Institute of Animal Breeding and Genetics
Georg-August Universität Göttingen

Assessment of Genetic Diversity of Zimbabwe Village Chicken Eco-types

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ASSESSMENT OF GENETIC DIVERSITY OF ZIMBABWE VILLAGE CHICKEN ECO-TYPES

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DEDICATION

To Edgar Farai and Kumba Damian Dzomba…my two blessings from above

To my mother and father… for the inspiration

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Introduction and Objectives and Hypotheses

Chapter 1

1.1 Introduction

The village chicken production system in Africa is mainly based on scavenging indigenous chickens (Kitalyi, 1998). In Zimbabwe, the village chicken population is estimated at 30 million (Mhlanga *et al.,* 1999; Kusina *et al.,* 2001). These chickens play an integral role in the smallholder farming systems. They are used to meet the multiple household social, economic and cultural needs. Of more importance to the rural communities worldwide is the role of indigenous chickens to biodiversity (Delany, 2003). Village chickens are part of the total poultry genetic diversity that comprises of chickens, turkeys, quails, ducks, goose, guinea fowls and pheasants. This diversity is needed for future advances and improvements in response to changing environments and consumer demands. Genetic variation enables both adaptive evolutionary changes and artificial selection. Local chicken populations are seen as an important genetic reservoir developed over thousands of years and successful in extreme and unusual environments with limited veterinary and management input (Hall and Bradley, 1995). The shift towards free range organic farming systems might see higher dependency on local chicken genotypes that already exist in similar production systems (Hall, 2004). Village chickens might have valuable genetic variation that could be transferred through marker assisted selection and genetic engineering to high performing commercial populations.

Despite their current importance and future potential, very little is known about the genetic composition of local chickens in Zimbabwe and most developing countries. Although village chickens are considered an important genetic reservoir (FAO, 1999; Delany, 2003; Hall, 2004), the genetic diversity contained in these populations and its distribution has not been comprehensively quantified. At present local chickens in Zimbabwe are commonly referred to as ‘village’ or just ‘indigenous’ chickens without differentiating them into any populations. The Zimbabwe chicken population, however, consists of different phenotypic variants raised by communal farmers in five agro-ecological zones (eco-zones) of the country. It is not known whether village chickens found in different eco-zones reflect pronounced population boundaries. In other countries such as Tanzania and Ethiopia, the term ‘eco-types’ has been adopted to describe chickens from different farming systems (Tadelle*,* 2003; Msoffe *et al.,* 2005).

There are several hypotheses why eco-regions should be used to define village chicken population boundaries. Chickens in different agro-ecological zones could have originated

from different founder populations. Geographical isolation of chicken populations in different eco-zones could lead to substructuring as each eco-type experiences different forces of evolution particularly drift, mutation and natural selection. The different climatic, social and economic factors determine the importance and the degree to which village chickens are integrated in contrasting agro-ecological zones. Chicken management is likely to differ between eco-zones depending on farmer production goals. Artificial selection for certain production traits influences the type of chickens that are kept or culled in different agro-ecological zones. In addition to these factors, differences in disease prevalence, nutritional supply and other environmental factors between agro-ecological zones can result in different genotypes being favoured or selected against in contrasting regions. In such instances eco-types would refer to populations adapted to local conditions within the agro-ecological zones.

The characterisation of village chicken populations requires a holistic approach. The production systems (intensive, extensive or semi-intensive) housing village chickens have a major influence on the extent to which they are integrated in farming communities (Steglich and Peters, 2004). The feasibility of breeding programmes and *in situ* conservation programmes depends on whether they are tailor made for the particular production systems. An understanding of the production systems should therefore be coupled with an assessment of the genetic diversity within and between assumed population boundaries. Within population diversity describes the genetic flexibility of a population and how it responds to different selection pressures. Between population diversity reflects the degree to which populations differ. Genetically distinct populations might carry unique genetic features due to unique alleles and allelic combinations.

Microsatellites are codominant, highly polymorphic markers that are commonly used for assessing genome-wide genetic diversity (Baumung *et al.,* 2005; Soller *et al.,* 2006). They are assumed to be neutral to selection and can therefore give an insight into both current and unknown future genetic value of populations. They have been used in many diversity studies and have been found to give reliable estimates of genetic diversity within populations as well as the level of differentiation between breeds (Weigend and Romanov, 2001). As a global initiative, the Food and Agricultural Organisation (FAO) has recommended use of microsatellites to assess genetic diversity in domestic animal genetic resources (FAO, 2004).

Chicken mitochondrial DNA (mtDNA) is maternally inherited (Watanabe *et al.,* 1985). The simple sequence organization, maternal inheritance and absence of recombination make mtDNA an ideal marker for assessing historical genetic structure and the geographical distribution of genetic diversity of populations (Avise *et al.,* 1987; Harrison, 1989). The distribution of mtDNA haplotypes can be used to investigate whether chickens from different agro-ecological zones originated from the same founder population. On the other hand, the rapid rate of sequence divergence allows differentiation of recently diverged lineages.

1.2 Justification

The population structure of the Zimbabwe chickens is not clearly defined. Currently, all the local chickens in Zimbabwe are considered as one population. The question whether different agro-ecological zones define distinct populations needs to be resolved. The recognised role of indigenous animal genetic resources (AnGR) to smallholder farming communities and even commercial agriculture has raised interest in the conservation of these local resources from extinction and displacement. The methods used to prioritise populations for conservation depend on pre-defined existing populations (eg Reist-Marti *et al.,* 2003; Mateus *et al.,* 2004; Simianer *et al.,* 2005) and not individual chickens. It is therefore necessary that accurate population boundaries are drawn. The poor inventory of local chickens in Zimbabwe is a sign of little regard of their value as an important genetic resource. This poses a big threat to local chicken populations because proper conservation and breed improvement programmes can not be initiated. Such a scenario is not unique to Zimbabwe but pertain to most developing countries in Africa and the rest of the world (Weigend and Romanov, 2001). The characterisation of the Zimbabwe local chickens will therefore be an important step towards establishing inventory data that might be used as case study for similar chicken production systems.

1.3 Objectives

The overall goal of this study was to characterise diversity of the local chicken population in Zimbabwe

The specific objectives were to:

(i) Characterise the farming systems in Zimbabwe agro-ecological zones and identify possible threats and opportunities to the existence of local chicken populations.

(ii) Investigate the existence of chicken strains and evaluate the breeding goals and strategies used by village chicken farmers in Zimbabwe.

(iii) Evaluate genetic variability within and between the chicken populations from the five agro-ecological zones of Zimbabwe, and

(iv) Determine the level of population differentiation between Zimbabwe and other village chicken populations from similar extensive systems of production and purebred closed populations.

1.4 Hypotheses

To achieve the goals of this study, the following hypotheses were tested:

(i) There is variation in the production systems across the agro-ecological zones of Zimbabwe. This variation in the climatic and socio-economic factors results in different chicken production goals and influences breeding practises.

(ii) Genetic diversity in the Zimbabwe chickens is high

(iii) The village chicken populations in Zimbabwe are genetically substructured according to agro-ecological zones.

(iv) The Zimbabwe chicken eco-types are a unique population, genetically distinct from other village chickens from similar production systems and from purebred lines.

Identifying and Characterising Genetic Diversity of Extensively Raised Chicken Populations

Literature Review

Chapter 2

2.1 Introduction

In Zimbabwe, almost every household in the communal areas owns local chickens *(Gallus gallus domesticus)*. These village chickens are reared within a mixed crop-livestock farming system (McAinsh *et al.,* 2004; Maphosa *et al.,* 2005). They are used to meet the multiple household objectives that include income generation, food and social security (Kitalyi, 1998, Muchadeyi *et al*., 2005). Indigenous chickens also contribute to genetic diversity (Delany, 2003).

Fewer efforts have been made to characterise and conserve the local chicken populations. In Zimbabwe as in most developing countries there is scant information on village chicken genetic resources. National statistics are based on estimations of human populations in the communal areas.

2.2 Objectives

The overall goal of this article was to review information available on role of chicken genetic resources in Zimbabwe and other developing countries, the village chicken production systems, and the definition of genetic diversity in light of village chicken production systems. The methods with which village chicken diversity can be assessed are discussed. Lastly alternative methods with which priorities are set to conserve chicken genetic resources and the possible conservation programmes are highlighted.

2.3 Role of chickens in the smallholder farming sector

Increasing affluence especially in the developing world is expected to increase meat demand from 200 million tonnes to 327 million tonnes in 2020 (Hall, 2004). There is a shift towards pig (in non-Muslim communities) and poultry meat in both the developed and developing world. The worldwide chicken population is estimated at 1.3 billion with major producers in Sub Saharan Africa being Nigeria and South Africa (FAOSTAT, 2005). Although Zimbabwe is a net exporter (FAOSTAT, 2005), all of the reported chicken meat and eggs are from the commercial sector that makes use of imported genotypes. These commercial hybrids also play an important role in urban areas. Indigenous birds provide the bulk of the poultry meat and egg requirements for the subsistence and smallholder communities (Mhlanga *et al.,* 1999).

There is however, no national censuses on the total meat and egg output from the indigenous chickens. The village chicken population in Zimbabwe is estimated at 15-30 million based on 1 million communal farmers each owning ~20birds (Mhlanga *et al.,* 1999; Kusina *et al.,* 2001). Surveys (Kusina and Kusina, 1999a), and monitoring studies (Pedersen, 2002; Maphosa *et al.,* 2005; Muchadeyi *et al.,* 2005) have revealed that village chickens are a readily available source of protein and income to smallholder communities whose livelihoods depend on farming. The situation is similar in other African countries for example in Ethiopia (Tadelle *et al.,* 2002), Malawi (Gondwe, 2004), Botswana (Badubi *et al.,* 2006) and Ghana (Aboe *et al.,* 2006). Surveys world wide have also shown that village chickens provide meat and eggs for home consumption in the rural communities (Gueye, 2002).

According to Anderson (2003) and Gueye (2002), livestock including chickens are often used as buffers to shield rural households from risks such as food insecurity and cash deficits. The rain-fed agricultural production system leaves a lot of rural households prone to seasonal starvation and malnutrition (Anderson*,* 2003). Village chickens have been shown to offset this seasonality by complementing with other enterprises and providing meat and eggs for consumption (Kitalyi, 1998; Muchadeyi *et al.,* 2004).

Village chicken production is one of the few agricultural enterprises used to address gender issues in developing countries (Kitalyi, 1998; Dolberg and Peterson, 2000). In Kenya, Roberts (1996) observed that women, young males between 6 – 15 years and the elderly (above 65) spend considerable time engaged in livestock activities. Unlike with large animals, women are reported to have more control and decision making powers on chickens (Pedersen, 2002). Muchadeyi *et al.* (2004) observed that the proportion of chickens owned by women and children in Zimbabwe was higher than for any other livestock species. Aboe *et al.* (2006) observed a significant effect of sex of household head on chicken flock sizes, management practises and uses in Ghana. Ngo Thim *et al.,* (2006) and Gondwe (2004) made similar observations in village chicken production systems of Vietnam and Malawi respectively.

2. 4 Chicken production systems

In general, there are three chicken management systems namely intensive, semi-intensive and extensive or free ranging. The socio-economic factors in a community determine the type of management system practised (Sonaiya, 1990).

2.4.1 Intensive system

The intensive system is based on specialized phenotypes (egg or meat producing strains). Flock sizes in this production system are normally in thousands (Appleby *et al.,* 1992). The stocks of chickens contributing to the global production of meat and eggs are managed and designed by a few primary breeders in response to market demands (Delany, 2003). Elite lines of birds are intensively selected for performance traits to create the grand parent or parent lines. The parental lines are then crossed to create commercial lines that are supplied to the market.

The intensive production system is a high input - high output system. To achieve optimum genetic potential, the specialized breeds require quality management and controlled environmental conditions (Sheldon, 2000). In sub-Saharan African countries, 30 percent of the total chicken population is reared under the intensive system of production (Kitalyi, 1998). In Zimbabwe, over 55 percent of the total chicken meat produced comes from the intensive sector (Faranisi, 1995). Most farmers in rural communities cannot meet the standard management practices due to limited physical and capital resources as well as lack of technical knowledge.

The intensive production system is based on a restricted genetic base (Delany, 2003). Information on the features and number of lines involved in the creation of industry populations is not in the public domain. However with the consolidation of breeder companies (Aurthur and Albers, 2003), it is suggested that the number of elite pure lines is on the decrease. The intensity and duration of selection could probably result in loss of genetic variation through loss of alleles. A reduction in population heterogeneity creates selection walls that result in reduced response to future selections (Delany, 2003).

2.4.2 Semi-intensive system

As with the intensive system, the semi-intensive production system is based on specialised breeds. Flock sizes in the semi-intensive system range from 50 to 1000 birds (Sonaiya, 1990; Kitalyi, 1998). More labour is required to manage flocks in the semi-intensive system compared to the intensive system. In both intensive and semi-intensive systems, keeping of

big flocks is a result of research in artificial incubation, nutrient requirements and disease control. The birds are fed on concentrate based feeds that are formulated to meet their specific needs. With the rising feed costs, most producers under this system have resorted to home made rations (Tadelle *et al.,* 2003). Birds are vaccinated against most diseases of economic importance such as Newcastle, Marek's, Infectious Bursal Disease and fowl coryza. Marketing of live birds is common under this system (Gueye, 2002). In areas where markets are a problem, farmers are forced to keep the birds longer and this increases the costs of production by increasing the amount of feed required to keep the birds alive.

A major constraint surrounding the semi-intensive chicken producers is the need to maintain high management practices that are beyond the capacity of most smallholder farming systems. The problem is born out the use of highly selected and less heterogeneous chickens that are imported from other countries. Ideally a less specialised and more flexible breed should be made available to semi-intensive producers. Such a breed will allow the producer to yield profits under the compromised production environment. Although crossbreeding exotic and indigenous breeds has been a solution in other livestock production systems (Mhlanga *et al.,* 1999), the lack of information and access to pure exotic lines has failed many chicken cross breeding programmes. Selection within the local populations has also been suggested (Pedersen, 2002). An inventory and characterisation of the variation within local populations will be of much benefit to any selection programmes.

2.4.3 Extensive or scavenging system

Households keep different poultry species and farm other livestock and crop species under the extensive system of production. Poultry species include chickens, guinea fowls, ducks, geese and turkeys (Sonaiya; 1990; Kitalyi, 1998). In most developing countries, chickens dominate in number and economic contribution (Sonaiya, 1990; Gueye, 2002). Chicken flock sizes range from 4 to 50 birds per household (Sonaiya, 1990; Muchadeyi *et al.,* 2005; Aboe *et al.,* 2006; Abdelqader *et al.,* 2007). Chickens kept are non-descript breeds utilised for both meet and egg production.

Poor management is one characteristic of the extensive system. Smallholder farmers have very few resources that they have to allocate to many enterprises. Housing for these chickens is at a rudimentary stage (Chitate and Guta, 2001). Field surveys have shown cases where no

housing or shelter is provided (Sonaiya, 1990; Ngo Thim *et al.,* 2006). In Zimbabwe, 95 percent of households were found to keep their chickens in poor fowl runs at night, three percent left them to stay in trees or open spaces, while 2 percent were kept in woven baskets (Kusina *et al.,* 2001).

Scavenging is the main feeding system (Gunaratne *et al.,* 1993). Poor understanding of disease epidemiology, poor infrastructure and inadequate diagnostic facilities compound the problem in disease and health control (FAO/IEAE, 2002; Aboe *et al.,* 2006). Interactions of different entities within and among the flocks, such as contacts while scavenging, and transmission of diseases from other poultry species and wild birds (Kitalyi, 1998; Otim-Onapa *et al.,* 2006), limit the development of sound health control programs.

Regardless of its shortcomings, the extensive or scavenging method is considered the most important in smallholder chicken production (Kitalyi, 1998; Hall, 2004). It is a low input production system that allows farmers to produce eggs and meat without resorting to expensive poultry feeds often unavailable to the rural people.

2.5 Biodiversity and its role in chicken production systems

Delany (2003) defined poultry diversity as the total genetic variants found within all domesticated birds. The main categories of poultry genetic resources are experimental research lines, industry stocks, domesticated and feral populations (Delany and Pisent, 1998; Weigend and Romanov, 2001). Of these, breeds and strains that are of economic, scientific and cultural importance to present and future agriculture are referred to as animal genetic resources (AnGR; FAO, 2001). Variation in chicken populations is displayed by many breeds and populations that differ in phenotypic characteristics and exist in different geographical and production systems.

Biodiversity allows for future advances and improvements in response to changing human and animal production needs (Notter, 1999). Variation at the genetic level enables both adaptive evolutionary change and artificial selection (Delany, 2003). Evolutionary changes and selection pressures include, change in consumer preferences, animal welfare concerns

such as housing requirements (Christman, 1998), new disease challenges and demands for a global society (Sheldon, 2000).

2.6 Forces that create, maintain and reduce chicken biodiversity

Genetic diversity relates to variation at the gene level. The diversity in chicken populations was brought about and is maintained by a number of evolutionary forces that include mutation, drift, natural and artificial selection and migration (Falconer and Mackay, 1995).

2.6.1 Drift, mutation and restricted gene flow

The present day chickens are thought to have originated from the Red jungle fowl species (Crawford, 1990; Akashinonomiya *et al.,* 1996; Liu *et al.,* 2006). Variation is observed in chicken populations found in different geographical locations and production systems across the world. It was suggested by Crawford (1990) that the start of domestication and diffusion of chickens from the centers of origin resulted in population substructuring. Discontinuity of interbreeding between subpopulations resulted in populations developing differently through drift and mutation.

2.6.2 Natural and artificial selection and non random mating

In the wild, chicken populations have always been under different natural selection pressures that included climatic stress, nutrition and disease challenges. Subsequent geographic isolation during domestication led to development of distinctive regional types through natural selection for adaptation to local environments and artificial selection to meet regional needs (Crawford, 1990). The human need to derive livelihood from chickens shaped the breeding practices and artificial selection during domestication (Diamond, 2002). In Japan many varieties of domesticated chickens were developed through specialized breeding and artificial selection for ornamental and religious purposes such as cock fighting and long crows (Komiyana *et al.,* 2004).

Most breeds and varieties existing today are from the hen craze era of the 19th century (Crawford, 1990). There was explosive growth of industry and agriculture in Europe and America during this period. Increased selective breeding focused on the cultural value of

chickens and the main objectives were perfecting feather colour and form. The 20th century saw the vast demand in poultry meat and eggs and an understanding of Mendelian genetics. The invention of trap nest facilitated measuring egg production and individual hen performance. Production poultry were first selected and bred as purebreds. This period was associated with many breed companies. Later in the 1930s and 1950s the selection for production traits was through the use of crossbreds at first and later chicken strains (Appleby *et al.,* 1992). This era saw a reduction in number of breeders and breed companies (Crawford, 1990). The number of breeds, varieties and strains used in the food production today has now declined and are represented by very few breed companies (Aurthur and Albers, 2003).

Selection pressures in today's chicken populations vary depending on production systems. Chickens in the intensive system of production are artificially selected for specific production traits that include egg laying and meat production. Egg layers are intensively selected to maximize egg production at reduced production cost (Groen, 2003). Meat lines on the other are under continuous selection for higher feed conversion efficiency resulting in high growth rates (Emmerson, 2003). Uniform performance within flocks is an important aspect in both the egg laying and meat production systems. Chicken flocks are raised in batches of thousands to hundred thousands. Less variability within flocks facilitates scheduling of events such as egg collection, culling and slaughtering. Common to all the intensively managed flocks is the absence or minimum role of natural selection. Chickens are not exposed to the outside environment and disease challenges are minimized by use of vaccination programs and strict bio-security measures.

The scavenging system of production on the other hand is characterized by minimum artificial selection and exposure of birds to various levels of natural selection pressures. The scavenging feed these chickens depend on is scarce and fluctuates with climatic factors, farming system and socio-economic environment (Gunaratne *et al.,* 1993). There is also variation in the extent to which farmers give supplementary feeding (Maphosa *et al.,* 2005). Bio-security measures and other health control programmes are almost non-existent (FAO/IAEA, 2002). As a result, disease challenges are a major selection pressure in village chicken production. Bacterial, viral, fungal, parasitic and nutritional diseases have been observed to be prevalent in Zimbabwe (Kusina *et al.,* 2001; Chitate and Guta, 2001) and most village chicken production systems in Africa (FAO/IAEA, 2002). Due to the influence of physical and biological factors, different disease pathogens exist under varying production

systems. Variations in Newcastle disease epidemiology (Kitalyi, 1998) and in parasite prevalence in village chickens sampled from different climatic regions of Zimbabwe (Mukaratirwa *et al.,* 2000; Poulsen *et al.,* 2000; Permin *et al.,* 2002) are good examples of the differences in selection pressures.

Selection is the differential survival and reproduction of phenotypes that are better suited to the environment or to obtaining mating success (Falconer and Mackay, 1995). The existence of the extensive system of village chicken production under various selection pressures has been attributed to a broad genetic base of locally adapted chickens. As a result village chickens are seen as a reservoir of unique genotypes developed in extreme environments (Hall and Bradley, 1995). Unsuitable and weaker genotypes are selected against whilst new and rare alleles are promoted as production environments fluctuate or vary between farming regions. It is therefore hypothesised that the extensive production system, house unique alleles and allelic combinations important for small-scale production (Delany, 2003).

The village chicken production system is also seen as a reservoir of genotypes, in which village farmers keep unselected birds which exhibit different phenotypic characteristics (Kitalyi, 1998; Pedersen, 2002). According to FAO (2001) the indigenous breeds have co-evolved with particular environments and farming systems and represent an accumulation of both genetic stock and management strategies that are relevant to biodiversity. There is however not much data to confirm the existing management practises and their influences on genetic diversity.

It is feared that diversity in local chickens is under threat (FAO-DAD-is; Weigend and Romanov, 2002). Table 2.1 is Hammond and Leitch's (1995) list of factors accelerating the erosion of livestock biodiversity. While these factors are generalised for all livestock species, Weigend and Romanov (2002) argued that the situation is worse when looking at poultry biodiversity. This is attributed to the hegemony structure of the poultry industry and the poor inventory of chicken and poultry genetic resources compared to other livestock species. Wollny (2003) identified indiscriminate crossbreeding and civil conflicts as the major causes of breed extinction in Africa.

Table 2.1: Factors accelerating the erosion of livestock biodiversity (Hammond and Leitch, 1995)

Factor	Description
Development interventions	Preference given to high-input, high-output breeds developed for benign environments. Commercial interests in donor countries promote use of relatively temperate-adapted breeds and create unrealistic expectations in developing countries
Specialization	Emphasis on a single productive trait, e.g. egg production, leading to exclusion of multipurpose animals
Genetic introgression	Cross-breeding and accidental introgression leading to loss of indigenous breeds
Technology	Machinery replaces work animals
Biotechnology	Cryopreservation equipment that is inadequate to store germplasm of threatened breeds. Artificial insemination and embryo transfer rapidly displace indigenous breeds
Political instability	Can eliminate local breeds owned by vulnerable populations
Natural disaster	Floods, drought and epizootics preferentially affect remote or isolated human and livestock populations

Diversity contributed by indigenous breeds is under threat from high performing exotic breeds that are freely available on the global market (Hall, 2004). The commercial broiler and egg laying industry make use of exotic and highly specialised breeds and supply the bulk of the meat and egg requirements for most developing countries.

2.7 Assessment of biodiversity

A critical point in the utilisation and conservation of animal genetic resources lies in the accurate assessment of the genetic biodiversity within and between populations of interest. There are two ways in which diversity can be measured.

2.7.1 Phenotypic diversity

Phenotypic diversity relates to the expressed genetic diversity and gives a quantitative measure of adaptation of breeds to the environmental aspects (Hall, 2004). Some morphological features that are not associated with adaptation to environment are also used to assess phenotypic diversity. In Ethiopia identification of chicken populations was at first made on the basis of plumage colour to give black (*tiku*), red (*Kei*), grey (*gebsima*) and white (*netch*) (Yami, 1995). Plumage colour has been used in most countries which include Zambia, Tanzania and Zimbabwe (Kitalyi, 1998). Phenotypic diversity is relatively easy to assess. However the phenotypic variation is due to both genetic and environmental effects (Crooijmans *et al.,* 1996). As such this measure will not reflect true genetic diversity (Eding and Laval, 1999). For phenotypic comparisons breeds should be assessed under uniform environment. Such a requirement make measuring phenotypic diversity more demanding as some animals have to be moved from their natural habitat. It is difficult to measure phenotypic diversity in village chicken populations that are raised under the scavenging production system. There are no production records to monitor performance and management practices are not consistent between farms and communities.

2.7.2 Genetic diversity

Genetic diversity refers to variation at the gene or chromosomal level. This diversity can either be expressed if it is at coding zones of the genome or neutral if it is at non-coding zones of the chromosome. Genetic diversity can be assessed using molecular markers. A marker is an identified genome site that exhibit polymophism (Crooijmans *et al.,* 1996). Genetic markers can be classified as either type I if they are associated with genes of known functions or type II if in anonymous genomic regions (O'Brien, 1991). The Type I and Type II markers can further be divided into fingerprint markers and clone or sequence based markers (Dogson *et al.,* 1997).

2.7.2.1 Microsatellites

Microsatellites are short tandem repeats, generally consisting of motifs of 1 to 6 bases. The polymorphic variants are thought to have been generated from unequal crossing over between repeat units during meiosis (Kaeser *et al.,* 1999). DNA slippage and point mutations in the flanking regions are also responsible of generating polymorphic variants. They are amplified by polymerase chain reaction (PCR) using primers in the flanking region on either side of the repeat sequence. Microsatellites are highly polymorphic, abundant and evenly distributed throughout the genome. These properties have made them suitable markers for mapping, paternity testing and population genetics (Weigend and Romanov, 2001). Advantages of microsatellites also include easy detection via PCR and their codominance nature. Microsatellites belong to the clone or sequences based type of markers. This is important because the unique sequence in the genome can be mapped and easily exploited for many genetic applications (Soller *et al.,* 2006).

Initial identification of microsatellites requires laborious screening of libraries or some other method of obtaining sequence information so that primers can be designed. In some cases, the marker information is not transferable between species (Inoue-Muyarama *et al.,* 2001). The availability of primer sets and sequences supplied by the US Poultry Genome Mapping Project and European Avian Diversity (AVIANDIV) Projects make use of microsatellites in chicken diversity studies more feasible (Delany, 2003). Genetic diversity measures using microsatellites yield reliable estimates of variability within and genetic relations between chickens populations (Weigend and Romanov, 2001; Delany 2003). Monolocus microsatellites have been shown to be suitable markers for assessing genetic variation between domestic fowls from different genetic backgrounds (Romanov and Weigend, 2001). Weigend and Romanov (2001) used microsatellites to analyse genetic relationships between various domestic chicken populations and the jungle fowls. Recent studies have shown the suitability of microsatellites for estimation of kinship coefficients in the absence of pedigree data (Toro *et al.,* 2002; Blouin, 2003; Eding and Bennewitz, 2007). Microsatellites have been used to assess genetic diversity of a number of native chicken populations in Africa (for example Tadelle, 2003; Msoffe *et al.,* 2005; Muchadeyi *et al.,* 2005; Olowofeso *et al.,* 2005) and Asia (Ngo Thim *et al.,* 2006; Shahbazi *et al.,* 2006). A major challenge that exist now is integrating these result to compare the population structures in different countries. Different studies have used different markers

and different sample sizes and this complicates between studies comparisons. Under the Measurement of Domestic Animal Diversity (MoDAD) project, FAO has recommended use of microsatellites to generate information on the uniqueness of breeds (FAO, 2004). A new set of 30 recommended markers are found on the website:
http://dad.fao/en/refer/library/guideline/marker.pdf

2.7.2.2 Single nucleotide polymorphism (SNPs)

These are single base pairs variations in DNA**.** They can be found in the coding region of the DNA (synonymous SNPs) or in non-coding regions (non- synonymous SNPs). Some SNPs are thought to have a putative function effect and are referred to as candidate SNPs. rSNPs are in the regulatory region of a gene and will cause a change in gene expression while pSNPs are those that cause a change in the phenotype (Aggrey and Okimoto, 2003). SNPs are a novel and promising marker whose advantages include their codominant nature, occurrence with high frequency such that many marker loci can be developed to generate highly saturated maps (Vignal *et al.,* 2002). They are mainly criticised for their biallelic nature, making them less informative than other types of markers, for example microsatellites. Vignal (2002) argues that this can be compensated by their occurrence at high frequency in most species. SNP frequencies ranging from 1.28 – 1.64 have been reported from a number of chicken diversity studies (reviewed by Soller *et al.,* 2006). SNPs are gaining popularity and a number of reviews have been made on their application to generate population parameters (e.g Jeffrey *et al.,* 2003; Hillel *et al.,* 2007). SNPs can be used in biodiversity studies as single locus or haplotypes that are more stable in time (Soller *et al.,* 2006).

The first chicken genome sequence draft was completed in 2004 (International Chicken Consortium, 2004). The availability of this genome sequences offers many opportunities in the use of SNPs. Based on the available sequence draft about 2.8 SNPs were identified by comparing the red jungle fowl, Chinese silkie, a broiler and layer line (International Chicken polymorphism Map Consortium, 2004). A total of 145 SNPs were observed when 6,952bp regions of non coding genes were sequenced in the AVIANDIV project. The frequency of SNPs observed in this project was higher (1 SNP per every 50bp) than was observed in the International Chicken Polymorphism Map Consortium (reviewed by Soller *et al.,* 2006). A combination of SNPs found in the different regions of the genome (coding non-coding or

regulatory) can be used to evaluate the role of different evolutionary forces in shaping genetic diversity.

2.7.2.3 Mitochondrial DNA sequences

Animal mitochondrial DNA (mtDNA) is gaining an increasingly important role as a genetic marker in population and diversity studies. The mtDNA is a circular molecule of 16, 785bp in size and is inherited maternally (reviewed by Soller *et al.,* 2006). The displacement loop (D-loop) region of the mtDNA contains elements that control the replication of the molecule and is highly polymorphic. The popularity of mtDNA derives, in part, from the relative ease with which clear homologous sequences can be isolated and compared (Watanabe *et al*., 1985; Harrison, 1989). The clonal transmission of the mtDNA sans recombinant noise making it possible to discern discrete maternal lineages in domestic populations (Harrison, 1989; MacHugh and Bradley, 2001). Rapid rate of sequence divergence allows discrimination of recently diverged lineages (Brown *et al.,* 1979). Mitochondrial DNA has been used to study phylo-geographic structure of avian species (Ronald *et al.,* 2003), goats (Luikart *et al.,* 2001), donkeys (Chen *et al.,* 2006). The mtDNA have been used to investigate chicken domestication events (Akishinonomiya *et al.,*1996; Niu *et al.,* 2002; Komiyana *et al.,* 2004; Liu *et al.,* 2006). Unlike the nuclear DNA, inheritance of the mtDNA is purely maternal and may therefore give insights into sex specific evolution and population history (Weigend and Romanov, 2001).

Other molecular markers were used starting in the early nineties but have become less popular as new and more reliable markers were introduced. Early studies using molecular markers were heavily influenced by the availability of methods and laboratory equipment. Availability of expertise, and budget constraints might influence the choice of markers in present day studies. Ideal markers should have codominant expression and should be found in an easily accessible tissue. High degree of polymorphism and random distribution throughout the genome makes markers more informative (Weigend and Romanov, 2002; Bruford *et al.,* 2003). A survey on genetic diversity studies revealed that microsatellites are the most preferred marker in chickens and other livestock species (Baumung *et al.,* 2004). FAO (2004) has recommended that diversity in chickens and other livestock should be assessed using microsatellite markers, making them almost a prerequisite for diversity studies. Other markers such as the mtDNA are not widely used but offer some of the answers to population genetics

and domestication. The completed chicken genome sequence draft offers new opportunities in evaluation of chicken genetic diversity using SNPs.

When resources are available, it should be beneficial to assess genetic diversity using different types of markers that have different modes of inheritance and locations in the genome. Comparing the structure of populations based on different markers will give more insight into the evolutionary forces shaping genetic variation.

2.8 Diversity measures

Generally diversity can be categorised into within and between population measures. There is variation in the diversity indices depending on the types of markers used (Kremer *et al.,* 1998). Diversity measures appropriate for microsatellites and DNA sequences are discussed in this review.

2.8.1 Within population diversity measures

2.8.1.1 Number of alleles

The simplest index is the number of alleles that exists within a given populations (Kremer *et al.,* 1998). Allelic diversity has been considered as the most relevant diversity measure (Petit *et al.,* 1998; Barker, 2001; Foulley and Ollivier, 2006). The high number of alleles implies more variation and more genetic flexibility. The limit to selection response is determined by the initial number of alleles in populations (Toro and Caballero, 2005). Allelic diversity is considered more sensitive to population bottlenecks and can be used to assess fluctuations in effective populations in temporal studies. The parameter is however sensitive to sample size such that the sampling strategies of each study should be taken into consideration before comparing results from different studies. Allelic diversity does not take into consideration the allele frequencies. As a result an inflated figure is observed in the presence of rare alleles. Different markers have different levels of polymorphisms. It is therefore difficult to compare the number of alleles/locus between studies where different microsatellite loci were used. These weaknesses can be overcome by adopting internationally recommended markers such as those suggested in the MoDAD (FAO) project and standard alleles to adjust for allele scoring between laboratories.

2.8.1.2 Gene Diversity

A second and most widely used index is expected heterozygosity or gene diversity (Kremer *et al.,* 1998; Toro and Caballero, 2005). Expected heterozygosity (H_E) is defined as the probability that two variants taken at random in the population are different:

$$H_E = n\left(\frac{1-\sum_{l=1}^{k} p_i^2}{n-1}\right)$$

where *pi* = the frequency of the i^{th} of *k* alleles and *n* = sample size (Nei, 1973). Expected heterozygosity ranges from 0 when there is no heterozygosity to nearly 1 when there are a large number of alleles with equal frequencies. While investigators have tried to relate heterozygosity at molecular markers to key components of fitness, simulations studies have shown that many marker loci (˜200) are needed to get the slightest correlation between heterozygosity and an individual inbreeding coefficient (Balloux *et al.,* 2004).

Gene diversity depends mostly on the frequency of the most frequent alleles. This is a major shortcoming as rare alleles which are an indication of diversity, do not contribute much to heterozygosity indices. When alleles or variants are represented in equal frequencies a direct relationship will be observed between allelic diversity and gene diversity. Effective number of alleles (A_e) measures the number of alleles that give the same H_E. and can be useful when the frequencies of alleles are different (Kremer *et al.,* 1998).

2.8.1.3 Inbreeding coefficient

The inbreeding coefficient (F_{IS}) is another measure used to describe within population diversity particularly for microsatellite markers (Balloux and Moulin, 2002). F_{IS} will measure the correlation of genes within individuals belonging to the same subpopulation:

$$F_{IS} = \frac{\overline{H}_S - \overline{H}_I}{\overline{H}_S}$$

Where H_S = mean expected heterozygosity of a subpopulation; $\overline{H}_I$ = mean observed heterozygosity of individuals within subpopulation (Wright, 1951). Estimated from empirical data, F_{IS} will assess whether there is random mating within samples and will give an indication of whether individuals have been sampled from one or several subpopulations (Balloux and Moulin, 2002).

2.8.1.4 Marker estimated kinship (MEK)

An alternative but less frequently used measure of within population diversity is the degree of relatedness or similarity. Kinship between individuals plays an important role in practical applications of animal genetics. In animal breeding coancestry coefficients are required to estimate genetic parameters and for genetic evaluation (Falconer and Mackay, 1995). According to Caballero and Toro (2000), minimising coancestry between breeding animals increases effective population size and is an effective tool in conserving live animals. Heritability of traits can be estimated by regressing pairwise estimates of phenotypic similarity index against kinship (Blouin, 2003). In a captive population one can reduce inbreeding by choosing mates based on kinship (Cunningham *et al.,* 2001).

Traditionally, coefficients of kinship are calculated from pedigree records. These pedigree records are missing in most village chicken production systems (Kitalyi, 1998). The Marker estimated kinship (MEK) (Eding and Meuwissen, 2001) can be estimated using codominant polymorphic markers such as microsatellites. MEK are estimated from Malecot's similarity index which is defined as the probability that an allele drawn from one individual is the same as an allele randomly drawn from the other individual:

$$S_{ij} = \sum (p_{i,x} p_{j,x})$$

Where $p_{i,x}$ is the x^{th} allele frequency in population i and $p_{i,x}$ if the x^{th} allele frequency in population j (Eding and Meuwissen, 2001). From the similarity index, kinships estimates can be estimated by accounting for the probability of alleles being alike in state. Eding and Meuwissen (2001) presented the weighted log linear model:

$$\log(1 - S_{ij,L}) = \log(1 - f_{ij}) + \log(1 - s_L)$$

where $S_{ij,L}$ is the average similarity between population *i* and *j* for *L* loci and f_{ij} is the kinship coefficient between population *i* and *j* and s_L is the probability of allele identical in state. Under this model the kinship between populations or individuals is expected to be constant over all loci, while the probability of alleles being alike in state is expected to be equal for all pairs of populations (Eding and Bennewitz, 2007). The weighted log linear model accounts for differences in the informativeness of different marker loci. Generally marker estimated kinships give a measure of within and between population diversity and this is an advantage for diversity studies of domestic animals.

2.8.2 Between population diversity measures

2.8.2.1 Wright`s fixation index

When comparing diversity between populations, Wright's F_{ST} statistic (Wright, 1969) provides an overall comparison of the degree to which populations are structured:

$$F_{ST} = \frac{H_T - \overline{H}_I}{\overline{H}_S} = 1 - \frac{\overline{H}_S}{H_T}$$

where $\overline{H}_S$ = mean expected heterozygosity of subpopulations; $\overline{H}_I$ = mean observed heterozygosity of individuals; H_T = expected heterozygosity in the whole population. F_{ST} measures the diversity between breeds that arises when subpopulations are isolated and get fixated for certain alleles (Eding and Laval, 1999). Alternative ways to calculate F_{ST} were suggested by Weir and Cockerham (1984) and Robertson and Hill (1984) who give more weight to rare alleles. The two estimators have been shown to agree when all alleles have equal frequencies (Eding and Laval, 1999). Slatkin's R_{ST} is analogous to the Wright's F_{ST} but assumes the stepwise mutation model of microsatellites. When $F_{ST} = 0$, it means there is no population structure, no differentiation, whilst an F_{ST} of 1 would mean existence of completely differentiated populations.

A major criticism of the F_{ST} as a distance measure is that it is only appropriate when populations differ slightly since F_{ST} never exceeds 1. High mutation rates as observed in

microsatellite markers decreases the probability of identity of two alleles and will deflate F_{ST} values even when populations are divergent (Balloux and Moulin, 2002). F_{ST} values however gives insight into the level of gene flow between populations which is not clearly given by other genetic distance measures (Rousset, 1997). According to Reynolds *et al.* (1983) F_{ST} provides the basis for a measure of genetic distance when divergence is caused by drift.

Although F_{ST} can be assessed between populations, the pairwise "distances" take into account the data of just the two populations involved, not all the data simultaneously. Other genetic distances can quantify the degree to which more than two populations differ simultaneously.

2.8.2.2 Distance methods with biological assumptions

Genetic distances can be categorised into distance with or without underlying biological models. The distances with no biological assumptions or model are also known as geometric distances. Such distances include the Cavalli-Sforza chord distance (Cavalli-Sforza *et al.,* 1967) and Rodgers' distance (Rodgers, 1972). Other distance measures incorporate assumptions about the importance of drift and mutation as forces of change. According to Goldstein *et al.* (1995), the mutation process of microsatellite occur in "stepwise" fashion by adding or deleting one of a series of repeat units. The $\delta\mu^2$ of Goldstein *et al.* (1995) uses a stepwise mutation model (SMM) and were specifically developed for microsatellites. However on simulation Goldstein *et al.* (1995) concluded that their method was better suited for phylogenetic reconstruction of taxa that are sufficiently diverged. Although specifically developed for microsatellites the $\delta\mu^2$ is not commonly used particularly with domestic animals that have not been separated for a long time.

Nei's standard genetic distance (D) is based on a classical mutation-drift model and is given by the formula:

$$D_a = -\ln\left(\frac{J_{xy}}{\sqrt{J_x J_y}}\right)$$

where $J_x = \frac{\sum_i \sum_u X_u^2}{r}$; $J_y = \frac{\sum_i \sum_u Y_u^2}{r}$; and $J_{xy} = \frac{\sum_i \sum_u X_u Y_u}{r}$; X_u = u^{th} allele frequency in population X and Y_u be u^{th} allele frequency in population Y and r = number of loci; 2 (Nei,

1972). The main assumption of Nei's standard genetic distance is that populations are in equilibrium with regard to random drift and mutation (Eding and Laval, 1999). Divergence between populations over time is therefore attributed to mutations accumulated over generations of time. Nei's standard genetic distance is an example of an infinite allele model that assumes that mutations can take any state and are unpredictable. Reynolds' distance (Reynold *et al.,* 1983), which was derived for allozyme data is another infinite alleles model based distance that assumes a primary role for drift:

$$\theta_w = \sqrt{\frac{\sum_i \sum_u (X_u - Y_u)^2}{2\sum_l (1 - \sum_u X_u Y_u)}}$$

where X_u = u^{th} allele frequency in population X and Y_u be u^{th} allele frequency in population Y. Reynolds' reliance on drift is considered not appropriate for microsatellites, that have a mutation rate larger than of allozymes. Reynolds' distance, and its neglect of the importance of mutation, however may work better in some species/populations. In small population there is high potential for genetic drift. Drift is a random process and does not result in ordered distribution of alleles and this fits well with the infinite allele model. Mutation and random drift based models are used more often to calculate genetic distances particularly for intra-species diversity studies.

Genetic distances estimated from polymorphic microsatellite markers have been the most popular method of choice to assess diversity between populations (Toro and Caballero, 2005). According to Laval *et al.* (2000), all distances depend on the number of generations since the divergence of populations and on the effective population size of the breeds. The short divergence times between domestic breeds makes it less reliable to infer true breed phylogeny from distance based trees (Eding and Bennewitz, 2007). No admixture is a major assumption for genetic distance phylogeny (Felsenstein, 1982). This assumption is often violated when dealing with domestic animals. Genetic distances for domestic animals have also been criticised for focusing on between breed diversity and ignoring the most important within breed diversity (Caballero and Toro, 2002; Eding and Bennewitz, 2007).

2.8.3 Clustering analysis

Genetic distance measures and other diversity measure have been criticised for relying on *a priori* groupings of individuals either based on phenotypes or sampling location. A clustering method (Pritchard *et al.,* 2000) constructs genetic clusters from a collection of multilocus genotypes by estimating for each individual the fractions of its genome that belong to each cluster. It is a purely genetic analysis that uses no external information and provides the most direct method of determining population structure (Rosenberg *et al.,* 2002). The clustering involves a Bayesian algorithm computed using Markov Chain Monte Carlo (MCMC) methods to cluster individuals probalistically to inferred populations based on multilocus genotypes without any *a priori* assumption of population affiliation (Pritchard *et al.,* 2000).

Rosenberg *et al.* (2002) suggested that genetically distinct populations can be identified based on how difficult it is to separate them from others. Populations that are easier to separate into clusters with only a small number of markers are considered more distinct. Based on this they suggested that the number of loci required for correct clustering of populations can be used as a way of identifying those that are genetically different**.** Recently a more objective method have been shown that compares solutions from many structure runs and take the most frequent solution as the most probable clustering (Rosenberg, 2004).

The utility of microsatellite data for clustering and assigning individuals to genetic groups was studied using 20 breeds from the AVIANDIV project (Rosenberg *et al.,* 2001). In this study most of the breeds were correctly assigned to their original population with a success rate of 98%. A large scale structure analysis including 2000 chickens from 65 populations further supported the reliability of STRUCTURE based clustering (Hillel *et al.,* 2007).

2.8.4 Nucleotide diversity measures

2.8.4.1 Analysis of molecular variance

Excoffier *et al.,* (1992) came up with a way to partition nucleotide diversity to within and between populations components using the analysis of molecular variance (AMOVA). AMOVA computations and interpretations of results are more or less similar to that of Wright's F-statistics. By defining groups of populations, the user defines a particular genetic structure that will be tested. A hierarchical analysis of variance partitions the total variance in allele or haplotype frequency into covariance components due to within and between

individuals in a subpopulation and between subpopulations. Inferences can therefore be made concerning the level of population substructuring at different population hierarchies.

2.8.4.2 Nucleotide distance measures

The critical area in nucleotide genetic distance measures is the weighing of the differences between sequences. Other problems include the unequal rates of mutations within and between sequences. There are at least 4 main nucleotide genetic distance measures that vary in the handling of transitions and transversions and the variance of mutations rates within sequences.

The proportions of differences between sequences are calculated simply by counting the number of nucleotide differences and dividing by the total length of the sequences. However, Jukes and Cantor (1969) noted that the probability of a second substitution at any nucleotide site increase as the time of divergence between sequences increases but the increase in the count differences is slowed. To correct for this Jukes and Cantor's correction result in divergence of sequences being a logarithmic function of time:

$$D_{jc} = -(\frac{3}{4})\ln(1-(\frac{4}{3})D$$

where D = proportion of differences between sequences (Jukes and Cantor, 1965). This correction factor holds true for short divergence time. Its variance however increases with time making the estimation unreliable as the distances between sequences exceed 0.75. To counter this problem, Tajima (1993) suggested a modified estimator that uses a series expansion versus Jukes and Cantor's logarithmic function:

$$D_{jc} = \sum_{i=1}^{k} \frac{k^{(i)}}{i(\frac{3}{4})^{i-1_{n^{(i)}}}} \quad where\ k^{(i)} = k! \big/ (k-1)!\ and\ n^{(i)} = n! \big/ (n-1)!$$

where k = number of nucleotide differences and n = length of sequence (Tajima, 1993).

Even the modification of Jukes and Cantors estimate by Tajima is deemed unreliable due to their failure to correct for the differences in rates of transitions and transversions. The Kimura-2-parameter model established by Kimura (1980) factors in rates of transitions and of transversions in the nucleotide distance measure:

$$D_{K2p} = -(\frac{1}{2})\ln(1-2P-Q) - (\frac{1}{4})\ln(1-2Q)$$

where P is the transitional differences and Q is the transversions differences. Hasegawa *et al* (1985) suggested a model that Tamura and Nei (1993) have extended. This modifies the Kimura-2-parameter model by giving different weights to purine transitions and pyrimidine transitions. This correction factor is called the Tamura-Nei correction.

Regardless of the correction factor used an additional problem arises if substitutions are not equally spread throughout the sequences (Kumar, 1996). In this case there are some spots that are hot (have many substitutions) and other spots that are cold (have few substitutions). As a result some parts of the sequences will require strong correction for multiple substitutions and an excess of transitions and transversions while other parts of the sequences may require only minor correction. The most common method to correct for this problem is to use a gamma distribution. The gamma distribution is a non negative continuous distribution and can take a variety of shapes (Yang, 1995). Variation in rate of mutations among sites is a major problem when comparing highly diverged taxa. As such gamma correction factor is not usually applied for intra-species diversity studies.

Nucleotide genetic distance measures have their own limitations. Like other genetic distance measures, the methods depend on predefined populations and this is a limitation particularly when applying it to village chickens whose breed boundaries are not clear. There is also the need to make assumptions and correct for different rates of transitions and transversions. It is not always certain that the right model have been used.

2.8.4.3 Networking analysis

Bandelt *et al.,* (1995) made use of the median network approach to portray mtDNA relations and infer about population expansion and domestication events. When a haplotype network is drawn, ancient haplotypes can be differentiated from young ones because: (1) they occur at higher frequencies and (ii) they are usually positioned at the center surrounded by derived haplotypes in a star like topology (McHugh and Bradley, 2001; Liu *et al.,* 2006). Templeton *et al.* (1995) suggested a nested clade analysis to tests the effects of gene flow versus historical events such as fragmentation, colonization or range expansion on the extant population

structures. The method uses a haplotype network similar to that of Bandelt *et al.,* (1995). The haplotype tree is then converted into a nested series of clades by hierarchically combining close haplotypes (Templeton *et al.,* 1995). The nested design is used to look for geographical associations and test hypothesis concerning restricted gene flow, population fragmentation, expansion and colonization of new territories. Ronald *et al.* (2003) used Templeton's method to show that both historical events and contemporary forces had influenced the population structure of Lesser prairie-chickens.

Compared to nucleotide genetic distances, networking has the advantage of not using predefined breed boundaries but cluster individuals based on the haplotypes they contain and how these haplotypes differ from those in other individuals. It is therefore possible, using the haplotype networking, to identify admixed populations that share ancestral lineages.

2.9 Conservation of chicken genetic resources

Further erosion of animal diversity invites disaster as options for long term productivity and sustainability are lost. Diversity within and between poultry populations need to be conserved in case of changes in consumer demands, production methods and environmental conditions (Weigend and Romanov, 2002). According to Notter (1999) and Delany (2003), the core objective of conservation of AnGR is to maintain access to the adaptive genetic potential of each species and to maintain the current collection of valuable resources for artificial selection. The accelerated loss of specialised research material for human and animal research, consolidation of poultry primary breeder companies (Arthur and Albers, 2003), possible loss of genetic potential in industry stock as a result of decades of intensive selection and the replacement of locally adapted chicken breeds found on small farms and in villages around the world with modern industry stock constitute other rationales for conservation.

Decision making regarding conservation of genetic variation relies on composite information including phenotype, historical records and molecular genetic variation. Conservation and preservation within a species exists at two fundamental levels that encompass variation between individuals within populations and genetic differences between populations. There are two approaches to conservation of animal biodiversity namely, ex-situ and in-situ conservation (Geerlings *et al.,* 2002). Ex-situ refers to conservation approaches outside of a breed's natural habitat, such as in zoos and in gene banks. In-situ is the conservation of

ecosystems and natural habitats. It involves the maintenance and recovery of viable populations in their natural surroundings where they have developed their distinctive properties.

Conservation of genetic resources is costly and will be done in most cases at the expense of high yielding animals. Resources to conserve animal genetic resources are limited and have to be allocated efficiently. The result is that not all indigenous animal genetic resources can be conserved. Ruane (1999) identified three areas of breed conservation as: (i) the promotion of animal genetic issues through awareness, (ii) documentation of animal genetic resources and (iii) breed conservation programmes.

A number of methods for prioritising populations for conservation have been suggested. Weitzman's approach uses a genetic distance matrix to identify conservation units (Weitzman, 1992). The contribution of an element to group diversity is measured as the reduction in tree length caused by the removal of that breed or population. Although the method has been used by many investigators (eg Laval *et al.,* 2000; Reist-Marti *et al.,* 2003), it has been criticised for ignoring within breed or species diversity (Caballero and Toro, 2002). According to Toro and Caballero (2005) all genetic distance based methods do not account for within breed diversity. The within breed diversity is however of importance particularly when considering domestic breeds. The Weitzman approach and other genetic distance based methods have in some cases been found to favour highly inbred populations with extreme allele frequencies (Mateus *et al.,* 2004).

Choosing distant relations based on kinship estimates is another tool for maximising diversity for conservation units (Eding and Meuwissen, 2001; Caballero and Toro, 2002; Blouin, 2003). Unlike genetic distance based methods, co-ancestry measures emphasise on within population diversity (Toro and Caballero, 2005) and would favour non-inbred populations with an even distribution of gene frequencies (Mateus *et al.,* 2004). Simianer (2005) suggested using number of alleles and the risk to extinction as a measure to define conservation units. Gandini and Villa (2003) argue that conservation decisions should also consider the cultural value of breeds to the existing and future generations.

A major shortcoming of almost all methods of prioritising populations is the dependency on predefined breeds or populations. While most commercial breeds and populations in

developed countries have known breed boundaries and are kept as closed populations with pedigree information, the situation is totally different for indigenous populations in developing countries and more so for chicken populations. There is therefore a need to accurately define these population boundaries to be able to set up effective conservation programmes.

2.10 Conclusion

FAO (1999) defined genetic resources as those populations that show the highest genetic differences within a species and or show unique alleles and allelic combinations. Village chickens could be among the few AnGR that can be used to improve the livelihoods of most households in developing countries. At present there is inadequate information on the characteristics and uniqueness of village chicken populations. The management of village chickens as single populations without recognition of between and within population diversity endangers their future existence.

Appropriate breeding and conservation strategies need to be put in place to avoid further erosion of these AnGR. Due to limited resources, cost-effective strategies are expected and these depend on accurate identification of unique populations. The characterisation of village chickens should be done within the context of the village chicken production systems. Although phenotypic characterisation is relatively easy and relevant to most village chicken farmers who are the custodians of these AnGR, it should be coupled with detailed and accurate genetic characterisation to identify unique population structures and estimate risk status. Molecular techniques facilitate evaluation of genetic diversity in the absence of pedigree records. Appropriate diversity measures that in-cooperate both within and between population diversity should be used as these generate the information needed for prioritisation of populations.

Study Design

3.1 Description of the agro-ecological zones in Zimbabwe

Zimbabwe has an area of 390 757 km^2 and extends from latitudes 15^o 47` S to $22^o$ 24` S from longitude 25^o 14`E to 33^o 04' E. It is a landlocked country and the altitude ranges from 197m to 2592m above sea level. The country can be divided into six physical regions which are the eastern highlands, the highveld, middleveld, Kalahari sandveld, Zambezi valley and the lowveld. The country has a tropical climate and experiences uni-modal rainfall patterns. Much of the highveld and eastern highlands, however, tend to have a subtropical to temperate climate due to the modifying effects of altitude. There are five agro-ecological or natural regions (Eco-zone I-V) that vary in rainfall distribution and temperatures. The rainfall, temperature, major topographic features and farming systems of each agro-ecological region are given in Table 3.1.

Table 3.1: The rainfall, temperature and farming systems of each agro-ecological region*

Region	Area (km^2)	Rainfall (mm yr^{-1})	Temperature (^oC)	Physical regions	Commercial Farming system
I	7 000	> 1000	10 –15	Eastern highlands	specialised
II	58 600	750 – 1000	20.5 – 30.0	Highveld	intensive
III	72 900	650 – 800	20.5 – 30.0	Middleveld	semi-intensive
IV	147 800	450 – 650	30.5 – 35	Lowveld	semi-extensive
V	104 400	< 450	> 35	Kalahari sandveld; Zambezi valley	extensive

Source: Government of Zimbabwe, 2000

Communal areas in the country practice mixed crop-livestock farming. The types of crops and livestock vary among agro-ecological zones. Five districts, Risitu, Hurungwe, Gutu, Gokwe-South and Beitbridge in eco-zones I, II, III, IV and V respectively were used for this study (Figure 3.1).

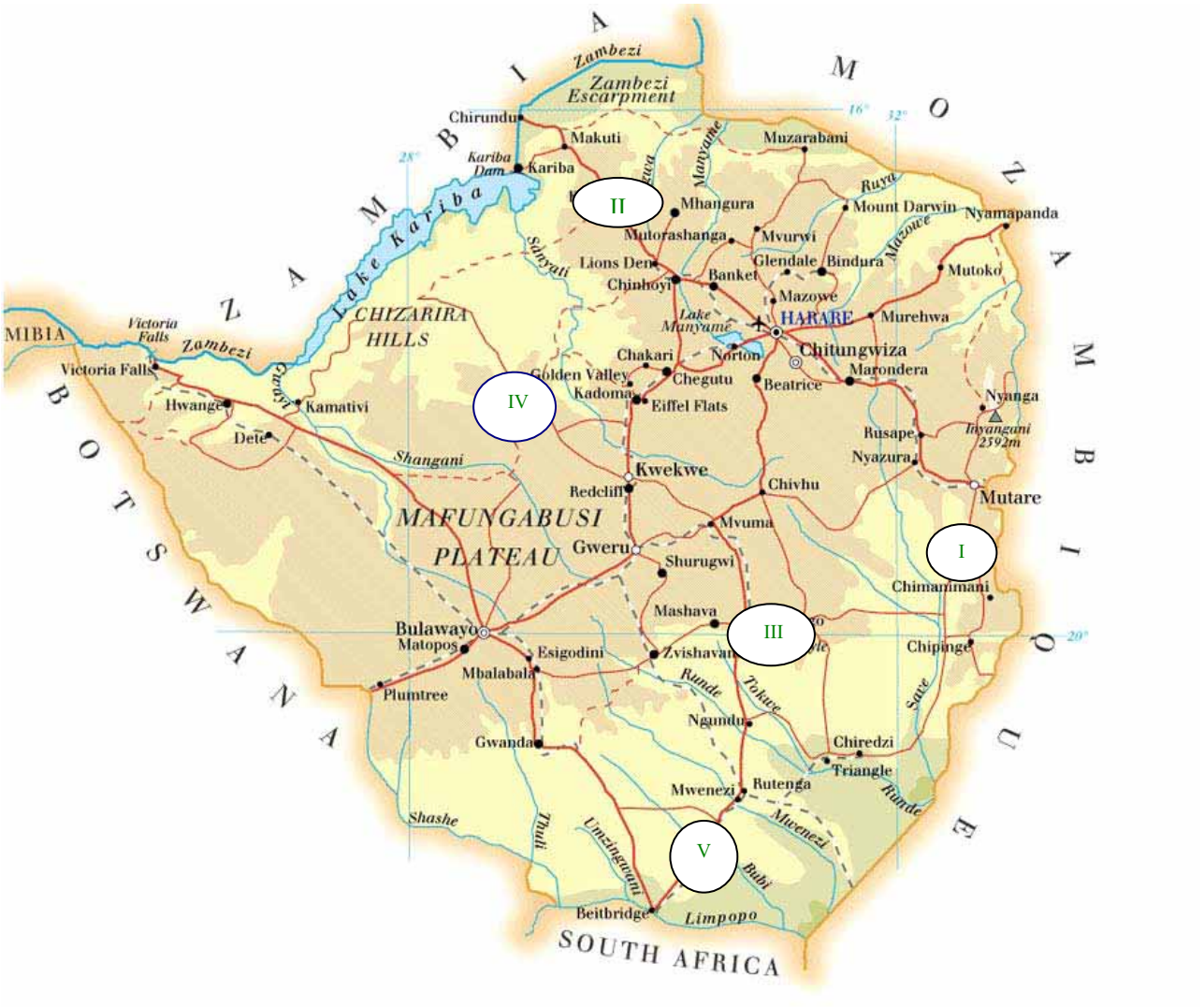

Figure 3.1: Map of Zimbabwe showing the selected districts

Scale: 1cm: 100km

3.2 Sampling of households

In each district, 7-10 villages remote from the growth point centres were randomly selected. Villages close to growth centres were avoided as they tend to have the influence of the urban farming community. Selected villages had the same agricultural production systems (production of similar crops and livestock) which are representative of the eco-zone. Distances between selected villages were, however, minimised to facilitate accessibility (villages were physically connected). List of households in each village were provided by the Agricultural Research and Extension (AREX) personnel. The first selection criterion for households was that they should own chickens and were willing to participate. From the

willing households, participants for this study were selected using stratified random sampling based on sex of head of household (male or female). Ninety-seven, 56, 70, 104 and 37 households were selected from eco-zones I – V respectively. The intended number of households was 100 per each district. Failure of some farmers to keep appointments and lack of good communication with some extension workers led to variation in sample sizes.

3.3 Chicken populations

3.3.1 Zimbabwe chicken eco-types

The local chickens in Zimbabwe are reared by communal farmers across the country under extensive systems of production. Under these production conditions, the chickens are exposed to the full variation in weather and environmental conditions. Management and productivity is heavily influenced by the physical, biological and socio-economic environment within each locality. The level of nutrition depends on the feeds available in the village, and the pathogen exposure on local disease situation. The scavenging feed resources and disease epidemiology varies among eco-zones.

Within household different age groups are raised as one flock. The communal ownership of the scavenging feed resources result in mixing of flocks from different households within communities. Although on average every household owns a cock, mixing of chickens during scavenging results in sharing of cocks among neighbouring flocks.

From each district 50 chickens were sampled except in eco-zone_V where 37 chickens were sampled. Chickens were sampled from the subset of the selected households (Section 3.2). One chicken was selected per household and 10 household were used per village. The number of villages per district ranged from 2-5. Unrelated and mature male (20%) and female chickens were sampled. A questionnaire asking on the source of chicken and households with which farmer shares breeding animals accompanied chicken sampling. This was done to ensure sampling of unrelated chickens.

3.3.2 Reference populations

Chicken populations from outside Zimbabwe were also included in the study. Broiler and layer purebred chicken lines were selected from those already analysed in the AVIANDIV[1] project, which is a European collaborative project for chicken biodiversity. Six lines were chosen, which were the Broiler dam line (BDL) Broiler Sire line (BSL), brown egg layers lines (BL_A and BL_C) and white egg layer lines (WL_A and LS_S). These purebred lines were managed as closed flocks with no migration from outside populations. They have a known breed history and pedigree and breeding is well controlled. These attributes made them ideal populations to compare and contrast with the extensively raised populations from Zimbabwe.

Scavenging chickens were sampled from a 50km radius in Lilongwe district of Malawi. The chickens in Malawi were raised under a similar scavenging system of production as described by Gondwe (2004). Village chickens from Sudan were also included. The Malawi, Sudan and Zimbabwe chickens are all non-descript populations not selected for any production trait. They are commonly referred to as village, scavenging or local chickens and are kept by smallholder farmers in the communal areas of the three countries. Both Malawi and Sudan are to the North of Zimbabwe. The geographical coordinates of Malawi are 13º30´ S and 34º00´E while Sudan is located at 15º00´ N and 30º00´ E.

3.4 Study layout

The study was carried out in two main stages:

(i) Analysis of the village chicken production systems in Zimbabwe

(ii) An assessment of genetic diversity of the Zimbabwe chicken eco-types using molecular markers

[1] AVIANDIV EC Contract No. BIO4-CT98-0342 (1998-2000); Weigend, S (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K. Wimmers, T. Burke, and A. Mäki-Tanila (http://w3.tzv.fal.de/aviandiv)

3.5 Research tools

(i) Phase I

Semi-structured questionnaires were used to capture information on production systems, chicken management and breeding practises. In addition, focused discussions with relevant farmers and key informants were used to get detailed qualitative information of management and breeding practises. Questionnaires were administered to the five districts in the five agro-ecological zones of Zimbabwe. Results from this study are presented in

(a) Chapter 4 which focused on the overall farming system in the 5 eco-zones and how it influences the existence of village chickens and;

(b) Chapter 5 that focused on the village chicken production mainly on the village chicken strains, selection of breeding stock and preferences of production traits

(ii) Phase 2

1. Twenty-nine microsatellite markers were used to determine within and between population genetic diversity. These microsatellite markers are the same set recommended by the Food and Agricultural Organisation (FAO, 2004) and the International Society of Animal Genetics (ISAG) for assessing chicken diversity. They are distributed over 15 chromosomes of the chicken genome and were used for assessing genome wide average relatedness of chicken populations. The twenty-nine markers were genotyped for chicken populations from the five eco-zones with 50 individuals sampled from Eco-zone I- IV and 37 from Eco-zone V). The reference populations had been genotyped in previous projects. Results from this analysis are presented in Chapter 6 of this thesis.

2. A total 455bp of the mtDNA D-loop region was also used to infer genetic diversity and phylogeographic structure of the chicken populations. The 455bp regions was sequenced for both the five Zimbabwe eco-types and the reference populations (N = 20 per population). Results for this analysis are presented in Chapter 7 of this thesis.

Chapter 4

Variation in Village Chicken Production Systems Among Agro-Ecological Zones of Zimbabwe[Ψ]

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4.1 Abstract

The degree to which village chickens are integrated in the smallholder farming systems would differ depending on the socio-economic, cultural and biological factors within each system. The objective of this study was to characterise the village chicken farming systems and identify possible threats to and opportunities for local chickens in the agro-ecological zones of Zimbabwe. A pre-tested questionnaire was administered to households randomly selected from five districts, Risitu (n = 97), Hurungwe (n = 56), Gutu (n = 77), Gokwe-South (n = 104) and Beitbridge (n = 37) in eco-zones I-V respectively. Age of head of household averaged 47 years (SD =14.28). Land holdings per household averaged 4.82 ha (SD = 3.6). Overall, 17.7 percent of the households ranked livestock as the major source of income compared to 70.8 percent who ranked crops as the main contributor. Chicken flock sizes averaged 16.74 (SD = 12.40). Highest flock sizes were observed in eco-zones I and IV. Households owning cattle, goats and other livestock assigned a less important rank to chickens. Chickens were used mainly for the provision of meat and eggs whilst the use of feathers and investing in chickens were uncommon practises. Results indicated that more support is necessary for village chicken production in the non-cropping regions of the country.

Key words: Zimbabwe, eco-zones, farming systems, village chickens

4.2 Introduction

Village chickens play an integral role in smallholder farming systems (Kitalyi, 1998; Mwalusanya *et al.,* 2002). They are used to meet the multiple social, economic and cultural needs of households. Local chickens serve as an important source of animal protein to the rural poor (McAinsh *et al.,* 2004). Households often sell chickens to generate cash. Unlike other livestock species particularly cattle, chickens are accessible even to the poor and landless households.

In Zimbabwe (McAinsh *et al.,* 2004; Muchadeyi *et al.,* 2004), as in other developing countries (Mwalusanya *et al.,* 2002; Tadelle *et al.,* 2003), local chickens are reared under an extensive system of production within a mixed farming set-up. Communal farmers have limited resources that they have to allocate to the different farming activities and in most cases chickens are left to scavenge for feed and drink unclean water. This exposes them to

predators and disease pathogens while farmers can only afford minimum interventions. When environmental conditions differ among farming systems, as is the case in Zimbabwe's agro-ecological zones (eco-zones), variation in production of village chickens becomes likely. It has been observed in other studies (Kitalyi, 1998; McAinsh *et al.,* 2004; Gondwe, 2004) that women and children are more involved in chicken production. This gender bias in chicken production implies some variation in the valuing and management of chickens in male and female headed households of the society. The degree to which these chickens are supported and integrated in the smallholder farming systems would therefore vary depending on the socio-economic, cultural and biological factors within each system. The objectives of this study were to characterise the farming systems in Zimbabwe agro-ecological zones and identify threats and opportunities to the existence of local chicken populations.

4.3 Materials and methods

4.3.1 Study Area

The study was carried out in five agro-ecological zones of Zimbabwe. The description of the agro-ecological zones and districts selected is given in Section 3.1.

4.3.2 Sampling procedure

Five districts mentioned in section 3.1 were selected. In each district, 7-10 villages located in 2 wards that were remote from the development centres (commonly known as the growth points) were randomly selected. Household were selected based on ownership of chickens and willingness to participate. Using this criterion, 97 households were selected from eco-zone I while 56; 77; 104 and 37 were chosen in eco-zones II, III, IV and V, respectively. In eco-zone I, III and IV households were chosen from 10 villages whilst 7 villages represented farmers in eco-zone II and V, respectively. The ratio of male to female headed households was 4:1 in all the eco-zones.

4.3.3 Questionnaire administration and participatory rural appraisals

Pre-tested questionnaires were administered to randomly selected households in each district. Data collected from questionnaires included farmer's sources of income and livelihood, crop

and livestock species kept by individual farmers and the respective hectares and animal herd sizes. The reasons why farmers produce the crops and rear the respective livestock species were given and further discussed during focus group discussions. Farmers ranked the sources of income and livelihoods on a scale from 1 (most important sources of income) to 6 (least important income sources) during questionnaire interviews. This was followed up qualitatively during focused discussions. A similar ranking system was also used for livestock species and uses of chickens and chicken by-products. Information on household chicken flock sizes, flock composition and management practises were captured during interviews. Threats to viability of chicken production were determined in terms of number of households accessing veterinary services, diseases and predators affecting the local chickens and health management practises in the face of diseases. Chicken farmers were also asked on the supplementary feed resources they give to chickens. Village chicken flock dynamics over the past twelve months were calculated as entries into (i.e. chickens bought, received as gifts and exchanged with other commodities) and exits (mortality, sales, exchanges and gifts) out of the flocks based on farmer recall information.

4.3.4 Statistical analysis

The generalised linear models procedure of SAS (2000) was used to analyse the effect of agro-ecological zone on farmers' sources of income, number of crop and livestock species, chicken flock sizes and composition, number of diseases and predators affecting the local chickens and the number of feed sources to the village chickens. The linear model used for this analysis was:

$$\mathbf{Y_{ijk} = \mu + Eco\text{-}zone_i + SHH_j + E_{ijk}}$$

where;

Y_{ijk} = dependent factors (farmers income sources, number of crop and livestock species, chicken flock sizes and composition, number of diseases and predators affecting the local chickens, number of chicken feeds and annual entries into and exits out of existing chicken flock;

μ = overall constant mean;

$Eco\text{-}zone_i$ = agro-ecological zone effects (where i = I, II, III, IV, V);

SHH_j = sex of head of household effect where j = male or female; and

E_{ijk} = random residual error.

Frequencies for the different household income sources and access to veterinary services were estimated using the frequency procedure of SAS (2000).

An ordinal logistic regression using PROC LOGISTIC (SAS, 2000) was used to determine the odds of ranking chickens as most important versus cattle, goats and other livestock in the five agro-ecological zones. The model used for this analysis was:

$$\ln\left[\frac{P}{1-P}\right] = \beta_0 + \beta_1 cattle + \beta_2 goats + \beta_3 otherlivestock + \beta_4 Eco - zone + \varepsilon$$

where:

P = probability of a household ranking chickens first;

β_0 = intercept;

$\beta_1 \ldots \beta_4$ = the regression coefficients of ownership of other livestock species on $\ln\left[\frac{P}{1-P}\right]$; and

ε = random residual error distributed as N (0, $I\sigma^2 e$).

$\left[\frac{P}{1-P}\right]$ = odds ratio, which referred to the odds of ranking chickens first. When computed for each estimator ($\beta_1 \ldots \beta_4$), the odds ratio was interpreted as the proportion of ranking chickens first in households without cattle (β_1), goats (β_2) and other (β_3) livestock species versus those that owned these animals, and in eco-zone V (β_4) compared to the wet to moderate eco-zones I-IV respectively.

A non-parametric Kruskal Wallis test (NPAR1WAY procedure of SAS) was used to analyse the ranking of the different sources of income, livestock species and the uses of chickens among the eco-zones by comparing the mean ranks from the five eco-zones.

4.4 Results

4.4.1 Household demographics and farming system

The average age of head of household was 47 years (SD =14.28) with no significant differences among agro-ecological zones. On average, a household was made up of 6.38 (SD

= 3.19) members, over 50 % of whom were adult males. There was no significant difference among eco-zones on the household size. Per household total income sources ranged from 1 to 4 and averaged 1.8 (SD = 0.63). Households in eco-zones II and III depended on significantly more (*P<0.05*) sources of income. The sources of income included livestock, crops, salaries and wages, home industries and remittances from relatives (Table 4.1).

Table 4.1: Frequencies[1] (% of households) in the five agro-ecological zones depending on livestock, crops, home industries, salaries and /or remittances for income

	Eco-zone					% Overall	Sig
	I	II	III	IV	V		
N (households)	97	56	77	104	37		
Livestock	38.2	67.2	61.9	52.9	**55.9**	51.6	***
Crops	**85.4**	**92.5**	**81.8**	**90.2**	2.9	79.8	***
Home industries	3.1	20.9	27.3	14.7	5.9	14.6	***
Salaries	27.1	13.4	16.9	18.6	20.6	19.7	***
Remittances	2.9	5.8	8.1	6.0	4.1	5.9	NS
Sig	***	***	***	***	***		

***frequencies of households depending on the different sources of income among eco-zones (rows) and among income generating activities (columns) are significantly different at *P<0.001*

[1] Multiple sources of income were observed in most households such that the frequencies within an eco-zone (column) or across eco-zone (row) will not add up to a 100.

Overall, 17.7 percent of the households ranked livestock as the major source of income compared to 70.8% who ranked crops as the main contributor. Salaries and home industries (included brick making, carpentry, basket and carpet weaving and black smith) were ranked first by 15.9 percent and 7.9 percent, respectively. Few people (4 percent) ranked remittances as the major source of income. In eco-zone I, the frequency of farmers who ranked livestock first was 1.04 percent whilst it was 20.9, 26, 11.8 and 50 percent in eco-zones II to V,

respectively. The mean ranks attached to the different sources of income are shown in Table 4.2. There was a significant difference in the ranks attached to income sources (*P<0.001*) with most households in eco-zones I to IV giving a higher rank to crops. In eco-zone V, livestock had a higher rank among the agricultural sources of income.

Table 4.2: Mean ranks (SD) attached to the different sources of income (1 = most important-up to 6 = least important) and significance levels based Kruskal-Wallis test

	Eco-zone					Sig[1]
	I	II	III	IV	V	
N (households)	97	56	77	104	37	
Crops	**1.9 (1.8)**	**1.7 (1.3)**	**2.5 (1.8)**	**1.9 (1.5)**	5.9 (0.7)	***
Livestock	4.8 (1.9)	3.2 (2.0)	3.3 (2.2)	3.8 (2.1)	**3.3 (2.5)**	***
Home industry	5.9 (0.8)	5.1 (1.8)	4.8 (2.0)	5.3 (1.7)	5.7 (1.2)	***
Salaries	4.7 (2.2)	5.4 (1.4)	5.2 (1.7)	5.1 (1.9)	4.8 (2.1)	NS
Remittances	5.8 (0.9)	5.7 (0.9)	5.6(1.4)	5.7 (1.1)	5.9 (0.9)	NS
Sig	***	***	NS	***	*	

[1]mean ranks of the different farming activities (columns) and agro-ecological zones (rows) are significantly different at *$P<0.05$; *** $P<0.001$)

Land holdings averaged 4.82ha (SD = 3.6) with a median of 3ha per household. On this land, households produced 2.3 (SD = 1.01) crop species and kept 2.3 (SD = 0.84) livestock species. Land size, number of crops and number of livestock species significantly varied (*$P<0.05$*) among eco-zones as shown in Table 4.3.

Table 4.3: Least square means (standard error) of household land holdings and number of livestock and crop species produced across the five agro-ecological zones

Eco-zone	Land size in ha (SE)	Crop species (SE)	Livestock species (SE)
I	2.4 (0.4)[a]	2.3 (0.1)[b]	1.9 (01)[a]
II	7.8 (0.5)[b]	2.9 (0.1)[c]	2.6 (0.1)[c]
III	2.1 (0.5)[a]	1.8 (0.1)[a]	2.3 (0.1)[b]
IV	7.4 (0.4)[b]	2.4 (0.1)[b]	2.3 (0.2)[b]
V	2.7 (0.7)[a]	1.9 (0.2)[a]	3.1 (0.2)[d]

[abc]values within a column with different superscript are significantly different ($P<0.05$)

The main livestock species kept by farmers across eco-zones were cattle, goats and chickens (Table 4.4). There was variation ($P<0.05$) among eco-zones in the type of crops produced (Table 4.4). While households in eco-zone I produced maize, citrus fruits and bananas, cotton and soyabeans were unique to agro-ecological zone II and small grains (sorghum and millet) dominated the few crops produced by farmers in eco-zone V.

Table 4.4: Least square means (standard error) of herd and flock sizes of livestock species reared and hectares of crops produced by households across the five eco-zones

	Eco-zone				
	I	II	III	IV	V
N (households)	97	56	77	104	37
Livestock species					
Cattle	0.3 (0.5)[a]	4.0 (0.6)[b]	3.9 (0.5)[b]	4.4 (0.5)[b]	5.0 (0.9)[b]
Goats	2.4 (0.6)[ab]	3.6 (0.8)[b]	1.1 (0.7)[a]	2.4 (0.2)[ab]	14.5 (1.2)[c]
Chickens	19.3 (1.3)[b]	16.1(1.5)[ab]	13.4(1.4)[a]	19.4 (1.2)[b]	12.0 (2.2)[a]
Other[1]	2.2 (0.4)[bc]	1.3 (0.5)[abc]	0.4 (0.4)[a]	0.7 (0.4)[ab]	2.8 (0.7)[c]
Crop species					
Maize	2.8 (0.6)[b]	5.7 (0.9)[c]	2.8 (0.9)[b]	2.7 (0.5)[b]	0.4 (0.9)[a]
Cotton	0[a]	0.5 (0.1))[b]	0[a]	0.4 (0.1)[b]	0[a]
Soyabeans	0[a]	0.3 (0.1)[b]	0.1 (0.1)[ab]	0.1 (0.1)[ab]	0[a]
Sunflower	0.1 (0.1)[ab]	0.2 (0.1)[ab]	0[a]	0.2 (0.1)[b]	0[a]
Small grain	0.5 (0.1)[bc]	0[a]	0.2 (0.2)[b]	0.6 (0.2)[bc]	0.8 (0.2)[c]
Other[2]	3.2(0.5)[b]	1.2 (0.2)[a]	0.5 (0.5)[a]	0.3 (0.5)[a]	0.6 (0.5)[a]

[abc]values within a row with the same superscript are not significantly different (*P>0.05*);

[1]other livestock species consisted of guinea fowls (n = 11), bees (n = 2 bee hives), pigs (n = 15), sheep (n = 79, pigeons (n = 39) turkeys (n = 6), donkeys (n = 23), rabbits (n = 7) and rock rabbits (n = 1) across all the agro-ecological

[2]other crop species consisted of citrus and banana plantations, sugar beans, groundnuts, round nuts, cowpeas and pumpkins.

4.4.2 Village chicken production system

The average chicken flock size was 16.74 (SD = 12.40) with a median of 13 birds per flock. Flock sizes varied significantly (*P<0.05*) among eco-zones as shown in Table 4.5. The flock compositions across the 5 zones are shown in the same Table. The lowest (*P<0.05*) number of chicks were observed in eco-zone V whilst eco-zone III had the least (*P<0.05*) growers and mature hens and cocks.

Table 4.5: Least square means (Standard error) of chicken flock sizes and composition in the 5 eco-zones

	Eco-zone				
	I	II	III	IV	V
N (households)	97	56	77	104	37
Chicks	7.2 (0.9)^{b}	7.3 (1.1)^{b}	7.2 (1.0)^{b}	8.5 (0.8)^{b}	1.6 (1.6)^{a}
Pullets	4.0 (0.5)^{c}	1.3 (0.6)^{a}	0.6 (0.5)^{a}	2.7 (0.4)^{b}	1.4 (0.8)^{ab}
Cockerels	0.7 (0.2)^{a}	0.8 (0.3)^{a}	0.8 (0.2)^{a}	1.0 (0.2)^{a}	0.6 (0.4)^{a}
Hens	6.0 (0.4)^{b}	5.5 (0.5)^{ab}	4.3 (0.5)^{a}	5.6 (0.4)^{b}	6.8 (0.7)^{b}
Cocks	1.3 (0.1)^{b}	1.0 (0.1)^{b}	0.8 (0.1)^{a}	1.5 (0.1)^{bc}	1.7 (0.2)^{c}
Total	19.3 (1.2)^{b}	16.1(1.5)^{ab}	13.4(1.4)^{a}	19.4 (1.2)^{b}	12.0 (2.3)^{a}

^{abc}values within a row with the same superscript are not significantly different (*P>0.05*).

Table 4.6 indicates the ranking of chickens as a major source of income and other livelihood needs compared to other livestock species. While goats and cattle were ranked least important in eco-zone I, they were considered more important sources of income and livelihood in eco-zone V. Chickens received a higher ranking in agro-ecological zone I and were ranked second to goats in eco-zone V.

Table 4.6: Mean ranks (SD) of chickens and other livestock species (1 = most important up to 7 = least important) across agro-ecological zones and significant levels according to Kruskal-Wallis test

	Eco-zone					Sig
	I	II	III	IV	V	
N (households)	97	56	77	104	37	
Cattle	4.7 (1.1)	**2.2 (1.8)**	2.1(1.8)	**2.0 (1.7)**	2.9 (2.0)	***
Goats	3.0(1.2)	3.4(1.5)	4.0 (1.3)	3.6(1.6)	**1.7 (0.8)**	***
Chickens	**1.4 (0.1)**	2.3 (2.0)	**1.9 (0.7)**	2.3 (1.1)	2.8 (1.0)	***
Other	2.2 (0.2)	3.5 (0.2)	2.5 (0.8)	3.1 (0.6)	3.0 (0.6)	***

*** Mean ranks from different agro-ecological zones are significantly different at *P<0.001*

Across all eco-zones, the odds of assigning a higher rank to chickens were higher (at 95% confidence) for households without other livestock species compared to farmers owning other animals (Table 4.7). The odds were highest for households without cattle, followed by those without goats and least for farmers without other livestock species such as donkeys, pigs and sheep.

Table 4.7: The odds ratio estimates, lower and upper 95% confidence interval (CI)) of ranking chickens first in households without cattle, goats and other livestock species compared to those owning these species and in eco-zone V compared to eco-zones I – IV

Parameter	Odds ratio	Lower CI	Upper CI
Eco-zone V vs I – IV	1.6	1.29	1.99
Households without cattle	178.2	74.69	425.03
Households without goats	72.8	33.56	157.75
Households without other livestock species	9.8	4.85	20.22

In all the eco-zones, chickens were used for provision of meat and eggs for consumption, income generation through sales, provision of manure for crop production, as an investment and source of security and for cultural reasons. As indicated in Table 4.8, the most important (*P<0.05*) role of chickens was in the provision of meat for household consumption while the use of chicken feathers and as a form of investment were uncommon practises. There was more utilisation of chickens reported in agro-ecological zones II–IV, while eco-zones II and IV were characterised by abundance of feed resources (Table 4.9). Overall very low entries (mean = 0.20, median = 0 and maximum = 38 chickens) characterised the village chicken flocks across all eco-zones while exits particularly mortality (mean = 12, median = 10 and maximum = 100 chickens) dominated the village chicken flock dynamics.

Table 4.8: Mean ranks (SD) of the uses of chickens (1 = most important - upto 7 = least important) across eco-zones and significance level based on Kruskal-Wallis test

	Eco-zone					Sig[1]
	I	II	III	IV	V	
N(households)	97	56	77	104	37	
Uses of chicken						
Meat	**1.3 (0.9)**	**1.6 (1.6)**	**1.4 (1.25)**	**1.6 (1.04)**	**1.4 (1.15)**	**
Eggs	2.9 (1.5)	3.7 (2.0)	3.1(1.7)	3.0 (1.2)	3.4 (1.5)	*
Feathers	6.8 (0.8)	6.7 (2.0)	6.6 (1.1)	6.7 (2.0)	6.9 (0.7)	
Manure	4.0 (1.5)	4.5 (1.6)	3.7 (1.6)	4.7 (2.0)	3.6 (1.3)	***
Cash	4.4 (2.4)	3.8 (2.1)	4.1 (1.9)	3.0 (1.8)	3.4 (2.0)	***
Investment	6.0 (1.8)	5.7 (2.1)	6.1 (1.3)	5.2 (1.9)	6.1 (1.7)	**
Other	6.9 (0.4)	6.9 (0.7)	7.0 (0.3)	7.0 (0.2)	7.0 (0.0)	*

[1]Mean ranks from different agro-ecological zones significantly different at **P<0.05*; ** *P<0.01* and *** *P<0.001*

Table 4.9: Least square means (standard error) of the number of perceived threats and opportunities to chicken production across agro ecological zones

	Eco-zone				
Opportunity/threat	I	II	III	IV	V
Uses	3.8 (0.1)[a]	4.6 (0.2)[b]	4.7 (0.1)[b]	4.4 (0.1)[b]	3.9 (0.2)[a]
Predators	2.6 (0.09)[b]	2.7 (0.1)[c]	2.5 (0.1)[ab]	2.3 (0.1)[a]	2.2 (0.2)[ab]
Diseases	1.3 (0.07)[ab]	2.2 (0.1)[c]	1.5 (0.1)[b]	1.4 (0.1)[ab]	1.1 (0.1)[a]
Feeds	2.3 (0.07)[b]	2.6 (0.1)[c]	2.2 (0.1)[ab]	2.5 (0.1)[c]	2.0 (0.1)[a]
[1]Veterinary services (%)	60	35.8	26.9	26.2	39.0

[abc]values within a row with the same superscript are not significantly different (*P>0.05*)

[1]Proportion of households that received livestock veterinary services

4.5 Discussion

The rearing of local chickens in Zimbabwe is typical of most village chicken production systems in Africa and other developing countries (Kitalyi, 1998; Mwalusanya *et al.,* 2002). Characteristics of such production systems are low or zero input of either housing, feeding and health care (Maphosa *et al.,* 2005) and there is exposure of chickens to the full variation in environmental factors (Kitalyi, 1998). This exposure causes variations in the level of production of chickens as different areas experience varying climatic, economic, cultural and social factors.

The rural areas are known to house the bulk of indigenous animal populations (CSO, 2000; Geerlings *et al.,* 2002). The young and economically active members in a society try and derive livelihoods from the available means of production such as livestock and crop production. The relatively young age of heads of households (47) observed is contrary to national reports (CSO, 2000), that had portrayed the rural areas as habitats of the economically dependent age groups (≤ 15 years and ≥ 65 years of age) of the society. Most of the household heads were also not formally employed but full time communal farmers that

depended mostly on crops and livestock (Table 4.1 and 4.2). Dependency on agricultural sources of income has also been observed in Rushinga District of Zimbabwe (Muchadeyi *et al.,* 2004) and other countries of southern Africa (Gueye, 2002). This observed dependency on agricultural activities for income and livelihood is a positive attribute for the utilization and conservation of animal genetic resources (Anderson, 2003). Resources are more secure if communities derive benefits from them than in situations where they do not play a role in the livelihoods of their custodians (Geerlings *et al.,* 2002). Community-based management of animal genetic resources works to promote this dependency on agricultural resources as a way to ensure their conservation (Wollny, 2003). Despite the reliance on agricultural resources, the observed over-dependence on crops and not livestock (Table 4.1 and 4.2) might, however, impact negatively on the use of livestock genetic resources.

There are several reasons that might explain the low dependence on livestock particularly in remote areas of developing countries. The low turnover of livestock species increases risks of production and is a major liability to rural farmers whose sole means of income is farming. With the exception of poultry and other smaller species, a farmer would have to wait for at least two years to yield returns whereas it takes six or less months to harvest and sell crops. Risk in livestock production is also worsened by the numerous disease outbreaks and inefficient health control strategies in communal areas (Chitate and Guta, 2001). We found in this study that mortality outweighs other productive exits such as sales and consumption. Low production turnover and high risks will dissuade farmers from investing more in livestock production even when environmental factors allow it. Marketing barriers (Omano, 1998; Tisdell, 2003) could be another reason of lower dependence on livestock. Whereas there are organized marketing channels for crops, no marketing channels exist for most livestock species. In areas where they exist, they are informally operating through the middle man (Kusina and Kusina, 1999a and b). Farmers revealed in this study that they hardly sell chicken meat or chicken by-products mainly because of the low flock sizes, poor growth rates and the low prices they fetch on selling (Table 4.9 and focused discussion). However, opportunities for utilizing livestock exist particularly in marginal agro-ecological zones where crop production is hindered by climatic conditions. This is confirmed by the lower dependence on crops and relatively higher utilization of livestock to meet livelihood needs in agro-ecological zone V (Tables 4.1, 4.2 and 4.4).

The lower number of livestock species in eco-zone I (Table 4.4) can be explained by the existence of specialized farming of citrus fruits and banana plantations in this region. Hence there was virtually no land left for grazing impacting negatively on the number of livestock species. Small stock, mainly chickens, that require less land for production (McAinsh *et al.,* 2004), are reared as compared to larger species like cattle. In contrast, the large land sizes and moderate climate support the occurrence of both livestock and crops species at high frequency in eco-zone II. Although eco-zone IV is more suitable for livestock production, interventions in the form of irrigation schemes and gardening activities put limits on the number of livestock. While other species such as sheep, pigs and guinea fowls might increase species diversity at farm level, their numbers are too small (Table 4.4) and their ranking is too low (Table 4.6) to become a major competition to local chicken populations across all the eco-zones.

Although livestock was considered to be the main source of income in the arid eco-zones (Table 4.1 and 3), relatively lower chicken flock sizes were observed in these regions (Table 4.4). In most village production systems, chickens and other livestock species depend on crops residues and household kitchen waste as the main source of feed (Gunaratne *et al.,* 1993). Thus, although farmers in marginal agro-ecological zones have more livestock species, the flock or herd sizes are less than of the farmers in the 'cropping' regions of the country. In the marginal agro-ecological zones, chickens depend mainly on feed from scavenging that in most cases is scarce and fluctuates with seasons (Roberts, 1992). Underfed and under-nourished birds are more prone to low growth rates, poor reproductive performances and vulnerability to diseases and mortality (Butcher *et al.,* 2002; Smith *et al.,* 2005). The relatively high temperatures particularly in eco-zone V also explain the low chicken flock sizes in these regions. High temperatures are known to cause reduced egg production, reduced feed intake and overall low level of production in chicken flocks (Jacob *et al.,* 2003). The low number of chicks in eco-zone V (Table 4.5) confirms the poor reproductive performance.

Another plausible explanation for the low flock sizes could be the need to maximize returns from livestock possibly forcing farmers to concentrate on larger species, and not chickens, in the more arid zones. Cattle and goats, in most cases, are important for bigger roles such as income generation, draught power, social security and investment. Chickens, on the other hand, are crucial for the day-to-day needs such as meat for consumption, petty cash through sales and cultural roles (McAinsh *et al.,* 2004; Muchadeyi *et al.,* 2005). In this study, results

indicated that farmers used chickens mainly for meat and egg consumption (subsistence needs) and less for income-generation or investment (Table 4.8). Whereas farmers in eco-zone I can derive all their cash and investment needs from cropping activities and use chickens for the petty needs, farmers in arid zones need to ensure the livestock species they keep are able to meet these livelihood needs. As a result, farmers will concentrate on large livestock species and in the process sideline chickens. This is supported by the low odds of attaching important ranks to chickens in households with cattle, goats and other livestock species (Table 4.7). The ownership of cattle, goats and chickens by farmers in all agro-ecological zones (Table 4.4) implies that chickens complement other livestock species in meeting the farmers multiple household objectives (Francis and Sibanda, 2001). The existence of these other livestock species however has a negative impact on the valuing of chickens at household level (Table 4.7).

An interesting observation was the high number of predators and diseases (Table 4.9 in eco-zones associated with high chicken flock sizes (Table 4.5). It could be that an increased number of chickens attract more predators and diseases at a higher rate than the expected impact of these threats on survivability of chickens. Thus baboons, wild preying birds and cats have a good feed source in areas with high chicken flock sizes. Dense vegetation cover is also thought to house more predators (Kusina *et al.,* 2001) compared to the sparsely covered forests in arid zones.

4.6 Conclusions

The village chicken production systems in Zimbabwe are characterized by variation across eco-zones. Between eco-zones chickens are of different importance and have varying nutrition sources. Differences in flock sizes were observed in addition to the marked variation in the climatic factors among agro-ecological zones. Limited land and availability of more feed resources in the cropping regions, support chicken production at the expense of larger livestock species.

Chapter 5

Choice of Breeding Stock, Preference of Production Traits of Village Chickens Among Zimbabwe Agro-Ecological Zones[Ψ]

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5.1 Abstract

The free ranging chickens reared by smallholder farmers represent genetic diversity suited for particular environments and shaped by the socio-economic and cultural values of the farming systems. This study sought to investigate the existence of chicken strains and evaluate the breeding goals and strategies used by village chicken farmers in Zimbabwe. A semi-structured questionnaire was administered to 97, 56, 77, 104 and 37 households randomly selected from five agro-ecological-zones I-V, respectively. Fifteen chicken strains mostly defined by morphological traits were reported in the five eco-zones. Production criteria such as body size, health and fertility were highly ranked (ranging from 1.3 – 2.6) by farmers across all the eco-zones, while cultural traits, mainly plumage colour and sex of the chicken, were the least preferred production traits. As a common breeding practice, farmers choose the type of hens and cocks to retain for breeding purposes and these randomly mix and mate with others from community flocks. Chicken body size was ranked the major determinant in choosing breeding animals followed by mothering ability, availability, fertility and other health and morphological traits respectively. Farmers used an average of 3 criteria to cull chickens. More household culled chickens associated with poor reproductive performance and poor growth rates. The focus on many production and health traits and the absence of farmer records compromises breeding strategies in these production systems.

Keywords: Free ranging chickens, breeding practices, production traits, Zimbabwe eco-zones

5.2 Introduction

Smallholder farmers rear free ranging chickens in the marginalized communal areas of Zimbabwe (Maphosa *et al.,* 2005; McAinsh *et al.,* 2004; Muchadeyi *et al.,* 2004). These chickens are generally referred to as local or indigenous chickens and consist of heterogeneous phenotypes all assumed to have originated within the country (Mhlanga *et al.,* 1999). As in most village chicken production systems there are no clearly defined breeds (Kitalyi, 1998). Farmers refer to the chickens using vernacular names that in most cases describe their phenotypic attributes.

Some efforts have been made to identify and characterize the free ranging local chickens in Africa. Some of these efforts have resulted in the classification of free ranging chickens into

eco-types (Msoffe *et al.,* 2001a and b). It is assumed that chicken eco-types have special characteristics to enable them to survive in specific habitats (eco-zones). These chickens form the bulk of most indigenous chicken genetic resources and minimum work has been done to characterize them. Due to different climatic and physical factors, farmers from different agro-ecological zones experience different production challenges and economic needs. For example, livestock production in the smallholder sector is carried out within a mixed crop and livestock farming system (Francis and Sibanda, 2001). The crops and livestock produced are used to meet the multiple household objectives that include food security, income generation, risk aversion and social security (Anderson, 2003; Reithmuller, 2003). Different farming activities compete with and complement each other in meeting the household needs. The types of enterprises farmers engage in are influenced by the environmental factors, such as climate, leading to a variation across eco-zones. The resulting eco-types are therefore defined by the breeding strategies set up to achieve the village chicken production goals under specific environmental conditions.

Apart from competition with other indigenous livestock species, farmers in the communal areas have the option to use high yielding commercial chicken breeds particularly when the need for income generation and increased food security is high. However, production constraints that include need for high quality feed concentrate and appropriate health control strategies might force farmers to focus on adaptability traits mostly associated with free ranging indigenous chickens (FAO/IAEA, 2002). These decisions to choose production traits in most cases are made by the heads of households at both household and community level (Curry, 1996). Differences are most likely to become apparent between males who have been shown to focus more on income and the female head of households that are known to prefer the actual food available to the household (Curry, 1996; Roberts, 1996). In addition to socio-economic environment, biological factors such as those that influence feed supply of village chickens and exposure to diseases play a role in defining the genetic merits of village chickens in a given population. The combination of all these factors may have a considerable impact on what genotypes are lost or conserved in local chicken genetic resources. The objectives of this study were to evaluate the breeding goals and strategies used by smallholder chicken farmers and to investigate the existence of any chicken strains associated with the farmer's production systems in the five agro-ecological zones of Zimbabwe.

5.3 Materials and methods

5.3.1 Study site

The study was carried out in five districts located in five agro-ecological zones of Zimbabwe (described in Section 3.1). Chicken farmers who were willing to participate in the study were randomly selected from 7-10 villages in each district as described in Section 4.3.2. All selected households owned chickens that were reared under the semi-extensive to extensive system of production. The chickens would scavenge for feed and water, while substandard housing was provided at night. Farmers did not keep records on chicken production and management and there were no conventional health control programmes.

5.3.2 Questionnaire administration

A semi-structured questionnaire was administered to selected households. Farmers were asked to list and describe the breeds or strains making up their flocks according to a given set of production and morphological traits that included body confirmation, body size, plumage colour, heat resistance, drought resistance, meat taste, egg quality, mothering ability and cultural attributes. In the second stage, farmers were asked whether they had a control over the type of breeding stock they used and the reasons for their choice of breeding hens and cocks. Farmers that practiced selection of breeding stock were asked to rank the criteria used in breed selection in order of importance from 1 being the most important criteria to 8 the least important. All farmers were asked to rank growth, survivability, disease susceptibility, reproductive performance and cultural suitability in order of importance. Lastly, farmers were asked through an open-ended question to which extent they practice culling and the criteria they use. For the listed culling criteria, farmers were asked to rank them in order of importance using the same ranks as for criteria used in trait selection.

5.3.3 Statistical analysis

The main variable under investigation in this study was the agro-ecological zone. The five eco-zones in Zimbabwe are potential sources of variation due to the different climatic factors and the resultant socio-economic environment. The mean number of mature birds of each

strain per household in the five agro-ecological zones was estimated using the statistical analysis software (SAS, 2002).

To determine the influence of eco-zone on breeding practices, a non-parametric Kruskal Wallis test (NPAR1WAY procedure of SAS, 2000) was used to test whether median ranks attached to each criterion used in choosing breeding stock, production traits and culling chickens varied among agro-ecological zones. The Kruskal Wallis test generated the median ranks whose significance was tested using a Chi square test (SAS, 2000).

A generalized linear model (GLM) procedure (SAS, 2000) was used to find the effects of eco-zone on the number of culling criteria in both male and female chickens. The model used for the analysis was:

$$\mathbf{Y_{ijk} = \mu + Eco\text{-}zone_i + SHH_j + e_{ijk}}$$

where:

Y_{ijk} = number of culling criteria

μ = overall mean

$Eco\text{-}zone_i$ = agro-ecological zone effect (i = I – V),

SHH_j = sex of household head effect (j = male or female)

e_{ijk} = random residual error

The frequencies of the number of households using different culling criteria were estimated in SAS (2000).

5.4 Results

5.4.1 Chicken strains

Fifteen strains were reported across the five eco-zones (Table 5.1).

Table 5.1: Mean ± standard deviation of the number of mature chicken of different strains per household in the five agro-ecological zones (Eco-zone I – V)

Strain	Eco-zone					Total
	I	II	III	IV	V	
Kazhumu (Crested)	5.9 ±3.4	3.1 ± 2.3	3.1 ± 2.0	4.6 ± 3.7	2	263 (75) *
Normal	4.9 ±2.2	4.7 ± 1.9	7.0	7.5 ± 4.5	6.2 ± 2.8	258 (58)
Naked neck	4.0 ± 3.4	1.9 ± 1.1	4.0 ± 1.7	7.9 ± 5.1	2.0	205 (98)
Hanga	12.7± 2.4	1.75 ± 1.5	3.5 ± 0.4	12.4 ±2.1	0	159 (24)
Zizi	3.0	1.0	5.0 ± 4.2	10.9 ± 5.7	0	120 (22)
Chideya	7.3±2.5	1.8 ± 1.2	1.0	5.5 ± 4.5	1	73 (21)
Zaradota (Grey)	0	7.3 ± 2.6	1.5 ± 0.7	0	0	39 (5)
Mbira (Rumpless)	0	1.6 ± 0.7	3.5± 1.5	0	3.0	24 (14)
India	0	0	0	16	0	16 (3)
Giant	6.0 ± 2.4	0	4.0	0	0	12 (3)
Majombo	0	0	0	12.0	0	12 (1)
Chena (White)	0	0	2.0	0	0	2(1)
Nhema (Black)	0	0	1.0	1.0	0	2(2)
Tsvuku (Brown)	0	0	1.0	0	0	1 (1)
Chematama	0	1.0	0	0	0	1 (1)

* Number in parenthesis is the total number of households reporting a particular strain

Key:

Chematama : bird with bulging cheeks

India : a small and slander bird thought to have originated from India

Majombo : bird with feathers at the shanks

Kazhumu : bird with head crest

Mbira/mushayabesu : bird with no tail

Chideya : small bird with blocky compact shape and short legs

Hanga : bird with mottled plumage pattern

Zizi : bird with brown and black barred plumage pattern

The '*Giant', 'Chideya' and 'India',* are defined by body weight and confirmation, and all the other strains are categorized on the basis of morphological traits. Six strains *(Nhema, Tsvuku, Chena, Zaradota, Zizi and Hanga)* are named after plumage colour and design while the *Chematama, Majombo, Musvuu, Mbira and Kazhumu* were associated with fat/inflamed like cheeks, feathers in the shanks, naked neck gene, rumpless tail and crested comb respectively. Any other chicken that did not possess any of the mentioned attributes was just a normal chicken commonly referred to as 'ye*chishona/yechivanhu'*. In this category were multicolored birds of average body weight. The *crested, normal, naked neck, hanga* and *zizi* were the most prevalent and widely distributed strains. *India* and *majombo* were unique to a few households in eco-zone IV while *giant, chena, nhema tsvuku* and *chematama* occurred at very low numbers (Table 5.1). The observed strains ranked different *($P<0.05$)* for body size, comb shape, broodiness, scavenging ability and fertility in eco-zones I –IV (Table 5.2).

5.4.2 Choice of breeding stock

In all households surveyed, the village chickens scavenged outside the homestead boundaries during the day. They randomly mix, and consequently mate with breeding stock from other flocks in the same community. At the household level, farmers chose the type of hens and cocks to retain for breeding purposes. In 86% of the households surveyed, the breeding chickens were retained from those hatched within the flocks. Less than 4% of the breeding chickens were purchased in the remaining 14% of the households.

Table 5.2: Variation in the preferred chicken strains for different production and morphological traits by farmers from the five agro ecological zones

Trait	Eco-zones of significance [1]	Preferred strains[2]	Unwanted strains[3]
Body size	III	black, white, giant, local, zaradota	chideya, hanga, zizi
Comb	IV	crested, black, naked neck	India, chideya,
Neck	I and IV	naked neck	hanga and zizi
Disease resistance	IV	India, majombo, crested, naked neck	black, chideya,
Broodiness	IV	India, black, chideya, hanga,	normal, majombo, nacked neck
Scavenging ability	II and IV	black brown hanga, naked neck, zaradota,	chematama, crested,, local,
Fertility	I and IV	hanga, zizi	crested, local and chideya

[1] Refers to agro-ecological zones in which significant differences in the ranking of strains was observed

[2] Strains whose average mean score was ≤ 1.5; with 1 = very good, 2 = average and 3 = not good

[3] Strains whose average mean score was ≥ 2.5; with 1 = very good, 2 = average and 3 = not good

Farmers chose from within their flocks fast growing birds that resulted in heavy mature hens and cocks (Table 5.3). Blocky and compact mature birds were preferred compared to angular and tallish ones. Mature hens that layed more eggs (≥ 15eggs/clutch) were retained in the flock. High hatchability and chick survivability resulted in hens being used as breeding stock for longer periods (≥ 2 years). Farmers would maintain a breeding cock for longer periods (≥ 2 years) if associated with fast growing offspring and to some extend higher chick survivability (±5 days after hatching). The priorities given to body size, body confirmation,

plumage colour, comb shape, mothering ability and fertility in breeding stock selection varied significantly (*P<0.05)* within the eco-zones (Table 5.3).

Table 5.3: Mean ranks (SD) of factors used in the choice of breeding stock (with 1 = major determinant and 8 = least important) among eco-zones and significant levels according to Kruskal-Wallis test

	Eco-zone					
Factor	I	II	III	IV	V	Sig
Body size	**1.3 (1.2)**	**3.0 (2.3)**	**2.1 (1.7)**	**1.8 (1.8)**	**1.4 (1.1)**	*
Body confirmation	6.3 (1.6)	6.1 (1.2)	6.2 (1.7)	4.8 (2.1)	6.6 (1.4)	NS
Plumage colour	5.1 (2.1)	4.6 (2.3)	5.1 (2.0)	5.5 (1.8)	4.8 (2.4)	NS
Comb shape	7.0 (0)	6.6 (1.3)	6.9 (0.5)	6.8 (0.8)	7.0 (0)	NS
Availability	6.0 (2.1)	6.8 (1.1)	5.8 (2.1)	5.8 (2.2)	6.2 (2.0)	NS
Mothering ability	3.8 (2.4)	3.4 (2.6)	2.4 (1.9)	2.7 (1.8)	4.6 (2.4)	NS
Fertility	7.0 (0)	6.9 (0.7)	6.9 (0.6)	7.0 (0)	7.0 (0)	NS
Sig	*	*	*	*	*	

* mean ranks of the different factors (columns) and agro-ecological zones (rows) are significantly different at *P<0.05*

5.4.3 Ranking of production traits

Table 5.4 shows the farmers' preferences for production traits across the agro-ecological zones. It illustrates that given a choice, farmers would prefer *(P<0.05)* birds that produced more offspring (high reproductive performance) to fast growing birds that are able to survive in the environment. Disease resistance came third in ranking while cultural traits, mainly plumage colour, and sex of the chicken were the least *(P<0.05)* preferred traits. There was no significant difference in the ranking of production traits among the five agro-ecological zones.

Table 5.4: Mean ranks (SD) of preferences for production traits (with 1 = most preferred and 6 = least preferred) among eco-zones (Eco-zone) and significant levels according to Kruskal-Wallis test

	Eco-zone					
Factor	I	II	III	IV	V	Sig
Growth	2.5 (1.0)	2.6 (1.5)	2.7 (1.2)	2.6 (1.9)	2.2 (2.0)	NS
Survivability	2.7 (0.8)	**2.1 (1.5)**	2.5 (1.0)	2.8 (0.9)	3.0 (0.9)	NS
Disease resistance	3.5 (0.9)	3.7 (1.7)	3.2 (0.8)	3.3 (0.9)	3.5 (0.8)	NS
Reproductivity	**1.3 (0.5)**	2.5 (2.0)	**1.6 (0.9)**	**1.4 (0.7)**	**1.4 (0.5)**	NS
Cultural significance	5.2 (0.5)	4.9 (1.2)	4.9 (0.8)	5.3 (0.6)	5.8 (0.6	NS
Significance	*	*	*	*	*	

* mean ranks of the different factors (columns) are significantly different at *P<0.05*

5.4.4 Culling of male and female chickens

On average households used 2.7 (SD = 0.77) and 2.9 (SD = 0.79) criteria to select male and female birds for culling respectively. Households in eco-zones I and IV used more *(P<0.05*) criteria for both male and female chickens (Table 5.5).

Table 5.5: Least square means (SE) of the number of culling criteria in male and female chickens across the eco-zones

	N(households)	Male chickens	Female chickens
Eco-zone I	97	2.93 (0.05)^{c}	2.89 (0.06)^{b}
Eco-zone II	56	2.17 (0.08)^{a}	2.46 (0.08)^{a}
Eco-zone III	77	2.55 (0.06)^{b}	2.86 (0.06)^{b}
Eco-zone IV	104	2.85 (0.05)^{c}	3.28 (0.05)^{b}
Eco-zone V	37	2.68 (0.08)^{bc}	2.64 (0.11)^{a}

^{abc}Least square means within a column with different superscripts were significantly different at $P < 0.05$

More households culled chickens with poor mothering ability, poor reproductive performance, that were in poor health and small sized hens and cocks (Figure 5.1). Few farmers used morphological traits such as comb shape, feather patterns and plumage colour to cull chickens.

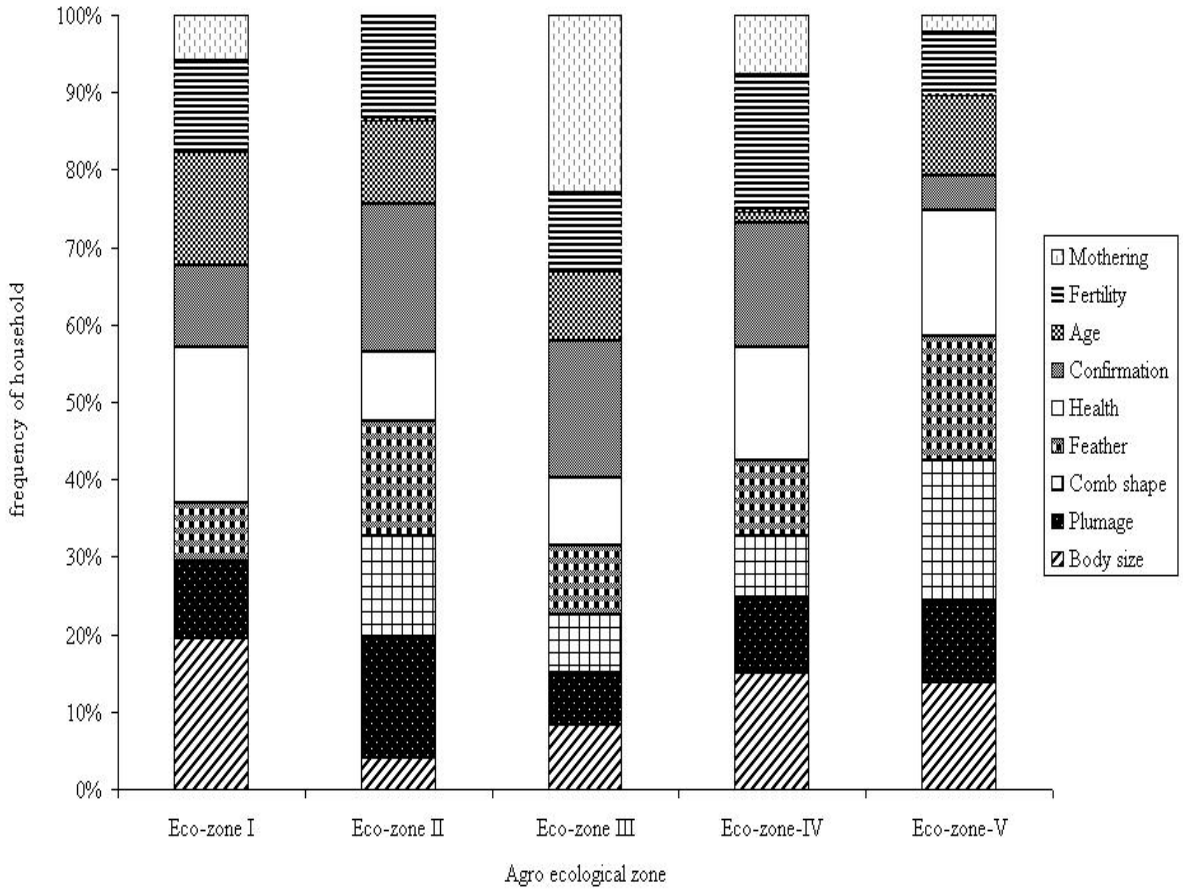

Figure 5.1: Frequencies of households using the different culling criteria in the five eco-zones (I – V)

[1]P value for frequencies of culling criteria*eco-zone = 0.010

In eco-zones I - IV, production criteria such as body size, health and fertility received higher ranks (Table 5.6). Plumage colour and pattern were also highly ranked as culling criteria for female chickens in eco-V.

Table 5.6: Mean rank sums (SD) [with 1 = most important down to 4 = least important] classified by culling criteria in male and female chickens

Culling criteria by sex	Eco-zone				
	I	II	III	IV	V
Male chickens					
Body size	1.3 (0.6)	1.7 (0.9)	1.5 (0.9)	1.4 (0.70)	1.2 (0.39)
Plumage colour	3.7 (0.6)	1.7 (0.6)	2.5 (0.6)	2.3 (0.5)	2.0 (0)
Comb shape	-*	-	2.7 (0.6)	3.0 (1.0)	2.0 (0)
Plumage type	2.0 (0)	2.5 (0.7)	2.0 (0)	3.0 (1.4)	2.0 (0)
Health	1.9 (0.6)	1.6 (0.7)	1.8 (0.9)	2.0 (0.9)	1.8 (0.6)
Body conformation	2.2(1.1)	2.7 (0.6)	2.0 (0.5)	2.4 (0.7)	3.0 (0)
Age	2.2 (0.8)	1.5 (0.6)	1.7 (0.7)	1.9 (0.8)	2.3 (1.0)
Fertility	2.9 (1.1)	1.5 (0.6)	2.1 (0.8)	2.7 (0.8)	3.0 (0)
Female chickens					
Body size	1.8 (1.0)	1.9 (1.2)	1.7 (1.1)	2.3 (1.3)	1.5 (1.0)
Plumage colour	-	2.3 (1.5)	2.7 (0.6)	3.3 (0.6)	1.0 (0)
Comb shape	-	3.0 (0)	2.3 (0.6)	-	3.0 (0)
Plumage type	-	-	2 (0)	2.0 (1.4)	1.0 (0)
Health	1.8 (0.6)	-	1.6 (0.9)	2.0 (0.8)	1.8 (0.5)
Body conformation	3.0 (0)	1.8 (0.8)	2.0 (0.9)	2.0 (1.2)	-
Age	1.8 (0.85)	1.8 (0.8)	2.0 (0.6)	1.8 (0.8)	2.0 (1.0)
Fertility	2.4 (1.0)	1.8 (0.7)	2.2 (0.9)	2.4 (1.2	2.1 (1.0)
Mothering ability	2.8 (1.3)	1.6 (0.8)	2.0 (1.1)	1.6 (0.9)	2.0 (0)

*Criterion not used for culling

5.5. Discussion

The village chicken eco-types are thought to be a source of environmentally adapted genetic diversity that help farmers overcome challenges in food security, risk aversion and social security. As with most local livestock genetic resources, they are supposed to have a socio-economical and a cultural value in the lives of their custodians. More information needs to be

generated regarding the environmental factors that shape these populations. Of particular importance are the breeding strategies because they are among the forces responsible for regulating diversity of these local chicken populations. Although no characterized village chicken breeds exist in most smallholder production systems, farmers have shown preferences to certain production traits and have used these to select for chickens that reproduce in subsequent generations. These traditional breeding systems, happening in the absence of written records and institutionalized structures have often been mistaken for absence of any breeding activities (Steglich and Peters, 2004). In this study we used open ended and closed questions to find out about the trait preferences and breeding practices of village chicken farmers in the five different agro-ecological zones of Zimbabwe.

Morphological features were also used in defining strains in Malawi (Gondwe *et al.,* 2000) South Africa (Marle-Köster and Nel, 2000) and Botswana (Badubi *et al.,* 2006). A possible explanation might be the existence of a relationship between these genotypes and the targeted production traits (Crawford, 1990). In Senegal, an association between dwarfism and frizzle genotypes and laying performance was observed (Missohou *et al.,* 2003). The naked neck gene is associated with efficient heat regulation and better reproductive and growth performance (Chen *et al.,* 2004). The preference of the naked neck in the hot and arid eco-zone IV (Table 5.2) was therefore expected. The ranking of *black, brown* and *zaradota* strains as efficient scavengers agrees with McAinsh *et al.,* (2004), who observed that chickens that have bright coloured plumage where more prone to predation during scavenging. The non-significant variations of most of the strains for the preferred production traits, however, indicate that the use of morphological features to define strains might just be an easy way for the farmers to recognize their chickens. There was no systematic ranking of strains for the different production and morphological strains among agro-ecological zones. This complexity in the definition and ranking of strains in this system could be attributed to the scavenging habits of village chickens. In all agro-ecological zones and most village chicken production systems (Kitalyi, 1998; Gondwe, 2004) the strains are raised as one flock and there is random intermixing and breeding of strains.

Regardless of the absence of clearly defined strains, consistent breeding practices were observed in all the agro-ecological zones. The observation that availability is one of the factors of least importance in breed selection (Table 5.3) implies that farmers did not just use available chickens or leave everything to random chance but made conscious decisions

concerning what breeding stock to use. The scavenging habit of village chickens does not allow farmers to directly influence the exact mates of the breeding stock. However, by selecting breeding birds within their individual flocks, farmers controlled the breeding system at the community level. Chickens that were not retained for breeding purposes were culled through sales and consumption ensuring that no random and uncontrolled mating will happen within connected flocks.

The higher ranking of chicken body size as both a factor in choosing breeding stock (Table 5.3) and a culling criteria (Table 5.6) is in contrast with the widely held opinion that smallholder farmers prefer adaptable but low performing chickens (FAO/IAEA, 2002). Although farmers ought to have healthy and environmentally tolerant breeds to survive in the harsh production environment (Delany, 2003), our results show that the main goal is to have meat and eggs for household consumption or sale to meet the multiple household needs, in accordance with Anderson (2003). Similar observations were made for smallholder dairy production in Kenya whereby farmers preferred larger exotic dairy breeds to smaller but locally adaptable indigenous and crossbreeds (Bebe *et al.,* 2003). In Nigeria there was a strong trend away from trypano-tolerant cattle in favour of high producing breeds (Jabbar and Diedhiou, 2003).

The high ranking of mothering ability and not fertility indicates that farmers are more concerned with the number of chicks reaching adulthood, than they are with the number of eggs hatched. High mortality of hatched chickens through predation, climatic stress and poor nutrition are the main constraints to chicken production (Kusina *et al.,* 2001). In a study on village chicken flock dynamics, Muchadeyi and co-workers (2005) observed that although there were high entries of hatched chicks into the flocks, mortality of these newly hatched chicks was higher resulting in either constant or decreasing flock sizes. By selecting for hens with good mothering ability, farmers aim to improve on chick survivability rates.

The ranking of growth, survivability, reproductive performance and disease resistance traits (Table 5.4) agrees with breed selection criteria (Table 5.3) and culling practices (Table 5.6) indicating some degree of consistency in farmers breeding strategies. The low ranking of the suitability of chickens for cultural ceremonies helps explain the observed insignificant role of morphological features such as plumage colour, comb shape and body stature in breed selection and culling. Discussions with farmers revealed that morphological traits, particularly

plumage colour, determined the cultural suitability of chickens and were not very important in these production systems. The implied less importance of Zimbabwe chickens in cultural ceremonies contrasts with some studies on most indigenous animal genetic resources (Henson, 1992, Patterson, 2003). Such a deviation from the expected should be further investigated as it has bearings on how conservation strategies are implemented.

The culling criteria used give an indication of the implicit farmers' breeding goals. The higher frequency of farmers culling chickens for productive rather than morphological traits, imply that village chickens are kept mainly for economic and food security reasons. Whereas fancy chicken breeders and those producing chickens for exhibition at agricultural shows and cultural ceremonies would concentrate on morphological traits such as feather colour and pattern, and chicken posture, our results show these traits were not as important to village chicken farmers in Zimbabwe. This trend was also observed in other sub-Saharan African countries (Gueye, 2002).

The greater attention given to the health of chickens also differentiates the village chicken production system from the large scale commercial system in which bio-security measures are set up and producers concentrate on the genetic merit of the animals for growth and reproduction traits. Diseases and parasites are a major threat in village poultry production (FAO/IAEA, 2002) and as such only those chickens that can survive in such environments are of importance. Disease prevalence usually varies across production systems (FAO/IAEA, 2002) so that although farmers in all eco-zones cull diseased and pest-susceptible birds, the genotypes removed from the populations should differ depending on the pathogen challenges in each system. Such information could, however, not be captured in this study because of the absence of farmers' records and the limited veterinary extension services to smallholder chicken producers.

The observed non significant differences in the ranking of growth, reproductive performance and disease resistance traits for both breeding animal as reasons for culling between eco-zones (Tables 5.3 and 5.6) indicate that there are little difference in the challenges faced by farmers between eco-zones. Regardless of variations in climatic and socio-economic factors among the eco-zones, chicken farmers across all farming systems experience poor chicken growth rates, low chicken reproductive fertility and disease challenges. As a result, farmers in different eco-zones show similar trait preferences and use of the same breeding practices.

The need to have high output animals while maintaining disease resistance in these compromised environments complicates the breeding strategies and genetic progress in the smallholder farming systems. Disease resistance and adaptability traits normally have a negative correlation with growth and fertility (Crawford, 1990). The simultaneous selection of both production and health traits by smallholders could therefore be counterproductive. Coupled to this limitation is the absence of farmer records which makes it difficult to accurately select individuals and assess the genetic progress thereafter.

Absence of Population Sub Structuring in the Zimbabwe Chicken Eco-Types Inferred Using Microsatellite Analysis[Ψ]

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6.1 Summary

The objective of this study was to investigate the population structure of village chickens found in the five agro-ecological zones of Zimbabwe. Twenty-nine microsatellites were genotyped for chickens randomly selected from 13 populations that included the five eco-zones of Zimbabwe (n = 238), Malawi (n = 60), Sudan (n = 48) and six purebred lines (n = 180). A total of 280 alleles were observed in the 13 populations. Forty-eight of these alleles were unique to the Zimbabwe chicken eco-types. The average number (± SD) of alleles/locus was 9.7 ± 5.10. Overall heterozygote deficiency in the Zimbabwe chickens (F_{IT} ± SE) was 0.08 ± 0.01, over 90% of which was due to within eco-type deficit (F_{IS}). Small Nei's standard genetic distances ranging from 0.02 to 0.05 were observed between Zimbabwe eco-types compared to an average of 0.6 between purebred lines. STRUCTURE software program was used to cluster individuals to $2 \leq K \leq 7$ assumed clusters. The most probable clustering was found at $K = 6$. Ninety-seven out of 100 STRUCTURE runs were identical, in which Malawi, Sudan and purebred lines split out as independent clusters and the five Zimbabwe eco-types clustered into one population. The within eco-type marker estimated kinships (mean = 0.13) differed only slightly from the between eco-type estimates. Results from this study lead to a rejection of the hypothesis that village chickens are substructured across agro ecological zones but indicated high genetic diversity within the Zimbabwe chicken population.

Keywords: chicken eco-types, population structure, genetic diversity, microsatellites.

6.2 Introduction

Indigenous chickens are an important contribution to the livelihoods of smallholder families in Africa (Anderson, 2003). In spite of their advantages to households, the local chickens' existence is threatened by a number of factors. In Zimbabwe, for example, commercial chicken production contributes 55 percent of the total chicken population and makes use of exotic genetic resources (Faranisi, 1995; Mhlanga *et al.,* 1999). The dependency on imported breeds sidelines the village chickens to communal small-scale subsistence farming. The lack of inventory data particularly for the indigenous chicken populations is a sign of negligence and poses a threat to poultry genetic resources (Weigend and Romanov, 2002).

The use of eco-type to describe village chicken populations is common in most village chicken production systems (Msoffe *et al.,* 2001b) and has been used as a sampling

framework in previous diversity studies (Wimmers *et al.,* 2000). The local chickens in Zimbabwe and other developing countries consist of different phenotypic strains (Mhlanga *et al.,* 1999; Tadelle *et al.,* 2003; Msoffe *et al.,* 2001b, McAinsh *et al.,* 2004) raised by communal farmers across distinct agro-ecological zones. Within eco-zones, subpopulations can be formed through selective breeding of distinct phenotypes. In addition, geographical isolation of the populations could lead to substructuring through drift, mutation and different natural selection. However, it is not known whether these eco-types represent genetically distinct populations. Characterisation of genetic structure and variation of local populations is an important step towards identifying unique and valuable genetic resources.

Polymorphisms that are revealed by genetic markers are a reliable way of assessing the genetic differences within and among chicken populations. Within population diversity is an important component of species variation particularly in domesticated species (Caballero and Toro, 2002). Between populations diversity is usually assessed using genetic distance measures (Nei, 1972; Reynolds *et al.,* 1983). Alternatively, mean kinships between populations (Eding and Meuwissen, 2001) provide a statistic that relates directly to quantitative genetic variation. Clustering individuals into populations based on their genotypic data (Pritchard *et al.,* 2000) allows one to interpret group relations without *a priori* definitions of breeds and lines.

The aim of this study was to characterize the genetic differentiation within and between Zimbabwe chicken populations sampled from different eco-zones and to relate the extent of differentiation to other African and purebred populations. Data on microsatellite genotypes in Zimbabwe chicken populations were compared with two other African chicken population and purebred lines. A number of alternative methods were used in this study to investigate differentiation among indigenous Zimbabwe chicken eco-types.

6.3 Material and methods

6.3.1 Zimbabwe eco-types

Five local chicken eco-types were obtained from Zimbabwe. Zimbabwe has an area of 390 757 km^2 and extends from latitudes 15^o 47` S to $22^o$ 24` S and from longitude 25^o 14`E to 33^o 04’ E. It is landlocked and altitude ranges from 197m to 2592m above sea level. The five

agro-ecological zones (I-V) vary in rainfall distribution (> 1000mm per annum in eco-zone I and <450mm per annum in eco-zone V) and temperatures (mean temperature = 15 ^{o}C in eco-zone I and > 35^{o}C in eco-zone V). Five districts, Risitu, Hurungwe, Gutu, Gokwe-South and Beitbridge in agro-ecological zones I through to V (ECO-I to ECO-V) respectively, were used for this study. Fifty chickens were sampled in eco-zones I, III and IV while fifty-one and thirty-seven chickens were sampled for eco-zones II and V, respectively. For each eco-zone, one chicken was sampled per household and 2-5 villages were selected for each district. Ten households were selected in each village. These chickens have not been formally selected for any commercial production traits and are raised by communal farmers under a scavenging system of production. They are characterised by high morphological variation.

6.3.2 Reference populations

Six populations were selected from the AVIANDIV[2] project, a European collaborative project on chicken biodiversity. These consisted of broiler dam (BRD) and sire (BRS) lines, two brown egg layers (BL_A and BL_C) and two white egg layers (LS_S and WL_A) with 30 individuals per population. The broiler dam and sire lines, brown egg layers and the white egg layer line A (WL_A) were commercial lines. The other white egg layer (LS_S) was the experimental White Leghorn line_Rs maintained at the Institute for Animal Breeding as a conservation flock (Hartmann, 1997). The purebred lines are managed as closed populations with known pedigree and breed history. These characteristics made them well suited to be used as reference populations in comparison with extensively raised chickens from Zimbabwe.

Sixty scavenging chickens that were sampled from a 50km radius in Malawi (MAL) and 48 Sudanese (SUD) chickens from a similar extensive system of production were also used. Similar to Zimbabwe chicken eco-types, Malawi and Sudanese chickens have not been selected for any particular production traits and show high levels of phenotypic heterogeneity. The geographical coordinates of Malawi are 13 º 30´ S and 34 º 00´ E while Sudan is located at 15º00´ N and 30º00´ E. The large geographic distances, mountains and rivers separating

2 AVIANDIV EC Contract No. BIO4-CT98-0342 (1998-2000); Weigend, S (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K. Wimmers, T. Burke, and A. Mäki-Tanila (http://w3.tzv.fal.de/aviandiv)

countries and more importantly official border post restrict the exchange of genetic material among the African countries.

6.3.3 Collection of blood samples and DNA isolation

A drop of blood was sampled from the wing vein of each bird onto Whatman FTA® filter cards (Whatman International Ltd), dried and stored in an aluminium foil envelope at room temperature awaiting analysis. DNA isolation was carried out using the phenol-chloroform method (Sambrook *et al.,* 2001).

6.3.4 DNA polymorphism

A set of 29 microsatellite markers (Table 6.1) were used to examine genetic variability. Twenty-eight of these are part of the 30 microsatellites recommended by the FAO (2004) MoDAD project for assessing chicken genetic diversity. *MCW80* is not included in the FAO list but had been previously used together with some of the FAO markers in the multiplex reactions for the AVIANDIV populations.

Multiplex polymerase chain reactions (PCR) were carried out according to FAO (2004) recommendations. Electrophoregram processing and allele-size scoring were performed with the RFLPscan software package (Scanalytics, Division of CSP, Billerica, U.S.A.). The reference populations were already typed in previous projects. However, the genotyping of the Zimbabwe eco-types was done in the same laboratory as the reference populations and standard alleles were used to adjust for allele scoring.

6.4 Statistical analyses

6.4.1 Marker polymorphism and within population diversity

Total number of alleles, allele frequencies, average number of alleles per locus, observed heterozygosity, expected heterozygosity and inbreeding coefficients (F_{IS}) per population were determined using the FSTAT (Goudet, 2001) software package. The Weir and Cockerham (1984) estimations of Wright's fixation indices (F_{IT}, F_{ST} and F_{IS}) were calculated in order to quantify the partitioning of variance between and within populations. Standard errors for the

fixation indices were generated using jacknifing over loci and populations using the FSTAT software.

6.4.2 Among population diversity

Pairwise F_{ST} (proportion of genetic variability due to population substructuring) was computed for all pairs of the 13 populations using the FSTAT software package. Nei's standard genetic distances (Nei, 1972) were estimated among pairs of populations using the PHYLIP software (Felsenstein *et al.,* 1995). Mean genetic distances among the groups (Zimbabwe, other African and the purebreds) were estimated using JMP software (JMP, Version 5.1, SAS Institute Inc.).

6.4.3 Assignment of individuals to populations

The algorithm implemented in STRUCTURE software was used to cluster individuals based on multilocus genotypes (Pritchard *et al.,* 2000). The analysis involved an admixture model with correlated allele frequencies. The model was tested using 20 000 iterations burn-in phase and 50 000 iterations for $2 \leq K \leq 8$ with 100 runs for each K value. K was the number of assumed clusters to be examined. A pair wise comparison of the hundred solutions was done using SIMCOEFF software (Rosenberg *et al.,* 2002). Solutions with over 95% similarity were considered identical. The most frequent solution was considered to be the most probable clustering and was visualised using DISTRUCT software (Rosenberg, 2004).

6.4.4 Marker estimated kinships

Similarity indices between and within populations were calculated from allele frequencies using Malecot's definition of similarity (Eding and Meuwissen, 2001):

$$S_{ij} = \sum (p_{i,x} p_{j,x})$$

where $p_{i,x}$ is the xth allele frequency in population i and $p_{j,x}$ is the xth allele frequency in population j. These similarity indices were subsequently used to calculate marker estimated kinships (MEK) among populations using a weighted log-linear model (Eding and Meuwissen, 2003):

$$\log(1 - S_{ij,L}) = \log(1 - f_{ij}) + \log(1 - s_L)$$

where $S_{ij,L}$ is the average similarity between population *i* and *j* for *L* loci, f_{ij} is the kinship coefficient between population *i* and *j* and s_L is the probability of alleles identical in state. In this model, observations on allele frequency similarities per locus and pairs of populations were weighted with the expected error variance of the similarity indices to account for variation in the informativeness of different loci. In order to construct a phylogenetic tree, the MEK were converted to kinship distance using the formula:

$$D(i, j) = \hat{f}_{ii} + \hat{f}_{jj} - 2\hat{f}_{ij}$$

where: $\hat{f}_{ii}$ and $\hat{f}_{jj}$ are kinship estimates within population *i* and *j* respectively. $\hat{f}_{ij}$ is the kinship estimate between population *i* and population *j* (Mateus *et al.,* 2004). A phylogenetic tree was constructed using the Neighbor–Joining method (Saitou and Nei, 1987) with the broiler sire line (BRS) as out-group using the PHYLIP software package (Felsenstein, 1995).

6.5 Results

6.5.1 Marker polymorphism, within and among population diversity

All microsatellite loci typed were polymorphic. The numbers of alleles per locus for the 13 populations, and for the five Zimbabwe eco-types alone, are given in Table 6.1. A total of 280 alleles were observed. The average number of alleles (± SD) was 9.7 ± 5.10 per locus. Expected heterozygosity (± SD) was 0.7 ± 0.02 while the observed heterozygosity (± SD) was 0.5 ± 0.04. The five eco-types of Zimbabwe yielded 240 alleles with an average (± SD) of 8.4 ± 4.72 allele/locus. Forty-eight of the observed alleles were unique to the Zimbabwe chicken eco-types. Twenty-eight of these unique alleles occurred at a frequency of less than one percent while allele frequency of the remaining 20 ranged from 1.3 to 10.0percent.

The average number of alleles per locus, expected and observed heterozygosity, and F_{IS} for each of the 13 populations are given in Table 6.2. Average number of alleles/locus (± SD) ranged from 2.8 ± 1.3 in the purebred line (WL_A) to 6.7 ± 3.8 in the Zimbabwe chicken Eco-type I. Higher expected and observed heterozygosity estimates were found in the Zimbabwe eco-types compared to the purebred lines.

Table 6.1: Observed allele size ranges and number of alleles in the all populations and the number and frequency of alleles unique to the Zimbabwe eco-types

Locus	All 13 populations		Zimbabwe population	
	Allele range[bp]	No of Alleles N = 526	No of Alleles N = 238	Unique alleles
ADL 112	122-134	7	6	
ADL 268	104-116	7	6	
MCW 330	256-290	9	6	
MCW 295	88-108	9	9	108 (0.42)[1]
MCW 248	207-223	6	3	
MCW 222	220-226	4	4	
MCW 216	137-149	7	7	137 (0.84)
MCW 206	221-249	14	11	233 (0.42); 249 (0.84)
MCW 183	296-326	15	12	297 (4.20); 309 (0.42); 326 (0.42)
MCW 165	114-118	3	3	
MCW 123	76-94	10	9	76 (2.10); 84 (0.84); 94 (5.46)
MCW 111	98-114	7	6	114 (0.42)
MCW 104	190-228	17	17	198 (0.42); 212 (1.26); 216 (0.42); 228 (0.84)
MCW 103	262-274	4	4	262 (0.42); 274 (0.42)
MCW 98	261-265	3	2	
MCW 081	112-145	11	10	141 (0.42); 131 (0.42); 133 (0.84); 145 (0.42)
MCW 080	266-282	14	11	272 (3.36); 273 (0.42); 282 (1.26)
MCW 078	135-145	6	5	
MCW 069	158-176	9	9	
MCW 067	176-190	8	7	182 (0.42) 188 (1.68)
MCW 037	154-160	7	6	157 (10.01); 159 (3.78)
MCW 034	214-246	15	13	214 (2.95); 244 (0.84)
MCW 020	179-185	4	5	
MCW 016	170-204	11	11	176 (0.84); 184 (1.68); 186 (2.10); 198 (0.84); 204 (0.84)
MCW 014	160-182	12	8	
LEI 234	216-368	24	22	256 (1.26); 260 (1.26) 311 (1.68); 368 (0.42) 356 (2.10)
LEI 166	350-366	7	5	354 (2.94)
LEI 094	245-289	20	18	245 (0.42); 253 (6.72); 273 (4.20); 277 (0.42)
ADL 278	114-123	10	5	115 (8.40); 117 (0.84); 121 (0.84)
Total		280	240	48

[1]Value in brackets indicate the absolute frequency (%) of the unique alleles found in the Zimbabwe chicken gene pool (N = 238)

Table 6.2: Mean number of alleles per locus, number of unique alleles, expected (H_E) and observed (H_o) heterozygosity and inbreeding coefficient (F_{IS}) per population

Population	N	Alleles/locus ±SD	Unique alleles	$H_E \pm SD$	$H_O \pm SD$	F_{IS}
Eco-I	50	6.7 ± 3.8	8 (2.0 – 8.0)[1]	0.642 ± 0.026	0.590 ± 0.013	0.083*
Eco-II	51	6.1 ± 2.9	5 (2.0)	0.650 ± 0.026	0.605 ± 0.013	0.070*
Eco-III	50	6.2 ± 3.2	5 (2.0 – 6.0)	0.647 ± 0.026	0.594 ± 0.013	0.083*
Eco-IV	50	6.4 ± 3.5	4 (2.0 – 4.0)	0.656 ± 0.024	0.598 ± 0.013	0.090*
Eco-V	37	6.2 ± 3.3	1 (2.7)	0.661 ± 0.023	0.625 ± 0.015	0.055*
MAL	60	5.9 ± 3.0	12 (1.7 –11.7)	0.607 ± 0.029	0.554 ± 0.012	0.088*
SUD	48	5.6 ± 2.5	4 (2.1 - 8. 3)	0.561 ± 0.025	0.517 ± 0.013	0.081*
LS_S	30	2.9 ± 1.1	1 (3.3)	0.355 ± 0.038	0.332 ± 0.016	0.067*
WL_A	30	2.8 ± 1.3	2 (2.3)	0.338 ± 0.039	0.309 ± 0.016	0.086*
BL_C	30	2.9 ± 1.1	0	0.393 ± 0.038	0.399 ± 0.017	-0.015
BL_A	30	2.9 ± 1.2	0	0.418 ± 0.039	0.391 ± 0.017	0.065*
BRD	30	4.8 ± 1.9	6 (3.3-20.0)	0.626 ± 0.023	0.614 ± 0.017	0.019
BRS	30	3.8 ± 1.5	0	0.547 ± 0.035	0.526 ± 0.017	0.039*

* Significantly different from zero at $P < 0.05$

[1]Minimum and maximum allele frequency (%) for the unique alleles in each population

The mean F_{IT}, F_{IS} and F_{ST} estimates per population of the five Zimbabwe eco-types, the three African populations and the six purebred lines, are given in Table 6.3. The overall population heterozygote deficiency (F_{IT} [± SE]) was 0.218 ± 0.014. A hierarchical analysis of the F_{IT} showed that heterozygote deficiency was highest in the purebred lines (F_{IT} [± SE] = 0.383 ±0.024) followed by the African (Zimbabwe, Malawi and Sudanese) and least in the Zimbabwe (F_{IT} [± SE] = 0.084 ± 0.012) population. A contrast in the distribution of within and between population variation (F_{ST} *vs.* F_{IS}) was observed between African populations, in particular between Zimbabwe eco-types and the purebred lines. For the purebred lines high

F_{ST} and low F_{IS} were found. In contrast, almost all of the F_{IT} was accounted for by the within eco-type heterozygote deficiency (F_{IS}) in the Zimbabwe population, with corresponding low F_{ST} estimates.

Table 6.3: [1]Overall population (F_{IT}), between populations (F_{ST}) and within population (F_{IS}) inbreeding coefficients of the Zimbabwe, African (Malawi, Sudan and Zimbabwe) and purebred populations

Population	F_{IT} ± SE	F_{ST} ± SE	F_{IS} ± SE
Zimbabwe	0.084 ± 0.012	0.008 ± 0.012	0.077 ± 0.012
African	0.115 ± 0.013	0.039 ± 0.004	0.079 ± 0.011
Purebred	0.383 ± 0.024	0.357 ± 0.020	0.041 ± 0.001
Overall	0.218 ± 0.014	0.159 ± 0.010	0.070 ± 0.009

* *P<0.05*

[1]The F statistics were calculated according to Weir and Cockerham (1984) estimations

6.5.2 Pairwise genetic distances

Low (0.01± 0.01) mean (± SD) pair wise F_{ST} values were observed between pairs of the Zimbabwe eco-types compared to a mean (± SD) of 0.36 ± 0.09 between purebred lines (Table 6.4). Nei's standard genetic distance estimates among the Zimbabwe chicken eco-types, brown egg layers, white egg layers and broiler dam and broiler sire lines are also given in Table 6.4. Small genetic distances ranging from 0.03 – 0.05 were observed between pairs of the Zimbabwe eco-types. The genetic distances were larger (mean [± SD] = 0.12 ± 0.037)) between the other African populations (Malawi and Sudan) and Zimbabwe eco-types and largest (mean [± SD] = 0.61 ± 0.183) between pairs of purebred lines.

Table 6.4: Mean Nei's standard genetic distances, pairwise F_{ST} and marker estimated kinships (MEK) within and between the Zimbabwe five eco-types, Malawi and Sudanese chickens and purebred lines

Population category	Nei's standard genetic distance	Pairwise F_{ST}	MEK
Within Zimbabwe eco-types	-	-	0.13 ± 0.04
Within Malawi and Sudanese	-	-	0.22 ± 0.06
Within purebreds	-	-	0.58 ± 0.04
Between Zimbabwe eco-types	0.04 ± 0.01	0.01 ± 0.01	0.11 ± 0.01
Between Malawi and Sudan	0.24	0.13	0.11
Between Purebreds	0.61 ± 0.18	0.36 ± 0.09	0.12 ± 0.02
Between Zimbabwe and Malawi and Sudan	0.12 ± 0.04	0.05 ± 0.03	0.11 ± 0.03
Between Zimbabwe and purebreds	0.35 ± 0.09	0.19 ± 0.07	0.08 ± 0.02

6.5.3 Cluster analysis

The results of the STRUCTURE clustering are displayed in Figure 6.1. At lower number of assumed clusters (K = 2 and 3*)*, the Zimbabwe eco-types clustered together with the Malawi, Sudanese and the two broiler lines. At *K = 2,* two solutions with approximately equal frequencies were observed. Both placed the white egg layers into one group and the two broiler lines, and African populations in the second cluster. At *K = 3* the most frequent (N = 71) solution showed the white and brown egg layers split to form two distinct gene pools while the broiler lines clustered with the African chickens. The solutions with the highest similarity coefficient (94 identical runs) were observed at *K = 4* and at *K = 6.* At *K = 4,* the purebred lines clustered into 3 distinct clusters (white egg layers, brown egg layers and broiler lines) separate from the African gene pool. At K = 6, the Malawi, Sudanese and purebred lines clustered as independent clusters and the five Zimbabwe eco-types gave one cluster. Above K = 6, the similarity coefficient dropped dramatically. The reference populations remained as distinct clusters, while individuals in the Zimbabwe eco-types were randomly

assigned to any of the added *K* clusters without showing any substructuring between eco-types.

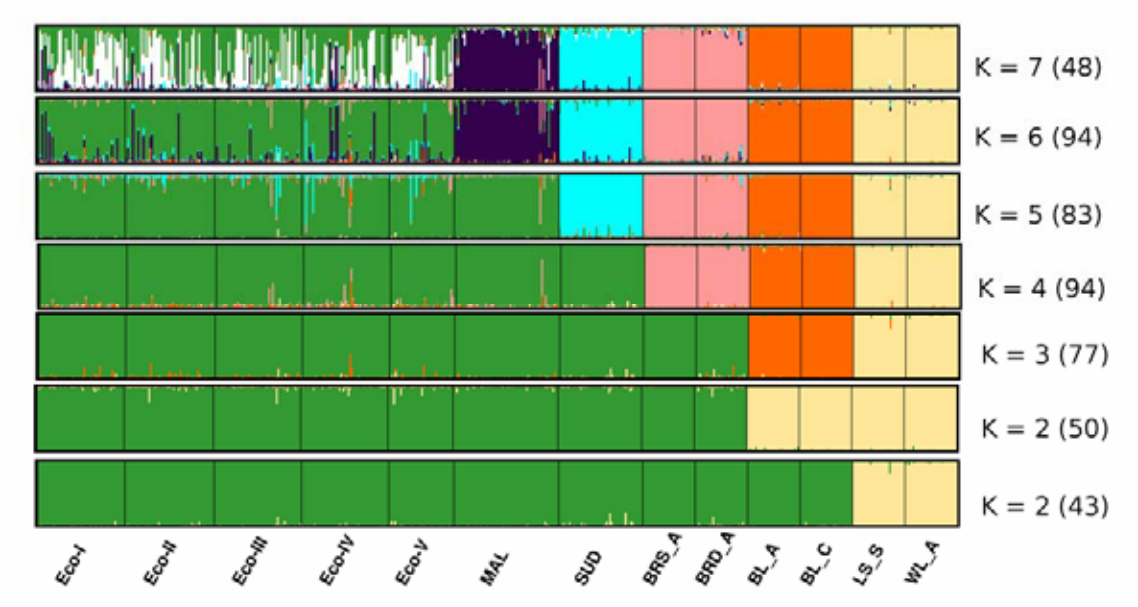

Figure 6.1: STRUCTURE clustering of Zimbabwe chicken eco-types in reference to the extensively raised Malawi and Sudanese chickens and purebred broiler, white and brown egg layers

Number in parenthesis indicates the number of identical solutions at 95% threshold.

Key:
Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; BRS_A = broiler sire line A; BRD_A= broiler dam line A; BL_A = brown egg layer line A; BL_C = brown egg layer line C; LS_S white egg layer experimental line and WL_A = white egg layer line A.

6.5.4 Marker estimated kinships

Marker estimated kinships within and between the populations is given in Table 6.4. The within population MEK for the Zimbabwe eco-types did not differ very much from the between eco-type MEK estimates. Mean MEK (± SD) value within eco-types was 0.130 ± 040, while the mean between eco-type estimate was 0.110 ± 0.005. The latter estimate was

slightly elevated in comparison to MEK estimates between eco-types and other populations. High between population kinship estimates were observed between pairs of purebred lines, particularly between the four egg layers.

A phylogenetic tree derived from the MEK estimates is given in Figure 6.2. The clustering indicates separation of the broiler lines from the layer lines, with the African populations clustered in between. Note the short branch lengths of the Zimbabwe eco-types.

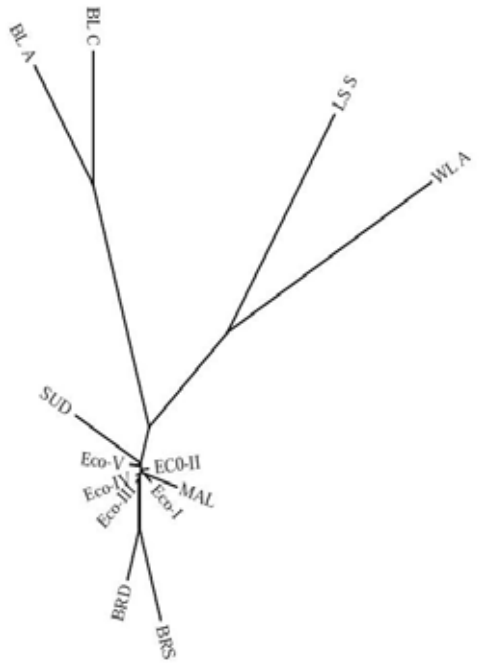

Figure 6.2: Neighbour-Joining tree derived from marker estimated kinships

Key:

Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; BRS_A = broiler sire line A; BRD_A= broiler dam line A; BL_A = brown egg layer line A; BL_C = brown egg layer line C; LS_S white egg layer experimental line and WL_A = white egg layer line A.

6.6 Discussion

Compared to the other eight populations used in this study, the Zimbabwe eco-types contributed more unique alleles and are thus a source of genetic diversity (Petit *et al.,* 1998). However, some of these alleles have low frequencies, contributing little to genetic variation

(Falconer and MacKay, 1996). In addition to the new alleles, the overall number of alleles/locus was higher in the Zimbabwe eco-types than in the purebred lines.

Both expected and observed heterozygosity estimates were high for the Zimbabwe eco-types together with the Malawi and Sudanese chickens (Table 6.2). Whereas purebred lines were founded on a limited number of breeds (Crawford, 1990) and selected for specific production traits, the Zimbabwe chicken eco-types have not been bred for any particular trait and roam freely during scavenging. The latter fact might result in migration of birds from one flock to a neighbouring one, causing a continuous gene flow between flocks, conserving a high number of alleles and heterozygosity in eco-type populations.

Zimbabwe eco-types raised under scavenging systems of production are highly polymorphic compared to the purebred lines (Tables 6.1 and 6.2). This agrees with other studies (Wimmers *et al.,* 2000; Hillel *et al.*, 2003; De Marchi *et al.,* 2006) in which wild and extensively raised chickens were found to be genetically diverse. Relatively high observed heterozygosity and allelic diversity have also been found in Tanzanian eco-types (Wimmers *et al.,* 2000) and free ranging village chickens from Mozambique and Botswana (Marle-Köster and Nel, 2000).

Contrary to what is implied by the large geographic distances between eco-types (300 - 800km), the low between eco-type (F_{ST}) variation (Table 6.3), indicated absence of clear substructuring of the Zimbabwe populations along agro-ecological zones. In fact, the observed total inbreeding (F_{IT}) was almost fully explained by within population inbreeding (F_{IS}, Table 6.3). Each Zimbabwe eco-type population seemed to represent the full range of genetic diversity present in Zimbabwe indigenous chickens. Although null alleles could lead to elevated F_{IS} values there was no indication of presence of null alleles in our analysis.
The relatively high F_{ST} estimates for commercial breeds indicate that each population represents a limited sample of the total gene pool. This high level of population divergence in purebred lines was expected because they are based on different founder breeds, raised as closed flocks and selected for different production traits (Delany, 2003).

STRUCTURE based clustering further supports the low among eco-type differentiation of the Zimbabwe chickens (Figure 6.1). The lack of observed substructuring among Zimbabwe eco-types at values of $K \geq 6$ suggests that Zimbabwe indigenous chickens essentially form one population. This finding agrees with observed Wright's fixation indices (Table 6.3).

Substructuring according to geographic location (eco-type) could not be observed. Furthermore, clustering of the Zimbabwe chickens was not related to phenotypic classes (data not shown). The separation of the purebred lines at $K \leq 4$ followed by the Sudanese and lastly Malawi populations emphasises the distinctiveness of the Zimbabwe population. The splitting of the Sudanese populations from the Zimbabwe populations at a lower *K* value (*K = 5)* than from the Malawian *(K = 6)* shows some geographical trend.

In Zimbabwe populations the mean within population kinships were only slightly higher than the mean between population kinships (Table 6.4). This observation could be due to either a very large effective population size or relatively strong and continuous gene flow between populations. Gene flow among populations would result in equal allele frequencies across all the five eco-types and give no cause of the inferred substructures. In addition to the lack of population substructuring, the MEK estimates showed low within population kinships in the Zimbabwe chicken eco-types compared to the purebred lines, in particular the white egg layers. The closer association of the Zimbabwe chicken eco-types with chickens from Malawi and Sudan (Figure 6.2) suggests that indiscriminate hybridisation with exotic commercial lines (Wollny, 2003; Hall, 2004) does not have a strong impact.

In conclusion, results from this study gave no indication that village chickens are substructured across agro-ecological zones. There is no evidence that the Zimbabwe chicken eco-types are locally adapted and restricted to their respective agro-ecological zones. The results did show high genetic variation within the Zimbabwe village chicken population.

Genetic Diversity and Phylogeographic Structure of the Zimbabwe Chicken Eco-Types Investigated Using Mitochondrial DNA D-Loop Sequences[Ψ]

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Chapter 7

7.1 Summary

This study sought to assess mitochondrial DNA (mtDNA) genetic diversity and phylogeographic structure of chickens from different agro-ecological zones of Zimbabwe. Furthermore, the degree to which Zimbabwe chickens shared haplotypes with chicken populations of different origins and management systems was determined. A 455bp fragment of the mtDNA D-loop region was sequenced for chickens from the five agro-ecological zones of Zimbabwe (n = 20 per eco-type), Malawi (n = 19) and Sudan (n = 20). In addition, two broiler, two white egg layer and two brown egg layer lines (20 chickens per line) were investigated for comparison. Thirty-one variable sites that defined 32 haplotypes were observed. Nine of the 32 haplotypes were unique to the Zimbabwe chicken eco-types. The major haplotype (A1) was present in all Zimbabwe eco-types and was also found in 18 out of 20 chickens from Malawi. The second major haplotype (C3) was widely distributed in four Zimbabwe eco-types (Eco-I – Eco-IV), three purebred lines (White Leghorn line LS_S, brown egg layer line BL_A and broiler dam line BRD_A) and in 80% of Sudanese chickens. Within Zimbabwe eco-type diversity accounted for 96.8% of the total variation while only 3.2% was due to between eco-type variations. The 259 individual chickens clustered into three clades that corresponded to (i) Zimbabwe and Malawi, (ii) purebred lines and (iii) mixture of Zimbabwe, Sudan and purebred lines. Results indicated a highly diverse Zimbabwe chicken population that is not substructured across agro-ecological zones, confirming findings based on autosomal markers. The Zimbabwe chickens shared some of the maternal lineages with other African and purebred chickens.

Keywords: chicken eco-types, genetic diversity, population structure, mtDNA,

7.2 Introduction

Village chickens in Zimbabwe are distributed over a wide geographical range. Due to the large distances and environmental differences, genetic variation is expected between indigenous chickens from contrasting agro-ecological zones of Zimbabwe. However, assessment of genetic diversity of these chicken eco-types using microsatellites revealed that they lack population substructure and make up one diverse population spread over a wide geographic range (Chapter 6). This lack of population substructuring might be due to either continuous gene flow among eco-types or the eco-types sharing many ancestral lineages from

domestication events. It is also possible that there was initially one single and diverse population which expanded into all the agro-ecological zones. Based on the microsatellite data Zimbabwe chickens are distinct from populations from Malawi, Sudan as well as purebred lines. Whether such population differentiation was due to genetic isolation and/or differences in the ancestral lineages is yet to be resolved.

Evolutionary relationships, level of variability and geographic substructuring can be assessed by comparing mitochondrial DNA (mtDNA) sequences (Avise *et al.,* 1987). The clonal transmission of mtDNA haplotypes avoids recombination noise and makes it possible to discern conserved maternal lineages. The D-loop region of the mtDNA is highly mutable and can therefore reflect genetic differences between recently separated populations (Harrison, 1989). Unlike nuclear DNA, inheritance of mtDNA is purely maternal and may therefore give insights into female specific evolution and population history (Weigend and Romanov, 2001). Such qualities of the mtDNA make it an appropriate marker to evaluate and explain the population structure of the Zimbabwe chickens. The objectives of this study were to (i) assess the genetic structure of the Zimbabwe chicken eco-types at the mtDNA level and to compare it to other extensively raised chicken populations and the purebred lines and (ii) to determine the degree to which Zimbabwe eco-types share haplotypes with other chicken populations raised in different production systems.

7.3 Materials and methods

7.3.1 Chicken populations

A total of 259 chickens were sampled from five Zimbabwe eco-zones, Malawi and Sudan and from purebred commercial and experimental lines.

7.3.1.1 Zimbabwe eco-types

Local chicken types (eco-types) were collected from five agro-ecological zones in Zimbabwe Geographical distances between agro-ecological zones ranged from 300km (Eco-III and Eco-V) to 800km (Eco-II and Eco-V). Details on the sampling framework and DNA isolation are described in the previous study (Chapter 6). Briefly, twenty chickens were sampled in eco-

zone I, II, IV and V, while 19 birds were selected from eco-zone III. One chicken was sampled per household.

7.3.1.2 Reference populations

Twenty chickens were sampled from each of the broiler dam (BRD_A) and sire (BRS_A) lines, from two brown egg layer (BL_A and BL_C) and two white egg layer (WL_A and LS_S) lines, respectively. These purebred lines were selected from the AVIANDIV project3, former European research cooperation on chicken biodiversity. In addition, 19 scavenging chickens sampled from Malawi (MAL), and another 20 from Sudan (SUD) kept in a similar extensive system of production were also included in this study.

7.3.2 mtDNA amplification and sequencing

Primers mtGlu-F (5′-ggcttgaaaagccattgttg-′3) and the mtGlu-R (5′-ccccaaaaagagaaggaacc-′3) were used to amplify a fragment of 455bp in size of the highly polymorphic D-loop region of the mtDNA. At the tails of these D-loop primers were universal primers M13-F (5′−gtaaaacgacggccag-′3) and M13-R (5′−caggaaacagctatgac-′3). PCR amplifications were based on HotStarTaq master mix (Qiagen GmBH, Hilden, Germany). The PCR products were purified by using ExoSAP-IT purification Kit (USB cooperation, USA) and then sequenced using fluorescently labelled primers complementary to the universal M13 sequence. Forward and reserve sequences were obtained using the Thermo Sequenase cycle sequencing kit (USB cooperation, USA). Sequencing products were visualized on 8% polyacrylamide gel using a LICOR DNA sequencer (LI-COR Inc., Nebraska, USA).

The forward and reverse DNA sequences were aligned using the AlignIR assembly and alignment software program (LICOR Inc., Nebraska, USA).

3 AVIANDIV EC Contract No. BIO4-CT98-0342 (1998-2000); Weigend, S (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K. Wimmers, T. Burke, and A. Mäki-Tanila (http://w3.tzv.fal.de/aviandiv)

7.4 Statistical analysis

7.4.1 Sequence variation and haplotype diversity

The position and number of variable sites and corresponding haplotypes were counted using MEGA version 3.1 (Kumar *et al.,* 2004). The number of unique haplotypes and their distribution in the samples was computed using the TCS software (Clement *et al.,* 2000).

7.4.2 Within population diversity

The 259 individual sequences were grouped according to their original population. Haplotype diversity *(h)*, which is the probability that two haplotypes sampled within populations are different, was calculated based on the formula:

$$h = \frac{\left(1 - \sum x_i^2\right)n}{n-1}$$

where x_i is the frequency of haplotype *i* and n is the sample size (Nei, 1973), using ARLEQUIN software (Excoffier *et al.,* 2006).

7.4.3 Determination of population structure using AMOVA

Analysis of molecular variance (AMOVA) was computed using the algorithms suggested by Excoffier *et al.* (1992) and implemented in the ARLEQUIN software (Excoffier *et al.,* 2006). Molecular variance components were computed for overall, between and within (i) all the 13 populations, (ii) the five Zimbabwe eco-types, (iii) the seven African populations (Malawi, Sudan and five Zimbabwe eco-types) and (iv) the six purebred lines.

7.4.4 Network analysis of haplotypes

To determine the relationships of haplotypes, median joining networks were constructed following the algorithms of Bandelt *et al.* (1995) using the NETWORK 4.1 software (www.fluxus-engineering.com/sharenet.html). Similar haplotypes cluster into clades. For each clade the number of individual chickens and unique haplotypes from each of the 13

populations were counted. The total number of individuals, number of unique haplotypes and haplotype diversity in each clade were computed using ARLEQUIN (Excoffier *et al.,* 2006).

7.5 Results

7.5.1 Sequence variation and haplotype distribution

In total, 31 variable sites that defined 32 haplotypes were observed. All the variable sites were due to substitution mutations, 94% of which were transitions (Supplementary Table S1).

7.5.2 Within population diversity

Nine of the 32 haplotypes were unique to Zimbabwe chicken eco-types (Table 7.1). The major haplotype (A1), which occurred at a frequency of 24 % across all populations, was common to all Zimbabwe eco-types (found in 52% of the Zimbabwe chickens), and was found in 90% of the Malawi chickens. The second major haplotype (C3) occurred at a frequency of 22% of the overall population and was widely distributed in four Zimbabwe populations, in three of the purebred lines and in 80% of the Sudanese chickens. Nineteen haplotypes (*i.e.* more than 50 % of the haplotypes found) occurred only once in the sample. The five Zimbabwe eco-types shared all the main haplotypes.

All 13 populations were polymorphic with the number of haplotypes ranging from two (LS_S line) to seven (Eco-IV) (Table 7.2). Haplotype diversity ranged from 0.29 – 0.78 and was low in the chickens from Malawi and Sudan, and white egg layers, respectively. In contrast haplotype diversity was high and averaged 0.65 in the Zimbabwe eco-types. Higher haplotype diversity were observed in the two brown egg layer lines (0.72 - 0.78), and in the broiler dam line (0.78).

Table 7.1: Distribution of mtDNA D-loop haplotypes in five Zimbabwe chicken eco-types, Malawi and Sudanese chickens and six purebred lines

Haplotype	ECO-I	ECO-II	ECO-III	ECO-IV	ECO-V	MAL	SUD	LS_S	WL_A	BL_A	BL_C	BRS_A	BRD_A	TOTAL
A1	12	9	11	6	8	16								62
A2	1			1										2
A3	1													1
A4	1													1
A5			1		2	2								5
A6				1										1
A7						1								1
B1				1										1
B2				1										1
B3									18					18
B4									1					1
B5										6	6			12
B6												12	7	19
B7											1			1
B8											1			1
B9													1	1
C1	3	1			8									12
C2	1	1	2		2				1	8				15
C3	1	8	5	9			16	9		5			4	57
C4		1												1
C5				1										1
C6							3							3
C7							1							1
C8								11				7	6	24
C9									1					1
C10										1				1
C11												1		1
C12											7			7
C13											1			1
C14											4			4
C15													1	1
C16													1	1
Total	20	20	19	20	20	19	20	20	21	20	20	20	20	259

Key:

Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; LS_S experimental white egg layer line; WL_A = commercial white egg layer line A; BL_A = commercial brown egg layer line A; BL_C = commercial brown egg layer line C; BRS_A = commercial broiler sire line A and BRD_A= commercial broiler dam line A.

Table 7.2: Number of polymorphic sites, number of mtDNA D-loop haplotypes and haplotype diversity of chickens populations from five Zimbabwe eco-types, Malawi, Sudan and six purebred lines

Population	N	Number of polymorphic sites	Number of haplotypes	Haplotype diversity (SE)
African				
ECO-I	20	12	7	0.64 ± 0.12
ECO-II	20	9	5	0.66 ± 0.07
ECO-III	19	9	4	0.61 ± 0.10
ECO-IV	20	13	7	0.73 ± 0.08
ECO-V	20	10	4	0.69 ± 0.06
MAL	19	2	3	0.29 ± 0.13
SUD	20	2	3	0.35 ± 0.12
Purebreds				
LS_S	20	1	2	0.52 ± 0.04
WL_A	21	13	4	0.27 ± 0.12
BL_A	20	9	4	0.72 ± 0.05
BL_C	20	11	6	0.78 ± 0.06
BRS_A	20	4	3	0.54 ± 0.08
BRD_A	20	13	6	0.78 ± 0.06

Key:

Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; LS_S experimental white egg layer line; WL_A = commercial white egg layer line A; BL_A = commercial brown egg layer line A; BL_C = commercial brown egg layer line C; BRS_A = commercial broiler sire line A and BRD_A= commercial broiler dam line A.

7.5.3 Population structure

Between population variation was 46.6% of the total variation while the remaining 53.4% was within population diversity (Table 7.3). Within Zimbabwe eco-type diversity accounted for 96.8% of the total variation while only 3.2% was between eco-types. In comparison diversity between populations accounted for 39% of the total variation in the group of purebred lines.

The total variance was higher for the six purebred lines compared to the African group and was least for the Zimbabwe chickens. High and significant (*P <0.001)* F_{ST} were observed for the purebred and the group of African chicken population.

Table 7.3: Partition of mtDNA D-loop variance within and between five Zimbabwe eco-types, seven African chicken populations (five Zimbabwe eco-types, Malawi and Sudan) and six purebred lines and the level of population substructuring (F_{ST})

Level of analysis	Components of variance (% variation)			F_{ST}
	Within population	Between population	Total	
Five Zimbabwe eco-types	2.03 (96.79)	0.07 (3.21)	2.11	0.03^{NS}
Seven African populations	1.51 (70.46)	0.63 (29.54)	2.14	0.30***
Six purebred lines	1.89 (60.98)	1.21 (39.02)	3.12	0.39***
All 13 populations	1.69 (53.42)	1.47 (46.58)	3.16	0.47***

NS = Non significant population substructuring *(P = 0.14)*

*** = Significant population substructuring *(P < 0.001)*

7.5.4 Network analysis

The network of 259 individuals is presented in Figure 7.1. Three main clades were observed. The number of individual chickens per clade and population are shown in Table 7.4. Clade A centred on haplotype A1 and was made up of haplotypes from Zimbabwe and Malawi chicken populations. Distances between haplotypes ranged from 1 to 4 mutations. Clade B consisted of individuals mainly from lines LS_S, BRS_A and BRD_A. Clade C was made up of haplotype C3 at the centre surrounded by haplotypes from a wide geographic range (Zimbabwe, Sudan and all purebred lines). The distance between haplotypes ranged from 1 to 5 mutations. Clades A and C were separated by 5 mutations and presented a star-like topology. Unlike the close clustering of haplotypes around A1 and C3, haplotypes in Clade B grouped into two small subclusters centred around haplotypes B5 (brown egg layers) and B6 (broiler dam and sire lines).

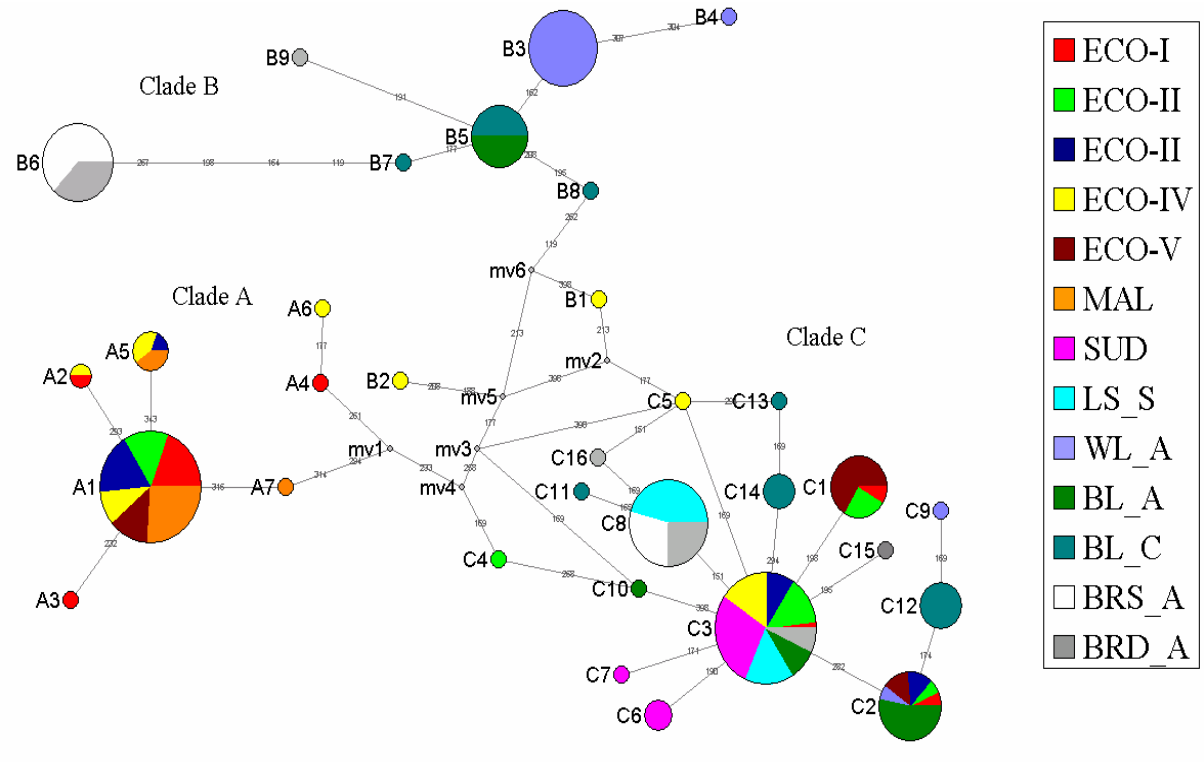

Figure 7.1: Median Network profile of the 32 mtDNA D-loop haplotypes observed in the five Zimbabwe eco-types, Malawi and Sudanese chickens and six purebred lines

The circle size corresponds to haplotype frequency. Numbers on the line correspond to mutational positions connecting haplotypes

Key:

Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; LS_S experimental white egg layer line; WL_A = commercial white egg layer line A; BL_A = commercial brown egg layer line A; BL_C = commercial brown egg layer line C; BRS_A = commercial broiler sire line A and BRD_A= commercial broiler dam line A.

7.5.5 Within and between clade diversity

Within clade diversity accounted for 16.1 % of the total variation and the remaining 83.9% was due to variation between clades. The total number of haplotypes and the haplotype diversity *(h)* of different clades are given in Table 7.4. Haplotype diversity was very low in clade A compared to clades B and C.

Table 7.4: The number of individuals per population, total number of haplotypes and haplotype diversity of the 3 mtDNA D-loop clades in Figure 1

	Clade A	**Clade B**	**Clade C**
ECO-I	15	-	5
ECO-II	9	-	11
ECO-III	12	-	7
ECO-IV	8	2	10
ECO-V	10	-	10
MAL	19	-	-
SUD	-	-	20
LS_S	-	-	20
WL_A	-	19	2
BL_A	-	6	14
BL_C	-	8	12
BRS_A	-	8	12
BRD_A	-	12	8
Total	73	53	133
Total haplotypes	7	9	16
Haplotype diversity	0.28 ± 0.07	0.74 ± 0.03	0.76 ± 0.03

Key:

Eco-I to Eco-V are the five Zimbabwe eco-types; MAL = Malawi; SUD = Sudan; LS_S experimental white egg layer line; WL_A = commercial white egg layer line A; BL_A = commercial brown egg layer line A; BL_C = commercial brown egg layer line C; BRS_A = commercial broiler sire line A and BRD_A= commercial broiler dam line A.

7.6 Discussion

All the 13 populations were polymorphic for the mtDNA D-loop region and had many haplotypes (Table 7.2). Multiple maternal origins of chickens during domestication (Liu *et al.,* 2006) could result in many haplotypes being initially introduced into the population. Maternal inheritance (Watanabe *et al.,* 1985) and clonal transmission of mtDNA (MacHugh & Bradley, 2001) allows that any of these ancestral haplotypes may persist in a population unless hindered by reproductive failure or other selective disadvantages of lineages (Harrison, 1989). However, over 50% of the haplotypes occurred at very low frequencies in the samples of 20 individuals per population. These infrequent variants may be products of new mutations (Figure 7.1). It is expected that haplotypes based on recent mutations are at lower frequency in a population compared to haplotypes from ancestral maternal lineages.

High haplotype diversity occurs when there is equal representation of the haplotypes in the population. Compared to the white egg layers, Malawi and Sudanese chickens, the Zimbabwe eco-types exhibited higher genetic variation (Table 7.2). The AMOVA results indicated that there was no substructuring of the Zimbabwe population. Within eco-type variation was high and accounted for over 90% of the total variation (Table 7.3). These findings agree with the lack of population substructuring and high diversity found in Zimbabwe chicken eco-types when microsatellite data were analysed for the same populations (Chapter 6). The purebred populations on the other hand, exhibited higher genetic variation that was caused by substructuring into separate and isolated lines.

Clustering of individual haplotypes resulted in three distinct clades (Figure 7.1). The Zimbabwe chickens were affiliated to two of these clades and all the five eco-types were equally represented in clades A and C (Table 7.4). This, like the AMOVA results, implies that the five eco-types of Zimbabwe are not substructured along agro-ecological zones. AMOVA results showed high between clade diversity (>80%) indicating that the clades were based on very distinct maternal lineages. Although gene flow cannot be ruled out, the observed networking of haplotypes clearly suggests that the high genetic diversity and population structure of Zimbabwe eco-types is due to the existence of multiple maternal lineages that are common to all the five eco-types. These results agree with the high level of

heterozygosity and the low between eco-type marker estimated kinships observed in the Zimbabwe eco-types based on microsatellite markers (Chapter 6).

Haplotypes A1, B5 and C3, which form the basis of the 3 clades, occurred at high frequency and presented a star-like branching structure with several derived haplotypes surrounding them. The star topology, which was more pronounced in clades A and C, is associated with ancestral haplotypes undergoing population expansion (Lopes *et al.,* 2005). The high level of population differentiation between purebreds and Zimbabwe eco-types observed with microsatellite analysis (Chapter 6) further suggests that Clade C is a reflection of an ancient genetic structure and not of a recent or ongoing interaction between populations. These results indicate that there could be at least three distinct maternal lineages from which these chicken populations were derived. The five Zimbabwe eco-types and the purebred lines each have a unique lineage plus one common maternal lineage among them (Clade C). In contrast, the Malawi and Sudanese populations are only aligned to single clusters. Unlike the five purebred lines which are subgrouped according to production systems (egg laying and broiler lines), the Zimbabwe eco-types were evenly distributed between the two clades and there was no evident eco-type based substructuring.

Contrary to the clear separation observed at the microsatellite level, the Zimbabwe chickens shared some of their haplotypes with purebred lines, Malawi and Sudanese populations (Tables 7.1 and 7.4). Unlike autosomal genetic structures, that can be altered as populations are separated, mtDNA genetic structures tend to be maintained amid genetic isolation or populations interbreeding. The genetic differentiation of the Zimbabwe and reference populations observed at the microsatellite level (Chapter 6) could therefore be explained by current genetic isolation and restricted gene flow among populations that shared some of their ancestral maternal lineages.

Supplementary Table S1: Variable sites of the 32 haplotypes from 5 Zimbabwe eco-types, Malawi, Sudan and 6 purebred lines.

	1111111111	1111222222	2222233333	3]
[	1566667778	9999013356	6689900114	9]
[	9124591478	0158832381	2723447453	8]
A1	TTCGGTCACT	GACCCTTGCT	TCCTGCTTTC	C
A2	....	....	...C......	.
A3	....	......C...	....	.
A4	....	C	....A..CC.	.
A5	....	....	.........T	.
A6	....	.......AT.	....A..CC.	T
A7	....	....	........C.	.
B1	........T.	.....C.AT.	....A..CC.	T
B2	........TC	....A..AT.	....A..CC.	.
B3	C.T.....T.	..T.TC.AT.	C...A..CC.	.
B4	C.T.....T.	..T.TC.AT.	C...ATCCC.	.
B5	C.......T.	..T.TC.AT.	C...A..CC.	.
B6	...A......	..TTTC.AT.	CT..A..CC.	.
B7	C.........	..T.TC.AT.	C...A..CC.	.
B8	C.......T.	.....C.AT.	C...A..CC.	.
B9	C.......T.	.GT.TC.AT.	C...A..CC.	.
C1	.....C....	...T...AT.	....A..CC.	T
C2	.....C....	.......AT.	..T.A..CC.	T
C3	.....C....	.......AT.	....A..CC.	T
C4	.....C....	.......A..	....A..CC.	.
C5	........T.	C	....A..CC.	.
C6	.....C....	C......AT.	....A..CC.	T
C7	.....CT...	.......AT.	....A..CC.	T
C8	.C...C....	.......AT.	....A..CC.	T
C9	.......G..	.......AT.	..T.A..CC.	T
C10	.....C....	.......AT.	....A..CC.	C
C11	.C..AC....	.......AT.	....A..CC.	T
C12	.....C.G..	.......AT.	..T.A..CC.	T
C13	....	.......AT.	.......CC.	T
C14	.....C....	.......AT.	.......CC.	T
C15	.....C....	..T....AT.	....A..CC.	T
C16	.C........	.......AT.	....A..CC.	T

Dots indicate nucleotide positions identical to those of Haplotype A1

Numbers at the top refer to variable sites and corresponds to the nucleotide positions of Haplotype A1.

The aligned sequences correspond to base pair positions 49 – 503 of mtDNA D-loop genebank sequence accession number: *AB294233*

General Discussion

8.1 General discussion

The overall goal of this study was to characterise diversity of the local chicken population in Zimbabwe. This is necessary because village chickens play a very important role in smallholder farming communities yet they seem to be neglected due to lack of inventory data. In addition, the available conservation strategies depend on pre-defined populations. It is almost impossible to set up breeding programmes and conservation schemes for uncharacterised or inadequately defined chicken populations. This study sought to understand the production systems in which the chickens are reared, and define and explain their population genetic structure.

As reviewed in Chapter 2, the Zimbabwe village chicken production systems are similar to those found in all African countries (Kitalyi, *et al.,* 1998; FAO/IEAE, 2002). The chickens are central to the livelihoods of smallholder farmers who depend on farming. The extensive systems of production under which these chickens are raised seem to be the most appropriate to these resource-limited farmers. Chickens are raised using fewer inputs but they still produce meat and eggs for household needs. In relation to biodiversity, village chickens are seen as a reservoir of genes that could be of future use. The harsh environmental conditions under which these chickens are raised were expected to produce, through natural selection, diverse alleles and allele combinations that are not present in the highly selected commercial or industrial lines. It was also assumed that village chicken genetic diversity is a product of different farming systems and is shaped by farmers' socio-economic circumstances.

The main objective of Chapter 4 was to characterise the farming systems in the different agro-ecological zones of Zimbabwe with focus on the production of village chickens. Results from this study presented the opportunities and threats to chicken production that arise in smallholder production systems. In all eco-zones, farmers depended on agriculture as a source of livelihood (Table 4.1 and 4.2). Such a dependency is considered an opportunity particularly for indigenous genetic resources that are found in most smallholder farming communities (Hall, 2004). Smallholder farmers will only keep and maintain resources that they are able to derive livelihoods from (Anderson, 2003; Geerlings *et al.,* 2002). A number of national and non-governmental organisations are further promoting the existence of indigenous genetic resources by incorporating them in developmental programmes. Chickens, for example, have

been used in a number of projects to alleviate poverty and achieve gender equality (Dolberg and Peterson, 2000).

Although agriculture was the mainstay of the rural economy, an over-dependency for income on crops and not livestock was observed in ecological zones I to IV (Tables 4.1 and 4.2). Climatic factors in these regions supported crop production and there was less opportunities to focus on commercial livestock production. Competition for resources is the main disadvantage and threat to AnGR in most mixed crop-livestock farming systems. Smallholder farmers have limited resources that they have to efficiently allocate among the farming activities in such a way that profits are maximised. As a result, fewer resources are allocated to less productive farming activities compared to the major enterprises that farmers depend on for livelihood. The lesser roles of livestock in ecological regions I to IV implied therefore that livestock received less attention in these eco-zones.

Although eco-zone I and II are generally referred to as cropping regions, this study revealed more support for chicken production in these areas. This was evidenced by the higher chicken flock sizes and higher ranks attached to chickens (Tables 4.5 and 4.6). The limited land available due to large crop plantations meant that chickens will be favoured against cattle that require large pieces of land for ranching. In addition, more crop residues in eco-zones I and II supported higher chicken flock sizes.

Even though livestock were the major source of income in eco-zone III and V, small chicken flock sizes were observed in these eco-zones. In addition, chickens were ranked second to cattle and goats as a source of income in agro-ecological zone II, IV and V. Since crop production is marginal in eco-zone V, competition and threats to chicken production come from other livestock species particularly cattle and goats. Households that owned cattle and goats assigned less important ranks to village chickens (Table 4.7). In eco-zone V, the threat was enhanced by over dependency of this region on livestock. Chickens are a small asset used for household subsistence and therefore are less preferred for income generation than goats and cattle.

Overall, Chapter 4 highlighted the support that village chicken production enjoys in the cropping regions of Zimbabwe. This variation in the importance of chickens could form a basis for substructuring of the village chickens in a number of ways. In one way, the

biological and socio-economic factors would define the breeding strategies and practices that would influence allelic distribution among the eco-zones. The lower flock sizes observed in villages where chickens were not so important imply some variations in effective population sizes. Differences in effective population sizes are a cause of population substructures particularly through drift (Caballero and Toro, 2000 and 2002). Based on findings in Chapter 4, it was therefore expected that the village chickens in Zimbabwe were substructured along agro-ecological zones.

Chapter 5 presented results that invariably supported the above hypotheses and in another vein, rejected variation among eco-zones. There was variation in the observed ranking of strains among eco-zones (Table 5.2). However, no eco-zone effect was observed in breeding animal selection criteria (Table 5.3), ranking of production traits (Table 5.4) and criteria used to cull chickens (Table 5.6). Regardless of variation in the climatic and socio-economic factors among the eco-zones, the focus of the farmers were to have meat and high chicken flock sizes through good reproductive performance. Farmers in the five agro-ecological zones also preferred chickens that were always healthy (Tables 5.4 and 5.6) particularly in the face of several disease challenges. These observations gave an indication that the production goals and challenges faced by village chicken farmers are similar in all agro-ecological zones. Although such similarities were observed, it could not be ascertained and therefore can not be guaranteed that the same genotypes were promoted in the different agro-ecological zones. The interactions between the environment and genotype give different phenotypes that the farmers use to select breeding animals in these systems. In addition, it could not be determined whether the disease pathogens experienced in the five agro-ecological zones varied.

While the preferences for higher production levels by smallholder farmers might threaten low performing village chickens, it is highly unlikely that exotic breeds would replace the indigenous populations in any of the agro-ecological zones of Zimbabwe. By using local chickens farmers ensure that they get both meat and eggs with minimum input requirements. Although exotic commercial breeds are high yielding, they are too specialised and require high levels of management. It is also unlikely that these chickens will produce optimally under the compromised village chicken production systems characterised by poor nutrition and disease challenges (FAO/IEAE, 2002). This study found that farmers still consider chicken's health, survivability and good mothering ability as important production traits

(Tables 5.3 and 5.4). Smallholder farmers would prefer local hens that are able to lay and incubate eggs to continuously regenerate their flocks.

The simultaneous consideration of several economically important traits could also be a positive feature for the village chicken diversity in Zimbabwe and similar countries. In intensive production systems, diversity is threatened by uni-directional selection for fewer production traits. Meanwhile, the extensive system of village chicken production is associated with poorly defined breeding practises characterized by many production and health parameters. Moreover, culling and selection of breeding animals was undertaken in the absence of management records. It is unlikely that farmers achieve considerable selection success under such conditions. The chances of chicken populations getting fixated for any alleles under such poorly defined breeding practices are low.

Inferring population structure from the production systems alone has its own shortcomings. When isolated, similar production systems can lose some alleles or support different ones through random genetic drift and mutations. Alternatively, two similar production systems could be based on two or more different base populations with a completely different set of alleles all suited to survive in those production systems. In this case, a conclusion would be wrongly drawn that production systems do not cause substructuring. Molecular technologies have opened up more reliable ways of investigating genetic diversity and population structures. Microsatellites are highly polymorphic codominant DNA markers that have been widely used in population genetics. Genetic diversity measures using microsatellites have also been shown to yield reliable estimates of genetic variation (Weigend and Romanov, 2001).

All five Zimbabwe chicken eco-types exhibited high genetic diversity. This was apparent from the findings which showed that the eco-types had more alleles, higher levels of observed and expected heterozygosity and higher within eco-type variation compared to purebred commercial and experimental lines (Table 6.2 and 6.3). The high level of population diversity is an indication of the variability of the village chicken production system. Unlike purebred commercial lines that are raised under controlled environments the village chicken eco-types are exposed to a wide range of environmental conditions (climate, nutrition, diseases). These environmental pressures fluctuate within and between eco-regions. High diversity increase ability to cope with many and sometimes fluctuating production challenges.

Although the eco-zones were geographically separated from each other (300-800km apart) and showed marked differences in the farming systems, chicken flock sizes and their importance (Chapter 4), the molecular assessment of population structure indicated that the village chickens in Zimbabwe were not substructured according to agro-ecological zones (Table 6.3, Figure 6.1). The microsatellites used in this study are assumed to be neutral markers and give an indication of overall population differentiation (Eding and Laval, 1999; Weigend and Romanov, 2001). The results therefore indicated that the eco-types were not so genetically isolated to be differentiated by genetic drift. There was no evidence that the Zimbabwe chicken eco-types are specialised populations restricted to their respective agro-ecological zones. Despite being raised in contrasting agro-ecological zones, the large geographical distances, different biological, social and economic factors among the farming systems have not played a significant role in the structuring of the populations.

The existence of chicken eco-types in Zimbabwe as one population over a broad geographical range could be an indication of their genetic capability to survive diverse production environments within and between agro-ecological zones. An individual eco-type contained all the alleles representative of the whole of Zimbabwe's chicken population. Such high levels of genetic diversity and lack of population substructure could also be seen as a product of the wide range of selection criteria that were imposed by farmers in all the agro-ecological zones (Tables 5.3, 5.4 and 5.6). The microsatellites used in this study were however assumed to be neutral to selection and it is not clear whether these eco-types have experienced similar selection pressures. The inference about adaptive genetic diversity of the Zimbabwe eco-types based on the microsatellite results is therefore not conclusive.

The results from this study also indicated that the Malawi and Sudanese chickens raised under similar production environments were genetically distinct from Zimbabwe gene pool. This implies that production systems can not be used to infer the genetic population boundaries of village chickens. Malawi, Sudan and Zimbabwe chicken eco-types are raised under the extensive system of production. The genetic differentiation between the Zimbabwe, Malawi and Sudan populations could be better explained by genetic isolation of chicken populations from different countries. Sudan which is more geographically distant from the Zimbabwe eco-types also came up to be more genetically different from the Zimbabwe eco-types compared to Malawi chickens. This finding agrees with Kitalyi (1998) who observed that the extensive systems of village chicken production are heterogeneous and depend on

biological and socio-economic factors of communities. Such factors vary between different countries resulting in different population structures. If genetically isolated, the populations would develop differently through random genetic drift or by natural and artificial selection.

In Ethiopia, Tadelle (2003) concluded that eco-types are genetically distinct populations, an observation that contradicts the findings from this study. Phenotypic (Msoffe *et al.,* 2001a) and genetic (Msoffe *et al.,* 2005) differences were also observed between Tanzanian eco-types. These results suggest differences in the chicken population structures of different countries. However, there were limitations in both Tadelle (2003) and Msoffe *et al.* (2005) sampling frameworks that should be considered before arriving at such conclusions. Low numbers of markers (ten) were used in assessing genetic diversity in the Ethiopian eco-types (Tadelle, 2003). Msoffe *et al.* (2005) used 13 individual chickens per population to assess within and between eco-type diversity. Sample sizes, number of markers and their polymorphism are critical factors for achieving accurate assessment of genetic diversity (Hillel *et al.,* 2007). The FAO MoDAD project has recommended that at least 30 polymorphic markers and 25 individuals per population should be used to characterize chicken genetic diversity.

Particularly important for conservation was the observation that purebred populations that are well managed and considered safe from extinction (Delany, 2003) were genetically distinct from the Zimbabwe chicken eco-types (Figures 6.1 and 6.2). Similarities between purebreds and eco-types would have made these village chickens redundant and allowed sampling from well defined purebred lines as representative populations for preservation. However, results indicated that these unique eco-types from diverse and low-resource production systems deserve consideration in conservation programmes.

The autosomal nuclear microsatellite loci used in this study are bi-parental markers whose inheritance is affected by recombination. Ancient population structures are therefore likely to be masked by generations of interbreeding when analysed at the microsatellite level. The mtDNA D-loop sequence on the other hand is a highly mutable marker that is clonally transmitted by female chickens. The absence of recombination at the mtDNA allows one to study conserved population structures. The current population structure can still be identified through recent mutations. The mtDNA sequence data can therefore be used to explain the

observed genetic structures particularly when used with other nuclear markers such as microsatellites.

The Zimbabwe chicken eco-types shared all the major haplotypes (Table 7.2) giving no cause (as with microsatellites, Table 6.3 and Figure 6.1) for population substructuring. These eco-types had many (Table 7.3) and distinct (Figure 7.2) haplotypes which corresponded to the many alleles, high observed and expected heterozygosity (Table 6.2) and low marker estimated kinships (Table 6.4) evident at the microsatellite level. The Zimbabwe chickens seem to be derived from at least 2 distinct maternal lineages (Figure 7.1 and 7.2). All eco-types were equally represented in these lineages (Table 7.4). Based on mtDNA and microsatellites findings, the high genetic diversity in the Zimbabwe populations can be associated with the diversity of the maternal lineages. The lack of population substructuring on the other hand, could be due to the absence of genetic isolation and the sharing of mtDNA haplotypes between all eco-zones.

The findings were slightly different for the population structure of the other African gene pool and also the purebred lines. In contrast to the Zimbabwe eco-types, Malawi and Sudanese populations were aligned to single mtDNA clades (Table 7.4). This observation further confirms that village chickens in different African countries are isolated from each other. Malawi and Sudan within population diversity parameters compared well to those of the Zimbabwe chicken eco-types at the microsatellite level (Table 6.2). However, it seems that these two other African populations have a narrow genetic base from the maternal lines (Table 7.3; Figure 7.1). These African populations are genetically isolated from the Zimbabwe eco-types and could have therefore lost some of its mtDNA diversity through drift and natural selection. Alternatively the Malawi and Sudanese populations could have originated from less diverse maternal lineages.

At the mtDNA level all the 13 populations shared some haplotypes. For example, 80% of the Malawi and Sudanese chickens belonged to single haplotypes found in all the five eco-types (Table 7.2). Haplotypes found in the Zimbabwe eco-types were also found in all the purebred lines. Haplotype sharing suggests that the 13 populations studied shared maternal lineages. These results however contrast with findings based on microsatellites whereby purebred lines were very different from the African gene pool (Table 6.4 and Figures 6.1 and 6.2). The genetic distinction of these populations observed at the microsatellite level could therefore be

explained by current genetic isolation and restricted gene flow between the populations. The sharing of haplotypes shows that the genetically isolated populations have some maternal lineages in common and this could date back to the time of domestication. The domestication of village chickens is an area that is still under investigation. The three distinct maternal lineages observed and the clustering of the Zimbabwe, Malawi, Sudan and purebred chicken haplotypes (Figure 7.1 and Table 7.4) seem to agree with the suggestion that there are multiple maternal origins of chickens (Liu *et al.,* 2006).

8.2 Conclusions

From this study several conclusions can be drawn:

(i) The village chicken production systems vary among the five agro-ecological zones of Zimbabwe. There is variation in the farming activities and the integration of chickens in the different eco-regions.

(ii) Farmers from the five agro-ecological zones show the same preferences for chicken production traits. They also use the same criteria to select breeding animals.

(iii) There is no evidence, from microsatellite and mtDNA analysis that chickens from the five eco-zones of Zimbabwe represents genetically distinct populations. There is no indication that the Zimbabwe chicken eco-types are genetically isolated or locally adapted to their respective agro-ecological zones to be considered as different populations.

(iv) The Zimbabwe chicken population as a whole is highly diverse and seems to have been derived from at least two maternal lineages. All eco-types were equally represented in the mtDNA lineages.

(v) At autosomal level, the Zimbabwe eco-types are genetically separated from chicken populations from Malawi, Sudan and six purebred lines.

(vi) Although clearly separated from the reference populations, the Zimbabwe chickens shares mtDNA haplotypes with chickens from Malawi, Sudan and six purebred lines indicating some common but ancient maternal lineages between these 13 populations.

8.3 Implications and recommendations

There should be a global initiative to intensify genetic characterization of population structures of indigenous chickens by including data from several countries. Results from this

study showed that the Malawi and Sudan chicken populations are genetically distinct from the Zimbabwe populations. In other studies population structures different from one observed in Zimbabwe were reported (e g. Tadelle, 2003). There is very limited gene flow between African populations and this would allow populations to evolve differently and have different population structures. Assessment of the chicken population structures should be conducted using sufficient sample sizes and possibly with the similar markers to allow unbiased comparisons. The use of standard alleles would make it possible to adjust for allele scoring between laboratories

Further research need to be conducted on whether the different eco-types have experienced different selection pressures. Although microsatellites are assumed to give average genome wide relatedness of populations, they do not show direct population differences in certain production traits. Analysis of polymorphism at genes known to code for specific traits, will add more information on the population structures of village chicken populations.

The high genetic diversity in the Zimbabwe chicken populations bears positive implications for both breeding programmes and conservation of poultry genetic diversity. Appropriate breeding programmes should be designed that take into consideration farmer interest and capacities as discussed in Chapters 4 and 5. If well designed, selection programmes will most likely yield good results due to high level of within population diversity (Chapters 6 and 7). At present there are no formal indigenous chicken breeding programmes and there is no infrastructure to support it (e.g. recording keeping). The aim of the breeding programmes should be, as reflected in Chapter 5, to produce a flexible breed that produces enough meat and eggs under the harsh extensive production systems that prevail in rural Zimbabwe.

The high number of alleles might prioritise the free ranging chicken eco-types of Zimbabwe for conservation (Simianer, 2005). In addition, populations with lower within population kinship estimates (as was observed in the Zimbabwe chicken eco-types) tend to have large contributions to the conservation core-sets (Mateus *et al.,* 2004). While individuals for conservation from the Zimbabwe chickens can be sampled from any one of the five eco-types, *in situ* conservation strategies depend so much on production systems on the ground. The variation in the agro-ecological zones should therefore be taken into consideration for such *in situ* programmes. It is also worthwhile to design conservation programmes that maximise on the high genetic diversity spread over a wide geographical range in Zimbabwe

and possibly other developing countries. The current conservation programmes are based on pre-defined breeds and this is a major limitation particularly for these village chickens. An alternative would be to consider diversity as a ‘continuum’ and not restrict it to within or between breed boundaries

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SUMMARY

The overall goal of this study was to characterise diversity of the local chicken population in Zimbabwe. Specifically the study sought to determine the production systems, breeding practices and genetic diversity between and within chicken populations from the five agro-ecological zones (eco-zones) of Zimbabwe. The level of genetic differentiation in the Zimbabwe chickens were compared to that of a set of reference populations from the purebred lines and other extensively raised African chickens.

In the first part of the study (Chapter 4), the village chicken farming systems and possible threats to and opportunities for local chickens in the five agro-ecological zones of Zimbabwe were characterised. Data were collected using a pre-tested questionnaire administered to households randomly selected from Risitu (n = 97, Hurungwe (n = 56), Gutu (n = 77), Gokwe-South (n = 104) and Beitbridge (n = 37) in eco-zones I-V, respectively. The results indicated a general dependence on agriculture as a source of income and livelihoods by communal farmers in all the agro-ecological zones. Overall, 17.7 percent of the households ranked livestock as their major source of income compared to 70.8 percent who ranked crops as the main contributor. Chicken flock sizes averaged 16.74 (SD = 12.40). Highest flock sizes were observed in agro-ecological zones I and IV. Households owning cattle, goats and other livestock assigned less important ranks to chickens. Chickens were used mainly for the provision of meat and eggs. Results indicated more support for village chickens in the cropping regions of the country compared to the arid agro-ecological zones. This was probably due to limited land for cattle ranging in the cropping regions particularly eco-zone I and the availability of crop residues as chicken supplementary feed.

In Chapter 5, the existence of chicken strains and breeding goals and strategies used by village chicken farmers in Zimbabwe were investigated. Fifteen chicken variants mostly defined by morphological traits were reported in the five eco-zones. Production criteria such as body size, health and fertility were highly ranked by farmers across all the eco-zones. As a common breeding practice, farmers choose the type of hens and cocks to retain for breeding purposes and these randomly mix and mate with others from community flocks. It was observed that agro-ecological zone had no effects on the trait preferences and culling criteria. Chicken body size was ranked the major determinant in choosing breeding animals followed by mothering ability, availability, fertility and other morphological traits respectively. More

households preferred chickens associated with good reproductive performance, fast growth rates and those tolerant to disease pathogens. The absence of farmer records to use in selection of breeding animals and the focus on many production and health traits could be a major compromise to making genetic progress in these production systems.

In Chapter 6, the objective was to investigate the population structure of village chickens found in the five agro-ecological zones of Zimbabwe. Twenty-nine microsatellites markers were genotyped for chickens randomly selected from the five Zimbabwe eco-zones (n = 238). Reference populations from Malawi (n = 60), Sudan (n = 48) and six purebred lines (n = 180) were also included in the study to give 13 populations in total. Results indicated a highly diverse Zimbabwe chicken population. Numbers of alleles per locus, expected and observed heterozygosity were high in the five eco-types. Within eco-type marker estimated kinship was low and comparable to between eco-type marker estimated kinship indicating that the level of genetic variation was high and very similar within and between eco-types. There was a rejection of the hypothesis that village chickens are substructured across agro-ecological zones. F_{ST} values were low and almost all the genetic variability was explained by within eco-type variation. The five eco-types remained as one cluster during STRUCTURE based analysis. The reference populations on the other hand formed distinct clusters separated from the Zimbabwe eco-types.

The mtDNA D-loop sequences were used to determine genetic diversity and the degree to which Zimbabwe chicken populations share haplotypes with other chicken populations raised in different production systems (Chapter 7). A 455bp region of the mtDNA D-loop region was sequenced for 259 chickens from the five Zimbabwe eco-types (n = 99); Malawi (n = 19; Sudan (n = 20) and six purebred lines (n = 121). Within Zimbabwe eco-type diversity accounted for 96.8% of the total variation while only 3.2% was due to between eco-type variation. The mtDNA haplotypes clustered into three clades that corresponded to (i) Zimbabwe and Malawi, (ii) purebred lines and (iii) mixture of Zimbabwe, Sudan and purebred lines. The five Zimbabwe eco-types were equally represented in two of these clades. Results indicated a highly diverse Zimbabwe chicken population that is not substructured across agro-ecological zones. At the mtDNA level, all the 13 populations shared some major haplotypes.

Overall, the study showed that the different climatic and socio-economic factors between agro-ecological zones do not influence farmer's preferences for chicken production traits and the genetic structures of chicken populations. Genetic diversity in the Zimbabwe population was high and could be attributed to gene flow between eco-types and the presence of highly diverse maternal lineages. This high level of genetic diversity was expected in extensively raised and unselected village chickens particularly when compared to highly specialised purebred lines. The Zimbabwe chicken population was not substructured along agro-ecological zones. There was no evidence that the Zimbabwe eco-types are genetically isolated, locally adapted and/or restricted to their respective agro-ecological zones. All the eco-types shared the mtDNA lineages observed.

Based on the autosomal microsatellites, the Zimbabwean population was separated from the reference populations particularly the purebred lines. The genetic differences between the Zimbabwe chicken eco-types and reference populations were probably caused by genetic isolation and restricted gene flow between populations. Between African populations gene flow is restricted by the large geographical distances and physical barriers between countries. This was evidenced by the observation that geographically distant Sudanese chickens were more genetically separated from the Zimbabwe eco-types compared to chickens from Malawi. The purebred lines are raised as closed populations in a way that controls gene flow between these commercial and experimental lines and the extensively raised village chickens. Alternatively, selection for specific production traits could have differentiated the purebreds from extensively raised chicken populations. The purebred lines were more genetically separated from the Zimbabwe chickens compared to the other African chickens. Although genetically different at the autosomal loci, the Zimbabwe chickens shared major mtDNA haplotypes with chickens from Malawi and Sudan and the purebred lines. This gives an indication that the 13 populations had some common maternal lineages. This sharing of ancestral lineages could not have been caused by recent interactions between populations but possibly represent an ancient genetic structure that could not be detected using microsatellite markers.

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APPENDICES

Appendix 1: Questionnaire to build an understanding on the environment housing the village chickens, the existing phenotypes and the farmers' management practises and perceived attributes of the phenotypes

Enumerator…….. Questionnaire number:.....

Date of interview ……

Household demography

1. Name of farmer……..
2. Village……. Ward…… District……Eco-zone……...
3. Sex of interviewed farmer (1 = male; 2 = female)…….. Tribe……
4. Household size 1. adult males……. 2. adult females……3. children (< 15 years)
5. **Land holding/farm size**

	Area (acres)
Crops	
Grazing*	
Forest	
Total	

* other than communal

6. **Livestock activity**

Is livestock the main activity on your farm?

Yes (1) ☐ No (2) ☐

7. **Source of income** *(Tick first column as appropriate and in second column rank importance where 1 = most important)*

1. Crops		
2. Livestock and products		
3. Home industries		
4. Salary/ wages*		
5. Remittances		

12. Livestock kept and Crops grown

Livestock	No of animals	Rank*	Reason of keeping
1. Cattle			
2. Goats			
3. Sheep			
4. Chickens			
5. Pigs			
6. Other (specify)			

Crops	Area (ha)	Rank	Reason of keeping
1. Maize			
2. Cotton			
3. Soya beans			
4. Tobacco			
4. Small grains			
5. Other (specify)			

*1 = most important

13. Chicken production system

Type of chickens kept	Number of chickens	Production systems (1 = Intensive, 2 = semi-intensive, 3 = extensive)
1.Local -Chicks -Growing pullets -Growing Cockerels -Mature hens -Mature cocks		
2. Exotic -broiler chickens -egg laying chickens		
3. Crossbreds -broiler X local -layer X local		

14. Purpose of keeping chickens

Ask an open question, tick any purpose considered in first column and then rank in the second

Function	Yes (1)/No (2)	Rank*
1. Meat		
2. Eggs		
3. Feathers		
5. Manure		
6. Cash from sales		
7. Investment		
8. Dowry		
9. Cultural		
10. Other (specify)		

*1= most important

15. Members of household who own chickens

(Tick 1 or more)

	Yes /No	Chicken numbers
Head		
Spouse		
Sons		
Daughters		
Others (specify)		

17. Access to veterinary services

(Tick as appropriate and rank importance in the second column (1= most important)

1. Government vet		
2. Private vet		
5. None		

18. What are the common predators

Predator	Rank*
1.	
2.	
3.	
4.	

***1 = most important**

19. Prevalent diseases that occur on farm

If none tick this box ☐

Local name or symptoms of diseases	Rank*
1.	
2.	
3.	
4.	

**Importance (1 = most important)*

20. Vaccinations/preventive treatments given

If none tick this box ☐

What is the frequency of vaccine use

1. Routine (indicate frequency in months)	
2. When need arise	

21. Influence of environment on nutrient supply

Feed supply	Type of feeds	Rank*
1. Scavenging feed	1. 2. 3.	
2. Supplementary feed	1. 2. 3.	

**1 = most important*

22. Movement of animals among flocks over the last 12 months

a. Was there any inflow of birds from other flocks (1 = Yes, 2 = No)

b. If yes what category, phenotype of bird and source of origin

Age category	Number of birds	Source	Birds used for breeding (1 = Yes, 2 = No)	Type of entry 2 = bought 3 = donated, 4 = exchanged
1.Chicks				
2. Pullets				
3. Cockerels				
4. Mature hens				
5. Mature cocks				
6. Total				

23. Number of exits within last 12 months

	Male	Female
1. Died		
2. Sold		
3. Slaughtered		
4. Exchanged		
5. Donated		
6. Stolen		
Total		

24. Reasons for choice of breeding

	Yes /No	Rank*
1. Size		
2. Confirmation		
3. Colour		
4. Comb		
5. Feathers		
6. Availability		
7. Mothering ability		
7. Other (specify)		

**1 = most important*

24. Reasons for culling *(tick reason in first column and rank in the second column, 1 = most important)*

	Males		Females	
1. Size				
2. Colour				
3. Comb				
4. Feathers				
5. Health				
6. Body conformation				
7. Poor growth				
8. Old age				
9. Poor fertility				
10. Mothering ability				
11.Other (specify)				

26. Important production traits

Production trait	**Rank**
1. Growth	
2. Survivability	
3. Disease susceptibility	
4. Reproductive performance egg production hatchability chick survivability	
4. Cultural value	
5. Other (specify)	

**1 = most important*

Breed (strains)

Number of strains *(tick)*	**0**	**1**	**2**	**3**	**4**	**5**

***Strain 1**

1. Description of strain……..

   Local breed name……

2. Number of adult birds

Male		Female	

3. **Origin/source of breed**

		Location
1. Within flock		
2. Communal area farm		
3. Commercial farm*		
4. Market*		

** specify location if known*

Strain 2

1. Description of strain ……

   Local breed name……

2. Number of adult birds

Male		Female	

3. **Origin/source of breed**

		Location
1. Within flock		
2. Communal area farm		
3. Commercial farm*		
4. Market*		

** specify location if known*

4. **Quality of traits as perceived by owner (Ask each question and for each trait tick one box, poor average, good or no opinion)**

Poor	Average	Good		Poor	Average	Good
			1. Size			
			2. Confirmation/shape			
			3. Feathers			
			4. Comb			
			5. Neck			
			6. Disease tolerance			
			7. Drought tolerance			
			8. Heat tolerance			
			9. Broodiness			
			10. Egg numbers			
			11. Meat taste/quality			
			12. Growth rate			
			13. Scavenging ability			
			14. Fertility			
			15. Other			
			16.			
			17.			

** A form to be filled according to the number of strains*

Appendix 2: Questionnaire to record phenotypic description of the chickens sampled for DNA analysis

1. Chicken ID……. Sex……..

2. Name of farmer…… Village…… District…….. AEZ……..

3. Date of sampling……4. Local name of phenotype …….5. Source

5. Any households that farmer exchanges breeding stock with

   1. same village……..

   2. outside villages……..

6. Phenotypic description (Cross or write down the applicable)

	1.	2.	3.	4.	5.	6.
Plumage colour						
Skin colour						
Shank(leg) colour						
Ear lobe colour						
Beak colour						
Plumage pattern (description)	spotted	mottled	pencil	barred	uniform	Other (specify)
Frizzle	absent	present				
Neck	feathered	naked				
Plumage density	full	sparse				
Head features	single	pea	rose	crested	v-shaped	Other (specify)
Length of shanks	short	medium	long			
Feathers in shanks	absent	present				
Spur size	rudimentary	medium	long			
Number of digits (toes)						
Body framework	blocky/compact	angular/tallish				
Feather Tail	absent	present				
Feather Tail	short	medium	long			
Egg shell colour	white	brown	tinted	N/A		Other (specify)
Egg size	small	medium	large	N/A		

CURRICULUM VITAE

Personal data

Surname (Family name):	Muchadeyi
Name:	Farai Catherine
Date of Birth:	18 October 1977
Place of Birth:	Harare, Zimbabwe
Gender:	Female
Nationality:	Zimbabwe
Marital Status:	Married

Educational background

2004-2007:	PhD fellow, Institute of Animal Breeding and Genetics, Georg-August Universität, Göttingen,
2000-2004:	MPhil in Agriculture, Department of Animal Science, University of Zimbabwe
1997-2000:	Bachelor of Science in Agriculture (Animal Science, Class 1.0), Department of Animal Science, University of Zimbabwe
1995-1996:	Advanced Secondary Education, St Davids' Bonda High School, Mutare, Zimbabwe
1991-1994	Ordinary Level Secondary Education, St Davids' Bonda High School, Mutare, Zimbabwe
1984-1990:	Primary Education, Sedze Primary School Nyanga, Zimbabwe

Working experience

2000-2003:	Graduate Teaching Assistant, Department of Animal Science, University of Zimbabwe
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Hobbies :	Scrabble, Tennis, Travelling.