

Charles Moses Lyimo

**Genetic Characterization of Tanzanian Indigenous Chickens in  
Relation to the Worldwide Spectrum of Chicken Diversity**

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# Genetic Characterization of Tanzanian Indigenous Chickens in Relation to the Worldwide Spectrum of Chicken Diversity

Dissertation  
to obtain the PhD degree  
in the International PhD Program for the Agricultural Sciences in Göttingen  
(IPAG) at the Faculty of Agricultural Sciences,  
Georg-August-Universität Göttingen, Germany



Presented by

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(Born in Dar-es-salaam, Tanzania)

A handwritten signature in blue ink, which appears to be "C. Lyimo".

Göttingen, November 2014

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**D7**

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**Date of Examination: 17<sup>th</sup> November, 2014**

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<sup>1</sup>The image on the cover page represent a map with pie charts showing distributions of STRUCTURE mean membership coefficients of the most probable clustering at  $K=3$  of pools of chicken populations across various regions. More details in Chapter 4.

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## DECLARATIONS

I hereby declare that this thesis is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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Göttingen, November, 2014

**Charles Moses Lyimo**

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---

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## ACRONYMS

|          |                                                                      |
|----------|----------------------------------------------------------------------|
| ACIAR    | Australia Centre for International Agricultural Research             |
| AFNSD    | Africa Food and Nutrition Security Day                               |
| AnGR     | Animal Genetic Resources                                             |
| CBD      | The Convention on Biological Diversity                               |
| DAAD     | Germany Academic Exchange (Deutsche Akademische Austausch Dienst)    |
| FAnGR    | Farm Animal Genetic Resources                                        |
| FAO      | Food and Agriculture Organisation of the United Nations              |
| FLI      | Friedrich-Loeffler-Institut                                          |
| GDP      | Gross Domestic Product                                               |
| IAEA     | International Atomic Energy Agency                                   |
| IFAD     | International Fund for Agricultural Development                      |
| IPAG     | International PhD Program for the Agricultural Sciences in Göttingen |
| ISAG     | International Society for Animal Genetics                            |
| MLD      | Ministry of Livestock Development - Tanzania                         |
| MLFD     | Ministry of Livestock and Fisheries Development - Tanzania           |
| MLHUD    | Ministry of Lands, Housing and Urban Development - Tanzania          |
| MoEVT    | Tanzania Ministry of Education and Vocational Training               |
| NBS      | National Bureau of Statistics - Tanzania                             |
| NCBI     | National Center for Biotechnology Information                        |
| SUA      | Sokoine University of Agriculture                                    |
| SYNBREED | Synergistic Plant and Animal Breeding                                |
| TCU      | Tanzania Commission for Universities                                 |
| UN       | United Nations                                                       |
| URT      | United Republic of Tanzania                                          |
| USAID    | United States Agency for International Development                   |
| WSFS     | World Summit on Food Security                                        |

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## SUMMARY

Poultry production is the most dynamic sector that signified the rapid growth of all meat sectors during the last decades (FAO, 2010). The global growth of poultry industry reflects an increase of world meat demand and consumption (de Haan *et al.*, 2001; Delego, 2003; Delgado *et al.*, 2008; Narrod *et al.*, 2008; Trostle and Seeley, 2013; FAO 2014). Among the poultry species, chickens are the humankind's most common and widespread birds. Chicken meat represents 88% of poultry meat output (Global Poultry Trends, 2014). Although the demand of chicken products has increased tremendously due to not only the fast growth of world population but also the increase of per capita global consumption of meat, nearly one third of chicken breeds are reported to be at risk of extinction (Pym, 2010). This is alarming and thereby calls for an assessment of the existing genetic resources, because many of the varieties which are at risk may have traits that can be valuable in future environmental challenges and marketing demands. Genetic variability in chickens is the best insurance for the adaptation and sustainable production for the future unpredictable needs (Simianer and Meyer, 2003). One of the important elements concerning sustainable use of animal genetic resources is to ensure that locally adapted populations remain a functional part of production systems.

Indigenous chickens are among the essential local assets of poor people living mainly in rural areas in Africa, and who make up between 65% and 80% of the total population in sub-Saharan Africa (Ndegwa *et al.*, 2000). In sub-Saharan Africa, 85% of all households keep chicken under free-range system, with women owning 70% of these birds (Sonaiya *et al.*, 2004; Abubakar *et al.*, 2007). In Tanzania, which is one of the countries in Sub-Saharan Africa about 80% of the human population lives in the rural area where majority of the indigenous chicken are kept. Since village chickens play numerous important roles in the rural households and village social welfares in Tanzania, efforts towards populations' characterizations, improvements, proper utilisation and conservation of the existing chicken genetic resources should be made.

The general objective of this study is to assess the genetic characterization of Tanzanian indigenous chickens in relation to the worldwide spectrum of chicken diversity. The specific objectives were to:

- (i) Investigate the maternal lineages and genetic diversity of Tanzanian indigenous chicken
- (ii) Investigate the genealogical patterns of chicken breeds sampled in Europe
- (iii) Evaluate global diversity and genetic contributions of chicken populations from African, Asian and European regions

**Key words:** *Farm animal genetic resources, Global chicken diversity, Chicken population structure, Genetic contribution, Tanzanian indigenous chickens, Microsatellite markers, Mitochondrial DNA, Nucleotide diversity, Haplotype diversity, Phenotypic traits*

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In the first objective of this study (Chapter 2), the genetic diversity and maternal lineages of five Tanzanian indigenous chickens (*Ching'wekwe*, *Kuchi*, *Morogoro-medium*, *Pemba* and *Unguja*) were examined. Phenotypic measurements and blood samples were collected in 196 individual chickens from eight regions of Eastern Zone, Central Zone, Lake Zone and Zanzibar islands. A principal component analysis of morphological measurements distinguished individuals most by body sizes and body weight. *Morogoro Medium*, *Pemba* and *Unguja* were grouped together, while *Ching'wekwe* stood out because of their disproportionate short shanks and *ulna* bones. *Kuchi* formed an independent group owing to their comparably long body sizes. Individual chickens were genotyped at 29 microsatellite loci, 28 of them taken from the 30 that have been suggested for biodiversity studies in chickens. From molecular genetic marker analyses, the expected ( $0.62 \pm 0.028$ ) and observed ( $0.62 \pm 0.017$ ) heterozygosity estimates in Tanzanian indigenous chickens, were more or less similar with other African chicken populations. Genetical differentiation between Tanzanian chicken ecotypes ( $F_{ST}$  0.048) was found to be smaller than between commercial chicken lines. Both STRUCTURE analysis and phylogenetic neighbour-net revealed three clusters of Tanzanian chicken populations. These clusters comprised i) *Morogoro-medium* and *Ching'wekwe* from Eastern and Central Zones ii) *Unguja* and *Pemba* from Zanzibar Islands and iii) *Kuchi* from Lake Zone regions, which formed an independent cluster. Sequence polymorphism of D-loop region was analyzed to disclose the likely maternal origin of Tanzanian chickens. According to Liu *et al.*, (2006) reference mtDNA haplotypes, the Tanzanian chickens that were sampled encompass two haplogroups of different genealogical origin. The haplotype network analysis indicates that Tanzanian chickens probably originated from the Indian subcontinent and from Southeast Asia.

In the second study (Chapter 3), sequencing data of 455bp fragment size of the control region (D-loop) in the mitochondrial genome was studied in 1256 individuals collected from 55 European chicken breeds. The 55 chicken breeds studied were categorized into six groups according to their historical background; *Mediterranean type*, *East European type*, *Northwest European type*, breeds based on introgression of Asian breeds into European breeds during their foundation 100 to 150 years ago (*Intermediate types and Game birds*), and breeds with recent Asian origin (*Asian type*). Median-joining networks were constructed to establish the evolutionary relationships based on the classification of haplotypes given by Liu *et al.* (2006) nomenclature. From the Median-joining network, haplogroup E was the predominant clade among the European chicken breeds. Genetic variation was assessed within breed categories using DnaSP v.5.10.01 program. Wide ranges of haplotype diversity and nucleotide diversity in European chickens ( $0.442 \leq H \leq 0.761$  and  $0.0030 \leq \pi \leq 0.0104$  respectively) suggest that European breeds have relative high genetic variation between them. High average haplotype number, haplotype diversity and nucleotide diversity were observed for Asian type breeds followed by intermediate type. East European and Northwest European breeds have lower haplotype and nucleotide diversity compared to Mediterranean type, intermediate type, Game bird, and Asian type breeds. This study revealed that haplogroup of Clade E are predominant

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in European breeds, and might have their roots mainly from the Indian subcontinent and some shown to have traces from East Asia.

In the third study (Chapter 4), global genetic diversity and genetic contributions of chicken populations from African, Asian and European regions were investigated by using multilocus microsatellite genotypes. A total of 3314 individuals from 113 chicken populations, which were sampled in different geographical regions, climatic conditions and population management were assessed using 29 autosomal microsatellite loci for estimating diversity and genetic contributions. The set of populations studied included 101 local populations with the breed history from three continents of Africa, Asia and Europe. Three populations of red and nine commercial purebred lines were included and used as reference populations in the analyses. The mean number of alleles per locus and population was higher in African ( $5.20 \pm 0.17$ ) and Asian chickens ( $5.12 \pm 0.16$ ), than in European breeds ( $3.20 \pm 0.11$ ) and commercial lines ( $3.28 \pm 0.26$ ). Higher estimates of expected heterozygosity were calculated for African ( $0.604 \pm 0.016$ ) and Asian ( $0.603 \pm 0.015$ ) chickens compared to European ( $0.455 \pm 0.011$ ) and commercial ( $0.453 \pm 0.026$ ) breeds. Traditional farming system in Africa of free-range management may have contributed to this higher genetic variation within the population. African and Asian chickens showed lower genetic differentiation ( $F_{ST}$   $0.108 \pm 0.004$  and  $0.120 \pm 0.005$ , respectively) compared to European and commercial breeds ( $0.320 \pm 0.011$  and  $0.327 \pm 0.022$ , respectively). The wider variation in diversity between European chicken populations is to some degree related to a varying degree of population sizes and isolation.

NeighbourNet phylogenetic network based on a kinship distances of 113 chicken populations revealed two main clusters of Asian and Northwestern European breeds at opposite ends of geographical representation. African populations were clustered with breeds from Eastern Europe, and Mediterranean regions, Broilers and Brown egg layers in between of these two main clusters. Among the commercial breeds, White egg layers were clustered with Northwestern European chickens. Red junglefowl populations provide a high contribution (0.019) in the optimal core set, followed by Asian chicken populations (0.0115). On average, European chicken population contributed least to the core set (0.007), whereas the average contributions of commercial line (0.009) is very similar to that of African chicken populations (0.009). Higher genetic differentiation and low genetic contributions have been observed in European breeds.

In general, Tanzania chickens are as highly diverse as other African chicken ecotypes. In terms of genetic compositions, Tanzania village chickens have the admixture of genetic material that is found in Asian and European chicken populations. However, the current efforts for improving productivity and disease resistance of Tanzania village chickens should go along with conservation efforts and Smallholder farmers' empowerment in rural areas in operating small-scale chicken breeding while retaining the diversity would help in implementing the conservation of chicken genetic resources. This study can potentially contribute to generating information on population structure and genetic diversity of the Tanzanian indigenous chickens, with regard to the appropriate utilization and conservation efforts of the existing chicken genetic resources.

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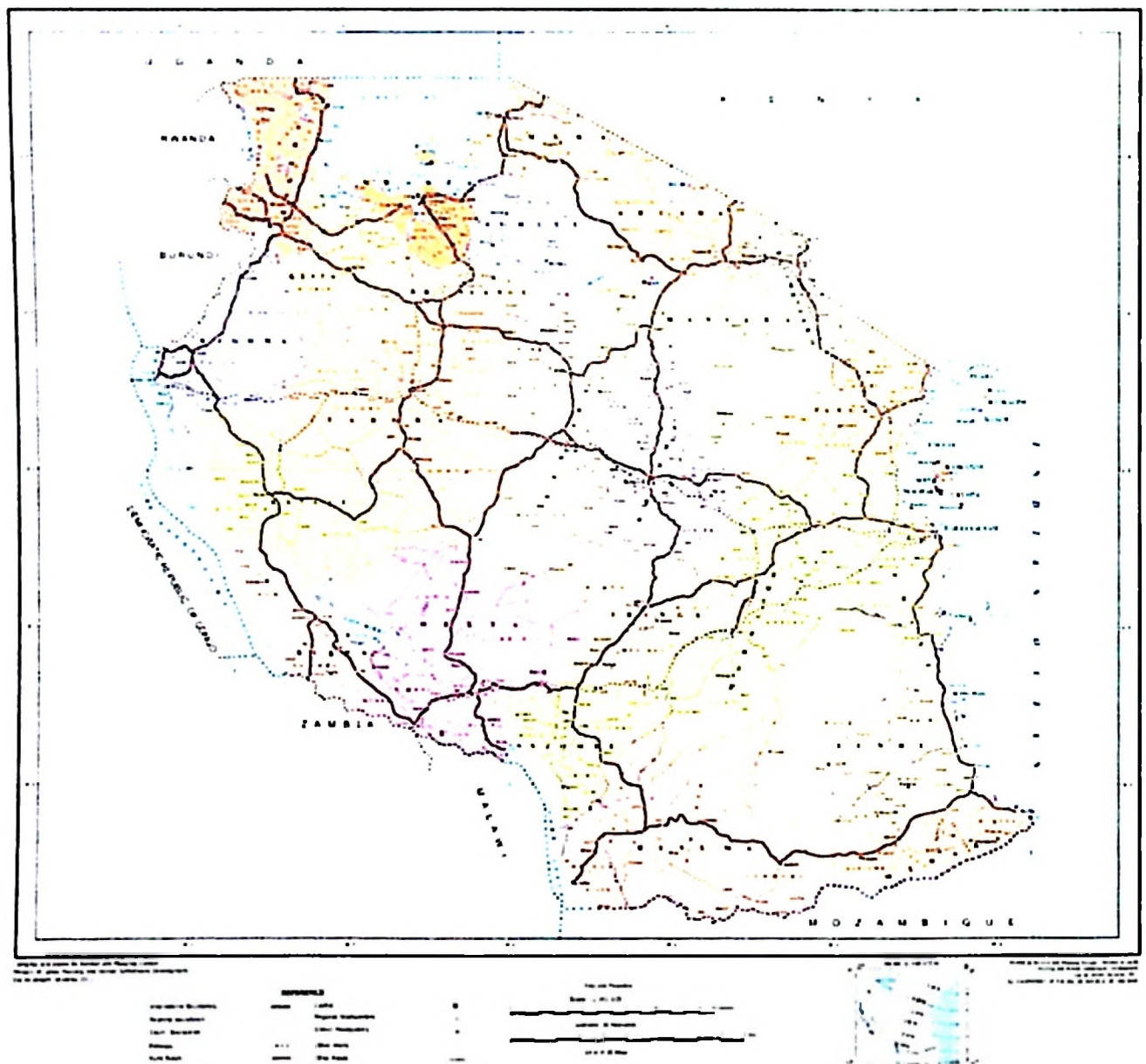
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## **CHAPTER ONE**

### **1.0 General introduction**

### 1.1. The United Republic of Tanzania

The United Republic of Tanzania is located in the Eastern coast of Africa just south of the equator between longitude 29° and 41° East, Latitude 1° and 12° South. It also incorporates several offshore islands, including Unguja (Zanzibar), Pemba, and Mafia. The country's eastern border is formed by the Indian Ocean coastline of approximately 1,400 km long. It has land borders with Kenya and Uganda to the north; Rwanda, Burundi and Democratic Republic of Congo to the west; and Zambia, Malawi and Mozambique to the south. Tanzania is the world's 31<sup>st</sup> largest country with a total area of 947,300 km<sup>2</sup> including 885,800 km<sup>2</sup> of the land surfaces and 61,500 km<sup>2</sup> of inland water bodies. It has a territorial sea limit of 22 km (12 nautical miles). Tanzania has a population of 45 million people with the annual growth rate of 2.7 % (NBS, 2013). There are over 120 ethnic groups, each with its own language or dialect. Swahili is the common and officially spoken language in all of Tanzania. However, English is the second official language and the language most often used in education, commerce and international affairs (Sa, 2007, Kanana, 2013; Development Education, 2014).



Source: Ministry of Lands, Housing and Urban Development (MLHUD, 2013)

Figure 1.1: Administrative map of the United Republic of Tanzania

### 1.1.1 Topography and General Climatic Condition of Tanzania

Tanzania is a country of highly varied physical-geographic features, including Africa's highest point at the snow-capped of Mount Kilimanjaro (5,895 m) in the northern border and Africa's lowest point at the floor of Lake Tanganyika (1,436 m) in the western border. Three of the African's Great Lakes are partly within the borders of Tanzania, namely Lake Victoria the African largest lake, Lake Tanganyika and Lake Nyasa. The country has a spectacular landscape of mainly three physiographic regions namely the Islands and the coastal plains to the east; the inland saucer-shaped plateau; and the highlands. The Central Plateau, covering over a third of the country, lies between the two branches of the Rift Valley. The East African Rift Valley runs from north to south leaving many narrow, deep depressions, often filled with lakes. A western branch of the Rift Valley runs along the western frontier and is marked by lakes Tanganyika and Rukwa. The eastern branch is the Great Rift Valley, from the Kenya border in the region of lakes Eyasi, Natron and Manyara to Lake Nyassa on the Mozambican border. Much of the country is above 900 meters and a small portion, including the islands and the coastal plains, lies below about 200 meters. The natural vegetation's are extremely varied, changing from coastal mangrove swamps to tropical rain forests; and from rolling savannas and high arid plateaus to mountain ranges. About a third of the country mainland is covered with wooded grassland savannas while two-thirds of Zanzibar Island is covered with bushes and grass.

Tanzania is characterized by a tropical climate although it experiences a great effect on its climatic conditions due to its geometrical location and physical features. The coastal area is tropical and humid with average temperatures of about 27°C (81°F). Further inland, the central plateau is hot and dry with temperatures that vary by season and time of day. High humidity in the lake regions and temperate conditions in the highlands can also be experienced. In the highlands, temperatures range between 10°C and 20°C, during cold and hot seasons respectively. The rest of the country has temperatures never falling lower than 20°C. The climate is governed by two monsoon periods. The north east monsoon from December to March brings the year's hottest temperatures, and when the winds shift to the south from March to May, they bring the heavy intermittent rains of the south west monsoon, which extends from June to September bringing relatively cool, dry weather. Generally Tanzania has two rainy seasons; a long heavy one from March to May, and a shorter, lighter one normally from November to January. Rainfall varies from an annual average of 1,250 mm in the wettest 3% of the land area, the south-eastern slopes of the great volcanoes, to below 600 mm in the central area of the country. The islands receive heavy rains in April and May with lighter rains in November and December. Drier weather occurs during the alternating monsoon seasons, which arrive from the northeast from December to March and from the southwest from June to October. Temperatures and rainfall are modified by altitude, with high elevations receiving more precipitation. In overall the rainfall period is usually short and is followed by a long dry season of six to eight months.

### 1.1.2 Tanzania Agro-ecological Zones

The country is divided into seven agro-ecological zones that are based on climate, physiography, soils, vegetation, land use and tsetse fly occurrence. All these factors are also the main physical agents, which influence opportunities and constraints for crop and livestock production. These agro-ecological zones are described as (i) Coastal, (ii) Arid Lands, (iii) Semi-arid Lands, (iv) Plateaux, (v) Southern, South-western and Western Highlands, (vi) Northern Highland and Granite Mountains and (vii) Alluvial Plains. Two of these zones, the arid and semi-arid zones together with the dry sub-humid areas constitute the dry-land areas of Tanzania. These dry-land areas cover about 61% of the total land area. The combinations of factors, which create the zones, are presented in the table (Table 1.1) below.

Table 1.1: Tanzania Agro-ecological Zones

| Zones ( <i>Altitude m</i> )                                                                                                                                  | Rainfall (mm)                                                                                                                                                                    | Soil and Topography                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Coastal Zone</b><br>( <i>Under 300</i> )                                                                                                                  | <i>North:</i> Bimodal, 750-1200<br><i>South:</i> Unimodal, 800-200                                                                                                               | <i>North:</i> Infertile sands on gently rolling uplands. Alluvial soils in Rufiji<br><i>South:</i> Sands and infertile soil, fertile clays on uplands and river flood plains                                                                                                                                                                                                               |
| <b>Arid Lands</b><br>( <i>North: 1300-1800</i> )<br>( <i>South: 500 – 1500</i> )                                                                             | <i>North:</i> Unimodal and unreliable, 500-800<br><i>South:</i> Unimodal and unreliable, 600-800                                                                                 | <i>North:</i> Volcanic ash and Sediments, Soils variable in texture and very susceptible to water erosion<br><i>South:</i> Rolling plains of reddish sandy clays of low fertility. Susceptible to water erosion. Pangani river flood plain with saline, alkaline soil                                                                                                                      |
| <b>Semi-arid Lands</b><br>( <i>Central: 1000-1500</i> )<br>( <i>Southern: 200 -600</i> )                                                                     | <i>Central:</i> Unimodal and unreliable, 500-800<br><i>Southern:</i> Unimodal 600-800                                                                                            | <i>Central:</i> Undulating plains, rocky hills and low scarps. Well drained soil low fertility. Alluvial hardpan and saline soils in Eastern Rift Valley and Lake Eyasi. Black cracking soils in Shinyanga<br><i>Southeastern:</i> Flat, or undulating plains w/rocky hills. Moderately fertile loams and clays in South (Morogoro), infertile sands in center.                            |
| <b>Plateaux</b><br>( <i>800 – 1500</i> )                                                                                                                     | <i>Western:</i> Unimodal, 800-1000<br><i>Southern:</i> Unimodal, very reliable, 900-1300                                                                                         | <i>Western:</i> Wide sandy plains and Rift Valley scarps. Flooded swamps of Malagarasi and Ugalla rivers have clay soil with high fertility<br><i>Southern:</i> Upland plains w/rock hills. Clay soils of low to moderate fertility in South, infertile sands in North                                                                                                                     |
| <b>Southern, South-western and Western Highlands</b><br>( <i>Southern: 1200-1500</i> )<br>( <i>Southwestern: 1400-2300</i> )<br>( <i>Western: 1000-800</i> ) | <i>Southern:</i> Unimodal, reliable, local rain shadows 800-1400<br><i>Southwestern:</i> Unimodal, reliable 800-1000<br><i>Western:</i> Bimodal 1000-2000                        | <i>Southern:</i> Undulating plains to dissected hills and mountains. Moderately fertile clay soils, with volcanic soils in Mbeya<br><i>Southwestern:</i> Undulating plateaux above Rift Valley(s) Sandy soils of low fertility<br><i>Western:</i> North south ridges separated by swampy valleys. Loams and clay soils of low fertility in hills, with alluvium and ponded clays in valley |
| <b>Northern Highland and Granite Mts.</b><br>( <i>Northern: 1000-2500</i> )<br>( <i>Granitic Mts: 1000-2000</i> )                                            | <i>Northern:</i> Bimodal, varies widely, 1000-2000<br><i>Granitic Mts:</i> Bimodal and very reliable, 1000-2000                                                                  | <i>Northern:</i> Volcanic uplands, Volcanic soils from lavas and ash. Deep fertile loams and clays. Soils in dry areas prone to water erosion<br><i>Granitic Mts:</i> Steep mountain sides to highland plateaux. Soil are deep, friable and moderately fertile on upper slopes; shadow and stony on steep slopes                                                                           |
| <b>Alluvial Plains</b>                                                                                                                                       | <i>Kilombero:</i> Unimodal, very reliable, 900-1300<br><i>Wami:</i> Unimodal 600–1800<br><i>Usangu:</i> Unimodal, 500-800<br><i>Rufiji:</i> Unimodal, often inadequate, 800–1200 | <i>Kilombero:</i> Central clay plain, with alluvial fans East and West<br><i>Wami:</i> Moderately alkaline black soils in East, and alluvial fans with well drained black loam in west<br><i>Usangu:</i> Seasonally flooded clay soils in North, alluvial fans in South<br><i>Rufiji:</i> Moderately alkaline black soils in East, and alluvial fans with well drained black loam in west  |

Sources: URT, (1999) and USAD, (2005).

### 1.1.3 Tanzanian natural resources and agriculture contributions

The country is endowed with abundant natural resources, which includes land, minerals, water bodies (rivers, lakes and ocean), waterfalls, gas reserves, forestry/woodlands, wildlife, forage and a large livestock resource base. The Gross Domestic Product (GDP) in Tanzania remained with an annual Growth Rate of 7% on average from 2002 until 2013 (The World Bank, 2014a; IFAD, 2014; NBS, 2014). Agriculture is still the foundation of the Tanzania economy that employs 80% of the Tanzanian workforce and it contributes half of the national income (URT, 2010). Agriculture also contributes around 80% of export earnings, and most industries in the country are linked to the agricultural sector in producing farm inputs and processing agricultural products. Tanzania agriculture is dominated by smallholder farmers (peasants) cultivating an average farm size between 0.9 to 3.0 hectares (ESRF, 2009). Smallholder farmers practice mainly subsistence farming and they commonly use rudimentary technologies which inevitably lead to the low yields. Only around 20% of farmers make use of animal traction for ploughing, and many are still using hand hoes for undertaking various operations such as land preparation and weeding. The use of advances in agriculture, such as improved seeds and fertilizer is still very low.

Tanzanian farmers grow a wide variety of food and cash crops. In terms of agricultural exports, coffee constitutes the most important cash crop (17.7%). Cotton is the second cash crop (16.3%), followed by cashew nuts (12.7%), tobacco (6.4%), tea (2.9%), and sisal (0.7%). The major importers of Tanzanian agricultural exports consist of the EU countries, especially the United Kingdom, Germany, and the Netherlands. For the food crop maize production dominates much of the country, particularly the highlands in the North and South. Bananas are grown much as a staple food in the Northern highland and in the Lake Zone at the western shore of Lake Victoria. The lush tropical coastal belt, always warm and humid, is dominated by cassava and rice. Drought resistant millet and sorghum are grown in the central plateau where temperatures and rainfall are highly variable. Tanzanian farmers also grow huge variety of fruits, vegetables and spices. Zanzibar, once a leading source of spices for the global market, continues to produce large amount of cloves.

Tanzania has vast animal genetic resources with a wide variety of indigenous farm animals. Available Animal Genetic Resources in 2012 included 22.8 million cattle, 15.6 million goats, 7.0 millions sheep, 2.1 million pigs and 60 million chickens (Njombe, 2013). Livestock industry contributes 18% of the total GDP, which is about 30% of the agricultural GDP and the poultry sub-sector contributes 16% of the livestock GDP (URT, 2010; PASS Trust, 2012). Within the livestock sector, village chickens are often the most common type of livestock and they are more frequent owned than larger livestock species. In the Tanzanian mainland, Shinyanga, Mbeya, Mwanza, Tabora, Morogoro, Iringa and Tanga regions accounted for 51.4% of the total chicken populations (NBS, 2012). According to FAO database the average consumption of chicken products in Tanzania is about 0.75 kg of meat and 13 eggs per year, which is far below the worldwide average consumption level of 6.8 kg of meat and 108 eggs capital per year (RLDC, 2010; PASS Trust, 2012).

## 1.2 Origin and dispersal of domestic chickens

The chicken (*Gallus domesticus*) is a domesticated fowl descending from wild junglefowls originating around Southeast Asia, the Southwestern part of China and the India subcontinent. Through domestication, migration, mutation, selection and management process, the specie has undergone several modifications from its wild ancestors in order to suit the adaptation to a new environment and its usefulness to humans. These processes led to remarkable evolutionary changes in morphology, physiology and behaviour of domesticated chickens (Price, 2002; Sawai *et al.*, 2010; Rubin *et al.*, 2010). The domestic chickens' ancestry can be traced back from the four species of wild junglefowls namely; Red junglefowl (*Gallus gallus*), Grey junglefowl (*Gallus sommeratii*), Ceylon junglefowl (*Gallus lafayetii*) and may be Green junglefowl (*Gallus varius*). Recent research studies suggested multiple domestication events of chickens over the last 8,000 years (West and Zhou, 1988; Fumihito *et al.* 1996; Liu *et al.*, 2006; Sawai *et al.*, 2010; Tixier-Boichard *et al.*, 2011), mainly from the wild ancestor red junglefowls (*Gallus gallus gallus*) in South-eastern Asia with some hybridizations of *Gallus sommeratii* and *Gallus lafayetii* (Groeneveld *et al.*, 2010; Tixier-Boichard *et al.*, 2011). Although domestic chickens are closely related to red junglefowls, the genetic contributions from other junglefowls remain uncertain (Sawai *et al.*, 2010). It is still not very clear how many of these subspecies have contributed to the origin of domestic chickens (Liu *et al.*, 2006). The absence of the yellow skin gene in the wild red junglefowl suggests the possibility of hybridization with the grey junglefowl (*Gallus sommeratii*) during the domestication of the species (Eriksson *et al.*, 2008; Clutton-Brock, 2012). The presence of yellow skin colour in many domestic chickens supports the theory of multiple progenitors as the foundation of several domestication events that occurred independently across Southeast Asia, Southwestern part of China and India subcontinent. A culturally significant hybrid between the red junglefowl and the green junglefowl in Indonesia is known as Bekisar (Hutagalung, 2000).

### 1.2.1 Classification and geographic distributions of wild junglefowls

The classifications of wild junglefowls are mainly based on the phenotypic traits and geographical distributions of the populations. The four species of junglefowl inhabited at different geographical regions of Asia (Figure 1.2) and display different morphological features (Sibley and Monroe, 1990; Nishibori *et al.*, 2005; Tixier-Boichard *et al.*, 2011). The conformation and body shape of *Gallus gallus* have the features of Mediterranean egg type breeds (Crawford, 1990). The vibrant male has long, golden-orange to deep-red crown and neck feathers, and a dark metallic-green tail with a white tuft at the base. The under-parts are a dull black while the upper-parts are a combination of glossy blue-green, rich dark red, maroon-red, fiery orange, rufous and blackish brown. The presence of a complete eclipse plumage in the summer and a shrill, short crow, which ends suddenly, are exclusively intrinsic in the male red junglefowl. Pure hens show neither comb nor wattles and both pure sexes hold

their tails almost horizontal (Appendix 1). The *Gallus sonneratii* has body plumage on a grey background colour and is endemic in India, distributed from Southwest to central India. *Gallus lafayetii* fowl inhabits only in Sri Lanka, morphologically resembles with RJF but exhibits an orange-brown colour of the breast with a purple spot on top of the neck, yellow spot on the comb and red legs. *Gallus varius* is morphologically distinct greenish plumage junglefowl limited to Java Island in Philippine and immediately vicinity of Bali and Lombok Islands. It thought to be the most primitive of the four gallus species, boasting sixteen tail feathers and short hackle feathers, whereas other species possess fourteen tail feathers and long pointed hackle feathers. *Gallus varius* also characterized by several morphological peculiarities including a single three-coloured wattle (red, yellow and blue), the lack of indentations of the comb and two additional feathers on the tail.

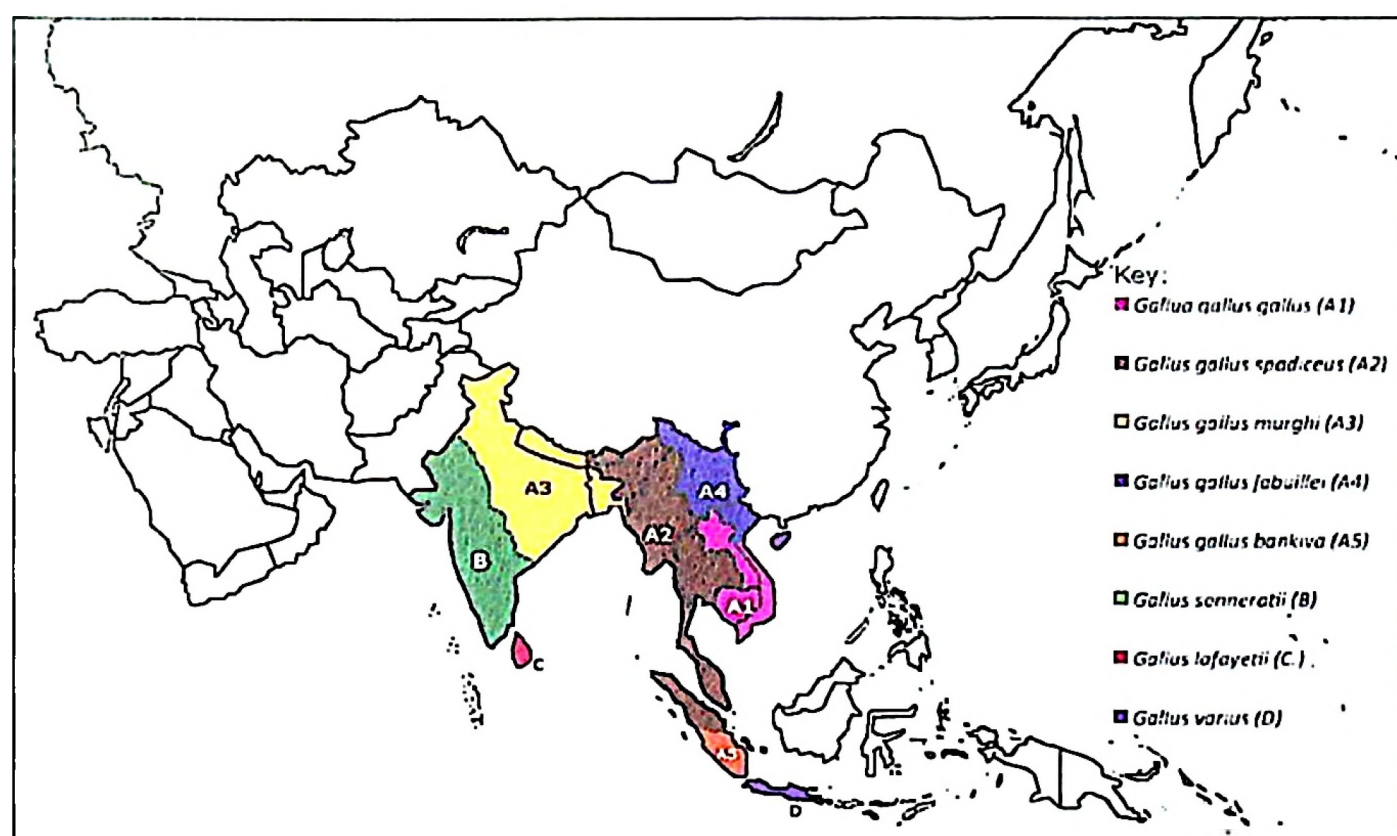


Figure 1 2: Origin distribution of the wild red junglefowl adopted from West and Zhou, (1988); and Tixier-Boichard *et al.*, (2011)

Red junglefowl is classified into five subspecies based on variation in home range, earlobes colour, comb size, facial wattles, length and colour of the male hackle feather. In general red junglefowl has a strong sexual dimorphism with males having red fleshy wattles (Sawai *et al.*, 2010) *Gallus gallus gallus* from South Vietnam, Cambodia, Thailand and Laos, characterized with white earlobes. *Gallus gallus spadiceus* from Myanmar, North Sumatra, Thailand, Malaysia and Southwest China, is characterized with red earlobes. *Gallus gallus murghi* from Northern India, Nepal, Bhutan and Bangladesh, possesses white earlobes. Males have extensive bare reddish skin on their face and throat,

dominated by large, deeply indented red fleshy corona combs. *Gallus gallus jabouillei* from South China and North Vietnam are characterized with red earlobes and have short facial wattles. *Gallus gallus bankiva* found in Java and Sumatra possess red earlobes. Beside the current wide dispersal and production of domestic chickens, wild populations of red junglefowl still exist in Southeast Asia, inhabiting in the forests areas and the domesticated chicken can be regarded as a subspecies (Collias and Collias 1967; Nishida *et al.* 1992; Fumihito *et al.* 1996; Potts, 2012).

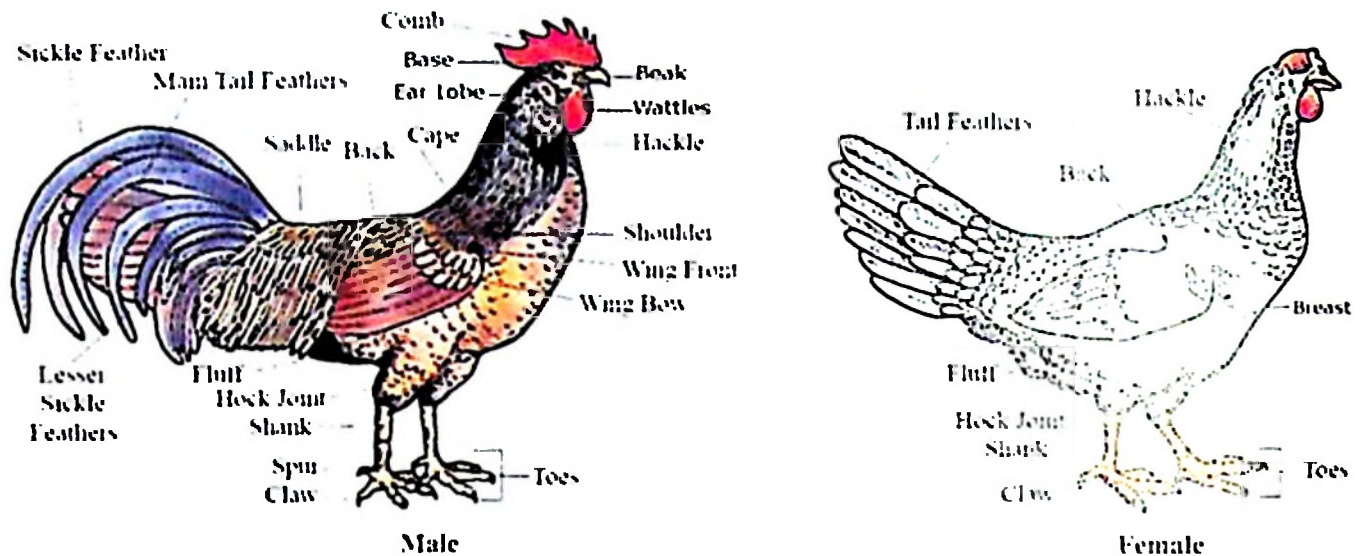


Figure 1.3. Male and female chickens body parts (Source: Extension, 2012)

### 1.2.2 Chicken dispersal from domesticated region

The domesticated chickens are thought to have then spread rapidly across Asia and then into Europe, Africa and America through human migration, along trade routes, colonization and expansion of agriculture (Liu *et al.*, 2006; Kanginakudru *et al.*, 2008; Groeneveld *et al.*, 2010; Wragg *et al.*, 2012; Storey *et al.*, 2012; Mwacharo *et al.* 2013a). Although some evidence illustrates the existence of chickens in Europe by the late Neolithic (4000 B.C.) and early Bronze Age, the Iron Age (3000 B.C.) was the main period for dispersion of chickens throughout Europe. They mainly came from China via Russia on the northern route, and from Indus Valley via Persia on the southern route (West and Zhou, 1988; Tixier-Boichard *et al.*, 2011; Flink *et al.*, 2014).

The chickens were introduced to Africa from Asia by way of the Indian Ocean, and from Europe and Arabian Peninsula via the Mediterranean and Red Sea (MacDonald and Edward 1993; Masonen, 1995; Boivin *et al.*, 2009). As early as 1500 B.C. depictions of chickens emerge in Egyptian hieroglyphic art. At this time a rooster is visible in a scene in the tomb of Rekhmara (Pott, 2012). Dabry *et al.* (1977) reported the existence of a painted limestone ostrakon from the tomb of Tutankhamun and other images that suggests the presence of chicken as exotic in Egypt during the New Kingdom era (1425-1123 B.C.). Chami (2001) has reported chicken bones from a Neolithic context on Zanzibar, dated to

800 B.C. Osteological and pictorial evidence for chickens in Africa become more common around 650 B.C. (Coltherd, 1966). Gifford-Gonzales and Hanotte (2011) reported two main waves of chickens being introduced to Africa. The first wave was from the Mediterranean Sea via Egypt during the Ptolemaic period (300 B.C.), later spreading through the Nile valley and to the West Africa along the Sudano-Sahelian corridor (MacDonald and Edward, 1993; Fuller *et al.*, 2011). The second wave came across the Indian Ocean when chickens were introduced to the East Coast of Africa by means of the existed trade networks during the beginning to middle of the 1<sup>st</sup> millennium A.D. (Adelaar, 1996; Chami, 2005; Blench, 2006; Boivin and Fuller, 2009; Fuller *et al.*, 2011). Chami (2001) and Chami and Kweakason (2003) have presented the archaeological evidence of chicken bones spread in the East African Islands as early as the 8<sup>th</sup> century B.C. The linguistic evidence strongly suggests multiple introductions of chickens across the Sahara, via the Berbers, on the east coast and possibly a separate introduction to Ethiopia via the Red Sea coast (MacDonald and Blench, 2000). Unlike cereals and other domestic plants, livestock are older and are apparently more linguistically stable in Africa. Species such as the chicken, introduced over 3000 years ago, have created a complex trail of loanwords that clearly indicate the routes whereby they entered and diffused across the continent (Blench, 2006). Williamson (2000) reported the linguistics and ethnographic evidence for domestic fowl in Africa that suggests chickens were moved from East to West Africa. In another pioneering study, Johnston (1886) used the words for chicken in Bantu languages to show that chicken cannot be reconstructed to Proto-Bantu because of its irregular reflexes and he considered it likely that the chickens were introduced into the Bantu area from the East.

Numerous suggestions have been made concerning the introductions of chickens to America. There is a postulation that chickens were brought to South America by Polynesians, which were first settled in South America in late 1200 A.D. (Lawler, 2010; Stores *et al.*, 2011). It was also believed that, the introduction of chickens to the East coast of South America, was brought by Portuguese or Spanish conquistadors around 1500 A.D. (Storey *et al.*, 2011). Other scholars indicated that; chickens were brought to America from Africa in the 16<sup>th</sup> Century as the results of Dutch and Portuguese slave trade (Caudill, 1975; Wasserman, 2008). Thomson *et al.* (2014) reported a controversies regarding the introduction of chickens to America from the evidences that indicates chickens were brought from the Polynesian Islands to Chile in 1350 A.D., and other evidence which suggesting chickens were brought by Spanish Conquistadors around 1500 A.D.

The present global genetic variation in chicken populations was influenced by the recent management practices and historic evolution process. Migration, gene flow, mutation, recombination, genetic drift, selection pressure, population size and breeding practices exercises by human are contributing factors to the existing population structure and genetic differentiation.

### 1.3 An overview of poultry production trend

The world's livestock sector is growing at an unprecedented rate and the driving force behind this enormous surge is a combination of population growth, rising incomes, increasing purchasing power, growth of intensive agriculture and urbanization. Poultry production is the most dynamic sector that signified the greatest growth of all meat sectors during the last decades (FAO, 2007; FAO, 2010). Currently, poultry production is characterized by faster growth in consumption and trading than any other major agricultural sectors in the world. FAO statistical reports described more than a 35% increase in poultry meat production from the year 2000 (66.9 mil. tons) to 2013 (106.8 mil. tons). Presently the total meat production is 308.3 million tons, where pork is the most widely consumed meat in the world (114.6 mil. tons), followed by poultry (106.8 mil. tons), beef (67.5 mil. tons), and mutton (13.7 mil. tons).

**Table 1.2: Worldwide meat production (million tons/year) from various species**

| Specie  | 2000  | 2005  | 2007  | 2008  | 2009  | 2010  | 2011  | 2012  | 2013  |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Poultry | 66.9  | 82.8  | 90.1  | 91.9  | 93.6  | 98.9  | 102.6 | 104.9 | 106.8 |
| Pork    | 91.3  | 104   | 99.8  | 104   | 106.3 | 109.3 | 109.2 | 112.7 | 114.6 |
| Beef    | 60    | 64.6  | 65.1  | 65.2  | 65    | 66.7  | 67.3  | 67.4  | 67.5  |
| Ovine   | 11.4  | 13.1  | 14    | 12.9  | 12.9  | 13.7  | 13.4  | 13.4  | 13.7  |
| World   | 233.9 | 269.7 | 274.4 | 279.4 | 283.6 | 294.2 | 298.1 | 304.1 | 308.3 |

Source: FAO (2011a; 2012a and 2013)

By the year 2050, the world population is projected to grow to 10 billion people. Most of the growth is expected to occur in poor developing countries, where income elasticity of demand for food continues to be high. The population increase, combined with moderately high-income growth, could result in more than a 70% increase in demand for food and other agricultural products (Wik *et al.*, 2008).

In developing countries, poultry meat consumption growth is much more pronounced than in developed countries (FAO, 2012a; FAO, 2013). The demand for meat in the developing world has been increasing annually due to growing populations, rising incomes and urbanization (de Haan *et al.*, 2001; Delego, 2003; Delgado *et al.*, 2008; Narrod *et al.*, 2008; Trostle and Seeley, 2013). The demand for livestock products is expected to double in sub-Saharan Africa and South Asia, from 200 kilocalories per person per day in year 2000 to some 400 kilocalories in the year 2050. The largest regional percentage increase in population will be Africa. The World Population Reference Bureau projected human population to double in Africa from 1.1 billion to about 2.3 billion by the year 2050 (PRB, 2013). Global Poultry Trend (2013ab) reported the expected increased for poultry demand in China, India, Thailand, Bangladesh and Africa as reflecting high population increases. However, in Eastern Asia an inadequate supply of pig meat observed starting in 2007, has resulted in a gradual shift in consumption from pork to poultry. In the Middle East the demand for poultry meat is more important where competition with pork is almost non-existing (FAO, 2010).

### 1.3.1. Social-economic contributions of chicken production

Chicken is the most prominent type among poultry species, which significantly provides humans with quality protein from both meat and eggs. Apart from being a source of food, chickens have also been used for many purposes around the globe, including their place in cultural mythologies, religious symbolism, rituals, traditional ceremonies, recreations and other cross-cultural beliefs fulfilment. Composting chicken manure has been extensively used in garden and crop productions, which potentially supply nitrogen, potassium, phosphorous and other elements. Furthermore, keeping chickens as pets has become increasingly popular over the years.

Chickens are among the most widespread domestic animals with a population of over 25 billions worldwide. That is the highest population of any bird in the world. Chicken meat represents 88% of global poultry meat output (Global Poultry Trends, 2013a). Chicken meat and eggs are the best source of quality protein to human beings. Protein deficiency is among the major contributing factors in malnutrition problems, largely prevailing in many developing countries of Africa, Asia, the Near East and Latin America. Furthermore, in Sub-Saharan Africa and South Asia, malnutrition and under-nutrition are closely associated with poverty. One quarter of the people in the developing world live below \$1.25 per day (table 1.3), whereas about half of the people in sub-Saharan Africa live below international poverty line (The World Bank, 2010). Poor nutrition is implicated in more than half of children deaths worldwide. Children who die of common diseases like diarrhea and malaria could have ultimately survived if they had not been malnourished in the first place (Hay and Elee, 2006). The United Nations Food and Agriculture Organization reported that nearly 900 millions people of the 7.1 billions people in the world, are suffering chronic undernourishment (FAO, 2012b). The World Bank report warns that malnutrition is costing poor countries up to 3% of their yearly GDP, while malnourished children are at risk of losing more than 10% of their lifetime earning potential (Hay and Elee, 2006, AFNSD, 2011). To effectively combat such malnutrition problem and under-nourishment, at least 20g of animal protein per person per day or 7.3 kg per year should be provided (FAO, 2008).

**Table 1.3: Poverty headcount ratio at national poverty line (% of population) in 2010**

| Region                       | % of population below lives \$1.25 per day |
|------------------------------|--------------------------------------------|
| Sub-Sahara Africa            | 48.5                                       |
| South Asia                   | 31.0                                       |
| East Asia and Pacific        | 12.5                                       |
| Latin America and Caribbean  | 5.5                                        |
| Middle East and North Africa | 2.4                                        |
| Europe and Central Asia      | 0.7                                        |

Source: The World Bank, (2010)

Livestock systems globally are changing rapidly in response to human population growth, urbanization and growing incomes (Thornton and Herrero, 2010). Despite with the decline in the overall population growth rate, the absolute size of the human population will continue to increase over the next several decades. However, most of the population growth is expected to occur in developing countries where growth rates are generally higher than developed countries (Figure 1.4).

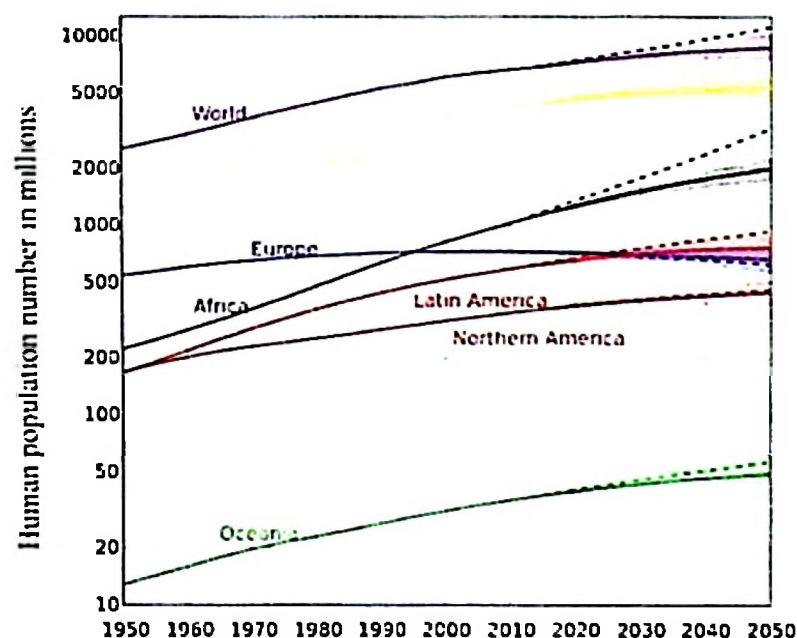


Figure 1.4: Estimate of population growth rate in different continents between 1950 and 2050 (UN, 2012)

Sub-Saharan Africa and South Asia show a potential increase in their annual Gross Domestic Product (Table 1.4). Low and middle-income countries are expected to consume more meat by 2050. Latin America, the Near East, North Africa and East Asia will have per capita food consumption similar to that of that of higher income countries in 1990 (Alexandratos and Bruinsma, 2012)

Table 1.4: Regional Gross Domestic Product (GDP) annual growth rate

| Region                       | 2012 | 2013 | 2014e | 2015f | 2016f |
|------------------------------|------|------|-------|-------|-------|
| Sub-Sahara Africa            | 3.7  | 4.7  | 4.7   | 5.1   | 5.1   |
| South Asia                   | 5.0  | 4.7  | 5.3   | 5.9   | 6.3   |
| Latin America and Caribbean  | 2.6  | 2.4  | 1.9   | 2.9   | 3.5   |
| Middle East and North Africa | 0.6  | -0.1 | 1.9   | 3.6   | 3.5   |
| Europe and Central Asia      | 1.9  | 3.6  | 2.4   | 3.7   | 4.0   |
| Developing countries         | 4.8  | 4.8  | 4.8   | 5.4   | 5.5   |
| High income countries        | 1.5  | 1.3  | 1.9   | 2.4   | 2.5   |

Source: The World Bank, (2014b)

Human population and economic growth have lead to an increase in world production of chicken meat and eggs in various continents, while Africa, Asia and South America have experienced the great increases in egg production (Daghir, 2008).

### 1.3.2. Global consumption of chicken products

Currently, chicken meat consumption is growing faster (2.5%) per year compared with pork (1.5%) and it was projected to overtake pork consumption before the year 2020 (The Economist, 2013). Global chicken meat output has increased by 39 million tons between 2000 and 2014 (Table 1.5).

**Table 1.5: Chicken meat production across the regions (million tons/year)**

| Region  | 2000 | 2005 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |
|---------|------|------|------|------|------|------|------|------|------|------|
| America | 27.1 | 32.7 | 35.0 | 37.4 | 36.7 | 38.6 | 39.9 | 40.2 | 41.1 | 42.2 |
| Asia    | 18.6 | 22.4 | 25.0 | 26.2 | 28.0 | 29.1 | 29.8 | 31.1 | 31.6 | 32.4 |
| Europe  | 9.3  | 10.9 | 11.6 | 12.1 | 13.3 | 13.9 | 14.9 | 15.2 | 15.4 | 15.5 |
| Africa  | 2.8  | 3.3  | 3.7  | 4.0  | 4.2  | 4.5  | 4.6  | 4.7  | 4.8  | 4.9  |
| Oceania | 0.7  | 0.9  | 1.0  | 1.0  | 1.0  | 1.1  | 1.2  | 1.3  | 1.3  | 1.4  |
| World   | 58.5 | 70.2 | 76.3 | 80.7 | 83.2 | 87.2 | 90.0 | 92.5 | 94.2 | 96.4 |

Source: Global Poultry Trends, (2014b)

Chicken meat contains important levels of proteins and other micronutrients, which are essential for growth and development. Trading and global consumption of chicken meat are continually growing especially in the developing world (Bevola *et al.*, 2012). Chicken meat consumption has increased by almost 40 million tons since year the 2000. African and Asian countries have the least chicken meat consumption (table 1.6).

**Table 1.6: Poultry meat consumption (Kg/person/ year)**

| Region  | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 |
|---------|------|------|------|------|------|------|------|------|------|------|
| America | 31.8 | 32.1 | 33.0 | 33.3 | 34.9 | 34.3 | 35.3 | 36.1 | 37.0 | 35.9 |
| Oceania | 30.2 | 30.4 | 32.4 | 33.6 | 33.5 | 35.7 | 35.8 | 37.0 | 35.5 | 35.7 |
| Europe  | 16.0 | 17.9 | 18.6 | 18.3 | 19.0 | 19.3 | 19.2 | 20.3 | 21.1 | 21.9 |
| Asia    | 6.7  | 6.6  | 6.8  | 7.0  | 7.0  | 7.5  | 7.7  | 8.2  | 8.6  | 8.8  |
| Africa  | 4.2  | 4.3  | 4.5  | 4.7  | 4.6  | 4.7  | 4.8  | 5.2  | 5.4  | 5.5  |
| World   | 11.1 | 11.3 | 11.6 | 11.8 | 12.1 | 12.3 | 12.6 | 13.1 | 13.6 | 13.6 |

Source: Global Poultry Trend, (2012).

Besides poultry meat production, egg production has been increasing, with an average increase of more than 2% p.a. (Table 1.7) between the years 2000 (51.1 mil. tons) and 2013 (66.7 mil. tons). Africa's population makes up more than 15% of the world's population but contributes less than 5% of the global egg production. Great potential for egg production growth is expected in the African region based primarily on the forecasts for increases in human population and per capita income (Global Poultry Trend, 2014).

**Table 1.7: World egg production trend (million tons/ year)**

| Region  | 2000 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 |
|---------|------|------|------|------|------|------|------|------|------|------|
| Asia    | 29.0 | 32.6 | 33.0 | 34.5 | 36.2 | 37.2 | 37.7 | 38.3 | 38.3 | 39.0 |
| America | 10.5 | 11.7 | 12.3 | 12.3 | 12.5 | 12.6 | 13.0 | 13.2 | 13.4 | 13.6 |
| Europe  | 9.5  | 9.9  | 10.1 | 10.0 | 10.1 | 10.3 | 10.5 | 10.6 | 10.6 | 10.8 |
| Africa  | 1.9  | 2.2  | 2.3  | 2.5  | 2.6  | 2.6  | 2.7  | 2.8  | 2.9  | 3.0  |
| Oceania | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  | 0.2  | 0.3  | 0.3  | 0.3  | 0.3  |
| World   | 51.1 | 56.6 | 57.9 | 59.5 | 61.7 | 62.8 | 64.1 | 65.2 | 65.5 | 66.7 |

Source: Global Poultry Trend, (2014c)

#### 1.4 Poultry production system in Africa

The common poultry production systems in Africa are mainly based on scavenging chickens, which are found in virtually all villages and households in rural Africa. These village chickens are characterized by small body size, slow growth rate, low egg production and late maturity in comparison to the commercial hybrids. Over 70% of the poultry products and 20% of the animal protein intake in most African countries comes from rural poultry sector (Kitalyi, 1998). In Africa chicken constituted around 98% of the total poultry population (Mengesha, 2013). Approximately 80% of the chicken populations in Africa are indigenous chickens and contribute significantly to the total meat production in the region (Gueye, 1998, 2000; Riise *et al.* 2004; Simainga, *et al.* 2011; Karim *et al.* 2013, Berima *et al.* 2013). Mwacharo *et al.* (2013b) reported the presence of scavenging village chickens in all agro-ecological zones of Africa. In sub-Saharan Africa, 85% of all households keep chicken under free-range system, with women owning 70% of it (Sonaiya *et al.*, 2004; Abubakar *et al.*, 2007). The traditional management of chickens in Africa provides for a flexible livestock production system, which allows their wide spreading across the region. Other factors, like low input requirements in terms of land, feed and labour, short generation intervals, the ability to survive on scavenging feeds, efficiency of converting poor quality feed into high quality protein, lack of religious or social restrictions, and the frequent use of chickens in traditional matters are some of the major reasons for most rural families to keep local chickens and favours their distribution in all parts of Africa (Kabatange and Katule, 1989; Melewas, 1989; ACIAR, 2009). There are few cultural or religious taboos that prevent the consumption of eggs and poultry meat in most African countries when compared to other livestock (Tadelle *et al.*, 2003). Village chicken productions require less start-up capital and can easily be raised even by poor people with limited land resources. Many institutions and development organizations in the rural areas promote the rearing of village chickens to improve income and nutritional status of the resources poor people (Randdorf *et al.*, 2007; Alders *et al.*, 2007). In the rural areas, livestock keepers are generally better off than those who depend entirely on crop agriculture (de Haan *et al.*, 2001). In many rural areas, poultry can provide some regular income through selling of eggs and chickens can also be sold immediately when the household need money (Saadullah *et al.*, 2006; Hilmi *et al.*, 2011). Therefore, it is obvious that putting an emphasis on local chickens would have an immediate impact on promoting sustainable animal protein intake and livelihood income.

African village chickens are non-descriptive, with a variety of morphological appearance. Generally the African village chickens are characterized by extensive phenotypic variations within and between different populations (Msoffe *et al.*, 2001; Muchadeyi *et al.*, 2007; Mwacharo *et al.*, 2007; Dana *et al.*, 2010; Mtileni *et al.*, 2011a; Leroy *et al.*, 2012; Lyimo *et al.*, 2013). Village chickens have been raised for thousands of years and selection pressure present in the village environments have resulted in local

breed that are well adapted to the local conditions (Copland and Alders, 2005). Natural selection can play an important role in shaping this variability through genetic hitchhiking (Andolfatto, 2001).

Local chickens remained predominant in African villages despite the introduction of exotic chickens and improved breeds. High-yielding chicken breed types were started to be introduced back in 1920s, but the farmers were not been able to afford the high input requirement of these introduced breeds (Kaiser, 1990; Safalaoh, 1997; Kitalyi 1998). Scavenging free range is the low input management system are characterized by low output per bird partly because of higher disease exposure, inadequate management intervention in terms of nutrition, veterinary health care and lack of selection for production traits. Despite these drawbacks these birds are apparently more suitable to the production system in the villages where there are limited resources, poor infrastructure and deficient veterinary services (Alfred *et al.*, 2012). In most African countries, the chickens have no regular health control programme, may or may not have shelter, and scavenge for most of their nutritional needs without any feed supplementations. Chickens reproduction is entirely based on uncontrolled natural mating and hatching of eggs are under natural incubation by brooding hens.

Indigenous chickens were rated to have superior merits for adaptation to the village environments with regard to traits such as disease resistance, tolerance to harsh tropical weather, ability to escape from predators, scavenging for food, natural incubation and broodiness characteristic (Badhaso, 2012; Suganthi, 2014). Normally, village chickens are defined according to their location and a few according to their physical appearances, without any other scientific acceptable character. Usually chicken names are established by the local ecotypes or varieties assuming local names depending on the locality and/or the local language. Only few can be termed as true breeds, such as the layers strains of Egypt namely *Fayoumi* and *Dandarawi*, whose plumage colour, size and productivity are well defined and uniform for each breed (Gueye, 1998; Hans, 2012).

Low genetic potential for production traits and frequent outbreaks of diseases, specifically Newcastle, Coccidiosis, Infectious Coryza, Fowl typhoid and Fowl cholera diseases, have been noted in a significant number of studies to be amongst the major factors limiting the productivity of the local chickens in the Africa both under intensive and extensive management systems (Katule and Mgheni 1990; Yongolo 1996; Alexander, 2001; Msoffe, 2003). Other factors leading to poor performances are poor bird management practices, ectoparasitosis, predators and a low level of literacy among farmers (Minga *et al.*, 1987, 1989; Katule 1988; Msoffe *et al.*, 2002; Salum *et al.*, 2002; Conroy *et al.*, 2005). Poor chick survival under extensive management has been noted by several researchers to be among the problems hindering further expansion of local chickens in the country. Chick mortality in a household under free-range conditions may vary from 0 to 100% with an average of 30% and more with most of it occurring in the first few weeks of life. Mwalusanya *et al.* (2002) reported that the average chick mortality under free-range conditions up to 10 weeks of age is about 40%.

### 1.5 Tanzanian poultry production

Traditional poultry farming in Tanzania is mainly dominated by chickens, which account for 94.1%, then followed by ducks and geese (5.3%), guinea fowl (0.4%) and turkey (0.2%). The traditional poultry system in Tanzania is the largest, supplying roughly 100% of the poultry meat and eggs consumed in rural areas, and 20% in the urban areas (Oleke and Isinika, 2011; NABC, 2012). Over 80% of Tanzania's human population live in rural areas where smallholders account for the bulk of farmers. More than 80% of rural households keep scavenging village chickens, which are important for their livelihood subsistence and income generations (Swai *et al.*, 2007). The available indigenous chicken breeds remain the predominant source of poultry breeding in Tanzania. In all 126 Ethnic groups existing in Tanzania, native chickens are kept under the traditional system where chickens are allowed the freedom to scavenge around the homesteads in the day time, picking up whatever feed is available in the environment, usually ranging from insects, seeds, kitchen wastes to grass (Sonaiya, 1990; Pedersen, 2002; Msolle, 2003; Mcainsh *et al.*, 2004; Riise and Meaish, 2004; Swai *et al.*, 2007).

Village chickens have been potentially used in several food security projects and/or poverty alleviation programs at the rural and peri-urban communities (Foeken *et al.*, 2004; Faiela *et al.*, 2007; Msami and Young, 2009; Copland and Alders, 2009; Pym *et al.*, 2009). The majority of rural communities regard chickens as “a walking bank” or “a bank on the hoof” because they provide immediately available petty cash in times of need (Moreki and Dikeme 2011). Village chickens are also used in different ritual ceremonies, traditional healing, as a quick exchange commodity and as valuable gifts to respected guests (Msami and Young, 2009; USAID, 2009; Moreki and Dikeme 2011). These chickens are primarily raised under free-range management systems that permit minimum or no care in terms of health, breeding management, housing and supplemental feed given to the birds (Msami and Young, 2009; Mammo and Wude, 2011; Mutayoba *et al.*, 2012).

#### 1.5.1 General Characteristics of the Tanzania indigenous chickens

Normally local chickens are found almost every part of Tanzania, although the majority of these local chickens (60%) are found in the Central Corridor regions of Tanzania. These indicate that indigenous chickens are highly important farm animals kept as a good source of animal protein, for social uses and as a source of income to most of the rural populations. Their widespread distribution all over the country indicates their adaptive potential to the local environmental conditions, diseases and other stresses. Tanzanian indigenous chickens are usually small in size that weigh 0.9 to 1.8 on average and lays a small number of eggs (80 – 120) per year. Research work on local chickens carried out in Tanzania shows that the number of clutches per year is 2-4; eggs per clutch is 15; egg weight is 44-49g; hatchability is 78% and chick mortality is 32.6% (Swai *et al.*, 2007).

### 1.5.2 Phenotypic characteristics of Tanzanian chickens

It is very difficult to classify Tanzania local chickens into breeds due to heterogeneous nature of the populations, the way they share the same basic anatomy and their physiological features are more or less the same (Lawrence, 1998). However, seven ecotypes of indigenous chicken were clearly identified basing on their geographical origin and prominent phenotypic characteristics (Table 1.8). Scavenging local chickens exhibit wide variations in plumage colours, comb types, skin and shank colour, shank length, adult body size, body conformation, as well as in performances (Payne 1990; Horst and Mathur, 1992; Lawrence, 1998; Msoffe, 2003; Fayeye *et al.*, 2005; Njenga, 2005). Breeding under this traditional management system is by random mating, since the village chickens are left outside for scavenging. The lack of a controlled breeding programme is responsible for the genetic heterogeneity found in the local chicken population (Minga *et al.*, 1996). In other study, Gwakisa *et al.* (1994) showed that the scavenging local chickens also show differences in immune response to Newcastle disease vaccine. Msoffe *et al.*, (2006) reported the divergent responses towards Newcastle disease vaccine in Tanzanian village chickens, while *Morogoro-medium* and *Mbeya* ecotypes shows resistance higher resistance. Almost all of Tanzanian local chickens have brooding characteristics, good at protecting themselves against predators, vigorous and active foragers. Frizzle feathers, slow feathering and a necked neck are features of some of them.

**Table 1.8: Characteristics of Identified Tanzanian Indigenous Chicken Ecotype and the place of their Origins**

| Ecotype                | Region found                   | Body size and shape                      |
|------------------------|--------------------------------|------------------------------------------|
| <i>Kuchi</i>           | Mwanza, Shinyanga, Tabora      | Big, upright posture, long legs          |
| <i>Singamagazi</i>     | Shinyanga                      | Big                                      |
| <i>Mbeya</i>           | Mbeya                          | Big, many feathers                       |
| <i>Morogoro medium</i> | Morogoro                       | Medium                                   |
| <i>Ching'wekwe</i>     | Morogoro, Tanga, Lindi, Mtwara | Compact/Stout, short legs                |
| <i>Pemba</i>           | Pemba Island                   | Medium, slender/ narrow, upright posture |
| <i>Unguja</i>          | Unguja Island                  | Medium, slender/ narrow, upright posture |

Sources: (Msoffe *et al.*, 2001, 2004 and 2006; Lyimo *et al.*, 2013)

The geographical distributions of Tanzanian chickens indicate that *Kuchi* and *Singamagazi* are the predominant ecotypes in Tanzania Lake Zone regions; *Morogoro-medium* and *Ching'wekwe* ecotypes found in East and Central Zones. *Mbeya* ecotype is found in Southern Highlands of Tanzania, and it is much mixed with Malawian chickens, which have *Black Australop* blood. The Northern part of Malawi is bordered to Mbeya region, with historical persistence of social-cultural interactions between cross-boarder tribes (Kalinga, 1983; Walsh, 1998; Walsh and Swilla, 2000; Woodward *et al.*, 2010). The *Black Australop* an exotic dual-purpose bird, reported to be introduced in Malawi back 1950's for crossbred with local chickens to improving eggs and meat productions (Gondwe *et al.*, 2001; Safalaoh, 2001; Gondwe and Wollnly, 2003). *Unguja* and *Pemba* ecotypes are found in two Islands of Zanzibar.

### 1.5.3 Commercial chicken production in Tanzania

The commercial chicken sector is based on rearing genetically improved exotic breeds of chickens raised under the high-input deep litter system. Flock size usually ranges from hundred to few thousands birds (Minga *et al.*, 1989; Yongolo, 1996; Mcainsh *et al.*, 2004). Farmers in this sector are located in urban and peri-urban areas where availability of feeds, drugs, vaccines, labour and markets for the products are ensured (Minga *et al.*, 1989; 1990; Kitaly, 1998). Since the introduction of commercial poultry farming in Tanzania, a visible growth in commercial production has been observed (Oleke and Isinika, 2011). In large-scale farms on the mainland, the population growth rate of layers was 12 % per year, whilst that of broilers was only 4.1% per year for the period from 1995 to 2003 (Msami, 2008). The number of layers in large-scale farms in Tanzania increased from 87,124 in 1995 to 216,474 in 2003, whilst the number of broilers only increased from 158,125 to 217,741 over the same period. On average, 5.5 million hatching eggs and one million day old chicks are imported annually to produce day old chicks for commercial purposes (MLD, 2008). In the urban centres, chicken products are marketed through agents and shops to household consumers, institutions, hotels and restaurants.

### 1.5.4 Marketing system for the village chickens

Tanzanian farmers have different marketing channels for the village chickens to the consumers. Normally chickens are directly purchased by intermediaries at the household level, in weekly village markets and at fortnightly livestock markets. The livestock markets involve a number of villages, and buyers include traders from town and city markets (Kitalyi, 1998; Mlozi *et al.*, 2003). The quantities of chickens supplied and sold in the markets are elastic and vary significantly in terms of sex and the month of the year. Normally, more cocks than hens are supplied in the markets. However, cropping seasons was reported to influence the supply of local chicken meat in the market. The supply is higher after the harvest, which marked the beginning of the dry season, and lower at the end of the dry season (Kitalyi, 1998). Village chickens go through a long chain from the village market until they reach to the urban market. The costs of transport, middlemen and traders make the local chickens relatively expensive in the urban market when compared to commercial chickens raised in urban areas. Despite the higher prices of local chickens, they are in higher demand than any other alternative protein sources, such as beef, broiler, fish and beans (Mlozi *et al.*, 2003). Middlemen play a major role in the local chickens marketing chain. However, middlemen benefited more and earn over 60% of the total profits generated in the local chicken market chain (Mlozi *et al.*, 2003; Oxfam, 2009). Marketing information reported as one of the major constraints for smallholder farmers (Moges *et al.*, 2010; Oxfam, 2011). Local chickens competed better than other alternative protein sources, implying that the marketing of local chickens will still remain unsaturated (Mlozi *et al.*, 2003; Oxfam, 2009). Commercializing indigenous poultry keeping is therefore timely for meeting the unmet market demand (RIU, 2012).

## **1.6 Characterization and Conservation of Chicken Genetic Resources in developing countries**

The genetic diversity comprised found in farm animal species is an important resource in livestock systems. The diversity of farm animal genetic resources significantly contributes towards achieving food security for the present time and into the future. Historical evidence and current observations show that biodiversity maintenance must be integrated with agricultural practices as a strategy that can have multiple ecological and socioeconomic benefits, particularly to ensure food security (Thrupp, 2000). Farm animal genetic resources (FAnGR) involve all breeds and strains of animals, particularly those of economic, scientific and cultural interest to mankind in agriculture either at present or potentially in the future (Alderson, 2010). The genetic composition of the animal population is linked to their origins with regards to geographic region, history and traditional uses.

In developing countries, indigenous breeds play an important role in the livelihoods of rural people and smallholder farmers toward the efficient utilization of the marginal ecological areas. Indigenous breeds have the ability to adapt and survive in often challenging environments. These adaption to a variety of ecological areas and represents an important genetic resource for the livelihood of rural inhabitants (Anderson 2003). A number of factors have contributed to the severe erosion of indigenous genetic resources and even extinction of indigenous breeds. The use of exotic breeds, changes in breeders or consumers preferences due to short-term social economic influence, development of genetically uniform livestock breeds, effective population size, degradation of the ecosystem in which the breeds were developed and natural disasters were reported to be contributing factors in genetic diversity erosion in domestic animals (Thrupp, 1998; FAO, 1998; 2000; Whiteley *et al.*, 2010). Drought and civil conflict have also decimated localized livestock populations in Africa region (Rage, 1999).

### **1.6.1 Loss of genetic variation in chickens**

Chicken biodiversity is an essential component in sustainable agriculture and food security. At first sight, the diversity within domestic chickens is extensive and should provide an excellent base for breeding animals that are well adapted to a variety of local environmental conditions. Though the demand of chickens and eggs has increased tremendously due to the fast growing world population, it has been reported that nearly one third of all chicken breeds are at risk of extinction (Pym, 2010). This is an essential alarm for assessing the existing genetic resources. Many of these risk varieties may have traits that can be valuable in meeting future environmental challenges and marketing demands. Genetic variability in chickens is the best insurance for the adaptation and optimum production for the future unpredictable needs (Simianer and Meyer, 2003). Poultry production has experienced a major shift toward intensification in recent years, which is claimed to be the major reasons for losing chicken genetic diversity. The indigenous breeds used in family poultry farming in the rural areas of developing countries contribute greatly to the genetic diversity of the world's poultry populations.

There are therefore significant concerns that the replacement of indigenous breeds with commercial strains of poultry could pose a real threat to the poultry genetic resources (FAO, 2014). Moreover, industrialization and globalization of chicken production in the 20<sup>th</sup> century adversely affected the distribution of chicken genetic resources worldwide, practically limiting the breed composition to commercial stocks of broilers and egg laying hens (FAO/IAEA, 2014). Consequently, many chicken breeds have already become extinct or are seriously threatened with extinction.

In order to cope with the unpredictable future, genetic reserves that can respond and adapt to a broad spectrum of environments must be conserved. Conservation of the genetic resources acts as reservoir house for genetic diversity, which forms the basis for selection (FAO, 2010). Therefore, there is a need to characterize indigenous breeds in order to understand the existing diversity, to facilitate the development of rational utilization and conservation strategies for these genetic resources (Hanotte and Jianlin, 2006).

### **1.6.2 Characterization of chicken genetic diversity**

Characterization of animal genetic resources refers to the process of identifying distinct populations and describing their characteristics and production environments (FAO, 2010). Description of the population size, geographical distribution, physical descriptions, adaptation characteristics, uses, prevalent breeding systems, population trends, predominant production systems, description of the environment in which they are predominantly found, indications of performance levels (meat, growth, reproduction, egg), genetic distinctiveness of the animal and within- and between -breed genetic diversity need to be realized (Weigend and Romanov, 2001; Groeneveld *et al.*, 2010). Accurate assessment of the genetic biodiversity within and between populations of interest is essential. Both morphological and molecular characterization can be used in assessing the diversity of chicken populations.

#### **1.6.2.1 Morphological characterization**

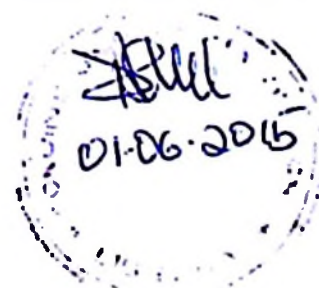
Morphological markers are external animal characteristics, which can be well described by visual observation and/or direct measurements. Morphological measurements are still an effective method for assessment of qualitative traits, for which it is easy to characterize phenotypic differences between populations or individuals through direct observation and measurement. The application of morphological markers is limited in evaluating quantitative traits. It is not easy to remove the effect of environmental factors (Yang *et al.*, 2013). In chickens, feather colour and shape, body shape, skin colour, shank colour, comb colour, earlobes colour, wattles colour, shank length, comb size, earlobes shape, shank length, keel length, wing length, tail length, shank thickness, number of toes and live body weight are the morphometric measurements which are used to characterise chicken populations (Dana *et al.*, 2010; Faruque *et al.*, 2010; Francesch *et al.*, 2010; FAO, 2012c; Aklilu *et al.*, 2013; Lyimo *et al.*, 2013).

### 1.6.2.2 Molecular characterisation

Molecular markers are playing vital roles in the evaluation of genetic diversity in the farm animals. These markers give an insight into breed history and provide information regarding both distinctiveness and diversity of a population (Boettcher *et al.*, 2010). Molecular characterizations are based on polymorphisms found in the nucleotide sequences across individual genome. These molecular markers can be used for investigating genetic variations at DNA level between different populations or individuals. Molecular markers have become essential tools for conservation biology, evolutionary, genomic selection and population studies as well as for mapping projects (Morin *et al.*, 2004; Kranis *et al.*, 2013; McMahon *et al.*, 2014). The availability of molecular markers in farm animals allows the detailed analyses and evaluation of genetic diversity and furthermore the detection of genes influencing economically important traits (Erhardt and Weimann, 2007). With the expansion of biotechnological techniques and computer innovations have led to more sophisticated analyses and even a whole genome SNP chips development.

#### (a) Microsatellite markers

A good class of genetic marker would have many scorable and highly variable loci with co-dominant alleles, and markers should cover a large part of the genome. Microsatellite markers meet these requirements, and they have become a marker of choice for biodiversity studies, mapping, forensic investigations, and population analyses as well as in ecological studies. What makes microsatellites useful is the fact that at the same location within the genomic DNA the number of times the sequence is repeated often varies between individuals, within populations, and/or between species. Microsatellites are highly polymorphic, abundantly and evenly distributed throughout the genome. These properties have made them substantial informative and potentially useful as a markers for mapping, paternity testing, forensic science, medical genetics, evolutionary biology and in the field of population genetics (Weigend and Ramanov, 2001; Mittal and Dubey, 2009). Until recently, microsatellites were the markers most widely used for genetic diversity studies, mapping quantitative trait loci for production, functional traits in farm animals and even in marker assisted selection practices (Yang *et al.*, 2013). FAO and the ISAG/FAO Advisory Group on Animal Genetic Diversity have proposed panel of 30 microsatellite markers for chicken biodiversity studies (FAO, 2011b). These set of markers are distributed across 15 chromosomes out of the 39 pairs of chicken chromosomes. Technical challenges for microsatellite analysis includes misclassifications as homozygosites when null alleles occurs, stutter bands may complicate scoring of polymorphisms and data generated in different laboratories can cause some variation in allele sizes making it sometimes difficult to merge data set.



### **(b) Mitochondrial DNA marker**

The haploid mitochondrial DNA is found in the mitochondria in the cell cytoplasm and is generally transferred to offspring only through the female line. In shape mitochondrial DNA has a circular molecule of 16,785bp size (Soller *et al.*, 2006). It is highly variable because of its elevated mutation rate, which can carry signals about population history over a short time frame (Galtier *et al.*, 2009). This, together with the absence of recombination makes mtDNA well suited marker for assessing historical genetic structure and the geographical distribution of populations. Mitochondrial haplotyping efforts typically focus on hyper-variable sites within the displacement region (D-loop), since high mutation rates within this region generate substantial haplotypic variation within the species (Buburuzan *et al.*, 2007). Mitochondrial DNA analysis has been used to investigate chicken domestication events (Liu *et al.*, 2006; Oka *et al.*, 2007). The mitochondrial DNA (mtDNA) polymorphism, especially the D-loop region has been applied to understand maternal lineage inheritance pattern and phylogenetic relationship in fowl species (Liu *et al.*, 2006; Lee *et al.*, 2007; Sawai *et al.*, 2010; Tixier-Boichard *et al.*, 2011; Storey *et al.* 2012). Liu *et al.* (2006) revealed nine divergent clades (A – I) that related to domesticated regions and geographical distributions of domestic chickens. According to Liu *et al.* (2006), clades A and B were the most frequent haplogroup in Yunnan province and other sorounded provinces in Southwest China. Clade C was mainly distributed in the southern part of China (esp. Guangxi and Guangdong provinces) and it was only constituted in domestic chickens. Clade D was the most frequent in Southeast Asia includings, Indonesia, India, Thailand and Vietnam. Clade E dominated in Indian sub-continent. Clades F and G are restricted found at Southwest China in Yunnan province. Clade H was only found in red junglefowls. Clade I was from Vietnam. Haplogroups from clades A, B and E were the most widely distributed. Although the molecular technique based on mitochondrial DNA has proven useful for identifying major evolutionary maternal lineages, the information derived represents only single mtDNA genome.

### **(c) SNP markers**

Single Nucleotide Polymorphisms (SNPs) are used as genetic markers in the applications of genetic diversity studies or genetic mapping. New technologies allow genotyping of hundreds of thousands of SNPs in a single reaction. SNPs are excellent markers for studying complex genetic traits and for understanding the genomic evolution. SNPs are less mutable and the low rates of recurrent mutation make them evolutionary stable (Jehan and Lakhanpaul, 2006). The use of SNP markers has become very popular and powerful investigative technique for many genomic studies and has become a widely used technique to study a range of genetic questions. Currently, SNP markers are most preferred for genotyping and adaptable to high-throughput analyses.

The whole-genomes of various livestock species have been sequenced, including chickens. The chicken has a genome size of around  $1.2 \times 10^9$  base pairs, and genome sequence estimated to contain around 20,000 - 23,000 genes (International Chicken Genome Sequencing Consortium, 2004; Furlong, 2005). Whole-genome sequencing in chickens led to the discovery of several millions of SNPs and technologies to analyze large data set of SNPs have been developed. A recent development of a high density 600K SNP genotyping array for chicken allows a wide spread usage in diversity studies, genomic selection, genome wide association studies, selection signature analyses, fine mapping of QTL and detection of copy number of variants (Kranis *et al.*, 2013). Heslot *et al.* (2013) insisting that ascertainment bias might arise when marker data were obtained from a random sample of the polymorphisms in the population of interest. Sample sizes and the populations in which SNPs are discovered affect the characteristics of observed variants. Ascertainment biases may affect the measurements of population divergence and distort population genetic inferences (Albrechtsen *et al.*, 2010; Lachance *et al.*, 2013).

#### **1.6.2.3 Choice of the marker type for biodiversity studies**

The advancement of biotechnology and increasing availability of molecular markers provides a universally applicable and objective approach for comparative genetic studies. However, there is a significant subjective element in the choice of the markers. The genetic marker and method of analysis proposed for diversity studies must consider; (i) the nature of the information required, (ii) cost effectiveness in development and use of different marker, (iii) availability of the method and data base for studying specific specie. Sharma and Gupta (2013) recommended the choice of genetic marker should depend mostly on mostly rate and mode of evolution of the genetic marker, and the mode of inheritance (maternal, biparental) and expression (dominant, co-dominant).

The nature of the information provided by different molecular genetic markers is very variable, and the features of the nature of the information, which are most desirable, vary according to the biological questions (Chenuil, 2006). When dealing with questions of contemporary gene flow, population isolation, and recent speciation events, a highly variable marker with a fast rate of evolution can increase resolution significantly (Peacock *et al.*, 2001). Genetic markers with slow rate of evolution are inappropriate markers to resolve relationship among more recently isolated populations (Okumuş and Çifçi, 2003).

### 1.6.3 Conservation of farm animal genetic resources

Maintaining livestock diversity has been proposed as the best strategy for insuarence on the sustainable use of animal genetic resources for agriculture and food production. The genetic variation that exists among farm animals is a basic requirement for efficient development and improvement of populations. One of the important elements concerning sustainable use of animal genetic resources is to ensure that locally adapted populations remain a functional part of the production system. The underlying objective for conservation of farm animal genetic resources is to maintain access to the adaptive genetic potential of a population. However, some of these adaptive fitness traits have not yet been discovered and might be of particular importance for the unpredicted future challenges and needs.

Normally the main idea of conservation of animal genetic resources focused on two separate but interlinking concepts. The first is the conservation of genes and the second is the conservation of populations (Hanotte, 2005). Both the conservation of genes and of populations is essential to meet future needs. One population can share large proportions of their genome with other populations, but each can possess a distinctive combination of genes. These may include distinctive traits particularly for their capacity to adapt to a specific environment. The wide distribution and geographical isolation of chicken populations in different eco-zones could lead to sub-structuring as each eco-type experienced different forces of evolution, particularly drift, mutation and natural selection. Chicken management is likely to differ between eco-zones, depending on farmer production goals. Sustainable chicken production in most of developing countries will depend on maintaining the local adapted populations. Although local chickens have been blamed for their poor performance in a number of production related traits when compared to the improved exotic birds, nevertheless these local chickens have been adapted to the prevailing harsh conditions through generations of natural selection (Pedersen, 2002). Differences in environmental factors between agro-ecological zones can result in different genotypes being favoured in contrasting regions. In such instances ecotypes would refer to populations adapted to local conditions within the agro-ecological zones. However, an accurate determination of genetic variation within and between chicken ecotypes is a fundamental step towards the conservation of genetic resources (Msoffe *et al.*, 2005).

#### 1.6.4 Tanzania's involvement in the conservation of farm animal genetic resources

The United Republic of Tanzania is one of the signatory to the Rio de Janeiro Convention on Biological Diversity adopted in 1992, according to which the signing countries are expected to conserve and improve livestock genetic resources. The provisions of the convention have now been translated into the relevant policies, legislations and other instruments to facilitate its implementation.

The global human population will continue to grow, in particular in developing countries (FAO, 2009; WSFS, 2009; Wise, 2013), and therefore an increased food production is needed in the near future. Tanzania is marked as one of the countries with a high population growth rate in Africa (2.7% p.a.). This will continue to create a burden on agriculture expansion and the entire ecosystem. In this instance, improvements in meat and egg production traits in the chicken will make a significant contribution towards supporting livelihood. Maintaining a rich diversity of chickens currently present in Tanzania, such as *Morogoro Medium* (Kawaida), *Chingwekwe*, *Unguja*, *Pemba* and *Kuchi*, their improvement in production should keep in consideration the need for retaining their genetic diversity.

In view of the importance of the livestock sector to the Tanzanian economy and to the people's livelihoods, the Ministry of Livestock Development and Fisheries in 2010 formulated a Livestock Sector Development Strategy (LSDS) for placing into effect the National Livestock Policy (NLP) of 2006. The Livestock Sector Development Strategy is an operation tool for the National Livestock Policy stipulates actionable interventions required to meet the livestock sector's Vision, Mission and Objectives for the short, medium and long term. The main objective regarding production is to increase quantity and improve quality of poultry and its products to satisfy domestic demand, increase exports and promote sustainable poultry production (MLD, 2006).

Regarding the poultry sector, the policy stated:

- (i) The Government will support and strengthen technical support services and use of appropriate technologies in poultry production.
- (ii) The Government will promote inventorisation, characterisation, evaluation and selection of the indigenous poultry breeds.
- (iii) In collaboration with other stakeholders the Government will promote improvement of genetic potential in the traditional flock in order to increase livestock productivity.
- (iv) Efforts will be undertaken to promote investment in poultry production, processing and marketing.
- (v) The Government will encourage the establishment of quality breeding farms and hatchery facilities.
- (vi) The Government will sensitise, encourage and promote establishment of poultry producers and traders associations.

## 1.7 Scope of the thesis

Genetic and phenotypic characterization of locally available farm animal populations provides essential information to make rational decisions for the improvement and the development of effective breeding programs (Thrupp, 2000; Wollny, 20013). Concerning Tanzanian village chickens, very limited information is currently available regarding the number per ecotypes and the amount of genetic variation. Based on the general concern that the genetic variation within chickens is quickly disappearing through breed substitution, indiscriminate crossbreeding and the absence of breed development programs, immediately steps must be taken to conserve the existing chicken diversity. Evaluation of Tanzanian chicken diversity is an important strategy in the conservation and utilization of chicken genetic resources, and will allows the accessibility of information to the policy makers and other stakeholders. Mainstreaming FAnGR in the policy will promote the participations of different stakeholder, decentralised community-based management, and foster the ability to the communities in decide and implement appropriate breeding strategies (Wollny, 2003).

The major scope of this thesis is to characterize the genetic diversity of five ecotypes of Tanzanian indigenous chickens, as compared with the worldwide spectrum of chicken diversity. Morphological measurements and molecular markers have been used to fulfill this general objective.

The specific objectives were to:

- (i) Investigate the maternal lineages and genetic diversity of Tanzanian indigenous chickens. Morphological measurements, microsatellite markers and mitochondrial DNA were used in these assessments. This is presented in Chapter Two.
- (ii) Investigating the genealogical patterns of chicken breeds sampled in Europe, and compared them to African and Asian chicken populations. This is presented in Chapter Three.
- (iii) Evaluate global diversity and genetic contributions of chicken populations from African, Asian and European regions. This was done to classify the Tanzanian chicken in a global view. Combined analyses of microsatellite data obtained in separate studies were done. This is because most population genetic studies using microsatellite markers were limited to small numbers of breeds, often representing a single country. In order to evaluate genetic diversity and relationships of Tanzanian chicken ecotypes in a wide sprectrum of chicken populations, it was necessary to use the common set of the microsatellites for analysis of the global genetic diversity. This is presented in Chapter Four.

From this research study, samples of wild chickens and commercial bred lines were used for comparison purposes. The inclusion of the commercial breeds in the study was also to crosscheck the possibility of genetic introgression of commercial lines in Tanzanian local chickens, which is claimed to be one of the major factors contributing in reducing chicken diversity.

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## CHAPTER TWO

### 2.0 Assessing the Genetic Diversity of Five Tanzanian Chicken Ecotypes Using Molecular Tools

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## 2.1 Abstract

The study aimed to evaluate the genetic diversity of Tanzanian chicken populations through phylogenetic relationship, and to trace the history of Tanzanian indigenous chickens. Five ecotypes of Tanzanian local chickens (*Ching'wekwe*, *Kuchi*, *Morogoro-medium*, *Pemba* and *Unguja*) from eight regions were studied. Diversity was assessed based on morphological measurements and 29 microsatellite markers recommended by ISAG/FAO advisory group on animal genetic diversity. A principal component analysis (PCA) of morphological measures distinguished individuals most by body sizes and body weight. *Morogoro Medium*, *Pemba* and *Unguja* were grouped together, while *Ching'wekwe* stood out because of their disproportionate short shanks and *ulna* bones. *Kuchi* formed an independent group owing to their comparably long body sizes. Microsatellite analysis revealed three clusters of Tanzanian chicken populations. These clusters encompassed i) *Morogoro-medium* and *Ching'wekwe* from Eastern and Central Zones ii) *Unguja* and *Pemba* from Zanzibar Islands and iii) *Kuchi* from Lake Zone regions, which formed an independent cluster. Sequence polymorphism of D-loop region was analysed to disclose the likely maternal origin of Tanzanian chickens. According to reference mtDNA haplotypes, the Tanzanian chickens that were sampled encompass two haplogroups of different genealogical origin. From haplotype network analysis, Tanzanian chickens probably originated on the Indian subcontinent and in Southeast Asia. The majority of *Kuchi* chickens clustered in a single haplogroup, which was previously found in *Shamo* game birds sampled from Shikoku Island of Japan in the Kōchi Prefecture. Analysis of phenotypic and molecular data, as well as the linguistic similarity of the breed names, suggests a recent introduction of the *Kuchi* breed to Tanzania.

**Keywords:** *Tanzanian indigenous chickens, genetic diversity, microsatellites, mitochondrial DNA*

## 2.2 Introduction

Tanzania is rich in indigenous farm-animal genetic resources of livestock species, including poultry. Traditional poultry farming is dominated (94.1%) by chickens (Swai *et al.*, 2007), which make a substantial contribution to the livelihoods of the most vulnerable rural households, which account for 80% of the Tanzanian human population (Swai *et al.*, 2007; Lwelamira *et al.*, 2008). The scavenging local chickens have been reared by the local community of Tanzania since time immemorial (Kabatange and Katule, 1989; Mutayoba *et al.*, 2012). Local chickens in Tanzania can be found in almost every place with human settlement, although most of the indigenous chickens are kept in the central corridor regions of Tanzania (FAO, 2007; RLDC, 2010).

Previous studies revealed genetic and phenotypic variability in Tanzanian indigenous chickens in terms of plumage colour and type, body shape and size, as well as productivity (Msoffe *et al.*, 2001; Minga *et al.*, 2004; Msoffe *et al.*, 2004; Msoffe *et al.*, 2006). In these reports, Tanzanian indigenous chickens were characterized based on their phenotypic traits and geographical origin in Tanzania (Msoffe *et al.*, 2005). Assessment of genetic differentiation between Tanzanian chicken breeds was based on a few microsatellite markers, with only one of the 20 microsatellite markers being in the recommended list of the markers proposed for chicken biodiversity studies by FAO (2011).

Several genetic studies have suggested multiple origins of African domesticated chickens. From mitochondrial DNA (mtDNA) analysis, Mwacharo *et al.* (2011) reported multiple introductions of chickens into East Africa, resulting in five distinct haplogroups of different maternal origin. Muchadeyi *et al.* (2008) found two distinct haplogroups from mtDNA sequence analysis in Zimbabwe village chickens, suggesting an origin of these chickens from southern Asian and the Indian subcontinent. Mtilem *et al.* (2011b) reported that conserved and field chickens in South Africa shared three major haplotypes, presumably originating from China, Southeast Asia, and the Indian subcontinent.

The aim of this study was to examine the existing diversity of five chicken ecotypes of Tanzania to obtain a more comprehensive picture of these genetic resources and their phylogenetic relationships, and to examine the historical background of Tanzanian local chickens by analysing the degree of shared mtDNA haplotypes with those of known origin to disclose probable maternal lineages of Tanzanian chickens.

### 2.3 Materials and Methods

A total of 196 individuals were used in this study, which represent five ecotypes of Tanzanian local chicken (*Ching'wekwe*, *Kuchi*, *Morogoro-medium*, *Pemba* and *Unguja*) from eight regions of Eastern Zone, Central Zone, Lake Zone and Zanzibar islands (Table 2.1). *Kuchi*, *Pemba* and *Unguja* ecotypes are characterized by upright posture, resembling game birds, while *Morogoro-medium* and *Ching'wekwe* ecotypes are of Bankiva type with very short shanks in the *Ching'wekwe* ecotype (Msoffe *et al.*, 2001, 2004). Forty-eight villages were randomly selected in 21 districts of these regions, which were chosen according to the predominant ecotype of indigenous chickens kept with less introgression from exotic populations. To avoid collecting closely related individuals, four chickens were sampled in each village and only one bird per household. The number of hens was higher than cocks, as farmers keep more breeding females than males.

### 2.3.1 Phenotypic traits measurements

The following morphological traits (figure 2.1) were collected to assess the phenotype of individual birds: 1) forearm length of the *ulna*, measured along the surface from the elbow (*olecranon*) to the wrist (*carpus*), 2) shank length (*tarso-metatarsus*) taken from the hock joint to the foot pad; 3) shank thickness measured from the top of an outstretched shank at the point right above the spur, 4) keel length, taken from the tip of the *chondral* across the keel/bone towards the sternum where the bones of the clavicle (*clavicula*) form a triangle; and 5) live body weight, assessed with a top-hanging weighing scale of 10 kg capacity with 10% margin of error and tolerance of 50 g (0.05 kg).

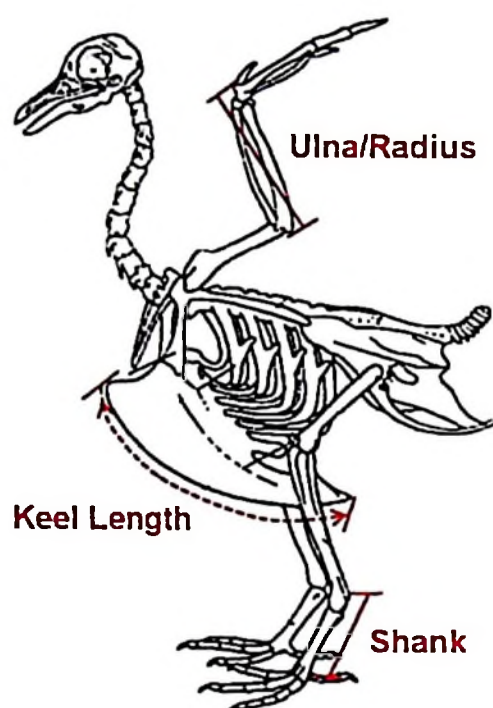


Figure 2.1: Anatomical orphological measurement of chicken skeleton

### 2.3.2 Genotyping

Blood samples were taken from the *ulna* vein of each bird and stored on Whatman filter paper (Whatman Biosciences, Brentford, UK). From the filter paper, approximately one cent coin was collected from the field. A half cent coin was extracted in the laboratory, which then provides an average of 25  $\mu\text{g}$  in a concentration of 250  $\text{ng}/\mu$ . Genomic DNA was isolated using the phenol-chloroform extraction method (Sambrook and Russell, 2001). Individuals were genotyped at 29 microsatellite loci, 28 of them taken from the 30 that have been suggested for biodiversity studies in

chickens (FAO, 2011). LEI 0192 and MCW0284 was not analysed, but microsatellite locus MCW0080 was added. PCR products were generated using primers labelled with fluorescent dyes (IRD700 and IRD800), and PCR products were visualized on 8% polyacrylamide gel using a LI-COR DNA analyser (LI-COR Inc. Nebraska, USA). Electropherogram and allele-size scoring were performed with RFLPscan plus software (Scanalytics, Division of CSP, Billerica, USA). Internal allele ladders and five DNA standard samples with known genotype were loaded on all gels and used to adjust the allele scoring between runs.

**Table 2.1:** List of Tanzanian indigenous chickens ecotypes used for genotyping

| Ecotype                | Number of birds |      | Region(s)                           | Districts                                                                                       |
|------------------------|-----------------|------|-------------------------------------|-------------------------------------------------------------------------------------------------|
|                        | Female          | Male |                                     |                                                                                                 |
| <i>Ching'wekwe</i>     | 20              | 6    | Morogoro and Tanga                  | Gairo, Kilindi and Mvomero                                                                      |
| <i>Kuchi</i>           | 20              | 10   | Mwanza, Shinyanga, Tabora and Geita | Misungwi, Magu, Shinyanga Rural, Kahama, Ushirombo/Bukombe, Geita, Sengerema, Nyegezi and Nzega |
| <i>Morogoro-medium</i> | 20              | 9    | Morogoro                            | Kilosa, Gairo, Morogoro Rural and Mvomero                                                       |
| <i>Pemba</i>           | 20              | 10   | Pemba Island                        | Chakechake, Wete and Mkoani                                                                     |
| <i>Unguja</i>          | 20              | 10   | Unguja Island                       | Magharibi, Kaskazini mashariki and Kaskazini                                                    |

### 2.3.3 Mitochondrial DNA amplification and sequence polymorphisms

The mtDNA was amplified and sequenced as described by Muchadeyi *et al.* (2008). DNA sequences were aligned using the AlignIR software (LI-COR Inc.). Extra nucleotide sequences that were outside the nucleotide sequences from 167 to 521 bp of the D-loop region were excluded from analysis.

### 2.3.4 Statistical analyses

#### 2.3.4.1 Morphometrics analyses

Least square means of phenotypic measurements of *ulna* length, shank length, shank thickness, keel length, and body weight for all ecotypes under study were compared with Tukey's HSD procedure using the JMP 9.0.2 statistical package. Pearson's correlation coefficients between all morphometric traits were estimated, and from the correlation matrix, principal component factor analysis (PCA) was done. The first two principal components (PC) were used to identify population clusters, and a variance maximization method (Varimax) was used for factor rotation (SAS/STAT, 2009).

#### 2.3.4.2 Genetic diversity

Allele frequency, mean number of alleles (MNA), polymorphic information content (PIC), expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity of the populations were estimated using Microsatellite-Toolkit (Park 2001). Wright's fixation indices were calculated using FSTAT 2.9.3.2 software (Goudet, 2002) to quantify within and between sub-population partitioning variances. Variance estimates were obtained by jack-knifing over loci and populations using the FSTAT software. The level of genetic differentiation was determined using Weir and Cockerham's (1984) estimation of Wright's (1951) fixation index. Analysis of molecular variance (amova) was done with the algorithms suggested by Excoffier *et al.* (1992), implemented in Arlequin software version 3.5.1.3.

#### 2.3.4.3 Genetic distance

Reynolds' genetic distance among Tanzanian chickens was estimated (Reynolds *et al.*, 1983), and 1000 bootstrapping replicates over loci were performed to test the robustness of the tree topology, using the PHYLIP software package (Felsenstein, 2005). The obtained tree was depicted using SplitsTree4 software version 4.12.3 (Hudson and Bryant, 2006).

#### 2.3.4.4 Cluster analysis

Population structure was determined by using a model-based clustering for assigning individuals from multilocus genotypes to a population with Structure 2.3.3 software (Pritchard *et al.*, 2000; Falush *et al.*, 2007; Hubisz *et al.*, 2009). The analysis involved an admixture model with correlated allele frequencies. Some 50 000 iterations in the burn-in phase were applied, followed by 100 000 iterations. The user-defined number of clusters ranged from  $2 \leq K \leq 5$ . Individuals were grouped into the predefined number of clusters with 100 independent Structure runs repeated for each K value. A pair-wise comparison of the 100 solutions using simCoeff (Rosenberg *et al.*, 2002) was carried out, and the solutions with over 95% similarities were considered identical. The most frequent solution was considered the most probable clustering and was visualized using Distruct 1.1 software (Rosenberg, 2004). In addition, the approach developed by Evanno *et al.* (2005) was applied from  $K = 1$  to  $K = 5$  to determine the optimal number of clusters.

#### 2.3.4.5 Analysis of mtDNA sequence polymorphisms

Median-joining networks were constructed to determine the evolutionary relationships of haplotypes following the algorithms of Bandelt *et al.* (1995), using Network 4.6.1.0 software (<http://www.fluxus-engineering.com/sharenet.htm>). Besides the sequences of the Tanzanian chicken populations, the network analysis included the most frequent haplotypes of nine clades from Liu's

network and of three clades from Oka's, which were used as a reference frame in haplotype analysis (Liu *et al.*, 2006; Oka *et al.*, 2007). The list of haplotypes and their GenBank accession numbers are given in Table 2.2. Haplotype diversity and Tajima's *D* value were analysed using DnaSP 5.10.01 software (Librado and Rozas, 2009)

**Table 2.2:** Liu and Oka's haplotypes names and their GenBank accession number

| Haplotype name     | Accession number | References               |
|--------------------|------------------|--------------------------|
| Liu A <sub>1</sub> | AB114069         | Liu <i>et al.</i> (2006) |
| Liu B <sub>1</sub> | AB007744         | Liu <i>et al.</i> (2006) |
| Liu C <sub>1</sub> | AB114070         | Liu <i>et al.</i> (2006) |
| Liu D <sub>1</sub> | AY588636         | Liu <i>et al.</i> (2006) |
| Liu E <sub>1</sub> | AB114076         | Liu <i>et al.</i> (2006) |
| Liu F <sub>1</sub> | AF512285         | Liu <i>et al.</i> (2006) |
| Liu G <sub>1</sub> | AF515588         | Liu <i>et al.</i> (2006) |
| Liu H <sub>1</sub> | D82904           | Liu <i>et al.</i> (2006) |
| Liu I <sub>1</sub> | AB009434         | Liu <i>et al.</i> (2006) |
| Oka D <sub>1</sub> | AB268535         | Oka <i>et al.</i> (2007) |
| Oka G <sub>1</sub> | AB268545         | Oka <i>et al.</i> (2007) |
| Oka F <sub>1</sub> | AB268543         | Oka <i>et al.</i> (2007) |
| Oka A <sub>1</sub> | AB268508         | Oka <i>et al.</i> (2007) |
| Oka A <sub>2</sub> | AB268509         | Oka <i>et al.</i> (2007) |

## 2.4 Results

### 2.4.1 Morphological traits

*Ulna* length, shank length, shank thickness, keel length and body weight were of larger size in male birds than in females in all ecotypes (Table 2.3). Highest mean values of all traits ( $P \leq 0.05$ ) were found in *Kuchi* ecotype, which is a game-type chicken, while *Ching'wekwe* ecotype had the lowest values. *Unguja*, *Morogoro* and *Pemba* ecotypes revealed no significant differences in all traits.

**Table 2.3:** Least square means ( $\pm$  SE) of phenotypic measurements in five ecotypes of Tanzanian local chickens

| Phenotypic traits            | Sex | Ecotypes                     |                              |                              |                              |                              |
|------------------------------|-----|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
|                              |     | <i>Ching'wekwe</i>           | <i>Morogoro</i>              | <i>Pemba</i>                 | <i>Unguja</i>                | <i>Kuchi</i>                 |
| <i>Ulna</i> bone length (cm) | M   | 7.53 <sup>a</sup> $\pm$ 0.31 | 9.92 <sup>b</sup> $\pm$ 0.24 | 9.47 <sup>b</sup> $\pm$ 0.23 | 9.78 <sup>b</sup> $\pm$ 0.24 | 11.5 <sup>c</sup> $\pm$ 0.21 |
|                              | F   | 7.01 <sup>a</sup> $\pm$ 0.12 | 8.28 <sup>b</sup> $\pm$ 0.11 | 8.15 <sup>b</sup> $\pm$ 0.11 | 8.26 <sup>b</sup> $\pm$ 0.11 | 10.1 <sup>b</sup> $\pm$ 0.11 |
| Shank length (cm)            | M   | 7.18 <sup>a</sup> $\pm$ 0.48 | 11.0 <sup>b</sup> $\pm$ 0.34 | 10.3 <sup>b</sup> $\pm$ 0.32 | 11.3 <sup>b</sup> $\pm$ 0.34 | 13.8 <sup>c</sup> $\pm$ 0.30 |
|                              | F   | 6.22 <sup>a</sup> $\pm$ 0.15 | 8.64 <sup>b</sup> $\pm$ 0.14 | 8.59 <sup>b</sup> $\pm$ 0.14 | 8.61 <sup>b</sup> $\pm$ 0.14 | 11.0 <sup>b</sup> $\pm$ 0.14 |
| Shank thickness (mm)         | M   | 10.1 <sup>a</sup> $\pm$ 0.66 | 11.9 <sup>b</sup> $\pm$ 0.51 | 11.4 <sup>b</sup> $\pm$ 0.49 | 12.5 <sup>b</sup> $\pm$ 0.51 | 15.6 <sup>c</sup> $\pm$ 0.45 |
|                              | F   | 9.92 <sup>a</sup> $\pm$ 0.16 | 9.34 <sup>b</sup> $\pm$ 0.14 | 9.65 <sup>b</sup> $\pm$ 0.14 | 9.59 <sup>b</sup> $\pm$ 0.14 | 12.3 <sup>b</sup> $\pm$ 0.14 |
| Keel length (cm)             | M   | 14.5 <sup>a</sup> $\pm$ 0.76 | 16.8 <sup>b</sup> $\pm$ 0.58 | 15.7 <sup>b</sup> $\pm$ 0.56 | 16.8 <sup>b</sup> $\pm$ 0.59 | 19.9 <sup>c</sup> $\pm$ 0.51 |
|                              | F   | 12.9 <sup>a</sup> $\pm$ 0.24 | 14.2 <sup>b</sup> $\pm$ 0.21 | 13.9 <sup>b</sup> $\pm$ 0.22 | 13.7 <sup>b</sup> $\pm$ 0.22 | 16.4 <sup>b</sup> $\pm$ 0.22 |
| Body weight (kg)             | M   | 1.65 <sup>a</sup> $\pm$ 0.17 | 2.42 <sup>b</sup> $\pm$ 0.14 | 1.59 <sup>a</sup> $\pm$ 0.14 | 2.36 <sup>b</sup> $\pm$ 0.14 | 3.29 <sup>c</sup> $\pm$ 0.13 |
|                              | F   | 1.34 <sup>a</sup> $\pm$ 0.06 | 1.52 <sup>b</sup> $\pm$ 0.05 | 1.25 <sup>a</sup> $\pm$ 0.05 | 1.53 <sup>b</sup> $\pm$ 0.05 | 2.57 <sup>b</sup> $\pm$ 0.06 |

NB: Means within a row with same letter are not significantly different at  $P \geq 0.05$ ; M: male; F: female.

Loading of shank length (0.857) and *ulna* length (0.851) were highest for the first PC, which explained 87.9% of the total variation present in all five phenotypic traits, while the second PC explained 5.13% of the total variance. Shank thickness (0.867), body weight (0.774), and keel length (0.697) contributed heavily to the second PC. The score plot of the first two PCs (Figure 2.2) showed *Ching'wekwe* chicken clustering separately from the other four ecotypes mainly owing to their disproportionately short legs. *Kuchi* chickens, on the other hand, were distributed more to the upper right because of greater shank thickness, longer keel length, and higher body weight, with a greater variation among individuals. The remaining ecotypes, *Morogoro*, *Unguja* and *Pemba*, cluster together in the centre of the plot, overlapping partly with *Kuchi*.

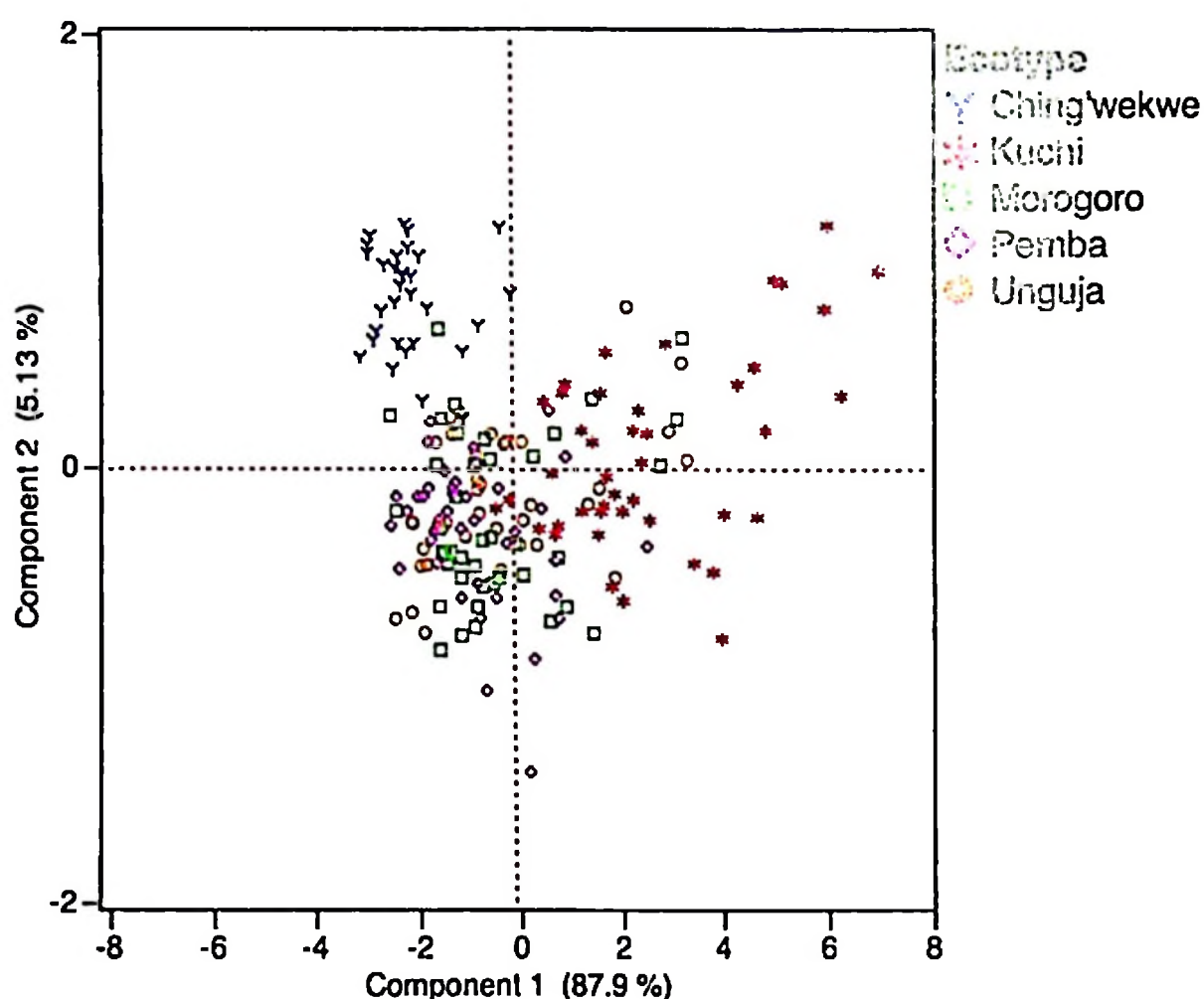


Figure 2.2: Principle component plot (PC<sub>1</sub> and PC<sub>2</sub>) of five Tanzanian chicken ecotypes based on five morphological traits

### 2.4.2 Genetic diversity

The overall means of expected and observed heterozygosity estimates were 0.62 and 0.62, respectively (Table 2.4). The expected heterozygosity was highest in *Unguja* ecotype (0.67) and lowest in *Kuchi* ecotype (0.58). None of the  $F_{IS}$ -estimates differed significantly from zero ( $P > 0.05$ ) indicating that the observed frequencies of heterozygotes were close to what is expected if populations were in Hardy-Weinberg equilibrium. The fixation index between Tanzanian chicken breeds ( $F_{ST}$ ) is 0.048, that is, the genetic diversity between the five ecotypes of Tanzanian chicken populations constituted 4.8% of the total genetic variance (Table 2.5).

Table 2.4: Genetic diversity within chicken population in Tanzania

| Population             | No. of birds | No. of loci | MNA $\pm$ SE    | HE $\pm$ SE      | HO $\pm$ SE      | $F_{IS}$ |
|------------------------|--------------|-------------|-----------------|------------------|------------------|----------|
| <i>Ching'wekwe</i>     | 26           | 29          | 5.41 $\pm$ 2.29 | 0.62 $\pm$ 0.027 | 0.65 $\pm$ 0.017 | -0.061   |
| <i>Kuchi</i>           | 30           | 29          | 5.10 $\pm$ 2.08 | 0.58 $\pm$ 0.034 | 0.56 $\pm$ 0.017 | 0.028    |
| <i>Morogoro-medium</i> | 29           | 29          | 5.69 $\pm$ 2.63 | 0.60 $\pm$ 0.026 | 0.58 $\pm$ 0.017 | 0.038    |
| <i>Pemba</i>           | 30           | 29          | 6.00 $\pm$ 2.80 | 0.65 $\pm$ 0.028 | 0.67 $\pm$ 0.016 | -0.029   |
| <i>Unguja</i>          | 30           | 29          | 6.28 $\pm$ 2.24 | 0.67 $\pm$ 0.027 | 0.63 $\pm$ 0.016 | 0.065    |
| Overall mean           | 29           | 29          | 5.70 $\pm$ 2.61 | 0.62 $\pm$ 0.028 | 0.62 $\pm$ 0.017 | 0.01     |

MNA: mean number of alleles,  $H_I$ : expected heterozygosity,  $H_o$ : observed heterozygosity, SE: standard error of the mean,  $F_{IS}$ : average inbreeding coefficient within subpopulation

Different  $F_{IS}$  estimates were not significantly different from zero at  $P \geq 0.05$ .

Table 2.5: Analysis of molecular variance (AMOVA) within and between five ecotypes of Tanzanian chicken population

| Source of variation | Sum of squares | Variance component | Percentage of variation |
|---------------------|----------------|--------------------|-------------------------|
| Between populations | 142.075        | 0.45848            | 4.81333                 |
| Within population   | 2575.917       | 9.06672            | 95.18667                |
| Total               | 2717.992       | 9.52520            |                         |

### 2.4.3 Population clusters and genetic distances

Genetic clustering based on STRUCTURE analysis of the five Tanzanian indigenous chicken ecotypes is shown in Figure 2.3. The most likely clustering appeared at  $K = 3$  as indicated by applying Evanno method (Evanno *et al.*, 2005). The maximum number of 100 identical runs were observed at  $K = 3$  and at  $K = 4$ , respectively. Clustering populations into more than three clusters did not change overall structure: *Ching'wekwe* clustered with *Morogoro-medium*, and *Unguja* clustered together with *Pemba* while *Kuchi* ecotype formed an independent cluster immediately at  $K = 2$ . *Unguja* and *Pemba* ecotypes which are the Island game birds split from *Ching'wekwe* and *Morogoro-medium* ecotypes at  $K = 3$ .

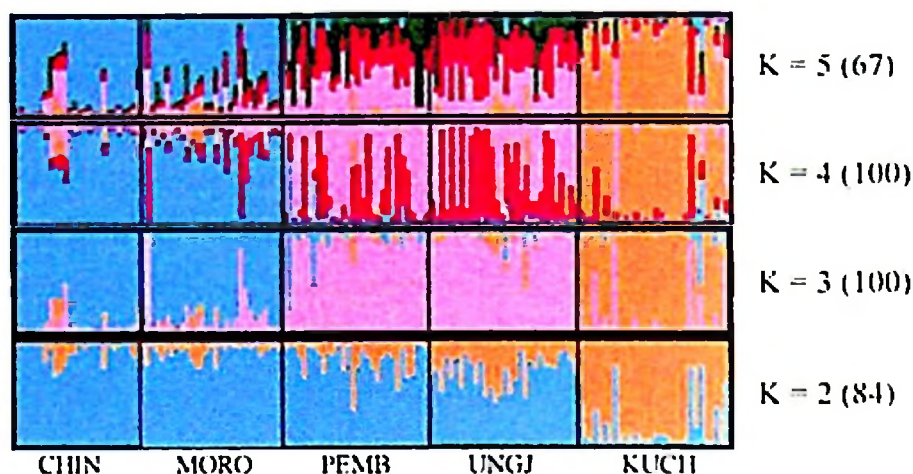


Figure 2.3: Clustering of five ecotypes of Tanzanian indigenous chickens. *Ching'wekwe* (CHIN), *Morogoro-medium* (MORO), *Pemba* (PEMB), *Unguja* (UNGJ), and *Kuchi* (KUCH) ecotypes. The numbers in brackets indicates the number of identical solutions at 95% threshold

Genetic distance estimates between Tanzanian indigenous chicken populations was used to form a neighbour net illustrating the relationship between breeds (Figure 2.4). The largest genetic distance was observed between *Kuchi* and *Ching'wekwe* ecotypes. *Unguja* and *Pemba* ecotypes exhibited the closest phylogenetic relationship, followed by *Morogoro-medium* and *Ching'wekwe* ecotypes.

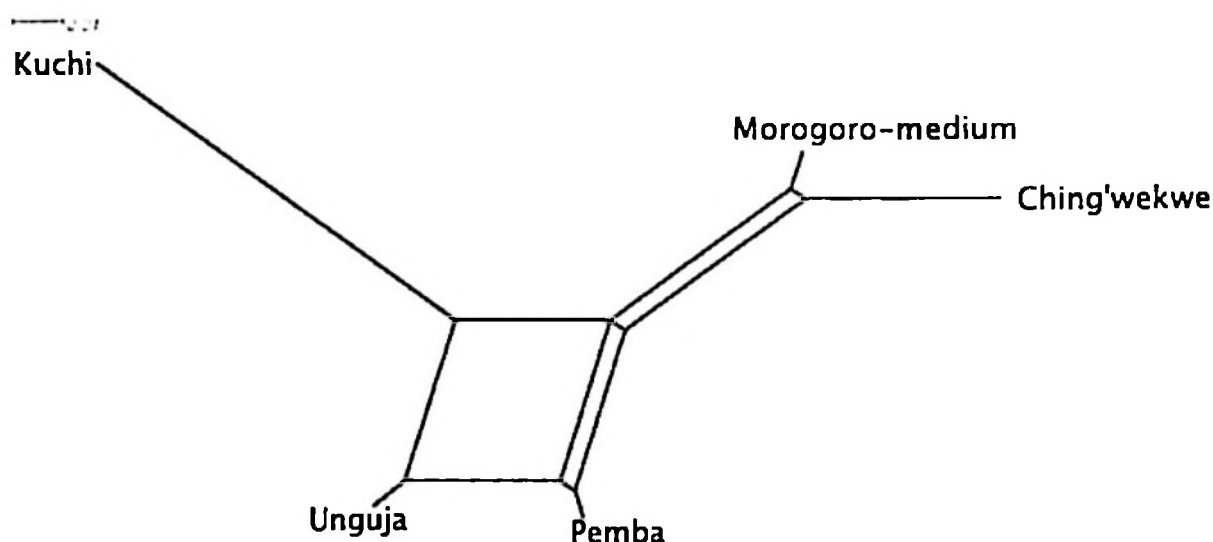
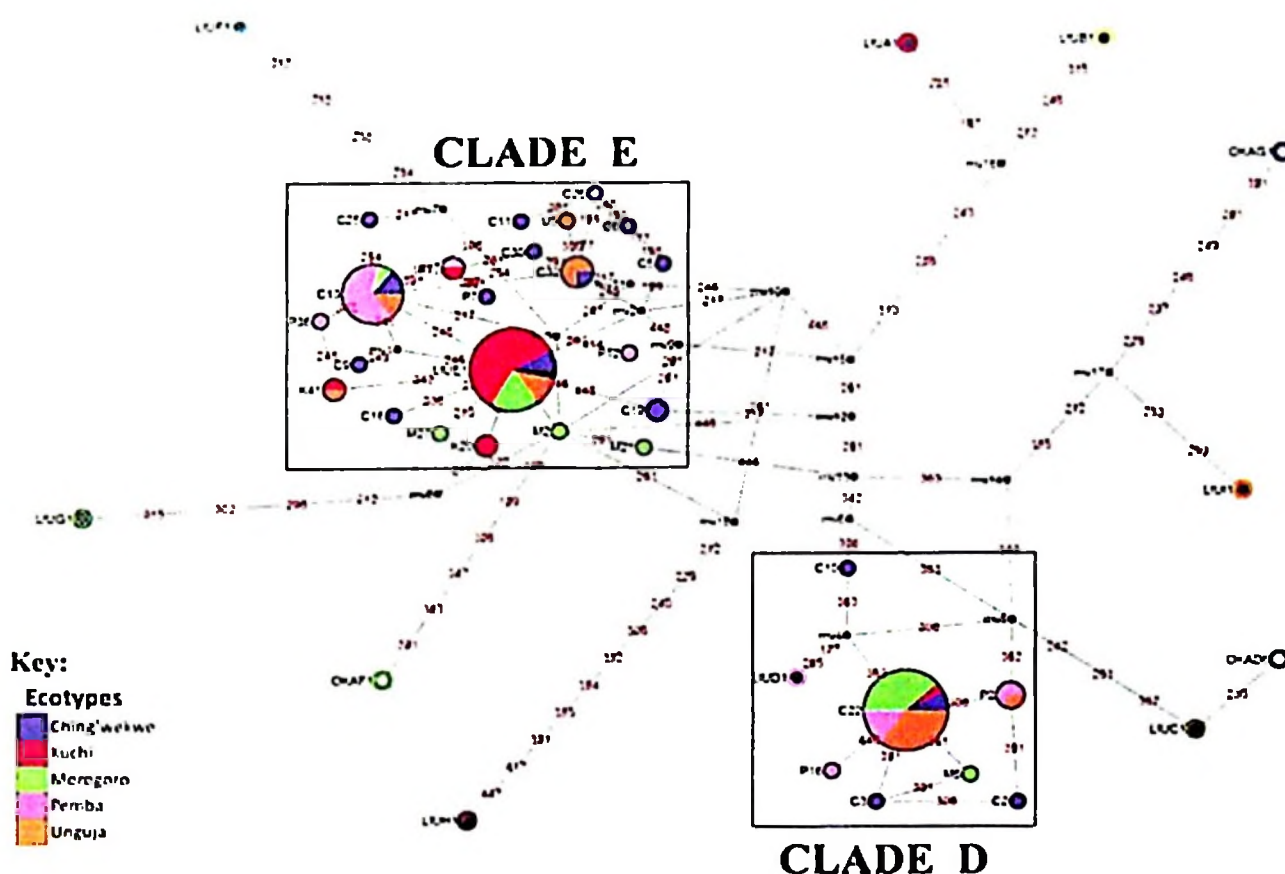


Figure 2.4: Neighbour Net of five ecotypes of Tanzanian indigenous chickens

#### 2.4.4 Network and polymorphic relationships

The median-joining (MJ) network analysis of the mtDNA D-loop haplotypes observed in Tanzanian local chickens, together with most frequently observed haplotypes from Liu *et al.* (2006) and Oka *et al.* (2007) as a skeletal frame reference, is shown in Figure 2.5. Twenty-three haplotypes were observed in Tanzanian chickens and were found to cluster with haplogroups D and E identified by Liu *et al.* (2006). The majority of *Kuchi* (95.2%) and *Ching'wekwe* (75.0%) clustered in clade E, while *Morogoro*, *Unguja*, and *Pemba* were distributed within clades E and D. Oka's haplotypes A3 and A4 clustered in clade E. Most of the *Kuchi* chickens (76.20%) clustered in haplotype Liu E1 in clade E. Analysis of sequence polymorphism revealed an overall haplotype diversity of 0.831, nucleotide diversity of 0.012, and Tajima's *D* value of 0.67475 ( $P > 0.10$ ) in Tanzanian chicken populations (Table 6). Among the Tanzanian chicken populations, *Kuchi* showed lowest haplotype diversity (0.424) and nucleotide diversity (0.003), respectively, while *Ching'wekwe* had highest estimates (respective values 0.916 and 0.012). Estimate of Tajima's *D* values were neutral in *Ching'wekwe*, *Morogoro*, and *Pemba* chicken populations.



**Figure 2.5:** Median-joining network profile of 23 haplotypes observed in Tanzanian indigenous chicken merged with the sequences of major haplotypes presented by Liu *et al.* (2006) and Oka *et al.* (2007). Note that the circle size corresponds to haplotype frequency

*Unguja* tested a significant positive value, while *Kuchi* recorded a significant negative value ( $P < 0.05$ ). *Kuchi* (95.2%) and *Chung'wekwe* (75.0%) clustered in clade E, while *Morogoro*, *Unguja*, and *Pemba* were distributed within clades E and D. Oka's haplotypes A3 and A4 clustered in clade E. Most of the *Kuchi* chickens (76.2%) clustered in haplotype L in E1 in clade E. Analysis of sequence polymorphism revealed an overall haplotype diversity of 0.831, nucleotide diversity of 0.012, and Tajima's  $D$  value of 0.67475 ( $P > 0.10$ ) in Tanzanian chicken populations (Table 2.6). Among the Tanzanian chicken populations, *Kuchi* showed the lowest haplotype diversity (0.424) and nucleotide diversity (0.003), respectively, while *Chung'wekwe* had highest estimates (respective values 0.916 and 0.012). Estimate of Tajima's  $D$  values were neutral in *Chung'wekwe*, *Morogoro*, and *Pemba* chicken populations. *Unguja* tested a significant positive value, while *Kuchi* recorded a significant negative value ( $P < 0.05$ ).

**Table 2.6:** Number of haplotypes, haplotype diversity, number of nucleotide diversity and Tajima's  $D$  test in mitochondrial DNA sequences of Tanzanian chickens

| Population             | Sample size | Number of Haplotypes (h) | Haplotype Diversity (Hd) $\pm$ S.E. | Nucleotide Diversity ( $\pi$ ) | Tajima's $D$ |
|------------------------|-------------|--------------------------|-------------------------------------|--------------------------------|--------------|
| <i>Chung'wekwe</i>     | 20          | 11                       | 0.916 $\pm$ 0.038                   | 0.01152                        | 1.13118      |
| <i>Morogoro-machum</i> | 20          | 7                        | 0.711 $\pm$ 0.089                   | 0.01131                        | 1.04689      |
| <i>Pemba</i>           | 20          | 8                        | 0.795 $\pm$ 0.071                   | 0.01225                        | 1.34791      |
| <i>Unguja</i>          | 20          | 7                        | 0.763 $\pm$ 0.079                   | 0.01286                        | 2.15115*     |
| <i>Kuchi</i>           | 21          | 5                        | 0.424 $\pm$ 0.131                   | 0.00317                        | -2.05611*    |
| Total                  | 101         | 23                       | 0.831 $\pm$ 0.023                   | 0.01147                        | 0.67475      |

\* $P < 0.05$  significant, indicating the rejection of the hypothesis neutral expansion.

## 2.5 Discussion

Molecular genetic information and morphological variation were used to achieve deeper insight into genetic diversity within and the relationship between five ecotypes of Tanzanian chickens. From molecular genetic marker analyses, the expected and observed heterozygosity estimates were higher in Tanzanian indigenous chickens compared with commercial breeds reported earlier (Granevitze *et al.*, 2007; Muchadeyi *et al.*, 2007; Bodzar *et al.*, 2009; Fosta *et al.*, 2011). Furthermore, the differentiation between Tanzanian chicken ecotypes ( $F_{ST}$  0.048) was found to be smaller than between commercial chicken lines. This is in agreement with several molecular studies, which revealed higher heterozygosity and lower  $F_{ST}$  values between African local chickens than between commercial lines (Muchadeyi *et al.*, 2007; Eltanany *et al.*, 2011; Fosta *et al.*, 2011; Goraga, *et al.*, 2011; Mtileni *et al.*, 2011a). In contrast to commercial lines, which have been managed as distant breeding populations for many generations, following a strict selection scheme, a higher genetic

diversity in Tanzania chickens can be expected, as they are managed in a free-range system with random breeding and no selection for performance traits.

Two maternal lineages in Tanzanian local chicken populations were revealed in the analysis of mtDNA sequences, which corresponded to haplogroups D and E described by Liu *et al.* (2006), who identified Southeast Asia and the Indian subcontinent as places of origin, respectively. Liu's clades D and E appear to be the common haplotypes in Eastern Africa. Muchadeyi *et al.* (2008) found the existence of two distinct maternal lineages of Liu's haplogroups D and E, which were evenly distributed among the five Zimbabwean chicken ecotypes. Mwacharo *et al.* (2011) reported the presence of haplotype E in chicken populations in Sudan and Ethiopia, and the presence of haplotype D in Kenya, Uganda, Sudan, and Ethiopia without frequent exchange of genetic materials.

Analyses of microsatellite and phenotypic data revealed population stratification among Tanzanian chicken populations. The results of the cluster analysis using the Structure software suggest that *Kuchi* might have originated from a different ancestral population than *Ching'wekwe*, *Morogoro*, *Unguja* and *Pemba* ecotypes. *Morogoro* and *Ching'wekwe*, which clustered together, were distributed in a closer geographical distance without a permanent boundary on the Tanzanian mainland. Although *Unguja* and *Pemba* are islands, 80 km apart, *Unguja* and *Pemba* chicken populations showed a higher degree of admixture among each other than with any of the other three Tanzanian chicken populations under study. This could be the result of a higher exchange of genetic materials between these islands, which form a sovereign state. Social and agriculture interrelationships between *Unguja* and *Pemba* were recorded in a Greco-Roman text from the first century AD, when these islands were used as a base for voyages between the Middle East, India, and other parts of Africa (Chami, 2005; Walsh, 2006). In the PC plot based on phenotypic traits, *Unguja* and *Pemba* chickens, which were characterized as island game birds, clustered with *Morogoro-medium* ecotype. Furthermore, mtDNA analysis results indicated that *Unguja*, *Pemba* and *Morogoro* chickens shared a rather equal distribution of haplotypes D and E. These results obtained from mtDNA and microsatellite analysis suggest that effects of genetic drift were stronger within these populations than gene flow between island and mainland populations (Johnson *et al.*, 2003). This is further supported by positive Tajima's *D*-value in *Unguja*, which might indicate a decrease in population size (Johnson *et al.*, 2007).

*Unguja* and *Pemba* game birds were both distributed in Liu's clade D and clade E. Oka *et al.* (2007) and Gongora *et al.* (2008) found Indian fighting birds in haplogroups that have been associated with

Liu's clade D and fighting birds from Western Asia and Japan in Liu's clade E (Liu *et al.*, 2006). Cockfighting was among the traditional sports in the Tanzanian islands of Unguja and Pemba, introduced by Austronesians in 945 - 946 AD, as reported by Walsh (2006; 2010). During the great maritime trade in the Indian Ocean between the tenth and eleventh centuries, Zanzibar was the main centre for trading with the mainland Swahili coast (Arsenat *et al.*, 2006; Vernet, 2009). The traders carried large amounts of ivory, slaves and animals to Zanzibar (Royer, 2000; UNESCO, 2012). This may possibly be another way in which chickens were introduced to the Zanzibar islands from the East African mainland.

## 2.6 Conclusions

Based on microsatellite information, Tanzanian chickens are clustered into three distinct groups which are related mainly to geographical distribution. *Unguja* and *Pemba* island game birds are clustered together, as well as *Ching'wekwe* and *Morogoro* ecotypes from the East and Central Zones of Tanzania mainland, while *Kuchi* from the Lake Zone forms an independent group. Based on body measurements *Ching'wekwe* ecotype formed an isolated group owing to their short legs and *ulna* bone length, while *Kuchi* ecotype with significant higher in body size formed another group, which overlapped partly with *Morogoro*, *Unguja* and *Pemba* ecotypes. Two maternal lineages were distributed among the five populations, although *Kuchi* ecotype was found to dominate in one haplotype. In all these analyses, *Kuchi* ecotype tended to remain in a distinct group. *Ching'wekwe*, *Morogoro-medium*, *Unguja* and *Pemba* might have been distributed to Tanzania with the two early main waves of introduction of chickens to Africa in which chickens were introduced along the African East Coast from the Indian Ocean or through Egypt from the Mediterranean before being spread inland through overland routes (MacDonald, 1992; Van Marle-Köster *et al.*, 2008; Gifford-Gonzales and Hanotte, 2011). In contrast, *Kuchi* seems to have been introduced recently and is highly associated with *Shamo* gamebirds from Japan.

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## CHAPTER THREE

### 3.0 Maternal Genealogical Patterns of Chicken Breeds Sampled in Europe

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### 3.1 Abstract

The aim of this study was to investigate the maternal genealogical pattern of chicken breeds sampled in Europe. Sequence polymorphisms of 1256 chickens of the hyper-variable region (D-loop) of mitochondrial DNA (mtDNA) were used. Median-joining networks were constructed to establish evolutionary relationships among mtDNA haplotypes of chickens which included a wide range of breeds with different origin and history. Chicken breeds which have their roots in Europe for more than 3000 years, were categorised by their founding regions encompassing *Mediterranean type*, *East European type*, and *Northwest European type*. Breeds which were introduced to Europe from Asia since the mid 19<sup>th</sup> century were classified as *Asian type*, while breeds based on crossbreeding between Asian breeds and European breeds were classified as *Intermediate type*. The last group of *Game birds* included fighting birds from Asia. The classification of mtDNA haplotypes was based on the Liu *et al.* (2006) nomenclature. Haplogroup E was the predominant clade among the European chicken breeds. The results showed, on average, the highest number of haplotypes, highest haplotype diversity and highest nucleotide diversity for *Asian type* breeds, followed by *Intermediate type* chickens. *East European* and *Northwest European* breeds had lower haplotype and nucleotide diversity compared to *Mediterranean*, *Intermediate*, *Game type* and *Asian type* breeds. Results of our study support earlier findings that chicken breeds sampled in Europe had their roots in the Indian subcontinent and East Asia. This is consistent with historical and archaeological evidence of chicken migration routes to Europe.

**Key words:** *chickens in Europe, mitochondrial DNA, nucleotide diversity, haplotype diversity*

### 3.2 Introduction

Historical records and archaeological discoveries disclosed the presence of domestic chickens in Europe since the earliest Iron Age around 3000 B.C. (West & Zhou, 1988). The diffusion of chickens from regions of domestication in Asia to Europe were mainly through two trading routes, a northern route via China and Russia, and a southern route from the Indus Valley via Persia and the Mediterranean Sea (West & Zhou, 1988; Crawford, 1995; Tixier-Boichard *et al.*, 2011; Storey *et al.* 2012). A frequent migration of chickens from Asia into the Mediterranean region continued until 1200 B.C., and then to Northwest Europe until 500 B.C. (Potts, 2012). Subsequently domestic chickens spread over the whole of Europe under the influence of the Romans (Benecke, 1993; Bejenaru *et al.*, 2008; Storey *et al.*, 2012). The *Mediterranean type* of chicken is considered to be the most ancestral type of domestic chickens in Europe (Tixier-Boichard *et al.*, 2011).

Based on archaeological studies, West & Zhou (1988) suggested domestication of chickens appeared first in Southeast Asia around 6000 B.C. and later it occurred in India either independently or by diffusion from Southeast Asia. In later studies using mitochondrial DNA (mtDNA) sequence information, Liu *et al.* (2006) and Oka *et al.* (2007) suggested multiple domestication events of chickens in South China, Southeast Asia and the Indian subcontinent. Liu *et al.* (2006) classified nine diverse clades (A – I) that were related to regions of domestication and geographical diffusions of domestic chickens. To illustrate the relationship between major haplogroups reported by Liu *et al.* (2006), a Median-joining network (Figure 3.1) was constructed based on the most frequent haplotypes of these nine clades taken from the NCBI database (Table 2.2), implemented in Network 4.6.1.0 software, following the algorithm of Bandelt *et al.* (1995).

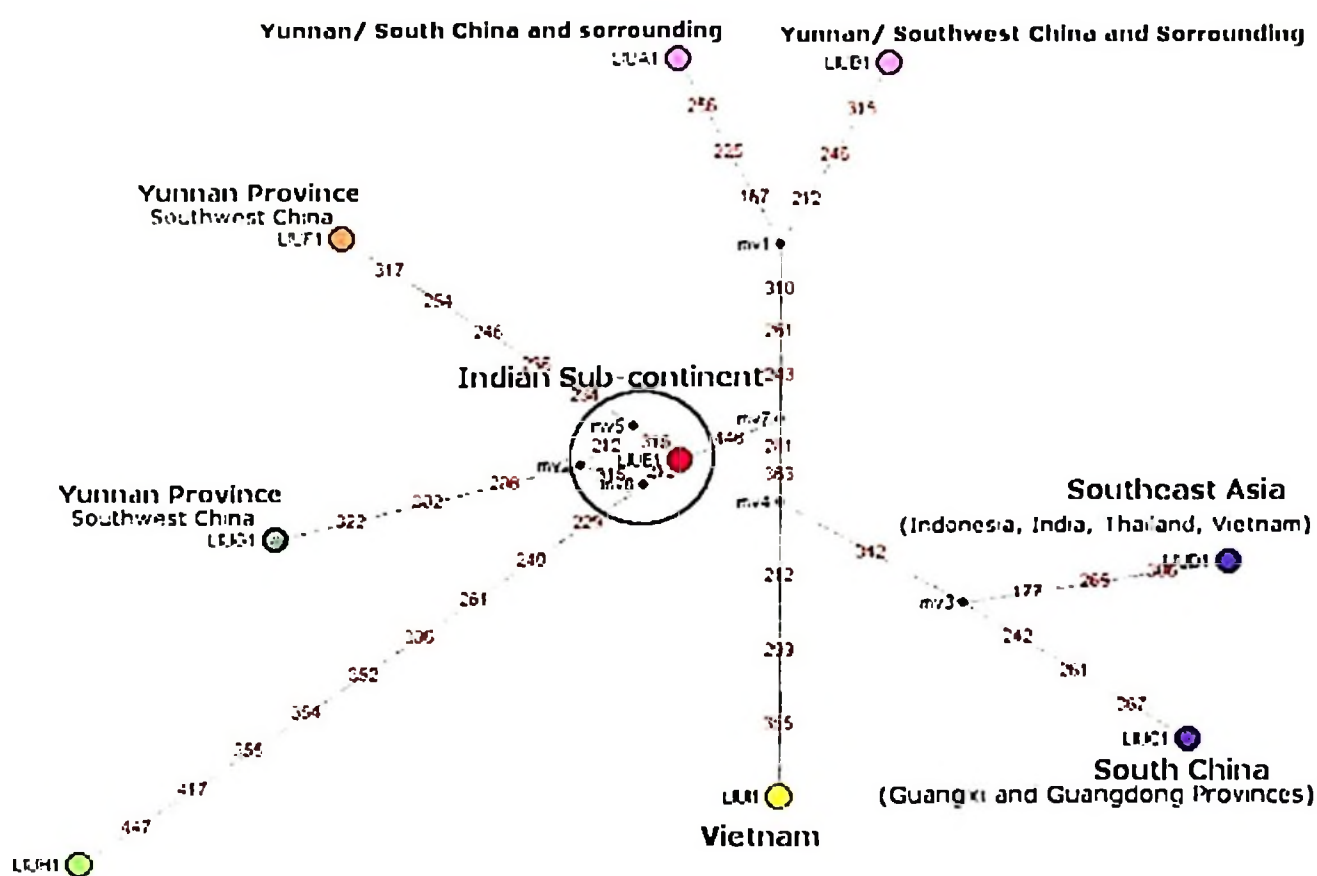


Figure 3.1. Nine most frequent haplotypes of Liu *et al.* (2006) suggesting origins of chickens

Several subsequent mtDNA studies have used the clades described by Liu *et al.* (2006) to characterize the maternal lineages of chickens from different populations. For European chickens, however, studies using mtDNA sequences were previously done with small sample sizes and without categorizing breeds according to their supposed origin (Liu *et al.*, 2006; Muchadeyi *et al.*, 2008; Dana *et al.*, 2010; Revay *et al.*, 2010; Ceccobelli *et al.*, 2013). For example, 58 chicken mtDNA

sequences sampled from European breeds were used by Liu *et al.*, (2006) to show that clade E dominated (91.38%) in Europe, followed by clade A (6.90%) while Clade B (1.72%) was least frequent. In our study, we aimed at analyzing mtDNA D-loop sequence polymorphisms to assess the maternal origin of a wide range of chicken populations sampled in Europe.

### 3.3 Material and Methods

Fifty-five chicken breeds sampled in ten European countries (Table S1), were categorized into six groups according to their historical background. Chicken breeds which have been in Europe for more than 3000 years were categorised into (i) *Mediterranean type*, (ii) *East European type* and (iii) *Northwest European type*, which are considered as the most original to Europe; (iv) *Intermediate type* encompasses populations which resulted from introgression of Asian breeds into European breeds during their foundation 100 to 150 years ago; (v) *Game birds* included introduced Asian fighting birds and breeds which retained game bird characteristics originating from crosses between European breeds and Asian fighting birds; and (vi) *Asian type* included breeds with recent Asian origin (Granevitze *et al.*, 2009; Lyimo *et al.*, 2014). Of these chicken breeds, 1256 individual sequences were generated of a 455 bp fragment of the (mtDNA) control region (D-loop). The haplotypes of these sequences have been submitted to NCBI database (accession numbers KP141230 - KP141745). In addition, mtDNA sequences of the most frequent haplotypes of nine clades reported by Liu *et al.* (2006) were used as a reference in haplotype analyses (Table 2.2). PCR was used to amplify a fragment of mtDNA positioned in the D-loop region of the mitochondrial genome by using primers located at the 16,739- to 16,775-bp (forward primer: mtGlu-F 5'-GGCTTGAAAAGCCATTGTTG-3') and 649- to 668-bp (reverse primer: mtGlu-R 5'-CCCCAAAAAGAGAAGGAACC-3') of the complete mtDNA sequence of domestic chickens (X52392; Desjardins & Morais, 1990). All the mtDNA sequences used in this study were truncated at positions 49 bp to 503 bp. The sequences were aligned using the ClustalW (1.6) program (Thompson *et al.*, 1994) and haplotypes were identified based on sequence identity. Median-joining networks were constructed to determine the evolutionary relationships of haplotypes using the algorithm developed by Bandelt *et al.* (1995), and implemented in NETWORK 4.6.1.2 software (<http://www.fluxus-engineering.com/sharenet.htm>). Haplotype diversity (*H*) and nucleotide diversity ( $\pi$ ) were analyzed using DnaSP 5.10.01 software (Librado & Rozas, 2009).

### 3.5 Results and discussion

The distribution of clades obtained in this study is shown in Figure 3.2. Five clades A to E were found among the chicken breeds sampled in Europe. Haplogroup E was the most dominant clade in all chicken breed types studied; *Mediterranean type* (96.9%), *East European type* (96.1%), *Northwest European type* (89.3%), *Intermediate type* (87.4%), *Game birds* (84.2%) and *Asian type* (67.4%)

breeds, followed by haplogroup A; *Asian type* (27.0%), *Intermediate type* (10.0%), *Northwest European type* (7.3%), *Game birds* (3.5%), *Mediterranean type* (3.1%), *East European type* (2.0%) breeds (Table 3.1). Clades B, C and D were found to be less widely distributed among the chicken breeds sampled in Europe. Clade B was distributed in *Northwest European type* (3.4%), *East European type* (2.0%), *Asian type* (1.8%) and *Intermediate type* (1.4%) chickens. Clade C was only found in *Asian type* (2.5%) and *Intermediate type* (0.3%) breeds. Furthermore, we found high frequency of haplogroup D in *Game birds* (12.3%) and to lesser degree in *Asian type* (1.4%) and *Intermediate type* (0.9%).

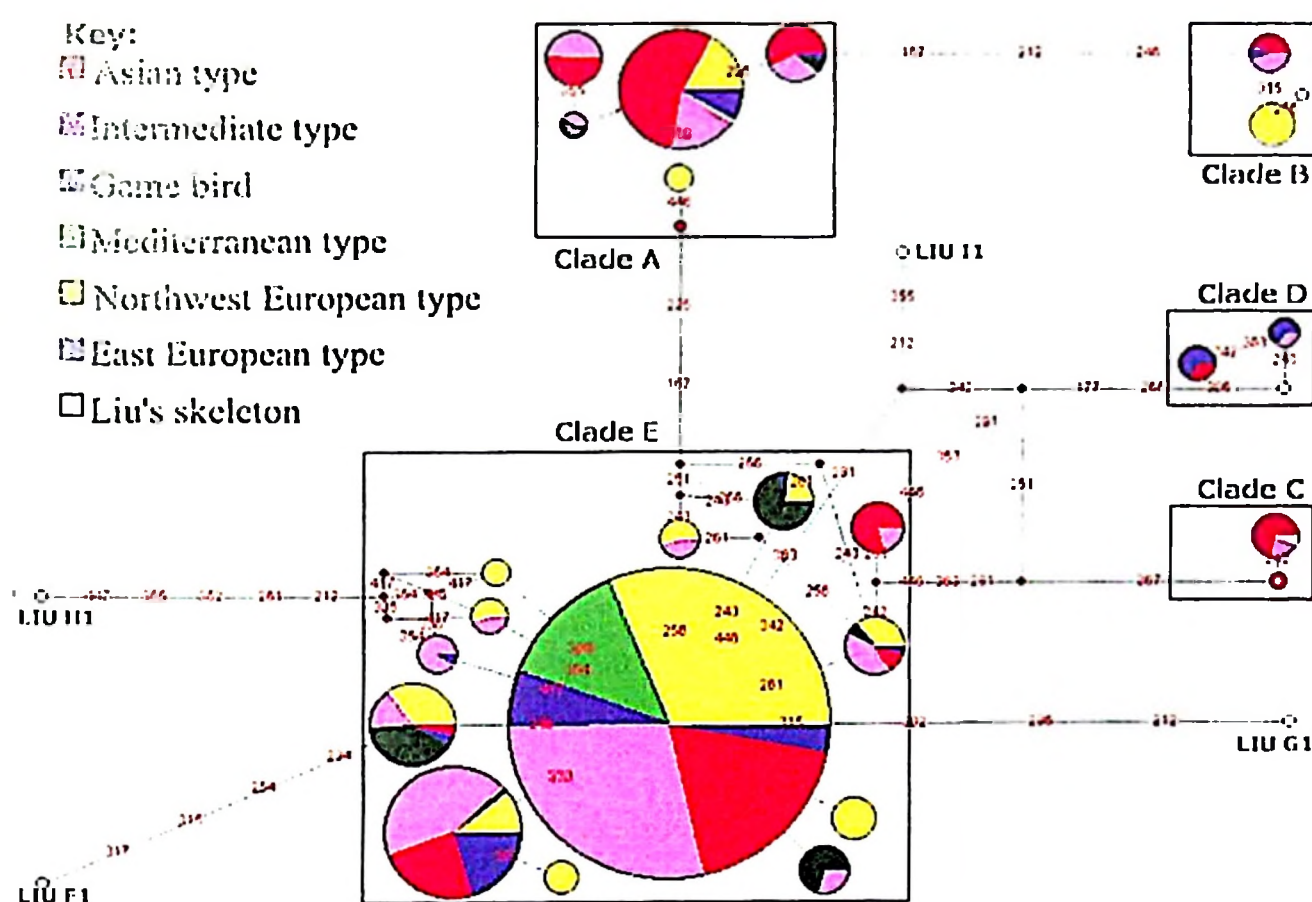


Figure 3.2: Median-Joining Network tree for 1256 chickens and 9 haplotypes. Clade definitions are from Liu *et al.* (2006). Different colours in the circles represent various categories of chicken breeds. Red diamonds are the media vectors produced by Network software representing a putative intermediate haplotype that was not observed in the samples. Size of circles corresponds to the number of haplotypes, and the number between the haplotype nodes refers to the position of nucleotide mutations.

The results of this study confirmed the findings from previous mtDNA studies in haplogroup distribution and diffusion of domestic chickens in Europe. Gongora *et al.* (2008) reported haplogroup E as the predominant clade among Indian, Middle Eastern, and European chickens. It was suggested

that most of the European chickens originated from the Indian subcontinent where Haplogroup E is the most frequent one. Haplogroup A which was found in our study at highest frequency in Asian type breeds, and haplogroup B are predominant haplogroups in the Southern and Eastern Chinese breeds, Yunnan and Japan (Liu *et al.*, 2006; Gongora *et al.*, 2008). The occurrence of Clade A, B and E among the European chickens is in line with historical records of dispersal routes of chickens from East and South Asian regions through the main two trade routes as indicated above (West and Zhou, 1988; Crawford, 1995; Tixier-Boichard *et al.*, 2011). Interestingly, in our study we did not find haplogroups C and D in the *Mediterranean type*, *Northwest European type* and *East European type* of chicken breeds. Liu *et al.* (2006) argued that the existence of clade D in red junglefowl in their study might either be due to gene flow from domestic into wild chickens or the result of a recent domestication event. In this context our findings might indicate that chickens that arrived to Europe early in history either did not carry mtDNA sequences of clade D or at a very low frequency only. In Liu's study, clade C was mainly distributed in Guangxi and Guangdong Provinces of China as well as in Japan but was absent in South Asia and red junglefowl. Furthermore, the presence of haplogroups C and D in *Intermediate type* chickens is consistent with intentional crossing of chicken breeds imported from Asia during breed development in the 19<sup>th</sup> century with breeds that have been settled in Europe for many centuries (Dana *et al.*, 2010; Flink *et al.*, 2014). Although haplogroup C is commonly found in East Asian chicken breeds, it exists in the East Asian region at low frequency compared to other haplogroups (Liu *et al.*, 2006; Oka *et al.*, 2007; Cue *et al.*, 2011; Dancause *et al.*, 2011; Miao *et al.*, 2013). Liu *et al.*, (2006) reported that the geographic distribution of clade D was closely related to the distribution of *game birds*, which is in line with this study where the *Game birds* showed a higher frequency of haplogroup D than *Asian type* and *intermediate type* chickens

Table 3.1: Average haplotype number (#ht), haplotype diversity (H), nucleotide diversity ( $\pi$ ) and the clade haplotype proportions of various breed categories of mtDNA sequences

| Breed category          | Pop. Size | Within breed diversity |                                 |                                   | Haplotype proportions in clades |       |       |       |       |
|-------------------------|-----------|------------------------|---------------------------------|-----------------------------------|---------------------------------|-------|-------|-------|-------|
|                         |           | (#ht)                  | (H)                             | ( $\pi$ )                         | A                               | B     | C     | D     | E     |
| <i>Asian type</i>       | 285       | 9.30                   | 0.761 $\pm$ 0.076 <sup>a</sup>  | 0.0104 $\pm$ 0.0040 <sup>a</sup>  | 0.270                           | 0.018 | 0.025 | 0.014 | 0.674 |
| <i>Intermediate</i>     | 349       | 8.23                   | 0.670 $\pm$ 0.067 <sup>ab</sup> | 0.0062 $\pm$ 0.0032 <sup>b</sup>  | 0.100                           | 0.014 | 0.003 | 0.009 | 0.874 |
| <i>Game bird</i>        | 57        | 7.00                   | 0.578 $\pm$ 0.107 <sup>b</sup>  | 0.0051 $\pm$ 0.0033 <sup>bc</sup> | 0.035                           | -     | -     | 0.123 | 0.842 |
| <i>Mediterranean</i>    | 160       | 4.17                   | 0.561 $\pm$ 0.091 <sup>b</sup>  | 0.0030 $\pm$ 0.0017 <sup>c</sup>  | 0.031                           | -     | -     | -     | 0.969 |
| <i>Northwest Europe</i> | 354       | 3.88                   | 0.472 $\pm$ 0.058 <sup>c</sup>  | 0.0041 $\pm$ 0.0019 <sup>c</sup>  | 0.073                           | 0.034 | -     | -     | 0.893 |
| <i>East Europe</i>      | 51        | 3.75                   | 0.442 $\pm$ 0.120 <sup>c</sup>  | 0.0030 $\pm$ 0.0009 <sup>c</sup>  | 0.020                           | 0.020 | -     | -     | 0.961 |
| 1256                    |           |                        |                                 |                                   |                                 |       |       |       |       |

Different superscript letters or numbers in a column indicate significant differences (Tukey's HSD,  $P < 0.05$ )

Haplotype diversity and nucleotide diversity lies between the ranges of  $0.442 \leq H \leq 0.761$ , and  $0.0030 \leq \pi \leq 0.0104$ , respectively suggesting that chicken breeds sampled in Europe displayed clear differences between breed categories in terms of diversity within populations. Breeds of *Asian type* and *Intermediate type* belonged to multiple haplotype clades and exhibit high numbers of haplotypes, haplotype diversity and nucleotide diversity (Table 3.1). *East European* and *Northwest European* breeds had the lowest haplotype diversity and nucleotide diversity compared to the *Mediterranean*, *Intermediate*, *Game* and *Asian type* breeds. The smallest haplotype and nucleotide diversity observed in *Northwest European* and *East European* chicken breeds may be related to the distant location from the primary chicken domestication region and the long existing management practices in which chicken breeds were managed under isolation with small effective population sizes (Granevitze *et al.*, 2007; Groeneveld *et al* 2010).

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## CHAPTER FOUR

### 4.0 Global Diversity and Genetic Contributions of Chicken Populations from African, Asian and European Regions

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#### 4.1 Abstract

Genetic diversity and population structure of 113 chicken populations from Africa, Asia, and Europe were studied using 29 microsatellite markers. Among these, three populations of wild chickens and nine commercial purebreds were used as reference populations for comparison. Compared to commercial lines and chickens sampled from the European region, high mean numbers of alleles and high degree of heterozygosity were found in Asian and African chickens as well as in red junglefowls. Population differentiation ( $F_{ST}$ ) was higher among European breeds and commercial lines than among African, Asian and red junglefowl populations. Neighbour-Net genetic clustering and STRUCTURE analysis revealed two main groups of Asian and Northwest European breeds, while African populations overlap with other breeds from Eastern Europe and the Mediterranean region. Broilers and Brown egg layers were situated between the Asian and Northwest European clusters. STRUCTURE analysis confirmed a lower degree of population stratification in African and Asian chickens than in European breeds. High genetic differentiation and low genetic contributions to global diversity have been observed for single European breeds. Populations with low genetic variability have also shown a low genetic contribution to a core set of diversity in attaining maximum genetic variation present from the total populations. This may indicate that conservation measures in Europe should pay special attention to preserving as many single chicken breeds as possible in order to maintain maximum genetic diversity since higher genetic variations comes from differentiation between breeds.

**Keywords:** *Genetic diversity, Chickens, Microsatellites, Population structure, Genetic contributions*

#### 4.2 Introduction

Domestic chickens are thought to result from multiple domestication events over the last 8,000 years (West and Zhou, 1988; Sawai *et al.*, 2010; Tixier-Boichard *et al.*, 2011), predominantly of red junglefowl (*Gallus gallus*) in Southeast Asia, and to a lesser extent involving *Gallus sonneratii* in Southwest India (Eriksson *et al.*, 2008) and *Gallus lafayetii* in Sri-Lanka (reviewed by Groeneveld *et al.*, 2010; Tixier-Boichard *et al.*, 2011). Later, the chicken spread to Europe and Africa through human migration and along trade routes (Liu *et al.*, 2006; Kanginakudru *et al.*, 2008; Groeneveld *et al.*, 2010; Storey *et al.*, 2012; Mwacharo *et al.* 2013a). The Iron Age (3000 B.C.) was the main period for dispersion of chickens through Europe. They mainly came from China via Russia on a northern route and from Indus Valley via Persia on a southern route (West and Zhou, 1988; Tixier-Boichard *et al.*, 2011; Flink *et al.*, 2014). The chickens were introduced to Africa from Asia by way of the Indian Ocean, and from Europe and Arabian Peninsula via the Mediterranean and Red Sea (MacDonald and Blench, 1993; Masonen, 1995; Boivin *et al.*, 2009). Archaeological, linguistic and ethnographic

evidence strongly suggests that the chicken moved to Africa in several waves from the Mediterranean region, Red Sea and the East coast of Africa with subsequent dispersion through overland routes across the Sahara, the Horn of Africa, central and West Africa (MacDonald and Edward, 1993; Williamson, 2000; MacDonald and Blench, 2000). Gifford-Gonzales and Hanotte (2011) reported two main waves of chicken introduction to Africa. The first wave was from Mediterranean Sea via Egypt during the Ptolemaic period (300 B.C.), later spreading through Nile valley and to the West Africa along the Sudano-Sahelian corridor (MacDonald and Edward, 1993; Fuller *et al.*, 2011). The second wave came across the Indian Ocean when chickens were introduced to the East Coast of Africa by means of the existing trade networks during the beginning to middle of the 1<sup>st</sup> millennium A.D. (Adelaar, 1996; Blench, 2006; Boivin and Fuller, 2009; Fuller *et al.*, 2011).

Genetic diversity within a species is defined by a large number of breeds and populations which exhibit a wide range of characteristics and variants. Genetic differentiation is expected to increase with both increasing geographic distance and demographic isolation (Wright, 1943; Parker *et al.*, 2004). The wide genetic variation between different breeds of domestic chickens which has been accumulated during domestication has several causes: founder effects at the time of domestication; subsequent isolation of breeds in different regions and under different environmental constraints that forced genetic drift and natural selection; selection imposed by man for breed standards; and most recently selection for improving production traits. Genetic diversity is now distributed among traditional local chickens, standardized breeds selected according to a given breed standard, and highly selected commercial lines (Tixier-Boichard *et al.*, 2011). It has been claimed that the loss of number of local populations, and hence a reduction in genetic variations due to the replacement of local chicken breeds in small farms and villages by modern industrial stocks are driven by the increasing demand for animal products (Besbes *et al.*, 2007; Halima *et al.*, 2007; Boettcher *et al.*, 2010; Hoffmann, 2011; Özdemir *et al.*, 2013). To counteract the loss of genetic diversity within the species, it is necessary to take conservation measures for animal genetic resources in order to retain the genetic potential of populations for flexibility in adapting to unpredictable future challenges. These conservation measures should be based on a comprehensive insight and understanding of the importance of genetic diversity within the species (Weigend *et al.*, 1995; Weigend and Romanov, 2002; Rege and Gibson, 2003; Simianer, 2005; Oldenbroek, 2007; Lenstra *et al.*, 2012). Adequate diversity analyses regarding the distinctiveness and demographic characterization of subpopulations are important when deciding conservation priorities (Groeneveld *et al.*, 2010).

In this study, regional patterns of intra- and inter-population genetic diversity of chicken populations from Africa, Asia and Europe were assessed using microsatellite variability. In this regard, the study

takes advantage of the availability of data of previous studies. The set of populations studied included 101 local populations from three continents as well as three red junglefowl populations and nine commercial pure breeds for comparison. A joint analysis of this comprehensive data set which represents a wide coverage in terms of geographical regions, climatic conditions and population management allows a global assessment of diversity within the species based on diversity at 29 autosomal microsatellite loci. In African and Asian countries chickens are mainly kept in free-range management systems (Abdelqader *et al.*, 2007; Gondwe and Wollny, 2007; Muchadeyi *et al.*, 2007; Rajkumar *et al.*, 2008), while non-commercial chicken breeds in Europe are of smaller population size and bred for standardized traits (Granevitze *et al.*, 2007; Siwek *et al.*, 2013). By including populations with roots in two subspecies of red junglefowl *Gallus gallus gallus* and *Gallus gallus spadiceus*, the study provides further insight into the degree of differentiation of domesticated chicken populations from their wild progenitors since domestication. The study aimed at evaluating genetic relationships within and between the 113 chicken populations included as well as assessing population stratification across regions of Africa, Asia and Europe. Then these populations were compared to wild populations on the one hand, and commercial lines intensively selected for egg or meat production and managed as isolated breeding populations for many selection generations on the other hand. Differences in the contribution of chicken breeds of different geographical regions to global diversity were investigated by using multilocus microsatellite genotypes.

### 4.3 Materials and Methods

#### 4.3.1 Chicken populations

Data used in this study were collected from earlier studies (Muchadeyi *et al.*, 2007; Granevitze *et al.*, 2009; Bodzsar *et al.*, 2009; Cuc *et al.*, 2010; Mtileni *et al.*, 2011a; Berima *et al.*, 2013; Lyimo *et al.*, 2013). Chicken populations included in this study encompassed various categories of management ranging from unmanaged backyard chickens to highly specialized commercial purebred chicken lines, sampled in various regions of Africa, Asia and Europe. All samples were genotyped at the same 29 microsatellite loci using the same laboratory protocol and standard samples to adjust for allele scoring between analyses. Twenty eight of the 29 loci were taken from the list recommended by the ISAG/FAO advisory group for chicken biodiversity (FAO, 2011).

Details of the sampling regions of breeds studied are shown in Appendix 3. In brief, chicken populations were categorized following different criteria. First, they were grouped in accordance to their breed history. The main geographical regions breeds originating from were sorted by continent. Within the continent, chicken populations were grouped into geographical sub-regions according to their breed history. UN Geoscheme map based on M49 coding classification were used to classify

sub-regions, which divides the world into macro-geographic regions and sub-regions (UNDATA, 2012). For Africa, chicken populations were grouped into three sub-regions of North Africa (NAF), East Africa (EAF) and South Africa (SAF). In Asia, chicken populations were divided into two sub-regions of East Asia (EAS) and Southeast Asia (SEA). The chicken populations sampled in Europe were classified into five groups: Northwestern European type (NWE), Eastern European type (EEU), Mediterranean (MED) type as well as a few breeds sampled in Germany that are of recent Asian origin (EAB). In addition, a few populations have been classified as population related to Brown layer (RBL) as New Hampshire and Rhode Island Red chickens, which formed a fifth, separate classification. Second, grouping was done according to sampling countries to represent the existing variations within geographic regions. Third, grouping was based on information on population management, i.e. field populations without population management, breeds selected for breed standard, conservation flocks and populations selected for quantitative performance traits.

A total of 3314 individuals from 113 chicken populations were included in this study. To limit over-representation of a breed, a maximum of 30 individuals per population was allowed. In cases where data of more than 30 individuals per breed were available, the number was reduced randomly using the research randomizer tool (<http://www.randomizer.org/form.htm>). The samples represented 21 countries in Africa, Asia and Europe including 22 ecotypes from Africa, 26 breeds from Asia and 53 breeds from Europe. In addition, three wild chicken populations and nine commercial Layer and Broiler purebred chicken lines were added to the study. Red junglefowls and commercial purebreds were used as reference populations in the analyses.

#### 4.3.2 Statistical Analyses

The presence of null alleles for each locus was estimated by the Expectation-Maximization (EM) algorithm of Dempster *et al.*, (1977) using FreeNA software (Chapuis and Estoup, 2007). The observed allele frequencies and allele frequencies estimated based on EM algorithm for each locus across populations were highly correlated ( $> 0.98$ ), and the estimated frequencies of null alleles per locus were below 5% (Appendix 4). According to Leroy *et al.*, (2012) and Pham *et al.*, (2013) frequencies of null alleles of below 20% are negligible. The percentage of the missing values of the microsatellite data was 0.63%. Allele frequencies, mean number of alleles (MNA), expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosity of the populations were estimated using Microsatellite-Toolkit (Park, 2001). Wright's fixation indices ( $F_{IS}$  and  $F_{ST}$ ) were estimated using FSTAT 2.9.3.2 software (Goudet, 2002).

### 4.3.3 Cluster analysis

The population structure was investigated using a model-based clustering approach as implemented in STRUCTURE 2.3.3 software (Pritchard *et al.*, 2000; Falush *et al.*, 2007; Hubisz *et al.*, 2009). The analysis involved an admixture model with correlated allele frequencies. The length of the burn-in period was set to 50,000 iterations followed by 100,000 iterations for Markov chain Monte Carlo sampling. User defined number of clusters ranged from  $2 \leq K \leq 40$ . Individuals were grouped into a predefined number of clusters with 100 independent runs for each K value. G' similarity coefficient based on *Large K Greedy* algorithm as implemented in CLUMPP software (Jakobsson and Rosenberg, 2007) was used to compare STRUCTURE runs within each K value. Solutions with a similarity higher than 95% were considered to be identical (Granevitze *et al.*, 2009). The most frequent solution was considered to be the most probable clustering, and a merger of these runs within each of the K-values obtained from CLUMPP software was visualized using DISTRUCT 1.1 software (Rosenberg *et al.*, 2004). In addition,  $\Delta K$  statistics suggested by Evanno *et al.* (2005) was applied to detect the number of clusters best reflecting the population structure (Figure 4.1). For the most likely clustering solution ( $K = 3$ ), distribution of membership coefficients obtained from STRUCTURE were subdivided into sub-regions according to breeds' historic geographical origin and displayed as pie charts (Figure 4.4).

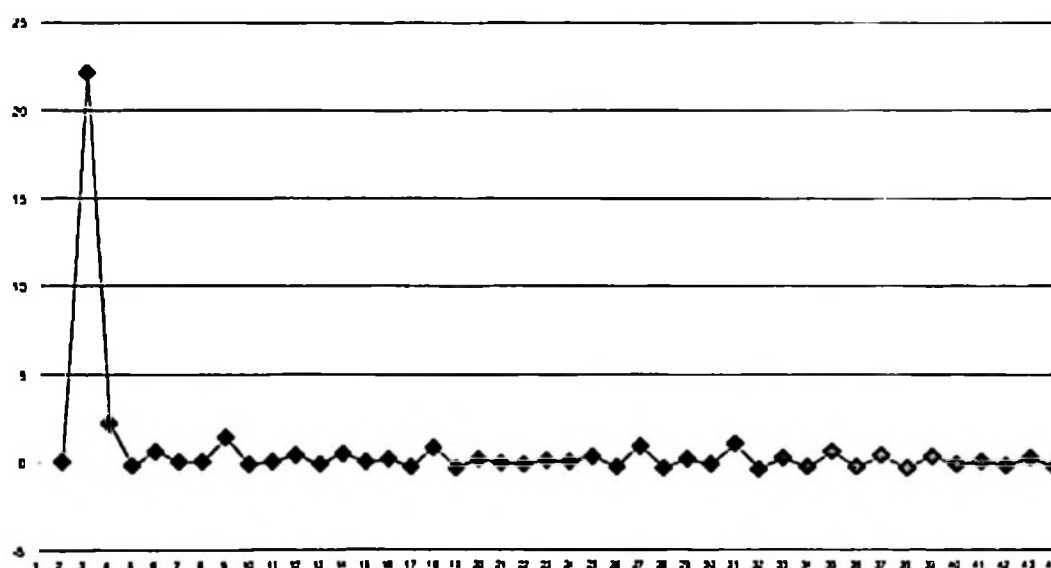


Figure 4.1: Estimation of most appropriate number of populations according to Evanno *et al.* (2005)

Similarity indices between and within populations were calculated from allele frequencies using Malecot's definition of similarity (Eding and Meuwissen, 2001). These indices were subsequently used to calculate marker-estimated kinship (MEK) among populations using weighted equal drift similarity (weds) to correct for alleles identical by state (Oliehoek *et al.*, 2006), which were executed

in MEKSAFE 1.0 software package (Eding *et al.* 2002). Mean kinship estimates within and between populations were obtained by averaging the corresponding values for all the within- and between-population pairs of individuals. The MEK estimates were converted to distances between populations (Eding *et al.*, 2002; Mateus *et al.*, 2004). The kinship based distance matrixes among populations were visualized in a Neighbour-Net using SPLITSTREE4 software (Huson and Bryant, 2006).

The genetic contributions of different chicken populations from each region to the total diversity were estimated according to Eding *et al.* (2002). Eding *et al.* (2002) proposed a core set analysis method, which is based on kinship estimates. This method accounts for both within and between populations diversity instantaneously. The genetic variation contribution of each breed was estimated with minimum overlap of the core set, and ranking of populations is achieved according to their contributions to the global diversity. In addition, total genetic diversity of the nine commercial breeds were set as fixed and the additional contributions of the local populations to the commercial gene pool were computed by adding breeds one by one to the fixed set (Eding *et al.*, 2002).

## 4.4 Results

### 4.4.1 Genetic diversity across regions

The mean number of alleles per locus and population was higher in African ( $5.20 \pm 0.17$ ) and Asian chickens ( $5.12 \pm 0.16$ ), than in European breeds ( $3.20 \pm 0.11$ ) and commercial lines ( $3.28 \pm 0.26$ ). The mean number of alleles ( $4.76 \pm 0.038$ ) and estimates of expected heterozygosity ( $0.610 \pm 0.035$ ) found in red junglefowls were comparable to African and Asian chicken populations (Table 4.1). Higher estimates of expected heterozygosity were calculated for African ( $0.604 \pm 0.016$ ) and Asian ( $0.603 \pm 0.015$ ) chickens compared to European ( $0.455 \pm 0.011$ ) and commercial ( $0.453 \pm 0.026$ ) breeds. Moreover, European breeds showed a wider variation of heterozygosity across populations than African and Asian chickens (Figure 4.2). The breeds from Eastern Europe ( $0.525 \pm 0.019$ ), the Mediterranean region ( $0.431 \pm 0.018$ ) and the group of breeds of Asian origins ( $0.489 \pm 0.028$ ) sampled in Europe displayed expected heterozygosity values which overlapped with Asian and African chicken populations at their upper end. The lowest estimates of European breeds were found in the Northwest subgroup which overlaps with commercial White layers at the lower part. African and Asian populations showed lower genetic differentiation ( $F_{ST}$   $0.108 \pm 0.004$  and  $0.120 \pm 0.005$ , respectively) compared to European and commercial breeds ( $0.301 \pm 0.007$  and  $0.327 \pm 0.022$ , respectively). Among the European chickens, the Northwest European breeds displayed, on average, the lowest expected heterozygosity ( $0.425 \pm 0.011$ ) within, and highest  $F_{ST}$  ( $0.315 \pm 0.010$ ) value between pairs of populations, respectively (Table 1).

Table 4.1: Global genetic diversity distribution among a wide range of chicken populations

| Sampling zones           | No. of popn. | H <sub>E</sub> ± std          | H <sub>D</sub> ± std          | MNA ± std                   | F <sub>ST</sub> ± std      | F <sub>IS</sub> ± std      |
|--------------------------|--------------|-------------------------------|-------------------------------|-----------------------------|----------------------------|----------------------------|
| <b>African chickens</b>  |              |                               |                               |                             |                            |                            |
| East Africa              | 6            | 0.625±0.026 <sup>a</sup>      | 0.613±0.026 <sup>a</sup>      | 5.66±0.27 <sup>a</sup>      | 0.053±0.005 <sup>f</sup>   | 0.019±0.027 <sup>a</sup>   |
| South Africa             | 12           | 0.624±0.018 <sup>a</sup>      | 0.590±0.018 <sup>a</sup>      | 5.23±0.19 <sup>ab</sup>     | 0.075±0.006 <sup>f</sup>   | 0.052±0.019 <sup>a</sup>   |
| North Africa             | 5            | 0.527±0.028 <sup>ab</sup>     | 0.490±0.029 <sup>abc</sup>    | 4.57±0.30 <sup>b</sup>      | 0.157±0.017 <sup>de</sup>  | 0.070±0.029 <sup>a</sup>   |
|                          | 23           | 0.604±0.016 <sup>(1)</sup>    | 0.574±0.018 <sup>(1)</sup>    | 5.20±0.17 <sup>(1)</sup>    | 0.108±0.004 <sup>(1)</sup> | 0.047±0.015 <sup>(1)</sup> |
| <b>Asian chickens</b>    |              |                               |                               |                             |                            |                            |
| Southeast Asia           | 16           | 0.640±0.019 <sup>a</sup>      | 0.600±0.020 <sup>a</sup>      | 6.04±0.21 <sup>a</sup>      | 0.053±0.006 <sup>f</sup>   | 0.063±0.211 <sup>a</sup>   |
| East Asia                | 10           | 0.590±0.016 <sup>ab</sup>     | 0.563±0.016 <sup>a</sup>      | 4.55±0.17 <sup>b</sup>      | 0.142±0.007 <sup>de</sup>  | 0.028±0.016 <sup>a</sup>   |
|                          | 26           | 0.603±0.015 <sup>(1)</sup>    | 0.576±0.017 <sup>(1)</sup>    | 5.12±0.16 <sup>(1)</sup>    | 0.120±0.005 <sup>(2)</sup> | 0.042±0.014 <sup>(1)</sup> |
| <b>European chickens</b> |              |                               |                               |                             |                            |                            |
| East Europe              | 12           | 0.521±0.018 <sup>ab</sup>     | 0.510±0.017 <sup>ab</sup>     | 3.45±0.19 <sup>d</sup>      | 0.226±0.011 <sup>e</sup>   | 0.021±0.019 <sup>a</sup>   |
| Asian background         | 5            | 0.489±0.028 <sup>bc</sup>     | 0.444±0.027 <sup>bcd</sup>    | 3.40±0.30 <sup>d</sup>      | 0.282±0.019 <sup>bc</sup>  | 0.091±0.029 <sup>a</sup>   |
| Related Brown layer      | 4            | 0.474±0.035 <sup>bc</sup>     | 0.442±0.037 <sup>bcd</sup>    | 3.09±0.19 <sup>d</sup>      | 0.258±0.035 <sup>bc</sup>  | 0.066±0.038 <sup>a</sup>   |
| Mediterranean            | 12           | 0.431±0.018 <sup>cd</sup>     | 0.400±0.019 <sup>cd</sup>     | 3.18±0.15 <sup>de</sup>     | 0.291±0.016 <sup>bc</sup>  | 0.081±0.029 <sup>a</sup>   |
| Northwest Europe         | 19           | 0.425±0.011 <sup>d</sup>      | 0.360±0.015 <sup>d</sup>      | 2.97±0.15 <sup>de</sup>     | 0.315±0.010 <sup>b</sup>   | -0.133±0.015 <sup>a</sup>  |
|                          | 52           | 0.455±0.011 <sup>(2)</sup>    | 0.419±0.012 <sup>(2)</sup>    | 3.20±0.11 <sup>(2)</sup>    | 0.301±0.007 <sup>(1)</sup> | 0.084±0.009 <sup>(1)</sup> |
| <b>Commercial lines</b>  |              |                               |                               |                             |                            |                            |
| Broilers                 | 4            | 0.563±0.031 <sup>ab</sup>     | 0.550±0.032 <sup>ab</sup>     | 3.98±0.33 <sup>cd</sup>     | 0.119±0.017 <sup>e</sup>   | 0.024±0.032 <sup>a</sup>   |
| Brown Layers             | 3            | 0.426±0.035 <sup>cd</sup>     | 0.414±0.037 <sup>c</sup>      | 3.02±0.38 <sup>d</sup>      | 0.177±0.047 <sup>d</sup>   | 0.027±0.038 <sup>a</sup>   |
| White Layers             | 2            | 0.275±0.045 <sup>d</sup>      | 0.264±0.045 <sup>d</sup>      | 2.26±0.47 <sup>e</sup>      | 0.470±0.057 <sup>a</sup>   | 0.026±0.047 <sup>a</sup>   |
|                          | 9            | 0.453±0.026 <sup>(2)</sup>    | 0.441±0.028 <sup>(2)</sup>    | 3.28±0.26 <sup>(2)</sup>    | 0.327±0.022 <sup>(1)</sup> | 0.026±0.023 <sup>(1)</sup> |
| Red Junglefowl           | 3            | 0.610±0.035 <sup>abc(1)</sup> | 0.581±0.037 <sup>abc(1)</sup> | 4.76±0.38 <sup>abc(1)</sup> | 0.175±0.014 <sup>(2)</sup> | 0.046±0.038 <sup>(1)</sup> |

Different superscript letters or numbers in a column indicate significant differences (Tukey's HSD, P&lt;0.05)

H<sub>E</sub> = Expected heterozygosity, H<sub>D</sub> = Observed heterozygosity, MNA = Mean number of allelesF<sub>ST</sub> = Average inbreeding coefficient within subpopulation, F<sub>ST</sub> = Differentiation between subpopulationsDifferent F<sub>IS</sub> estimates were not significantly different from zero at P>0.05

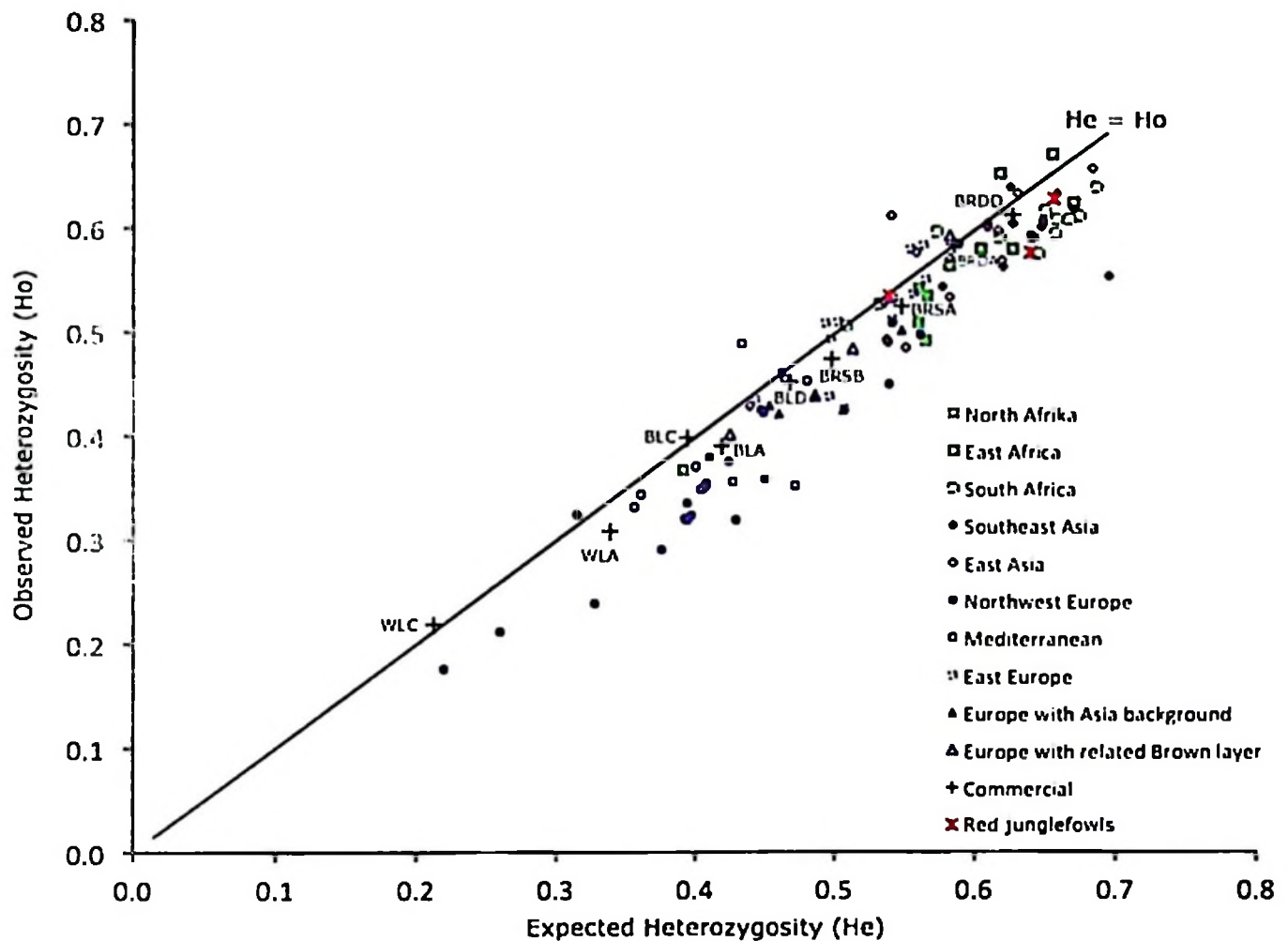


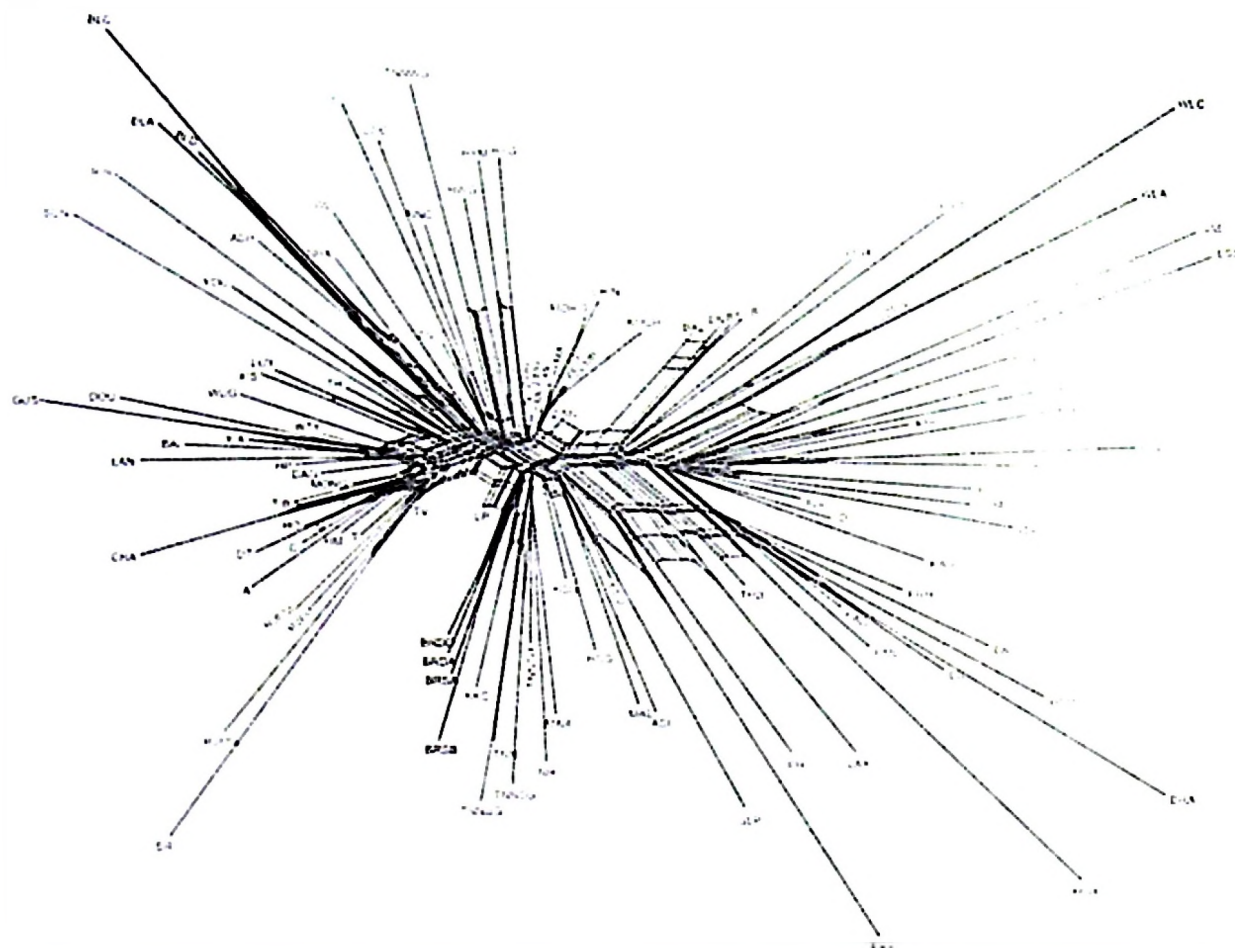
Figure 4.2 Distribution of heterozygosity among the regional chicken populations

#### 4.4.2 Population stratifications

Population stratifications of all 113 chicken populations were evaluated. A model based clustering algorithm implemented in STRUCTURE and a pair-wise MEK distances visualised in Neighbour-Net analysis were used to assess the population structure of the total pool of chicken breeds at various levels (Figure 4.3). In STRUCTURE analysis, at  $K=2$  and  $K=3$  respectively, all pairwise comparisons of runs showed  $G'$  similarity coefficient of  $> 95\%$  and were considered as identical. Likewise,  $K=3$  were the most likely number of genetic clusters as inferred by the method described in Evanno *et al.* (2005). Clustering found at these levels of resolution ( $K=2$  and  $K=3$ ) is comparable to the distribution of chicken breeds found in phylogenetic tree. Both STRUCTURE and the analyses of kinship distances revealed two main clusters of Asian and Northwest European chickens at opposite ends. African populations clustered between these two clusters together with breeds sampled from Eastern Europe, Mediterranean region, populations related to Brown layer, Broilers [BRDA, BRDD, BRSA and

BRSB] and Brown egg layers [BLA, BLC and BLD]. However, chicken breeds sampled in Europe but originated recently from Asia clustered together with Asian chickens and red junglefowls [RJFG, RJFSC and RJFST]. Within Asian chicken populations, red junglefowl (RJF) had smaller genetic distances to chicken populations from Southeast Asia (SEA) than to East Asian (EAS) populations.

(a)



(b)

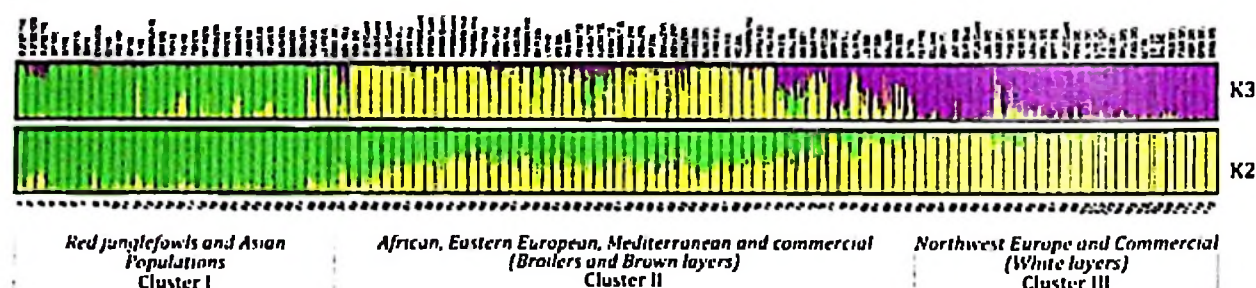


Figure 4.3: (a) Phylogenetic network tree of the chicken populations derived from MEK of 113 chicken populations from various origins and (b) Population structure at  $K=2$  and  $K=3$  of 113 chicken populations from various origin

Clustering of commercial chicken lines revealed obvious differences between White layers and Brown layers which both formed edges of the total spectrum of chicken populations at opposite sides. In contrast, the group of diverse Broiler lines were found more in a central position of the spectrum. White egg layers [WLA and WLC] clustered with Northwest European chickens. More specifically, White egg layers clustered together with other *White Leghorn* (i.e. *Line Sarcoma Susceptible* [LSS] and Scandinavian reference population [SCP]), as well as *Jaerhoens* [JAE] and *Padovana* [PDV]. The fancy breeds sampled in Germany (*Italiener rebhuhnfarbig* [ITAR], *Italiener schwarz* [ITAS], *Italiener Triesdorf* [ITAT], *Kastilianer* [KAS] and *Paduaner* [PAD]) which originated from Italy, clustered close to commercial white egg layers and other *White Leghorn* breeds. An Egyptian origin breed *Fayoumi* [FAY] which was sampled in France, clustered closely with Mediterranean chickens *Prat* [PII] from Spain and *Bedouin* [BED] from Israel. Commercial Brown egg layers clustered with *Rhode Island Red* [RIR], *AB\_High line* [ABH] which is based on *Isa Warren* cross, *Gödöllő NHX* [NHX] a *New Hampshire* cross maintained as a conservation flock in Hungary, and *Orlov* [ORL] chicken breed. Vietnamese breeds (*Te* [T], *H'mong* [HM], *Ac* [A], *Choi* [C], *Mai* [M], *Dong Tao* [DT], *Ho* [HO]) clustered closely to red junglefowls and *Brahma* [BR] chickens, a breed of Asian origin but sampled in Germany. In addition, *Sundheimer* [SUN] sampled in Germany, a breed with influences of populations from Asia was grouped in the East Asian main cluster. Chickens of South African conservation flocks of breeds *Koekoek* [KKC], *Venda* [VC], *Naked neck* [NNC] and *Ovambo* [OVC] clustered away from other African populations and shared a cluster with Eastern European breeds and Broilers chickens.

Means for the geographical subgroups (Table 4.2) of chicken populations were calculated from the individual membership coefficients of the most probable STRUCTURE clustering (i.e.  $K=3$ ). Red junglefowl (RJF), East Asian (EAS) and Southeast Asian (SEA) chicken populations showed very similar patterns with one cluster dominating in Asian breeds (Figure 4.4). Breeds of Asian background sampled in Germany (EAO) had a higher membership coefficient in the cluster of Asian chicken populations and represented a very low proportion in the Northwest European (NWE) cluster. The distribution of membership coefficients for commercial Brown layers (BLY) was similar to non-commercial populations related to Brown layers (RBL). Among African chickens, East African (EAF) chickens had higher membership coefficients in the cluster dominated by Asian chicken breeds, while South African (SAF) chickens showed a higher membership proportion in the cluster encompassing East European (EEU) breeds. North African (NAF) breeds showed a high membership coefficient in the cluster dominated by chickens from the Mediterranean region (MED) and Northwestern Europe (NWE).

Table 4.2: Average membership coefficients for the regional and sub-regional chicken populations described by historical origin of the breeds

| Region/Sub-region Chicken Populations      | Population number | Cluster I (CMC 1)           | Cluster II (CMC 2)          | Cluster III (CMC 3)        |
|--------------------------------------------|-------------------|-----------------------------|-----------------------------|----------------------------|
| <b>African Chicken populations</b>         |                   |                             |                             |                            |
| - South Africa                             | 12                | 0.1211±0.008 <sup>c</sup>   | 0.7517±0.011 <sup>b</sup>   | 0.1273±0.008 <sup>d</sup>  |
| - East Africa                              | 6                 | 0.2686±0.012 <sup>d</sup>   | 0.5352±0.016 <sup>c</sup>   | 0.1962±0.012 <sup>c</sup>  |
| - North Africa                             | 5                 | 0.0560±0.014 <sup>e</sup>   | 0.2361±0.017 <sup>c</sup>   | 0.7079±0.013 <sup>b</sup>  |
|                                            | 23                | 0.1449±0.007 <sup>a1</sup>  | 0.5810±0.013 <sup>a1</sup>  | 0.2742±0.012 <sup>a2</sup> |
| <b>Asian Chicken populations</b>           |                   |                             |                             |                            |
| - Southeast Asia (Vietnam)                 | 16                | 0.9157±0.024 <sup>d</sup>   | 0.0533±0.012 <sup>e</sup>   | 0.0310±0.009 <sup>f</sup>  |
| - East Asia (China)                        | 10                | 0.7702±0.007 <sup>b</sup>   | 0.2005±0.010 <sup>c</sup>   | 0.0293±0.007 <sup>f</sup>  |
|                                            | 26                | 0.8261±0.007 <sup>11</sup>  | 0.1439±0.012 <sup>11</sup>  | 0.0299±0.115 <sup>11</sup> |
| <b>European Chicken populations</b>        |                   |                             |                             |                            |
| - Northwest Europe                         | 19                | 0.0372±0.007 <sup>2</sup>   | 0.1175±0.009 <sup>f</sup>   | 0.8454±0.007 <sup>b</sup>  |
| - Mediterranean                            | 12                | 0.04479±0.009 <sup>12</sup> | 0.0950±0.012 <sup>f</sup>   | 0.8571±0.009 <sup>b</sup>  |
| - East Europe (Russia, Poland and Hungary) | 12                | 0.0825±0.009 <sup>2</sup>   | 0.7906±0.012 <sup>b</sup>   | 0.1269±0.008 <sup>d</sup>  |
| - Europe with recent Asia background       | 5                 | 0.4437±0.0013 <sup>c</sup>  | 0.3633±0.017 <sup>d</sup>   | 0.1929±0.013 <sup>e</sup>  |
| - Europe with related Brown layers         | 4                 | 0.0306±0.014 <sup>e</sup>   | 0.9296±0.020 <sup>f</sup>   | 0.0399±0.014 <sup>f</sup>  |
|                                            | 52                | 0.0895±0.005 <sup>11</sup>  | 0.3628±0.009 <sup>11</sup>  | 0.5477±0.008 <sup>11</sup> |
| <b>Commercial Chicken Breeds</b>           |                   |                             |                             |                            |
| - Broiler                                  | 4                 | 0.0441±0.014 <sup>12</sup>  | 0.8683±0.019 <sup>a</sup>   | 0.0876±0.014 <sup>e</sup>  |
| - Brown Layers                             | 3                 | 0.0362±0.016 <sup>e</sup>   | 0.9482±0.022 <sup>a</sup>   | 0.0156±0.016 <sup>f</sup>  |
| - White Layers                             | 2                 | 0.0238±0.020 <sup>e</sup>   | 0.0299±0.027 <sup>c</sup>   | 0.9463±0.019 <sup>a</sup>  |
|                                            | 9                 | 0.0367±0.011 <sup>11</sup>  | 0.7086±0.020 <sup>11</sup>  | 0.2544±0.019 <sup>12</sup> |
| <b>Red Junglefowl</b>                      | 3                 | 0.8455±0.017 <sup>a11</sup> | 0.0526±0.023 <sup>a11</sup> | 0.102±0.017 <sup>a11</sup> |

Different superscript letters or numbers in a column indicate significant differences (Tukey's HSD,  $P < 0.05$ )  
CMC is the cluster membership coefficients

#### 4.4.3 Genetic contributions

The contributions of the chicken populations across regions to an optimal core set of global diversity are shown in Table 4.3. Red junglefowl populations provided the highest contribution (0.0191±0.0016) to an optimal core set, followed by Asian chicken populations (0.0115±0.0006) and Broilers (0.0106±0.0014).

Table 4.3: Average contribution of various categories of chicken groups to the core set and additional contribution to the safe set when commercial lines were fixed as reference populations

| Sampling region/<br>Chicken type | Optimal core set<br>$c(i)$ | Contribution to the safe set<br>$c(i)'$ |
|----------------------------------|----------------------------|-----------------------------------------|
| Red junglefowls                  | 0.0191±0.0016 <sup>a</sup> | 0.3877±0.056 <sup>a</sup>               |
| Asia                             | 0.0115±0.0006 <sup>b</sup> | 0.3571±0.019 <sup>a</sup>               |
| Africa                           | 0.0089±0.0006 <sup>c</sup> | 0.3646±0.021 <sup>a</sup>               |
| Europe                           | 0.0069±0.0004 <sup>d</sup> | 0.1312±0.014 <sup>b</sup>               |
| Broilers                         | 0.0106±0.0014 <sup>b</sup> | -                                       |
| Brown layers                     | 0.0085±0.0028 <sup>c</sup> | -                                       |
| White layers                     | 0.0061±0.0019 <sup>d</sup> | -                                       |

Different superscript letters in a column indicate significant differences (Tukey's HSD,  $P < 0.05$ )

$c(i)$  is the relative contribution to the core set for breed  $i$ .

$c(i)'$  is the core set contribution of population  $i$  when added to the Safe set

On average, the contribution to the core set of a single European population ( $0.0069 \pm 0.0004$ ) and White layers ( $0.0061 \pm 0.0019$ ) was lowest. The correlation between core set contributions and average kinship estimates was highly negative ( $r = -0.962$ , Figure 4.5) implying that higher kinship levels between individuals within a population result in smaller genetic contribution to the core set.

Considering diversity of commercial lines as a fixed set, with exception of *Deutsche Sperber* [DSP] and *Bergische Schlatterkaemme* [BS], all populations showed a positive contribution to this set (Appendix 5). On average, European breeds contributed significantly less ( $0.1262 \pm 0.014$ ) to the commercial gene pool compared to African ( $0.3646 \pm 0.021$ ) and Asian ( $0.3571 \pm 0.019$ ) chicken population.

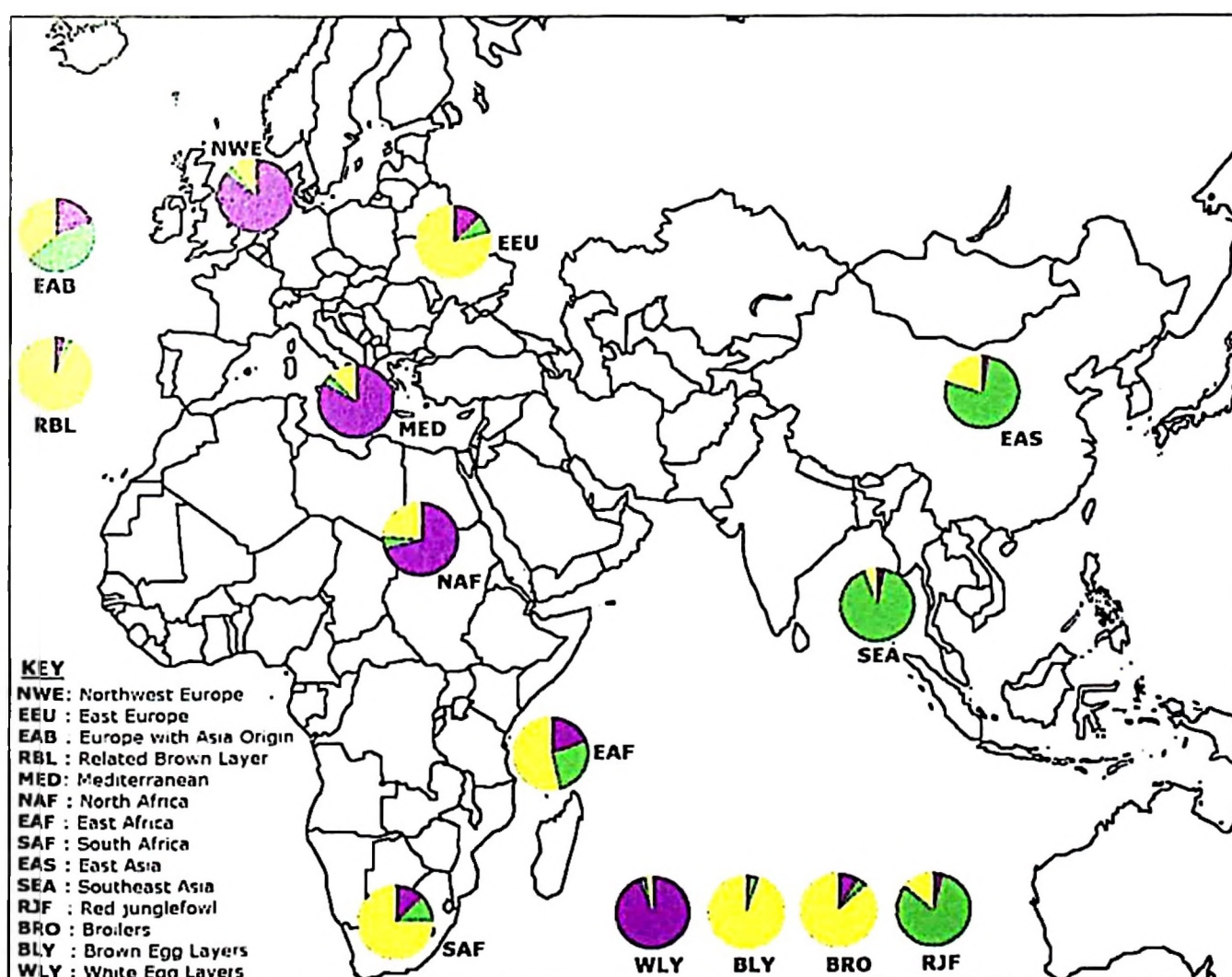


Figure 4.4: A map showing distributions of STRUCTURE mean membership coefficients of the most probable clustering at  $K=3$  of pools of chicken populations across various regions

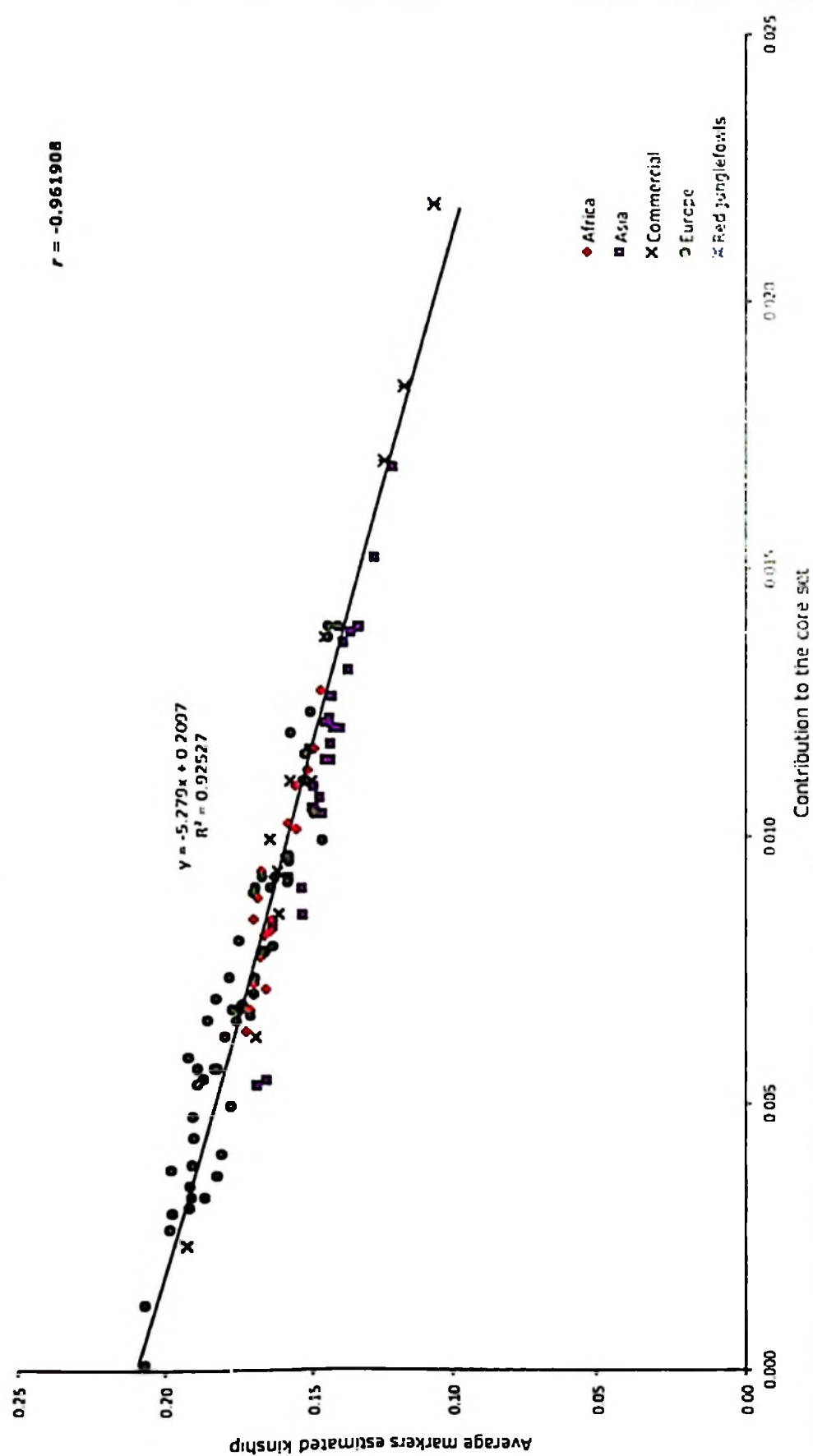


Figure 4.5: The correlation between marker estimate kinship and genetic contribution to the core set for 113 chicken populations

## 4.5 Discussion

Comprehensive knowledge of population stratification and the distribution of genetic variability in breeds and strains are important factors when considering conservation measures with the aim of maintaining sufficient genetic diversity within a species for future generations. In this study, the level of diversity of chicken populations from various continents and with differing population histories, their genetic relationships and the contributions of populations to global diversity were studied.

### 4.5.1 Genetic diversity across regions

Cluster analyses identified three main groups of populations. Phylogenetic network and STRUCTURE analysis showed clearly distinct gene pools of Asian and Northwestern European chicken populations. African, Southeast European and Brown layer breeds as well as Broiler lines formed a third group between the Asian and Northwestern European chicken gene pool. In comparison to European breeds mainly kept by fancy breeders, African and Asian chickens exhibited a higher degree of heterozygosity and a higher mean number of alleles per locus and population. According to many reports, local chickens from Africa and Asia are characterized by extensive phenotypic variations within and between different populations (Msoffe *et al.*, 2001; Muchadeyi *et al.*, 2007; Mwacharo *et al.*, 2007; Chen *et al.*, 2008; Rajkumar *et al.*, 2008; Dana *et al.*, 2010; Cuc *et al.*, 2010; Mtileni *et al.*, 2011a; Leroy *et al.*, 2012; Lyimo *et al.*, 2013). The traditional management system still in use in the present is defined by the absence of selection practice and by uncontrolled breeding management. This may have contributed to higher variation within the populations of this type (Abdelqader *et al.*, 2007; Gondwe and Wollny, 2007; Rajkumar *et al.*, 2008).

Although European breeds displayed a lower average heterozygosity compared to African and Asian breeds, they exhibited a wider distribution of heterozygosity estimates across populations than African and Asian chickens. This indicates a wider variation in diversity between European fancy populations, which is to some degree related to a varying degree of population sizes.

### 4.5.2 Asian gene pool

According to Phylogenetic Neighbour-net, RJF clustered closely with Southeast Asian chicken populations. The close association of Southeast Asia chicken populations with RJF was also reported in previous studies (Collias and Saichuae, 1967; Berthouly *et al.*, 2009; Cuc *et al.*, 2010). Berthouly *et al.* (2009) indicated admixture of H'Mong chickens from the Northern Vietnam with wild junglefowls indicating gene flow from wild to domestic population of the Southeast Asia. The chickens sampled in Europe with recent Asian background (*Brahma*, *Cochin* and *Sundheimer*) were closely related to the Asian gene pool. Even though these populations have been kept in Europe for

more than 150 years either directly originating from Asian breeds or from crosses of local strains with Asian breeds, as in case of *Sundheimer*, still they retain their origins.

#### 4.5.3 Northwest European gene pool

The standardized breeds of chickens sampled from the Northwestern Europe formed a gene pool distant from Asian gene pool. This pool encompasses also commercial White egg layers, fancy breeds of Mediterranean origin (*Italiener rebhuhnfarbig*, *Italiener schwarz*, *Italiener Triesdorf*, *Kastilianer* and *Paduaner*) and other White Leghorn breeds (*Line Sarcoma Susceptible* and Scandinavian reference population). *Icelandic landrace "landhamshaena"* is the native breed of Iceland situated more than 2000 km away from the European mainland. Although isolated from others, they clustered together with Northwest European chicken populations. Several reports indicated that the *Icelandic Landrace* might have originated from Old Norwegian Jadar and North German chicken breeds (Kirby and Hainkkanen, 2000; Ball-Gisch, 2009; Heinrichs, 2010). *Icelandic Landrace* clusters closely with the old German breeds *Bergische Schlotterkaemme*, *Ostfriesische Möwen* and *Italian black* sampled from German fancy breeders, although to the best of our knowledge no historic records are available explaining this immediate relationship. The Icelandic chickens were brought to the island between the 9<sup>th</sup> and 10<sup>th</sup> century by Norse settlers who established Viking settlements on Iceland (Grote, 2006; Ball-Gisch, 2009; Aviculture-Europe, 2010).

#### 4.5.4 African - Southeast European gene pool

These groups of chicken populations clustered in the middle of the Phylogenetic Network between the Asian and Northwest Europe gene pools. STRUCTURE analysis revealed an admixture of Asian and Northwest at K=2 (Figure 1b). The admixture of African, Mediterranean and Southeast Europe chickens could be related to the geographical junction of Asia, South Europe and Northern Africa through immediately interactions surrounding the Mediterranean Sea. Moreover, colonization and early human interactions through cross regional trade routes and prehistoric migration trails could also contribute to this admixture. A map (Figure 3.3) showing the distributions of STRUCTURE membership coefficients (at K=3) indicated that North African chickens share more of their ancestral gene pool with European breeds than Asian chicken populations. This finding is in line with historical records of the introduction of chickens to Africa and is also confirmed by mitochondrial DNA analysis. Several mtDNA studies showed that chicken populations from North Africa are commonly described with haplotypes more frequent in Europe than those from chickens sampled in Eastern Africa (Muchadeyi *et al.* 2008, Mwacharo *et al.* 2011; Mwacharo *et al.*, 2013b). Coltherd (1966) reported that the chicken arrived in Africa for the first time mainly from Europe through Egypt via land trade routes and/or sea born trade across the Mediterranean Sea. Mwacharo *et al.*

(2011) reported another link between North Africa and the Arabian Peninsula which were connected by a terrestrial route.

Cluster analyses visualized as Neighbour-Net tree derived from MEK distances and the STRUCTURE results indicate that South African breeds maintained as conservation flocks clustered closely with the European breeds and commercial purebreds. *Ovambo*, *Naked Neck* and *Venda* South African conserved breed were clustered closely to Hungarian breeds and Brown layers, and *Koekoek* conserved breed were clustered with Broilers. This suggests that these chickens maintained as conservation flocks in South Africa may partly possess the genetic make-up of European breeds and commercial purebred lines. Mwacharo *et al.*, (2013b) reported the arrival of European settlers to Africa in 15 century A.D. opening the opportunities for influx of European chickens to Africa. Van Marle-Köster *et al.* (2008) reported that chickens were introduced to Southern Africa during the 1600's by early settlers and traders from Europe and Sub-Saharan Africa. *Black Australorp*, *White Leghorn* and *Plymouth Rock* chickens were introduced to the former Potchefstroom Agricultural Research Institute during the late 1940's and used as parental stock for *Koekoek* breed (Van Marle-Köster and Nel, 2000). The clustering of *Koekoek* chickens with Broilers chickens might be due to introgression of *Plymouth Rock* chickens, which were also used as a parental stock for Broiler purebred. In contrast, South African chicken populations sampled from the field clustered together with East African scavenging chickens. Based on mtDNA studies, East African chickens have their roots to large degree in Southeast Asia and the Indian subcontinent (Muchadeyi *et al.* 2007; Mwacharo *et al.* 2011; Lyimo *et al.* 2013). Mtileni *et al.* (2011b) found the majority of South African village chickens also originated from the Indian subcontinent and Southeast Asia.

#### 4.5.5 Influence of Mediterranean chickens

Several reports confirmed the vast dispersal of Mediterranean chickens in different regions. This might be influenced by earlier (3000 B.C.) introductions of chickens from Asia (West and Zhou, 1988; Tixier-Boichard *et al.*, 2011), geographical location of the Mediterranean region and the region being among the earliest centres for the historical cross-cultural and trade link (Curtin, 1984; Sherratt and Sherratt, 1993; Tracy 1993). West and Zhou (1988) provided archaeological evidences of chickens' existence in Iran, Turkey, Syria, Greece, Romania and Ukraine on the earlier date before Mohenjo-Daro settlement (2600 B.C.). Mediterranean region is crucial to understanding the origins and development of many modern societies, and it was an important route for merchants and travellers of ancient times that allowed for trade and cultural exchange between emergent peoples of the region the Mesopotamian, Egyptian, Phoenician, Carthaginian, Iberian, Greek, Macedonian, Illyrian, Thracian, Levantine, Gallic, Roman, Albanian, Armenian, Arabic, Berber, Jewish, Slavic

and Turkish cultures (Encyclopedia, 2011). MEK and STRUCTURE analyses revealed that chickens of Mediterranean origin were wide spread in Europe. Historical and archaeological evidence suggests that indigenous chickens from the East and central Europe might have dual origins with a northern diffusion from the black sea (Revay *et al.* 2010) and possibly arrival from China through Russia route (West and Zhou, 1988, Tixier-Boichard *et al.*, 2011).

#### 4.5.6 Commercial lines and population related to Brown layer

The differences between classes of commercial lines were clearly observed in phylogenetic Neighbour-Net tree derived from MEK distances. The commercial lines of Broilers and Brown layers were clustered separately in the African - Southeast European gene pool. Breeding of both Broilers and Brown layers were developed from Asian and Mediterranean chickens (Crawford, 1990). The commercial broiler birds are the crosses between *Plymouth Rocks*, *Cornish* Indian game bird, *Langshans*, *Brahma* and *New Hampshire* (Hathaway *et al.*, 1953; Crawford, 1990). Early attempts to produce hybrid chicken for meat were in the 1930's and the intensification of the broiler industry started in the late 1950's (Gyles, 1989; Griffin and Goddard, 1994). Broiler chickens have been intensively selected for rapid growth rate, feed conversion efficiency and meat yield. Brown layer lines were developed in Rhode Island in the USA from a cross between *Cochin*, Red *Malay* game fowl, *Brahma*, *Shanghai*, *Wyandotte* and Mediterranean breed *Brown Leghorn*. The breed *Rhode Island Red* was originally a dual-purpose fowl and admitted to the American Poultry Association standard of perfection in 1904 (Anderson, 2013; Garrigus, 2014). Since 1940's, the *Rhode Island Red* has been selected for high egg production (Potts, 2012). The populations related to Brown layers [RIR, ABH, NH and NHX] clustered with commercial brown layers since they have their roots, to some degree, in the *Rhode Island Red* breed (Aviandiv, 2000).

Both STRUCTURE and phylogenetic clusters indicated that non-commercial *White Leghorn* chicken breeds [LSS, SCP] sampled in Northwest Europe clustered closely with commercial White egg layer [WLA, WLC] which are based on the same breed (Crawford, 1990). The breed *White Leghorn* originated from a native breed in central Italy (*Livorno*) and has been developed into commercial egg layers in United States since 1865. Today, a single comb *White Leghorn* is the most popular breed leading in producing white eggs worldwide (Global Poultry Trends, 2013; Garrigus, 2014). Majority of egg laying breeds of chickens have ancestries that trace them to the Mediterranean class of chickens (Crawford, 1990). Layers breeds have been genetically selected for high egg productivity.

Regarding population differentiation, European populations showed  $F_{ST}$  estimates very similar to those of commercial lines, which are kept as closed breeding populations with limited gene flow.

This confirms that European chickens have been kept as isolated breeds with small effective population sizes as reported previously (Granevitze *et al.*, 2007). Populations with low genetic variability have a low genetic contribution to the core set. Although the majority of the European chicken breeds (96.23%) add genetic diversity to the commercial gene in a core set analysis, their contributions are significantly lower than that of Asian and African populations. Higher genetic differentiation and low genetic contributions have been observed in European breeds. Since the genetic variation is higher between European chicken populations, attention should be drawn to conservation of some European breeds in order to maintain maximum genetic diversity.

#### 4.6 Conclusions

Merging data of several studies analysed with the same set of molecular markers provided comprehensive insights into genetic diversity of chickens across continents. Even though the number of loci is low compared to contemporary molecular tools such as high density SNP arrays or genome sequences, some general conclusions can be drawn from this study. Assuming that the two RJF populations originated in a straight line from wild ancestors, which formed the founder gene pool of domestic chickens, phylogenetic analyses in this study illustrated that a wide variation has accumulated during domestication. Forces such as genetic drift, natural and man induced selection accompanied by isolation as a result of human migration have led to separated gene pools generally corresponding to geographical locations. The Asian gene pool which is more closely related to wild ancestors is more polymorphic than the European gene pool. As reported for other species as well, this might be related to diversity decreasing when one moves outwards from the centres of domestication (Groeneveld *et al* 2010; Wiener and Wilkinson, 2011), but also to current breeding practice in European chicken breeds and low size of these populations. As a consequence, a single European breed contributes only little to global diversity, but in sum, they display a considerable part of the global diversity of the species. Furthermore, European chicken breeds which have been influenced by Asian breeds imported to Europe since the middle of the 19<sup>th</sup> century (EAO) are still clearly distinguishable from European breeds originating from chickens introduced to Europe over 3000 years ago during the Iron Age (NWE). African breeds, introduced in several waves to the continent, make up a cluster originating from Asia but less distant than European breeds. Furthermore, close relationships to Europe may explain the influence of European breeds on North African and South African chicken breeds. In contrast to European breeds, these chicken populations have not been selected for breed standards and management in free-range systems has maintained a high degree of variation with low differentiation between them.

Finally, commercial purebred layer lines made up outstanding clusters of the total spectrum separating White layers from Brown layers. Although both types of lines have been selected for high laying performances, they are very distinct from each other according to their different breed origins, while Broilers cluster more inside the spectrum of diversity. More importantly, these populations contribute only little global diversity, and replacing local populations by commercial lines will lead to tremendous reduction of diversity within the species.

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## **CHAPTER FIVE**

### **5.0 General Discussion**

### 5.1 General findings

The diversity of farm animal genetic resources contributes to achieving food security at the household level for the present time and into the unpredictable future. Currently, the rapid growth of human population is associated with an ever increasing demand for livestock products, which is foreseen to be a momentous constraint on society, especially in developing countries. Concurrently, the challenge of increasing livestock production requires an efficient exploitation of genetic diversity, among and within the populations of different livestock species. The most productive and adapted animals for each environment must be identified for breeding purposes and for conserving the diversity of such animals into the future.

The United Republic of Tanzania is one of the signatory countries to the Rio de Janeiro Convention on Biological Diversity adopted in 1992, and thereafter ratified by the Convention on Biological Diversity (CBD) concerning farm genetic resources in 1996 (URT, 2010). The CBD aims at maintaining food security and preserve the variation within species, including farm animals. Consequently, when a country becomes a signatory nation, it commits itself to taking national responsibility for conserving farm animal genetic resources. In Tanzania, the provisions of the convention have now been translated into the relevant policies, legislations and other instruments to facilitate its implementation (MLD, 2006). However, data and other types of information on livestock in the country have been a limiting factor among policy makers and other stakeholders in spearheading the appropriate livestock management aimed at conserving farm animal genetic resources. Livestock Information Services in the policy document (MLD, 2006) reveals that information on livestock is constrained by inadequate infrastructure and facilities, the high cost of data collection, insufficient expertise and the absence of a centralized database management system.

Keeping in mind the significance of chickens for human nutrition as well as the existing risk of extinction for many chicken populations worldwide, the importance of a study of global chicken diversity and regional genetic contribution should be addressed. There is a real and current need to outline a global picture of chicken population structures and genetic contributions in order to permit proper decisions concerning diversity management and conservation measures. A study in a single region cannot assess the global value of chicken breeds of that region, since many diverse forms of production are found in smallholder societies across the regions.

The general objective of this study was to characterize Tanzanian indigenous chickens in relation to the worldwide spectrum of chicken diversity. The specific objectives were:

- (i) To study maternal lineages and genetic diversity in five ecotypes of Tanzanian indigenous chickens to fill the limited information gap on the molecular diversity and the origin of Tanzanian chickens.
- (ii) To investigate the genealogical patterns of European chicken breeds, and compare them with the studies of African and Asian chicken populations.
- (iii) To evaluate global diversity and genetic contributions of chicken populations from African, Asian and European regions by using multilocus microsatellite genotypes. To achieve this, microsatellite data obtained in separate studies were combined for global analysis.

In the first study (Chapter 2), the genetic diversity and maternal lineages of five Tanzanian indigenous chickens (*Ching'wekwe*, *Kuchi*, *Morogoro-medium*, *Pemba* and *Unguja*) were examined. A total of 196 individual chickens from eight regions of the Eastern Zone, Central Zone, Lake Zone and Zanzibar islands were collected. Genetic diversity of the chickens within and between Tanzanian indigenous chickens was assessed at the autosomal level based on microsatellites. Higher genetic variations were observed between individual chickens within each of the populations compared to a variation between different Tanzanian chicken populations. Similar genetic diversity characteristics have been observed in other African scavenging chickens. This might be as a result of the common traditional chicken management free-range system, which allows random breeding without selection for performance traits (Muchadeyi *et al.*, 2007; Eltanany *et al.*, 2011; Fosta *et al.*, 2011; Goraga, *et al.*, 2011; Mtileni *et al.*, 2011a). In previous studies, the same set of 29 microsatellite markers were also used to assess the genetic diversity of South African chickens, Zimbabwean chickens and Malawian chickens. A similar and high degree of genetic diversity as in Eastern and South African chicken ecotypes has been observed in Tanzanian chickens (Muchadeyi *et al.*, 2007; Mtileni *et al.*, 2011a; Lyimo *et al.*, 2013).

Constructing a Neighbor Joining tree and STRUCTURE analysis, in both types of clustering Tanzanian indigenous chickens formed three groups, which were related to geographical distribution. The *Kuchi* ecotype from Tanzanian Lake Zone formed an independent cluster, while *Morogoro-medium* and *Ching'wekwe* ecotypes from East and Central Zones formed the second cluster, and the third cluster included *Unguja* and *Pemba* ecotypes from Tanzanian Islands of Zanzibar. *Unguja* and *Pemba* ecotypes from Tanzania Islands and *Kuchi* ecotype from Tanzania mainland are game bird type chickens. In the Lake Zone of Tanzania mainland where *Kuchi* game birds are found, there are no historical records for cock fighting games. In Zanzibar Islands, the culture of cock-fighting has existed since 945 A.D., and was introduced by Austronesians during trans-oceanic contact with

Southeast Asian (Boivin *et al.*, 2013). As Walsh (2010) reports both bull-fighting and cock-fighting in Unguja and Pemba Islands were introduced to the islands from Southeast Asia in the early to mid part of the first century A.D. Taro (*Colocasia esculenta*), water-yams (*Dioscorea esculenta*), and banana (*Musa spp.* including the wild banana of Pemba Island) all came together with fighting cocks found in the East African coast from Southeast Asia (Blench 2006; Walsh 2010). From the STRUCTURE analysis, high degrees of admixture between *Unguja* and *Pemba* chicken populations were found. Although the Tanzanian islands of Unguja and Pemba are about 80 km apart from each other, the similarity of both chicken types may indicate a long history of exchanging chickens between the two islands. Both morphological and genetic analyses showed an isolation of the *Kuchi* ecotype from *Morogoro-medium* and *Ching'wekwe* chickens as well as from the *Unguja* and *Pemba* ecotypes.

Two maternal lineages were distributed among Tanzanian chickens, which suggest that Tanzanian indigenous chickens originate both from the Indian Subcontinent and from Southeast Asia. With the exception of the *Kuchi* ecotype, all ecotypes possess both maternal origins of Southeast Asia and the Indian subcontinent. Tanzanian chickens also were found to share maternal lineages with other Eastern and South African village chickens, and this reflects the existence of a direct influence of the Indian Ocean on the dispersal of chickens to Africa (Muchadeyi *et al.*, 2008; Mtileni *et al.*, 2011b; Mwacharo *et al.*, 2011; Lyimo *et al.*, 2013). As Boivin *et al.* (2013) report, the emergence of the Indian Ocean-oriented trading culture of the Swahili along the East African coast encouraged the translocation of a number of new species into the region in the first half of the first millennium A.D. Gifford-Gonzalez and Hanotte (2011) reported the second main wave of chicken introduction to Africa via the East coast. The chickens were introduced in the Eastern coast of the region across the Indian Ocean by means of trade networks which existed during the beginning to the mid of the 1<sup>st</sup> millennium A.D. (Adelaar, 1996; Blench, 2006; Boivin and Fuller, 2009; Fuller *et al.*, 2011). The discoveries of the classical sailors using the monsoon winds in the first Century B.C. subsequently led to major changes in the scale and conduct of the Indian Ocean trades (Bovin *et al.*, 2009). As Bovin *et al.* (2013) and Al-Qamashoui *et al.* (2014) observe, the introduction of chicken to the East African coast also might reflect the existence of contacts with northeastern Africa, though the Arabian Peninsula, Persian Gulf, South Asia and Southeast Asia. Bovin *et al.* (2009) describe the vessel transport routes during marine time trade by the use of regular monsoon winds for long distance sea transportation between Southeast Asia, South Asia, Persian Gulf, Arabian Peninsular and the Northeastern coast of Africa.

Relatively low genetic diversity within, and large genetic distances of the *Kuchi* ecotype from all other Tanzanian chicken populations have been observed. This indicates that among the Tanzanian mainland chickens, *Kuchi* ecotype belongs to a distinct group. Moreover, the results of the analysis of mitochondrial DNA sequence polymorphism suggest the possibility of a recent introduction of *Kuchi* chickens into the Tanzanian mainland. There is no recorded history or the origin of the name “Kuchi” after this game bird ecotype. Nevertheless a clue from this study suggests that majority of *Kuchi* chickens were clustered in a single haplogroup, which was previously found in *Shamo* game birds sampled from Shikoku Island of Japan in the Kōchi Prefecture. The analysis of phenotypic and molecular data, as well as the linguistic similarity of the breed names, suggests a recent introduction of the *Kuchi* breed to Tanzania. The name “Kuchi” which is similar to prefecture Kōchi implies that *Kuchi* ecotype might have been imported to Tanzania from Japan.

In the second study (Chapter 3), 55 European chicken breeds were categorized into six groups according to their historical background; Chicken breeds which have been in Europe for more than 3000 years were categorised into (i) *Mediterranean type*, (ii) *East European type* and (iii) *Northwest European type*; breeds based on introgression of Asian breeds into European breeds since the middle of the 19<sup>th</sup> century was categorized as (iv) *Intermediate type*; introduced Asian fighting birds and breeds which retained game bird characteristics originating from crosses between European breeds and Asian fighting birds was classified as (v) *Game bird*; and breeds with recent Asian origin was classified as (vi) *Asian type*. A total of 1256 individual sequences of a fragment of 455bp in the size of the control region (D-loop) in the mitochondrial genome (mtDNA) were investigated. Haplogroup E was the dominant clade in European chicken breeds, followed by Haplogroup A. Gongora *et al.* (2008) considered Haplogroup E as the predominant clade among Indian, Middle Eastern, and European chickens, which is an indication that most of the European chickens may have originated from the Indian subcontinent. The occurrence of Clade A haplotypes in European chickens is in line with the historical records of the dispersal route of chickens from China to Europe (West and Zhou, 1988; Crawford, 1995; Tixier-Boichard *et al.*, 2011).

Wide ranges of haplotype diversity and nucleotide diversity suggest that there is high genetic variation within the population and between classes of chicken breeds sampled in Europe. Haplotype diversity and nucleotide diversity lies between the ranges of  $0.442 \leq H \leq 0.761$ , and  $0.0030 \leq \pi \leq 0.0104$ , respectively suggesting that chicken breeds sampled in Europe displayed clear differences between breed categories in terms of diversity within populations. Breeds of Asian type and Intermediate type breeds have multiple origins of haplotypes and exhibit a high number of haplotypes, haplotype diversity and nucleotide diversity. Similar haplotype and nucleotide diversities

were observed between Asian type breeds with East African and South African chickens in previous studies. Recent studies reveal that East and South African village chickens possibly might have their roots in the Indian subcontinent and Southeast Asia (Muchadeyi *et al.*, 2008; Mwacharo *et al.*, 2011; Mtileni *et al.*, 2011; Lyimo *et al.*, 2013). Mediterranean chicken types displayed similar nucleotide diversity as the North African and West African chickens (Adebambo *et al.*, 2010, Mwacharo *et al.*, 2011, Wani *et al.*, 2014). Furthermore, the dominance of clade E was reported in many North African chicken populations which have many influenced from Mediterranean (Coltherd, 1966; Muchadeyi *et al.*, 2008; Mwacharo *et al.*, 2011).

In the third study (Chapter 4), global genetic diversity and genetic contributions of chicken populations from African, Asian and European regions were investigated by using multilocus microsatellite genotypes. Twenty-eight of the 29 loci used were taken from the list recommended by the ISAG/FAO advisory group for chicken biodiversity (FAO, 2011). The possibility of merging data of various studies allowed a wide insight into the existing diversity of the species across countries and regions. This merged set of data included 101 local populations with the breed history from Africa, Asia and Europe. The samples represented 22 chicken populations from Africa, 26 chicken populations from Asia and 53 chicken breeds sampled in Europe. In addition, three populations of red junglefowl and nine commercial purebred lines were added and used as reference populations in the analyses.

African and Asian chickens exhibited a higher degree of heterozygosity and a higher mean number of alleles per locus and population as compared to European breeds. The existence of traditional farming systems in Africa, prevailing extensive management of chicken flocks, and a limitation of selection practices may have contributed to this higher genetic variation within the African and Asian chicken populations (Kabatange and Katule, 1989; Abdelqader *et al.*, 2007; Gondwe and Wollny, 2007; Rajkumar *et al.*, 2008; ACIAR 2009). Regarding population differentiation, African and Asian populations showed lower genetic differentiation when compared to European and commercial breeds. European populations had a very similar  $F_{ST}$  estimate as commercial lines, which are kept as closed populations without admixture. This suggests that European breeds have been bred as rather isolated breeds with small effective population sizes; and these results are consistent with previous reports (Granevitze *et al.*, 2007). Although European breeds displayed a lower average heterozygosity compared to African and Asian breeds, the former exhibited a wider distribution of heterozygosity estimates across populations than is the case with African and Asian chickens. This indicates a wider variation in diversity among European populations, which is to some degree related to a varying degree of population sizes. A comparable pattern of chicken populations' diversity

distribution has been observed when these populations were analyzed using 600K SNP array for genotyping (Weigend *et al.*, 2014).

Three main gene pools were observed from the Phylogenetic network and STRUCTURE analysis: two distinct clusters of Asian and Northwestern European chicken populations at opposite ends, and the third cluster in-between including African chickens overlapping with breeds from Southern Europe, broilers and brown layers. In addition to the analysis of the most likely genetic clustering ( $K=3$ ) done in Chapter four, deeper insight into the structure of the studied population was achieved by going up to the next most likely number of clusters in the STRUCTURE analysis (next maximum peak of delta  $K$  at  $K=9$ ). Red junglefowls and Southeastern Asian chicken populations shared the same cluster at all levels (Figure 5.1). Compared to African and Asian chicken populations, the highest degree of population stratification was observed in the gene pool of European chicken populations and commercial breeds. Current breeding practices for European chicken breeds and the low size of these populations might contribute to the higher differentiations between breeds (Groeneveld *et al* 2010). The Asian gene pool is more closely related to wild ancestors and consistently shares the cluster with Southeast Asia with the least population stratification. As reported for other species as well, this might be related to a diversity decrease as when one moves away from the centres of domestication (Groeneveld *et al* 2010; Wiener and Wilkinson, 2011).

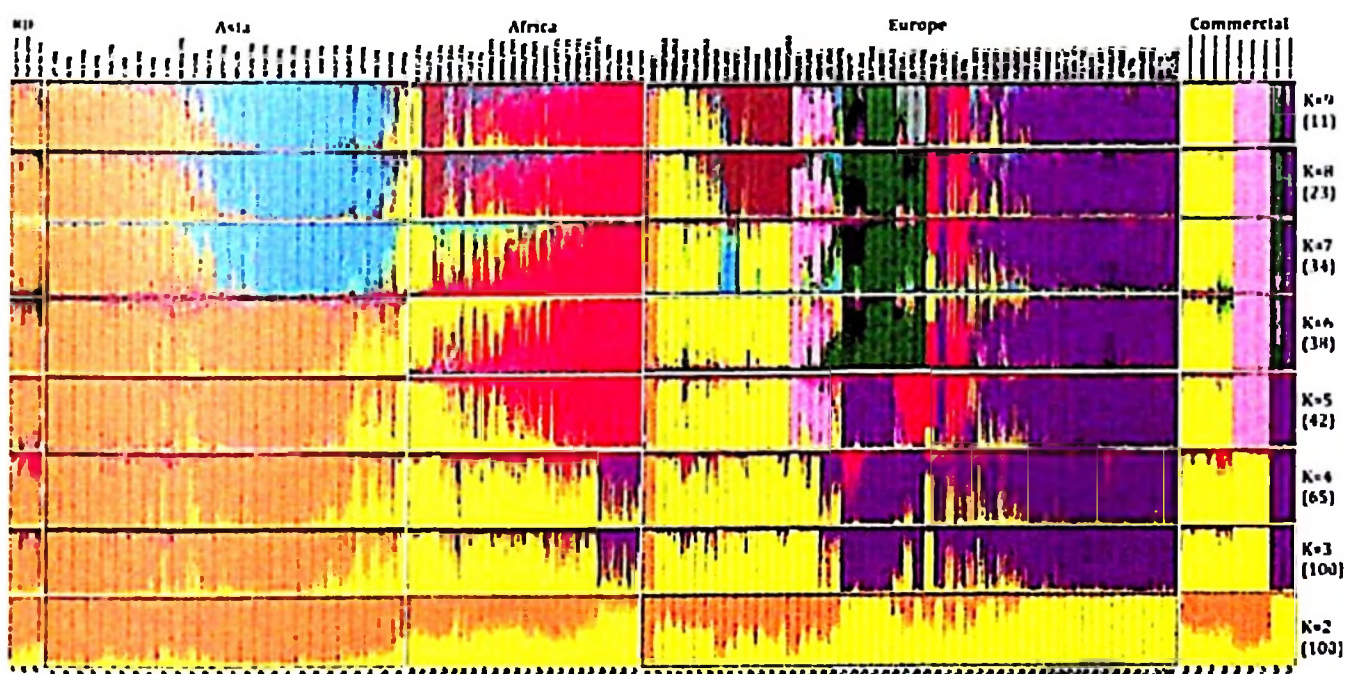


Figure 5.1: Population structure of 113 chicken populations grouped per sampling regions

NeighborNet analysis showed a high genetic distance between the red jungle fowl and European chicken breeds indicating that European populations developed independently from wild ancestors for many generations since domestication, due to the fact that they have been isolated from regions of origin for more than 3000 years. Historical records and archaeological discoveries disclose the presence of domesticated chickens in Europe since the earliest Iron Age around 3000 B.C. (West and Zhou, 1988; Fixier-Bouchard *et al.*, 2011; Hink *et al.*, 2014). Tanzanian indigenous chickens, together with other East African and South African village chickens cluster at the middle of the NeighborNet tree. The commercial chicken lines bred for eggs productions revealed obvious differences between Brown layers and White layers which both formed an edge of the total spectrum of chicken populations at opposite sides.

The genetic contributions of different chicken populations from each region to the total diversity were estimated according to Eding *et al.* (2002). Core set method was used to assess both within and between populations diversity based on kinship estimates. The genetic variation contribution of each breed was estimated, with minimum overlap of the core set. The total genetic diversity of the nine commercial breeds was set as fixed and the additional contributions of the local populations to the commercial gene pool were computed by adding breeds one by one to the fixed set (Eding *et al.*, 2002). From this analysis, the genetic contribution of the chicken populations from each region was assessed. Generally, the populations with low genetic variability have been found with low genetic contribution as compared to the populations with higher variability. Although red jungle fowl populations provided the highest contribution, followed by Asian and African chicken populations, only a few populations can contribute to the total diversity since these populations show overlapping variability. Low genetic contributions have also been observed for single European breeds, however all breeds together make a noticeable contribution to the total genetic diversity.

## 5.2 General conclusion

Tanzanian indigenous chickens share a gene pool with other East and South African Village chickens reflecting common routes of origin. Furthermore, the concordance in the degree of diversity among these chicken ecotypes may reflect similarities of the existing traditional farming systems in Africa. The genetic overlap of the African chicken gene pool with European breeds and Asian chickens might have been influenced by geographical locations, interactions at the Mediterranean Sea, Indian Ocean trade links and colonization effects that allowed the influx of chickens from both Asia and Europe. North African chicken populations are genetically closely related to European chicken breeds from the Mediterranean regions than to the East and South African chicken populations.

High genetic variations between Tanzanian chicken ecotypes imply the possibilities of the selection for improvement of some production traits and signify their importance in the conservation of genetic diversity. Since there is limited information about the existing farm animal genetic resources, this study will objectively contribute to Tanzanian indigenous chicken genetic resources information in the conservation programmes. Considering the limitations of information regarding population structure and production performances in Tanzanian farm animals in conservation plans, genetic information obtained from the assessment of Tanzanian chickens could be a starting point towards the conservation of farm animal genetic resources, as stipulated in the Livestock Sector Development Strategy (LSDS) for implementing the National Livestock Policy (NLP) of 2006 (MLD, 2006; URT, 2010; MLFD, 2011; Njombe, 2013). This information will be useful to policy makers and other stakeholders in spearheading planning and decision-making towards livestock development and genetic resources conservation strategies. Furthermore, by re-sampling the same ecotypes and analyzing their diversity with same set of molecular markers, monitoring of development and trend of genetic variation in these chicken populations could be established.

Tanzanian indigenous chickens have been found to be distantly related to commercial chickens. Tanzanian chickens clustered at the middle of the Neighbor-Net, while commercial purebred layer lines formed the outstanding clusters of the total spectrum separating White layers from Brown layers. Although both types of lines have been selected for high laying performances, they are very distinct from each other according to their different breed origins, while broilers cluster is more inside the spectrum of diversity.

In this study of global chicken diversity, the sampling of 101 chicken populations across the regions of Africa, Asia, and Europe assumed a fair representation for a global diversity assessment of domesticated chicken using multilocus microsatellite genotypes. With the exception of South America, all the diffusion roots of chicken dispersion to the Europe and Africa from Asia have been represented.

Overall, the study gives an insight of global chicken diversity using 29 microsatellite markers. Since high-density SNP arrays have become available for chickens as for other farm animal species, more detailed genome wide characterization of variation across a broad range of populations can be performed. This will facilitate the direct exploitation of the specific genetic structure of a global diversity in domesticated chickens and allow future management of the breeds to be based on an improved knowledge of their genetic structure and the relationships between breeds for conservation and breeding purposes.

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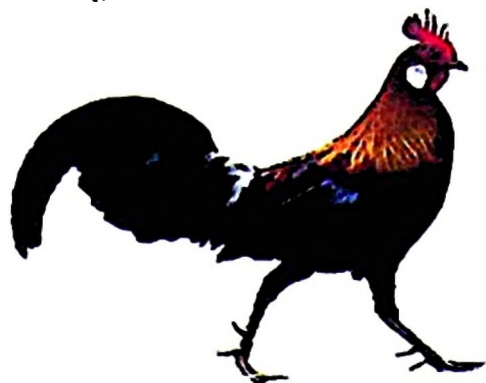
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## APPENDICES

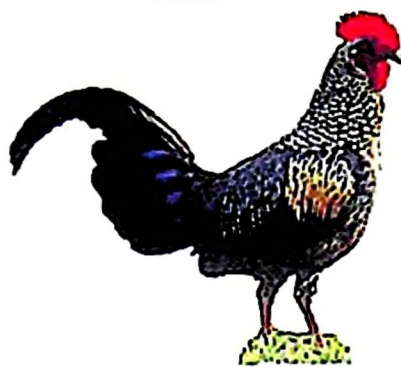
## Appendix 1: Physical appearance of four wild species of junglefowls

*Gallus gallus*

Male



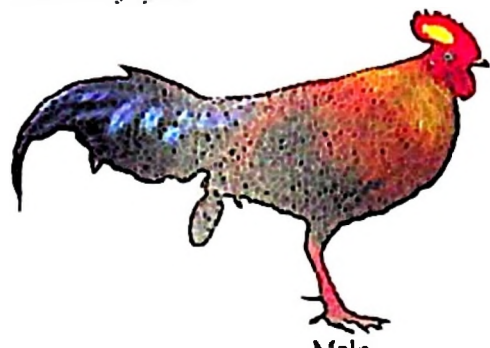
Female

*Gallus sonneratii*

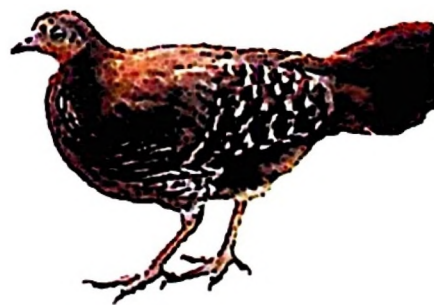
Male



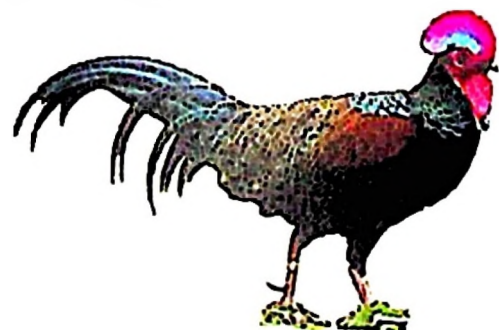
Female

*Gallus lafayetii*

Male



Female

*Gallus varius*

Male



Female

Sources: Krishnappa (2006), Garvie (2009), Tan (2010), Vasanthan (2011), Silva (2011), Sindagi (2013), Cabedo (2013)

Appendix 2. Breed classifications of the fifty-five European chicken populations

| SN | Common breed name                              | ID   | Sampling region | Breed Classification     |
|----|------------------------------------------------|------|-----------------|--------------------------|
| 1  | Appenzeller <sup>*</sup>                       | APP  | Great Britain   | Northwest European breed |
| 2  | Bergische Krahler <sup>*</sup>                 | BK   | Germany         | Northwest European breed |
| 3  | Bergische Schlotterkamme <sup>*</sup>          | BS   | Germany         | Northwest European breed |
| 4  | Brabanter <sup>*</sup>                         | BB   | Germany         | Northwest European breed |
| 5  | Brakel <sup>*</sup>                            | BRA  | Germany         | Northwest European breed |
| 6  | Deutsche Sperber <sup>*</sup>                  | DSP  | Germany         | Northwest European breed |
| 7  | Friesenhuhn <sup>*</sup>                       | FRH  | Germany         | Northwest European breed |
| 8  | Hamburger Lackhuhn <sup>*</sup>                | HLH  | Germany         | Northwest European breed |
| 9  | Hamburger Spierkel <sup>*</sup>                | HSP  | Germany         | Northwest European breed |
| 10 | Black Hamburg <sup>†</sup>                     | HBL  | Great Britain   | Northwest European breed |
| 11 | Jaerhoens <sup>*</sup>                         | JAE  | Norway          | Northwest European breed |
| 12 | Kruper <sup>*</sup>                            | KRP  | Germany         | Northwest European breed |
| 13 | Lakentelder <sup>*</sup>                       | LAK  | Germany         | Northwest European breed |
| 14 | Old English Pheasant Fowl                      | OEPP | Great Britain   | Northwest European breed |
| 15 | Ostfriesische Mowen <sup>*</sup>               | OFM  | Germany         | Northwest European breed |
| 16 | Polish <sup>†</sup>                            | POL  | Great Britain   | Northwest European breed |
| 17 | Westfälische Fotleger <sup>*</sup>             | WFT  | Germany         | Northwest European breed |
| 18 | Hungarian Speckled <sup>*</sup>                | HS2  | Hungary         | East European breed      |
| 19 | Hungarian White Goldilocks <sup>*</sup>        | HWG  | Hungary         | East European breed      |
| 20 | Hungarian Yellow <sup>*</sup>                  | HY2  | Hungary         | East European breed      |
| 21 | Transylvanian Naked Neck <sup>*</sup>          | TNN  | Hungary         | East European breed      |
| 22 | Favonit <sup>*</sup>                           | FAY  | France          | Mediterranean breed      |
| 23 | Italiener Triesdorf <sup>*</sup>               | ITAT | Germany         | Mediterranean breed      |
| 24 | Leghorn <sup>†</sup>                           | LGH  | Great Britain   | Mediterranean breed      |
| 25 | Padovana <sup>*</sup>                          | PAD  | Italy           | Mediterranean breed      |
| 26 | Paduaner <sup>*</sup>                          | PDU  | Germany         | Mediterranean breed      |
| 27 | Scandinavian Reference population <sup>*</sup> | SCP  | Scandinavia     | Mediterranean breed      |
| 28 | Spanish <sup>†</sup>                           | SPN  | Mediterranean   | Mediterranean breed      |
| 29 | Brahma <sup>†</sup>                            | BRH  | Great Britain   | Asian type               |
| 30 | Buff Orpington <sup>†</sup>                    | BOR  | Great Britain   | Asian type               |
| 31 | Cochin <sup>†</sup>                            | COC  | Great Britain   | Asian type               |
| 32 | Croad Langshans <sup>†</sup>                   | CRL  | Great Britain   | Asian type               |
| 33 | Light Sussex (L. Success) <sup>†</sup>         | SSL  | Great Britain   | Asian type               |
| 34 | Marans <sup>†</sup>                            | MRN  | Great Britain   | Asian type               |
| 35 | Modern Langshans <sup>†</sup>                  | MDL  | Great Britain   | Asian type               |
| 36 | Rhode Island Red <sup>†</sup>                  | RIR  | Great Britain   | Asian type               |
| 37 | Silkie <sup>†</sup>                            | SLK  | Great Britain   | Asian type               |
| 38 | Sussex <sup>†</sup>                            | SSS  | Great Britain   | Asian type               |
| 39 | Dark and Jubilee Indian Game <sup>†</sup>      | GDJI | Great Britain   | Game birds               |
| 40 | Indian Cornish Game <sup>†</sup>               | GCOR | Great Britain   | Game birds               |
| 41 | Malay <sup>†</sup>                             | MLY  | Great Britain   | Game birds               |
| 42 | Old English Game <sup>†</sup>                  | GOE  | Great Britain   | Game birds               |
| 43 | Araucana <sup>†</sup>                          | ARC  | Great Britain   | Intermediate type        |
| 44 | Buff Lincolnshire <sup>†</sup>                 | BLS  | Great Britain   | Intermediate type        |
| 45 | Derbyshire Redcap <sup>†</sup>                 | DRC  | Great Britain   | Intermediate type        |
| 46 | Dorking <sup>†</sup>                           | DRK  | Great Britain   | Intermediate type        |
| 47 | Icelandic Landrace <sup>*</sup>                | ICL  | Iceland Island  | Intermediate type        |
| 48 | Ixworth <sup>†</sup>                           | IXW  | Great Britain   | Intermediate type        |
| 49 | Marsh Daisy <sup>†</sup>                       | MIR  | Great Britain   | Intermediate type        |
| 50 | Norfolk Grey <sup>†</sup>                      | NG   | Great Britain   | Intermediate type        |
| 51 | Ramelsloher <sup>*</sup>                       | RML  | Germany         | Intermediate type        |
| 52 | Rheinlander <sup>*</sup>                       | RHL  | Germany         | Intermediate type        |
| 53 | Scots Dumpy <sup>†</sup>                       | SCD  | Great Britain   | Intermediate type        |
| 54 | Scots Grey <sup>†</sup>                        | SCG  | Great Britain   | Intermediate type        |
| 55 | Vorwerk <sup>*</sup>                           | VOR  | Germany         | Intermediate type        |

\* Samples obtained from AVIANDIV project (AVIAN, 2000) and

† Samples obtained from UK (Wilkinson et al., 2011)

Appendix 3: Source of data, sampling country, breed history, geographical distances from Southeast Asia (SEA), core set contributions, genetic diversity between and average (K=3) cluster membership coefficients (CMC) for 113 chicken populations

| S/N | Sampled population name   | Label | Sampling Country | Country of history of breed origin | Population Management | Sample size | H <sub>d</sub> ± std | H <sub>d</sub> std | lnNA1std  | F <sub>ST</sub> | CMC1  | CMC2  | CMC3  | MEK    | c(i)   | Previous study references |
|-----|---------------------------|-------|------------------|------------------------------------|-----------------------|-------------|----------------------|--------------------|-----------|-----------------|-------|-------|-------|--------|--------|---------------------------|
| 1   | African populations       | CHW   | Tanzania         | Tanzania                           | Field                 | 26          | 0.62±0.027           | 0.65±0.017         | 5.41±2.25 | -0.0609         | 0.023 | 0.727 | 0.155 | 0.1551 | 0.0127 | Lyimo et al. (2013)       |
| 2   | Ching we're               | UCH   | Tanzania         | Tanzania                           | Field                 | 30          | 0.58±0.034           | 0.55±0.017         | 5.1±2.05  | 0.0275          | 0.403 | 0.436 | 0.161 | 0.1654 | 0.0071 | Lyimo et al. (2013)       |
| 3   | Kuchi                     | MA    | Malawi           | Malawi                             | Field                 | 30          | 0.63±0.028           | 0.58±0.017         | 5.48±2.30 | 0.0719          | 0.141 | 0.705 | 0.153 | 0.1536 | 0.0283 | Muchadevari et al. (2007) |
| 4   | Morogoro                  | MORO  | Tanzania         | Tanzania                           | Field                 | 29          | 0.60±0.026           | 0.58±0.017         | 5.09±2.63 | 0.0365          | 0.059 | 0.693 | 0.205 | 0.1572 | 0.0102 | Lyimo et al. (2013)       |
| 5   | Pemba                     | PEMB  | Tanzania         | Tanzania                           | Field                 | 30          | 0.65±0.038           | 0.67±0.036         | 6.00±2.80 | -0.0291         | 0.373 | 0.403 | 0.223 | 0.1546 | 0.0101 | Lyimo et al. (2013)       |
| 6   | Unjua                     | UNJG  | Tanzania         | Tanzania                           | Field                 | 30          | 0.67±0.027           | 0.62±0.016         | 6.78±3.24 | 0.0643          | 0.478 | 0.277 | 0.245 | 0.1431 | 0.0115 | Lyimo et al. (2013)       |
| 7   | Large Baladi              | BAL   | Sudan            | Sudan                              | Field                 | 30          | 0.56±0.035           | 0.51±0.017         | 5.07±2.2  | 0.0877          | 0.063 | 0.282 | 0.655 | 0.1745 | 0.0068 | Berima et al. (2013)      |
| 8   | Bare neck                 | BN    | Sudan            | Sudan                              | Field                 | 30          | 0.56±0.027           | 0.54±0.017         | 5.28±2.42 | 0.0286          | 0.069 | 0.252 | 0.679 | 0.1683 | 0.0053 | Berima et al. (2013)      |
| 9   | Behtol                    | BT    | Sudan            | Sudan                              | Field                 | 30          | 0.57±0.029           | 0.53±0.017         | 4.79±1.80 | 0.0513          | 0.061 | 0.256 | 0.643 | 0.1659 | 0.0093 | Berima et al. (2013)      |
| 10  | Large Beladi              | LB    | Sudan            | Sudan                              | Field                 | 30          | 0.56±0.023           | 0.49±0.017         | 5.14±2.26 | 0.1259          | 0.046 | 0.330 | 0.625 | 0.1697 | 0.0084 | Berima et al. (2013)      |
| 11  | Mixed chickens from field | EC    | South Africa     | South Africa                       | Field                 | 26          | 0.69±0.030           | 0.64±0.038         | 5.72±2.81 | 0.0642          | 0.194 | 0.712 | 0.094 | 0.1591 | 0.0096 | Mtsheni et al. (2011)     |
| 12  | Koeloe (consrv)           | KKC   | South Africa     | South Africa                       | Consrv                | 29          | 0.53±0.035           | 0.52±0.017         | 4.00±1.65 | 0.0056          | 0.063 | 0.857 | 0.080 | 0.1693 | 0.0072 | Mtsheni et al. (2011)     |
| 13  | Hooked neck (consrv)      | HNC   | South Africa     | South Africa                       | Consrv                | 29          | 0.57±0.026           | 0.59±0.017         | 3.72±1.13 | -0.0455         | 0.051 | 0.934 | 0.015 | 0.1496 | 0.0112 | Mtsheni et al. (2011)     |
| 14  | Ovamba (consrv)           | OVC   | South Africa     | South Africa                       | Consrv                | 26          | 0.62±0.026           | 0.59±0.018         | 4.86±1.87 | 0.0405          | 0.099 | 0.600 | 0.300 | 0.1626 | 0.0092 | Mtsheni et al. (2011)     |
| 15  | Ovamba (field)            | OVP   | South Africa     | South Africa                       | Field                 | 30          | 0.67±0.022           | 0.61±0.017         | 6.00±2.83 | 0.0892          | 0.242 | 0.645 | 0.112 | 0.1643 | 0.0082 | Mtsheni et al. (2011)     |
| 16  | Venda (consrv)            | VC    | South Africa     | South Africa                       | Consrv                | 30          | 0.51±0.027           | 0.50±0.017         | 3.52±1.09 | 0.0000          | 0.043 | 0.898 | 0.059 | 0.1533 | 0.0109 | Mtsheni et al. (2011)     |
| 17  | Venda (field)             | VF    | South Africa     | South Africa                       | Field                 | 30          | 0.67±0.024           | 0.61±0.017         | 5.97±1.30 | 0.0841          | 0.137 | 0.737 | 0.175 | 0.1640 | 0.0083 | Mtsheni et al. (2011)     |
| 18  | Zimbabwe 1                | ZIM1  | Zimbabwe         | Zimbabwe                           | Field                 | 30          | 0.64±0.026           | 0.59±0.017         | 6.07±2.28 | 0.0734          | 0.132 | 0.667 | 0.201 | 0.1682 | 0.0077 | Muchadevari et al. (2007) |
| 19  | Zimbabwe 2                | ZIM2  | Zimbabwe         | Zimbabwe                           | Field                 | 30          | 0.65±0.027           | 0.63±0.017         | 5.66±2.72 | 0.0478          | 0.110 | 0.713 | 0.157 | 0.1720 | 0.0067 | Muchadevari et al. (2007) |
| 20  | Zimbabwe 3                | ZIM3  | Zimbabwe         | Zimbabwe                           | Field                 | 30          | 0.64±0.028           | 0.57±0.017         | 5.59±2.69 | 0.1040          | 0.096 | 0.795 | 0.110 | 0.1733 | 0.0063 | Muchadevari et al. (2007) |
| 21  | Zimbabwe 4                | ZIM4  | Zimbabwe         | Zimbabwe                           | Field                 | 30          | 0.66±0.025           | 0.59±0.017         | 6.00±1.2  | 0.0915          | 0.155 | 0.662 | 0.182 | 0.1667 | 0.0081 | Muchadevari et al. (2007) |
| 22  | Zimbabwe 5                | ZIM5  | Zimbabwe         | Zimbabwe                           | Field                 | 30          | 0.66±0.024           | 0.61±0.017         | 5.90±1.14 | 0.0715          | 0.134 | 0.761 | 0.103 | 0.1638 | 0.0084 | Muchadevari et al. (2007) |
| S/N | Asian populations         |       |                  |                                    |                       | 645         |                      |                    |           |                 |       |       |       | 0.1627 | 0.0089 |                           |
| 1   | Baier                     | BAI   | China            | China                              | Consrv                | 30          | 0.54±0.032           | 0.49±0.017         | 4.17±2.27 | 0.0857          | 0.945 | 0.039 | 0.016 | 0.1450 | 0.0107 | Granevite et al. (2009)   |
| 2   | Chahua                    | CHA   | China            | China                              | Consrv                | 30          | 0.55±0.041           | 0.48±0.017         | 4.45±2.15 | 0.1164          | 0.900 | 0.037 | 0.074 | 0.1358 | 0.0136 | Granevite et al. (2009)   |
| 3   | Dagu                      | DAG   | China            | China                              | Consrv                | 30          | 0.63±0.033           | 0.635±0.016        | 5.07±2.22 | -0.0095         | 0.773 | 0.195 | 0.032 | 0.1424 | 0.0122 | Granevite et al. (2009)   |
| 4   | Dou                       | DOU   | China            | China                              | Consrv                | 30          | 0.53±0.035           | 0.53±0.017         | 3.83±1.83 | 0.0075          | 0.960 | 0.028 | 0.012 | 0.1249 | 0.0152 | Granevite et al. (2009)   |
| 5   | Gushi                     | GUS   | China            | China                              | Consrv                | 30          | 0.44±0.041           | 0.43±0.017         | 3.34±1.37 | 0.0183          | 0.939 | 0.049 | 0.011 | 0.1647 | 0.0054 | Granevite et al. (2009)   |
| 6   | Huainan Partridge         | HP    | China            | China                              | Field                 | 30          | 0.62±0.031           | 0.57±0.017         | 5.58±2.86 | 0.0777          | 0.920 | 0.060 | 0.020 | 0.1416 | 0.0114 | AVIANDIV (2000)           |
| 7   | Langshan                  | LAN   | China            | China                              | Field                 | 30          | 0.54±0.033           | 0.61±0.017         | 3.83±1.69 | -0.1373         | 0.860 | 0.119 | 0.021 | 0.1408 | 0.0117 | Granevite et al. (2009)   |
| 8   | Luyuan                    | LUY   | China            | China                              | Consrv                | 30          | 0.58±0.032           | 0.535±0.017        | 4.34±2.02 | 0.0792          | 0.509 | 0.459 | 0.032 | 0.1475 | 0.0109 | Granevite et al. (2009)   |
| 9   | Tibetan                   | TIB   | China            | China                              | Consrv                | 30          | 0.62±0.035           | 0.599±0.017        | 5.34±2.69 | 0.0260          | 0.920 | 0.047 | 0.033 | 0.1476 | 0.0105 | Granevite et al. (2009)   |
| 10  | Wannan Three-yellow       | WTY   | China            | China                              | Field                 | 30          | 0.65±0.027           | 0.609±0.017        | 6.24±3.17 | 0.0537          | 0.867 | 0.107 | 0.025 | 0.1356 | 0.0131 | AVIANDIV (2000)           |
| 11  | Wuzhu                     | WUZ   | China            | China                              | Consrv                | 30          | 0.58±0.033           | 0.572±0.017        | 4.31±1.91 | 0.0155          | 0.915 | 0.066 | 0.019 | 0.1429 | 0.0171 | Granevite et al. (2009)   |
| 12  | Xianju                    | XIA   | China            | China                              | Consrv                | 30          | 0.54±0.035           | 0.494±0.017        | 3.97±2.11 | 0.0784          | 0.916 | 0.039 | 0.025 | 0.1683 | 0.0053 | Granevite et al. (2009)   |
| 13  | Xiaoshan                  | XIS   | China            | China                              | Consrv                | 30          | 0.61±0.023           | 0.602±0.017        | 4.45±1.90 | 0.0099          | 0.509 | 0.457 | 0.034 | 0.1407 | 0.0126 | Granevite et al. (2009)   |
| 14  | You                       | YOU   | China            | China                              | Consrv                | 30          | 0.56±0.027           | 0.578±0.017        | 4.28±1.67 | -0.0377         | 0.723 | 0.260 | 0.018 | 0.1430 | 0.0114 | Granevite et al. (2009)   |
| 15  | Ac                        | A     | Vietnam          | Vietnam                            | Field                 | 30          | 0.61±0.034           | 0.606±0.017        | 5.41±2.37 | 0.0033          | 0.973 | 0.013 | 0.014 | 0.1186 | 0.0169 | Cuc et al. (2010)         |
| 16  | Choi                      | C     | Vietnam          | Vietnam                            | Field                 | 30          | 0.62±0.036           | 0.641±0.016        | 5.57±2.77 | -0.0772         | 0.954 | 0.021 | 0.025 | 0.1311 | 0.0139 | Cuc et al. (2010)         |

| 17 | Dong Tao                   | DT   | Vietnam | Vietnam          | Field | 30  | 0.58±0.035 | 0.545±0.017 | 5.24±2.28 | 0.0538 | 0.967 | 0.017 | 0.017 | 0.1516 | 0.0085 | Cuc et al. (2010)    |
|----|----------------------------|------|---------|------------------|-------|-----|------------|-------------|-----------|--------|-------|-------|-------|--------|--------|----------------------|
| 18 | H'mong                     | HM   | Vietnam | Vietnam          | Field | 30  | 0.66±0.028 | 0.635±0.016 | 6.45±2.98 | 0.0335 | 0.936 | 0.017 | 0.017 | 0.1318 | 0.0139 | Cuc et al. (2010)    |
| 19 | Ho                         | HO   | Vietnam | Vietnam          | Field | 30  | 0.62±0.034 | 0.564±0.017 | 5.72±2.66 | 0.0889 | 0.964 | 0.018 | 0.018 | 0.1446 | 0.0104 | Cuc et al. (2010)    |
| 20 | Luong Phuong               | LP   | Vietnam | China            | Cons  | 30  | 0.63±0.023 | 0.659±0.016 | 5.21±2.23 | 0.0337 | 0.116 | 0.819 | 0.045 | 0.1497 | 0.0116 | Cuc et al. (2010)    |
| 21 | Mia                        | M    | Vietnam | Vietnam          | Field | 30  | 0.65±0.033 | 0.693±0.017 | 6.62±3.48 | 0.0666 | 0.968 | 0.016 | 0.016 | 0.1382 | 0.012  | Cuc et al. (2010)    |
| 22 | H'mong                     | MONG | Vietnam | Vietnam          | Field | 30  | 0.67±0.03  | 0.62±0.016  | 6.59±3.31 | 0.0732 | 0.933 | 0.050 | 0.027 | 0.1346 | 0.0138 | Grasse et al. (2009) |
| 23 | Ri                         | R    | Vietnam | Vietnam          | Field | 30  | 0.65±0.031 | 0.605±0.017 | 6.97±3.65 | 0.0643 | 0.933 | 0.036 | 0.031 | 0.1526 | 0.009  | Cuc et al. (2010)    |
| 24 | Te                         | T    | Vietnam | Vietnam          | Field | 30  | 0.64±0.029 | 0.592±0.017 | 5.55±2.32 | 0.0750 | 0.903 | 0.051 | 0.047 | 0.1482 | 0.0105 | Cuc et al. (2010)    |
| 25 | Tam Hoang                  | TH   | Vietnam | China            | Cons  | 30  | 0.63±0.023 | 0.608±0.017 | 4.45±1.7  | 0.0320 | 0.784 | 0.116 | 0.101 | 0.1522 | 0.0092 | Cuc et al. (2010)    |
| 26 | Tau Yang                   | TV   | Vietnam | Vietnam          | Field | 30  | 0.69±0.022 | 0.555±0.017 | 6.31±3.04 | 0.2003 | 0.725 | 0.236 | 0.039 | 0.1412 | 0.012  | Cuc et al. (2010)    |
| 27 | European populations       |      |         |                  |       | 780 |            |             |           |        |       |       |       | 0.1431 | 0.0115 |                      |
| 1  | Beldoun                    | BED  | Israel  | Israel           | Field | 30  | 0.59±0.035 | 0.586±0.017 | 4.81±2.39 | 0.002  | 0.169 | 0.152 | 0.679 | 0.1637 | 0.009  | Grasse et al. (2009) |
| 2  | Padovana                   | PDV  | Italy   | Italy            | Field | 30  | 0.40±0.043 | 0.349±0.016 | 2.83±1.28 | 0.134  | 0.010 | 0.043 | 0.947 | 0.1769 | 0.0073 | Grasse et al. (2009) |
| 3  | Prat                       | PH   | Spain   | Spain            | Field | 30  | 0.43±0.039 | 0.40±0.017  | 3.38±1.52 | 0.134  | 0.149 | 0.458 | 0.393 | 0.1733 | 0.0067 | AVIAN DIV (2000)     |
| 4  | Denizli                    | D    | Turkey  | Turkey           | Field | 30  | 0.48±0.041 | 0.454±0.017 | 3.45±1.21 | 0.052  | 0.059 | 0.185 | 0.757 | 0.1834 | 0.0036 | AVIAN DIV (2000)     |
| 5  | Genç                       | G    | Turkey  | Turkey           | Field | 30  | 0.36±0.033 | 0.344±0.016 | 2.66±1.01 | 0.044  | 0.017 | 0.027 | 0.957 | 0.1882 | 0.0056 | AVIAN DIV (2000)     |
| 6  | Fayoumi                    | FAY  | France  | Egypt            | Stand | 30  | 0.39±0.045 | 0.365±0.016 | 2.59±1.30 | 0.056  | 0.042 | 0.020 | 0.937 | 0.1395 | 0.0119 | Grasse et al. (2009) |
| 7  | Marais                     | MAR  | France  | France           | Stand | 30  | 0.56±0.026 | 0.499±0.017 | 3.76±1.35 | 0.109  | 0.173 | 0.554 | 0.273 | 0.1482 | 0.0121 | Grasse et al. (2009) |
| 8  | Asteri (Aul)               | ASL  | Germany | India & Pakistan | Stand | 30  | 0.51±0.037 | 0.476±0.017 | 3.67±1.45 | 0.156  | 0.482 | 0.107 | 0.411 | 0.1650 | 0.0078 | Grasse et al. (2009) |
| 9  | Brabant                    | BB   | Germany | Germany          | Stand | 14  | 0.42±0.041 | 0.376±0.024 | 2.77±1.03 | 0.111  | 0.027 | 0.031 | 0.942 | 0.1973 | 0.0017 | Grasse et al. (2009) |
| 10 | Brigische Krieger          | BK   | Germany | Germany          | Stand | 30  | 0.38±0.043 | 0.291±0.015 | 2.72±1.03 | 0.274  | 0.012 | 0.035 | 0.952 | 0.1972 | 0.0026 | Grasse et al. (2009) |
| 11 | Brahma                     | BR   | Germany | China            | Stand | 30  | 0.46±0.039 | 0.427±0.017 | 3.48±1.12 | 0.081  | 0.881 | 0.101 | 0.018 | 0.1477 | 0.0079 | Grasse et al. (2009) |
| 12 | Brakel                     | BRA  | Germany | Belgium          | Stand | 30  | 0.26±0.038 | 0.273±0.014 | 2.77±0.97 | 0.181  | 0.006 | 0.008 | 0.986 | 0.1902 | 0.0058 | Grasse et al. (2009) |
| 13 | Brigische Schläger/Laermie | BS   | Germany | Germany          | Stand | 25  | 0.41±0.039 | 0.38±0.018  | 2.83±0.85 | 0.071  | 0.015 | 0.034 | 0.951 | 0.2052 | 0.0001 | Grasse et al. (2009) |
| 14 | Cochin                     | COC  | Germany | China            | Stand | 27  | 0.55±0.031 | 0.503±0.018 | 3.55±1.27 | 0.080  | 0.229 | 0.759 | 0.011 | 0.1465 | 0.0104 | Grasse et al. (2009) |
| 15 | Deutsche Spelter           | DSP  | Germany | Germany          | Stand | 30  | 0.39±0.038 | 0.32±0.016  | 2.83±1.2  | 0.186  | 0.025 | 0.190 | 0.785 | 0.1907 | 0.003  | Grasse et al. (2009) |
| 16 | Friesenhuhn                | FRH  | Germany | Germany          | Stand | 30  | 0.45±0.045 | 0.476±0.017 | 3.00±1.31 | 0.045  | 0.075 | 0.068 | 0.887 | 0.1821 | 0.0056 | Grasse et al. (2009) |
| 17 | Hamburger Lackhuhn         | HLH  | Germany | Germany          | Stand | 30  | 0.22±0.037 | 0.176±0.013 | 2.45±0.74 | 0.196  | 0.015 | 0.055 | 0.930 | 0.2040 | 0.0012 | Grasse et al. (2009) |
| 18 | Hamburger Sperl            | HSP  | Germany | Germany          | Stand | 24  | 0.33±0.041 | 0.239±0.016 | 2.31±0.93 | 0.269  | 0.011 | 0.015 | 0.973 | 0.1878 | 0.0033 | Grasse et al. (2009) |
| 19 | Italiener reibhühn         | ITAR | Germany | Italy            | Stand | 29  | 0.39±0.038 | 0.321±0.016 | 2.83±1.00 | 0.181  | 0.012 | 0.030 | 0.953 | 0.1898 | 0.0037 | Grasse et al. (2009) |
| 20 | Italiener schwarz          | ITAS | Germany | Italy            | Stand | 30  | 0.41±0.043 | 0.352±0.016 | 2.90±1.18 | 0.133  | 0.014 | 0.026 | 0.960 | 0.1676 | 0.009  | Grasse et al. (2009) |
| 21 | Italiener Friesdorf        | ITAT | Germany | Italy            | Stand | 21  | 0.40±0.042 | 0.371±0.02  | 2.76±1.12 | 0.070  | 0.030 | 0.052 | 0.917 | 0.1820 | 0.0059 | Grasse et al. (2009) |
| 22 | Kastelaner                 | KAS  | Germany | Italy            | Stand | 30  | 0.43±0.039 | 0.357±0.016 | 2.76±0.99 | 0.162  | 0.034 | 0.369 | 0.597 | 0.1502 | 0.0032 | Grasse et al. (2009) |
| 23 | Kurper                     | KRP  | Germany | Germany          | Stand | 17  | 0.46±0.043 | 0.467±0.022 | 2.74±1.21 | 0.002  | 0.027 | 0.035 | 0.924 | 0.1900 | 0.0043 | Grasse et al. (2009) |
| 24 | Lakenfelder                | LAK  | Germany | Germany          | Stand | 30  | 0.43±0.037 | 0.32±0.016  | 2.86±0.92 | 0.257  | 0.036 | 0.059 | 0.875 | 0.1903 | 0.0034 | Grasse et al. (2009) |
| 25 | Line Sarcoma Susceptible   | LSS  | Germany | Italy            | Cons  | 30  | 0.36±0.035 | 0.333±0.016 | 2.66±1.13 | 0.065  | 0.028 | 0.023 | 0.948 | 0.1671 | 0.0039 | Grasse et al. (2009) |
| 26 | Malay                      | MAL  | Germany | India            | Stand | 30  | 0.48±0.038 | 0.439±0.017 | 3.69±1.69 | 0.033  | 0.458 | 0.075 | 0.465 | 0.1865 | 0.004  | Grasse et al. (2009) |
| 27 | New Hampshire              | NH   | Germany | Mixed            | Cons  | 30  | 0.49±0.032 | 0.44±0.017  | 3.13±1.03 | 0.053  | 0.023 | 0.024 | 0.947 | 0.1703 | 0.0066 | Grasse et al. (2009) |
| 28 | Ost-Morven                 | OFM  | Germany | Germany          | Stand | 30  | 0.41±0.04  | 0.353±0.016 | 2.62±0.94 | 0.128  | 0.012 | 0.020 | 0.968 | 0.1863 | 0.0054 | Grasse et al. (2009) |
| 29 | Paduaner                   | PAD  | Germany | Italy            | Stand | 26  | 0.47±0.033 | 0.353±0.017 | 3.31±1.63 | 0.204  | 0.012 | 0.027 | 0.950 | 0.1856 | 0.0035 | Grasse et al. (2009) |
| 30 | Rheinländer                | RHL  | Germany | Germany          | Stand | 30  | 0.39±0.045 | 0.336±0.016 | 2.86±1.03 | 0.145  | 0.031 | 0.042 | 0.927 | 0.1759 | 0.0038 | Grasse et al. (2009) |
| 31 | Rhode Island Red           | RIR  | Germany | Mixed            | Stand | 30  | 0.42±0.04  | 0.402±0.017 | 2.72±1.10 | 0.072  | 0.030 | 0.058 | 0.912 | 0.1553 | 0.0055 | Grasse et al. (2009) |
| 32 | Ramelsloher                | RRL  | Germany | Germany          | Stand | 30  | 0.45±0.036 | 0.359±0.016 | 3.21±1.03 | 0.260  | 0.038 | 0.103 | 0.858 | 0.1970 | 0.0079 | Grasse et al. (2009) |
| 33 | Sundheimer                 | SUN  | Germany | China            | Stand | 30  | 0.45±0.04  | 0.410±0.017 | 2.66±0.97 | 0.049  | 0.147 | 0.313 | 0.039 | 0.1492 | 0.0115 | Grasse et al. (2009) |
| 34 | Thür. Barchthener          | THB  | Germany | Germany          | Stand | 30  | 0.54±0.028 | 0.451±0.017 | 3.97±1.45 | 0.162  | 0.050 | 0.286 | 0.664 | 0.1912 | 0.0038 | Grasse et al. (2009) |
| 35 | Vorwerk                    | VOR  | Germany | Germany          | Stand | 30  | 0.45±0.04  | 0.423±0.017 | 3.21±1.11 | 0.056  | 0.119 | 0.155 | 0.776 | 0.1750 | 0.0055 | Grasse et al. (2009) |

| 36  | Westf. Totiger                           | WFT   | Germany     | Stand   | 30   | 0.40±0.035  | 0.374±0.016 | 2.79±0.9  | 0.182   | 0.013 | 0.036 | 0.951 | 0.1757 | 0.0067 | Grancvitz et al. (2009) |
|-----|------------------------------------------|-------|-------------|---------|------|-------------|-------------|-----------|---------|-------|-------|-------|--------|--------|-------------------------|
| 37  | AB line high                             | ABH   | Netherlands | Select  | 30   | 0.51±0.036  | 0.485±0.017 | 3.41±1.38 | 0.053   | 0.023 | 0.936 | 0.041 | 0.1671 | 0.0079 | Grancvitz et al. (2009) |
| 38  | Jaerthens                                | JAE   | Norway      | Stand   | 30   | 0.31±0.051  | 0.325±0.016 | 2.62±1.4  | -0.035  | 0.012 | 0.036 | 0.952 | 0.1534 | 0.0119 | Grancvitz et al. (2009) |
| 39  | Green-legged Partridge                   | GLP   | Poland      | Stand   | 30   | 0.50±0.03   | 0.439±0.017 | 3.31±1.03 | 0.113   | 0.052 | 0.634 | 0.307 | 0.1507 | 0.011  | Grancvitz et al. (2009) |
| 40  | Orlov (Orloff)                           | ORL   | Russia      | Stand   | 30   | 0.56±0.031  | 0.585±0.017 | 3.72±1.67 | -0.11   | 0.035 | 0.221 | 0.783 | 0.1382 | 0.0139 | Grancvitz et al. (2009) |
| 41  | Scandinavian Ref. Population             | SCP   | Denmark     | Stand   | 30   | 0.46±0.035  | 0.456±0.017 | 3.55±1.40 | 0.015   | 0.015 | 0.015 | 0.955 | 0.1653 | 0.0032 | Grancvitz et al. (2009) |
| 42  | Hungarian Specified Goshawk              | HSG   | Hungary     | Stand   | 30   | 0.56±0.033  | 0.538±0.017 | 3.73±1.21 | 0.031   | 0.002 | 0.735 | 0.122 | 0.1659 | 0.007  | Grancvitz et al. (2009) |
| 43  | Hungarian Specified Hofmeester's variety | HSH   | Hungary     | Stand   | 30   | 0.56±0.033  | 0.552±0.017 | 4.17±1.56 | 0.021   | 0.147 | 0.622 | 0.160 | 0.1832 | 0.0036 | Grancvitz et al. (2009) |
| 44  | Hungarian White Goshawk                  | HWG   | Hungary     | Stand   | 30   | 0.54±0.033  | 0.513±0.017 | 3.93±1.44 | 0.050   | 0.033 | 0.872 | 0.095 | 0.1614 | 0.0032 | Grancvitz et al. (2009) |
| 45  | Hungarian Yellow Goshawk                 | HYG   | Hungary     | Stand   | 30   | 0.50±0.036  | 0.511±0.017 | 3.07±1.16 | -0.020  | 0.015 | 0.939 | 0.046 | 0.1731 | 0.0063 | Grancvitz et al. (2009) |
| 46  | Hungarian Yellow Magyar                  | HYM   | Hungary     | Stand   | 30   | 0.50±0.034  | 0.495±0.017 | 3.03±1.09 | 0.002   | 0.035 | 0.933 | 0.032 | 0.1655 | 0.0073 | Grancvitz et al. (2009) |
| 47  | Goshawk Mx                               | GMx   | Mixed       | Conserv | 30   | 0.58±0.034  | 0.594±0.017 | 4.41±1.86 | -0.022  | 0.040 | 0.901 | 0.059 | 0.1570 | 0.0091 | Grancvitz et al. (2009) |
| 48  | Transylvanian Naked Neck                 | TNN   | Hungary     | Stand   | 30   | 0.51±0.033  | 0.477±0.017 | 3.48±1.62 | 0.156   | 0.049 | 0.793 | 0.152 | 0.1773 | 0.0049 | Grancvitz et al. (2009) |
| 49  | Tri-Belt Goshawk                         | TNBG  | Hungary     | Stand   | 30   | 0.44±0.033  | 0.436±0.017 | 2.93±0.96 | 0.014   | 0.018 | 0.921 | 0.060 | 0.1859 | 0.0032 | Grancvitz et al. (2009) |
| 50  | Tri-Belt Specified Goshawk               | TNBGS | Hungary     | Stand   | 30   | 0.54±0.029  | 0.533±0.017 | 3.74±1.18 | 0.015   | 0.011 | 0.927 | 0.062 | 0.1416 | 0.0137 | Grancvitz et al. (2009) |
| 51  | Tri-Belt Specified Hofmeester's variety  | TNBHS | Hungary     | Stand   | 30   | 0.55±0.032  | 0.581±0.017 | 3.76±1.55 | -0.051  | 0.021 | 0.903 | 0.076 | 0.1671 | 0.0078 | Grancvitz et al. (2009) |
| 52  | Tri-Belt White Goshawk                   | TNBWG | Hungary     | Stand   | 30   | 0.49±0.032  | 0.514±0.017 | 2.93±0.84 | -0.037  | 0.043 | 0.852 | 0.105 | 0.1558 | 0.0096 | Grancvitz et al. (2009) |
| 53  | Iceland Landrace "Landnámshæna"          | ICL   | Iceland     | Field   | 30   | 0.54±0.036  | 0.51±0.017  | 4.17±1.73 | 0.056   | 0.039 | 0.043 | 0.918 | 0.1799 | 0.0062 | Grancvitz et al. (2009) |
| S/N | Commercial purebred                      |       |             |         | 1533 |             |             |           |         |       |       |       | 0.1734 | 0.007  |                         |
| 1   | Broiler dam line A                       | BRDA  | Mixed       | Select  | 30   | 0.58±0.028  | 0.585±0.017 | 3.79±1.03 | -0.0017 | 0.025 | 0.831 | 0.094 | 0.1558 | 0.011  | Grancvitz et al. (2009) |
| 2   | Broiler dam line D                       | BRDD  | Mixed       | Select  | 30   | 0.63±0.023  | 0.614±0.017 | 4.79±1.93 | 0.0192  | 0.059 | 0.884 | 0.057 | 0.1431 | 0.0137 | Grancvitz et al. (2009) |
| 3   | Broiler sire line A                      | BRSA  | Mixed       | Select  | 30   | 0.55±0.035  | 0.576±0.017 | 3.79±1.52 | 0.0384  | 0.042 | 0.907 | 0.051 | 0.1630 | 0.0031 | Grancvitz et al. (2009) |
| 4   | Broiler sire line B                      | BRSB  | Mixed       | Select  | 30   | 0.50±0.042  | 0.475±0.017 | 3.55±1.38 | 0.0443  | 0.050 | 0.801 | 0.149 | 0.1599 | 0.0091 | Grancvitz et al. (2009) |
| 5   | Brown egg layer A                        | BLA   | Mixed       | Select  | 30   | 0.42±0.039  | 0.391±0.017 | 2.91±1.18 | 0.0646  | 0.036 | 0.950 | 0.014 | 0.1674 | 0.0062 | Grancvitz et al. (2009) |
| 6   | Brown egg layer C                        | BLC   | Mixed       | Select  | 30   | 0.39±0.038  | 0.369±0.017 | 2.91±1.11 | -0.0151 | 0.051 | 0.937 | 0.013 | 0.1460 | 0.011  | Grancvitz et al. (2009) |
| 7   | Brown egg layer D                        | BLD   | Mixed       | Select  | 30   | 0.47±0.038  | 0.453±0.017 | 3.73±1.33 | 0.0300  | 0.022 | 0.958 | 0.021 | 0.1590 | 0.0085 | Grancvitz et al. (2009) |
| 8   | White egg layer A                        | WLA   | Italy       | Select  | 30   | 0.34±0.039  | 0.309±0.016 | 2.76±1.27 | 0.0858  | 0.020 | 0.026 | 0.954 | 0.1611 | 0.0094 | Grancvitz et al. (2009) |
| 9   | White egg layer C                        | WLC   | Italy       | Select  | 30   | 0.21±0.039  | 0.219±0.014 | 1.78±0.64 | -0.0130 | 0.037 | 0.014 | 0.939 | 0.1898 | 0.0073 | Grancvitz et al. (2009) |
|     |                                          |       |             |         | 270  | 0.453±0.036 | 0.441±0.017 | 3.78±1.33 | 0.0258  | 0.037 | 0.709 | 0.255 | 0.1606 | 0.0089 | Grancvitz et al. (2009) |
| S/N | Red junglefowls (RJF)                    |       |             |         |      |             |             |           |         |       |       |       |        |        |                         |
| 1   | Gallus gallus gallus                     | RJFG  | Thailand    | Wild    | 30   | 0.66±0.028  | 0.630±0.017 | 5.76±2.52 | 0.018   | 0.792 | 0.063 | 0.140 | 0.1040 | 0.0218 | Grancvitz et al. (2009) |
| 2   | Gallus gallus spadiceus                  | RJFSC | China       | Wild    | 30   | 0.54±0.033  | 0.536±0.017 | 3.79±1.37 | 0.004   | 0.846 | 0.035 | 0.119 | 0.1205 | 0.017  | Grancvitz et al. (2009) |
| 3   | Gallus gallus spadiceus                  | RJFST | Thailand    | Wild    | 26   | 0.64±0.031  | 0.578±0.016 | 4.77±1.75 | 0.094   | 0.907 | 0.055 | 0.038 | 0.1142 | 0.0184 | Grancvitz et al. (2009) |
|     |                                          |       |             |         | 86   |             |             |           |         |       |       |       | 0.1129 | 0.019  |                         |

CMC is the cluster membership coefficients

MEK is the Marker-estimated Kinship

c(i) is the relative contribution to the core set for breed i

H<sub>1</sub>, H<sub>2</sub>, MNA and F<sub>1</sub> have been defined at the footnote of table 1

Population management category: Field = No management or selection; Conserv = Conserved flock; Stand = Standard breed and Select = Selected for quantitative traits

Appendix 4: Estimated and observed allele frequencies, correlation coefficients and estimated null allele frequency based in EM algorithm (Dempster *et al.* 1977) for 29 microsatellite markers used in analysing 113 chicken populations

| Locus | Marker  | Estimated       | Observed        | Corr. coeff. of estimated and observed allele freq. | Estimated frequency of null allele ( <i>r</i> ) |
|-------|---------|-----------------|-----------------|-----------------------------------------------------|-------------------------------------------------|
| 1     | MCW0103 | 0.3353 ± 0.0177 | 0.3353 ± 0.0177 | 0.99370                                             | 0.031                                           |
| 2     | MCW0295 | 0.1735 ± 0.0084 | 0.1735 ± 0.0084 | 0.98360                                             | 0.047                                           |
| 3     | MCW0222 | 0.2609 ± 0.0135 | 0.2609 ± 0.0135 | 0.98852                                             | 0.038                                           |
| 4     | ADL0268 | 0.2007 ± 0.0089 | 0.2007 ± 0.0089 | 0.99183                                             | 0.025                                           |
| 5     | MCW0183 | 0.1743 ± 0.0085 | 0.1743 ± 0.0085 | 0.98347                                             | 0.044                                           |
| 6     | MCW0014 | 0.2205 ± 0.0120 | 0.2205 ± 0.0120 | 0.99092                                             | 0.039                                           |
| 7     | MCW0067 | 0.2336 ± 0.0106 | 0.2336 ± 0.0106 | 0.99386                                             | 0.026                                           |
| 8     | MCW0098 | 0.3363 ± 0.0182 | 0.3363 ± 0.0182 | 0.99554                                             | 0.023                                           |
| 9     | LEI0166 | 0.2410 ± 0.0116 | 0.2410 ± 0.0116 | 0.99341                                             | 0.032                                           |
| 10    | MCW0069 | 0.1773 ± 0.0084 | 0.1773 ± 0.0084 | 0.99604                                             | 0.020                                           |
| 11    | MCW0081 | 0.2240 ± 0.0120 | 0.2240 ± 0.0120 | 0.99514                                             | 0.027                                           |
| 12    | ADL0112 | 0.2360 ± 0.0117 | 0.2360 ± 0.0117 | 0.99346                                             | 0.028                                           |
| 13    | MCW0034 | 0.1407 ± 0.0058 | 0.1407 ± 0.0058 | 0.99375                                             | 0.023                                           |
| 14    | MCW0111 | 0.1997 ± 0.0096 | 0.1997 ± 0.0096 | 0.99157                                             | 0.034                                           |
| 15    | MCW0078 | 0.2266 ± 0.0115 | 0.2266 ± 0.0115 | 0.99306                                             | 0.029                                           |
| 16    | MCW0206 | 0.1830 ± 0.0084 | 0.1830 ± 0.0084 | 0.99067                                             | 0.033                                           |
| 17    | LEI0094 | 0.1357 ± 0.0059 | 0.1357 ± 0.0059 | 0.97847                                             | 0.043                                           |
| 18    | MCW0248 | 0.2670 ± 0.0153 | 0.2670 ± 0.0153 | 0.99379                                             | 0.027                                           |
| 19    | LEI0234 | 0.1111 ± 0.0045 | 0.1111 ± 0.0045 | 0.97811                                             | 0.049                                           |
| 20    | MCW0330 | 0.2069 ± 0.0100 | 0.2069 ± 0.0100 | 0.99169                                             | 0.032                                           |
| 21    | MCW0016 | 0.1762 ± 0.0082 | 0.1762 ± 0.0082 | 0.98950                                             | 0.028                                           |
| 22    | MCW0104 | 0.1514 ± 0.0075 | 0.1514 ± 0.0075 | 0.99528                                             | 0.021                                           |
| 23    | MCW0020 | 0.2175 ± 0.0095 | 0.2176 ± 0.0094 | 0.99330                                             | 0.024                                           |
| 24    | MCW0165 | 0.2689 ± 0.0124 | 0.2688 ± 0.0124 | 0.97879                                             | 0.055                                           |
| 25    | MCW0123 | 0.1800 ± 0.0091 | 0.1800 ± 0.0091 | 0.99383                                             | 0.027                                           |
| 26    | ADL0278 | 0.2007 ± 0.0098 | 0.2007 ± 0.0098 | 0.99267                                             | 0.033                                           |
| 27    | MCW0037 | 0.2139 ± 0.0099 | 0.2139 ± 0.0099 | 0.98514                                             | 0.041                                           |
| 28    | MCW0080 | 0.1556 ± 0.0075 | 0.1557 ± 0.0075 | 0.99009                                             | 0.038                                           |
| 29    | MCW0216 | 0.2266 ± 0.0118 | 0.2266 ± 0.0118 | 0.99145                                             | 0.034                                           |
|       |         | 0.1971 ± 0.0018 | 0.1971 ± 0.0018 | 0.99105                                             | 0.033                                           |

Appendix 5: Diversity contributions of 104 chicken populations when commercial breed lines were considered safe from extinction

| S/N  | Sampling Region | Sub-region     | Population | Div(S) | c(i)  | d(i)*E-3 |
|------|-----------------|----------------|------------|--------|-------|----------|
| 1    | Wild            | Southeast Asia | RJFG       | 0.842  | 0.456 | 40.817   |
| 2    | Wild            | Southeast Asia | RJFSt      | 0.840  | 0.413 | 39.694   |
| 3    | Wild            | East Asia      | RJFSc      | 0.828  | 0.294 | 27.445   |
| Mean |                 |                |            | 0.837  | 0.388 | 35.985   |
| 4    | Asia            | Southeast Asia | LP         | 0.823  | 0.612 | 22.651   |
| 5    | Asia            | Southeast Asia | TV         | 0.842  | 0.560 | 40.869   |
| 6    | Asia            | Southeast Asia | MONG       | 0.841  | 0.498 | 39.784   |
| 7    | Asia            | Southeast Asia | HM         | 0.839  | 0.470 | 38.591   |
| 8    | Asia            | East Asia      | WTY        | 0.835  | 0.464 | 34.228   |
| 9    | Asia            | Southeast Asia | M          | 0.835  | 0.435 | 33.824   |
| 10   | Asia            | Southeast Asia | R          | 0.828  | 0.429 | 27.171   |
| 11   | Asia            | Southeast Asia | C          | 0.838  | 0.409 | 37.256   |
| 12   | Asia            | East Asia      | DAG        | 0.828  | 0.404 | 27.072   |
| 13   | Asia            | Southeast Asia | A          | 0.842  | 0.401 | 40.941   |
| 14   | Asia            | Southeast Asia | HP         | 0.829  | 0.383 | 28.680   |
| 15   | Asia            | Southeast Asia | HO         | 0.826  | 0.363 | 25.100   |
| 16   | Asia            | Southeast Asia | T          | 0.818  | 0.361 | 17.437   |
| 17   | Asia            | East Asia      | TIB        | 0.828  | 0.356 | 26.842   |
| 18   | Asia            | East Asia      | WUG        | 0.827  | 0.317 | 25.732   |
| 19   | Asia            | East Asia      | XIS        | 0.822  | 0.314 | 21.485   |
| 20   | Asia            | Southeast Asia | TH         | 0.813  | 0.294 | 11.790   |
| 21   | Asia            | East Asia      | DOU        | 0.825  | 0.289 | 24.647   |
| 22   | Asia            | East Asia      | LUY        | 0.816  | 0.273 | 15.130   |
| 23   | Asia            | Southeast Asia | DT         | 0.818  | 0.272 | 17.468   |
| 24   | Asia            | East Asia      | CHH        | 0.824  | 0.272 | 23.418   |
| 25   | Asia            | East Asia      | LAN        | 0.821  | 0.257 | 20.255   |
| 26   | Asia            | East Asia      | YOU        | 0.817  | 0.251 | 16.318   |
| 27   | Asia            | East Asia      | BAI        | 0.817  | 0.250 | 16.345   |
| 28   | Asia            | East Asia      | XIA        | 0.813  | 0.227 | 12.222   |
| 29   | Asia            | East Asia      | GUS        | 0.806  | 0.124 | 4.770    |
| Mean |                 |                |            | 0.826  | 0.357 | 25.001   |
| 30   | Africa          | South Africa   | EC         | 0.830  | 0.588 | 29.278   |
| 31   | Africa          | South Africa   | OVF        | 0.824  | 0.547 | 23.085   |
| 32   | Africa          | East Africa    | UNGJ       | 0.835  | 0.527 | 34.481   |
| 33   | Africa          | South Africa   | VF         | 0.823  | 0.526 | 22.703   |
| 34   | Africa          | South Africa   | ZIMv       | 0.823  | 0.494 | 21.940   |
| 35   | Africa          | South Africa   | ZIMiv      | 0.819  | 0.486 | 17.885   |
| 36   | Africa          | East Africa    | PEMB       | 0.828  | 0.464 | 27.266   |
| 37   | Africa          | South Africa   | ZIMii      | 0.816  | 0.447 | 15.646   |
| 38   | Africa          | South Africa   | ZIMi       | 0.819  | 0.429 | 18.323   |
| 39   | Africa          | South Africa   | ZIMiii     | 0.813  | 0.404 | 12.324   |
| 40   | Africa          | East Africa    | CHIN       | 0.823  | 0.363 | 22.019   |
| 41   | Africa          | East Africa    | MORO       | 0.818  | 0.328 | 17.414   |
| 42   | Africa          | East Asia      | MA         | 0.813  | 0.327 | 12.623   |
| 43   | Africa          | South Africa   | OVC        | 0.815  | 0.318 | 14.311   |
| 44   | Africa          | East Africa    | KUCH       | 0.815  | 0.272 | 14.177   |
| 45   | Africa          | North Africa   | BT         | 0.816  | 0.256 | 15.049   |
| 46   | Africa          | South Africa   | NNC        | 0.815  | 0.254 | 14.187   |
| 47   | Africa          | North Africa   | BN         | 0.814  | 0.247 | 13.460   |
| 48   | Africa          | North Africa   | BAL        | 0.812  | 0.232 | 11.115   |
| 49   | Africa          | North Africa   | LB         | 0.809  | 0.195 | 8.223    |
| 50   | Africa          | South Africa   | VC         | 0.813  | 0.194 | 12.651   |
| 51   | Africa          | South Africa   | KKC        | 0.804  | 0.124 | 3.056    |
| Mean |                 |                |            | 0.818  | 0.365 | 17.328   |

|      |        |                  |       |       |       |        |
|------|--------|------------------|-------|-------|-------|--------|
| 52   | Europe | Mediterranean    | BED   | 0.816 | 0.314 | 14.783 |
| 53   | Europe | East Europe      | ORL   | 0.812 | 0.231 | 10.764 |
| 54   | Europe | Northwest Europe | MAR   | 0.812 | 0.227 | 10.731 |
| 55   | Europe | East Europe      | TNNSG | 0.814 | 0.226 | 13.136 |
| 56   | Europe | Northwest Europe | SCP   | 0.807 | 0.223 | 6.353  |
| 57   | Europe | Northwest Europe | ASI   | 0.814 | 0.219 | 13.222 |
| 58   | Europe | East Europe      | TNNSH | 0.809 | 0.218 | 8.633  |
| 59   | Europe | East Europe      | GLP   | 0.817 | 0.212 | 16.337 |
| 60   | Europe | East Europe      | HSG   | 0.810 | 0.210 | 9.034  |
| 61   | Europe | East Europe      | HWG   | 0.811 | 0.206 | 10.525 |
| 62   | Europe | East Europe      | HSI   | 0.807 | 0.204 | 6.160  |
| 63   | Europe | Northwest Europe | COC   | 0.810 | 0.192 | 8.930  |
| 64   | Europe | East Europe      | NHX   | 0.807 | 0.189 | 5.731  |
| 65   | Europe | Mediterranean    | FAY   | 0.817 | 0.184 | 15.897 |
| 66   | Europe | East Europe      | TNNWG | 0.811 | 0.180 | 10.708 |
| 67   | Europe | East Europe      | HYG   | 0.808 | 0.166 | 7.140  |
| 68   | Europe | Northwest Europe | NH    | 0.808 | 0.165 | 7.260  |
| 69   | Europe | Northwest Europe | MAL   | 0.807 | 0.160 | 6.662  |
| 70   | Europe | Northwest Europe | RIR   | 0.809 | 0.154 | 8.555  |
| 71   | Europe | Northwest Europe | BR    | 0.809 | 0.153 | 8.521  |
| 72   | Europe | Northwest Europe | SUN   | 0.809 | 0.149 | 8.022  |
| 73   | Europe | Northwest Europe | ABH   | 0.805 | 0.149 | 4.539  |
| 74   | Europe | Northwest Europe | PAD   | 0.806 | 0.145 | 5.408  |
| 75   | Europe | Mediterranean    | PH    | 0.806 | 0.135 | 5.705  |
| 76   | Europe | East Europe      | HYM   | 0.805 | 0.133 | 4.135  |
| 77   | Europe | Northwest Europe | FRH   | 0.805 | 0.125 | 4.254  |
| 78   | Europe | East Europe      | JAE   | 0.808 | 0.120 | 6.817  |
| 79   | Europe | Northwest Europe | BK    | 0.806 | 0.116 | 5.428  |
| 80   | Europe | Mediterranean    | D     | 0.804 | 0.112 | 2.788  |
| 81   | Europe | Mediterranean    | G     | 0.805 | 0.102 | 3.909  |
| 82   | Europe | East Europe      | TNN   | 0.803 | 0.102 | 2.318  |
| 83   | Europe | Northwest Europe | THB   | 0.803 | 0.102 | 2.031  |
| 84   | Europe | Northwest Europe | VOR   | 0.803 | 0.098 | 2.585  |
| 85   | Europe | Northwest Europe | ICL   | 0.802 | 0.098 | 1.565  |
| 86   | Europe | Northwest Europe | BB    | 0.804 | 0.097 | 3.414  |
| 87   | Europe | Northwest Europe | ITAS  | 0.804 | 0.097 | 2.776  |
| 88   | Europe | Northwest Europe | LSS   | 0.804 | 0.088 | 3.238  |
| 89   | Europe | Northwest Europe | LAK   | 0.803 | 0.082 | 2.075  |
| 90   | Europe | Northwest Europe | KRP   | 0.803 | 0.081 | 1.726  |
| 91   | Europe | Northwest Europe | RHL   | 0.802 | 0.064 | 1.453  |
| 92   | Europe | Northwest Europe | ITAT  | 0.802 | 0.061 | 1.168  |
| 93   | Europe | Northwest Europe | BRA   | 0.802 | 0.059 | 1.493  |
| 94   | Europe | Mediterranean    | PDV   | 0.802 | 0.058 | 0.994  |
| 95   | Europe | Northwest Europe | WFT   | 0.802 | 0.055 | 0.961  |
| 96   | Europe | East Europe      | TNNBG | 0.801 | 0.051 | 0.692  |
| 97   | Europe | Northwest Europe | HSP   | 0.801 | 0.043 | 0.631  |
| 98   | Europe | Northwest Europe | RML   | 0.801 | 0.041 | 0.429  |
| 99   | Europe | Northwest Europe | ITAR  | 0.801 | 0.036 | 0.379  |
| 100  | Europe | Northwest Europe | HLH   | 0.801 | 0.035 | 0.637  |
| 101  | Europe | Northwest Europe | KAS   | 0.801 | 0.016 | 0.058  |
| 102  | Europe | Northwest Europe | OFM   | 0.801 | 0.006 | 0.007  |
| 103  | Europe | Northwest Europe | DSP   | 0.000 | 0.000 | 0.000  |
| 104  | Europe | Northwest Europe | BS    | 0.000 | 0.000 | 0.000  |
| Mean |        |                  |       | 0.776 | 0.126 | 5.297  |

Different superscript letters in a column indicate significant differences (Tukey's HSD,  $P < 0.05$ )

$Div(S+i)$  is the total diversity of the safe set ( $S$ ) and the population added to the fixed set ( $i$ )

$ct(i)$  is the core set contribution of population  $i$  when added to the fixed set

$d(i)$  is the different genetic diversity between  $Div(S+i)$  and  $Div(S)$

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## CURRICULUM VITAE

### Personal particulars

Name: Charles Moses Lyimo  
Nationality: Tanzanian

### Main research interests

Molecular genetics in farm animals

- Genetic resources conservation in farm animals
- Population genomics

Poultry development programs for smallholder farmers

Livestock production in the tropics

Transfer of agricultural knowledge to the farmers/ interpreneurs

Statistical analyses related to agriculture and molecular genetics



### Academic qualification

- 2010 – 2014: **Ph.D. fellow**, Department of Animal Sciences, Animal Breeding and Genetics Group, Georg-August-Universität, Göttingen in collaboration with the Department of Breeding and Genetic Resources, Institute of Farm Animal Genetics of the Friedrich-Loeffler-Institute, Neustadt - Mariensee, Germany.
- 2000 – 2003: **MSc. Tropical Animal Production**, from Department of animal Science and Productions, Sokoine University of Agriculture (SUA), Tanzania.
- 1994 – 1998: **BSc. Animal Science**, from Department of animal Science and Productions, Sokoine University of Agriculture (SUA), Tanzania.

### Employment/ Working experience

- 2006 – to date: Employed at Sokoine University of Agriculture (SUA). Specialized in Genetics and Biochemistry. Stationed at the Faculty of Science, Department of Biological and Ecological Studies.
- 2004 – 2006: **Livestock tutor II** at Livestock Training Institute – Morogoro, under the Ministry of Water and Livestock Development, Tanzania. Working in the department of Animal Sciences, taught Animal Breeding, Biochemistry, Beef and Dairy cattle, Pig and Poultry production. Conducting Farmers Training, Supervising students in Special Research Projects and Entrepreneurship projects

### Current professional organisation membership

- 2012 – to date: World Poultry Science Association (WPSA)
- 2001 – to date: Tanzania Society for Animal Production (TSAP)
- 2000 – to date: Tanzania Graduate Farmers Association (TGFA) – Among the founder member

### Publications from this work

- Lyimo C.M., Weigend A., Msoffe P.L., Hocking P.M., Simianer H. and Weigend S. (2015). Maternal Genealogical Patterns of Chicken Breeds Sampled in Europe. Manuscript accepted for publication in *Animal Genetics*
- Lyimo C.M., Weigend A., Msoffe P.L., Eding H., Simianer H. and Weigend S. (2014). Global diversity and genetic contributions of chicken populations from African, Asian and European regions. *Animal Genetics* 45(6), 836-848
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