis of genetic variation in the same species (Eenennaam, 2009). The studies of effective population size, population history, population structure and various genetic diseases are due to genetic variation (Mburu and Hannote, 2005).

Genotype is the molecular code of the DNA that can also lead to the study of phenotype of an organism while genotyping is the process of analyzing the variation of an individual's genotype by examining the DNA sequence from its biological assay and also by comparing it with other individual's sequences. Genotyping makes it easy to identify the inherited properties of the individual within its family and also how they differ between other species (Eenennaam, 2009).

2.3 Genetic Diversity

Braganholi *et al.* (2010) using RAPD determined the genetic variability among natural populations of *Zaprionus indanus* (Drosophilidae) in the states of Sao Paulo and Minas Gerais, Brazil. F_{ST} value was high (0.524), low gene flow value (0.3727), genetic variability ranged from 14.1 to 52.1%, genetic distance range was from 0.0804 to 0.6086, the correlation coefficient for genetic and geographic distances, obtained was significant ($r = 0.52$; $P < 0.05$ with 5000 permutations) suggesting isolation by distance**.**

According to Luciana *et al.*(2012), the genetic diversity of *D. ornatifrons* from the Atlantic forest biome in South America by electrophoretic analyses of 9 isoenzymatic systems resulted in 7 loci and 46 alleles, *Ho* was between 0.149 and 0.406, *He* ranged between 0.488 to 0.733, F_{IS} was 0.4896, F_{IT} was 0.5621, F_{ST} was 0.1420, Nei's genetic distance analyses showed variation between 0.3145 and 0.7166, Nei genetic identities ranged between 0.4884 and 0.7302, the effective number of migrants (*Nm*) was calculated as 1.51 and all loci showed significant departure from the expected Hardy-Weinberg equilibrium.

Genetic variation has been determined with 7 isozyme system analysis in 8 natural house fly (*Musca domestica* L.) populations from the western and southern coasts of Turkey. The proportion of polymorphic loci in populations ranged from 44.44% to 66.67%. The overall mean number of observed alleles per locus (*Na*) was 1.778 ±0.878, the overall mean number of effective alleles per locus (*Ne*) was 1.314±0.454. The overall mean observed heterozygosity (*Ho*) was 0.205±0.278 while the mean estimated expected heterozygosity (*He*) of populations was 0.177±0.228. F_{IS} was –0.2002 indicating random mating, F_{ST} was 0.0630. The average level of gene flow (*Nm*) was calculated to be 3.7182 per generation among all populations studied while Genetic distance coefficients (*DN*) ranged from 0.0026 to 0.0487 among population pairs .A very high proportion (93.70%) of genetic diversity was due to variation within populations while a relatively small portion (6.30%) was due to variation among populations (Taskin *et al.,* 2011).

Population genetic analysis was performed in 45 (forty- five) natural Indian populations of *D. ananassae* using cosmopolitan inversions as marker. *Ho* ranged from 0.15 – 0.61, *He* ranged from 0.15 – 0.45, F_{IS} ranged from -0.53 to 0.47, F_{ST} values ranged from -0.04 to 0.64, F_{IT} ranged from -0.41 to 0.68 and Pairwise genetic distance (*D*) values among populations range from 0.000 to 0.436. Genetic distance and geographic distance were insignificantly correlated ($r = 0.200$; $p > 0.05$). The study revealed that *D. ananassae* showed a strong sub- structured population due to genetic drift (Pranveer and Bashisth, 2010).

Tripathi *et al*. (2013) assessed four populations of the common house fly *Musca domestica* using allozymes at four gene enzyme systems. Number of alleles ranged from 1.85 to 2.07 with a mean of 1.97, the percentage of polymorphic loci ranged from 50.00% to 75.00% with a mean of 68.75% and the *Ho* ranged from 0.250 to 0.447, with a mean of 0.356. The *He* ranged from 0.483 to 0.534, with a mean of 0.510. Nei's genetic identity (*I*) values were between 0.558 and 0.955 and the Nei's genetic distance (*D*) values were between 0.046 and 0.584. F_{IS} mean value was 0.314 and F_{ST} mean was 0.135.

Genetic variability in local Bulgarian Honey bees (*Apis mellifera rodopica*) was analysed using six enzyme systems (allozymes) and nine microsatellite loci. It was calculated that the average number of alleles per locus was 3, the percent of polymorphic loci (P=0.95) was 83.3 and the *Ho* and *He* was 0.24 and 0.259, respectively for the enzyme system. It was found that the *Ho* varied between 0.444 and 0.567 and the *He* between 0.435 and 0.548 for the microsatellite loci. There were no significant deviations of genotype frequencies from Hardy-Weinberg expectations at most of the loci in population ($0.99 > P > 0.1$) (Nikolova, 2012).

Genetic diversity of a threatened butterfly (*B. aquilonaris*) was carried out using three markers (microsatellites, RAPD and allozymes) in order to compare their relative utility, reliability and relevance in designing species-specific conservation measures. The results revealed a polymorphic loci ranging between 0.692-1 for 15 microsatellites, 0.526-0.895 for

RAPD, and 0.50 ±0.13 for allozymes. *He* ranged from 0.294- 0.576, 0.239- 0.388 and 0.188±0.029 respectively. Mean number of alleles was 3.103±0.615 and allelic richness was 1.250±0.171 for microsatellite loci. Significant inbreeding was detected in two of the population with F_{ST} of 0.125 at regional and 0.130 at landscape for microsatellites, F_{ST} of 0.129 at regional and 0.082 at landscape for RAPD and F_{ST} of 0.171 at regional and 0.091 at landscape for allozymes. 3 clusters were detected using microsatellites while 2 genetically distinct clusters were seen using RAPD. Genetic similarity decreased with increasing distance at regional (r = 0.599, p = 0.022) (r= 0.706, p = 0.004) for microsatellites and RAPD respectively but wasn't significant for allozymes (r = 0.414, p = 0.092), the genetic diversity (*He*) was 0.485, 0.315, and 0.188 for microsatellites, RAPD and allozymes respectively indicating a low genetic diversity in this species (Turlure *et al.*, 2014).

Onyia *et al*, (2013), reported that *Clarias anguilaris* and *Heterobranchus bidorsalis* from three ecological zones in Nigeria have a common ancestor though variations due to distance in relationship exist among them. It was also observed that estimated genetic distances between the populations were directly correlated with geographical distances.

Genetic diversity in five populations of Nigerian local breeds of goat was investigated using Random Amplified Polymorphic DNA markers. Nei's genetic similarity was 0.9505 and 0.9995 while the genetic distance was 0.0005 and 0.0507 as a result of only two clusters. The five populations showed high similarity due to loss of heterozygosis which may have been as a result of uncontrolled mating and consanguinity (Udeh *et al.*, 2015). Ajayi *et al*, (2015) investigated genetic variability among three cattle breeds in Nigeria using. Thyroid Hormone Response Spot 14 Alpha Gene through PCR. The allelic frequency ranged from 0.3600 to 0.6400, average heterozygosity ranged from 0.4608 to 0.4861, genetic distance also ranged from 0.0010 to 0.0058 and the genetic identity was from 0.9942 to 0.9990. The result demonstrated variation among the breeds.

Sushila and Jaya (2013) analysed the Genetic diversity in eleven natural populations of *Salvadora oleoides* by isozyme electrophoresis using seven enzyme systems. The genetic analysis demonstrated that *S. oleoides* maintain relatively high genetic diversity (p was 0.62, *Na* was 1.75 and *Ho* and *He* were 0.184 and 0.199 respectively) when compared with other plant taxa. Genotypic proportions at most loci in most population's fit Hardy-Weinberg expectations, the coefficient of genetic differentiation among populations based on F_{ST} equalled 0.023 and genetic identities between population's pairs were high (mean *I*= 0.98).

Population structure and genetic diversity in four natural populations of *Theobroma speciosum* belonging to the Amazon rainforest located in the State of Mato Grosso were analysed using 15 ISSR molecular markers. A total of 101 loci were found, of which 54.46% were polymorphic at the species level. The value of genetic diversity at the species level obtained by Nei's diversity (*H*) was 0.167, while the Shannon's diversity index (*I*) was 0.254, *Nm* was 0.404 and F_{ST} value was 0.60, expected total heterozygosity (*HT*) was 0.172, which indicated that the species has a reserve of genetic variability in these populations, average genetic diversity within populations (*HS*) was 0.076 and genetic divergence between populations (G_{ST}) was 0.552. The findings suggested that genetic isolation might be in progress in these populations (Giustina *et al.*, 2014).

2.4 Application of Microsatellite Markers

D. melanogaster according to John, *et al.* (2012) have a total of 390,873 microsatellite repeats satisfying minimum length and purity specifications out of which 92,047 (24%) were mononucleotides, 58,153 (15%) were dinucleotides, 95,234 (24%) were trinucleotides, 78,264 (20%) were tetranucleotides, and 67,175 (17%) were pentanucleotides. The median repeat length was 11 bases (range 7–651), and 90% of repeats were shorter than 23 nucleotides. Over 98% of microsatellites were accessible to the shortest reads employed in the DGRP sequencing libraries (45 bases), while only 165 repeats (0.04%) were beyond the

reach of the longest reads (110 bases). Katti *et al*. (2001), stated that (GT)n microsatellites are the predominant repeat type in mammals and *D. melanogaster*, while (AT)n repeats are the most abundant in *Arabidopsis thaliana* and yeast.

African and non- African *Drosophila melanogaster* populations were studied using 49 microsatellite markers. Zimbabwe and Kenya harboured the most genetic variation (*He* = 0.77) for the African populations while for the non- African populations, American *D. melanogaster* was the most variable (*He* = 0.58), followed by European *D. melanogaster* (*He* = 0.53) and Asian *D. melanogaster* (*He* = 0.48). The study concluded that *D. melanogaster* in Asia are highly structured and further suggested that *D. melanogaster* originated from sub-Sahara Africa (Schlotterer *et al.*, 2005).

Seven microsatellite loci were analysed in three populations of *Drosophila antonietae* from xerophytic vegetation in South American Environment. *Ho* was between 0.413 to 0.566, *He* ranged between 0.611 to 0.639, F_{IS} was 0.2561 and F_{IT} was 0.2927(which is considered a very high within and among populations heterozygote deficiency), F_{ST} was 0.0491, Reynolds genetic distance was between 0.0725 and 0.0321, most of the populations were deviated from Hardy- Weinberg equilibrium (Luciana *et al.*,2012).

A study on helmeted guinea fowl in Nigeria using 31 set of microsatellite loci, for the 31 polymorphic loci, the mean observed and expected heterozygosity ranged from 0.012 to 0.102 and -0.063 to 0.591 respectively, with the genetic distance ranging from 0.4472 to 1.0. A 100% variation was observed among population and 0.00% within population. All populations were in equilibrium except for a single locus. The study indicated that a rich genetic diversity existed among the five strains of the Guinea fowl (Umar *et al.*, 2015). Mohy- eldein *et al.* (2013), investigated population structure and genetic diversity of five Sudanese native chicken using 29 microsatellites. Two hundred and one (201) alleles were detected in all populations, with a mean number of 6.93 ± 3.52 alleles per locus. The mean

observed and expected heterozygosity across 29 loci was 0.524 and 0.552, respectively. Total inbreeding coefficient (F_{IT}) was 0.069±0.112, while differentiation of subpopulations (F_{ST} 0.026±0.049) was low indicating the absence of clear sub-structuring of the Sudanese native chicken populations. The inbreeding coefficient (F_{IS}) was 0.036±0.076. The study revealed the absence of population sub-structuring of the Sudanese indigenous chicken populations.

Ben- Larbi *et al,* (2014) studied the first detailed analysis of genetic diversity of the Tunisian rabbit populations using 36 microsatellite markers, out of which 294 were genotypes. The genetic differentiation among the population implies that 98.9% of the total genetic variation was explained by individual variability with heterozygosity ranging from 0.3 to 0.53.

Hoda and Marsan (2012) characterized the genetic diversity and evaluated the genetic relationship and structure of three important local sheep breeds, using 31 microsatellite markers. The genetic diversity ranged from 0.74-0.77, *PIC* ranged from 0.495 to 0.856, *Ho* was 0.72 while *He* was 0.75.The F_{IS} parameter, ranged between −0.081to 0.458, the global heterozygosity deficit (F_{IT}) was estimated to be 0.052 and global breed differentiation evaluated by F_{ST}, was 0.011. The populations deviated significantly from Hardy- Weinberg equilibrium ($P < 0.01$), with low genetic differentiation and a high level of breed admixture.

Genetic characterization of four Romanian Local Horses breeds using 12 microsatellites markers was conducted by Georgescue and Costache (2012). A total of 119 different alleles were seen, *Ho* ranged from 0.662 to 0.676, *He* ranged from 0.759 to 0.741, F_{ST} values ranged from 1.8% to 9.7%, Reynold's genetic distance ranged from 0.096 to 0.194 and all four populations were in equilibrium based on the Hardy- Weinberg test. Georgescue *et al.* (2009) also conducted genetic diversity using five local Romanian cattle breeds based on allelic frequencies of 11 microsatellite loci. 125 distinct alleles were detected, number of alleles varied between 4 and 12, *Ho* ranged from 0.580 and 0.711, *He* ranged from 0.690 to 0.778, the Hardy-Weinberg equilibrium test showed that all the five breed populations are in

equilibrium, F_{ST} 93% correspond to differences among individuals and Reynold's genetic distance ranged from 0.056757 to 0.131480.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out using *Drosophila melanogaster* populations in Savanna zones of Nigeria (Figure 1). The zone which occupies about 80% of the total land surface of Nigeria is divided into Guinea, Sudan and Sahel zones based on the type of vegetation and climatic conditions:

The Guinea Savanna: This zone consists of the larger part of the savanna zone and is divided into the southern Guinea savanna and northern Guinea savanna. It covers an area that has 100 – 150 cm of annual rainfall and where the wet season lasts for 6 - 8 months. The false balsam Copaiba (*Daniellia oliveri*), Almond (*Terminalia spp*), Dwarf red wood (*Lophira*), pod mahogany (*Afzeila africana*), African copailba basalm (*Daniellia oliveri*), the poor mahogany (Khaya senegalensis) are the common species found in the southern Guinea savanna. In the northern guinea savanna species such as Doka (*Isoberlinia doka*) and *I. tomentosa* form the bulk of the scattered woodland. Also found are Elephant grass, locust bean tree (*Parkia filicoidea*), shea butter tree (*Butyrospermum parkii*) and mangoes (*Mangifera indica*). Comparatively, there are fewer trees in the northern Guinea savanna than in the southern Guinea savanna and the trees are not as tall as those found in the southern Guinea savanna. The appearance of this zone differs from season to season. During the rainy season, the whole zone is green and covered with tall grasses that grow and reach maturity rapidly and thus become fibrous and tough. In the dry season they tend to die and disappear and one can see for kilometers. This clearing is due to several periodical bush-

burning that occurs during the dry season between November and April, carried out to either assist in farm clearance or hunting (Agabi, 1995: Ileoje, 2001: FGN, 2002).

The Sudan savanna: This vegetation belt is found in the north-west stretching from the Sokoto plains in the west, through the northern sections of the central highland. It spans almost the entire northern states bordering the Niger Republic and covers over one quarter of Nigeria's total area. The low annual rainfall of usually less than 1000 mm and the prolonged dry season (6-9 months) sustain fewer trees and shorter grasses than the Guinea savannah. It is characterized by abundant short grasses of 1.5-2m and few stunted trees hardly above 15m.It is by far the most densely human populated zone of northern Nigeria. The grass vegetation is interspersed with farms and thick bush trees such as shea butter tree (*Butyrospermum parkii*), Apple-ring acacia (*Acacia albida*), locust bean tree (*Parkia filicoidea*), tamarind tree (*Tamarindus indica*) and mango (*Mangifera indica*) (Agabi, 1995: Ileoje, 2001: FGN, 2002).

The Sahel savanna: Occupies about 18 130 km^2 of the extreme northeast corner of Nigeria and is the last vegetation zone of the savanna type between the Sahara and the northern frontier of the Sudan savanna where the dry season lasts for up to 9 months and the total annual rainfall is hardly up to 700mm. It is characterized by very short grasses of not more than one metre high located in-between sand dunes. The area is dominated by several varieties of the acacia and date- palms. Here the vegetation is not only sparse but the grasses are very short. This zone is characterized by plants such as Sorghum grass, Indian sandbur (*Cenchrus biflorus*), and Twisted acacia (*Acacia raddiana*). The shrubs that are predominantly scattered in the zone are African myrrh (*Commiphora africana*) and *Leptadenia spartum*. (Agabi, 1995: Ileoje, 2001: FGN, 2002).

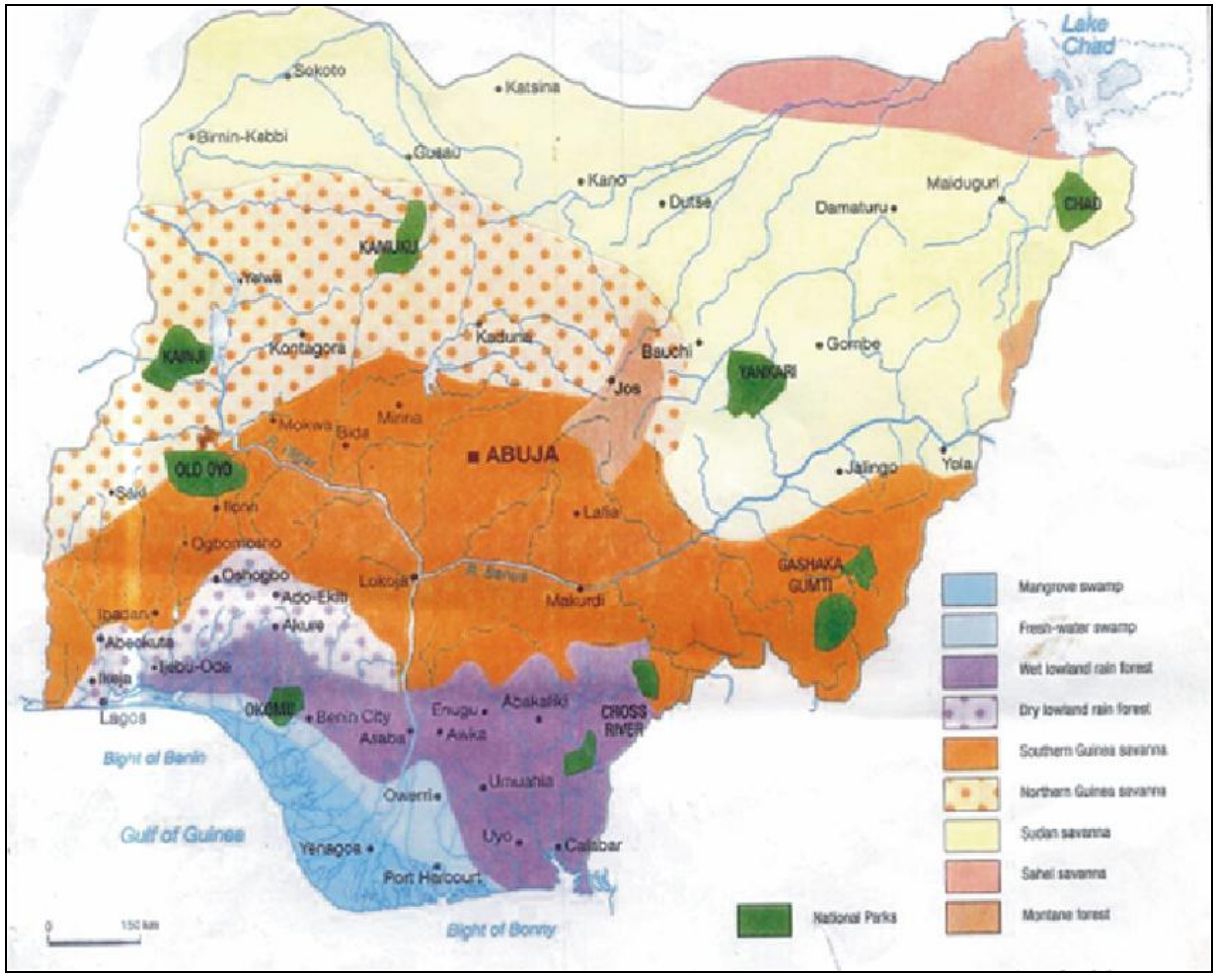


Figure 1: Vegetation Map of Nigeria

Source: FGN, 2002

3.2 Sampling Locations

Samples were collected from two localities in each of the zones using Random Convenient Sampling method. Kaduna (Anguwan Dosa (Urban) and Dan hono (Rural)) , Kano (Hotoro (Urban) and Gundun wawa (Rural)), Azare (Kasuwan Azare (Urban) and Kazuru (Rural)) (Table 3.1, Figure 3).

Table 3.1: Sampling Locations

Location	**Vegetation type**	**State**	**Latitude**	**Longitude**	**Altitude**	**Sample size**
Anguwan-Dosa	Northern Guinea	Kaduna	10˚33′46.4′′	007˚27′02.4′′	632m	66
Danhon	Northern Guinea	Kaduna	10˚31′36.3′′	007˚29′24.6′′	608	26
Hotoro	Sudan	Kano	11˚58′05.2′′	008˚34′47.5′′	492	47
Gunduwawa	Sudan	Kano	12˚00′47.2′′	008˚37′49.1′′	467m	18
Kasuwan-Azare	Sahel	Azare	11˚40′34.3′′	010˚11′26.4′′	420m	8
Kuzuru	Sahel	Azare	11˚39′59.0′′	010˚08′56.5′′	402m	19

Source: Author's Fieldwork, 2016

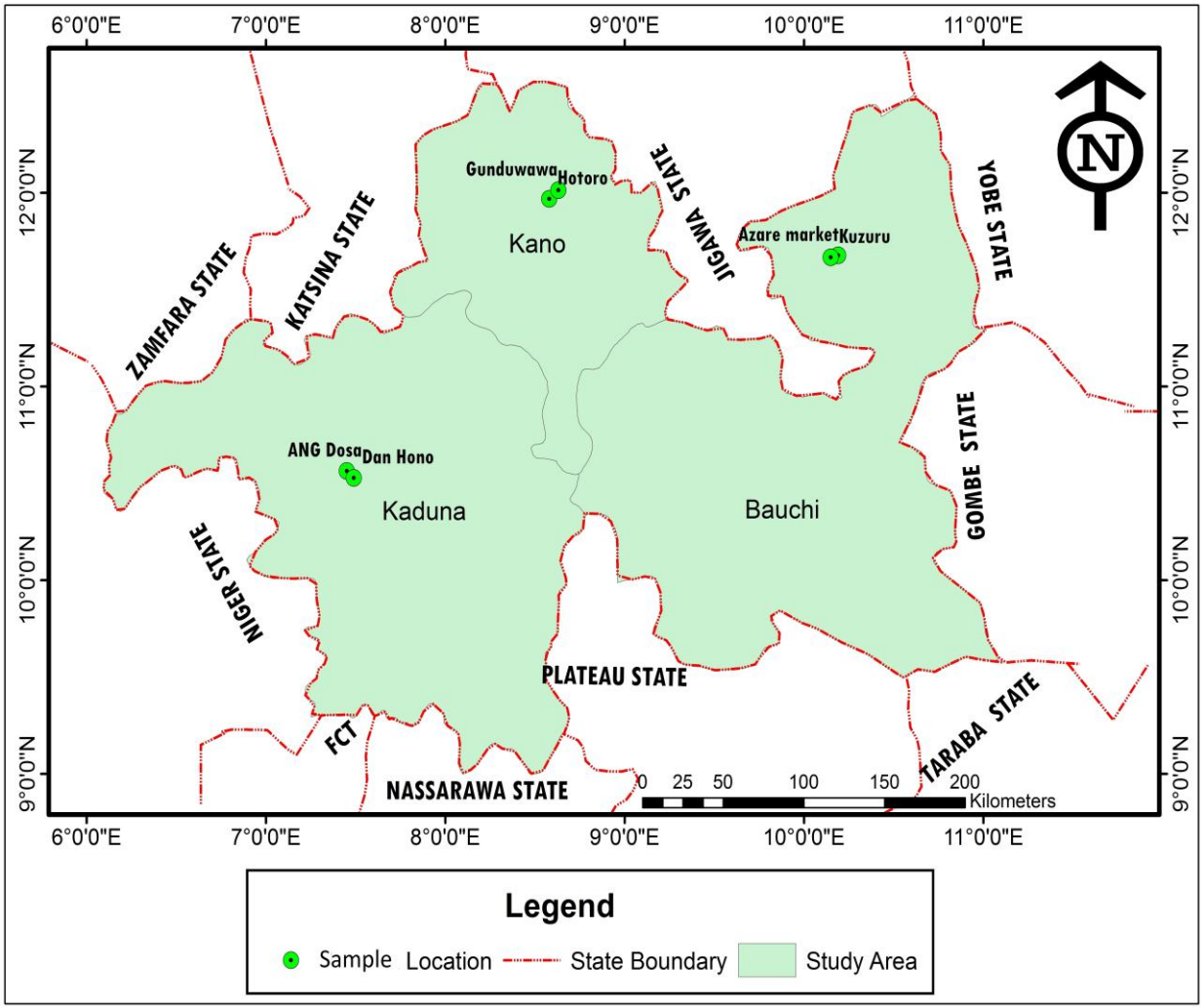


Figure 3 Sampling Locations

Source ; Adapted and modified from map of Nigeria, Ministry of Survey, 2016

3.3 *Drosophila melanogaster* Collection

Flies were collected by bottle trap method; the traps were hand made using 70cl plastic beverage bottles, 1000ml pipette tips, string and masking tape. The bottles were cut into two unequal parts with the upper part smaller than the lower part, the caps were tightly screwed and pierced in the center to suit the size of the pipette tips, the tip of the 100ml pipette tips were cut to a size capable of enabling the entrance of the flies, the pipette tips were then inserted in the hole crested on the cap.

The bait was prepared using Banana fruits, water and Baker's yeast. Peeled Bananas and dry active yeast were placed in a plastic container and mixed with water and allowed to ferment for 48 hours with periodic stirring after 24 hours. The mixture was transferred into the handmade bottle traps and sealed with masking tape. The bottle traps were then transported to the collection sites and hung on tree branches on which there was minimum exposure to sunlight to avoid death of the flies due to high temperature for 48 hours (Srinath and Shivanna, 2014). A total of ten replicate bottles were tied per sites for collection, the collections were carried out from November 2015 to March 2016, once a day (at the time of their maximum activity, in the morning), during 3 days within a single month (Sofija and Kekic, 2014).

The fruit flies were identified using *Drosophila* identification key by Markow and O'Grady (2006) with an aid of a dissecting microscope. A total of 184 *D. melanogaster* were collected, sexed (based on the pigmentation and presence/ absence of sex combs) and preserved in separate vials containing 70% ethanol and stored at -20^{o}C until analysis (Machado *et al.*, 2003).

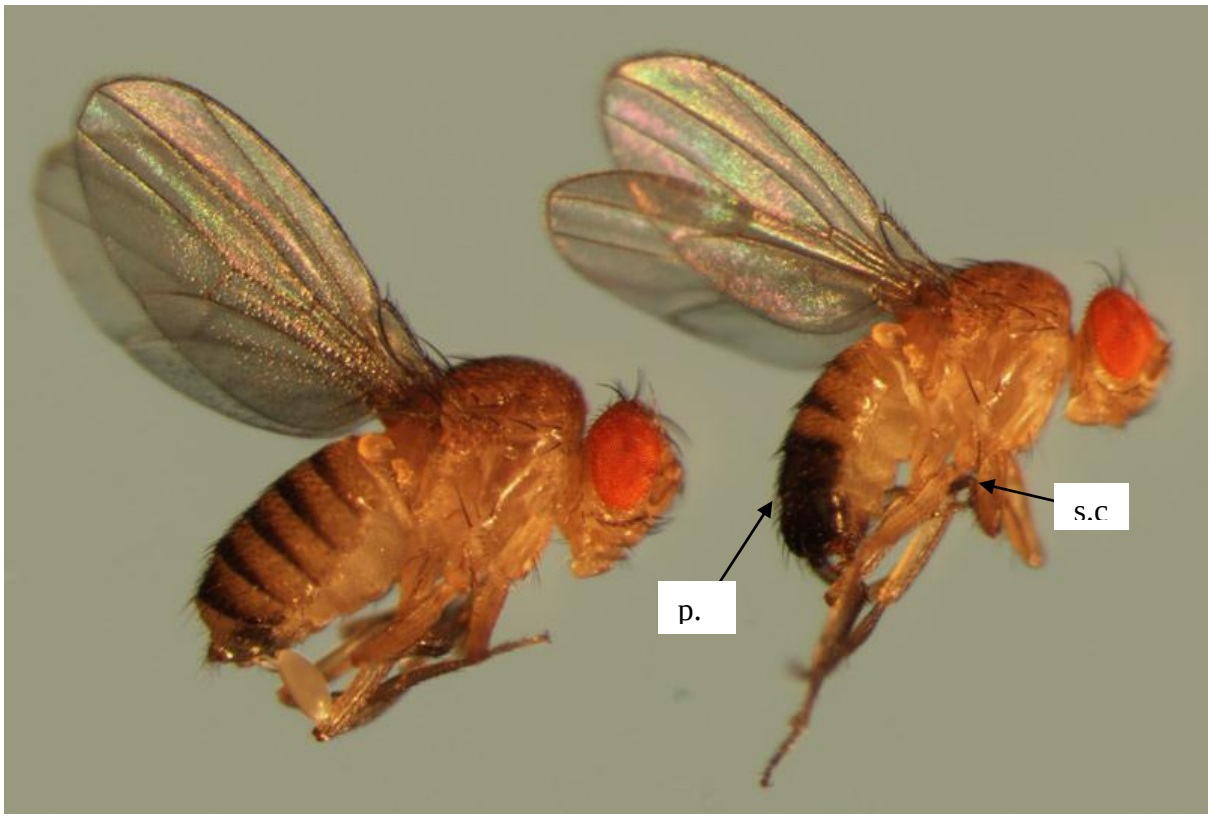


Plate I. *Drosophila* melanogaster: female (left), male (right).

Magnification × 800.

Source: the Exploratorium

Note the pigmentation (p.g) on the lower dorsal surface of the male; this pigmentation is absent in the female. Also the sex comb (s.c) which is only present in the male

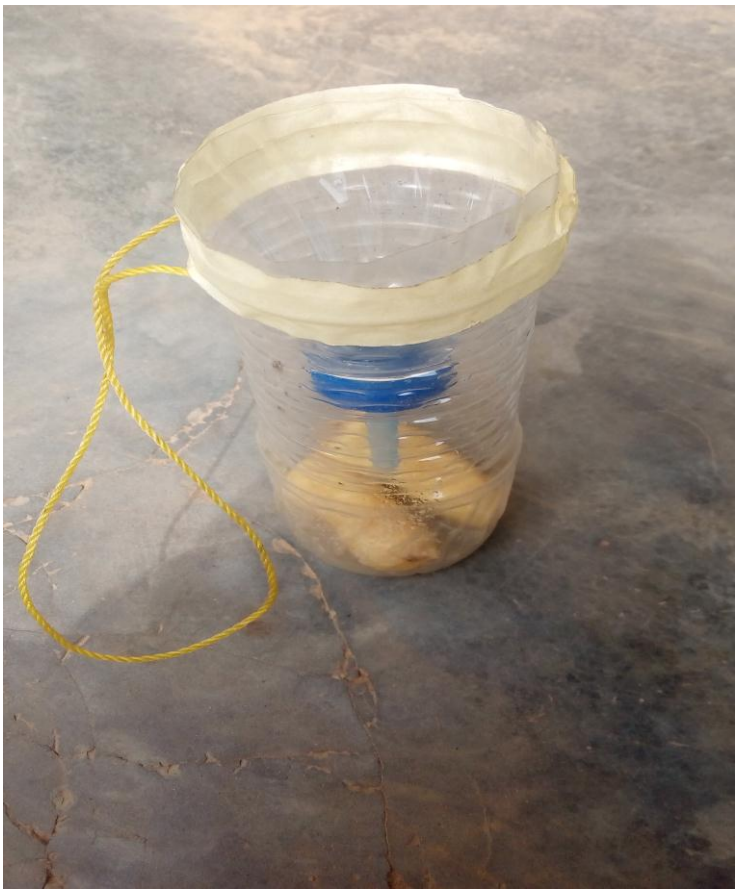


Plate II. Hand-made *Drosophila* trap (Srinath and Shivanna, 2014)

3.4 Loci Sequenced Selection

The microsatellite loci surveyed in this study were DMPROSPER, Antp1 (DRO15DC96Z), DROACS2, and DMWHITE (Irvin *et al.*, 1998; Schug *et al.*, 1998) and DM18, DM30 and DMtena (Harr and Schlotterer, 2000), (Table 3.4) Primers were designed on the basis of release 6.07 of *D. melanogaster* genome (http:// www.flybase.org) and PRIMER 3 OUTPUT (http://www-genome.wi.mit.edu/genome-software/other/primer3.html).

Table 3.2. *Drosophila melanogaster* Primers for Genetic Diversity

Locus	Primers	Fragment Size(Bp)	Annealing Tempt.(^{O}c)	GC %
DM18		**205**	59.47,59.96	45.00
Forward	$^{5'}$GCCGGCCAAACTTAACAATA$^{3'}$			
Reverse	$^{3'}$GCCGGCCAAACTTAACAATA$^{5'}$			
DMPROSPER		**201**	60.01,60.05	50.00
Forward	$^{5'}$AGGCAAACAAAGGTGTGTCC$^{3'}$			
Reverse	$^{3'}$GGGAGGTCACTCATCTTGGA$^{5'}$			
Antp1		**199**	59.99,59.93	55.00
Forward	$^{5'}$CAAGGACTTGCGTTCTCTCC$^{3'}$			
Reverse	$^{3'}$CACCTACGCGTTCGACTACA$^{5'}$			
DROACS2		**201**	59.83,59.80	50.00
Forward	$^{5'}$TGTTTGGATGAGTCCAGCAG$^{3'}$			
Reverse	$^{3'}$ATCTCCACCTGGTACGGATG$^{5'}$			
DM30		**203**	59.94,59.93	45.00
Forward	$^{5'}$TTTGGGTTTCTATCGCCAAC$^{3'}$			
Reverse	$^{3'}$AGGGAACTGTCCATGAATGC$^{5'}$			
DMWHITE		**207**	59.59,59.98	45.00
Forward	$^{5'}$GGTAAGCAGGGGAAAGTGTG$^{3'}$			
Reverse	$^{3'}$ATTTTTGTGGGTCGCAGTTC$^{5'}$			
DMtena		**200**	59.97, 59.97	40.00
Forward	$^{5'}$ACAATTTGCGTTGGGAAAAG$^{3'}$			
Reverse	$^{3'}$ACGGACAGGACCTCAATCAC$^{5'}$			

Source: Irvin *et al.,* 1998; Schug *et al.,* 1998; Harr and Schlotterer, 2000; Flybase, 2015

3.5 Laboratory Analysis

The Laboratory analysis was done at DNA Laboratory, Off Katuru Road, Unguwan Sarki, Kaduna, Kaduna State.

3.6 Genomic DNA Extraction Using Phenol Chloroform Method

Genomic DNA was extracted from pooled male flies; seven from each population were ground in ependorf tubes with loop. 400µl of lysis buffer and 4µl of proteinase K were added, vortexed for 20sec. then incubated for an hour with a 20 minutes interval of vortexing. 400µl of phenol was added and centrifuged at 14,000 rmp for 10 minutes after which the supernatant was decanted and 400µl of chloroform added and centrifuged at 13,000 rmp for 5 minutes. The supernatant was discarded, 400µl of absolute ethanol and 10µl of 4M sodium acetate were added and left overnight at -20˚C. The samples were then centrifuged for 10mins at 4˚C. The pellet formed was washed with 400µl of cold 70% ethanol to remove salt then centrifuged for another 5 minutes. The DNA pellet was then air dried on a flow bench, after which it was dissolved in Tris EDTA (TE) buffer or sterile double distilled water (ddH_20). According to the procedure described by (Machado *et al.,* 2003; Preiss *et al.,*1988). Genomic DNA was extracted from only male flies because they do not undergo meiotic recombination and are therefore preferred for use in genetic studies (Adams *et al*., 2000).

3.7 Polymerase Chain Reaction and Simple Sequence Repeat Protocol

20µl Reaction Mix: 1µl of genomic DNA, 0.5µl of each forward and reverse primers and 18 µl dNTPs. The tube was tapped gently and centrifuged at 14,000rpm for 5 seconds.

3.7.1 PCR Amplification

A conventional simplex PCR was carried out using the 7 microsatellites (Table 3.2) because of the closeness of the primer sizes by a PX2 Thermo hybrid thermal cycler. A typical cycling profile consisted of 30 cycles with pre-denaturation at 94˚C for 5 minutes,

denaturation at 94˚C for 1 minute, annealing at 60˚C for 1 minute, extension at 72˚C for 30 seconds and final extension at 72˚C for 5 minutes, according to the procedure of Schlotterer *et al.*(2005) with modifications (Conventional PCR, base sizes of primers).

3.8 Electrophoresis

The PCR products were subjected to electrophoresis on Bio-Rad gel electrophoresis unit in 1.5% Agarose gel prepared in 1xTBE buffer (1.5g Agarose dissolved in 100ml distilled water and 1xTBE buffer). Ethidium bromide (2µL / 200mL) was added to the gel for visualizing DNA bands. PCR products were sized by loading a "slippage ladder" that produced bands every 100 bp and a known size standard next to them. The Gel was run at 110 mA current for 45 minutes. After electrophoresis, the product was visualized by gel documentation and analysis system (Molecular Imager® Gel Doc™ XR+ system with image Lab™ Software of BIO-RAD) calibrated to low sensitive level (25%) (Jean-Louis, 2000; Umar, 2016).

3.9 Data Analyses

The bands in the electrophoregram (Plate III to V) were analyzed and scored using the image Lab™. Observed allelic frequencies, Number of Alleles, observed and expected heterozygosity, Deviations of the locus/population from Hardy-Weinberg Equilibrium (HWE), Analysis of Molecular Variance, (AMOVA), Polymorphic Information Content (PIC), Nei's genetic distance and identity, F- Statistics and Gene Flow were computed using GenAlex version 6.501 (Dieringer & Schlotterer, 2003; Peakall & Smouse,2012; Umar,2016). Phylogenetic analysis was constructed using MEGA 6 (Koichioro *et al.*, 2011).

3.9.1 Standard diversity indices

The following diversity indices were computed using GenA1ex 6.501:

*3.9.1.1Allele frequency***:**

Estimates gene frequencies at each locus from raw data, which are expressed as a fraction or percentage. Missing values are excluded from such estimation. It is calculated using the equation:

$$F_x = \frac{2N_{xx} + N_{xy}}{2N}$$

Where N = number of samples, xx = alleles for homozygous individuals, xy = alleles for heterozygous individuals.

3.9.1.2 Number of Allele:

Is the number of alleles with nonzero frequency ($F_X > 0.05$). It is determined by direct count.

3.9.1.3 Effective allele number:

Estimates the reciprocal of homozygosity. It enables meaningful comparison of allelic diversity across loci with diverse allelic frequency distribution. It is calculated as:

$$N_e = \frac{1}{1 - H_E}$$

Where H_E = expected heterozygosity

3.9.1.4 Observed heterozygosity:

Estimates proportion of observed heterozygosity at a given locus within a population. It is calculated using the equation:

$$Ho = \frac{No.\_of\_Hets}{N}$$

Where No. of Hets = number of heterozygotes by direct count, N= sample size

3.9.1.5 Expected heterozygosity:

Estimates proportion of expected heterozygotes under random mating only for co-dominant markers. Two estimates are given, the first is heterozygosity (Saitou and Nei, 1987) and the second is unbiased heterozygosity (Nei, 1978). It is defined as the probability that two randomly chosen haplotypes are different in the sample and is referred to as genetic diversity within a population This is calculated by:

$$H_E = 1 - \sum p_i^2$$

Where $\sum P_i^2$ = sum of the squared allele frequency

3.9.1.6 Hardy- Weinberg Equilibrium test:

Hardy-Weinberg Equilibrium Test explains that both the gene and genotype frequencies will be constant from generation to generation (Labate, 2000). Hardy-Weinberg Equilibrium assumes that mating is random, no selections, no mutation on any allele, no migration and large population size Deviation from any of the assumptions results in disequilibrium and the usual tool for determination of whether the allelic frequencies at each locus are in HWE is the chi square test tool (Maritte and Kremer, 1999). If X^2 cal $\leq X^2$ tab then Ho is accepted, it means that the allele frequencies for loci in a given population are in HWE, else X^2 cal $\geq X^2$ tab means disequilibrium, then Ho is rejected (Hartle and Clark, 1989). Chi square is calculated using the equation;

$$X^2 = \sum_{i=1}^{k} \frac{(O - E)^2}{E}$$

Where O_i = observed number of individual with ith genotype, E_i = expected number of individual with degree of freedom $DF = [Na(Na-1)]/2$, Na = number of alleles at that locus.

3.9.1.7 Polymorphic Loci:

Percentage of all loci which are polymorphic regardless of allele frequencies. It is of limited value for DNA based markers such as SSRs where comparisons make little sense, because

the selection of markers is often based on their high degree of polymorphism. May be useful for multi locus DNA markers such as AFLPs.

3.9.1.8 Polymorphic Information Content:

The value of PIC indicates the degree of polymorphism and gene diversity equivalent to Shannon Weaver Index in Ecology (Peakall and Smouse, 2012). A site is highly polymorphic when PIC > 0.5 and lowly polymorphic when PIC < 0.25. It is calculated as:

$$I = \sum p_i \ln p_i$$

Where $\sum P_i$ = sum of frequency of ith allele, ln = the natural logarithm

3.9.1.9 Genetic Distance and Identity:

Estimates Nei (1972) genetic identity and genetic distance and also Nei's (1978) estimated unbiased genetic identity and genetic distance. The estimation is made for Groups and Multiple populations which are used to show relationships between and among populations. They are estimated using the equation;

$$D = -\ln(I)$$

Where ln = natural logarithm, I = genetic identity

$$I = \frac{J_{xy}}{\sqrt{(J_x J_y)}}$$

Where J is calculated as:

$$J_{xy} = \sum_{i=1}^{k} p_{ix} p_{iy},$$

$$J_x = \sum_{i=1}^{k} p_{ix}^2, J_y = \sum_{i=1}^{k} p_{iy}^2$$

Where P_{ix} and P_{iy} = frequency of the ith allele in populations x and y

3.9.1.10 F- Statistics:

Wright's F- Statistics which is used to characterize population genetic structure is calculated using AMOVA. These statistics allow the partitioning of genetic diversity within and among populations. The parameters include the F_{IS}, which is the inbreeding coefficient for individuals, the F_{IT} which is the inbreeding coefficient for total population and F_{ST} which is the genetic differentiation. Values close to zero are expected under random mating, while positive values indicate inbreeding and a negative value indicates excess of heterozygotes. It is estimated via:

$$F = \frac{H_E - H_O}{H_E}$$

Where H_E and H_O are the expected and observed heterozygosity respectively

3.9.1.11 Analysis of Molecular Variance (AMOVA):

This is a method used to detect population differentiation utilizing molecular markers. It allows for heirachical partitioning of genetic variation among populations and regions.

3.9.1.12 Genetic Differentiation (Pairwise F_{ST}):

This provides a measure of the genetic differentiation among populations. That is, the proportion of the total genetic divergence that seperates the populations, where $F_{ST} < 0.05$ means little genetic differentiation, $F_{ST} < 0.5$ means moderate differentiation, $F_{ST} < 0.25$ means great differentiation and $F_{ST} > 0.25$ means very great differentiation (Wright, 1978; Hartl and Clark, 1997).

. It is calculated as thus:

$$F_{ST} = \frac{H_T - \bar{H}_e}{H_T}$$

Where H_T = total heterozygosity and He = mean of expected heterozygosity

3.9.1.13 Gene Flow:

This is the number of migrants in a population and is calculated from the gentic differentiation, that is,

$$Nm = \left[(1/F_{ST}) - 1\right]/4$$

Where F_{ST} = degree of population genetic differentiation.

According to Wright, (1931) a $Nm < 1$ means a limited level of gene flows which indicates genetic isolation.

3.9.1.14 Construction of phylogeny:

The Neighbor- joining (NJ) methodology (Saitou and Nei, 1987) was used to construct the phylogenetic tree of population genetic characteristics. Phylogenetic tree shows the degree of relationships between different species and provide information on the closeness within the same species (Palti *et al.*, 1997; Mojekwu, 2014).

The NJ is known to be more efficient than many other methods in obtaining the correct tree (Rzhetsky and Nei, 1992), because it does not assume an equal rate of evolution between lineages unlike the un- weighted pair group- method with arithmetic mean (UPGMA), (Nei and Chesser, 1983).

3.9.1.15 Spatial Autocorrelation and Heterogeneity Testing:

This test is used to detect sex- biased dispersal patterns by calculating the correlation coefficient between genetic and geographic distances. It provides information on the genetic structure of a species. The GenA1ex 6.501 uses Bootstrapping (n = 1000) to calculate a 95% envelope bars and permutation test (n = 999) to calculate confidence envelope.

CHAPTER FOUR

4.0 RESULTS

4.1. Amplification of Microsatellite Loci of *D. melanogaster* populations from Northern Guinea Savanna.

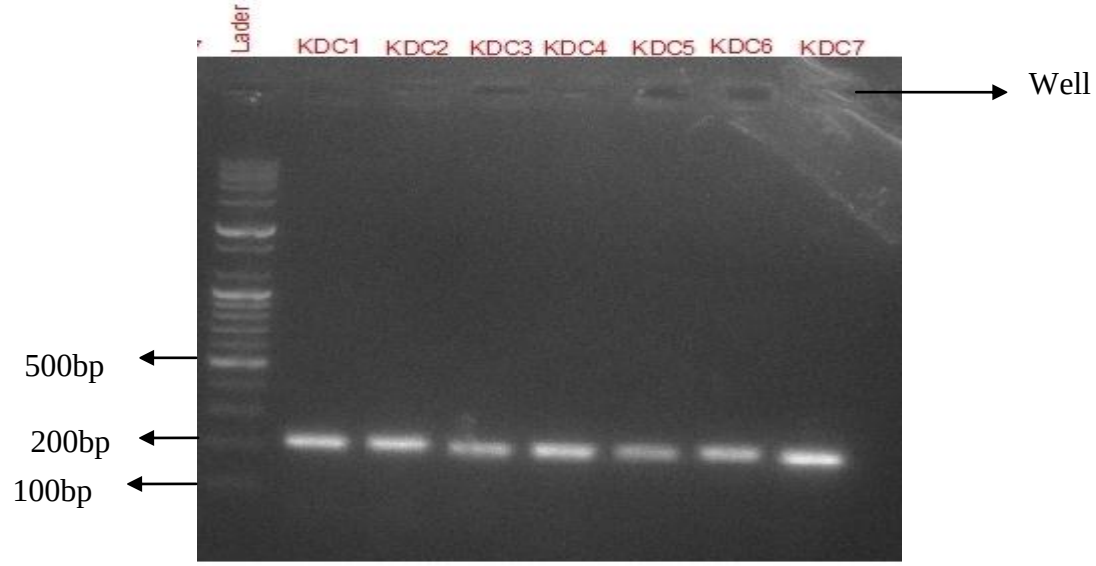
Plates III(a) and (b) shows the amplicons of the microsatellite loci. The band sizes of Plate III(a) ranged from 147.8 to 174.0bp while the sizes of Plate III(b) ranged from 202 to 203.

4.2 Amplification of Microsatellite Loci of *D. melanogaster* populations from Sudan Savanna.

Plates IV(a) and IV(b) shows the amplicons of the microsatellite loci. The band sizes of Plate IV(a) ranged from 181.2 to 213.3bp while the sizes of Plate IV(b) ranged from 109.1 to 136.9bp.

4.3 Amplification of Microsatellite Loci of *D. melanogaster* populations from Sahel Savanna.

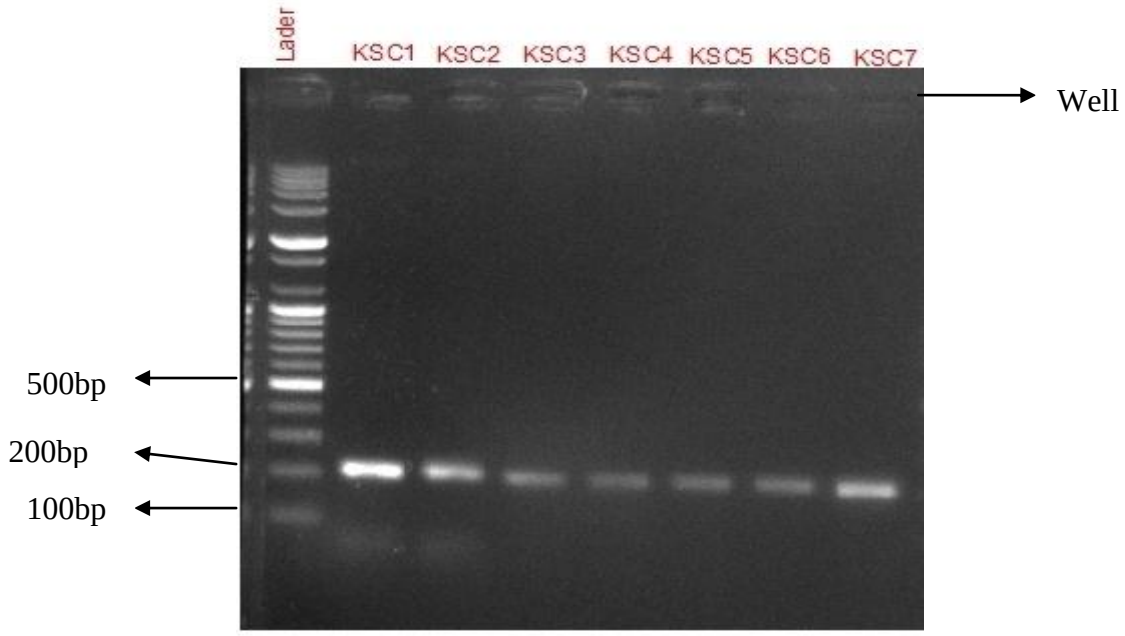
Plates V(a) and V(b) shows the amplicons of the microsatellite loci. The band sizes of Plate V(a) ranged from 86.3 to 104.5bp while the sizes of Plate V(b) ranged from 200 to 202bp.



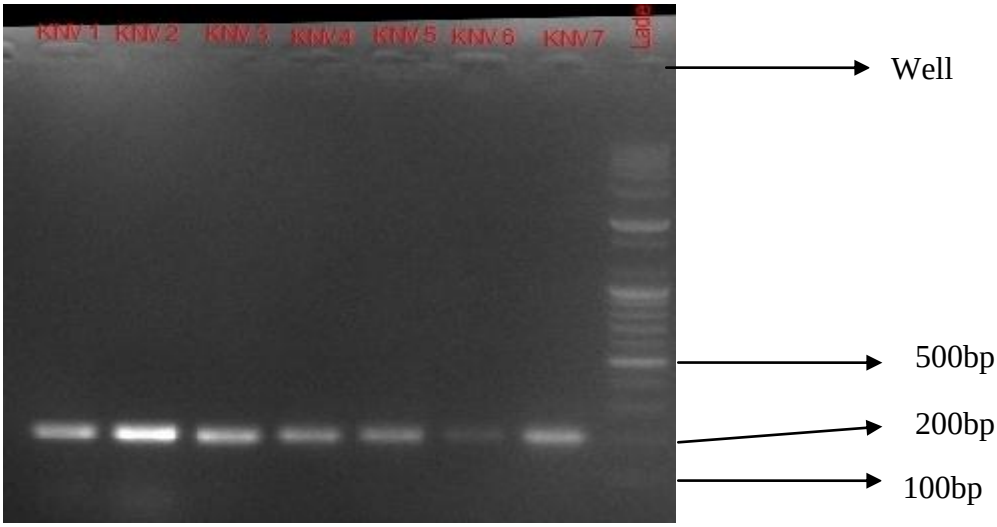
(a)

(b)

Plate III (a) and (b): Amplicons of PCR analysis of *D. melanogaster* from Anguwan-Dosa (a) and Danhono (b) (Northern Guinea Savanna).
Lane 1-8: Ladder, DM18, DMPROSPER, Antp1, DROACS2, DM30, DMtena

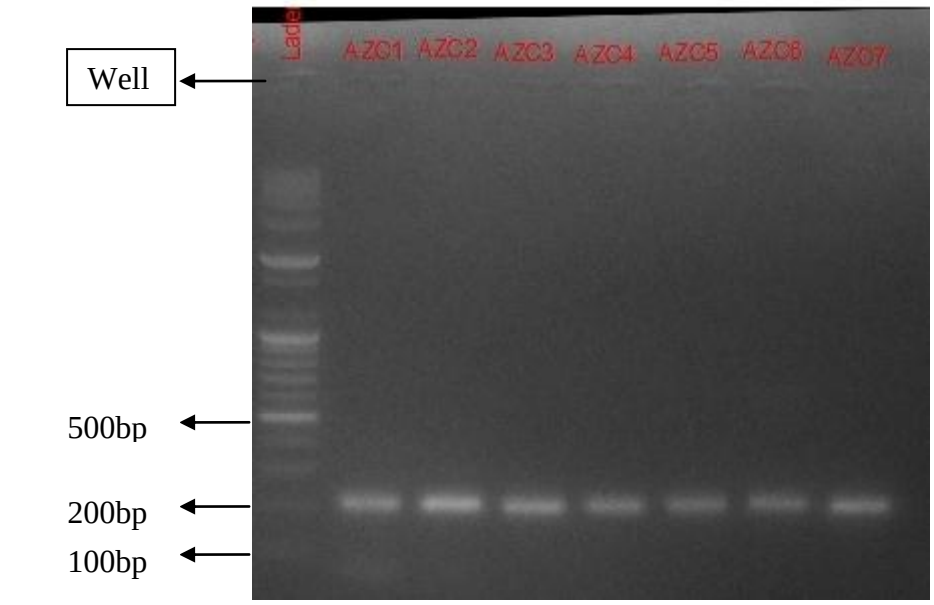


(a)

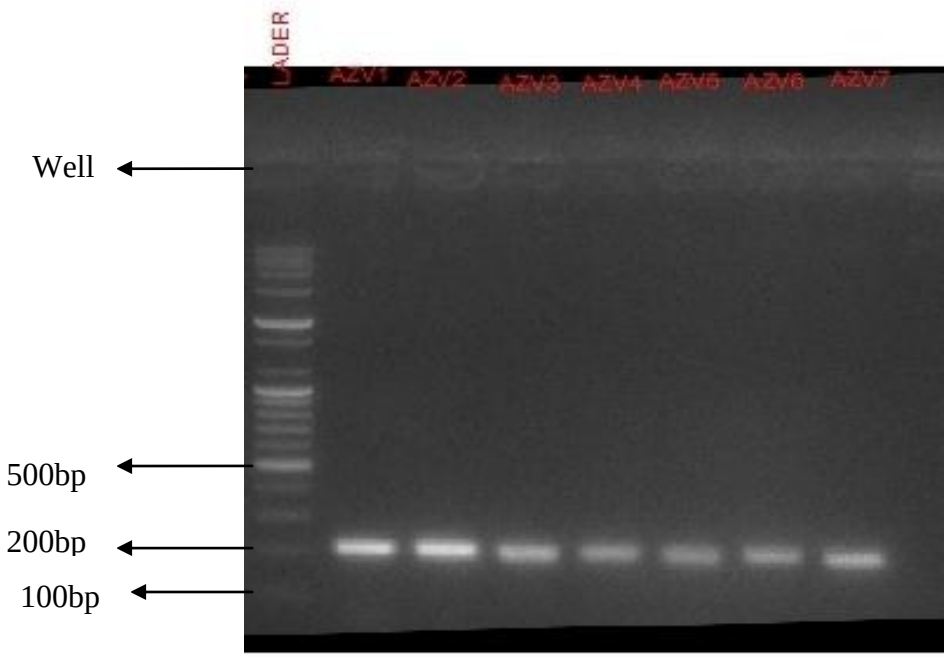


(b)

Plate IV (a) and (b): Amplicons of PCR analysis of *D. melanogaster* from Hotoro (a) and Gundun wawa (b) (Sudan Savanna)
Lane 1-8: DM18, DMPROSPER, Antp1, DROACS2, DM30, DMtena, Ladder



(a)



(b)

Plate V (a) and (b): Amplicons of PCR analysis of *D. melanogaster* from Kasuwan Azare (a) and Kazuru (b) (Sahel Savanna)
Lane 1-8: Ladder, DM18, DMPROSPER, Antp1, DROACS2, DM30, DMtena

4.4 Genetic Diversity of *D. melanogaster* populations from Northern Guinea Savanna

Effective number of allele (Na), observed (Ho) and expected (He) Heterozygosity, polymorphism information content (PIC) and Hardy- Weinberg Equilibrium (HWE) of *Drosophila* melanogaster in Northern Guinea Savanna was presented in Table 4.4. The effective number of allele as such was two for all the markers. PIC estimate was 0.693 for all markers. The Ho and He were 1.000 and 0.500 respectively for all studied loci. All loci had Hardy- Weinberg Equilibrium (HWE) value of 0.157.

4.5. Genetic Diversity of *D. melanogaster* populations from Sudan Savanna.

Table 4.5 shows the genetic diversity of *Drosophila melanogaster* populations in Sudan Savanna. The result indicated that the effective number of allele was two for all the markers PIC estimate was 0.693 for all markers. The Ho and He were 1.000 and 0.500 respectively for all studied loci. All loci had Hardy- Weinberg Equilibrium (HWE) value of 0.157.

4.6. Genetic Diversity of *D. melanogaster* populations from Sahel Savanna.

Table 4.6 shows the genetic diversity of *Drosophila melanogaster* populations in Sudan Savanna. The result indicated that the effective number of allele was two for all the markers PIC estimate was 0.693 for all markers. The Ho and He were 1.000 and 0.500 respectively for all studied loci. All loci had Hardy- Weinberg Equilibrium (HWE) value of 0.157.

4.7. Analysis of Molecular Variance (AMOVA) and Fixation Indices

The AMOVA estimated (Table 4.4) showed that 91% (3373.877) of all variation in *D. melanogaster* is found within populations, while 9% (338.786) genetic variation resides among vegetation zones and 0% for among populations within a zone. The F_{IS} estimate was -1.000, F_{IT} estimate was – 0.675 while the F_{ST} was 0.162.

Table 4.1 Genetic Diversity of *D. melanogaster* populations from Northern Guinea Savanna.

LOCUS	Ne	PIC	Ho	He	HWE
DM18	2	0.693	1.000	0.500	0.157
DMPROSPER	2	0.693	1.000	0.500	0.157
Antp1	2	0.693	1.000	0.500	0.157
DROACS2	2	0.693	1.000	0.500	0.157
DM30	2	0.693	1.000	0.500	0.157
DMWHITE	2	0.693	1.000	0.500	0.157
DMtena	2	0.693	1.000	0.500	0.157
Mean	**2±0.00**	**0.693±0.00**	**1.000±0.00**	**0.500±0.00**	**0.157±0.00**

Effective number of alleles, (He), Polymorphic Information Content (PIC), Observed (Ho) and Expected (He) heterozygosity and Hardy- Weinberg Equilibrium (HWE) with Level of Significance at ($P< 0.05$).

Table 4.2 Genetic Diversity of *D. melanogaster* populations from Sudan Savanna.

LOCUS	Ne	PIC	Ho	He	HWE
DM18	2	0.693	1.000	0.500	0.157
DMPROSPER	2	0.693	1.000	0.500	0.157

Antp1	2	0.693	1.000	0.500	0.157
DROACS2	2	0.693	1.000	0.500	0.157
DM30	2	0.693	1.000	0.500	0.157
DMWHITE	2	0.693	1.000	0.500	0.157
DMtena	2	0.693	1.000	0.500	0.157
Mean	**2±0.00**	**0.693±0.00**	**1.000±0.00**	**0.500±0.00**	**0.157±0.00**

Effective number of alleles, (He), Polymorphic Information Content (PIC), Observed (Ho) and Expected (He) heterozygosity and Hardy- Weinberg Equilibrium (HWE) with Level of Significance at ($P< 0.05$).

Table 4.3 Genetic Diversity of *D. melanogaster* populations from Sahel Savanna.

LOCUS	Ne	PIC	Ho	He	HWE
DM18	2	0.693	1.000	0.500	0.157
DMPROSPER	2	0.693	1.000	0.500	0.157
Antp1	2	0.693	1.000	0.500	0.157
DROACS2	2	0.693	1.000	0.500	0.157
DM30	2	0.693	1.000	0.500	0.157
DMWHITE	2	0.693	1.000	0.500	0.157
DMtena	2	0.693	1.000	0.500	0.157
Mean	**2±0.00**	**0.693±0.00**	**1.000±0.00**	**0.500±0.00**	**0.157±0.00**

Effective number of alleles, (He), Polymorphic Information Content (PIC), Observed (Ho) and Expected (He) heterozygosity and Hardy- Weinberg Equilibrium (HWE) with Level of Significance at ($P< 0.05$).

Table 4.4 Analysis of Molecular Variance (AMOVA) for *Drosophila melanogaster* populations in Savanna zone, and the fixation indices.

Source of Variation	Degree of freedom	Sum of Squares	Mean Sum of Squares	Estimated Variance.	Percentage variation (%)	Fixation indices
Among zones	2	17306.821	8653.411	2163.353	9	F_{IT}:-0.675
Among populations within zone	3	0.000	0.000	0.000	0	F_{IS}:-1.000
Within populations	6	133920.835	22320.139	22320.139	91	F_{ST}: 0.162
Total	11	151227.656		24483.492	100	

4.8 Genetic distance and genetic identity (genetic relationship)

Table 4.5 shows a pairwise genetic distance (*D*) estimate of 0.000 between Northern Guinea Savanna vs. Sudan Savanna and Sudan Savanna vs. Sahel Savanna and an estimate of 2.639 between Northern Guinea Savanna vs. Sahel Savanna.

Pair-wise population matrix of Nei's genetic identity (*I*) estimates of *D. melanogaster* from Savanna zone of Nigeria showed the highest genetic identity (1.000) exists between Northern Guinea Savanna vs. Sudan Savanna, 0.071, between Northern Guinea Savanna vs. Sahel Savanna and least identity of (0.000) between Sudan Savanna vs. Sahel Savanna.

Genetic correlation (r) ranged from – 0.141 to 0.212. *D. melanogaster* displayed positive genetic correlation values in distance classes up to 4km (Figure 4.1).

4.9. Genetic differentiation (F_{ST} Estimates) and gene flow (*Nm*)

The pairwise F_{ST} estimate between Northern Guinea Savanna vs. Sudan Savanna had the higher value of 0.333 than between Northern Guinea Savanna vs. Sahel Savanna (0.314) but the same value (0.333) was estimated between Sudan Savanna vs. Sahel Savanna.

The pairwise *Nm* estimate between Northern Guinea Savanna vs. Sudan Savanna and Sudan Savanna vs. Sahel Savanna had the same lower value of 0.500 while a higher *Nm* value (0.545) was observed between Northern Guinea Savanna vs. Sahel Savanna as presented in Table 4.6.

Table 4.5 Genetic distance above diagonal and genetic identity estimates below diagonal of *D. melanogaster* populations.

	Northern Guinea Savanna	Sudan Savanna	Sahel Savanna
Northern Guinea Savanna	_	0.000	2.639
Sudan Savanna	1.000	_	0.000
Sahel Savanna	0.071	0.000	_
Mean Genetic Distance	0.880		
Mean Genetic Identity	0.357		

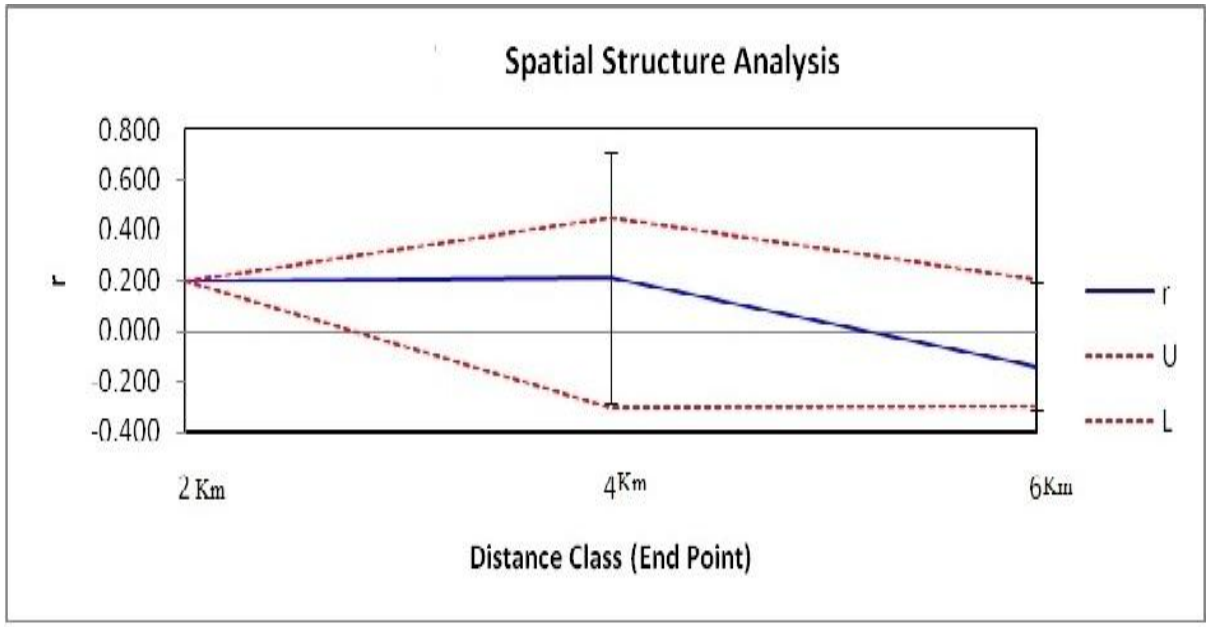


Figure 4.1 Correlation between geographic distance (Km) and genetic distance in natural population of *Drosophila melanogaster*

Key
x- axis = geographic distance
y- axis = genetic distance
r = correlation coefficient
U = upper limit
L = lower limit

Table 4.6 Pairwise F_{ST} estimates above diagonal and *Nm* (gene flow) below diagonal across zones.

	Northern Guinea Savanna	Sudan Savanna	Sahel Savanna
Northern Guinea Savanna	_	0.333	0.314
Sudan Savanna	0.500	_	0.333
Sahel Savanna	0.545	0.500	_

4.10 Cluster Analysis for *D. melanogaster* Populations

The dendrogramme (Figure 4.4) based on the seven Microsatellite markers used on the basis of Nei's genetic distance indicated that the studied populations formed two clades at various degree of similarities with Sudan savanna and Sahel Savanna forming a cluster at 0.21. The first clade then grouped with Northern Guinea Savanna to form the second clade at 0.78.

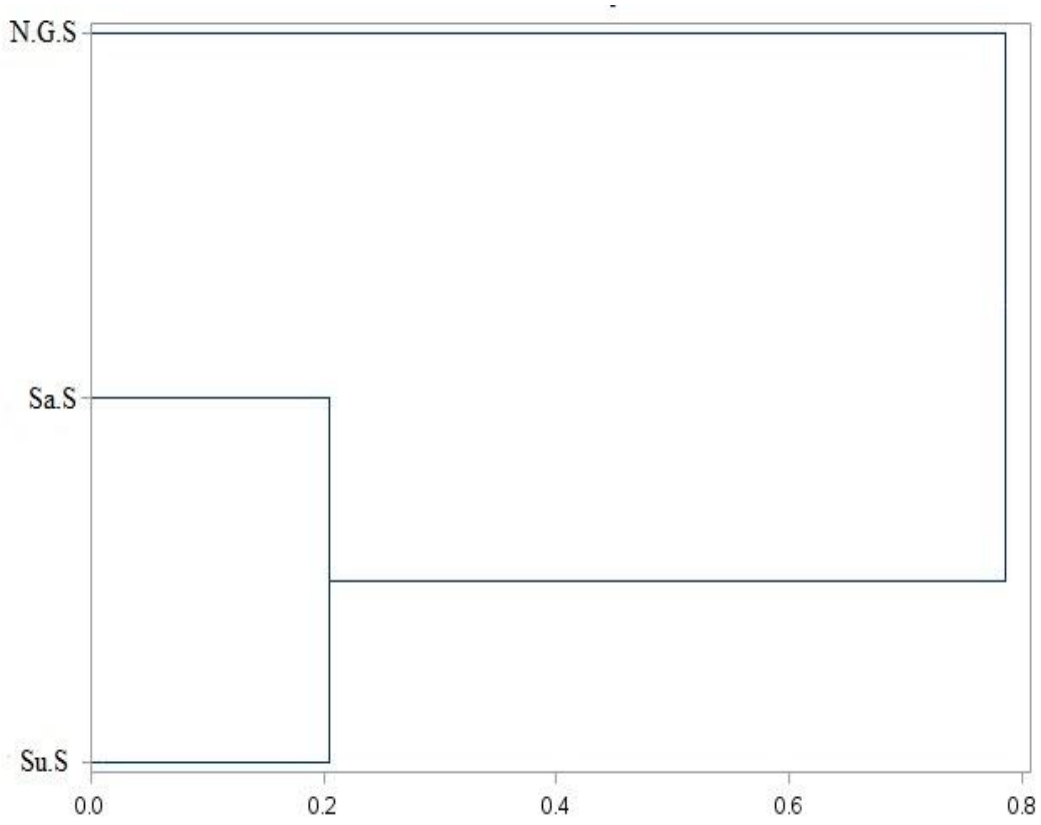


Figure. 4.2. Dendrogram representing phylogenetic relationship of *Drosophila melanogaster* from Savanna zone of Nigeria. (N.G.A = Northern Guinea Savanna, Su. S = Sudan Savanna, Sa.S = Sahel Savanna)
Key
x-axis = genetic distance classes
y-axis = Savanna zones

CHAPTER FIVE

5.0 DISCUSSION

5.1. Amplification of Microsatellite loci of *Drosophila melanogaster* Populations from Savanna Zone of Nigera.

The amplicons revealed that the DNA fragments were of various sizes both within and among populations with varying degree of brightness. The brightness or faintness of a band could be an indication on the quantity of genetic material in the sample (Milhaven, 2016).

5.2 Genetic Diversity in *Drosophila melanogaster* Populations from Savanna Zone of Nigeria.

The effective number of alleles, polymorphic information content, observed and expected heterozygosities both among loci and populations gave the same values throughout which could be due to the small sample size, the sizes of the primers (very close base sizes), the essential functions of the microsatellite loci in the development of *D. melanogaster,* (the DM18 prevents amyliod- beta neurotoxicity, DMPROSPER for brain and central nervous system, Antp1 for repressing antennal genes in the leg, DROACS2 for peripheral nervous system development, DM30 prevents sensory neurons degeneration, DMWHITE for male courtship behavior, and DMtena for the D- fan shaped body formation) and might be due to the type of technique used (Conventional simplex PCR).

The result revealed that all primers were highly polymorphic, mean PIC = 0.693 (PIC >0.5), Percentage polymorphic loci for all population were 100%, which indicated a high gene diversity among the studied markers (Chang *et al.,* 2007; Sushila and Jaya, 2013) and can therefore be used for molecular genetic studies on this species. The mean Ho > the mean He, indicates outbreeding as is the case of most natural populations and also indicates a decrease in heterozygosity. The decrease in heterozygosity in the studied populations may be due to population sub-structuring which could be understood as interpreting each sub population as sort of an "extended family". The results are in consonance with those of Pranveer and Bashisth, (2010), who also reported a decrease in heterozygosity for 45 Indian populations of *Drosophila ananassae* using cosmopolitan inversions as markers. The authors attributed the decrease to population sub structuring. According to Hartl and Clark, 2007, Organisms in the same population often share one or more recent or remote common ancestors, and so mating

between organisms in the same subpopulation will often be mating between relatives hence a decrease in heterozygosity. The genetic variation parameter (He) obtained in this study (0.50) was higher than the genetic variation obtained for indian *D. ananassae* (He = 0. 303) reported by Pranveer and Bashisth, (2010), *D. simulans* from Africa (He = 0.373) as reported by Stephan *et al,* (1998) but was lower than what was observed in African populations of *D. melanogaster* (0.81) reported by Kaurer *et al,* (2003).

The populations did not deviate from Hardy- Weinberg Equilibrium (HWE) which means that mating is random, mutation has no effect on allele frequency, no natural selection therefore the difference between the observed and expected heterozysities are likely due to chance alone (Umar, 2016).

5.3 Population Structure of *Drosophila melanogaster* from Savanna zones of Nigeria

5.3.1 Analysis of Molecular Variance (AMOVA) and F-Statistics

Most of the genetic variation at these microsatellite loci for *D. melanogaster* was concentrated among individuals within the same vegetation zone. The result showed that each zone was relatively isolated from others, but within any one zone there was extensive gene flow which could be attributed to geographic distance and was similar to the report of Ross and Markow (2006) for *Drosophila mojavensis*.

The negative value of F_{IS} (-1.000) indicates random mating and excess of heterozygotes in the sub-populations. The value of F_{IT}, the most inclusive measure of inbreeding was also negative (-0.675) which means the total populations are outbreeding. The F_{ST} which is the major determinant of the magnitude of random changes in allele frequency and which is usually affected by sample size was found to be high (F_{ST} = 0.162) indicating a high degree of genetic differentiation among all populations. The F_{IS} and F_{IT} deviated from zero confirming the observation that all mean Ho was higher than all mean He. This result

reinforce outbreeding in these populations as earlier stated, as $F_{IS} < F_{IT}$ (Table 4.4). This result is similar with that of Indian *D. ananassae* (F_{IS} = - 0.53 to 0.47, F_{IT} = -0.41 to 0.68, F_{ST} = 0.04 to 0.64) (Pranveer and Bashisth, 2010).

5.3.2. Genetic Distance and Genetic Identity (genetic relatedness)

The low genetic distance (0.000) and high genetic identity (1.000) between Northern Guinea Savanna and Sudan Savanna showed that the *Drosophila melanogaster* populations from these zones are closely related and must have had a very recent common ancestor while the high genetic distance and low genetic similarity between Northern Guinea and Sahel Savanna indicated that *D. melanogaster* populations from these zones are divergent populations. This could be as a result of the geographic distance among the zones (Kaduna to Kano = 234km, Kano to Azare = 203km, Kaduna to Azare = 410km). According to Bader, (1998), $I > 0.9$ and $D < 0.1$ means closely related species while divergent populations have $I < 0.8$ and $D > 0.2$. The mean genetic distance (0.880) for *D. melanogaster* from savanna zone of Nigeria was high, while the mean genetic identity (0.357) was low indicating high genetic divergence among the populations which may be due to average population densities. This genetic relatedness is similar to the report of Malviya *et al.* (2011) for Indian *Musca domestica* (0.322) using RADP markers

Isolation by distance (Wright, 1943) effect was not conformed statistically as the (GSA) revealed genetic distance and geographic distances were insignificantly correlated in *D. melanogaster* populations (r = 0.212, P > 0. 05). It is evident from genetic distance values that populations separated by greater geographic distance have lower genetic similarity than those situated close to each other.

5.3.3 Genetic Differentiation and Gene Flow

Pairwise F_{ST} estimate revealed very great genetic differentiation (divergence) among the populations especially between Northern Guinea vs. Sudan Savanna. It might be possible that

the high divergence between Northern Guinea vs. Sudan Savanna reflects multiple, independent colonization events. This great genetic differentiation indicates a highly sub-structured *D. melanogaster* populations in Savanna zones which could be due to geo-climatic heterogeneity of the zones as each population adapts to its environment (David *et al.,* 1985; Gilbert, 2004), it could be due to small number of founders starting their colony afresh and during that precarious bottleneck period random genetic drift might have played its role in causing differentiation, it could also be due to the mating system of this species (Praveer and Bashisth, 2010). This is in line with the genetic differentiation (0.3) observed in Asian *D. melanogaster* by (Scholotterer *et al,* 2005) who attributed the differentiation to low dispersal capabilities and genetic drift. The F_{ST} values also provide additional perspective on population divergence

The low pairwise gene flow values (0.500 and 0.545) and conversely high F_{ST} values are surprising because *Drosophila* has a low dispersal capacity but since it is co-transported via agency of human travel along with fruits and vegetables so geographic barriers or habitat discontinuity of any kind hardly hinders its movement (Walker, 2000; Mcrae *et al*., 2005). Despite the gene flow, it maintains very high level of genetic differentiation and exists as sub-structured semi-isolated populations, Sympatric divergence could also be the explanation for these observations as was hypothesized for *D. anannasae* (Pranveer and Bashisth, 2010).

The limited level of gene flow further supports that the genetic divergence was due to genetic drift. The very great genetic differentiation and moderate gene flow further reinforces strong sub structuring in natural populations of *D. melanogaster* in Savanna zone of Nigeria. This agrees with the findings of Scholotter *et al*.(2005) and Pranveer and Bashisth (2010).

5.3.4 Cluster Analysis for *D. melanogaster* populations

The resulting clades further support population subdivision in *D. melanogaster* with Northern Guinea and Sudan Savanna populations closely related. This is collaborated by several other

studies on *Drosophila melanogaster* from other countries (Hale and Singh, 1987; Schlotterer *et al.,* 1997**;** Scholotter *et al.,* 2005).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS, RECOMMENDATIONS AND CONTRIBUTIONS TO KNOWLEDGE

6.1 Summary

The study revealed the seven (7) set of microsatellite markers were all highly polymorphic (PIC > 0.5) and therefore can be used for molecular genetic studies. The mean observed heterozygosity (Ho = 1.000) for all populations was higher than the mean expected heterozygosity (He = 0.500) which indicates outbreeding in the populations. None of the population deviated from Hardy- Weinberg Equilibrium which meant that the difference observed between Ho and He was due to chance. A high genetic diversity was observed in the studied populations (He = 0.500, P = 100% and PIC = 0.639).

Each vegetation zone was relatively isolated from each other but within each zone there was high gene flow. Mating is random and excess of heterozygotes within the sub populations. The low mean genetic identity (0.357), high mean genetic distance (0.880), high mean FST (0.327) and moderate mean gene flow (0.515) indicated a high genetic divergence which was due to genetic drift in the studied populations. Homogeneity of the diversity indices (He = 0.500, P = 100% and PIC = 0.639) suggests that the species has sufficient capacity to oppose the natural loss of genetic variability due to drift.

The very great genetic differentiation and moderate gene flow indicates strong sub-structuring in natural populations of *D. melanogaster* in Savanna zone of Nigeria. The strong genetic differentiation is what keeps the species populations in sub-structure and semi- isolated fashion. The consistent clusters formed helped to further quantify the degree of relatedness between the vegetation zones.

6.2 Conclusions

- The markers are highly polymorphic (Ne > 1, PIC >0.5).
- There is high genetic diversity in natural populations of *Drosophila melanogaster* from Savanna zone of Nigeria (He = 0.5, PIC >0.6),
- The populations are out breeding with high genetic divergence due to drift (F_{IS}: -1.000 < F_{IT}: -0.675, Nm < 1).
- Natural populations of *D. melanogaster* from Savanna zone of Nigeria are strongly sub- structured and semi- isolated (F_{ST} > 0.25, Nm < 1),
- *Drosophila melanogaster* from Northern Guinea and Sudan Savanna are more closely related.

6.3 Recommendations

- Markers such as SNPs should be used to also evaluate the genetic diversity of this species.
- Genetic diversity in natural populations in *Drosophila melanogaster* from other vegetation zones in Nigeria should be evaluated so as to have an overall picture of the population structure of *D. melanogaster*.
- A *Drosophila melanogaster* stock center should be established to help preserve the genetic resources, and for use in researches.

6.4 Contributions to Knowledge

- The study revealed that there was high genetic diversity in natural populations of *Drosophila melanogaster* from Savanna zone of Nigeria (He = 0.5, PIC >0.5).

- The natural populations of *Drosophila melanogaster* from Savanna zones of Nigeria exhibit a high genetic divergence attributable to genetic drift (*$_{FIS}$*: -1.000 < *$_{FIT}$*: -0.675, *Nm* < 1)**.**
- *Drosophila melanogaster* from Northern Guinea and Sudan Savanna have a recent common ancestor (genetic distance = 0.00 and genetic identity = 1.00).

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