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GENETIC VARIABILITY IN POPULATIONS OF DROSOPHILA MELANOGASTER ACROSS NIGERIAN SAVANNA ZONES

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ABSTRACT

Drosophila melanogaster has been considered the best biological model for the studies in areas such as genetics, behaviour, evolution, development, molecular biology, ecology and population biology. We studied the Genetic variability of *Drosophila melanogaster* sampled from Savanna zones of Nigeria using seven random microsatellites from previously characterized markers. The amplified fragments of 42 *D.melanogaster* from six populations across the three zones were analysed and scored using the Image Lab™and GenAlex 6.05 software. The three zones revealed high gene diversity (He = 0.5) which could be due to the high gene flow observed within a zone, Polymorphism Information Content (PIC) of 0.693, indicating a good discrimination power of the selected markers. The populations also did not deviate from Hardy-Weinberg Equilibrium (0.157) as shown by the difference between observed and expected heterozygosities. The genetic variation observed as indicated by AMOVA was mostly variations within the populations (91%), 9% among zones. The negative fixation indices (F_{IS} of -1.000 and F_{IT} of -0.675) indicated an excess of heterozygotes in the sub- populations and outbreeding in the total populations respectively. The study therefore reveals that the used markers are highly polymorphic (PIC>0.5), the populations of *D. melanogaster* are highly genetically diversified, outbreeding and highly sub-structured.

Keywords: Fruitfly, Microsatellites Markers, Gene flow, sub- populations

INTRODUCTION

The leading evolutionary forces such as mutation, natural selection, and genetic drift have created diversity of organisms leading to delineation of species with different levels of performance (Mahmut, 2012). To understand the genetics of speciation, identifying populations of the same species is the most essential step. While interspecific studies are informative about the relationship between genetic differentiation and the strength of a given isolating mechanism after speciation has occurred, intraspecific studies are ideal for identifying prior speciation by measuring both genetic divergence and presence of reproductive isolation mechanisms (Ross and Markow, 2006). Organisms in their natural habitat are faced with constantly changing pressure from natural forces such as temperature, light, competition, predation, or from human impacts such as pollution, habitat destructions resulting 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). 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). Information about genetic structure and historical demography of natural populations is crucial to the understanding of how natural selection changes genomes (Schug et al., 2007).

Populations are usually characterized using morphological, biochemical or molecular markers with the most accepted been the molecular markers as 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 provides 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 analyse and score.

Drosophila melanogaster (Meigen, 1830) of the Order: Diptera, Family: Drosophilidae, generally referred to as fruit fly or vinegar fly, have a phenotypic appearance of yellow-brown body colouration, brick red eyes and transverse black rings across the abdomen (Flybase, 2015). The flies are sexually

dimorphic, with females about 2.5 millimeters (0.098inches) 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, 2015).The body coloration of *D.melanogaster* has been found to be an adaptation to varying ecological conditions (Pool and Aquadro, 2006). The fruit fly is speculated to have originated from sub- Sahara Africa (Capy et al., 2004), but evaded temperate climate regions due to the spread of beneficial mutations in non-Africa populations (Kirby and Stephan, 1996; Kauer et al., 2003) and recent selection pressure imposed by human activities such as resistance to insecticides (Daborn et al., 2001). Genetic studies on African and Non-African *D. melanogaster* population by Schlotterer et al. (2005), revealed a high genetic diversity in Africa and low genetic diversity Asian *D. melanogaster* population which further reinforces the origin of *D. melanogaster* to be Africa.

The wild type fruit flies *D. melanogaster* serves as a multiple model organism with its embryo and pupa stages been modeled for developmental and toxicological studies; the larval stage in physiological and behavioral studies, while the adult fly is bestowed with organs functionality that mimic the mammalian reproductive system, heart, kidney, and lung (Nichols et al., 2002; Wolf and Herbelein, 2003; Andretic et al., 2008). 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 (Meeta et al., 2008; Acurio et al.,2010; Penariol and Madi- Ravazzi,2013). There is dearth of information on the genetic variability in Nigerian Savanna populations of *Drosophila melanogaster*. The study was undertaken using microsatellite markers to assess the genetic variation among six *D. melanogaster* populations collected from three Savanna zones of Nigeria.

MATERIALS AND METHODS

D. melanogaster Collection

The *Drosophila melanogaster* were collected from two locations in each Savanna zone (Fig. 1) using hand-made bottle traps baited with rotten bananas (Srinath and Shivanna, 2014). The Northern guinea Savanna is characterized by tall trees ≥ 15m, annual rainfall greater than 1500mm; the Sudan Savanna is characterized by abundant short grasses of 1.5-2m and few stunted trees hardly above 15m and low annual rainfall of usually less than 1000 mm; the Sahel Savanna is characterized by very short grasses of not more than 1m high located in-between sand dunes and total annual rainfall of hardly up to 700mm (FGN, 2002). Males were directly identified to species using the identification key by Markow and O'Grady (2006), isolated from the females and preserved in separate vials containing 70% ethanol and stored at -20ºC for later use. The collections were carried out from November 2015 to March 2016.

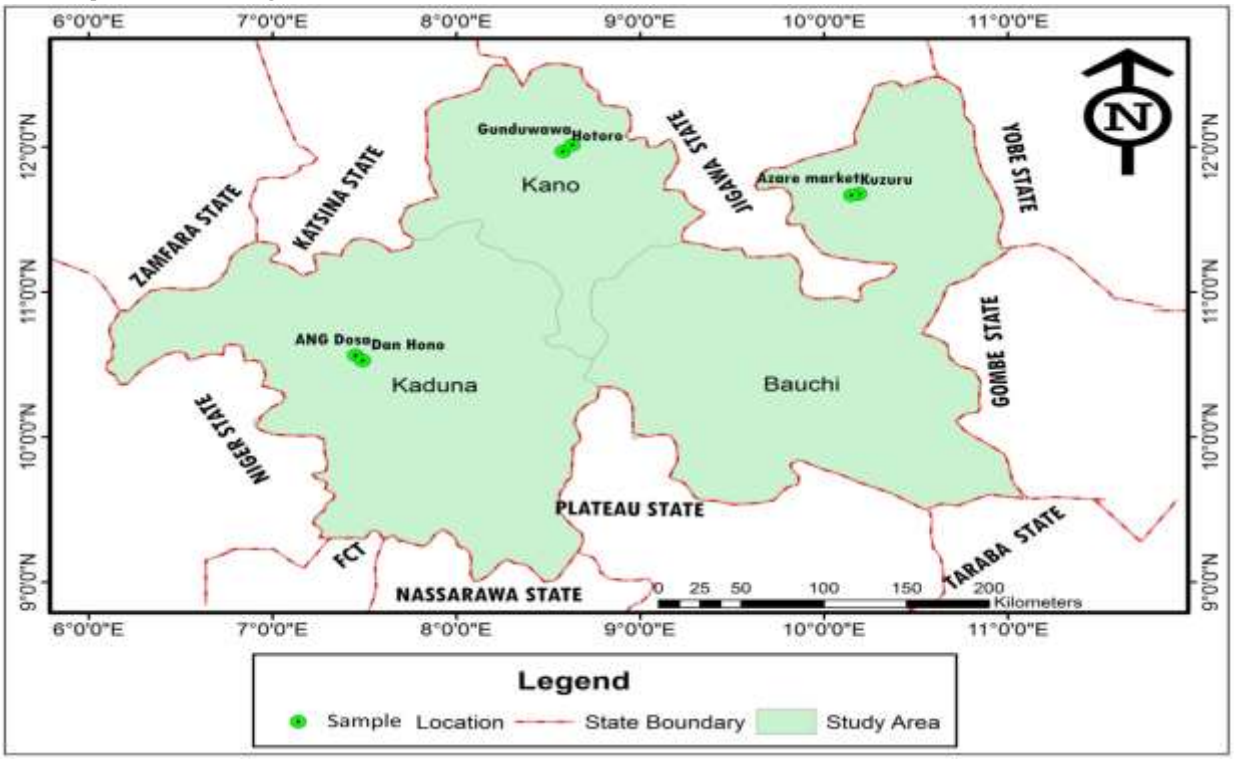

Fig. 1: Map of Sample Locations

DNA Isolation and Microsatellite Genotyping

Genomic DNA used as a template for PCR reaction was extracted from 42 males by phenol- chloroform method. Briefly, the samples were rinsed in distilled water, 400 µL of lysis buffer and 4 µL of Proteinase K were added to the samples in eppendorf tubes and pulverized. The homogenate was vortexed for 20 seconds then incubated for an hour with a 20 minute interval of vortexing. 400 µL of phenol was added and centrifuged at 14,000 rmp for 10 minutes, supernatant was decanted and 400 µL of chloroform was added and centrifuged at 13,000 rmp for 5 minutes. Supernatant was again decanted and 1000 µL of absolute ethanol and 10 µL of 4M sodium acetate were addede and left overnight at -20˚C. samples were then centrifuged at 14,000 rmp for 10 minutes, 400µL of 70% ethanol was added and centrifuged for 5 minutes. The supernatant was decanted and the samples air-dried (Machado et al., 2003[d1]). A conventional singlet Hot start PCR was done using seven (7) sets of primers designed by the authors from Flybase *Drosophila genome release sequence* and PRIMER 3 over the internet (Table 1). A 20µl PCR reaction was performed using 5µL of genomic DNA, 0.5µl of each forward and reverse primers, 3.2 µL of PCR buffer, 2.0 µL of $MgCl_2$, 0.5 µL of dNTPs and 0.4 µL of hot start *Taq* polymerase with an initial Denaturation at 96ºC (5 min), Annealing at 53ºC (1 min), extension at 72ºC (30 seconds) and final extension at 72ºC (5 min) for 30 cycles (Schloterrer, 2005) with modifications. PCR products were run on 1.5% agarose gel stained with Ethidum bromide, visualized and scored using Molecular Imager®GelDoc^{TM}XR + system with Image Lab^{TM} software of BIO-RAD caliberated at 25%.

Statistical Analysis

The bands in the electrophoregram were analysed and scored using the image Lab^{TM}. 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),F- Statistics and Gene Flow were computed using GenAlex version 6.501 (Peakall and Smouse, 2012)

Table 1 ***Drosophila melanogaster*** **primers for genetic diversity assessment**

LOCUS	FORWARD PRIMERS REVERSE PRIMERS	BASE SIZE(bp)	ANNEALING TEMPT.(ºC)	GC %
DM18	GCCGGCCAAACTTAACAATA GCCGGCCAAACTTAACAATA	**104- 214**	59.47;59.96	45.00
DMPROSPER	AGGCAAACAAAGGTGTGTCC GGGAGGTCACTCATCTTGGA	**102- 211**	60.01;60.05	50.00
Antp1	CAAGGACTTGCGTTCTCTCC CACCTACGCGTTCGACTACA	**90- 201**	59.99;59.93	55.00
DROACS2	TGTTTGGATGAGTCCAGCAG ATCTCCACCTGGTACGGATG	**92- 202**	59.83;59.80	50.00
DM30	TTTGGGTTTCTATCGCCAAC AGGGAACTGTCCATGAATGC	**92- 202**	59.94;59.93	45.00
DMWHITE	GGTAAGCAGGGGAAAGTGTG ATTTTTGTGGGTCGCAGTTC	**96- 201**	59.59;59.98	45.00
DMtena	ACAATTTGCGTTGGGAAAAG ACGGACAGGACCTCAATCAC	**87- 201**	59.97;60.00	40.00

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

RESULTS AND DISCUSSION

Genetic diversity

The 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, Sudan and Sahel Savanna zones for the seven microsatellite markers are presented in Table 2. The effective number of allele as such was 2 for all the markers. PIC estimate was 0.693. The Ho and He were 1.000 and 0.500 respectively for all studied loci. All loci and populations had Hardy-Weinberg Equilibrium (HWE) value of 0.157.

Table 2. Genetic Diversity of *D. melanogaster* populations from Savanna zones

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 (2) represents the combined genetic diversity observed in the Northern, Sudan and Sahel Savanna zones as values obtained weren't different.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).

Genetic structure and gene flow

The AMOVA estimate (Table 3) indicated 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.

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.

Table 3: Analysis of Molecular Variance (AMOVA) for *Drosophila melanogaster* populations in Savanna zone, and the fixation indices

Source of Variation	Df	SS	MSS	EV.	PV (%)	FI
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	

DF Degree of Freedom, SS Sum of Squares, MSS Mean Sum of Squares, EV Estimated Variance, PV Percentage Variance, FI Fixation Indices

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 reinforces outbreeding in these populations (F_{IS}<F_{IT}). 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). 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 which is similar to population structure of 45 Indian natural populations of *Drosophila ananassae* carried out by Singh and Singh (2010).

CONCLUSION

The findings of this study which is the first of its kind in Nigeria imply that the identified Microsatellite markers can be successfully employed in evaluating and identifying the DNA polymorphism in natural populations of *Drosophila melanogaster* from Savanna zone. There is high genetic diversity and the 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 populations are out breeding with high genetic divergence due to drift. The very great genetic differentiation and moderate gene flow indicates strong sub-structuring and reproductive isolation in natural populations of *D. melanogaster* in Savanna zone of Nigeria. The present estimates of genetic diversity, and the potential for current gene flow via human traffic, suggest that Savanna populations of *D. Melanogaster* will be able to cope with environmental changes and may provide additional insight into the impact of colonization, migration, and adaptive evolution on genome variation in very recently established populations.

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