ESTIMATION OF GENETIC RELATIONSHIPS AMONG SOME POULTRY SPECIES USING CYTOCHROME OXIDASE b GENE AND MICROSATELLITE MARKERS

BY

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA.IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE.

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NOVEMBER, 2015

DECLARATION

I declare that the work in this thesis entitled "ESTIMATION OF GENETIC RELATIONSHIPS AMONG SOME POULTRY SPECIES USING CYTOCHROME b GENE AND MICROSATELLITE MARKERS" has been carried out by me in the Department of Animal Science, Ahmadu Bello University, Zaria. Theinformation derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATTION

This thesis **entitled " ESTIMATION OF GENETIC RELATIONSHIPS AMONG SOME POULTRY SPECIES USING CYTOCHROME OXIDASE b GENE AND MICROSATELLITE MARKERS" by MOHAMMED AWWAL GALADIMA** meets the regulation governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

To the memory of my father Mohammed Galadima, for his relentless effort to make me a useful member of the society.

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ABSTRACT

This thesis observed evolutionary relationships, compare the effectiveness of mitochondrial DNA (cytochrome oxidase b gene) among some species of poultry, 30 birds per breeds or species (Rhode Island Red, Rhode Island White, Shikabrown, Francolins, Guinea Fowls and Japanese Quails) using mitochondrial DNA`s cytochrome b oxidase gene and chicken microsatellite markers. It has also evaluated some quantitative trait loci (QTL) affecting egg quality traits on chromosome 1 to 5 of 205 Shikabrown F_2 crossed using 25 microsatellites markers. In the mitochondrial analysis, a total of 83 haplotypes were identified from 414 polymorphic sites. Rhode Island White (RHIW) had the highest haplotypes 26, while Rhode Island Red (RHIR) had the least haplotype number 3. The highest number of transition was observed in Japanese quails (JPQ) 78. RHIW had the highest mismatch observed mean value 18.94. The value of Tajima`s D and Fu`s Fswere negative in all the species. The probability sum of squared difference P(Sim. SSD $\geq$ Obs. SSD), and probability Harpending`s raggedness index P(Sim.r $\geq$ Obs.r) 0.92, 0.33, 0.40, 0.68 and 0.79, 0.76, 1.00, 0.42 were not significant in RHIW, RHIR, Shikabrown (SHBR) and Guinea Fowl (GFW), but significant 0.001, 0.05 and 0.0.03, 0.05 in JPQ and Francolins (FRN). Phylogenetic tree and Median network analysis grouped RHIW, RHIR, SHBR, and GFW together. Analysis of molecular variance (AMOVA) showed that among population maternal genetic variance was 58.23%, while within population maternal genetic variance was 41.77%. Fixation index (F_{ST}) among population was 0.58. SHBR had the highest mean number of alleles (MNA) 5.37±0.43, and mean number of effective alleles (MNEA) 3.1±0.29 in the microsatellite analysis, the mean number of alleles in the whole population was 4.67±0.20, while the mean number of effective allele in the whole population was 2.70±0.0.13.The mean Shannon information index (I) across the populations was 1.1±0.04. The mean observed heterozygosity (Ho) across population was 0.58±0.03, while the mean expected heterozygosity across population (He) was 0.5±0.02. AMOVA indicated that 20% of variation was among population, 78% of variation was among individuals and 2% was within individuals.QTLs affecting, shell thickness, yolk length, egg weight, yolk weight, albumen weight, shell weight and albumen length were identified on these chromosomes. The effect of genotype on some of these traits were additive while others were dominant. Two QTLs located on chromosome 2 (shell thickness and shell weight), and three QTLs located on chromosome 3 (albumen weight, yolk weight and egg weight) showed pleiotropic effect, correlations between these QTLs were positive, negative and significant.It was concluded that all the species studied showed genetic variation at cytochrome b gene. Variation within microsatellite was only observed in the chicken breeds. Chicken microsatellite markers amplified the Japanese Quails, Guinea Fowl and Francolins DNA poorly. Chromosome 1 to 5 contain genes affecting egg quality traits. Mitochondrial DNA is highly recommended in studies involving species because less problem of genome amplification was encountered, while due to the problem of amplifying other species genome using microsatellites they are recommended for analysis within the chicken species only. Further analysis should be carried out using higher density markers to fine-tune positions of markers linked with these loci controlling egg quality.

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ABBREVIATIONS

AA	Rhode Island Red genotype
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular variance
AnGR	Animal genetic resource
ANOVA	Analysis of Variance
BB	Rhode Island White genotype
cM	Centimorgan
DNA	Deoxyribonucleic acid
EST	Expressed sequence tag
FAO	Food and Agriculture organization
F_{IS}	Coefficient of inbreeding
FRN	Francolin
FSD	Deviation from Hardy-Weinberg expected values
F_{ST}	Fixation index
GFW	Guinea Fowl
GnRH	Gonadotropic-releasing hormone
H	Heterozygosity
He	Expected heterozygosity
Ho	Observed heterozygosity
I	Shannon information index
IAR	Institute for Agricultural Research,
JPQ	Japanese Quails

LOD	Logarithm of odd
MAS	Marker assisted selection
MNA	Mean number of alleles
Mt-DNA	Mitochondrial DNA
NEA	Number of effective alleles
Nm	Estimated gene flow
OPU	Operational taxonomic unit
PCR	Polymerase chain reaction
PIC	Polymorphism information content
QTL	Quantitative trait loci
r	Harpending`s raggedness index
RAPD	Randomly amplified polymorphic DNA
RFLP	Restricted fragment length polymorphism
RHIR	Rhode Island Red
RHIW	Rhode Island White
SHBR	Shikabrown
SNP	Single nucleotide polymorphism
SSD	Sum of squared deviation

CHAPTER ONE

1.0 Introduction

The chicken, whose genome has been sequenced, is the most studied species in poultry.The significant progress in developing a higher density chicken genetic map has brought about a better understanding of the chicken genome. Knowledge about the chicken`s genome continue to increase at a rapid pace (Groenen *et al*., 2000). Increase in knowledge of the chicken genome however, has not been used to develop information needed to construct potentially useful DNA markers and to understand the genome of other important poultry species through comparative analysis (Smith *et al*., 2000).

The importance of game birds such as Francolin (*Francolin bicalcaratus*), Japanese quails (*Coturnix japonica*), and Helmeted Guinea fowl (*Numida meleagris*), in providing the needed animal protein in human diet, which is inadequate in most developing countries cannot be overemphasized. The unique organoleptic properties (Adeyinka, 2002; Kaye, 2005; Audu, 2008), of the *Francolinus*, *Numida*and *Coturnix*, made up of different species of wild birds found around the world, have become delicacies and favorite among the avian species hunted as game food. Unlike the chicken which was domesticated about 8000 years ago (Romanov *et al.*, 2001) and the focus of contemporary research, the knowledge about the genome of the wildspecies is scanty (Romanov and Weigend, 2001).Poultry species such as Japanese quails, Guinea fowls and domestic Chickens along with the wild species for example *Francolins* belong to the same order (galliforms). The importance of keeping genetic diversity in domestic livestock is advocated worldwide by the Food and Agriculture Organization (FAO 2007 a). In recent years in the poultry industry, only limited number of species and breeds, which match economic demands, are reared on a large scale. This

limitation in the number of breeds probably resulted in a decrease of the genetic diversity in domestic chickens because these commercial breeds or their hybrids are generated during a considerably short time to satisfy economic benefit (Tadano *et al.*, 2006). On the other hand, other breeds and species not used for commercial purposes seem to have more genetic diversity as compared with recent commercial breeds, because they have been improved and established in their long breeding history through processes, greatly different from those used for commercial (Tadano *et al*., 2006). In his book "*On The Origin of Species by Natural Selection*" Darwin proposed by giving overwhelming evidence from his extensive comparative analysis of living specimen and fossils, all living organisms descended from a common ancestor through the formation and branching of linages .

Organisms that share a recent common ancestor have extensive homologies among them, both in their morphologies and genetic materials (DNA sequences) than those from distant ancestors.

Armed with this information, transferring economically important characters such as disease resistance and production trait from wild to commercially closely related species may be feasible, especially with contemporary techniques such as genetic engineering and recombinant DNA technologies. Therefore conservation of other breeds and species of poultry as a genetic resource is important to fill unanticipated breeding demands in the future (Tadano *et al.*, 2006).

1.1 Statement of the Research Problem

Knowledge about the genetic resources of domestic animals and their immediate wild ancestors are important component for a successful production programme as required by world food security. The benefits derived by impoverished and poor societies on livestock

such as poultry can only be underscored, in environments that are less conducive for mix-farming, livestock production is the only alternative available. According to the Food and Agriculture Organization of the United Nation (FAO 2007 b), livestock including poultry currently contributes about 30% of Agricultural gross domestic product in developing countries, with a projected increase to 40% by 2030.

However little is known about the genetic resources of livestock, and the diversity of animal genetic resource is continuously decreasing due to increase in usage of commercial breeds. If the available Animal genetic diversity are not characterized and cataloged, a time will come when there would be a complete erosion of diversity in the poultry population, and that is going to have catastrophic effect on production. Variation the only tool that both natural and artificial selection depends upon, would be depleted hence there would be no room for improvement of livestock, both in terms of production and resistance to diseases. Inbreeding depression characterized by manifestations of various maladies, such as infertility low disease resistance and low production rate could set in.

1.2 Justification

Poultry is an important component of global animal diversity. It provides meat, egg and aesthetic values. Poultry industry is crucial to the global food security. The domestication of the present chicken ancestor, the wild jungle fowl (*Gallus gallus*) give rise to a very wide array of breeds sprouting up due to intensive selection. Each breed is bestowed with unique trait either in the form of production, organoleptic and tenderness, or the beauty of its plumage, which are all desired by man.

In the last century, commercial poultry production has revolutionized this sector by producing few specialized breeds that have dominated the industry across the world.

Obsession with these breeds if not checked would result in erosion of poultry diversity, increasing inbreeding depression, of which its consequence will have a devastating effect on the industry. Selection is an important tool for livestock improvement and it operates only on diversity. Preserving the extant poultry breeds is very important for the survival of the industry. Characterizing and cataloging at molecular level as required by Animal Genetic Resource (AnGR) is very importantin the conservation of the poultry genetic resource. The characterization of Animal genetic resource is necessary for proper assessment of breeds and to guide decision making in livestock development and breeding programme.

One of the major goals of agricultural research is the identification of genes controlling the expression of economically important traits. Most of these traits display a wide variation in the expression of genes at distinct loci (QTL). The currently availability of highly polymorphic DNA markers in many species renders, possible the elaboration of well saturated genetic maps and consequently, genetic dissection of complex quantitative trait (Wardecka *et al., 2003*).

Conventional selection methods, using half sibs and pedigree information to estimate heritability and selection response is expensive, time consuming and cumbersome. Selection at molecular level is straightforward, needs fewer number of mapping population, less expensive and takes a shorter time to be accomplished. Information from these molecular markers linked to QTL of interest can be used in Marker Assisted Selection. Such procedure will bring faster gain in livestock improvement within the shortest time.

1.3 Aim and Objectives of the Study

The present work aimed at:

1. Observing the genetic variations between Francolin, commercial chickens (in this case, Rhode Island "Red and white" and Shikabrown), Guinea fowl and Japanese quails by using mitochondrial DNA (cytochrome oxidase b gene) and chicken microsatellites markers.
2. Establishing phylogenetic relationship between the species.
3. Comparing the effectiveness of mitochondrial DNA and microsatellite markers in studying relationships between species.
4. Evaluate quantitative trait loci (QTL) that affects egg quality traits on chromosome 1 to 5 of the chicken, using microsatellite markers.

1.4 Research Hypotheses

I. H_o = there are no significant differences in the molecular profile and phylogenetic relationships among the species studied.

H_a = there are significant differences in the molecular profile and phylogenetic relationships among the species studied.

II. H_o=there are no significant differences between mitochondrial and microsatellite markers in analyzing relationships among species.

H_a = there are significant differences between mitochondrial and microsatellite markers in analyzing relationships among species.

III. H_o = Chromosome 1 to 5 of Rhode Island Red and Rhode Island White F_2 intercross (RHIR X RHIW F_2) do not carry alleles for egg quality traits.

H_a = Chromosome 1 to 5 of Rhode Island Red and Rhode Island White F_2 intercross (RHIR X RHIW F_2) carry alleles for egg quality traits.

IV. H_0 = there are no linkages existing between markers and QTLs controlling egg quality traits along the genome.

H_a = there are linkages existing between markers and QTLs controlling egg quality traits along the genome.

CHAPTER TWO

2.0 Literature Review

The chicken genome consists of thirty eight pairs of autosomes and sex chromosomes Z and W. The chromosomes can be classified into two size groups, nine macrochromosomes and thirty microchromosomes. In chickens the male is homogametic sex ZZ, while the female is heterogametic ZW (Tuiskula-Haavisto *et al*., 2002). The size of the chicken genome is estimated to be 1.2 $x10^9$ base pairs and approximately 4,000 cM in length (Emera *et al.,* 2003). The concerted effort by international investigators to develop a consensus genetic map for the chickens was a major advancement in avian genomics. In addition, several other important projects are worth noting. Four hundred(400,000) expressed sequences tag (EST) have been identified from a variety of chickens tissues, including activated T cells, oviduct fat, liver, skeletal muscles, intestine, bursa, embryo, pituitary and other(Boardman *et a*l., 2002, Buerstedde *et al.,* 2002) conserved known regions call markers. A marker is a DNA sequence with known location that can be used to identify individuals or species. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism) or a long one, like minisatellite repeats. Two types of DNA markers are used in molecular analysis in chickens, type I which are genes with known function and type II which are noncoding DNA segment, this group includes the highly conserved or repetitive microsatellites, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and CR1 retrotransposons (Emera *et al.,* 2003). However, with the isolation of novel genetic polymorphism in these genes, type 1 markers are expected to receive wider application in the chicken genomics and QTL identification. In contrast, the type II markers have received considerable more attention

and have been the markers of choice for genetic mapping and QTL researches. Microsatellites markers are referred to as "second generation markers" for gene mapping studies (Weissenbach, 1998). Microsatellites are tandem repeats of 1-6 bases. The polymerase chain reaction primers (PCR) that are complementary to these markers are used to amplify sequences of interest that are flanked by the microsatellites repeats, this is a way of length polymorphism identification. The length polymorphism can be easily scored by automated DNA sequencers when fluorescently labeled PCR primers are incorporated. The ability to automate the genotyping along with the high likelihood of finding a DNA polymorphism have made microsatellites markers the primary genetic markers to map and use in genome-wide QTL search (Hans, 1997). The microsatellites markers are extensively used in assessing genetic structures, diversities and relationships because of many advantages, such as being numerous throughout the genome, showing a high degree of polymorphism and co-dominant inheritance (Roushdy *et al.,* 2008). Others include Randomly amplified polymorphic DNA(RAPD) and Restriction fragment length polymorphim(RFLP). RAPD are short primers of 8 to 12 nucleotides created arbitrarily. No knowledge of the DNA sequence for the targeted gene is required as primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had attention of scientific community or relatively few DNA sequences are compared (not suitable for forming gene bank). Due to the fact that it relays on large, intact DNA template sequence, it has some limitations in the use of degraded DNA samples. Its removing power is less than targeted, species specific DNA comparing method such as short tandem repeats (Mbwana-Mbwana, 2006). AFLP are differences in restriction fragment length caused by SNP and

insertion/deletion of markers that create or abolish restriction endonuclease recognition sites. The AFLP technique is based on selective PCR amplification of restriction fragments from a total digest of genome DNA. It has the following short comings: propriety technology is needed to score heterozygotes and homozygotes. Developing locus specific markers from individual fragment is difficult. There is the need to use different kits adapted to the size of the genome being analyzed (Vos, 2009). RFLP is a difference in homologous DNA sequences that can be detected by the presence of fragment of different length after digesting of DNA samples in question with specific restriction endonuclease. RFLP as a molecular marker is specific for a single clone/restriction enzyme combination. Most RFLP markers are co-dominants (both alleles in the heterozygous and homozygous samples can be detected) and highly locus specific. However isolation of sufficient DNA for RFLP analysis is time consuming and labor intensive, which is a major setback.

2.1 Phylogenetics

Molecular phylogenetics applies a combination of molecular and statistical techniques to infer evolutionary relationships among organisms or genes. The similarities of biological function and molecular mechanisms in living organisms strongly suggested that species descended from a common ancestor. Molecular phylogenetics uses the structure and function of molecules and how they change over time to infer these evolutionary relationships. This branch of study emerged in the early twentieth century but did not begin in earnest until the 1960s, with the advent of protein sequencing, PCR, electrophoresis, and other molecular biological techniques. Over the past thirty years, as computers have become more powerful and more generally accessible, and computer algorithms more sophisticated, researchers have been able to tackle the immensely complicated stochastic and probabilistic problems that

define evolution at the molecular level more effectively. Within the past decade, this field has been further reenergized and redefined as whole genome sequencing for complex organisms has become faster and less expensive. As mounds of genomic data becomes publically available, molecular phylogenetics is continuing to grow and find new applications (Dowell 2009). Phylogenetics promotes the understanding of evolution of species, how diseases are spread, the understanding of trait evolution and conservation of livestock genetic resources.

2.2 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a biochemical techniques in molecular biology to amplify a single or a few copies of DNA across several order of magnitudes, generating thousands to million copies of particular DNA sequences (Carr *et al.*, 2012). PCR is now a common and often indispensable method used in medical and biological research laboratories for a variety of applications. These include DNA cloning for sequencing, DNA-based phylogenetic analysis or functional analysis of genes; genetic mapping and QTL analysis, diagnosis of hereditary diseases and identification of genetic finger-print (Schwartz *et al., 2012*). The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers, containing sequences complementary to the targeted regions along with DNA polymerase are key components to enable selective and repeated amplifications. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.

2.3 Quantitative Trait Loci (QTL)

Quantitative trait loci refer to phenotypes that vary in degree and can be attributed to polygenic effect and their environment. Quantitative trait loci are stretches of DNA containing or linked to the genes that underlie a quantitative trait. Mapping regions of the genome that contain genes involved in specifying a quantitative trait is done by using molecular tags such as microsatellite, single nucleotide polymorphism and amplified fragment length polymorphism (Ricky, 2003).

Most economically important traits are under the influence of polygenes. Identifying those loci would underscore the kind of progress that would ensue in improving production of poultry and livestock at large (Hocking *et al*., 2002). The knowledge on position and effect of quantitative trait loci (QTL) is lacking for most traits of interest to animal breeders. Identification and use of quantitative trait loci (QTL) in selection programmes offer the potentials for more rapid improvement, particularly traits that are difficult to measure. In order to enhance these potentials, identification of loci having large effects on these economically important traits and the origin of potentially beneficial alleles is necessary (Hocking *et al.,* 2002). Derived information on QTL is useful for marker assisted breeding, thereby improving the understanding of the biological background (that is which genes are involved and their effect) of traits (Vankaam *et al.,* 1999).

2.4 Methods to Detect QTLs

There are three methods used to detect QTLs, they are single-marker analysis, simple interval method and composite interval method. Single-marker analysis is the simplest method for detecting QTLs associated with single markers. The statistical method used for single-marker

analysis include-t-test, analysis of variance (ANOVA) and linear regression. Linear regression is commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variations arising from the QTL linked to the marker. This method does not require a complete linkage map and can be performed with basic statistical software programs.

Simple Interval Mapping (SIM)

The simple interval mapping takes full advantage of a linkage map. The method evaluates the target association between the trait values and the genotype of a hypothetical QTL (target QTL) at multi analysis point between pair of adjacent marker loci (the target interval). Presence of a putative QTL is estimated if the odds ratio (LOD) exceeds a critical threshold. SIM can be easily accessed through statistical packages such as MAPMAKER/QTL. By using tightly linked markers for analysis it is possible to compensate for recombination between markers and the QTL, thereby increasing the probability of statistical detecting the QTL, and providing an unbiased estimate of QTL effect.

Composite Interval Mapping (CIM)

Composite interval mapping combine interval mapping for a single QTL in a given interval with multiple regression analysis on marker associated with other QTL. It considers a marker interval plus a few other well-chosen single markers in each analysis, so that n-1 test for interval-QTL associations are performed on a chromosome with n markers. The advantages of CIM are as follows; (i) mapping of multiple QTLs can be accomplished by the search one dimension;(ii) by using link markers as cofactors, the test is not affected by QTL outside the region, thereby increasing the precision of QTL mapping;(iii) By eliminating much of the

genetic variance by other QTLs, the residual variance is reduced, thereby increasing the power of detection of QTL (Collard and Mackill, 2007)

2.5 Marker Assisted Selection

Marker assisted selection (MAS) is a process whereby a marker (morphological, biochemical, or one based on DNA/RNA variation) is used for indirect selection of a genetic determinant or determinants of a trait of interest for example (productivity, disease resistance, antibiotic resistance and quality). This process is used in animal and plant breeding. Considerable development in biotechnology have led animal breeders to developed more efficient selection method to replace traditional phenotypic-pedigree-based selection systems (Collard and Mackill, 2007).

MAS is an indirect selection process where a trait of interest is selected, not based on the trait itself, but on a marker linked to it (Rosyara *et al., 2009*).For example if MAS is being used to select individuals with a disease, the level of the disease is not quantified but rather a marker allele which is linked with the disease allele is used to determine disease presence. The assumption is that linked alleles associate with the genes and/or quantitative traits of interest. MAS can be useful for traits that are difficult to measure, exhibit low heritability, and are expressed late in development.

2.6Neighbor-joining

This program constructs a tree by successive clustering of lineages, setting branching length as the lineages join. The tree does not assume an evolutionary clock, so that it is in effect an unrooted tree. The program cannot evaluate the user tree nor can it prevent branch length from becoming negative. However the algorithm is faster than FITCH or KITSC. This makes

it much accepted than those two programs for large studies or for bootstrap or jackknife reassemble studies (Felsenstein 2005)

2.7 Tajima`s D and Fu`s Fs

These are test of selective neutrality of DNA sequence haplotypes under the infinite site model. The Tajima`s D is a statistical test created and named after the Japanese researcher Fumio Tajima. The purpose of the test is to distinguish between DNA sequence evolving randomly (neutral) and one evolving under a non-random process, including directional selection or balancing selection, demographic expansion or contraction, genetic hitchhiking, or introgression. Randomly evolving DNA sequence contains mutation with no effect on the fitness and survival of an organism, and therefore is called neutral. Thus Tajima`s D can be used as a test statistics to assess whether data are consistent with the population being a neutral mutation-drift equilibrium. The values of D under the following scenarios are as follows;

- If the variation is neutral, D is = zero.
- If there is overdominant selection, D would be positive.
- If the population have undergone a bottleneck D would be positive.
- If there is population expansion D would be negative.

Similarly a positive Fu`s Fs indicates population bottleneck, while a negative Fs is an evidence of population expansion or genetic hitchhiking.

2.8Harpending`s Raggedness and Sum of Square Deviation

Harpending`s raggedness(r) and the sum of square deviation (SSD) are two statistics that are used to assess the validity of a model on neutrality test.

The probabilities (p-value) of the statistics were computed as the proportion of simulated cases that show values larger than the original.

P(sim.r >= obs.r) = p-value of r

P(sim.SSD>= obs. SSD) = p-value of SSD

A significant r or SSD value is taken as an evidence of departure from the estimated demographic model, which can either be a model of population expansion or population stationary.

2.9 Arlequin

The goal of the Arlequin is to provide the average user in population genetics with quite a large set of basic methods and statistics test, in order to extract information on genetic and demographic features of a collection of population samples. The graphic interface is designed to allow users to rapidly select the different analysis they want to perform on their data. The statistical test implemented in Arlequin have been chosen such as to minimize hidden assumptions and to be as powerful as possible. Thus, they often take the form of either permutation test or exact test.

2.10Some Important Poultry Traits

2.10.1 Layer chicken

2.10.1.1 *Pelvic bone arch and the keel bone*

The size of area between the pelvic bones and the keel is a good egg laying trait. It is an indication of ample space for eggs and reproductive organs.The size of this area should be at least three or more fingers wide (Don-Schrider, 2007).

2.10.1.2 *Molting ability*

Molting is the shedding of old fathers and growth of new ones. Chicken molt in a predictable order beginning at the head and neck, proceeding down the back, breast, wings and tail. While molting occurs at fairly regular intervals for each chicken, it can occur at any time due to lack of water, food and normal lighting conditions. Birds should be selected for their molting abilities. Individuals that drop feathers almost at once are more preferred over birds that takes longer time to molt. The quicker the molt, the quicker the birds get back to egg laying business (Mormino 2012).

2.10.1.3*Body conformation*

The space within the body of a chicken is filled with various organs. When one section of the body is narrow or lacks space, the organs of that area must be reduced for space and thus tend to function below optimum. Birds with good width between their legs often are better layers, as this is one indicator of body capacity.

Birds with full, well-rounded breast and with legs set well back tend to be more productive. This is another trait that indicates body capacity. The body of a good layer hen is not rectangular when viewed from the side, it is deeper at the rear than in the front of the bird. This gives more room for the reproductive organ (Melvin, 2008).

2.10.1.4*Egg quality trait*

In the past, feed efficiency and increasing egg number was the primary goal and only recently has egg quality traits received more attention. Nowadays with the improvement of laying persistency and therefore continuously lengthened production cycle, egg quality has become more and more crucial in the breeding program. It is well established in general that

egg quality for example, egg shell and Haugh unit decrease as the hen ages. Consequently to further improve egg mass, it is necessary to reduce the number of downgraded eggs by selecting hens for higher egg quality at the end of the laying period (Burlot *et al.*, 2012). In addition, as different egg industries and consumer demands are getting more specific and more important the uniformity of the external and internal egg quality becomes one of the major traits in selection.

Singh *et al.*(2012), Sarika*et al.* (2012) and Parmar *et al.* (2006) studied the most effective variables for egg quality traits in chicken genotypes. They found significant difference in external quality traits such as egg weight, shape, breaking strength, shell thickness and egg specific gravity and internal quality traits such as albumen height, albumen index, yolk height, egg specific gravity and yolk color across the genotypes.

2.11 Genetic Diversities and Phylogenetic Relationships in Poultry

Landari *et al.* (2008) studied the molecular characterization of Indonesian indigenous chickens based on mitochondrial DNA displacement loop (mt DNA d-loop) sequences and identified 54 polymorphic sites with polymorphism between 167 and 397 contributing to 94.5% of the sequence variation. Analysis of molecular variance (AMOVA) indicated that 67.85% of the total sequences variation was within population and 32.15% was between populations.

Adebambo *et al.(*2009) analyzed the mitochondrial d-loop segment in domestic chicken from South-Western Nigeria and discovered seventeen haplotypes. Analysis of molecular variance indicated that 97.32% of variation was within the population and 2.68% was between population. They also discover total haplotype diversity of 0.39±0.08 in the population.

Molecular characterization of Sudanese and Southern Sudanese chicken breeds were studied using mt-DNA d-loop region, (Charles *etal.,* 2014). They identified 14 haplotypes in the populations. AMOVA showed that genetic variation within the population accounted for 88.6% and the difference among population accounted for 11.4%. They also found an average fixation index of 0.38 in the population they studied.

Pairot *et al. (*2013) studied the genetic diversities of Thai native chicken using mt DNA sequences, they reported a total haplotype number of 30, a total haplotype diversities of 0.970±0.013 and a total nucleotide diversityof 0.015±0.0.082.

Eltanany *et al.* (2011) assessed the genetic diversities of three Egyptian local chicken strains (Fayoumi, Dandarawi and Sinai) and six synthetic breeds derived from Fayoumi and Sinai by intercrossing with Barred Plymouth Rock, Rhode Island Red or White Cornish, using 29 microsatellite. They identified three main clusters of chicken populations, encompassing selected Fayoumi lines and DOKI-4 (cluster-1), native Dandarawi and Sinai (Cluster-2) and all six synthetic breeds (Cluster-3). The between population marker-estimated kinship was lower than within-population estimates.

Qu *et al.* (2006), investigated 78 indigenous chicken breeds in China. They discovered number of alleles range from 6 to 51 per locus with a mean of 18.74. Heterozygosity (H) values of the 78 chicken breeds were all more than 0.5. The average H value (0.622) and polymorphism information content (PIC) 0.573 of these breeds suggested that the China indigenous chickens possessed more genetic diversity than those reported in many countries.

Masroor *et al.*(2011), observed a total of 91 alleles in 4 varieties of Aseel Chickens with an average of 9.1 alleles per locus. They reported variations in number of alleles between 4 to 8 in Lakha, 4 to 9 in Mushki, 3 to 10 in Mianwali and 3 to 7 in Pashawani. They reported mean

polymorphic information content value of 0.67, 0.69, 0.71 and 0.65 in individual varieties respectively. Mean observed and expected Heterozygosity index values of 0.3941 and 0.7376 were recorded in Lakha, 0.4105 and 0.7468 for Mushki, 0.4105 and 0.7718 Mianwali and 0.3692 and 0.7191 for Peshawmani, whereas its value was minimum (0.3533) between Lakha and Mushki.

Hassen*et al.* (2009) studied indigenous Ethiopian chicken populations using microsatellites markers to determine genetic diversity and variations. They included three local lines of South African chicken and two commercial chicken strains for the comparison. They discover that the Ethiopian chicken population Gassay/Farta had the highest number of alleles per locus (10) for microsatellite markers MCW158. MCW154 was the most polymorphic marker across all populations with an average of seven different alleles. A high genetic diversity was observed in overall loci for all populations with heterozygosity (Ho) value of 0.77. The highest heterozygosity (0.93) across all markers was observed in the Mecha chicken population, while the lowest heterozygosity across all loci (0.66) was observed in the White Leghorn breed.

Hui-Fang *et al.* (2009) Table: 2.2, reported a genetic diversity study of Qingyuan partridges chickens using 30 microsatellites markers. They distinguished a total of 154 alleles. All the microsatellites were polymorphic with mean allelic number of 5.1, ranging from 2 to 9 per locus. The expected mean heterozygosity in the population ranged between 0.500 and 0.839, with mean of 0.685, indicating considerable genetic variation in the population.

Seo *et al.*(2013) Table: 2.1, evaluated the genetic diversity among five Korean chicken lines. They reported an expected heterozygosity (He), observed heterozygosity (Ho) and

polymorphic information content (PIC) range of 6 to 2, 0.466 to 0.852, 0.709 to 0.882 and 0.648 to 0.865, respectively.

Choi *et al.* (2015) characterized commercial Korean native chicken population (KNC, WM1 and WM2) using microsatellite markers. They reported mean number of alleles (MNA), mean expected heterozygosity (Ho) and mean polymorphic information content (PIC) of 5.162, 0.641, and 0.612.

Wei *et al.* (2013) evaluated the genetic diversities of four protected indigenous chicken breeds in China (Dongan, Xuefen black-bone Xianghuang and Taoyuan) using microsatellite markers and reported average heterozygosity values of 0.514, 0.581, 0.567 and 0.589, PIC , 0.455, 0.581, 0.557 and 0.576, respectively for the four chicken populations.

Choi *et al.* (2012) study the genetic variations in five Korean native chickens using 30 microsatellites markers and reported mean number of alleles 4.57. They found expected heterozygosity, observed heterozygosity and Polymorphic information content of 0.310 to 0.86, 0.145 to 0.699 and 0.268 to 0.847, respectively.

Ceccobelli *et al.* (2013) characterized Italian local chickens using microsatellite markers. They reported lower inbreeding coefficient of 0.08 among the native Italian chicken breeds compare to commercial breeds.

Zheng-Hua *et al.* (2010) analyzed genetic diversity by using 10 microsatellites loci in Chai chicken breeds. Allele frequencies, effective number of alleles, heterozygosity, polymorphism information content, F-statistics, migration rate were calculated. The result showed that the average observed heterozygosity (Ho) and PIC amongst population ranged from 0.5353-0.5614 and 0.5108-0.5199, respectively. The genetic diversity was very rich and

was of high selective potency. Pair-wise F_{ST} coefficient explained only 8.4 % variability from the breed difference.

Alipanah *et al.(*2011) evaluated genetic diversity among three native chicken breeds (Dashtiari, Khazak, And Zabol) in Iran using 10 microsatellites markers. (The mean Heterozygosity was 0.77, 0.79 and 0.52 for Khazak, Dashtiari and Khazaz respectively. The Heterozygosity was consistent with polymorphism information content.

Lyimo *et al.* (2012) evaluated the genetic diversity of Tanzanian chickens population through phylogenetic relationship, and traced the history of Tanzanian indigenous chickens. The microsatellite analysis revealed three clusters of Tanzanian chickens population with a Shannon information index (I) of 0.98 ± 0.032. Chin´wekwe clustered together with Morogoro Medium, Unguja and Pemba made up a common cluster distinct from Morogoro Medium and Ching´wekwe cluster, while Kuchi formed an independent cluster.

Eight genomic DNA were isolated from three indigenous chickens populations-Normal feathered (NF), Frizzle feather (FF) and Naked Neck (NN) maintained in south-south region of Nigeria by Ohwojakpor *et al.,*(2012). They reported mean allele number (Na) for all loci to range from 5.625 (NN) to 9.875 (NF) and mean effective number of alleles (Ne) to range from 5.0133 (NN) to 7.1637 (NF). Mean PIC was between 0.7588(NN) and 0.8282(NF). Averages of observed heterozygosity among loci were 0.5068, 0.5236 and 0.5755 for NN, NF, and FF. Chicken populations, respectively.

Table 2.1: The Statistical Analysis of Heterozygosity(Ho and He), Polymorphic Information Content and F-Statistics Value Using Selected 15 Microsatellite Markers among Five Native Korean Chicken Lines.

Locus	Chro	No. of alleles	He	Ho	PIC	F_{ST}	F_{IT}	F_{IS}
LEI107	1	8	0.77	0.716	0.739	0.1076	0.1001	-0.0083
MCW0145	1	7	0.791	0.727	0.739	0.1141	0.0774	-0.0413
MCW0063	2	8	0.712	0.648	0.665	0.0956	0.1142	0.0206
MCW0087	2	9	0.830	0.761	0.806	0.1219	0.1074	-0.0165
MCE0264	2	6	0.709	0.713	0.648	0.0309	0.0013	-0.0306
MCW0261	3	8	0.842	0.830	0.817	0.1324	0.0455	-0.1002
ADL0292	5	7	0.821	0.716	0.791	0.1345	0.1610	0.0306
MCW0029	5	11	0.789	0.739	0.770	0.1215	0.0761	-0.0517
ADL0021	9	8	0.849	0.795	0.825	0.1111	0.0892	-0.0246
ADL0259	9	9	0.846	0.773	0.826	0.0890	0.0923	0.0036
GCT0016	9	8	0.804	0.466	0.773	0.2473	0.4513	0.2710
LEI0251	13	12	0.822	0.852	0.865	0.0927	0.0538	-0.0429
MCW0104	13	11	0.846	0.659	0.823	0.1965	0.2349	0.0478
ADL0304	18	8	0.768	0.678	0.735	0.1004	0.1449	0.0495
ADL324	20	6	0.767	0.568	0.723	0.2174	0.2853	0.0868
Total/Mean	8	126/8.4	5.162	0.641	0.612	0.1290	0.1370	0.0093

Sources:Seo *et al* (2013) He= expected heterozygosity, Ho= observed heterozygosity, PIC= polymorphic information content, F_{ST}= fixation index, F_{IT}= global fixation index, F_{IS}= heterozygosity deficit between lines.

Table 2.2: Information from 30 Microsatellites Markers Used In Characterizing Qingyuan Partridges In China.

Markers	Chr.	Alleles	No. Alleles	Size	Ho	H
ADL136	9	9	6.200	117-163	1.000	0.839
ADL166	5	8	4.383	114-151	1.000	0.772
ADL185	2	9	4.891	113-155	1.000	0.796
ADL195	1	4	2.921	95-124	0.893	0.658
ADL210	11	5	2.325	108-121	1.000	0.570
ADL212	2	8	3.363	83-110	1.000	0.703
ADL225	13	7	5.725	118-158	0.915	0.825
ADL123	11	2	2.000	86-98	1.000	0.500
ADL201	2	2	2.000	118-128	1.000	0.500
ADL176	2	6	4.976	172-202	1.000	0.796
LEI166	3	4	2.439	254-290	1.000	0.490
LEI066	14	6	3.674	298-360	1.000	0.726
LEI094	4	7	4.388	158-223	1.000	0.772
MCW295	4	3	2.649	80-965	1.000	0.622
MCW014	6	4	3.372	164-193	1.000	0.704
MCW067	10	4	2.138	181-204	1.000	0.532
MCW081	5	4	3.279	96-126	0.983	0.695
MCW183	7	4	2.563	287-351	0.683	0.610
MCW294	2	3	2.388	311-359	1.000	0.582
MCW330	17	4	3.060	257-289	0.729	0.673
MCW085	4	6	2.632	275-311	1.000	0.620
MCW085	4	6	2.632	275-311	1.000	0.620
MCW264	2	4	3.905	208-263	1.000	0.744
MCW134	9	4	2.601	275-311	1.000	0.620
MCW104	13	8	3.104	182-245	0.847	0.678
MCW145	1	2	2.000	186-195	1.000	0.500
MCW004	3	5	4.181	107-207	1.000	0.500
Mean		51	3.474		0.967	0.685

Source: Hui-Fang (*et al 2009*)

Genetic diversity among nine Vietnamese and two Chinese local chicken breeds were studied by Cuc *et al.*(2010), using 29 microsatellite markers. Cluster analysis using structure software suggest six clusters as the most likely grouping of the eleven breeds studied . The groups encompassed four homogenous clusters, one formed by the two Chinese breeds and the other three representing a single breed each: The Mekong delta breed AC, the South central coast breeds Choi, and the Red River Delta breed Dong-Tao. The six remaining breeds formed two additional admixed clusters.

Microsatellite based genetic diversity was studied in Dahlem Red stocks, selected strain of Rhode Island Red (RIR-B) and their cross (Dahlem X RIR) using ten microsatellites marker loci(Chattopadhyay *et al*.,2009). Polymorphism between different strains was observed for different microsatellites marker loci. MCW216 marker showed highest polymorphism across all the population with an observed average number of alleles (15), while MCW222 showed least polymorphism with only 7 alleles. Expected Heterozygosity across the strain ranged from 0.77(MCW222) to 0.92(MCW216).While average heterozygosity varied from 0.67(MCW222) to 0.86(LEI 0192).

Clementino *et al.*(2010) studied genetic diversity in Brazilian chicken ecotypes using 20 microsatellite markers. The analysis revealed a total of 191 alleles, ranging from 3 to 18, with an average of 9.6 alleles per locus. The average observed heterozygosity was 0.785, while the mean expected heterozygosity was 0.688. The mean polymorphic information content value was 0.731.

Wilkinson *et al.*(2012) assessed genetic diversity and genetic structure in 265 individual genotyped samples from 24 British chicken breeds. They found a total of 239 alleles across 30 microsatellite loci, with a mean number of 7.96 alleles per locus. The breeds were highly

differentiated with an average F_{ST} of 0.25, average expected heterozygosity of 0.49, ranging from 0.20 in Spanish to 0.62 in Araucana. Average observed heterozygote frequency was 0.3, it ranged from 0.15 in Spanish to 0.94 in Cochin.

Mtileni *et al.*(2011) compared the level and distribution of genetic variation between South African conserved and Village chicken population using 29 microsatellite markers. They found village chicken population more diverse than conserved flocks. Cluster analysis indicated a clear subdivision of each of the conservation flocks that were different from the three village populations.

In a study conducted by Osei-Amponsah *et al.*(2010), 22 microsatellite markers were used to genotype 114 local chickens from the Forest and Savanna eco-zones of Ghana and the result compared to those of the ancestral Red Jungle Fowl and two European commercial chicken population (Broiler and White Leghorn) A total of 171 alleles were observed with an average of 7.8 alleles per locus. The local Ghanaian chicken showed higher diversity in terms of observed number of alleles per locus (6.6) and observed heterozygosity (0.568) compared with the combined control population (6.0 and 0.458 respectively).

The genetic structure and diversity of 15 Chinese indigenous chicken breeds was investigated using 29 microsatellites markers by Chen *et al.*(2008). The total number of birds examined was 542. They observed a total of 277 alleles (mean number 9.55 alleles per locus ranging from 2 to 25). All populations showed high levels of heterozygosity with the lowest estimates of 0.44 for the Gushi chickens, and the highest of 0.644 observed in Wannan Three-Yellow chicken. About 16% of the total genetic variability originated from differences between breeds, with all loci contributing significantly to these differences.

Genetic variation at 20 microsatellite loci and genetic diversity were examined in Yangzhou chicken by Liu *et al.*(2008). The observed and effective number of alleles across the microsatellite loci varied from 2 to 6 with an overall mean of 3.778 and 2.404, respectively (Table 2.3).The observed and expected heterozygosity varied from 0.129 to 0.755 with an average of 0.422 and 0.517, respectively. Average polymorphism information content was 0.464. The population showed fairly high level of inbreeding (F_{IS}= 0.184) and global heterozygosity deficit.

Mtileni *et al.*(2010) determined genetic diversity within South African indigenous chicken populations. Two Chicken populations were genotyped for 29 autosomal microsatellite markers. All microsatellites typed were found to be polymorphic. A total of 213 alleles were observed for all four populations. The mean number of alleles per population ranged from 3.52±1.09 to 6.62 ±3.38. Mean observed heterozygosity and expected heterozygosity in the conservation flocks were 0.55 and 0.57 respectively.

Wu *et al.*(2004) evaluated allele frequency, heterozygosity (H), mean heterozygosity, polymorphic information content (PIC) and genetic relationships in Luyan , Gushi , Tibetan, Bailer, Xianju, Chahua, Dagu, Beijing Fatty, Langshan, Henan Game, Taihe Silki and Xiaosham chicken using seven microsatellites DNA markers. They discover variations among allele frequencies of seven microsatellites loci in 12 Chinese native chicken breeds. Among the 12 Chinese native chicken breeds, mean heterozygosity of Luyan chickens was the highest (0.5929), and that of Chahua chicken was the lowest (0.3514). There was a similar result in PIC. Cluster analysis showed that the genetic relationship between Taihe Silkies chickens and Henan game chicken was the nearest, and that between Gushi and the other chickens breeds was the most distant.

Table 2.3: Genetic Variations in Yangzhou Chicken

Locus	Chromosome	A	Ho	He	PIC	F_{IS}
ADL156	3	1.172	0.129	0.147	0.13	0.124
ADL183	1	4.058	0.745	0.755	0.712	0.013
ADL185	2	2.443	0.551	0.592	0.544	0.069
ADL201	2	1.987	0.795	0.492	0.544	-0.601
ADL217	2	1.508	0.345	0.337	0.310	-0.022
ADL292	5	2.963	0.907	0.665	0.603	-0.367
LEI166	5	2.882	0.387	0.655	0.579	0.409
LEI194	4	1.193	0.147	0.165	0.156	0.106
LEI166	3	1.757	0.330	0.432	0.382	0.235
MCW58	1	3.089	0.238	0.683	0.620	0.651
MCW85	4	2.574	0.280	0.613	0.541	0.543
MCW120	7	2.719	0.247	0.634	0.571	0.611
MCW154	2	1.388	0.267	0.263	0.251	-0.018
MCW170	4	3.555	0.581	0.721	0.668	0.194
MCW180	4	3.650	0.609	0.727	0.678	0.162
MCW258	2	2.637	0.258	0.622	0.549	0.585
MCW264	2	1.232	0.211	0.189	0.171	-0.115
MCW330	17	2.493	0.560	0.600	0.516	0.067
Mean	2.404	0.422	0.517	0.464	0.184	

Source: Liu *et al.,(2008*)

Li *et al.*(2004) used twenty microsatellites markers to analyze the genetic relationship among four lines (A,B,C, and D) of egg-typed chickens. The genetic heterozygosity, polymorphism information content, effective allele number, and genetic distance among lines were calculated and dendrogram was constructed to evaluate the genetic variability within lines and genetic relationship among lines. They detected 65 alleles in 20 microsatellites markers. Average allele number for microsatellite markers is 3.250 and average effective allele number for those markers were 2.395. The polymorphic information content of the microsatellite markers averaged at 0.454, and ranges from 0.102 to 0.729. The heterozygosity of microsatellites markers ranged from 0.108 to 0.765. The genetic distance between A and B lines was 0.005 to 0.016, while between C and D lines was around 0.900.

Dorji *et al.* (2012) used microsatellites markers to examine the genetic diversity of Bhutanese chickens. They discovered all loci to be polymorphic, with number of alleles ranging from 6 (MCW0111) to 23(MCW0183). Substantial genetic variation was observed in all populations, with Bhutanese native chicken Yuebjha Narb showing the lowest genetic variability. A neighbor-joining tree revealed the genetic relationships.

Vanhala *et al.* (2009) evaluated the genetic diversity and variability of eight chicken lines using microsatellite markers and found all the microsatellites to be polymorphic with number of alleles varying from 4 to 13 per locus and one to ten per line. The observed heterozygosity ranged from 0.00 to 0.91. The highest (0.67) and lowest (0.29) mean heterozygosity per line was observed in the broiler and in White leghorn (Table 2.4).

Roushdy *et al.* (2008) assessed the genetic characteristics of three population generated from Egyptian Fayoumi native breed (P, G and R). They detected a total of 106 alleles. The lowest heterozygosity was found in P (20%) and the highest (27%) was found in G population.

The genetic variability of three poultry breeds namely, Aseel, Miri, and Nicobari taken from different geographical location of India were evaluated using 15 microsatellites loci by Pandey *et al.*(*2002*). They discover that number of alleles varied from 3 to 9 in Aseel, 3 to 8 in Miri and 2 to 7 in Nicobari. Mean PIC in Aseel was 0.6, average expected heterozygosity and observed heterozygosity were 0.64 and 0.59.

Lang-Hui *et al.,*(*2006*) studied the genetic diversity of Nixi chicken using 33 microsatellites markers from 24 chromosomes. The result showed that 122 alleles, were identified in 50 individuals and the mean value was 3.7 per locus. The average Heterozygosity and PIC of 33 microsatellite markers were 0.635 and 0.551, respectively, indicating that Nixi chicken breed has high level of genetic variability.

2.12 Studies on Quantitative Trait Loci in Poultry

2.12.1 Egg quality traits:

Genome-wide scan of QTL for egg quality and productivity have been conducted by Tuiskula- Haavistor *et al.* (2002) using a reference population. 14 genome wide significant and six suggestive QTL located in chromosome 2,3,4,5,8,and Z were located.

Wardecker *et al.*(2002) demonstrated 16 traits with significant genotypic effect (QTL) in Green-legged partridges and Rhode Island Red on egg production and egg quality traits, based on analysis using 23 microsatellite markers.

Table 2.4: The Expected and Observed Heterozygosity for Each Locus Line, Identified In EightBroiler and Leghorn Chicken Lines.

Locus		LSL (30)	M (31)	J (20)	RIR (30)	FJ (19)	FS (27)
HUJ7	H(exp.)	0.488	0.554	0.512	0.728	0.478	0.815
	H(obs.)	0.000	0.387	0.280	0.633	0.263	0.731
	P	0.000	NS	NS	NS	NS	NS
HUJ5	H(exp.)	0.499	0.647	0.724	0.747	0.152	0.621
	H(obs.)	0.600	0.355	0.621	0.690	0.053	0.704
	P	NS	NS	NS	NS	NS	NS
	H(exp.)	0.515	0.627	0.593	0.747	0.569	0.662
HUJ10	H(obs.)	0.033	0.000	0.240	0.276	0.105	0.500
	P	0.000	0.000	0.000	0.000	0.000	NS
HUJ12	H(exp.)	0.364	0.444	0.440	0.253	0.563	0.706
	H(obs.)	0.467	0.581	0.345	0.207	0.526	0.667
	P	NS	NS	NS	NS	NS	NS
MCW03	H(exp.)	0.000	0.000	0.000	0.339	0.000	0.604
	H(obs.)	0.000	0.000	0.000	0.217	0.000	0.522
	P	NS	NS	NS	NS	NS	NS
MCW05	H(exp.)	0.705	0.589	0.665	0.737	0.602	0.477
	H(obs.)	0.700	0.600	0.630	0.862	0.737	0.519
	P	NS	NS	NS	NS	NS	NS
	H(exp.)	0.000	0.643	0.409	0.729	0.496	0.752
MCW07	H(obs.)	0.000	0.129	0.182	0.320	0.053	0.593
	P	NS	NS	NS	NS	NS	NS
MCW14	H(exp.)	0.589	0.236	0.668	0.509	0.522	0.453
	H(obs.)	0.767	0.258	0.586	0.360	0.526	0.593
	P	NS	NS	NS	NS	NS	NS
MCW16	H(exp.)	0.499	0.135	0.354	0.687	0.149	0.642
	H(obs.)	0.667	0.143	0.448	0.483	0.152	0.615
	P	NS	NS	NS	NS	NS	NS

Source: Vanhalla *et al* (2009)

Table 2.5: Observed Heterozygosity (Ho) and Expected Heterozygosity (He) and their Means Estimated for Each Locus in Egyptian Fayoumi Native Breed (P, R and G)

Locus		P	R	G	Mean
	(observed)	0.16	On	058	0.28
1	(expected)	0.59	0.49	0.63	0.57
	P value	0	0	0.005	—
	FIS*	0.73	0.7S	009	0.45
	(observed)	0.32	0.5	0.24	0.35
	(expected)	0.S6	0.S6	0.S8	0.87
1	P value	0	0.001	0	—
	Ns	0.63	0.43	0.73	0.62
	(observed)	0.24	0.11	0.32	0.22
3	(expected)	0.49	0.4	0.55	0.48
	P value	0013	0.001	0 009	—
	Ns	0.52	0.73	0.42	0.51
	(observed)	0.3	0.33	0.26	0.29
	(expected)	0.75	0.72	0.74	0.74
4	P value	0	0	0	—
	Ns	0.6	0.54	0.65	0.61
	(observed)	0.16	0.2S	0.19	0.21
5	(expected)	0.85	0.74	086	0.82
	P value	0	0	0	—
	Ns	0.82	0.63	0.78	0.76
	(observed)	0	0	0	0
6	(expected)	0.75	0.69	0.63	0.69
	P value	0	0	0	—
	Ns	1	1	1	1
Mean (H_E)		0.72	0.65	0.72	069
Mean (H_0)		0.2	0.22	0.27	0.23

Source: Roushdy *et al., (2008*)

Table 2.6:Theshold p-value for genome-wise QTL anlysis for Egg Quality and Productivity in Green –Legged Partridge and Rhode Island Red.

Significance level	**Traits**	**P-value**	**Traits**	**P-value**	**Traits**	**P-value**	**Traits**	**P-value**
0.1		12.3851		12.4713		12.9502		13.2319
0.05	SG33	14.0008	SW33	14.6013	SG53	14.2942	SW53	14.6185
0.01		16.8038		19.6956		18.5063		18.2477
0.1		12.7509		12.9955		13.3255		13.2216
0.05	SG33	14.1597	SD33	15.0747	SS53	14.8999	SD53	15.3849
0.01		19.1018		19.9337		17.7742		18.3651
0.1		13.5141		12.5342		13.6760		12.6794
0.05	SC33	14.5763	ST33	13.8237	SC33	15.3230	ST53	14.3963
0.01		19.8742		16.8468		20.0591		16.7848
0.1		12.5833		12.0423		12.9066		16.7848
0.05	EW33	14.3098	YW33	13.2971	EW53	14.7752	YW53	15.3215
0.01		17.6079		17.2604		18.9988		21.7459
0.1		13.1197		12.6586		13.1153		13.3460
0.05	HU33	15.0394	AW33	13.9797	HU53	15.0501	AW53	14.8469
0.01		19.1495		18.0169		18.5606		17.8389

Wardecker *et al. (2002)*

Genetic mapping of QTL affecting egg production, egg quality trait and body weight in F_2 White leghorn- Rhode Island Red intercross chickens was done by Sasaki *et al.* (2004) using 123 microsatellite markers. They assigned 96 markers to 25 autosomal linkage groups and 13 markers to 2 chromosomes, including eight previously unmapped markers. Significant QTL were discovered for body weight on chromosome 4 and 27, egg weight on chromosome 4, the albumen height on chromosome 4 and egg shell color on chromosome 11. A significant QTL for age at first lay was found on chromosome z. Overall 6-19% of the phenotypic variation found in the F_2 population was explained by these QTLs.

Chatterjee *et al.* (2008), conducted a study on six crossbred chicken populations of white leghorn to estimate variability of microsatellites and their association with egg production traits. They used five microsatellites markers located on chromosome 1, 2, 5, and 30, and discovered ADL023 marker to be significantly associated with egg production up to 64 and 72 weeks, and egg weight at 28 weeks of age.

A scan of the chicken genome using 72 microsatellite markers was carried out on meat-type and egg-type resource population measured for production and egg quality traits by Hansen *et al.* (2005). Evidence of eight QTL regions associated with a total of eight traits (specific gravity, albumen height, Haugh unit score, shell thickness, shape index, total number of eggs, final body weight gain, and feed efficiency were found.

Liu *et al.* (2011) performed a genome wide study to identify QTL responsible for egg production trait such as age at first egg (AFE), first egg weight (FEW), and number of eggs (EN) from 21 to 56 weeks of age, and egg quality trait such as egg weight (EW), egg shell weight (ESW), egg shell strength (ESS), yolk weight (YW), egg shell thickness (EST),albumen height (AH), and Haugh unit (HU) on white leghorn female and brown-egg

dwarf dams, using single nucleotide polymorphism and discovered many chromosome wise significant SNP located in previously reported QTL regions. The genome-wide scan also revealed 8 SNPs showing genome-wide significant association with egg production trait.

Lahva *et al*. (2006) made simulation analysis on marker assisted selection based on multitrait economic index in chickens using a computer program written specifically for this purpose and observed that response was higher when the mapped distance between the marker and the quantitative trait gene was small, and also a large number of distantly located markers, spread 10 centimorgan (10 cM) apart, yield higher response to selection than a small number of closely located markers spread 3cM apart, suggesting the effect of polygenes on quantitative traits.

Schreiweis *et al.* (2005) identified eleven QTL using microsatellite markers on chromosome 2 and 4 influencing egg color, egg and albumen weight , percentage shell weight, body weight, and egg production trait at 5% genome-wide significance threshold in white leghorn and broiler(Ceobb-ceobb) F2 cross.

Honkatukia (2010) performed a whole genome scan on Rhode Island Red and White leghorn F2 cross using microsatellite markers and discover two distinct QTL areas on chromosome 2 affecting albumen quality. A quantitative loci area on chromosome z was associated with blood and meat spots, and QTL on chromosome 8 was found to be associated with fishy taint disorder.

Xu *et al.* (2011) looked at variations that could be associated with chicken egg number at 300 days of age (EN300) in seven genes of the hypothalamic-pituitary gonadal axis, including gonadotrophin-releasing hormone-1(GnRH-1), GnRH receptor(GnRHR), neuropeptide-y (NPY), dopamine D_2 receptor(DRD_2), vasoactive intestinal polypeptide(VIP),

VIP receotor-1(VIPR-1), prolactin (PRL), and the QTL region between 87 and 105 cM of the Z chromosome, using microsatellite marker-trait association analyses in a population comprising 1310 chickens.

They found C1704887T of VIPR-1 highly significantly associated with EN300. T5841629C of DRD2 and the C1715301T of VIPR-1 were significantly associated with EN300. A highly significant association was found between the C1704887T and C1715301T haplotypes of VIPR-1 and EN300.

Tuiskula-Haavisto *et al.* (2010) used an F2 population of 688 female chickens to map QTL affecting eggshelltraits(eggshell deformation, breaking force, and weight) using 160 microsatellite markers on 27 chromosomes. They found 11 genome-wide and 15 suggestive QTL for egg shell traits measured at different times during production. Loci affecting the deformation were found on chromosome 1,2,6,10,14, and Z. Loci affecting the breaking force were detected on chromosome 2,3,10,12, and Z. Loci affecting the shell weight were detected on chromosome 6,12,24 and Z. Each QTL explained between 1.5% and 4.6% of the phenotypic variance.

The effect of using marker information in estimating breeding value was studied by Van derBeck *et al.* (2010). They found that cumulative response to selection increased proportionately by 0.06 to 0.13 if a marker linked to a QTL that explained 0.2 of the genetic variance was used. Cumulative response increased up to 0.28 if the QTL explained 0.8 of the genetic variance.

Table 2.7: Genome-Wise QTL for Egg Production, Egg Shell Quality and Body Weight in White Leghorn and Ceobb-ceobb F2 cross.

Linkage	Trait	F-ratio	Location	Flanking Markers	Confidence Interval	Additive Effect	Dominance Effect
2	EC35	8.76	254	ADL026-LEI0147	34-334	2.9±0.8	-3.0± 1.4
2	EC55	8.69	230	BCL2-ADL0267	178-297	2.6±0.7	-2.8±1.2
4	EW35	33.38	206	LEI0081-UMA4034	197-213	2.74±0.34	-0.75±0.53
4	AW35	35.83	209	UMA4034	201-214	2.33±0.27	-0.69±0.39
4	PSH35	11.35	209	UMA4034	122-216	0.23±0.05	0.19±0.08
4	EC35	8.27	219	UMA4034-ADL0260	54-236	2.7±0.8	-2.9±1.3
4	EW55	18.10	204	LEI0081-UMA4034	196-214	2.48±0.32	0.02±0.81
4	AW55	30.28	206	LEI10081-UMA4034	198-218	2.48±0.32	-0.67±0.5
4	BW4	1030	209	UMA4034	124-217	27.6±6.1	-5.6±9.0
4	BW5	16.19	212	UMA4034-ADL0260	122-220	45.5±8.0	-9.5±12.6
4	EPI	10.44	200	LEI0081-UMA4034	132-221	-3.2±0.7	2.6±1.2

Source: Schreiweis *et al.(2005*)

Quantitative trait loci on chromosome 9 influencing the quality of egg shell were identified by analyzing an intercross between two parent lines developed from the same founder population by a two-way selection for egg shell strength with non-deformation conducted over 14 generations in a study carried out by Takashi *et al.* (2009). Chromosome-wise highly significant($p<0.01$) QTL associated with egg weight (EW), short length of egg(SLE),long length of egg(LLE) and eggshell weight were mapped to the distal region of chromosome 9.

A genome-wide scan was performed to detect chromosome regions that affect egg production traits in reciprocally crossed chicken lines; partially inbred New Hampshire (NHI) and inbred White leghorn (WL77) using microsatellite markers by Goraga *et al.* (2012). The result showed a highly significant region on chromosome 4 with multiple QTLs for egg production trait between 19.2 to 82.1 map unit (MU). This QTL region explained 4.3 and 16.1% of the phenotypic variance for number of egg and egg weight in the F2 population, respectively. Genome-wide suggestive QTLs for egg weight were found on chromosome 1, 5 and 9, and number of eggs on chromosome 5 and 7.

Goto *et al.* (2011) identified main-effect QTL for age at first egg lay (AFE) on chromosome 1 and 4 main-effect QTL for egg production rates (EPR) on chromosome 1 and 11(three on chromosome1 and one on chromosome11) at genome-wide 5% significant level, in a population of 421 F2 hens derived from an intercross between Oh-shomo (Japanese large breed) and White leghorn breeds of chickens.

De-ji *et al.* (2010) detected QTL affecting egg shell quality in a study on 262 hens obtained by crossing a strong egg shell line with a weak egg shell lines of the White Leghorn bred, using microsatellite markers. They discovered QTLs associated with 7 traits i.e. body weight

, short length of egg, long length of egg, egg shell strength (ES), egg shell thickness (EST) , egg shell weight (ESW) and egg weight (EW). Highly significant QTLs association with EST and ESW and a significant QTL association with EW were mapped to region flanking ABRO545 and ABRO362 on chromosome 9.

Wembo *et al.* (2011) performed a genome-wide association studies in White leghorn and Brown-egg dwarf layers. The genome-wide scan revealed 8 genome-wide significant association with egg production and quality trait.

Wolc *et al*.(2012) identified a QTL region on chromosome 4 linked to egg laid at specific ages, egg mean weight on chromosome 2,5,6,8,20,23,and Z, and a region on chromosome 13 linked to egg weight at 26-28 weeks of age.

Wright *et al.* (2007) using 160 microsatellite markers on an F_2 population of White leghorn X Red jungle fowl intercross, found a total of nine significant QTLs and three suggestive QTLs affecting egg size , egg shell thickness, egg taste and meat quality on chicken chromosomes 1,2,4,5,7,8,10 and 12.

2. 13 Characteristics of Some Poultry Species

i. **Double Spurred Francolin (***F. bicalcaratus*): This is a game bird belonging to the pheasant family phasianidae and order Galliforms. Like most Francolins that are restricted to Africa, it is most common in tropical West Africa, but there is a small and declining isolates in Morocco (Hall, 1963). The bird is found in open habitats with trees. It nests in lined ground scrape laying 5–7 eggs. Double spurred Francolin takes a wide variety of plants and insect food (Ezealor *et al.,* 2005). The male is mainly brown, sparingly streaked and spotted dark and cream above, chest and flank feathers are dark brown edge and centrally spotted cream (Hall, 1963). The face is

pale cream finely flecked with dark brown, and the head features a chestnut crown and white supercillium. It has a chestnut neck collar, white cheek patches and brown wings. The male usually have two spurs on each leg. The female is similar but usually lack spurs and is slightly smaller and less robustly built.

ii. **Japanese quails** (C. *Japonica*): This belongs to the order Galliforms, family phasianidae and genera *Coturnix*. Quails are small terrestrial birds, and seed eaters but will take insect and smaller prey when available. They are migratory and fly long distances (Johnsgard and Henry 1988). Japanese quails are hardy birds that thrive in small cages and are inexpensive to keep. They mature in about six weeks and are usually in full egg production at fifty days of age (Kaye, 2005). The adult male quail weigh about 100 – 140g while the females are slightly heavier, weighing between 120 – 160g. The females are characterized by light tan feathers with black speckling on the throat andupper breast with males having rusty brown throat and breast feathers

ii. **Helmented guinea fowl** (*N. Meleagris*) This belongs to the subfamily *Numidiae* and the family Phasianidae (Martinez, 1994). Guinea fowls are native to Africa, but helmeted guinea fowls are commonly held in captivity and had been introduced elsewhere in the world. They are large birds measuring about 40 -71cm, and weighing between 700-1600g (Elliot and Sargatha, 1999). Most species of guinea fowl have a dark grey or blackish plumage with dense white spots. Thehelmeted guinea fowl generally occur in open or semi-open habitats such as savannah or semi-desert. It breeds in warm, fairly dry and open habitats with scattered shrubs and trees such as savannah or farm land. It lays normally 6–12 clutches of eggs, which the female

incubates for 26–28 days. Their diet consists of a variety of animals and plant food, seeds, fruits, snails, spiders, worms and insects (Birds Life International, 2008).

iv. **Rhode Island Red** (*Gallusgallus domesticus)*:This is a breed of chicken that has red color plumage. It is utility bird raised for meat and eggs and also as show bird. It is a popular choice for backyard flocks because of its egg laying abilities and hardness. Rhode Island Red feathers could be rusted colored, but dark shades are also common, including maroon bordering on black. Its eyes are red-orange and it has yellow feet, withreddish brown beaks. Chicks are a light red to tan color, with two dark brown bars running down their backs. The Rhode Island Red chicken was originally bred in Adamsville a village which is part of Little Compton, Rhode Island (Raymond, 2001).

v. **Rhode Island White:** Thisis a breed of chicken developed in Peacedale, Rhode Island U. S. A. It is a dual purpose fowl, suitable for both meat and egg production. It has a Rose comb, with pure white plumage, Red wattle and ear lobes. Despite their very similar names and shared place of origin, the Rhode Island Red and Rhode Island White chickens are distinct breeds. Rhode Island White Chicken was developed from crosses of Partridge Cochins, White Wyandotte and White Leghorn) while Rhode Island Red was developed through crosses involving (Buff Cochin, Langsh and Black Malay).

vi. **Shikabrown** Gallus gallus *domesticus b*): The Shikabrown the only indigenously bred breed of chicken, was the result of over thirty years of active breeding and selection work by scientists at National Animal Production Research Institute (NAPRI) of Ahmadu Bello University, Zaria Nigeria. Shikabrown was developed to

cope with hash tropical environment (Oni *et al.*, 2002). Shikabrown has been tested and has very good performance record in all the ecological zones of the country. The bird is well adapted to tropical climate and gives good economic returns. It is hardy and resistant to many diseases (Shikabrown Management Guide, 2005).

CHAPTER THREE

3.0. Materials and Methods

3.1. Experimental Location and Management

The samples and data on Rhode Island Red, Rhode Island White and Shikabrown layers were collected from the Poultry Research Programme farm of National Animal Production Research Institute Shika.Shika is located between latitude 11^{o}Northand Longitude 12^{o} East at an altitude of 640 meters above sea level. It lies within the Guinea Savannah zone where the average annual rainfall is 1100mm, most of which fall during May to October. Peak rainfall is recorded during the wet season (June to September), when the relative humidity and daily temperature averages 72% and 25^{o}Crespectively. Following the rainy season is a period of dry, cool weather called "harmattan" which marks the onset of the dry season. This extends from mid-October to January. The hot dry season (February to May) is characterized by very hot weather condition. At this period, daily temperature ranges from 21^{o}C to 30^{o}Cwhile mean relative humidity is 21%.(Meteological Unit, I.A.R, 2005),

Francolins were captured at Kamuku National Park, Birnin Gwari Kaduna State. Kamuku National Park is located at coordinate 10^{o} 40^{-} North, 6^{o} 33^{-} East. It lies within the Northern Guinea Savannah.

Japanese Quails were purchased from National Veterinary Research Institute Vom, and Guinea Fowl were purchased from Sabon Gari market.

The experimental birds obtained from National Animal Production ResearchInstitute Shika were managed under deep litter system. The required vaccines and medications were administered from day old to maturity.

Breeder mash containing 2699 ME Kcal/Kg and 18-20% crude protein were fed to the birds and water was given *ad-libitum.* All feeds were formulated at the Institute.

3.1.1 Experimental population

The information on population sources, specific features and number of individuals examined per population in the characterization studies is presented in Table (3.1).

3.1.2 Experimental stock used in characterization studies

A total of 180 birds of four species and three breeds were used for the study. The populations included in this study are as follows.

i. Double spurred Francolin (*F. bicalcaratus*).
ii. Japanese quails (*C. Japonica*).
iii. Helmeted Guinea fowl (*N. meleagris*).
iv. Commercial Chicken Breeds.
    a. Rhode Island Red (*Gallus gallus domesticus 'a'*)
    b. Shikabrown (*Gallus gallus domesticus 'b'*)
    c. Rhode Island White*(Gallus gallus domesticus 'c')*

3.1.3 Experimental stock used in QTL affecting egg quality traits

The mapping population consisted of 205 F_2 generation obtained from crossing two chicken breeds. The sire line contained 252 hens and 25 cocks, and the dam line contained 250 hens and 25 cocks, in the ratio of 1:10. The cocks of the sire line were crossed with the hens of the dam line using the same ratio. The chicks hatched were Shikabrown (F_1) that were identified, with pullets appearing brownish in color and the cockerels were white or silvery (auto-sexing). The F_1 Shikabrown chickens were crossed in the same ratio of 1:10 to produce the F_2 mapping population.

Table 3.1: Description of the Breed and species used in Characterization study

Population	Source	Specific Feature	Number
Double spurred francolin	Captured in the wild around Birnin Gwari Forest	Brown sparingly streaked and spotted darker and cream above, mature males have double spur. Used as game bird.	30
Japanese quails	National Veterinary Research Institute vom.	Light tan feather with black speckling on the throat and upper breast, weigh between 110-160g.	30
Guinea fowl	Bought from market	Dark grey or blackish plumage with dense white spots, 40-71cm length. Weigh between 700-1600g.	30
Rhode Island Red	Obtained from Poultry Research Programme NAPRI Shika	Rusted color feather, red eyes and yellow feet. A utility birds, raised for meat and egg. Medium size.	30
Rhode Island White	Obtained from Poultry Research Programme NAPRI Shika	White color feather, red eyes and yellow feet. A utility birds, raise for meat and egg. Medium size.	30
Shikabrown	Obtained from Poultry Research Programme NAPRI Shika	Brown egg layers, medium size, excellent egg shell quality, production rate and resistance to disease.	30

The birds were mated at the age of 40 weeks and the egg traits were monitored at the age of 45 weeks. Seven egg quality traits were recorded during the laying period. The traits were as follows: albumen weight, shell thickness, albumen length, egg weight, shell weight, yolk length and yolk weight.

3.2 Data Collection and Analysis

3.2.1Characterization study

DNA isolation: Blood samples (1.5 ml) were taken from the brachiocephalic vein with 1.5 inch injection needle. Blood samples were dried and stored on FTA classic cards. DNA was extracted as per the manufacturer´s instruction. Discs (1.2 mm) of dried blood were punched from the FTA cards. The discs were incubated for 5 min at room temperature with 200 μL of FTA purification reagent. This incubation steps were repeated three times with fresh purification reagent each time. Next, the discs were washed for 5 min with 200 μL of TE buffer. These washing steps were repeated once. After washing, the discs were placed directly into the PCR tubes.

3.2.1.1 *PCR conditions*

The primers used in this study are presented in Tables 2 and 3. The PCR was carried out in a total of 20μL, containing 50ng genomic DNA , PCR buffer (10mM Tris-HCl, pH 8.8; 1.5 mM $MgCl_2$; 50mM KCL; and 0.1% Triton-X), 200 mM dNTP, 0.25 U Dynazyme II DNA polymerase and 10 pmol of each primer. The PCR was performed for 5 minutes. at 95^{o}C and subsequently with 25 cycles consisting of the following steps: 30 sec at 95^{o}C, 45 sec at an annealing temperature that varied from 43^{o}C to 64^{o}C depending on the marker, and 60 sec at 72^{o}C, followed by a final elongation step of 5 minute. at 72^{o} C. Amplified products were

multi-loaded on a 6 or 5.5% gel with ALF[7]or ALF Express[7]respectively. A mixture of 1μL of PCR product, 1 μL of internal standard, 4 μL of loaded dye (formamide) were denatured at 95^{o}C for 2 min. Fragments analyses were performed with Fragment Manager Version 1.2.Software (Tuiskula-Haavisto *et al.,* 2002).

3.2.2 Quantitative trait loci study(Data Collection)

Egg quality traits (yolk length, yolk weight, albumen weight, albumen length, Shell weight, shell thickness, and egg weight) were measured. A total of 15 eggs per hen were collected. For each hen one egg was collected every seven days. Quality traits were measured on each egg and an average computed for each hen. The measurement were done using vernier caliper for (Yolk length and Albumen length), micrometer screw gauge for (Shell thickness) and sensitive electronic scale (KERRO BL20001, 0.05 gram sensitivity) along with petri dishes for (Egg weight, Yolk weight, and Shell weight). The unit used in measuring Yolk length Albumen length and Shell thickness is millimeter, while Yolk weight, Albumen weight, Egg weight and Shell weight were measured in grams.

3.3.0 Statistical analysis

3.3.1 Characterization Studies

3.3.1.1 *Sequencing of cytochrome b Gene.*

Purified fragment with a concentration of at least 35ng/μL, were sequenced to determine the precise nucleotide sequenced. Sequencing of the cytochrome b gene was done using two internal primers L15662 (forward) and H160659 (reversed), Table (3.2). Multiple alignment of the cytochrome sequences were done using clustal X software version 1.81(Thompson *et al* 1997). The analysis of pair wise distance using K2P model (Kimura 1980) was performed

using MEGE version 4 software. Pair wise genetic distance was carried out in order to evaluate the degree of sequences dissimilarities among the species. Phylogenetic tree was constructed using neighbor-joining method (NJ). The phylogenetic tree inferred from cytochrome b sequence was rooted with FRN as an out group.

Cytochrome b diversity indices, genetic differentiation and mismatch distribution were determined using DNaSP version 4.0(Rozas *et al.*, 2003). Maternal genetic diversity was quantified using computer Arlequin version 3.01.

The following formulae were used to compute Pairwise distance, Diversity indices, Tajima`s D, Fu`s Fs, and Theta values.

Pairwise distance;

$$(\delta\mu)^2 = (\mu_A - \mu_B)^2$$

Where μ_A and μ_B are average number of allelic size difference within population A and B.

Diversity indices;

$$H = n/n\text{-}1\left(1-\sum_{i=1}^{k} P^2\right)$$

Where n is the number of gene copies in the sample, k is the number of haplotypes and Pi is the sample frequency of the i^{th} haplotype.

Mismatch distribution;

$$Fs(\ddot{\imath}\ ,\ \Theta_0,\ \Theta_1) = Fs(\Theta_1) + (-\ddot{\imath}\ ,\ \Theta_1 + 1/\ \Theta_1 \sum_{j=o}^{s} /j\ [Fs\text{-}j(\Theta_0) - Fs\text{-}j(\Theta_1)]$$

Where $\mathbf{Fs}\ \Theta = \Theta^{s}/(\Theta + 1)^{s+1}$ is the probability of observing two random haplotypes with s difference in a stationary population, $\Theta_0 = 2uN0,\ \Theta_1 = 2uN1,\ \check{\imath} = 2ut$ and **u** is the mutation rate for the whole haplotypes.

Theta values;

$\Theta_H = 1/(1\text{-}H)^2 - 1$, where **H** is the expected heterozygosity.

$\Theta_S = S/a_1,$ where $a_1 = \sum_{i=1}^{n-1} 1/i$

$\Theta_K = \Theta\sum_{I=0}^{n-1} 1/\Theta{+}1$

$\Theta_\pi = \Theta$, where 2Neu for haploid individuals and 4Neu for diploid individuals.

Tajima`s D;

$D = \frac{\pi - S}{\sqrt{Var(\pi - S}}$, where $\Theta_{\pi} = \pi,\ \Theta_{S} = S\sum_{i=0}^{n-1} 1/1$, and **S** = number of segregating sites in the sample.

Fu`s Fs;

$Fs = \ln(S/_{1\ -\ S}),$ where $S = prob.\ (K \geq Kobs./_{-\pi}),$ where **K** = number of alleles, π = number of pairwise difference.

Table 3.2: List of Primers and Their Sequences Used in the Cytochrome Analysis

Primer	Primer Sequence	GC content %
L15662(forward)	5`-CTAGGCGACCCAGAAAACTT-3`	50
H16065(reversed)	5`-TCAGTTTTTGGTTTACAAGAC-3`	32

Table 3.3: List of Primers Used in Microsatellite Analysis

1) ADL2068	16)MCW0111
2) MCW0206	17) MCW0020
3) LEI0166	18) MCW0034
4) MCW0295	19) LEI0234
5) MCW0081	20) MCW0103
6) MCW0014	21) MCW0222
7) MCW0183	22) MCW0016
8) ADL0278	23) MCW0037
9) MCW0067	24) MCW0098
10) MCW0104	25) LEI0094
11)MCW0123	26) MCW0284
12) MCW0330	27) MCW0078
13) MCW0165	28) LEI0192
14) MCW0069	29) ADL0112
15) MCW0248	30) MCW0216

Table 3.4: List of Primers Used in QTL Studies

1)MCW0132	12) MCW0115	23) MCW0115
2)MCW0247	13) MCW0252	24) MCW0040
3)MCW0082	14) MCW0037	25) MCW0139
4)MCW0095	15) MCW0001	26) MCW0107
5)MCW0003	16) MCW0063	27) MCW0114
6)MCW0018	17) MCW0131	28)MCW0047
7)MCW0120	18) MCW0220	29) MCW0081
8)MCW0068	19) MCW0206	30) MCW0217
9)MCW0128	20) MCW0041	31) MCW0029
10)MCW0145	21) MCW0127	32) MCW0032
11)MCW0252	22) MCW0126	33) MCW0193

3.3.1.2*Microsatellite analysis*

Statistical analysis was computed according to Tedano *et al. (*2006). Genetic diversity of each species was assessed by calculating the number of alleles per locus and its mean (MNA), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E) Nei, (1987), and polymorphic information content (PIC) Botstein *et al*,, (1980), using the CERVUS version 2.0 software package (Marshall *et al.*, 1998). Additionally F-statistics [fixation coefficient of an individual within a subpopulation (F_{IS}), fixation coefficient of an individual within the total population (F_{IT}), and fixation coefficient of a subpopulation within the total population (F_{ST}) per locus across the 3 breeds of chickens (Tadano *et al.*,2006) were calculated using GENEPOP version 3.4 software (Raymond and Russet, 1995).

Genetic distances among the breeds were computed using Cavalli-Sforza chord distance (D_A; Nei *et al.*, 1983). A phylogenetic tree was constructed based on the D_A genetic distance by using neighbor-joining (NJ) method (Saitou and Nei, 1987).

The following formulae were used to calculate distance matrix and F-Statistics;

Distance Matrix;

$\mathbf{Q(i,\ j)\ =\ (n\text{-}2)d(i,j)\text{-}\sum_{k=1}^{n} d(i,k) - \sum_{k=1}^{n} d(i,k),}$ where $\boldsymbol{d(i,k)}$ is the distance between taxa i and j, Q = matrix of distance.

F-Statistics;

$$\mathbf{F = 1\text{-}\ \frac{O(f(Aa))}{E(f(Aa))}\ ,}$$

Where *O(f(Aa*)) = Observed heterozygosity, and *E*(*f*(*Aa*)) = Expected heterozygosity.

3.3.2. Linkage analysis

Linkage analysis was performed according to Tuiskula-Haavistor *et al.*(2002) using the CRIP-MAP program version 2 (Green *et al.*,1990). All pair wise combination of markers was first analyzed by the TWOPOINT option. An LOD scored was assigned to indicate linkage and was used for configuration of a linkage group. Option FLIPS was used to check optional orders between adjacent markers. CHROMPIC was used to detect double or triple recombination and potential genotyping errors. Significance threshold was also determined. The chromosome-wide -P-value for suggestive linkage of a specific chromosome equals the contribution (r) of that chromosome to the total genome length, which was obtain by dividing the length of the chromosome by the total analyzed length of the genome (Tuiskula-Haavisto *et al.*, 2002)

3.3.2.1 *Quantitative trait loci analysis*

The mapping of QTL was performed using the program QTL cartographer version 1.13 (Basten *et al.*, 1999). The program uses linear regression, composite interval mapping (Zeng,1993, 1994) method to dissect the underlying genetics of the quantitative traits. Composite interval mapping combines interval mapping with multiple regression (Wardecka *et al.*, 2003).

Significance thresholds were calculated using the permutation test (Churchill and Doerge, 1994). This is done by random shuffling of corresponding markers genotypes, for each trait. Any relationship between a marker and QTL is broken and reshuffled. The distribution under the null hypothesis of no QTL is constructed in this way.

CHAPTER FOUR

4.0 Result

4.1. Characterization Studies

4.1.1. Molecular diversity indices

A total of 83 haplotypes were identified from 414 polymorphic sites with polymorphism (Table 4.1). Rhode Island White had the highest haplotypes 26 and Rhode Island Red had the least haplotypes 3. The highest haplotype diversity value 1.00±0.01 was observed in Rhode Island White, while the least value 0.13±0.08 was observed in Rhode Island Red. The highest number of transition 78 was observed in Japanese Quails, while Rhode Island Red had the least number of transition 1. Transversion ranged from 145 to 1, with the highest in Japanese Quails and the least in Rhode Island Red. Japanese Quails had the highest number of substitution 223 while Rhode Island Red had the lowest 2. The highest number of polymorphic site 204 was in JPQ while the least 2 was in RHIR. JPQ had the highest nucleotide diversity 0.14±0.07 while RHIR had the least 0.01±0.001. The highest number of pair-wise difference 37.64±0.072 was observed in JPQ, while the least number 0.13±0.02 was observed in RHIR, JPQ had the highest value of theta based on expected heterozygosity (Θ_H), theta value based on number of alleles(Θ_K), theta value based on segregating side(Θ_S)and theta value based on pairwise difference($\Theta\pi$) while RHIR had the lowest values.

Table 4.1 Molecular diversity indices Computed for RHIW, RHIR, SHBR, JPQ, GFW and FRN Species

	RHIW	**RHIR**	**SHBR**	**JPQ**	**GFW**	**FRN**
Sample size	26	30	27	22	27	29
Haplotypes	26	3	9	21	15	9
Haplotypes diversity (Hd)	1.00±0.01	0.13±0.08	0.56±0.11	0.99±0.02	0.86±0.06	0.65±0.10
Sum of square frequency	0.04	0.87	0.46	0.05	0.17	0.37
Number of observed transitions	29	1	16	78	43	6
Number of observed transversions	56	1	26	145	41	5
Number of substitutions	85	2	42	223	84	11
Number of observed indels	0	0	0	0	0	0
Number of polymorphic sites	83	2	38	204	76	11
Number of observed nucleotide sites	264	264	264	264	264	264
Nucleotide diversity ± standard deviation	0.07±0.04	0.01±0.00	0.02±0.01	0.14±0.07	0.07±0.04	0.05±0.00
Mean number of pairwise differences	18.95±8.67	0.13±0.20	3.89±2.02	37.64±17.02	19.58±8.94	1.07±0.73
θ_H	-	0.11±0.08	0.96±0.45	228.03±815.42	5.22±3.01	1.42±0.63
θ_k	-	0.61±0.18	4.31±1.92	216.78±55.85	13.10±6.32	4.08±1.84
θ_s	21.75±7.22	0.51±0.37	9.86±3.43	55.96±18.71	19.72±6.53	2.80±1.18
θ_π	18.94±9.66	0.13±0.23	3.89±2.25	37.64±18.99	19.58±9.96	1.07±0.81
C (%)	10.07	5.32	5.43	10.55	5.58	7.59
T (%)	29.50	31.06	30.95	29.25	31.02	31.14
A (%)	23.43	24.24	24.20	24.38	24.62	21.17
G (%)	37.00	39.38	39.42	35.81	38.78	40.10

θ_H: Theta value based on expected homozygosity; θ_k: Theta value based on number of alleles; θ_s: Theta value based on number of segregating sites; θ_π: Theta value based on the average number of pairwise differences; C: Cytosine; T: Thymine; A: Adenine; G: Guanine: RHIW: Rhode Island White: RHIR: Rhode Island Red; SHBR: Shika Brown; JPQ: Japanese Quail; GFW: Guinea Fowl and FRN:

Table (4.2) contains the result of mismatch distribution analysis. RHIW had the highest mismatch nucleotide with a mean value of 18.94 and variance of 146.56 while FRN had the least value of mismatch nucleotide 1.07 and variance of 1.41. The sum of square deviation (SSD) which is the maximum differences in nucleotide sequences had a high value of 0.38 in SHBR, and a lowest value of 0.00 in RHIR. The probability of SSD, P(sim.SSD>=obs.SSD) ranged from 0.92 in RHIW to 0.001 in JPQ. Harpending`s raggedness index had the highest value of 0.56 in RHIR and lowest value of 0.008 in RHIW. The probability of` Harpending`s raggedness index, P(sim.r >= obs.r) ranged from 1.00 in SHBR to 0.35 in FRN. Tajima`s D, a test statistics used to distinguished between nucleotide sequences evolving under a random process from a non-random process had a lowest negative values of, -1.99 in FRN and -0.30 in GFW , while the values of Tajima`s D in RHIW, RHIR, SHBR, and JPQ were all negative -0.50, -1.51, -2.26, and-1.34 respectively. The probability of Tajima`s D, P(sim.D >= obs.D) ranged from 0.56 in GFW to 0.01 in FRN. Fu`s Fs values ranged from -12.40 in RHIW to -0.03 in SHBR. The probability of Fu`s Fs, P(sim.Fs >= obs.Fs) ranged from 0.8 in GFW to 0.02 in FRN.

Analysis of molecular variance (AMOVA) (Table 4.3) shows that among populations maternal genetic variance was low 8.58, within maternal genetic variance was also low 6.15. The percentage genetic variation among population was high 58.23% while the genetic variation within population was moderate 41.77%. Fixation index among population was 0.58.

Figure (4.1) shows the dendrogram of the evolutionary relationship between the species(RHIW, RHIR, SHBR, GFW, JPQ and FRN). RHIR and RHIW form a subclade which was joined by a clade containing SHBR. This is suggesting that RHIR, RHIW and

SHBR are more related than the rest of the species. A third clade formed by GFW was between JPQ and SHBR, while FRN was an-outgroup. Bootstrap values are presented at the nodes.

Figure (4.2) is a median joining network drawn for the species to show the relationship between them. The median joining network indicated that the relationship between RHIW, RHIR, SHBR and GFW was stronger than the relationship with JPQ and FRN. Appendix 1 to 5 are mismatch distribution chart constructed for the six population.

4.1.2 Microsatellite analysis

The Guinea Fowl, Francolins and Japanese Quails amplified poorly with microsatellite markers, thereby producing no result from these species. However the Gallus *gallus domesticus* species produced results. For the Gallus *gallus domesticus* species the basic indicators of polymorphism resulting from microsatellites analysis are shown in Table (4.4), Shikabrown had the highest mean number of alleles (MNA) 5.370 ± 0.43, and mean number of effective alleles (MNEA) 3.104 ± 0.29, followed by Rhode Island Red, that had a mean number of alleles 4.556 ± 0.26, and a mean number of effective alleles 2.585 ± 0.18. Rhode Island White had the least 4.111 ± 0.31 and 2.438 ± 0.19. The mean number of alleles in the whole population was 4.679 ± 0.20, while the mean number of effective alleles in the whole population was 2.709 ± 0.13. The values of mean number of alleles and mean number of effective alleles are high among the three breeds of chicken, indicating allele richness in the populations.

Table 4.2 Mismatch distribution Table of RHIW, RHIR, SHBR, JPQ,GFW and FRN

	RHIW	**RHIR**	**SHBR**	**JPQ**	**GFW**	**FRN**
Mismatch observed Mean	18.94	0.13	3.90	37.64	19.58	1.07
Mismatch observed Variance	146.56	0.12	41.97	1292.43	493.70	1.41
T	10.23	3.00	0.00	7.91	51.58	0.98
θ_0	11.22	0.00	0.00	19.33	0.00	0.00
θ_1	67.55	0.16	99999.0	135.70	8.04	99999.0
Sum of square Deviation	0.003	0.00	0.38	0.01	0.03	0.01
P (Sim. SSD > = Obs. SSD)	0.92	0.33	0.40	0.001	0.68	0.05
Harpending's Raggedness index	0.008	0.56	0.08	0.01	0.05	0.10
P (Sim. Rag. > = Obs. Rag.)	0.79	0.76	1.00	0.03	0.42	0.05
Tajima's D	-0.50	-1.51	-2.26	-1.34	-0.3	-1.99
P (Sim. D < Obs. D)	0.35	0.44	0.38	0.05	0.56	0.01
Fu`s Fs	-12.40	-2.35	-0.03	-3.21	-1.99	-5.08
P (Sim. Fs < Obs Fs)	0.46	0.51	0.53	0.04	0.80	0.02

T: time of expansion; θ_0 andθ_1: mutation parameter: P(Sim.D>=Obs.D), P(Sim.SSD>=Obs.SSD) and P(Sim.Fs< Obs Fs) are probability values.

Table 4.3 Analysis of molecular variance on the Population of FRN, RHIR, RHIW, SHBR, JPQ and GFW

Source of variation	**d. f.**	**Sum of squares**	**Variance components**	**Percentage of variation**	F_{ST}
Among populations	5	1180.105	8.58183 V_a	**58.23**	0.582
Within Populations	155	954.187	6.15604 V_b	**41.77**	
Total	160	2134.292	14.73787		

V_a = variance component among populations, V_b = variance components within populations, F_{ST} = fixation index.

The Shannon information index (I) ranged from 1.192 ± 0.09 for Shikabrown, 1.056 ± 0.06 Rhode Island Red to 0.965 ± 0.082 for Rhode Island White, while the mean Shannon index across all the populations was 1.071 ± 0.04. These values were high, indicating species abundancy and evenness within the populations.

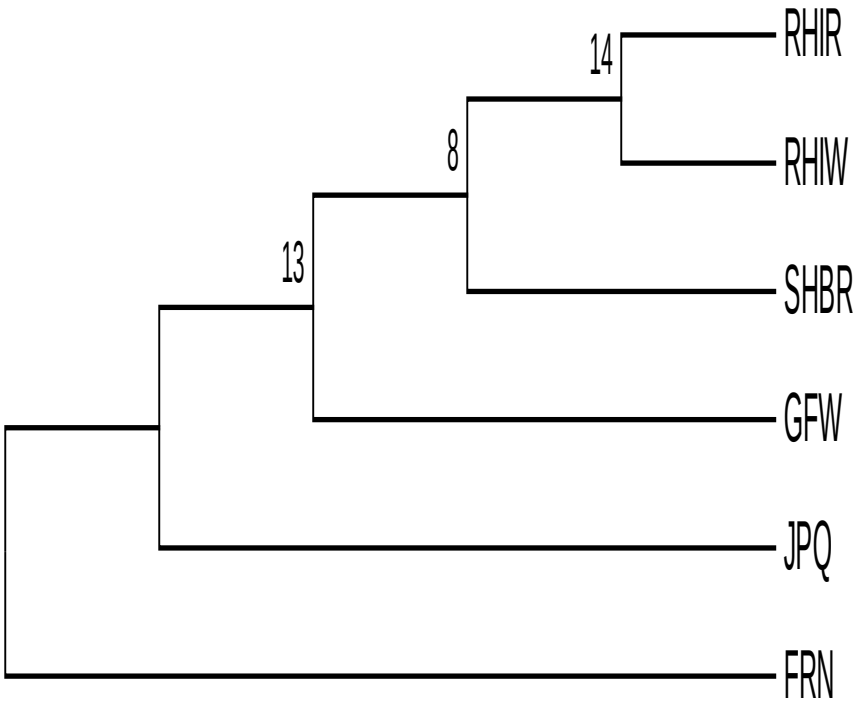


Figure 4.1: Dendrogram of six avian species consensussequenceshaplotypes based on the polymorphic sites of the mitochondrial Cytochrome Oxidase b gene.

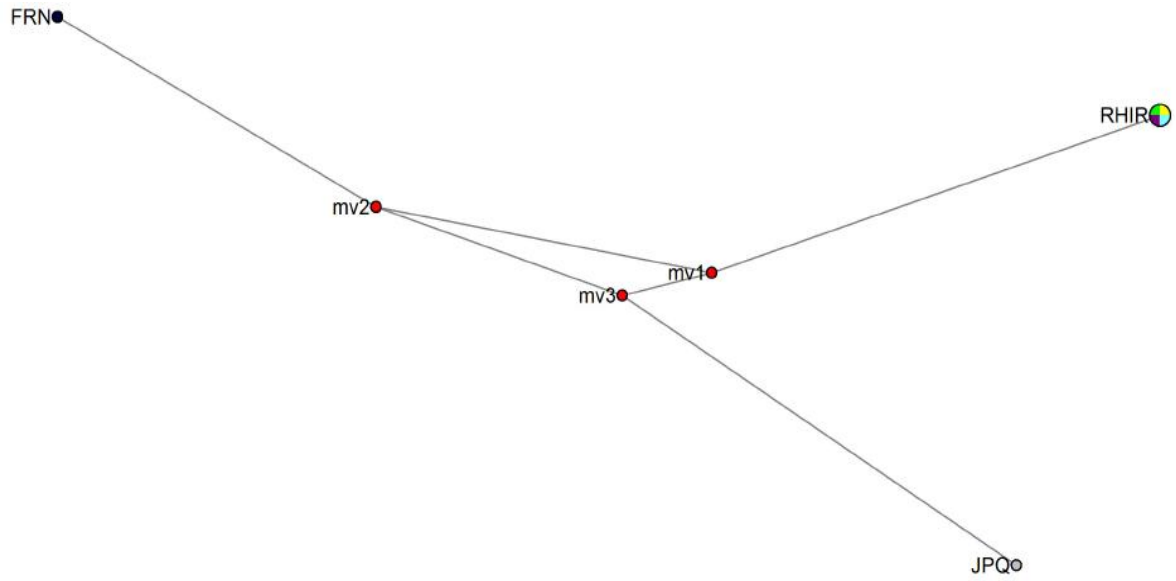


Figure 4.2: Median-joining network (ε = 0) of six Avian Species consensus sequence haplotypes based on the polymorphic sites of the mitochondrial Cytochrome b gene.

Area of each circle is proportional to the frequency of the corresponding haplotype. Different classes of haplotypes are distinguished by use of colour codes (grey = JPQ, black = FRN, violet = RHIR, green = RHIW, yellow = SHBR, blue = GFW). The red colour between the haplotype nodes refer to the positions of median vector.

Shikabrown had the highest mean observable heterozygosity (Ho) 0.481 ± 0.01, among the whole population, followed by Rhode Island Red 0.452 ± 0.01, then Rhode Island White 0.422 ± 0.01. The mean observable heterozygosity across population was 0.582 ± 0.03. The mean expected heterozygosity (Ho) was 0.606 ± 0.04 for Shikabrown, 0.566 ± 0.03 for Rhode Island Red and 0.530 ± for Rhode Island White, while the mean expected heterozygosity across population was 0.557 ± 0.02. The difference between the mean observed and the mean expected heterozygosity was small indicating considerable level of allele diversity among the three populations. All the indicators of polymorphism (mean number of alleles,mean number of effective alleles, Shannon information index and mean observed and expected heterozygosities) were high, this is expressing a high level of polymorphism among the breeds.

The fixation index (F_{IS}) or inbreeding coefficient was 0.192 for Shikabrown, 0.188 for Rhode Island White and 0.122 for Rhode Island Red.

The diversity indices of microsatellite markers are presented in Table 4.5. All the thirty microsatellites used in the study were found to be highly polymorphic. The mean polymorphic information content across the whole microsatellites was 0.586. The number of alleles per locus (K) ranged from 5 in marker LEI0166 to 90 in marker MCW0295. Among population genetic diversity (Ho), marker ADL0278 had the highest frequency 0.989, while marker ADL0268 had the least frequency 0.230. The overall mean of genetic diversity among microsatellite markers was 0.582. Marker MCW0104 had the highest frequency of 0.784 within genetic diversity (Hs), while marker MCW0067 had the least frequency 0.242. The mean within genetic diversity across all markers was 0.557.

Table 4.4: Basic indicators of genetic variation in RHIR, RHIW and SHBR Chicken

Pop	Sample size	MNA	MNEA	I	Ho	He	F_{IS}
RHIR	30	4.556 (0.263)	2.585 (0.188)	1.056 (0.066)	0.452 (0.018)	0.566 (0.031)	0.122***
RHIW	30	4.111 (0.313)	2.438 (0.199)	0.965 (0.082)	0.422 (0.018)	0.530 (0.039)	0.188***
SHBR	30	5.370 (0.437)	3.104 (0.290)	1.192 (0.093)	0.481 (0.018)	0.606 (0.041)	0.192***
Mean		**4.679 (0.206)**	**2.709 (0.135)**	**1.071 (0.047)**	**0.582 (0.030)**	**0.557 (0.022)**	

MNA – Mean number of alleles; MNEA – Mean number of effective allele; I – Shannon information index; Ho – Mean observed heterozygosity; He – Mean expected heterozygosity; F_{IS} – Fixation index;*** $P < 0.001$

Marker ADL0278 had the highest value under Global observed heterozygosity (OBS) 0.787, while marker MCW0014 had the least value of 0.127. The marker with the highest polymorphic information content (PIC) was marker MCW0104 0.812, while the markers with the least values were markers MCW0014 and MCW0067 with 0.239 each, the mean polymorphic information content across all markers was 0.586. The marker with the lowest within loci heterozygosity deficit (F_{IS}) was marker MCW222 with -0.154, while the marker with the highest value was marker ADL0268 0.623. The mean within loci heterozygosity deficit across all markers was 0.187. Marker ADL0278 was the marker with the lowest between loci (F_{IT}) heterozygosity deficiency -0.018, while marker MCW0295 had the highest between loci heterozygosity of 0.671. The mean between loci heterozygosity deficit across all microsatellite markers was 0.284. The marker with the lowest deviation from the Hardy-Weinberg expected values (F_{SD}) was marker ADL0268 with a value of 0.006, while marker MCW0206 had the highest 0.465. The mean value of deviation from Hardy-Weinberg Expected values across all the markers was 0.118. The marker with the highest estimated gene flow (Nm) was marker ADL0268 (44.197), while marker MCW0206 had the lowest value 0.288. The mean value of gene flow across the microsatellites markers was 5.433.

The markers in (Table 4.5) were the operational taxonomic units (OPU),they were the basis of comparison between the breeds. These markers were found to be polymorphic (mean PIC value of 0.59), with a high number of alleles per locus ranging from 5 in marker LEI0166 to 90 in marker MCW0295.Estimated values of among population genetic diversity (Ho), mean genetic diversity, mean within population genetic diversity, global observed heterozygosity, mean within and mean between loci heterozygosity deficitacross all the markers were found to range from low, moderate and high. The mean value of deviation from Hardy-Weinberg

expected values (F_{SD}) across all markers was small. These findings are indicating that genetic variations at molecular level among the breeds are high.

The lowest genetic distance 0.2 was observed between Shikabrown and Rhode Island Red, while the highest genetic distance 0.442 was observed between Rhode Island Red and Rhode Island White. The lowest genetic identity 0.634 was between Rhode Island Red and Rhode Island White, while the highest genetic identity 0.819 was between Shikabrown and Rhode Island Red, these facts are portraying that the relationship between Shikabrown and Rhode Island Red is closer than the relationship between Shikabrown and Rhode Island White Table 4.6.

The analysis on molecular variance (Table 4.7), indicated that 78% of the variations in the chicken population studied were among individuals,20% of among populations and 2% within individuals.

The dendrogram, of genetic distance and neighbour-joining method, indicated that the Rhode Island Red and Shikabrown are on one cluster, while Rhode Island White was on a separate lineage (Figure 4.3), this means that Shikabrown is more related to Rhode Island Red than Rhode Island White.

4.2. Quantitative Traits Loci Analysis.

Table (4.8) shows the egg quality traits analysis for F_2 RHIRXRHIW crosses. Albumen weight had mean of 31.03± 0.01 with a coefficient of variation of 16.85%, the variation within this trait and egg weight 10.03% is low. The variation in Shell thickness20.83%, albumen length 25.25%, yolk length 21.24% and yolk weight 20.71% were moderate. While shell weight 34.17% was high,

Table 4.5: Diversity indices for microsatellite markers used in Study on RHIR, RHIW and SHBR Chicken

Locus	*K*	Ho	Hs	Ht	OBS	EXP	PIC	F_{IS}	F_{IT}	F_{ST}	*Nm*
ADL0112	25.000	0.433	0.543	0.614	0.433	0.618	0.543	0.202	0.295	0.116	1.902
LEI0094	67.000	0.654	0.657	0.750	0.618	0.746	0.705	0.041	0.162	0.126	1.735
MCW0037	37.000	0.743	0.645	0.690	0.618	0.693	0.646	0.039	0.102	0.065	3.587
MCW0104	77.000	0.480	0.786	0.834	0.468	0.837	0.812	0.407	0.441	0.057	4.164
MCW0295	90.000	0.465	0.656	0.830	0.276	0.832	0.810	0.584	0.671	0.210	0.943
ADL0278	56.000	0.989	0.672	0.770	0.787	0.775	0.737	-0.167	-0.018	0.127	1.717
MCW0067	35.000	0.495	0.242	0.261	0.239	0.255	0.239	-0.007	0.061	0.067	3.460
MCW0123	69.000	0.600	0.604	0.669	0.598	0.676	0.638	0.006	0.103	0.098	2.306
MCW0216	54.000	0.865	0.677	0.687	0.523	0.691	0.638	0.224	0.235	0.014	17.725
MCW0034	58.000	0.549	0.696	0.737	0.389	0.745	0.696	0.444	0.475	0.056	4.222
MCW0069	55.000	0.471	0.575	0.646	0.408	0.649	0.585	0.268	0.348	0.110	2.027
MCW0081	43.000	0.326	0.331	0.339	0.303	0.341	0.330	0.081	0.103	0.025	9.873
MCW0103	32.000	0.380	0.472	0.482	0.356	0.486	0.366	0.243	0.260	0.022	11.085
MCW0183	61.000	0.471	0.657	0.709	0.425	0.713	0.676	0.361	0.409	0.074	3.117
ADL0268	56.000	0.230	0.606	0.609	0.230	0.614	0.534	0.621	0.623	0.006	44.197
LEI0192	74.000	0.737	0.698	0.729	0.621	0.735	0.683	0.111	0.150	0.043	5.565
MCW0020	48.000	0.751	0.584	0.660	0.512	0.665	0.594	0.131	0.230	0.115	1.930
MCW0078	49.000	0.776	0.395	0.425	0.352	0.425	0.406	0.101	0.165	0.071	3.284
MCW0248	67.000	0.813	0.631	0.680	0.598	0.686	0.651	0.058	0.126	0.073	3.191
LEI0166	5.000	0.418	0.476	0.488	0.424	0.494	0.431	0.120	0.142	0.024	10.139
MCW0014	40.000	0.366	0.257	0.282	0.127	0.247	0.239	0.434	0.483	0.086	2.662
MCW0165	61.000	0.325	0.518	0.652	0.307	0.648	0.615	0.396	0.520	0.206	0.965
MCW0009	63.000	0.460	0.419	0.667	0.447	0.663	0.611	-0.096	0.310	0.371	0.423
MCW0206	45.000	0.752	0.378	0.706	0.508	0.689	0.632	-0.068	0.428	0.465	0.288
MCW0330	9.000	0.515	0.757	0.807	0.536	0.814	0.782	0.320	0.362	0.062	3.796
MCW0222	31.000	0.658	0.522	0.637	0.607	0.643	0.586	-0.154	0.054	0.180	1.140
MCW0111	68.000	0.982	0.570	0.692	0.560	0.696	0.638	0.023	0.187	0.167	1.245
Mean	**50.926**	**0.582**	**0.557**	**0.632**	**0.454**	**0.632**	**0.586**	**0.187**	**0.284**	**0.118**	**5.433**

K: number of alleles per locus; Ho: among genetic diversity; Hs: within genetic diversity; Ht: total genetic diversity; OBS: global observed heterozygosity; EXP: global expected heterozygosity; PIC: Polymorphic information content; F_{IS}: Within loci heterozygote deficit (Nei, 1987) F_{IT}:Between loci heterozygote deficiency (Nei, 1987); F_{ST}: Deviation from HWE as revealed by heterozygous deficit (Nei, 1987); Nm: Estimated gene flow (Nei, 1987)

Table 4.6: Genetic distance (below diagonal)and genetic identity (above diagonal) Computed on RHIR, RHIW and SHBR Chicken

	RHIR	**RHIW**	**SHBR**
RHIR	-	0.643	0.819
RHIW	0.442	-	0.791
SHBR	0.200	0.235	-

RHIR= Rhode Island Red, RHIW = Rhode Island White SHBR= Shikabrown

Table4.7: Analysis of molecular variance on RHIR, RHIW and SHBR Chicken

Source	Df	SS	MS	Est. Var.	%
Among Population	2	3088929.718	1544464.859	22809.889	20%
Among Individual	87	15300821.262	175871.509	87062.175	78%
Within Individual	90	157244.220	1747.158	1747.158	2%
Total	179	18546995.200		111619.223	

Df: degree of freedom; SS: sum of Square; MS: Mean square; Est. Var.: Estimated Variation

Table (4.9) contains a linkage analysis of 25 microsatellite markers.Distances between loci were in kosambi scale within the mapped populations. The 25 microsatellites markers mapped on chromosome 1 to 5, gives a ratio of 5 haplotypes per chromosome. The polymorphism information content (PIC) of markers were on average 0.586. The linkage groups covered 913 centimorgan (cM), with an average spacing of 36.52cM between markers.

Phenotypic correlation was computed to evaluate the relationship between the egg quality traits (Table 4. 10), Egg Wt was highly correlated to Yolk Wt (0.85), Alb Wt (0.93) and Alb Lth (0.82). There is a negative correlation between Yolk Length and Shell Thc (-0.006), the correlation between Yolk Wt and Shell Thc was also negative (-0.089). The correlation between the rest of the traits ranged from moderate to low.

The threshold p-values for chromosome–wise QTL analysis of egg quality traits in the F_2 RHIR X RHIW (Table 4.11), show that all the seven traits were significant at 1, 5 and 10% levels of significance. However, only the 1% and 5% significant levels showed evidence of linkage between traits and markers.

The positions of QTLs affecting egg quality traits in cM on chromosome 1-5, are shown in Table 4.12. In total 10 chromosomes-wise QTL areas were identified. On chromosome 1, three QTLs affecting shell thickness, yolk length and shell weight were identified. Shell thickness was linked to marker MCW145, yolk length was linked to marker MCW68 and shell weight linked with marker MCW145. A QTL affecting Albumen weight was discovered on chromosome 2 linked with marker MCW206 at about 138.9 cM.

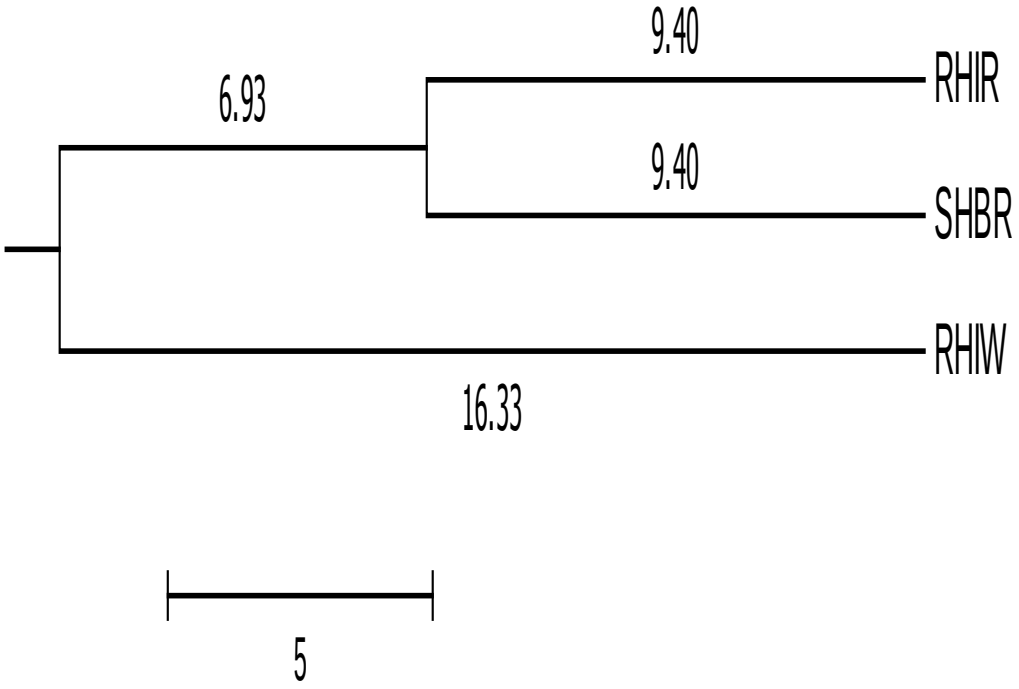


Figure 4.3: Dendrogram of microsatellite analysis on the three chicken genotypes using genetic distance.

Table 4.8: Egg Quality Traits of F2 RHIR X RHIW Chickens

Trait	N	Mean±SE	CV %
Albumen weight	205	31.03±0.01	16.85
Shell weight	205	0.24±0.003	20.83
Albumen length	205	9.25±0.16	25.52
Egg weight	205	56.21±0.39	10.03
Shell weight	205	5.60±0.13	34.17
Yolk length	205	14.73±0.21	21.24
Yolk weight	205	15.45±0.22	20.71

Alb Wt=Albumen Weight, Shell Thc=Shell thickness, Alb Lth=Albumen Length,Egg Wt= Egg weight, Shell Wt= Shell Weight, Yolk Lth= Yolk Length, Yolk Wt=Yolk Weight.

Table4.9: Linkage Analysis of 25 Microsatellite Markers on Chromosome 1-5 of F2 RHIR X RHIW Chicken

Position	Chromosome 1	Chromosome 2	Chromosome 3	Chromosome 4	Chromosome 5
Locus 1	MCW18	MCW63	MCW127	MCW107	MCW81
(cM)	0.00	0.00	0.00	0.00	0.00
Locus 2	MCW120	MCW131	MCW126	MCW170	MCW217
(cM)	100.00	28.61	43.99	100.66	21.93
Locus 3	MCW68	MCW220	MCW115	MCW114	MCW29
(cM)	141.16	95.16	92.10	182.66	38.44
Locus 4	MCW120	MCW206	MCW40	MCW47	MCW32
(cM)	172.13	138.98	134.42	241.36	57.11
Locus 5	MCW145	MCW41	MCW139	MCW298	MCW193
(cM)	266.24	188.36	222.22	283.50	68.61

Table4.10: Correlations between Egg Quality Traits Perform on F2 RHIR X RHIW Chicken

Traits	Alb Wt	Shell Thc	Alb Lth	Egg Wt	Shell Wt	Yolk Wt
Alb.wt						
Shell thc	0.30					
Alb lth	0.53**	-0.21				
Egg wt	0.94***	0.30	0.82***			
Shell wt	0.51**	0.84***	0.55**	0.52**		
Yolk lth	0.47*	-0.06	0.69***	0.46*	0.22	
Yolk wt	0.79***	-0.09	0.53**	0.85***	0.17	0.55**

*=P<0.05, **=P<0.01, ***=P<0.001

Alb wt=Albumen Weight, Shell thc=Shell thickness, Alb lth=Albumen Length,Egg wt= Egg weight, Shell wt= Shell Weight, Yolk lth= Yolk Length, Yolk wt=Yolk Weight

Three QTL affecting albumen weight, yolk weight and egg weight were found on chromosome 3. All the 3 QTLs were linked with marker MCW139 at 222.2 cM.

QTLs affecting albumen length and albumen weight were located on chromosome 4, albumen length was connected with marker MCW170 at 100.6 cM, while albumen weight was linked with marker MCW47 at 241cM.QTL affecting shell thickness was found on chromosome 5 linked to marker MCW81 at 0.00cM.

The additive and dominance effect of genotypes across significant QTLs or Traits along with their linked markers on chromosome 1-5 are presented in Table 4.13 The AA (Rhode Island Red) genotype had an additive effect of 0.57 and dominance effect of -0.25 on shell thickness in chromosome 1, while the effect on yolk length had additive negative value -2.5 and a dominant positive value of 3.42. The effect of the AA genotype on shell weight is additive negative -1.96 and the dominance effect is positive 2.77. In chromosome 2 the effect of AA genotype on albumen weight was positive with a value of 2.21 while the dominance effect was negative -1.95. The AA genotype has an additive effect of 3.09 and negative dominance effect of-0.88 on albumen weight , a negative additive effect of -2.21 and a positive dominance effect of 3.21 on yolk weight and a positive additive effect of 2.84 with negative dominance effect of -1.59 on egg weight in chromosome 3. The effect of the AA genotype on albumen length in chromosome 4 is additive negative-0.69 and dominance positive 2.95. While the effect of the genotype on albumen weight on the same chromosome is additive positive 3.38 and dominance negative -1.99. The BB (Rhode Island White) genotype had a positive additive effect of 0.61and a negative dominance effect of -0.092 on shell weight in chromosome 5.

Table 4.11: Threshold P-values for Chromosome-wise QTL Analysis of Egg Quality Traits in RHIR X RHIW F2 Chicken.

Trait	Threshold P-value		
	0.1	0.05	0.01
Egg weight (g)	8.67	18.98	35.01
Albumen wt. (g)	9.68	15.75	24.31
Albumen lth (mm)	10.55	17.04	25.94
Yolk weight (g)	10.45	14.98	26.47
Yolk length (mm)	8.78	17.04	25.94
Shell weight (g)	11.21	15.37	24.31
Shell thc. (mm)	10.37	19.77	23.40

Albumen wt=Albumen Weight, Shell thc.=Shell thickness, Albumen lth.=Albumen Length, Egg weight= Egg weight, Shell weight= Shell Weight, Yolk length= Yolk Length, Yolk weight=Yolk Weight

Table 4.12: Positions of QTLs (in cM) on Chromosome 1-5 and Their Corresponding, F-ratios, P-values and R^2 of Markers Attached to The QTLs

Trait	Chrom	QTL Position	Marker	Marker Position	P-value	R^2	Level of sig. %
Shell thic.		265.03	MCW 145	266.24	0.037	0.07	*
Yolk. lth.	1	140.96	MCW 068	141.16	0.020	0.21	*
Shell. wt.		265.07	MCW 145	266.24	0.001	0.09	**
Alb. wt.	2	136.99	MCW 206	138.94	0.016	0.23	*
Alb. wt.		219.71	MCW 139	222.22	0.031	0.26	*
Yolk. wt.	3	219.54	MCW 139	222.22	0.05	0.21	*
Egg. wt.		218.93	MCW 139	222.22	0.001	0.16	**
Alb. lth.		97.63	MCW 170	100.66	0.003	0.18	**
Alb.wt.	4	240.00	MCW 047	241.30	0.002	0.20	**
Shell.thc.	5	0.00	MCW081	0.00	0.008	0.09	**

* = $P< 0.05$, ** = $P < 0.01$

Alb wt=Albumen weight, Shell thc. =Shell thickness, Alb. lth.=Albumen length, Egg wt= Egg weight, Shell wt.= Shell Weight, Yolk lth= Yolk length, Yolk wt=Yolk weight

Table 4.13: QTLs, Linked Markers, Genotypes and Their Corresponding Significant Additive and Dominance Effect on Chromosome 1-5 of F2 RHIR X RHIW Chicken

QTLs	Chromosomes	Marker Id	Genotypes	Additive Effect	Dominance Effect
Shell Thc	1	MCW145	AA	0.57	-0.25
Yolk Lth	1	MCW68	AA	-2.25	3.42
Shell Wt	1	MCW145	AA	-1.96	2.77
Alb Wt	2	MCW206	AA	2.21	-1.95
Alb Wt	3	MCW139	AA	3.09	-0.88
Yolk Wt	3	MCW139	AA	-2.21	3.21
Egg Wt	3	MCW139	AA	2.84	-1.59
Alb Lth	4	MCW170	AA	-0.69	2.95
Alb Wt	4	MCW047	AA	3.38	-1.99
Shell Thc	5	MCW81	BB	0.61	-0.09

Alb.wt=Albumen Weight, Shell the.=Shell thickness, Alb. lth.=Albumen Length, Egg wt.= Egg weight, Shell wt.= Shell Weight, Yolk lth.= Yolk Length, Yolk wt.=Yolk Weight.

CHAPTER FIVE

5.1. DISCUSSION

5.1.1 Relationship Study

5.1.1.1*Cytochrome b analysis*

The large numbers of haplotypes observed in this study is consistent with the findings of other studies on Galliforms (Paul *et al.*,2004, Sansook *et al.*, 2009).The high haplotype diversity could be due to the hypervariable nature of the mitochondrial DNA in avian species that had a mutation rate of 5-10 times faster than the nuclear genome (Charles *et al.*,2014). The number of transversion observed among the species was higher than number of transition and this would not come as a surprise because this study was not conducted on only one species, but it was conducted on different species (RHIW, RHIR, SHBR, GFW, JPQ and FRN). Sequence divergence are more among species than within a single species. Paul *et al.*(2004) had reported higher rate of transversion than transition rate among birds species of North-America studied using DNA Barcoding.

The negative values of Tajima`s D observed were not significant in RHIW, RHIR, SHBR and GFW. This is suggesting that there is a weak evidence of any departure from drift-mutation equilibrium, implying that these populations have not under-gone expansion nor have they under-gone purifying selection. Therefore the populations are under constant growth, which was consistent with what was reported by Adebambo *et al.*(2009) among south-western Nigerian chicken. These values are however negative and significant in JPQ and FRN, suggesting a population expansion in these two species. Similarly Fu`s Fs was negative and not significant in RHIW, RHIR, SHBR and GFW, also implying a constant

population growth in these species. The negative and significant values of Fu`s Fs in JPQ and FRN indicates a population expansion.

To test the validity of the models, Tajima`s D and Fu`s Fs , sum of square deviation and Harpending`s raggedness indices were used (Harpending *et al.*, 1998), the P(sim.SSD>= obs.SSD) and P(sim.r >= obs.r) were not significant in ( RHIW, RHIR,SHBR and GFW), but are significant in JPQ and FRN, also vindicating the models (Tajimas`s D and Fu`s Fs) that RHIW, RHIR, SHBR, and GFW were undergoing a constant population growth, while there is a population expansion in JPQ and FRN.

In the analysis of molecular variance genetic variation within population and among population accounted for 41.77% and 58.23% of the total genetic variation. This indicates that majority of the genetic variation occurred among the sampled population, with less variation within the sampled population, which may be due to specific differences, difference in sampled breeds, and size. The fixation index (Fst) value of 0.58 indicates high rate of out breeding among the sampled populations, that is the population are outbred (the population are inbred or higher rate of inbreeding if Fst<= 0, the population are highly out bred if Fst is close to 1) (Holsinger and Bruce, 2009).

The phylogenetic analysis of the six genetic group, illustrated their evolutionary and genetic diversities as well. The dendrogram showed that RHIR and RHIW formed a cluster; a second cluster was formed between RHIW-RHIR and SHBR. The GFW formed a clade closer to the Gallus *gallus domesticus* species (RHIW, RHIR and SHBR). The JPQ formed a third clade next from GFW, while FRN stood out as an-outgroup. Charles *et al*.(2014) and Landari *et al*. (2008) have reported similar grouping among Sudaness native chicken and Indonesian chicken breeds.

Median joining network showed the relationship between the species. The network showed that there were three dominant clades represented as follows: grey= JPQ, black= FRN, the Gallus *gallus domesticus* species were lumped along with GFW into the third clad with (violet= RHIR, green= RHIW, yellow= SHBR and blue = GFW). The network also revealed an evidence of convergence or reverse mutation among the three clades. Charles *et al*. (2014) had observed similar median network with convergent mutation among Sudanese Chicken breeds.

The mismatch distribution chart, was smooth with a peak in JPQ and FRN, illustrating population expansion in these species, but ragged in RHIW, RHIR, SHBR and GFW, implying a constant population growth which also supported the result from Tajima`s D and Fu`s Fs analysis, which was consistent with the report of Kumar *et al.* (2013) on the evolutionary history and phylogenetic relationship between *Auxis thazard* and *Auxis rochei*inferred from cytochrome oxidase 1.

5.1.1.2 *Microsatellite analysis*

All the 30 loci were found to be polymorphic. The mean number of effective alleles in the whole population was 2.709 ± 0.13 suggesting allele richness; this was in agreement with what was reported by Parmar *et al.*(2007), Alipanah *et al.*(2011) andSeo *et al.* (2013) who reported values of 2.9 and 3 among Dashtiari, and Kadaknath breeds of chickens.

Shannon diversity index (I) is used to characterize species diversities in a community. It accounts for both abundance and evenness of species present. The mean Shannon index for the whole population was 1.071 ± 0.047, thus indicating that the three breeds used in the study are not only in abundance but are even as well(Lyimo *et al*., 2012). This did not come as a surprise because the number of birds used per breed in this study were 30 across the

three breeds. The mean observed heterozygosity 0.582 ± 0.03 deviated by only 0.015 from the mean expected heterozygosity 0.557 ± 0.022, which could be due to an error either from the personnel or equipment used during the analysis. The negligible difference between the observed and expected Heterozygosity indicates that the populations are at Hardy-Weinberg equilibrium. This is so because the data used for the analysis came from populations kept at Animal Production Research Institute, where all the management practices are done by professionals; hence factors such as effective population size, mating ratios, random mating and other factors that cause inbreeding were kept under check. However these mean values of observed 0.582 ± 0.03 and expected 0.550 ± 0.022 heterozygosities were within the range reported by Masroor *et al.*(2011), and Wei *et al.* (2013) of between 0.422 ± 0.23 and 0.517 ± 0.20 expected heterozygosity.

The inbreeding coefficient (F_{IS}), measures the relative heterozygosity deficit and non-random mating within population. Its values ranged between (-1 all the individuals are heterozygotes, 0 random association of alleles and 1 all individuals are homozygotes) (Holsinger and Bruce 2009). The value of F_{IS} in the three populations ranges from 0.122 for Rhode Island Red, 0.188 Rhode Island White and 0.192 Shikabrown. These values were close to 0, suggesting that controlled matings to check the effect of inbreeding was done when selecting for egg production traits. Although intense selection for egg production traits (egg weight, egg number and age at first egg) were carried out on the population, at what may be interpreted as it was done on only three loci, the total variation in the whole genome is colossal, especially when one consider the number of genes within the chicken genome, about 23,000 (Human Genome Project., 2004). Selection at these loci may exhaust them along with other loci that may be under pleiotropic effect or linkage-disequilibrium with the loci out of variation, but

not the enormous variability existing at more than 20,000 loci in the genome, because of this the value of F_{IS} in the population showed considerable variations.

Polymorphism information content (PIC) was estimated with the intention of accessing how informative the markers were in assessing the heterogeneity of the populations. It is an index that reflects genetic variation of microsatellite loci. A locus is highly polymorphic if PIC is larger than 0.5, medium polymorphic if it is between 0.25 and 0.5 and low polymorphic if it is less than 0.25 Bolstein *et al*. (1980). The overall mean of PIC (0.586) indicates that all the markers were highly polymorphic. The values obtained in this study is between 0.464 and 0.68 as reported by Liu *et al.* (2008) Masroor *et al.* (2011), and Choi *et al.* (2015) on Yangzhou, Lakha, Mushki, Mianwali, Pashawani and commercial Korean chickens respectively.

The marker that had the highest gene flow in the populations was marker ADL0268 with a value of 44.197. For such a marker to have this high value it must have been introduced into this population from a different population and selection may be quite intense on this marker either because it influences production trait or because it affects survival trait Kimura (1983). According to Kimura (1983), newly introduced allele became fixed in population and the frequency of the introduced allele increases drastically if it favours the survival of individuals.

The genetic distance is the furthest between Rhode Island Red and Rhode Island White. This is so because the two breeds, despite their shared place of origin (Rhode Island), they were developed from entirely different breeds. Rhode Island White was developed from crosses of (Partridge Cochins, White Wyandotte and White Leghorns) while Rhode Island Red was

developed from crosses involving (Buff Cochins, Langsh, and Black Red Malay) breeds (Raymond, 2001)

The genetic identity or genetic similarity study showed that Shikabrown was more related to Rhode Island Red than to Rhode Island White. In the dendrogram, Rhode Island Red and Shikabrown formed a cluster, while Rhode Island White formed a different lineage, further indicating the close similarity of Rhode Island Red and Shikabrown, than the similarity between Rhode Island White with Shikabrown.

5.1.2 Quantitative trait loci study

The orders of 25 microsatellite markers in the linkage analysis were in agreement with the chicken consensus linkage maps (Groenen *et al*., 2000).

Two chromosome-wise significant QTLs affecting shell thickness and shell weight were found at exactly the same position 265cM on chromosome 1. The two traits were highly and positively correlated 0.838. The additive effect of the BB genotype on shell thickness is positive and the dominance effect is negative. The additive effect of BB genotype on shell weight is negative while the dominant effect is positive. This suggested that the two QTLs are not additive. The two traits were attached to a single QTL; thus pleotropic effect cannot be rule out at this QTL. Sasaki *et al*. (2004) had reported a region on chromosome 1 affecting both shell thickness and shell weight.

Another QTL affecting yolk length located at 140.9 cM on chromosome 1 was discovered. The effect of the AA genotype on this QTL is negative additive-2.25 and the dominance effect is positive 3.42. This is suggesting that on the average, a chicken of the heterozygote genotype AB will have 1.17mm longer yolk than a chicken with a homozygote genotype of

either AA or BB. This QTL accounted for 21% of the phenotypic variation in the trait, which was in agreement with the findings of Hansen *et al.,* (2005).

On chromosome 2 a QTL situated at 136.9 cM affecting albumen weight was located. The effect of the AA genotype on this QTL is positive additive 2.21 and negative dominance -1.95. This should not be surprising, because some QTLs affecting the same traits were identified on chromosome 3 and 4 with positive additive effect and negative dominance effect, signifying that each of the three loci, located on the different chromosomes (chromosome 2, 3, and 4) have contributed additively in affecting albumen weight. The three loci accounted for 20-26% of the phenotypic variance, which is consistent with what was reported by Schreiweis *et al.* (2005) and Sasaki *et al.* (2004). Three traits, albumen weight, yolk weight and egg weight were significantly linked with marker MCW139 at the same locus (219 cM) on chromosome 3 and the traits were highly correlated. The effect of the AA genotype on albumen weight is positively additive3.09, while the dominance effect was negative -0.88. The effect of the same genotype on yolk weight is negatively additive -2.21, and positively dominant 3.21. On egg weight the effect is additively positive 2.84, and negatively dominant -1.59 implying that these traits are not additive in effect, but are influenced by only one QTL which has pleotropic effect on these traits.

The QTL on chromosome 5 affecting shell thickness, located at 0.00cM was influenced by the BB genotype that had a positive additive effect 0.61 and a negative dominant effect -0.09. The effect of this genotype on the trait is additive.

CHAPTER SIX

6.0.Summary, Conclusion and Recommendation

6.1.Summary

Evolutionary relationship among four species and three breeds of poultry was estimated using mitochondrial cytochrome b gene and chicken microsatellite markers. The species were able to be characterized with cytochrome b gene, and dendrogram of relationships along with median joining network were computed. The dendrogram and the network indicated that Guinea Fowl is closely related to the Gallus *gallus domesticus* species than to either Francolins or Japanese Quails. The chicken microsatellite did not amplify Francolin, Guinea Fowl and Japanese Quails genome effectively, however result was obtain for the Gallus *gallus domesticu*s species, which indicated that Shikabrown was more related to Rhode Island Red than to Rhode Island White.

Chromosome 1 to 5 of Shikabrown F_2 crossed were scanned for the presence of QTL affecting egg quality traits. Some QTL affecting shell thickness, were found on chromosome 1 and 5 while QTL affecting albumen weight were discovered on chromosome 2, 3 and 4. QTL affecting yolk length and yolk weight were found on chromosome 1 and 3 while a QTL affecting albumen length was discovered on chromosome 4.

6.2. Conclusion

All the species studied have shown genetic variations at the cytochrome b gene (mitochondrial DNA). Variation within microsatellite was only observed in the chicken breeds.

1. The species have been characterized based on their evolutionary relationships. The three chicken breeds(Rhode Island Red, Rhode Island White and Shikabrown) shared a predominant clad with Guinea Fowl, suggesting a close relationship between chicken species and Guinea Fowl (bootstrap values 13). Fixation index among the populations was 0.58 indicating that there was substantial variation in the population.
2. Shikabrown was found to be more related to Rhode Island Red than it is to Rhode Island White in the microsatellite analysis with bootstrap values of 9.40 each. The boot strap value between Shikabrown and Rhode Island White was 16.33.
3. Microsatellite markers amplified the Japanese Quails, Guinea Fowl and Francolins DNA poorly, however it was able to assess variation within and between the chicken populations.
4. Chromosome 1-5 of the chicken genome contain genes affecting egg quality traits. The effect of some of these genes are additive, and some are dominant, while others are pleiotropic indicating their dominant or additive values. Inbreeding coefficient were found to be 0.12 in Shikabrown, 0.18 in Rhode Island White and 0.19 in Rhode Island Red.

6.3. Recommendation

1. The closeness between Guinea Fowl and the chicken breeds means that gene introgression between the two species would be possible. This would give an opportunity of transferring noble traits such as breaking season egg laying in Guinea Fowl, introducing alleles that has an effect on cholesterol level, and trait that influences shell strength from Guinea Fowl to domestic chicken species. Further research between

the two species is recommended so that adequate knowledge on how to transfer traits of importance between the species would be acquired.

2. In interspecies studies, mitochondrial DNA is highly recommended as less amplification problems were encountered compared with microsatellite markers, however microsatellites are recommended in studies involving the chicken species because of the importance attached to the within and between population levels of variation it gives.
3. Further analysis should be carried out using higher density markers to fine tune positions of markers linked with these loci controlling egg quality traits so that stronger resolution of QTLs to be used in marker assisted selection can be achieved.

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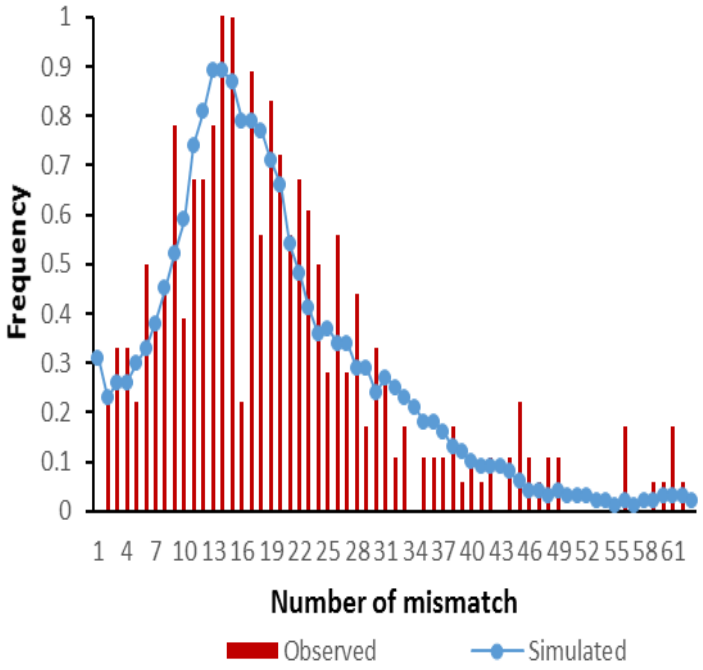
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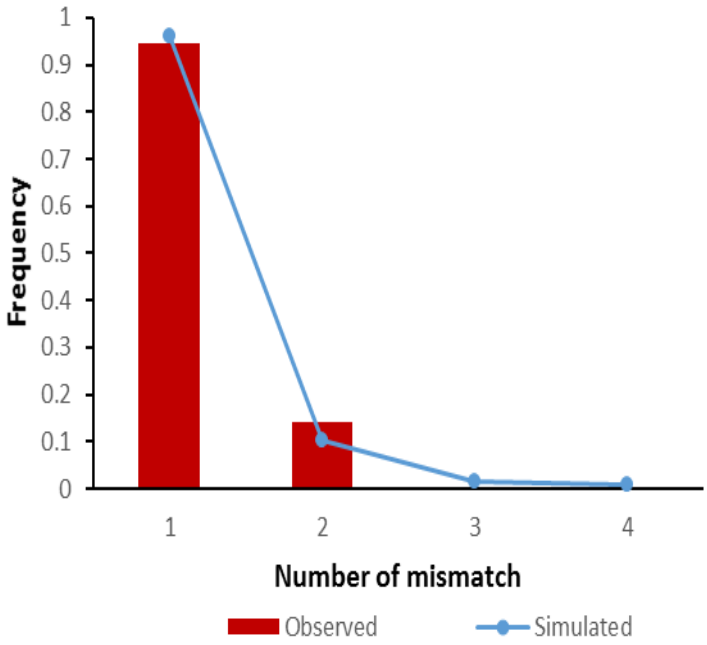
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APPENDIX 1



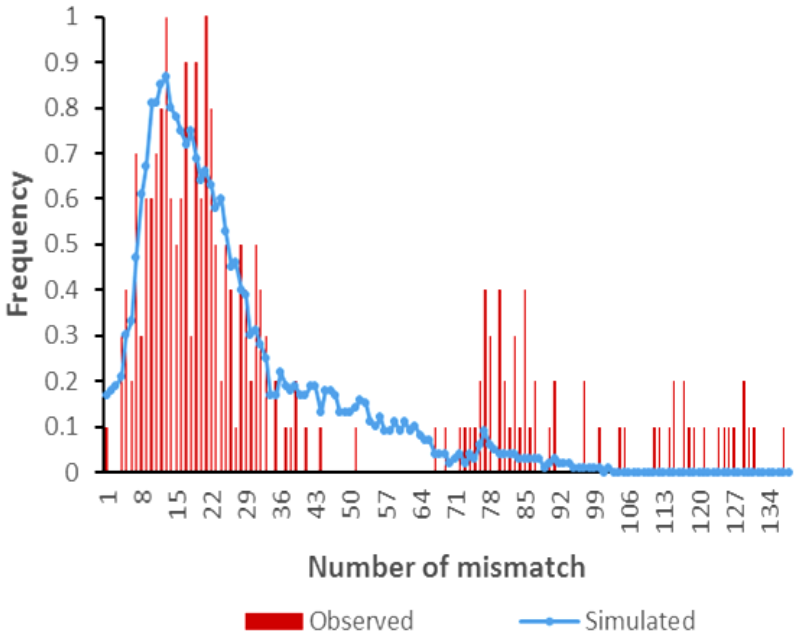
Mismatch Distribution Chart, Japanese Quails

APPENDIX 2



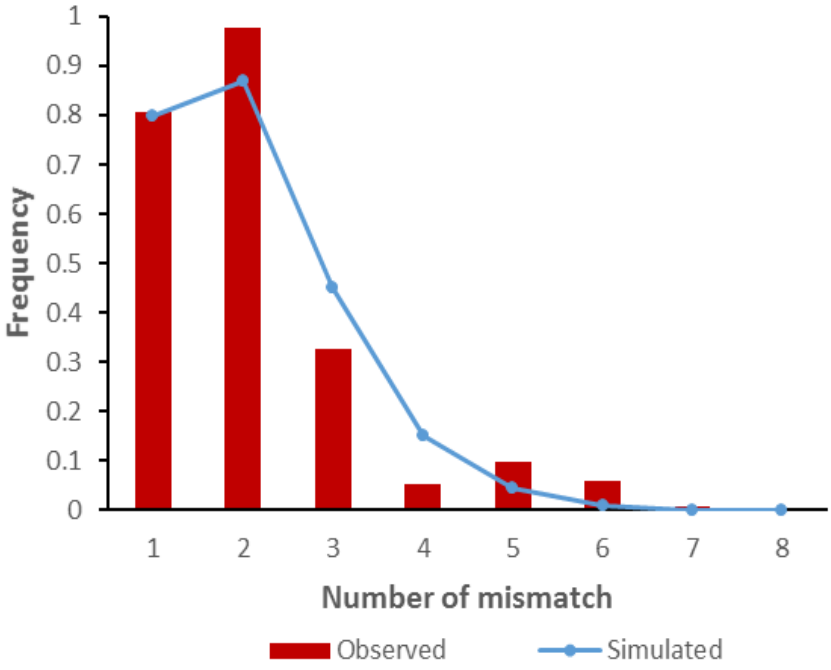
Mismatch Distribution Chart Francolin

APPENDIX 3



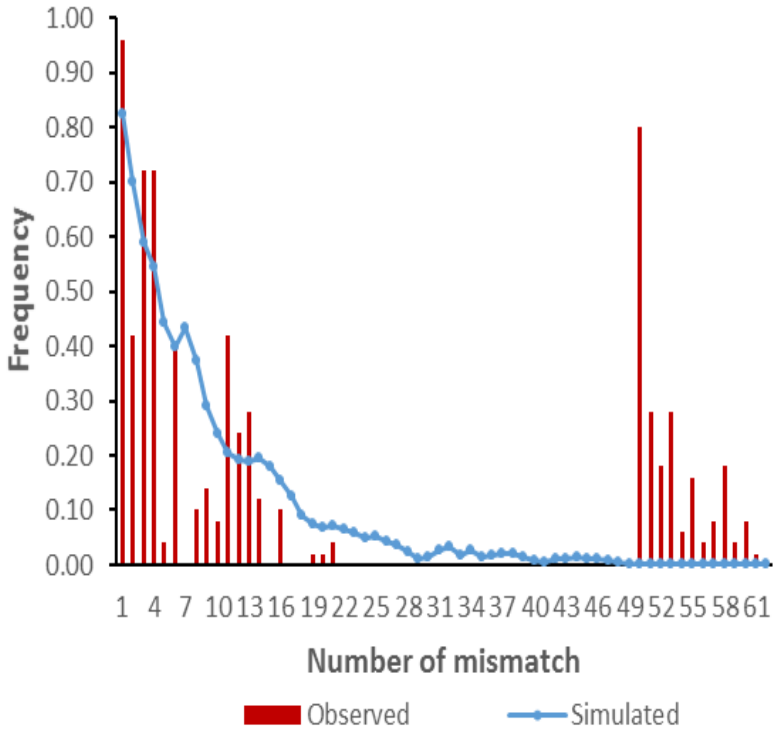
Mismatch Distribution Chart Rhode Island White

APPENDIX 4



Mismatch Distribution, Rhode Island Red

APPENDIX 5



Mismatch Distribution Guinea Fowl

APPENDIX 6

Analysis of Variance on Albumen length: Markers Significant at 5 and 1% with their Respective Chromosomes.

Trait	Chrom.	Markers	Hypothesis	DF	SS	MS	F	Prob
Alb.Wt	4	MCW0170	1	2	57.96	25.94	28.98	0.01

APPENDIX 7

Analysis of Variance on Egg Weight: Markers Significant at 5 and 1% with their respective Chromosomes.

Trait	Chrom	Markers	Hypothesis	DF	SS	MS	F	Prob
Egg.Wt	2	MCW0206	1	2	1114.82	557.41	18.98	0.008
Egg.Wt	3	MCW0139	1	2	1361.36	680.68	35.07	0.001

APPENDIX 8

Analysis of Variance on Albumen Weight: Markers Significant at 5 and 1% with their Respective Chromosomes.

Trait	Chrom	Markers	Hypothesis	DF	SS	MS	F	Prob
AlbWt	2	MCW0206	1	2	1758.35	879.17	24.31	0.01
Al.Wt	3	MCW0139	1	2	1331.30	665.65	26.23	0.009
Al.Wt	4	MCW0170	1	2	1340.17	670.08	25.31	0.03

APPENDIX 9

Analysis of Variance on Shell Weight: Markers Significant at 5 and 1% with their Respective Chromosomes.

Trait	Chrom	Markers	Hypothesis	DF	SS	MS	F	Prob
Shell.Wt	1	MCW0145	1	2	116.16	58.08	15.77	0.05

APPENDIX10

Analysis of Variance on Yolk Length: Markers Significant at 5 and 1% with their Respective Chromosomes.

Trait	Chrom	Markers	Hypothesis	DF	SS	MS	F	Prob
Yolk.Lth	1	MCW0068	1	2	339.64	169.82	17.04	0.003

APPENDIX 11

Analysis of Variance on Shell Thickness: Markers Significant at 5 and 1% with their Respective Chromosomes.

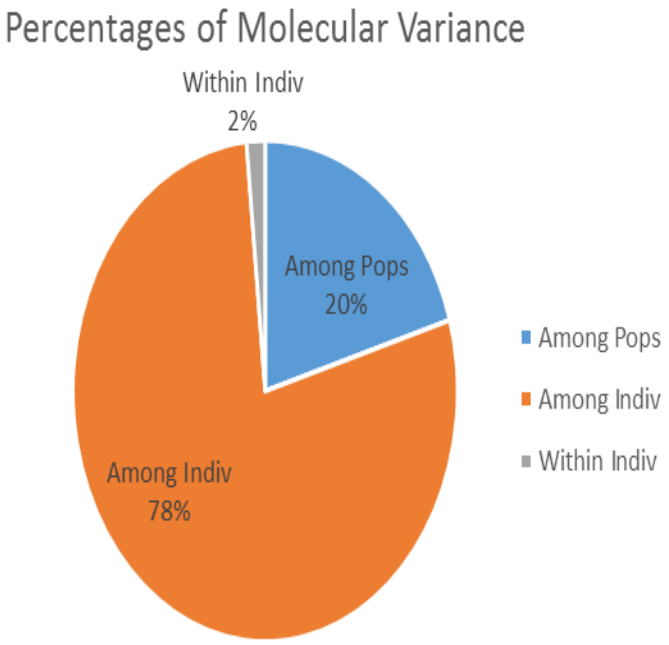
Trait	Chrom	Markers	Hypothesis	DF	SS	MS	F	Prob
Shell.Thc	1	MCW0145	1	2	0.12	0.06	23.40	0.01
Shell.Thc,	5	MCW0081	1	2	0.086	0.04	19.77	0.02

APPENDIX 12

Global *F*-statistic estimates for Nigerian indigenous chicken populations

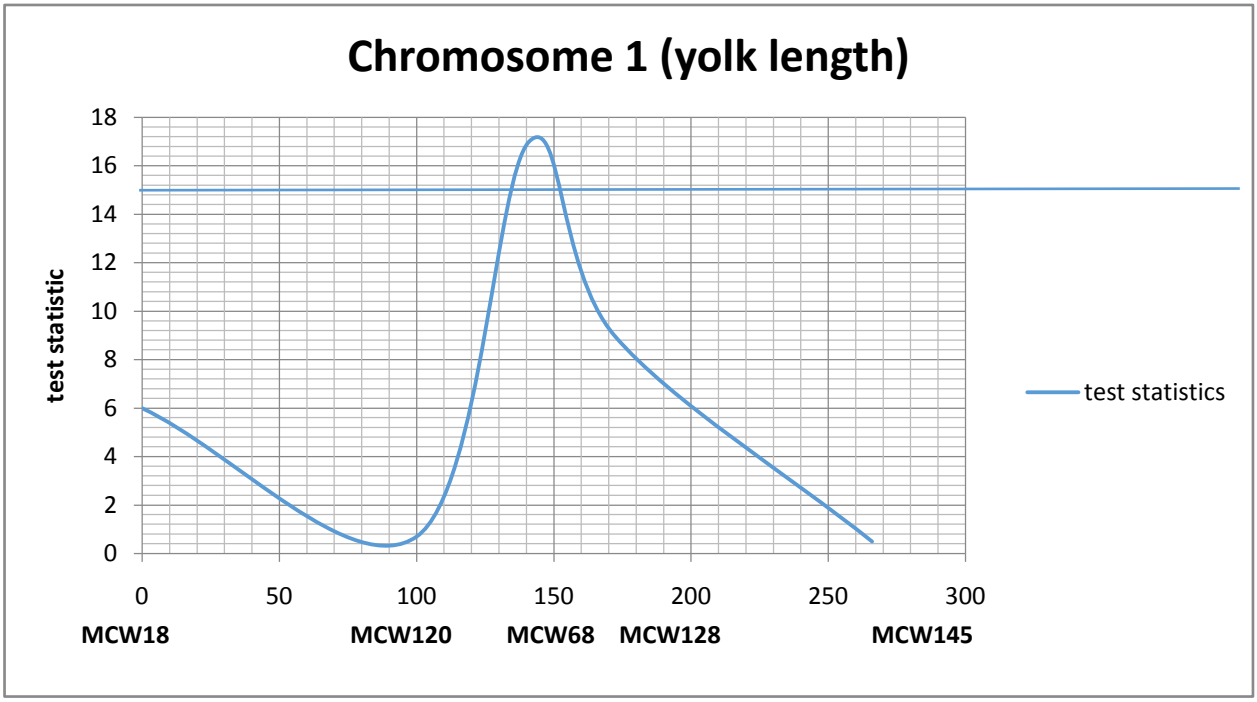
Global	Sample size	F_{IS}	F_{IT}	F_{ST}
RHIR	30	-0.128 (0.102)	-0.088 (0.096)	0.033 (0.008)
RHIW	30	-0.025 (0.085)	0.002 (0.085)	0.029 (0.009)
SHBR	30	-0.170 (0.117)	-0.130 (0.107)	0.030 (0.005)
		-0.044 (0.075)		0.118 (0.022)

APPENDIX 13



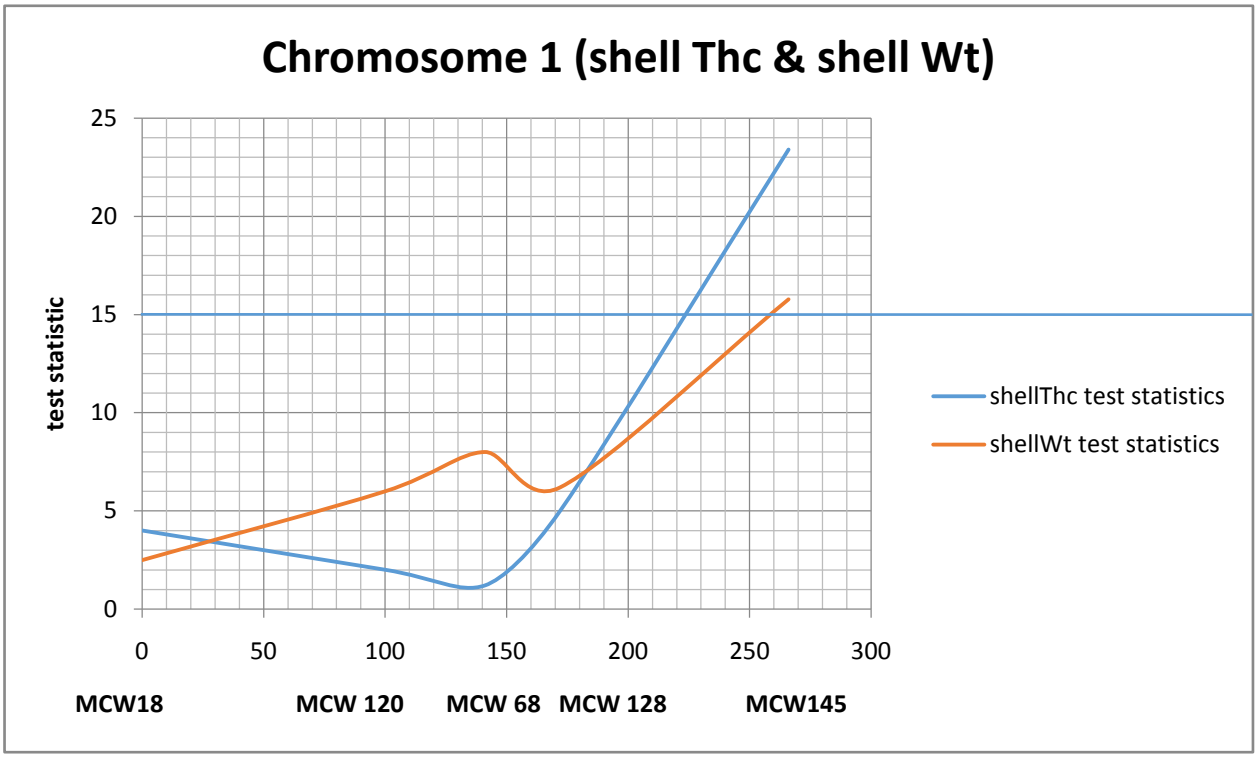
Percentage of Molecular Variance among Shika Brown, Rhode Island Red and Rhode Island White.

APPENDIX 14



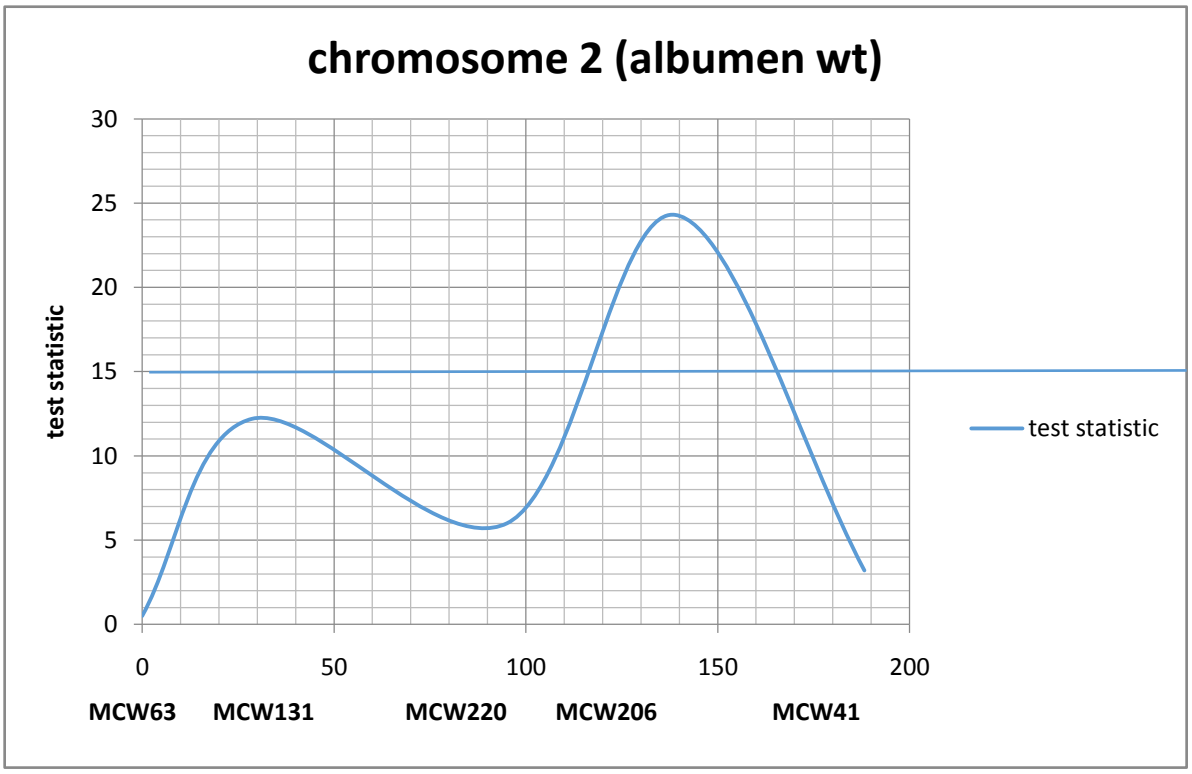
Test statistics value from the analysis of yolk length on chromosome 1, showing the position of QTL/marker (MCW0068) at 141.1cM, the 5% chromosome-wise significance threshold was indicated.

APPENDIX 15



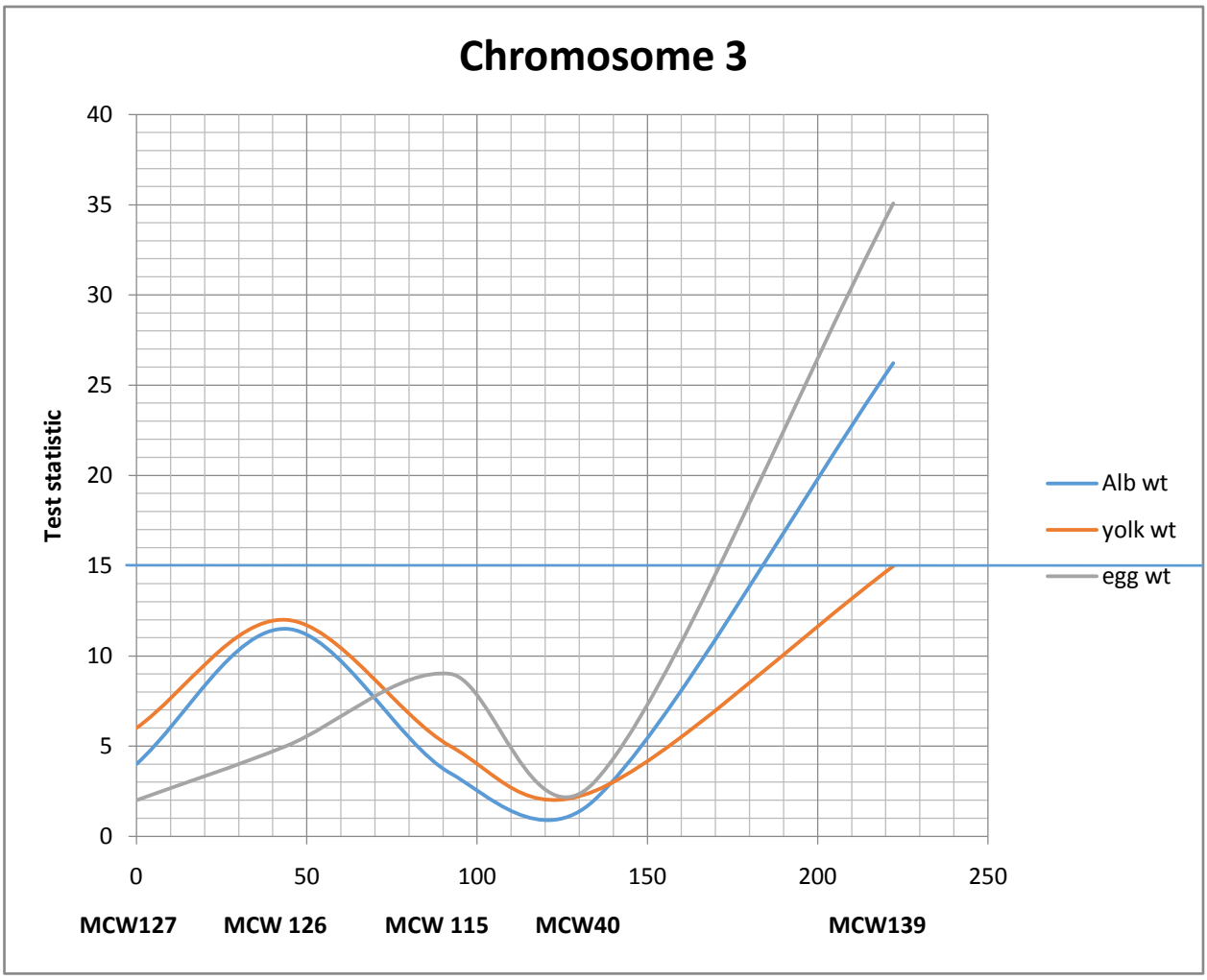
Test statistics value from the analysis of shell thickness and shell weight on chromosome 1, showing the position of QTL/marker (MCW0145) at 266.2cM. The two traits are linked to the marker and occupied the same locus. The 5% chromosome-wise significant threshold was included.

APPENDIX 16



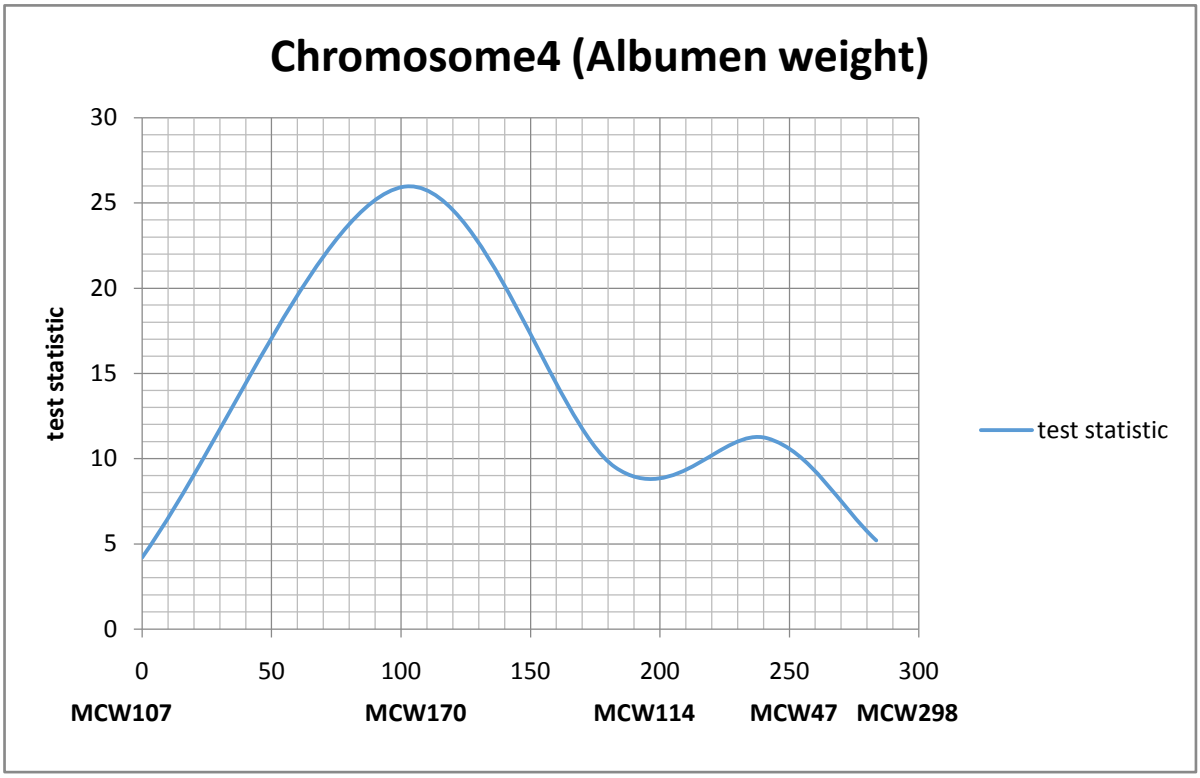
Test statistics value from the analysis of albumen weight on chromosome 2, showing the position of QTL/ marker (MCW0206) at 138cM. The 5% chromosome-wise significant threshold was included.

APPENDIX 17



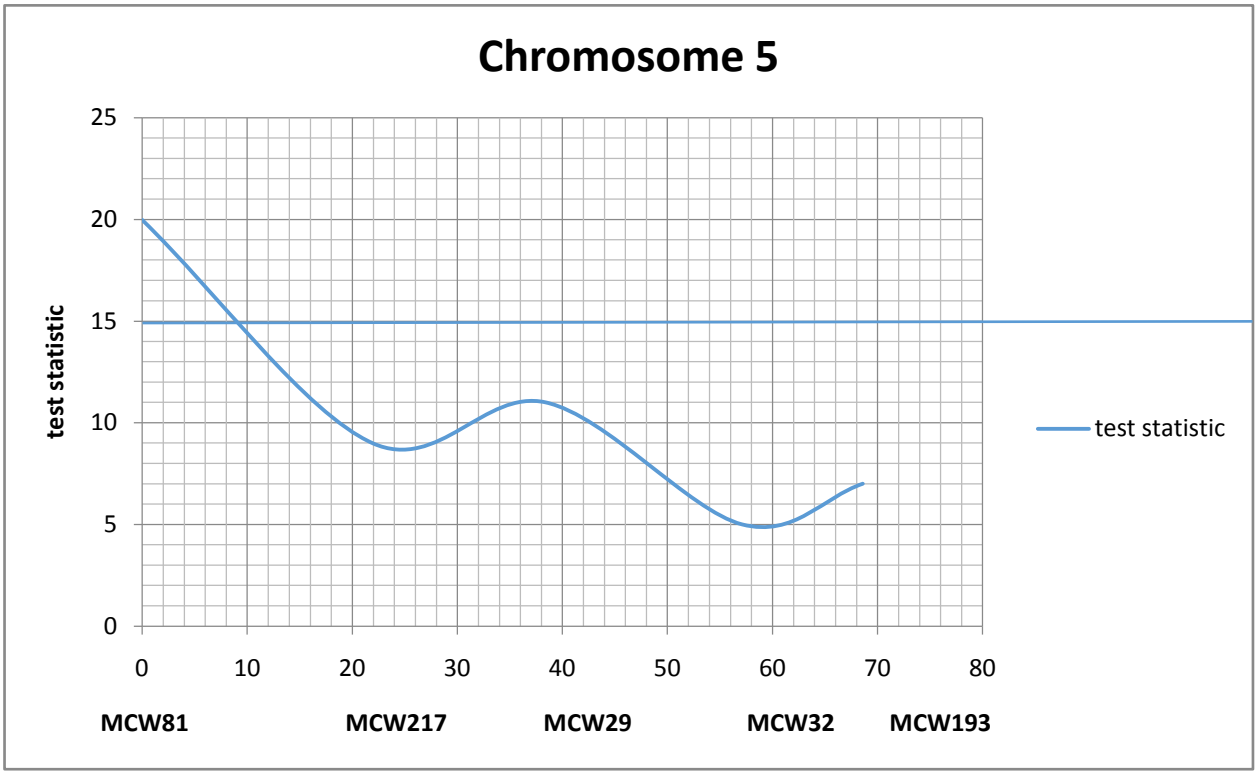
Test statistics value from the analysis of albumen weight, yolk weight and egg weight on chromosome 3, showing the position of QTL/marker (MCW0139) at 222.2cM. The three traits are attached to the same marker and therefore occupied the same locus. The 5% chromosome-wise significance threshold was included.

APPENDIX 18



Test statistics values from the analysis of albumen weight on chromosome 4, showing the position of QTL/marker (MCW0170) at 100.6cM. The 5% chromosome wise-significance threshold was included.

APPENDIX 19



Test statistics values from the analysis of shell thickness on chromosome 5, showing the position of QTL/marker (MCW0081) at 0.00cM. The 5% chromosome-wise significant threshold was included.