Genetic and phenotypic characterization of native fowl populations in South Africa

by

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CONTENTS

		Page
Abstract		(i)
Samevatting		(ii)
List of abbreviations		(iii)
List of Tables		(iv)
List of Figures		(v)
List of publications and conference proceedings		(vi)
CHAPTER ONE:	Introduction	1
CHAPTER TWO:	Literature overview	
2.1	Definition of indigenous, native & local fowl	6
2.2	Conservation of native fowl populations	7
2.3	Origin of the native fowl in South Africa	9
2.4	Markers for studying genetic variation in farm animals	15
2.4.1	Genetic markers	16
2.4.2	DNA–based markers	18
2 4.2.1	Restricted Fragment Length Polymorphism	20
2.4.2.2	Microsatellites	21
2.4.2.3	Minisatellites	22
2.4.2.4	Random Amplified Polymorphic DNA	23
2.4.2.5	Amplified Fragment Length Polymorphism	23
2.4.2.6	Single nucleotide polymorphism	24
2.5	Mapping of the chicken genome	25
2.6	Genetic markers and variability in chickens	27
2.7	Measurement of variation	29
2.7.1	Gene diversity (heterozygosity)	29
2.7.2	Measures of population structure	30
2.7.3	Genetic distance	31
CHAPTER THREE:	Genetic characterization of native fowl in South Africa	
3.1	Introduction	34
3.2	Materials and methods	35
3.3	Results	39
3.4	Discussion	57
CHAPTER FOUR:	Phenotypic characterization of native fowl populations in South Africa	
4.1	Introduction	65
4.2	Materials and methods	67
4.3	Results	70
4.4	Discussion	74
CHAPTER FIVE:	Critical review and recommendations	83
CONCLUSION		89
REFERENCES		90
ADDENDUM A		

Abstract

Native fowl populations in South Africa were characterized genetically and phenotypically. Four South African native populations, two dual-purpose breeds, and two populations from Mozambique and Botswana were included for genetic analysis. For phenotypic characterization, two commercial lines were also included as a benchmark. Twenty-three microsatellite markers were selected and tested to obtain genetic data for estimation of genetic variability and distance. Growth (weight gain) and egg production were included for phenotypic characterization. A relatively high (53% – 64%) genetic variation was found among the populations, which suggests conservation as a genetic resource for future use. The lowest genetic variation (53%) was found for the Koekoek and Australorp populations, which are the two populations that were subjected to formal selection, while the highest variation was observed in the Naked Neck population (64%). The New Hampshire has often been included in upgrading programs and this is evident from the close relationship with both the Lebowa-Venda and Naked Neck fowls. Phenotypic trials indicated significant differences among the populations included for growth, carcass and egg production traits. The Koekoek and New Hampshire populations had the best performance for egg production and growth (weight gain) in the study. Genetic and phenotypic differences indicate that the populations can be distinguished as different breeds or groups of fowl. The results of this study may contribute to selection for improved performance for household food production, as well as conservation of the populations as a genetic resource.

Samevatting

Inheemse pluimveepopulasies van Suid-Afrika is fenotipies en genotipies gekarakteriseer. Vier Suid-Afrikaanse inheemse populasies, twee dubbeldoel rasse en twee populasies van Mosambiek en Botswana is ingesluit vir genetiese analise. Vir fenotipe karakterisering is ook twee kommersiële lyne as 'n verwysing ingesluit. Drie-en-twintig mikrosatelliet merkers is geselekteer en in die populasies getoets om data vir beraming van genetiese variasie en afstand te verkry. Groei (massatoename) en eierproduksie is vir fenotipe karakterisering ingesluit 'n Relatiewe hoë (53% – 64%) genetiese variasie is tussen populasies aangetref, wat dui op bewaring van die populasies as genetiese bron vir toekomstige benutting. Die laagste genetiese variasie (53%) is gevind vir die Koekoek en Australorp populasies, die enigste twee populasies wat formele seleksie ondergaan het, terwyl die hoogste variasie in die Kaalnek populasie (64%) gevind is. Die New Hampshire is dikwels ingesluit in opgraderingsprogramme en dit word weerspieël in hulle nou verwantskap met beide die Lebowa-Venda- en Kaalnek hoenders. Betekenisvolle verskille in die groei-, karkas- en eierporduksie-eienskappe is gevind tussen die populasies met fenotipe proewe. Die Koekoek en die New Hampshire het die beste prestasie vir eierproduksie en groei (gewigstoename) gehad. Genetiese en fenotipe verskille dui aan dat die populasies as verskillende rasse of pluimveegroepe onderskei kan word. Die resultate kan 'n bydrae maak tot seleksie vir verhoogde produksie vir huishoudelike voedselsekuriteit, asook bewaring van die populasies as bron van genetiese materiaal.

List of Abbreviations

AFLP	amplified fragment length polymorphism
AU	Australorp
bp	basepairs
BS	Botswana
cDNA	complementary DNA
CRI	chicken repeat element
CSB	clone/sequence based markers
DAD-IS	Domestic Animal Diversity Information System
DFP	DNA fingerprint
D_m	Nei minimum genetic distance
dNTP	deoxynucleoside triphosphate
D_R	Roger's distance
Ds	Nei standard distance
EDTA	ethylenediamine tetracetric acid
FAO	Food and Agricultural Organization
FST	fixation index
H	heterozygosity
IAM	infinate alleel model
LV	Lebowa-Venda
kb	kilobase
KK	Koekoek
MAS	marker assisted selection
Mb	megabase
MHC	major histocompatibility complex
MS	Mozambique
NN	Naked Neck
NH	New Hampshire
NJ	Neighbour-joining tree
OV	Ovambo
PCR	polymerase chain reaction
PIC	Polymorphic information content
QTL	quatitative trait loci
RAPD	randomly amplified polymorphic DNA
RE	restriction enzyme
RFLP	restricted fragment length polymorphism
SMM	stepwise mutation model
SNP	single nucleotide polymorphism
STS	sequence tagged sites
UPGMA	unweighted pair wise group method with arithmetic mean
VNTR	variable number tandem repeats
ZM	Zimbabwe

List of Tables **Page**

Chapter two
Table 2.1
Table 2.2 Properties for DNA-based markers 19
Table 2.3 Fingerprint markers used in estimation of genetic variability in chickens 28
Table 2.4 Equations for F-statistics 30
Distance measures 32

Chapter three
Table 3.1 Final mixes of three sets of microsatellite for automated analyses .. 37
Table 3.2 Number of samples per population for the different microsatellite markers obtained from genescan analysis..... 43
Table 3.3 Characteristics of microsatellite loci: Expected and observed range and number of alleles..... 44
Table 3.4 Polymorphic information content (PIC) for microsatellite markers tested in the different fowl populations 48
Table 3.5 Heterozygosity values for microsatellite markers tested in different fowl populations 49
Table 3.6 Grouping of native populations for microsatellite markers shared for calculation of genetic distances 46
Table 3.7 Genetic variability for 11 loci for all nine populations (Group I) ... 50
Table 3.8 Genetic variability for 18 loci in the South African populations (Group II) 51
Table 3.9 Genetic variability for 15 loci in the South African populations, Botswana and Mozambique (Group III) 51
Table 3.10 Genetic distances for all populations and 11 loci (Group I)..... 52
Table 3.11 Genetic distances for the South African populations and 18 loci (Group II) 53
Table 3.12 Genetic distances for the South African populations and 18 loci, Mozambique and Botswana (Group III) 54

Chapter four
Table 4.1 Productive traits of native chickens 66
Table 4.2 Initial and final weights, cumulative feed intake and feed conversion for the different fowl populations..... 71
Table 4.3 Least square means for growth (weight gain) traits for the different fowl populations 72
Table 4.4 Least square means and standard error for carcass traits for the different fowl populations 73
Table 4.5 Least square means and standard error for chemical analyses of carcasses of the different fowl populations 73
Table 4.6 Differences in fatty acid composition for the different fowl populations..... 73
Table 4.7 Egg production traits for the different fowl populations 74

List of Figures **Page**

Chapter two
Figure 2.1a A Koekoek cock and two hens 10
Figure 2.1b Koekoek fowls..... 10
Figure 2.2a A New Hampshire hen..... 11
Figure 2.2b New Hampshire fowls..... 11
Figure 2.3a A heterozygous and homozygous Naked Neck fowl..... 12
Figure 2.3b Naked Neck fowls..... 12
Figure 2.4a A Lebowa-Venda hen..... 13
Figure 2.4b Lebowa-Venda fowls..... 13
Figure 2.5a Ovambo hens 14
Figure 2.5b Ovambo fowls..... 14
Figure 2.6 Black Australorp fowls..... 15

Chapter three
Figure 3.1 Automated genescan for three populations and seven microsatellite markers..... 40
Figure 3.2 Alleles observed for three New Hampshire individuals with genotyper analysis and microsatellite marker MCW0216 41
Figure 3.3 Standard neighbour-joining tree for Group I..... 55
Figure 3.4 Standard neighbour-joining tree for Group II..... 55
Figure 3.5 Standard neighbour-joining tree for Group III..... 56
Figure 3.6 UPGMA-tree for Group I..... 57

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CHAPTER ONE

Introduction

The domestication of fowl took place between 8000 to 3000 years BC. Archaeological evidence indicates that fowls were domesticated in the Indus River Valley (present Pakistan) as early as 3250 BC (Moiseyeva, 1998). The distribution from east to west most probably was along the silk route during the 6th and 8th Centuries BC, via Turkistan, Iran and then to the Mediterranean region and central Europe. The wild species of *Gallus* that may have contributed to the domestic fowl include the Red Jungle Fowl (*Gallus gallus*), Grey Jungle Fowl (*Gallus sonnerrati*), Ceylon Fowl (*Gallus lafayettei*) and the Green Fowl (*Gallus varius*). Studies on morphological characteristics such as comb and feathers have shown similarities between the Red Jungle Fowl (*Gallus gallus*) and the domestic fowl. Geneticists therefore generally accept the Red Jungle Fowl as the common ancestor of the domesticated chicken (Crawford, 1990).

The historical records indicate that the initial domestication of the fowl was primarily for cultural and religious purposes. Fowls were kept for feathers, white and black magic and fighting, which must have influenced the selection of the birds, especially in terms of colour and morphology (Crawford, 1990). From the centers of domestication, chickens were distributed to other continents, cultures and environments, that led to the development of specific regional types. During the 19th Century, poultry in general became very popular in Europe and America as a hobby and a fair amount of money was invested in acquiring breeding stock. Crawford (1990) refers to the "hen craze of the 19th Century" as the time when most varieties, which still exist, were developed. It was only during the 20th Century that the poultry industry developed into a commercial industry and attention was directed to eggs and meat as products. Poultry, especially chickens, were now selected for improved production and the advances in breeding and genetics were applied for developing strains for egg (layer) and meat (broiler) production.

The introduction of the domesticated chicken to Africa and South Africa is not well documented. It is believed that chickens were used in rituals in Egypt during the Greco-Roman period (332 BC). Recent findings of skeletal remains in Qasr Ibrim by MacDonald & Edwards (1993) now indicate that the earliest known remains of the fowl in Sub-Saharan Africa can be traced to the late 5th Century AD. However, faunal samples have shown that fowls were associated with Early Iron Age communities (ca. 1000 BC) in southern Africa (Plug, 1996). Various domesticated chicken breeds were introduced from Europe during the era of African colonization, leading to extensive mixing of local and domesticated chicken populations.

The poultry industry in South Africa developed into a major livestock industry over the past 20 years. A major factor that contributed to this expansion was the higher demand for poultry meat, as it has become the "healthier" choice of meat world-wide. Other contributing factors include the advances made in monogastric nutrition, selection of more efficient, higher producing broiler and layer lines as well as improvement in poultry housing.

The industry is characterized by primarily large commercial enterprises, making use of high technology inputs such as environmentally controlled housing and intensive feeding and management systems. Forty-seven percent of all layers are owned by only two percent of all the egg producers in South Africa and eighty percent of the producers own less than 50000 layers (Deiner, 1999). A similar situation exists for the broiler industry where a few large companies produce approximately 80 percent of all broilers. The commercial broiler industry produces 928000 tons of meat and the layer industry 316000 tons of eggs per year. *Per capita* consumption of chicken and eggs are estimated at 22.9 and 6.59 kg per year (http://www.nda.agric.za).

Despite the extent of the commercial industry, fowls are found in most rural and peri-urban households in South Africa. These birds consist mostly of dualpurpose lines or what is also referred to as indigenous, local, native or "village chickens". They survive on a few kitchen scraps and the occasional handfull of maize, but for most of the time

they are left to scavenge. Flock size may vary between 5-20 birds. These native birds should be a valuable source of protein in terms of eggs and meat for rural populations, although their exact contribution to household food protein and or food security will be relatively difficult to estimate.

The changes in the political and socio-economic scene the past four to six years also brought challenges for agriculture in South Africa. In the past very little scientific research has been carried out on rural farming and alternative farming practices for household food security. Since 1994, agricultural policies have been directed towards rural and small scale farming systems and animal scientists are required to pay more attention to breeds that may lead to improvement of rural production. Indigenous and native breeds are now being re-evaluated as alternatives to the exotic breeds for application in rural farming systems.

During 1994, a project was launched by the Poultry Supply Unit of the Animal Improvement Institute of the Agricultural Research Center at Irene, to conserve the native chicken populations found in South Africa and to promote their re-introduction to rural agriculture (Joubert, 1996). The project referred to as "Fowls for Africa" include a number of different native populations, such as Lebowa-Venda, Ovambo, Naked Neck and Koekoek, as well as middle-level breeds for example New Hampshire, Rhode Island Red, Australorp and Cornish (Honeyborne [personal communication], 2000).

The native fowl populations that formed the conservation population at ARC (Irene) were originally collected from rural areas of the Northern Province, KwaZulu-Natal, the Free State and eastern- and southern Cape. The fowls from the Ovambo population were collected in Ovamboland in Namibia. The New Hampshire population included in the program came from the population, which was kept as a benchmark in the egg performance tests at the ARC (Irene) for many years. All these populations are being kept as a conservation population, according to the FAO guidelines in terms of population size to control inbreeding (Honeyborne [personal communication], 2001). The Koekoek, Lebowa-Venda, Naked Neck and Ovambo populations, as well as New

Hampshire and Australorp from the program were included to determine genetic variation and relatedness.

The interest in the native fowl in South Africa and southern Africa is of a binary nature. There is firstly a need to genetically characterize these populations for guidelines on their conservation. Genetic characterization is essential in estimating the relatedness among the populations which will assist in breed identification, selection for improved lines and planning of future conservation of these populations. Secondly, a phenotypic characterisation for growth and egg production will also assist in selection and improvement of these populations for application in rural agriculture.

Aims of study

There is a world-wide tendency to conserve and preserve native livestock species. Many species in the wild have reached the point of extinction with the corresponding loss of valuable genetic resources. In farm animals, especially chickens, breeders are continuously selecting for improved production, resulting in the reduction of genetic variation and the loss of unique alleles associated with disease resistance. A better understanding of the genetic variation and potential of the stock native to South Africa are therefore essential for informed decision making in conservation efforts.

No research has been done on the production potential and genetic make-up of the different native populations commonly seen in the rural areas of South Africa. For many years the emphasis was on the commercial production of poultry and native fowls were disregarded as research subjects. It was generally assumed that these fowl are all related and because of their poor production not worth conserving. It was decided to evaluate the native populations from the "Fowls for Africa" project with particular reference to their production traits and their genetic relatedness and variability.

In particular, this study represents the first genotypic analyses and comparison of native fowl populations in South Africa. For this purpose, a number of markers have been identified, evaluated and applied. Phenotypic trials were conducted only for baseline

data. Evaluation of the populations in different production systems was beyond the scope of this study.

It is my belief that the native fowl have an important role to fulfill in rural agriculture as well as contributing to genetic biodiversity of fowl in South Africa. The study of these fowl was driven by the questions that have been raised on their conservation and the recognition that they deserve in their contribution to securing food for the rural household.

CHAPTER TWO

Literature overview

2.1 Definition of indigenous, native and local fowl

This study refers to the characterization of fowl, which is found primarily in rural areas of South Africa, associated with backyard farming and or extensive small-scale agriculture. The terminology used for these birds is often confusing, as they are referred to as "indigenous", "native", "local" or even a "village chicken". Therefore, it was necessary to decide which term would be most appropriate to describe these fowl populations. The Concise Oxford Dictionary (1990) provides appropriate definitions for the following terms:

- Indigenous : occurring or living naturally in an area; not introduced; native
- Native : belonging by birth or origin to a specified country or place; inborn; indigenous as opposed to foreign or exotic; unaffected by artificial influences
- Local : a native inhabitant; of or pertaining to a place

The word indigenous, originating from the Latin: *indigena*, implies that the individual and its ancestry originated from a specific country or continent. As the common ancestor of the fowl is dated back to the Indus Valley 3250 BC (Moiseyeva, 1988) and was introduced to South Africa by settlers and traders (Ramsey *et al.*, 2000), the fowls could hardly be labelled as "indigenous" fowl. It could be argued that "local" fowl might be the most correct term for South African birds, but it could then mean that the locally bred Ross and other broiler lines (originating from imported stock) are now also "local" birds. The definition for native refers to place of birth and distinguishes the inhabitant from exotic or foreign but it also includes indigenous (*vida supra*).

Most of the fowls associated with rural and small-scale agriculture are found in the developing world and terminology ranges from indigenous fowl in Asia (Mukherjee, 1990) to local Malawi fowl in Africa (Safalaoh *et al.*, 1996). All breeds of fowl in this study originated from birds introduced to South Africa at least 350 years ago, left to

scavenge around the household and were not subjected to artificial selection over the years. Therefore, it was decided to use "native" in the characterization of the fowls in this study.

2.2 Conservation of native fowl populations

It is inevitable that selection, inbreeding and various crossbreeding systems may lead to the loss of genetic variation within breeds and that the breed itself may become extinct. For this reason, the scientific community identified the need for conservation of livestock resources, including poultry, during the late 1980's. During 1992 the Food and Agricultural Organization (FAO) launched a program for Global Management of Farm Animal Genetic Resources, with the main objective being to stimulate conservation activities and create an awareness of possible losses of genetic resources on an international basis (Scherf, 1995; Gandini & Oldenbroek, 1999). An information system, namely the Domestic Animal Diversity Information System (DAD-IS), forms one of the main components of the program and provides a list and description of all breeds in existence. During 1999 there was 332 cattle, 407 sheep, 123 goat, 156 pig and 213 horse breeds listed in DAD-IS from 37 European countries (Gandini & Oldenbroek, 1999). The biodiversity of 52 chicken breeds from Europe were assessed in a European Union project and a chicken DNA bank and poultry biodiversity database have been established at INRA Jouy-en-Josas (Weigend, 2000). In South Africa the Farm Animal Conservation Trust (FACT) was established in 1994 to facilitate and promote conservation of native animal genetic resources. Three South African native fowl breeds and one locally developed breed are listed by FACT (Ramsey *et al.*, 2000).

In order to make informed decisions on conservation of poultry breeds, Crawford & Christman (1992) emphasized that one should take into account the diverse groups of poultry and the reasons why they are kept. These authors identified the following categories:

- Food producers: indigenous types, middle-level and industrial.
- Fancy or exhibition poultry: kept by the hobbyists.

- Fighting stock: although illegal in many countries, these still exist.
- Wild ancestors of poultry: mostly endangered and the concern of aviculturalists.
- Inbred and specialised lines: developed for research purposes.

In this study only the food producers are of interest, as native fowls are mainly applied for household food production. A few companies in the world are responsible for breeding commercial stock that is bred from a relatively narrow genetic base. As these broilers and layers are selected for maximum production, they are replacing most of the middle-level poultry, the dualpurpose predecessor breeds, as well as the indigenous birds. Although the indigenous or native stock has a poor performance, relative to highly selected commercial lines, they do have the ability to survive in challenging environments. Very little is known about their potential to disease resistance and adaptation mechanisms (Crawford & Christman, 1992).

Most of the research efforts towards the conservation of genetic animal resources in Africa have been on large (cattle) and small (sheep and goats) livestock. Native chickens were mostly disregarded and no data on breeds or lines have been reported (Setshwaelo & Adebambo, 1992; Hofmeyr *et al.*, 1998). One reason for this lack of data is that the need for the conservation of livestock resources is still an issue of debate among scientists. Two different approaches to conservation are recognised: the utilizationist versus the preservationist (Mason & Crawford, 1993). The utilizationist is more concerned with the usefulness of the genetic resource and the loss of breed identity is of lesser importance, while the preservationist views the conservation of the breed on the long term as the main objective. In the developing countries, where food security is a problem, the approach for conserving the native fowl should be more towards utilisation, rather than preservation of breed characteristics.

The project launched by the Poultry Supply Unit of the Animal Improvement Institute of the Agricultural Research Centre at Irene, was not only to conserve the native fowl populations found in South Africa, but to promote their role in rural agriculture (Joubert, 1996). As described in chapter one the native fowl populations included in the project

was originally collected from rural areas of most parts of South Africa and the fowls referred to as Ovambo's were collected in Ovamboland in Namibia. FAO guidelines are followed in terms of population size to control inbreeding. The "Fowls for Africa" include populations, such as the Lebowa-Venda, Ovambo, Naked Neck and Koekoek, as well as middle-level breeds for example the New Hampshire, Rhode Island Red, Black Australorp and Cornish (Honeyborne [personal communication], 1999). Except for the Koekoek, New Hampshire and Australorp, the other populations have not been described according to phenotype or included in the breed standards of the South African Poultry Society. The Lebowa-Venda is in the process of being described for inclusion in the breed standards (Honeyborne [personal communication], 2001).

2.3 Origin of the native fowl in South Africa

The Potchefstroom Koekoek has for many years widely been used by South African farmers for egg production for household purposes. The breed originated from a cross between Black Australorp and the White Leghorn during the 1950's, at the former Potchefstroom Agricultural College. The Plymouth Rock was later included into the breeding program and eventually the Koekoek was registered as a South African breed with the South African Poultry Association in 1976 (Viljoen, 1986). These birds have a characteristic black and white speckled colour pattern, which is present in as many as nine different poultry breeds. The males inherit the bar gene, a sex-linked gene and they are easily distinguished, having light grey bars on the feathers, while the females are darker (Figure 2.1a & b). Koekoek is classified as a heavy breed, with the average adult body weight varying between 3-4 kg for cocks and 2.5 - 3.5 kg for hens (Viljoen, 1986). The average egg weight is 55.7 g and the colour of the eggs are brown (Ramsey *et al.*, 2000).

The New Hampshire originated in the United States, bred from Rhode Island Reds. The first importation to South Africa was in 1947. These birds are also classified as a heavy breed with adult body weight varying between 3.9 kg for cocks and 3 kg for hens. Plumage colour is a chestnut red with a light salmon colour on the breast area (Figure

2.2a & b). Egg colour is light brown. Although the New Hampshire is not native to South Africa, it was included as it is very often kept as a dualpurpose line in rural areas.

Figure 2.1a: A Koekoek cock and two hens.

Figure 2.1b: Koekoek fowls.

Figure 2.2a: A New Hampshire hen.

Figure 2.2b: New Hampshire fowls.

Traders from Malaysia and settlers that came with the Dutch East Indian Company most probably introduced the Naked Neck chicken to South Africa (Ramsey *et al.*, 2000). These chickens carry the major gene *Na-* for Naked Neck. The gene is inherited autosomal with incomplete dominance and was mapped on chromosome 1 of the chicken

genome (Pitel *et al.*, 2000). Chickens that are homozygous for the *Na*-gene have no feathers on the neck area, while the heterozygous have a little tuft of feathers on the lower portion of the neck. The *Na*-gene is associated with significantly less plumage cover than chickens not carrying the Naked Neck gene. Naked Neck chickens were found to have better heat resistance (Pech-Waffenschmidt *et al.*, 1995). Naked Necks of South Africa are very colourful - white, red and black feather combinations are found (Joubert, 1996) (Figure 2.3a & b). Egg colour varies from cream to light brown.

Figure 2.3a: A heterozygous and homozygous Naked Neck fowl.

Figure 2.3b: Naked Neck fowls.

The first recording of the Lebowa-Venda chickens was made during 1979 in Venda in the Northern Province. They are quite common in this area and the name Lebowa-Venda is associated with the two largest ethnic groups residing in the Northern Province. These chickens have white and black or white and brown plumage with shades of dark green on the feather tips (Joubert, 1996) (Figure 2.4a & b). The colour of the eggs is cream and also sometimes tinted (Ramsey *et al.*, 2000).

Figure 2.4a: A Lebowa-Venda hen.

Figure 2.4b: Lebowa-Venda fowls.

The Ovambo was originally found in the rural areas of Namibia and Ovamboland and the name refers to the geographical area where they were first distinguished by the local people as a group of chickens native to the area. These chickens have predominantly dark feathers. They tend to be aggressive if their young is in danger and they often roost in trees to avoid predators (Joubert, 1996) (Figure 2.5a & b).

Figure 2.5a: Ovambo hens.

Figure 2.5b: Ovambo fowls.

The Black Australorp was imported to South Africa from Australia during 1925, where they have been developed from the Black Orpington, which has been selected for egg production. Over the years different strains of Australorp were developed in South Africa with feather colours varying from white, golden "wheaten laced" and black. All the strains were primarily used as dualpurpose breeds, with an average mature body weight varying from 3.6 – 4.5 kg for cocks and 2.7 – 3.9 kg for hens (Viljoen, 1986). The Black Australorps in the "Fowls for Africa" program are a pure line, which originates from the former Poultry Research Unit at Potchefstroom.

Figure 2.6 The Black Australorp fowl

2.4 Markers for studying genetic variation in farm animals

Biochemical systems have been extensively applied over the years in genetic and physiological research of farm animals. Discoveries made by Landsteiner in the early

1900's on human blood group variability and Erlich & Morgenroth and Todd & White on blood groups in farm animals, set the scene for research in immuno genetics and genetic differences among animals (Hines, 1999). Stormont did further work during 1950 on the blood group systems in cattle. Biochemical polymorphisms, historically, have often been used in detection of genetic differences in farm animals. As early as 1940, Irwin and co-workers at the University of Wisconsin used blood group antigens for parentage verifications in the Holstein Friesians (Hines, 1999). The applications of blood groups proved to be a powerful tool in detection of incorrect parentage. Biochemical systems consist of a variety of proteins found in blood plasma, serum and milk such as haemoglobin, transferrin, albumin casein kappa and erythrocyte GSH, K and Na-concentrations. More recently biochemical polymorphisms were also applied in genetic characterization of cattle and goats (Baker & Manwell, 1980; Tucker, *et al.*, 1983; Arranz *et al*, 1996a). However, the development of molecular biology techniques during the late eighties has, opened up new ways for the study of genetics. DNA has become the alternative for the research of various genetic, breeding and physiological questions in farm animals.

2.4.1 Genetic markers

PCR (Polymerase Chain Reaction) technology

PCR technology has become an essential tool in molecular biology. It is a relatively easy *in vitro* method for amplification of a specific DNA-sequence from genomic DNA or a cDNA population (Strachen & Read, 1996). A PCR consists of a number of cycles of denaturation, annealing and extension. A basic PCR cycle will include the following steps:

i. DNA template is placed in a tube with specific primers (synthetic oligo-nucleotides), deoxynucleotides (dNTP's) and a heat stable polymerase enzyme, a buffer and $MgCl_2$.

ii. The mixture is heated (± 95°C) to denature or separate the two DNA strands.

iii. Temperature is decreased to ± 55°C to allow primers to bind/anneal to form a complementary strand. The annealing temperature will depend on the primer length and sequence.

iv. Temperature is increased to ± 72°C for DNA polymerase to act and add nucleotides to the 3' end of each primer, allowing for extension of new strands.

v. Cycle two will then commence by heating the reaction again.

A typical cycle takes approximately 5 minutes and amplification is repeated for 30-35 cycles on most PCR-machines. The product synthesized in one cycle serves as a template in the next, so that the original DNA is doubled in every cycle. The amplicon or PCR-product can then be visualized on an agarose or polyacrylamide gel (Nicholas, 1996; Turner *et al.*, 1998; Erlich, 1991). Often PCR requires optimization to ensure a good amplicon, distinguished as a defined band on the gel. Usually the annealing temperature and or $MgCl_2$ concentration may be adjusted towards obtaining optimal amplification.

The primers in a primer pair are designed to be of similar lengths (usually 18-30nt in length) with a similar G + C content, in order to anneal to the complementary DNA at approximately the same temperature. The primers will extend towards each other by the addition of the enzyme (Taq polymerase) and nucleotides (DNTP's) to the 3' end. A suitable heat stable DNA-polymerase enzyme is an essential component in PCR, as the enzyme initiates the synthesis of the new strands and must survive the first heating step at 95°C. *Taq* polymerase, derived from the thermophilic bacteria *Thermus aquaticus*, is most often used. Incorrect copying may occur using *Taq*, because it has no proof reading function. This refers to the presence of a 3' - 5' exonuclease activity, which reduces the chance of incorrect copying of nucleotides during the synthesis of the new strand. Other polymerase enzymes are available that can be used if a higher accuracy is required (Strachan & Read, 1996; Turner, 1998).

The source of DNA used for PCR can be genomic DNA (whole blood or tissue), or forensic specimens and ancient biological samples (Turner *et al.*, 1998). The aim of PCR is to multiply the given small segment of template DNA, to provide a fragment large

enough for cloning, sequencing or detection on a polyacrylamide gel. Prior knowledge is required of the sequence of at least a segment of the DNA to be amplified. This information is used to synthesize an oligonucleotide, or referred to as a primer, which is used to prime the synthesis of the new DNA strands (Nicholas 1996; Turner *et al.*, 1998).

PCR technology is used to amplify known sequences of a sample of DNA or for arbitrary priming of variable regions of the genome. It is therefore possible to use PCR for Variable Number Tandem repeats (VNTR's), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restricted Fragment Length Polymorphism (RFLP) (Erlich, 1991). All these markers can be used for estimation of genetic variability. PCR has the advantage of being a relatively fast, sensitive and reliable method. It cannot only amplify very small amounts of DNA, but also amplify degraded or poor sources of DNA (Strachen & Read, 1996; Erlich, 1991).

2.4.2 DNA-based markers

Repetitive DNA

The structure and composition of DNA provides the basis for the understanding of the genetic information stored, coded and transmitted for production of the proteins necessary for all metabolic functions. Genomic DNA consists of non-coding and coding DNA. Coding DNA encode for the synthesis of proteins, a process, which involves the translation of RNA, which is transcribed from the DNA template. Non-coding DNA makes up a large proportion of the genomes of the eukaryotes. These non-coding regions contain regulatory elements such as promoters and enhancers, but can in many cases also contain repetitive elements, eg. multiple repeats (Turner *et al.*, 1998). These repetitions include satellite DNA, which are thousands of tandem repeats in one site, as well as mini- and microsatellite DNA depending on the number of repeats. Many satellites usually range in size from 10-100 bases and microsatellites from 1-6 bases (Nicholas, 1996; Turner *et al.*, 1998). Although both minisatellites and microsatellites occur throughout the genome, the minisatellites tend to be concentrated in the telomere regions and sites associated with a high frequency of recombination (Nicholas, 1996). The number of

repeats in the satellite DNA is highly hypervariable and differs among individuals of a species. For this reason these repeats may be applied as DNA markers in the study of genetic differences.

Classification

Genetic markers associated with DNA are commonly grouped into Type I and Type II markers (O'Brien, 1991). Type I markers are usually associated with a gene with a known function, while Type II markers refer to anonymous gene segments. Alternatively, DNA-based markers are also grouped as clone/sequence-based markers (CSB) such as microsatellites and fingerprint markers (Dodgson *et al.*, 1997).

CSB markers require isolation of a DNA fragment and the determination of the sequence of the fragment. For Fingerprint markers the sequence of the DNA region is unknown and the markers include randomly amplified polymorphic DNA (RAPD) (Dodgson *et al.*, 1997). Table 2.1 provides a summary of the properties of DNA-based markers, which are often used in the studies of genetic variability in farm animals.

Table 2.1 Properties of DNA-based markers (Dodgson *et al.*, 1997)

	Clone Sequence Based (CSB)		**Fingerprint (FP)**		**Single Nucleotide Polymorphism (SNP)**
Variable	RFLP	Microsatellites	RAPD	Mini-satellite	
Genome distribution	Ubiquitous	Ubiquitous	Ubiquitous	Heterochromatien	Ubiquitous
Genome surveyed	sc & mr	Sc	Sc & mr	mr-hr	Sc
Typical PIC	Low	High	moderate	High	Low
Typical allele number	2	2-10	2	2	2
Inheritance mode	Co-dominant	Co-dominant	Dominant	Dominant	Co-dominant
Type of loci (O'Brien)	I and II	II > I	II	II	I and II
Reliability	High	High	Low	High	High
Speed of assay	Low	High	High	Low	High
Initial investment	Moderate	High	High	Low-moderate	High

Sc = single copy, mr = moderately repetitive, hr = highly repetitive and
PIC = polymorphic information content.

2.4.2.1 Restricted Fragment Length Polymorphism (RFLP)

RFLP generally refers to the differences in banding patterns obtained, from DNA fragments, after digestion with restriction enzymes. Restriction enzymes (RE) bind to specific sequences and cut the DNA at a specific cleavage site. The DNA fragments of different lengths are then subjected to electrophoresis and fragments will migrate according to their weights, the smaller fragments faster and the larger fragments slower (Nicholas, 1996). This application of RFLP is the more conventional use for example for detection of diseases. Restriction enzymes are enzymes that are produced by bacteria, which protects the bacterial cell against foreign DNA by cutting it into fragments. Restriction enzymes are usually named after the bacteria where they were found, for example in BamH1, the **Bam** refers to the **B**acillus **amy**loliquefaciens where it was found and **H** refers to the strain and **1** indicates that it was the first restriction enzyme obtained from that bacteria. Various Restriction enzymes have already been shown (BamHI, EcoRI SacI and TaqI) to be useful in obtaining RFLP patterns for haplotype identification in individuals (Spike *et al.*, 1996; Smith *et al.*, 1996). It is often required, that many enzymes need to be tested in the initial phase to be able to identify the polymorphism, but even then it is still an easy and relatively cheap marker to use (Dodgson *et al.*, 1997). Potential disadvantages of the RFLP technique are the dimorphic nature, since a RFLP only indicates the presence or absence of a cleavage site, and therefore does not provide a great deal of genotypic information. Large amounts of DNA are also required for RFLP analyses and the technique is relatively time consuming.

RFLP techniques are also used in DNA fingerprinting which is based on the detection of Variable Number of Tandem Repeats (minisatellites) by firstly digestion of the DNA with a RE, followed by Southern analysis and hybridization techniques. The steps required for producing a DNA fingerprint for an individual are briefly as follows (Nicholas, 1996):

i. Isolation of the DNA template from for e.g. blood, tissue or hair.
ii. Digestion of the DNA with a Restriction enzyme.
iii. Separation of the fragments with gel electrophoresis.

iv. Transfer of the fragments onto a nylon membrane (blotting).

v. Membrane is " baked" to allow for DNA fragments to attach to membrane (hybridization).

vi. The solution with the probe (labeled DNA) is added. The probe is single stranded and will attach to any complementary DNA fragment on the membrane.

vii. Fragments can then be visualized by autoradiography.

A number of bands can be observed as a result and each individual has a unique set, referred to as the DNA fingerprint (DFP), which makes this technique very useful for parentage verifications.

2.4.2.2 Microsatellites

Microsatellites consist of tandem repeats between one and six bp, repeated up to 60 times and referred to as simple sequence loci. These domains were first demonstrated by Hamada and colleagues, during the early eighties (Tautz & Renz, 1984; Tautz, 1989; Smeets *et al.*, 1989). Repeat units may consist of $(A)_n$, $(TG)_n$, $(CA)_n$ or $(AAT)_n$ repeat. For example in most vertebrates the $(CA)_n$ repeat is the most common motif (Beuzen *et al.*, 2000). Microsatellites are highly polymorphic due to the variation in the number of repeats. It is not uncommon to find up to 10 alleles per locus and heterozygosity values of 60% in a relatively small number of samples (Goldstein & Polack, 1997). Microsatellites are well-distributed in animal genomes and are multi-allelic, co-dominant and can be detected by PCR-technology (Tautz, 1989).

The function of these simple regions of short sequence motifs is not clear. Tautz (1989) indicated that they could be involved in gene regulation or act as signals for recombination, as a certain amount of crossing over takes place within $(GT)_n$ repeat sequences. However, tandem repeat loci may also have a function in the packaging and condensing of the DNA in eukaryotic chromosomes (Stallings *et al.*, 1991). It was for example found that $(GT)_n$ repeat sequences are much more frequent in euchromatin than heterochromatin and the $(GT)_n$ repeat could therefore be an important determinant in

distinguishing between hetero- and euchromatin (Stallings *et al.*, 1991). Microsatellites are found in both coding and non-coding regions of eukaryotic genomes and are generated by a mechanism referred to as slippage (Tautz *et al.*, 1986). Slippage occurs when normal pairing of repeats is altered during replication. A backward slippage causes an insertion of a repeat in the new strand and a forward slippage a deletion (Strachan & Read, 1996).

Microsatellites can be isolated by various methods. The development of microsatellite markers requires the construction of a genomic library. Briefly, the construction of a genomic library involves cloning the DNA of a specific species (e.g. chicken DNA) as follows: firstly, the genomic DNA is digested using restriction enzymes, which yield small DNA fragments. The DNA fragments are then cloned into vectors such as phages or plasmids, which allow proliferation in bacterial cells. The next step involves hybridization where thousands of clones are screened with synthetic polynucleotides such as $(TG)_{13}$, $(CAC)_5$, and $(GAT)_4$ which are labeled radio-actively with $^{32}P\gamma$-ATP. Positive clones are then isolated and sequenced (Crooijmans *et al*, 1993). The sequence information is used to synthesize PCR primers, which are then also tested on a panel of unrelated animals. This step is essential to ensure that primers work optimally and that there is no cross reactions (Crooijmans *et al*, 1993; Crooijmans *et al*, 1997). Primers are developed in pairs and usually labeled with a fluorescent dye for application on automated sequencing machines.

Microsatellites have been mapped for various species, including humans, mice, fruit flies, cattle, sheep, pigs and chickens (Goldstein & Pollack, 1997). As a consequence these elements have become most valuable markers in studies on genetic variability, parentage verifications and genome mapping projects.

2.4.2.3 Minisatellites

Minisatellites were first described as hypervariable tandem repeats, when found in the human genome. They were found to be longer repeats than microsatellites, consisting of

up to 200bp. This led to the use of the term Variable Number of Tandem Repeat loci (VNTR's) in reference to repetitive units that include mini- and microsatellites. Minisatellites are also well distributed through the eukaryotic genome (Bruford & Wayne, 1993). Minisatellite markers have certain limitations, as they do not uniformly mark the genome, the marker fragment is difficult to clone and they are dominant markers if the repeat is used as a probe, which reduces the potential information for genotyping (Dodgson *et al.*, 1997).

2.4.2.4 Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) is also a technique that can be applied in evaluation of genetic diversity (Smith *et al.*, 1996; Nicholas, 1996). The RAPD technique is based on the use of a number of short, arbitrary primers in one PCR reaction. These random primers will generate several amplification products, which will differ in size and may be characterized by simple agarose electrophoresis. RAPD markers have the advantage that they can be obtained at a reasonable cost and will generally amplify a range of fragments of most DNA and show polymorphism. The polymorphic bands obtained from a RAPD can be cloned for further analysis. A major disadvantage is that the RAPD primers are very sensitive to PCR conditions and may lead to poor reproducibility (Dodgson *et al.*, 1997).

Minisatellites, Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) have also been used widely for parentage determinations, genomic diversity and measurement of kinship (Gilbert *et al.*, 1991).

2.4.2.5 Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) is a DNA fingerprinting technique that is based on the detection of DNA fragments, subjected to restriction enzymes, followed by selective PCR amplification. The DNA is cut with two restriction enzymes and double stranded adapters are then ligated to the ends of the DNA fragments to

generate the template for PCR. The specific adapter, ligated to the DNA fragment, determines the sequence of AFPL primers. Fragments are then separated and analyzed using gel electrophoresis (Vos *et al.*, 1995).

Two restriction enzymes are used, the one usually a rare and the other a frequent cutter. There are various reasons for using two enzymes. The frequent cutter is important for producing small fragments that will amplify well and have an optimal size for denaturation on a gel, while the rare cutter will reduce the number of fragments to be amplified. A further reduction in the number of fragments to be amplified is achieved by extending the PCR primers with 1 to 3 bp. This results in a further four fold reduction for every bp extension of the primer. Two enzymes will optimize the amplification and a large number of fingerprints may be produced with a small number of primers (Vos *et al.*, 1995).

The AFLP technique can be done at a reasonable cost and has extensively been used, particularly in the genome mapping of plants. It also has the advantage of a higher reproducibility than RAPD (Vos *et al.*, 1995).

2.4.2.6 Single Nucleotide Polymorphisms (SNP's)

SNP refers to the substitution of one nucleotide for another. It could also be an addition or deletion of one or more nucleotides, causing the polymorphism (Beuzen *et al.*, 2000). SNP's are bi-allelic markers, indicating a specific mutation (polymorphism) in two alleles only of a population. In order to obtain information from a SNP marker, which is similar in complexity to that obtained from a microsatellite marker, at least five SNP markers will be required (Beuzen *et al.*, 2000).

SNP's are also found in coding regions directly associated with the protein function and the inheritance pattern is more stable, making them more suitable markers for selection over time (Beuzen *et al.*, 2000). Most RFLP and AFLP markers are also the result of a

SNP in a restriction enzyme recognition site, which confirms the importance of SNP markers.

Microsatellite markers are currently the most reproducible and applicable method for genotyping and genome mapping in farm animals. It is however envisaged that the use of SNP's will increase as soon as optimization of arrays and application for specific species are confirmed.

2.5 Mapping of the chicken genome

The chicken genome has 39 pairs of chromosomes, eight large macro-chromosomes, 30 pairs of micro-chromosomes and two sex chromosomes (♀ ZW, ♂ ZZ) (Bitgood & Somes, 1990). The size of the chicken genome is estimated at 1.2 x 10^9 bp, which is small in comparison to the mammalian genome of 3 x 10^9 bp (Stevens, 1986; Crooijmans, 2000). The macro-chromosomes and micro-chromosomes account for approximately 130Mb and 12.5Mb respectively (Fillion, 1998). The chromosome lengths were found to be linear to the DNA content, the larger marco-chromosomes (one to eight) have the highest DNA content and represent up to 82% of the haploid genome together with the sex chromosomes (Smith & Burt, 1998). It was reported by Fillion (1998) that the micro-chromosomes represent up to 30% of the genome. The micro-chromosomes have a high gene density, even higher than the macro-chromosomes, which confirms their genetic importance (McQueen *et al.*, 1998).

The chicken is an ideal model for genetic mapping technology, because of its economic importance, a short generation interval and the potential to generate large full sib families (Crooijmans, 1993 *et al*; Fillon, 1998). Hutt published the first attempt for a genetic map of the chicken during 1936. Since then three different linkage maps were developed. The International Workshop on the Poultry Genome initiated the detection of polymorphic markers towards the mapping of the chicken genome. The choice and establishment of the reference families (East Lansing and Compton families) were also decided in this process (Crittenden *et al.*, 1993). The Compton population was

established at the Institute for Animal Health in Compton in the United Kingdom and the first map based only on DNA markers were published by Bumstead & Palyga (1992). The Compton line was based on a cross between an outbred and inbred White Leghorn line. These lines differ in susceptibility to different diseases. A single F_1 female was backcrossed to the male to generate the progeny for the reference family (Bumstead & Palyga, 1992). The East Lansing family was initiated in 1988 at the Michigan State University and was bred from a cross between a single, inbred male of Jungle Fowl origin and females from a highly inbred line of White Leghorn, to produce the F_1-progeny (Crittenden *et al.*, 1993; Cheng 1997; Cheng *et al.*, 1998). F_1-males were then backcrossed with the inbred Leghorn females to produce the BC1 (F_2-generation). A chicken genome map was constructed consisting of RFLP, RAPD and CR1 markers (Cheng 1997; Cheng *et al.*, 1998). The Wageningen Agricultural University generated a third population of 10 full sib families, using two commercial broiler lines from Nutreco BV. These lines are originally from the Plymouth Rock breed (Groenen *et al.*, 1998). The map generated was based primarily on microsatellite and AFLP markers (Herbergs *et al.*, 1999). A consensus linkage map combining the genotyping data of the East Lansing, Compton and Wageningen reference populations have been completed, consisting of 1889 loci, covering approximately 3800cM (Groenen *et al.*, 2000). The Chicken Database listed 586 genes, 2349, loci, 1251 microsatellites and 2959 available primers during September 2000 (www.Ri.bbsrc.av/chickmap/chickgbase/html).

As the commercial poultry industry is focused on improvement of quantitative traits such as growth, reproduction and disease resistance, genome mapping is aimed at discovering genetic markers, genes and Quantitative Trait Loci (QTL) (Cheng, 1994; Lamont *et al.*, 1996). These discoveries will also aid in assays for marker-assisted selection (MAS). Traits associated with growth have been so far been empasized and the most likely position for a QTL affecting body weight was found to be on chromosome 1 at 240cM (Groenen *et al.*, 1998; Van Kaam *et al.*, 1998). The HMGI-C gene also has been under investigation as a candidate gene for autosomal dwarf gene (*adw*) in the chicken, which is of importance in the broiler breeding stock programs (Ruyter-Spira *et al.*, 1998). Quantitative trait loci affecting the susceptibility to Marek's Disease are also being

studied. Vallejo *et al.* (1998) have indicated the mapping of a non-major histo-compatibility complex QTL affecting the susceptibility to Marek's disease.

Another important application of genome mapping is identifying genetic markers that are associated with disease resistance. For example, genotypes with a lower susceptibility to Marek's disease, were identified by Lamont *et al.* (1996). Markers are also mapped for application in evolutionary studies. A further important aspect of genome mapping is the comparison of maps of different species, to identify DNA markers that amplify in both species. Already, comparative mapping between chickens and humans is expanding as more chicken genes are found with a human homologue. A total of 154 autosomal conserved segments have been identified between the chicken and human which may be very useful in human genetic and disease studies (Schmid *et al.*, 2000). The most exciting application will surely be in what is referred to as functional genomics, where a connection is sought between the genotype and the physiological mechanism involved in the final expression of the trait (Dodgson & Cheng, 1999).

2.6 Genetic markers and variability in chickens

Genetic variability in chickens has mostly been studied by using fingerprint markers such as DFP's and RAPD (see Table 2.2). DFP's also have been applied in evaluation of potential QTL's in chickens (Dunnington *et al.*, 1993; Lamont *et al.*, 1996). Various commercial strains and indigenous types have been studied. Both types of markers were found to be useful tools in the estimation of genetic variability.

Until recently genetic characterization of chicken breeds, especially native and indigenous populations were limited. However, it can be expected that this situation will rapidly change with more microsatellite markers becoming available. Over a thousand microsatellite markers are available which is more than adequate for biodiversity studies.

Table 2.2 Fingerprint markers used in estimation of genetic variability in chickens

Aim of study	Breed/line of chicken	Markers used	Reference
DFP's for individual identification and linkage studies in poultry	Broilers: Cornish x White Rock breed, Layers: Leghorn Muscovy Duck, Turkey & Goose	DFP's	Hillel *et al.*, 1989
Genetic variation	Two White Leghorn lines, 3 commercial broilers lines, Rhode Island Red, & 8 exotic breeds	DFP's	Ponsuksili *et al.*, 1994
Determination of genetic distance	French broiler breed, random bred and inbred lines of White Leghorn	DFP's	Kuhnlein *et al.*, 1989
Genetic characterization of chicken lines	Ten lines of White Leghorn, 2 Fayoumi & 1 Spanish breed	DFP's & RAPD	Plotsky *et al.*, 1995
Genetic variability among layers and their correlation with performance	Nine different lines bred from commercial strains imported form Romania & USA	DFP's	Meng *et al.*, 1996
Relatedness & diversity in Chickens & turkeys	Rhode Island Red, White Plymouth Rock, single comb White Leghorn & Araucona & Turkeys	RAPD	Smith *et al.*,1996
Estimation of relatedness in White Leghorn lines	White Leghorn	RAPD	Deepak *et al.*, 1998

In a study of genetic variability with native lines from Egypt, India, Indonesia and Thailand, a higher heterozygosity was found among the lines using microsatellite markers, compared to DFP's (Ponsuksili *et al.*, 1996). Microsatellite markers have been successfully applied in characterization of waterfowl (Fields & Scribner, 1997) and are frequently used in studying genetic variability in other mammalian species such as sheep, pigs and cattle (Buchanan *et al.*, 1994; Van Zeveren *et al.*, 1995; MacHugh *et al.*, 1997). Microsatellite polymorphism has been found to be applicable for determination of genetic variation in commercial broiler and layer lines (Crooijmans *et* al., 1996b). Vanhala *et al.* (1998) also applied microsatellite markers for evaluation of variability and genetic distance in commercial egg and broiler lines. Genetic relationships among Japanese native chicken breeds based on eight microsatellite markers were also shown (Takahashi *et al.*, 1998). Microsatellite markers were also shown to be accurate and reliable for studies of genetic biodiversity in a study by Zhou & Lamont (1999) on genetic characterization of highly inbred chicken lines. There is a tendency towards

microsatellite markers as the preferred markers in genetic studies of chicken and other mammalian species.

2.7 Measurement of genetic variation

Animal populations differ in size and may change as they are continuously subjected to the forces of natural selection, migration and mutation. In livestock populations, artificial selection plays a major role in population changes. Selection, migration and mutation all may lead to non-random or directional changes in the allele frequencies of the population (Hartl, 1988).

Estimations of genetic variation include gene diversity or heterozygosity and genetic distance when using genetic markers such as microsatellites. Phylogenetic trees can be constructed from sequence data or microsatellite data to illustrate genetic relationships among the populations studied (Nei, 1987; Weir, 1996).

2.7.1 Gene diversity (Heterozygosity)

The average heterozygosity (H) over all loci in the genome as described by Nei (1987) takes into account the number of loci and number of individuals per locus. It is assumed that sampling was done from the loci and of the genes at each locus. For a single locus h can be estimated as follows:

$$\mathbf{h = 2n\,(1 - \sum x^2_i)/(2n - 1)}$$

and for more than one locus:

$$\mathbf{H = -\sum\, h_k\, /r}$$

Where: h_k = the value of h for k_{th} locus

n = the number of individuals sampled

x^2 = the population frequency of the genotype at the locus

r = the number of loci studied

The number of individuals studied will influence the variance. Intralocus variance will be influenced by the number of individuals sampled at each locus and can be reduced by increasing the number of individuals, while the interlocus variance can be reduced by increasing the number of loci studied (Nei, 1987). This is an important aspect to consider in estimation of genetic variance and it is recommended to increase the number of loci, rather than the number of individuals (Nei, 1987; Goldstein & Pollock, 1997).

2.7.2 Measures of population structure

Population structure is often measured according to the changes in the proportion of heterozygous individuals. The complexity of heterozygosity (H) in a population can be described by using the hierarchical F-statistics, originally proposed by Wright in 1943 (Hartl 1988; Nei, 1987; Weir, 1996). There are three parameters applied for measuring reduction in H or deviations of genotype frequencies. These are the F_{IS}, F_{IT} and F_{ST} values, also referred to as Fixation Indices (Table 2.3).

The fixation index (F_{ST}) is widely used as a measure of genetic difference between populations. F_{ST} may vary between a theoretical minimum of 0 (no genetic change) to a maximum of 1 (fixation of the allele). Guidelines for interpretation suggest that 0.0 - 0.05 indicate little genetic difference, 0.05 - 0.15 moderate difference and 0.15 - 0.25 a large difference. Values above 0.25 will indicate a very large difference, but is not common (Hartl, 1988). It should be noted that low values do not imply that the difference is negligible.

Table 2.3 Equations for F-statistics (Hartl, 1988; Nei, 1987)

Parameter	Equation	Significance
$\mathbf{F_{IS}}$	$\mathbf{F_{IS}} = H_S - H_I / H_S$	Comparable with inbreeding coefficient – H relative to sub-population
$\mathbf{F_{IT}}$	$\mathbf{F_{IT}} = H_T - H_I / H_T$	Effect of sub population on total population
$\mathbf{F_{ST}}$ (Fixation Index)	$\mathbf{F_{ST}} = H_T - H_S / H_T$	Reduction in individual H relative to total population

Where: H_I = H of an individual in a sub-population
I_S = expected H of an individual in a sub population with random mating
H_T = expected H of an individual in a total population with random mating

Original statistics for measuring genetic variation was based on biochemical markers (allozyme loci) (Nei, 1987). Goldstein *et al.* (1995) and Goldstein & Pollock (1997) suggested that statistical measures for microsatellite data should be adapted due to the mutation rate, distribution of mutation size, number of repeats and asymmetry during mutation, associated with microsatellites. The major difference for microsatellite loci is a stepwise mutation process usually in one or two steps, but larger mutations have been observed. The size of the new mutant allele is often dependent on the size of the allele that has mutated (Slatkin, 1995). To prevent a biased estimate of genetic variability in the populations studied, it is critical to consider the process of mutation in microsatellite markers. An estimate $R_{ST,}$ was proposed as an alternative to F_{ST} by Slatkin (1995), where the R_{ST} is based on a fraction of the total variance of the allele size between populations.

$$\mathbf{R_{ST} = \tilde{S} - S_W / \tilde{S}}$$

Where: S_w and $\tilde{S}$ are proportional to the within-population total variances.

In simulations performed by Slatkin (1995) using F_{ST} and R_{ST}, it was concluded that R_{ST} performed better as it takes stepwise mutation into account. If the time-scale of interest for the population studied is short, F_{ST} is still an acceptable measure, but for studies over a longer period of time F_{ST} may lead to biased estimations. Pérez-Lezaun *et al.* (1997) also indicated R_{ST} as an alternative for microsatellite analyses, as F_{ST} does not take mutational relationships among alleles into consideration.

2.7.3 Genetic distance

Genetic distance is used to explain genomic differences or similarities between two populations, which is usually a function of allele frequencies (Nei, 1987). The distance could be zero, if no differences were observed or set to a maximum of 1, if there were no common alleles found at a common locus (Weir, 1996). Genetic distance is often used to estimate time of divergence in evolutionary studies. Depending on the study, different distances may be required, as it is used in construction of phylogenetic trees. The

distance measures proposed by Nei & Roychoudhury (1974) and Nei (1978) are most widely used, where I refers to the genetic identity and D to the genetic distance (Weir, 1996). Table 2.4 summarizes a few of the distance measures that can be applied in analyzing molecular data.

Table 2.4 Distance measures

Distance	Equation	Reference
Nei standard genetic distance D_S	$\mathbf{I} = J_{XY}/\sqrt{J_X J_Y}$	Nei (1987)
	$\mathbf{D_S} = -\ln[J_{XY}/\sqrt{J_X J_Y}$	Takezaki & Nei (1996)
Nei minimum genetic distance D_M	$\mathbf{D_M} = (J_X + J_Y)/2 - J_{XY}$	Takezaki & Nei (1996)
Roger's distance D_R	$\mathbf{D_R} = \frac{1}{\gamma} \Sigma\sqrt{\frac{\Sigma^{mj}_{I}(x_{ij} - y_{y})^2}{2}}$	Takezaki & Nei (1996)
Goldstein's distance $(\delta_\mu)^2$	$(\delta_u)^2 = \Sigma^{r}_{j}(\mu_{xi} - \mu_{yj})^2/\gamma$	Takezaki & Nei (1996)

Estimations using classical measures of genetic distance are usually based on the infinite alleles model or IAM that is not based on an evolutionary model. These distances, including the chord distance (D_c) and minimum distance (D_m) make use of allele frequencies shared between populations. The distances do not increase linearly with time and are mostly useful for closely related populations or taxa. They are less accurate for estimation of larger distances (Goldstein & Pollock, 1997).

Microsatellites may have certain limitations in phylogenetic reconstruction due to the nature of their mutation process (Goldstein & Pollock, 1997). According to Bruford & Funk (1999) changes are observed in allelic frequency of microsatellites between populations and it is estimated that mutation rates may vary form 10^{-4} and $5x10^{-6}$. New alleles may occur because of polymerase slippage during replication. The majority of the mutations is in one or two steps and therefore requires alternative statistical

analysis for estimation of distance and phylogeny. For microsatellites three distances are suggested that is based on a stepwise mutation model (SMM) that allows linear increases with time.

Distance based on SMM is dependent on the variation within populations and if population sizes vary, which is often the case, there is also intra-population variance to consider. Goldstein *et al.* (1995) defined a distance measure $(\delta_\mu)^2$ that deals with the variance term and was suggested for tree construction when studying distant populations. The distance increases linearly with time, but has a lower variance. The $(\delta_\mu)^2$ distance described by Goldstein *et al.* (1995) and Goldstein & Pollock (1997) takes differences in allele size into account and a stepwise mutation in each direction of samples x and y (Table 2.4).

Phylogeny is used to describe relationships as a genetic tree. Beside the distance measure applied, the choice for the tree will depend on the aim of the tree, whether only grouping of populations are of interest or describing evolutionary relationships (Nei, 1987).

Takezaki & Nei (1996) evaluated classical distance measures and concluded that estimates for 10 loci tend to be low, irrespective of a high or low H. Phylogenetic trees based on a less than 10 loci may be questionable. The efficiency of a tree will depend on the sample error and linear relationship with time. Takezaki & Nei (1996) suggested that different distance measures might be required for construction of branches of trees. When studying current populations, where evolutionary pathways are unknown, the standard unbiased genetic distance was found to be acceptable and both the NJ and UPGMA methods can be used for tree construction.

In conclusion, it could be stated that there are a wide range of statistical programs available for the analysis of molecular genetic data (some described under 2.10). Choosing the most appropriate program will depend on the nature of specific data set and the final goal of the study. A summary of programs can be found at the following websites: http://www.lcp.ucl.ac.be and corba.ebi.ac.uk/Biocatalog/Population.genetics.

CHAPTER THREE

Genetic characterization of native fowl in South Africa

3.1 Introduction

Native fowl populations in South Africa have received very little scientific recognition over the years. As described in Chapter One, it was only during 1994, that the "Fowls for Africa" program was established to conserve and promote native fowl populations in South Africa (Joubert, 1996). Although a phenotypic characterization contributes to breed definition and description of their production potential, a genetic characterization of the native chicken based on DNA information, is essential for long term conservation of the genetic resource. Genetic characterization provides information on the relationships and variation in the populations that may determine how the populations should be conserved as a genetic resource.

Various methods for the study of genetic variation in farm animals were reviewed in Chapter Two. Initially, blood protein polymorphisms were applied to estimate genetic variation (Hines, 1999). With the development of molecular techniques during the late eighties, specifically the Polymerase Chain Reaction, it became possible to target the DNA directly in genetic studies, which led to intensive studies of the genome and development of various DNA-markers including RFLP, DFP, mini - and microsatellites. These markers are widely used to describe variation and genetic relationships among and within populations (Zhou & Lamont, 1999). Microsatellites were decided on as the most appropriate DNA- marker for this study, as a large number of microsatellite markers are already mapped on the chicken genome, with a high degree of polymorphism.

This chapter describes the selection of appropriate polymorphic microsatellite markers for the study, the evaluation of the markers in the native populations as well as the application in the genetic characterization of the South African native fowl populations.

3.2 Material and Methods

Source of DNA

Blood samples were collected from the Potchefstroom Koekoek, New Hampshire, Naked Neck, Lebowa-Venda, Ovambo and the Black Australorp populations kept in the "Fowls for Africa" project at the ARC at Irene. Between forty and fifty venous blood samples of each population were collected in 2 ml tubes containing 80 µl EDTA (final concentration 0.5 M). Twenty blood samples from native chicken populations were donated by the University of Zimbabwe, Botswana Agricultural College and the Eduardo Mondlane University in Mozambique. The origin of the Potchefstroom Koekoek, New Hampshire, Naked Neck, Lebowa-Venda and Ovambo fowls was described in Chapter Two. The Black Australorp population was only included in the genetic characterization, as these birds were very often used in rural areas as dualpurpose breeds (eggs and meat) and may have genetic similarities with the other native populations. The Australorp was also often applied in upgrading of native fowl in other African countries. The blood samples from Botswana and Mozambique were collected from native populations kept at the respective universities and the samples from Zimbabwe were collected from rural native chicken populations on routine testing for New Castle disease. These chicken populations are not yet described as lines or breeds and vary greatly in colour and conformation.

After collection the blood samples, were frozen in Eppendorf tubes and kept at -70°C. DNA was extracted from the blood samples using a Puregene DNA-isolation kit (Gentra Systems, Minneapolis). Avian blood contains erythrocytes that are nucleated and only a small volume of blood is required for DNA-extraction. The concentration of the DNA was quantitated by spectrophotometry and diluted to a concentration of 10 ng/µl.

Selection and testing of microsatellite markers

Twenty-seven fluorescently labeled polymorphic microsatellite markers were selected from the collection of markers made available by Dr Martien Groenen (Department of Animal Breeding, Wageningen Agricultural University, The Netherlands). The selection

was based on the degree of polymorphism and genome coverage (Crooijmans *et al.*, 1996a & b; Crooijmans *et al.*, 1997). The characteristics of the markers used, including the chromosome location, expected range in base pairs and numbers of alleles, as reported by Groenen *et al.* (1998), are summarized in Table 3.1. These markers were all tested in the reference population kept at the Wageningen Agricultural University.

PCR conditions and gel analyses

PCR reactions were carried out in a volume of 12 µl, containing 30-60 ng target DNA, 200 µM dNTP's, 1 mM Tetramethylammoniumchloride (TMAC), 10 mM TrisHCl (pH = 9.0), 1.5 mM $MgCl_2$, 50 ml mM KCl, 0.01% gelatine, 0.1% Triton X-100, 0.2 U, Taq enzyme and 300 ng/µl of each primer (microsatellite marker). Preparation of samples were followed by thermal cycling in a Thermal Controller (Perkin Elmer) using the following programme: 5 minutes at 94°C followed by 35 cycles consisting of 30 sec at 94°C, 45 sec at 55°C, 90 sec at 72°C and an extension step of 10 min at 72°C. The microsatellite amplicons were then tested on an agarose gel to ensure a good product before a mix was prepared and analyzed on an automated DNA-sequencer (ABI 373A). Some primers required further optimization and PCR conditions and temperatures were adapted until amplicons of a desirable quality were obtained. Annealing temperatures varied between 50°C and 58°C for the different primers.

In order to make the most economical use of the ABI Automated sequencer, primers were divided into in three sets according to differences in size and fluorescent labels namely HEX (yellow), FAM (blue) or TET (green) (Table 3.1). First, a mix containing the microsatellite amplicons were prepared for each set according to the expected signals. Then a loading buffer containing the GENESCAN-350 TAMRA internal standard and formamide (3.2µl) was mixed with 1µl of the pooled PCR amplicons, denaturated and loaded onto a polyacrylamide sequencing gel (ABI 377 sequencing machine). The gel data were transferred for analysis with Genescan software.

Table 3.1 Final mixes of the three sets of microsatellites for automated analyses

Set 1		Set 2		Set 3	
Microsatellite	**Volume (µl)**	**Microsatellite**	**Volume (µl)**	**Microsatelite**	**Volume (µl)**
MCW0037	4 (F)	MCW0014	2 (F)	ADL0112	2 (F)
MCW0067	1 (T)	MCW0034	2 (F)	ADL0268	4 (T)
MCW0098	1 (T)	MCW0069	5 (H)	LEI0192	2 (T)
MCW0078	3 (H)	MCW0103	2 (T)	LEI0194	3 (T)
MCW0183	2 (T)	MCW0111	2 (H)	MCW0081	2 (H)
MCW0284	6 (T)	MCW0216	2 (T)	MCW0226	2 (F)
MCW0294	3 (H)	MCW0248	2 (T)		
MCW0295	3 (H)				
MCW0330	1 (F)				

Dye colour is indicated in brackets (F) = blue; (T) = green and (H) = yellow.

Statistical analyses

The Genescan version 2.0 and Genotyper for MacIntosh were used to determine the fragment sizes in base pairs. From Genotyper, data files were exported to Microsoft Xcel, for preparation of input files for statistical analyses. The statistical programs of the SAS Institute (1992) and BIOSYS-1 program package (Swofford & Selander, 1989) were used for calculations of allele frequencies and heterozygosities. Allele frequencies were calculated and a Chi-square test was performed to test for Hardy-Weinberg equilibrium. There were several unique alleles among the populations and therefore, alleles were grouped according to homozygotes for the most common allele, the heterozygotes for the most rare or common alleles and rare homozygotes. Heterozygosity per microsatellite marker was calculated according to Nei (1978):

$$H_l = [2n/2n-1][1-i\acute{O}^{ml}(pl_i^2)]$$

Where: n = the number of individual chickens per population,
ml = the number of alleles at locus 1
pl_I = the frequency of the I^{th} allele at locus 1.

The standard error was calculated from the total variance at each locus and total variance over all the loci studied. An analysis of variance (Tukey's Studentized Range) was performed to test for significant differences in H among the lines (SAS, 1992).

The Polymorphic Information Content (PIC) values were also estimated according to Botstein *et al.* (1980) using SAS (1992). PIC values were for all the microsatellites per chicken population:

$$PIC = 1 - (\Sigma_{i=1}^{n-1} p_i^2) - \Sigma_{i=1}^{n-1} \Sigma_{j=i+1}^{n} 2 p_i^2 p_i^2$$

Where: k = number of different alleles for the specific locus
p_i^2 and p_i^2 = the population frequencies of the i^{th} and j^{th} allele

F_{ST} values were calculated as estimators of genetic subdivision for each microsatellite marker across all the populations. The R_{ST} was calculated as an alternative to F_{ST} for describing population subdivision.

R_{ST} was calculated using MSAT (MICROSAT: hhtp://lotka.stanford.edu/microsat/microsat.htm/) based on the fraction of the total variance of allele size between populations as proposed by Slatkin (1995).

$$R_{ST} = \frac{\bar{S} - S_w}{\bar{S}}$$

Where: S_w is proportional to the within-population variance
$\bar{S}$ is proportional to the total variance

Genetic distances were calculated according to Nei (1978), unbiased standard genetic distance D_S, using BIOSYS-1 (Swofford & Selander, 1989) and DISPAN (Ota, Institute of Molecular Evolutionary Genetics, Pennsylvania State University PA, USA). DISPAN was also applied to resample allele frequencies, with 1000 bootstrap replicates and D_S

calculated for a phylogenetic consensus tree. Both the neighbour-joining method (NJ) and unweighted pair-group method with arithmetic mean (UPGMA) were used in the calculations for tree construction. Both these methods are considered to be useful in obtaining the correct tree topology, using standard genetic distances (Takezaki & Nei, 1996).

The standard genetic distance D_S, according to Nei (1978):

$$\mathbf{Ds = (1 - J_{xy}) - ½ \{(1 - J_x) + (1 - J_y)\}}$$

$$\mathbf{D_S = \ln [J_{xy}/\sqrt{J_x J_y}]}$$

Where: $J_x = (2n_x \Sigma x^2_i - 1)/2n_x - 1)$

$J_y = (2n_y \Sigma y^2_i - 1)/2n_y - 1)$

$J_{xy} = \Sigma xy$

n = population size (number of individuals in sample)

$x_i y_i$ = allele frequencies for x^{th} allele in population x and y

3.3 Results

The nine native chicken populations were tested with microsatellite markers shown in Table 3.1 and Table 3.3. Although a total number of at least 36 samples were tested for most of the lines (except the Botswana, Mozambique and Zimbabwe populations), some samples had to be discarded if the results obtained from the genescan gel run were not satisfactory. The quality of the blood samples varied, especially for the chicken populations from Botswana, Mozambique and Zimbabwe, which influenced the final number of individuals available for statistical analyses. The number of samples included for the different populations in the final analyses are presented in Table 3.2.

Figure 3.1 is an example of one of the Automated (ABI 377) gel runs containing samples of the Ovambo, Australorp and Zimbabwe populations and six microsatellite markers from set 2 (Table 3.1).

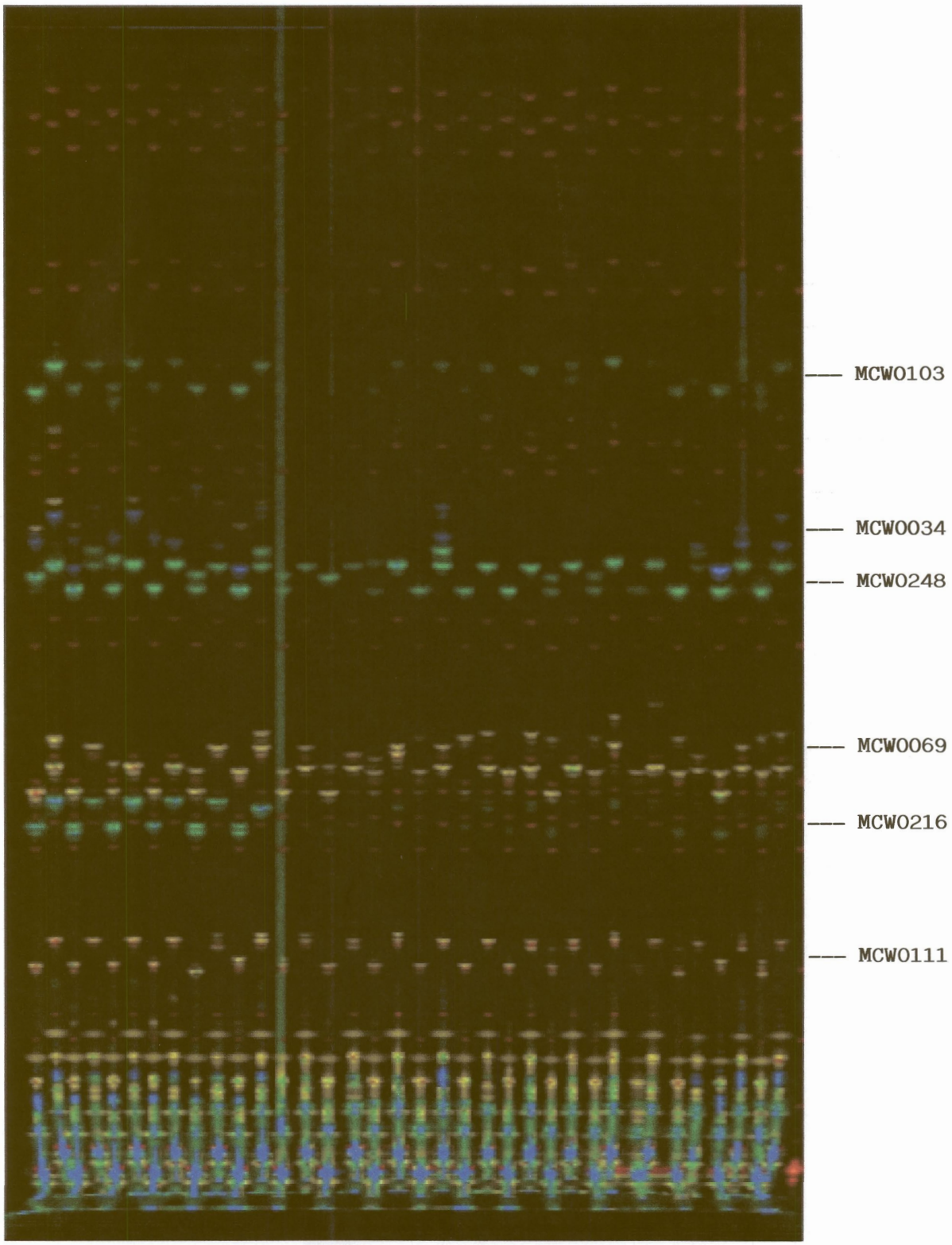

Figure 3.1 **Automated Genescan for three populations and six microsatellite markers.**

Microsatellite markers

Twenty-six microsatellite markers were tested in the nine different populations. (Table 3.3). Three of these markers (LEI0166, MCW0150, MCW0222) did not amplify well and were not included in the final sets. LEI0192 of set three was also left out in the final analysis, as only accurate results were observed for the Koekoek population. Figure 3.2 illustrates the alleles observed when analyzing with Genotyper. It can be seen that the three individuals were heterozygotes and 3 different alleles were observed for the specific locus.

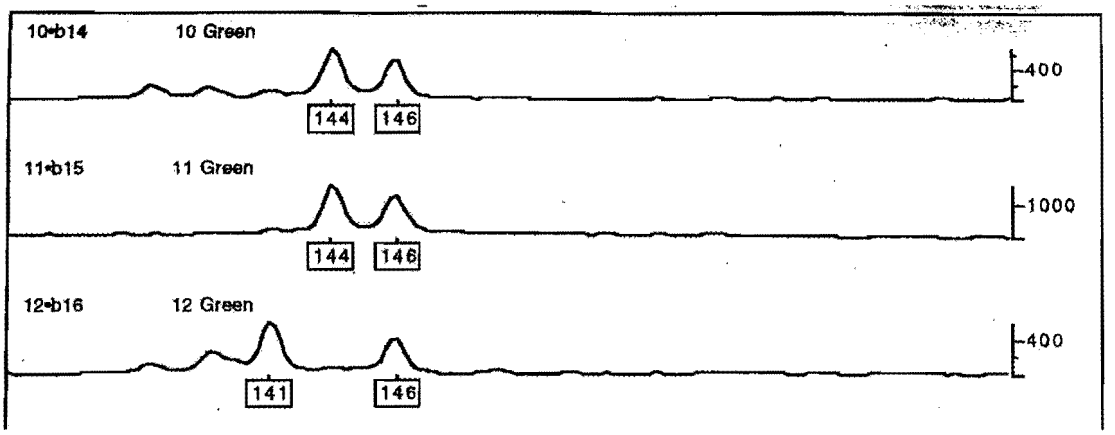

Figure 3.2 Alleles observed for three New Hampshire individuals with Genotyper analysis and microsatellite marker MCW0216.

The different number of alleles observed for the different markers tested in all the populations is presented in Table 3.3. Microsatellites tested were found to be highly polymorphic. Only three loci were found to be monomorph. These were LEI0194 and MCW0222 in the Koekoek and MCW0294 in the New Hampshire population. The number of alleles per loci varied from three to fourteen different alleles (Table 3.3). Allele size ranged from a seven bp difference (220-227 bp) for locus MCW0222, to a difference of 47 bp (153-200 bp) for locus MCW0067. Except for the Zimbabwe population, alleles specific to a population were observed for ten of the markers. The allele frequencies estimated for all loci and populations are shown in Addendum A 1.

There were eight microsatellite markers that deviated from Hardy-Weinberg equilibrium. Three of the markers (MCW0067, MCW0216, MCW0069) tend to show a consistent deviation in all the populations, except for the Botswana, Mozambique and Australorp populations. Microsatellite markers (MCW0330) deviated in the Koekoek, New Hampshire and Naked Neck population, while the four other markers that deviated were specific to one population only.

Polymorphic Information Content (PIC) and Heterozygosity (H)

In order to describe the polymorphic nature of the microsatellite markers tested, the PIC values for the different markers were calculated as discussed in the methods and is shown in Tables 3.4. Lower PIC values were observed throughout for all the microsatellites tested in all the populations, when compared to the H values for the same markers and populations. The highest PIC and H values were observed for the markers MCW0295, MCW0330, MCW0069, MCW0067 and ADL0268. These markers also had the highest different number of alleles. PIC values always tend to be lower than H values, as the PIC is calculated for the number and the frequency of the different alleles. Loci with a large number of different alleles may have a high PIC value, but if one or two alleles dominate, then the PIC may still be relatively small (Buchanan *et al.*, 1994). It was found that although some of the markers for example, markers MCW0078 and MCW0081 had six and nine different alleles respectively, their PIC values were as low as the values estimated for markers, MCW0014 and MCW0098, which only had four different alleles among the populations. For the markers MCW0078 and MCW0081, two alleles dominated for the respective microsatellite markers. In the Koekoek population, for example, the dominant allele represented 80% of the five different alleles observed for marker MCW0078. A dominant allele for the same marker (MCW0078) was also observed in the Naked Neck population where the allele accounted for 68% of the four different alleles. On average, the PIC per marker varied from as low as 0.33 to as high as 0.66 for the different microsatellite markers.

Table 3.2 **Number of samples per population for the different microsatellite markers obtained from genescan analysis**

Micro-satellites	Koekoek		New Hampshire		Naked Neck		Lebowa-Venda		Ovambo		Australorp		Botswana		Mozambique		Zimbabwe	
	samples	alleles	samples	alleles	samples	alleles	samples	alleles	samples	alleles	samples	alleles	samples	alleles	samples	alleles	samples	alleles
ADL0112	24	6			9	2	18	3	2	1	12	2					8	3
ADL0268	17	4	13	5	14	5	34	5	36	6	18	6	13	4	14	5	14	3
ADL0278	16	3	14	3	9	3	12	3	10	3			13	3	16	3		
LEI0194	6	1	44	5	18	5	46	2	38	4	32	5					24	3
MCW0014	16	1	14	3	9	3	12	2	12	2	0		13	3	14	3		
MCW0034	24	5	9	5	19	5	18	2	8	4	20	5					7	5
MCW0037	36	4	33	4	31	6	39	3	39	6	20	3						
MCW0067	37	4	36	6	31	9	38	6	38	8	22	4	12	3	16	3	18	4
MCW0069	50	4	47	7	44	8	33	6	27	4	25	5	13	6	16	5	20	6
MCW0078	26	5	15	2	22	4	34	4	38	3	18	3	9	4	16	4		
MCW0081	16	6	25	3	14	3	26	3	25	5	21	3					11	4
MCW0098	46	3	22	3	41	3	26	3	23	2	3	3	13	2	16	2	14	4
MCW0103	36	2	36	4	13	4	37	4	17	5	29	2	13	2	27	3	16	3
MCW0111	47	8	46	7	42	6	18	5	30	4	25	3	11	5	14	5	17	3
MCW0183	35	3	21	6	41	8	31	4	38	8	22	6	11	7	15	5	17	7
MCW0216	47	5	46	3	40	4	37	3	28	3	26	4	10	4	9	4	13	3
MCW0222	16	1	13	3	11	3	12	3	12	4			12	4	16	4		
MCW0226	21	4			11	4	11	4	4	3	14	3					17	3
MCW0248	37	2	46	10	39	7	32	4	23	5	19	2	11	3	14	4	21	3
MCW0284	13	2	11	3	11	2	17	2	8	3	10	3						
MCW0294	25	2	19	1	12	3	17	3	24	4	19	2					20	4
MCW0295	41	5	13	4	28	10	34	8	33	7	18	4	13	6	14	5	9	4
MCW0330	31	5	31	5	36	6	30	5	30	7	11	7	12	4	16	4	18	5

Table 3.3 Characteristics of microsatellite loci: Expected and observed range and number of alleles

Microsatellite	*Chromo-some	*Expected range (bp)	*Expected n of alleles	N of alleles observed	Range and different alleles
ADL0112	10	120 – 150	4	6	125 127 131 133 135 145
ADL0268	1	90 – 130	7	10	**104** 106 108 **110** 112 114 116 120 **123** 127
ADL0278	8	100 – 130	3	3	114 120 123
LEI 0166	3	250 – 280	3	-	-
LEI0192	6	256 – 292	5	-	-
LEI0194	1 & 4	120 – 160		9	121 127 129 132 134 138 146 151 157
MCW0014	6	160 – 190	8	4	173 177 183 187
MCW0034	2	220 – 250	12	10	222 224 226 228 230 232 234 242 245 250
MCW0037	3	140 – 180	3	8	143 148 153 155 157 161 163 165
MCW0067	10	140 – 200	6	11	**153** 155 **167** **172** 176 178 180 182 184 186 **200**
MCW0069	26	145 – 185	6	14	**146** **151** **153** 157 159 161 163 165 167 169 171 173 175 177
MCW0078	5	130 – 150	6	6	136 139 142 144 146 148
MCW0081	5	105 – 145	6	9	108 112 114 119 125 127 132 134 139
MCW0098	4	250 – 270	2	4	258 262 264 266
MCW0103	3	260 – 290	2	7	**262** **264** 268 270 272 274 280
MCW0150	3	215 – 250	-	-	-
MCW0111	1	90 – 120	5	13	**90** **93** **97** 99 101 103 105 107 110 **112** **114** 118 **120**
MCW0183	7	280 – 320	9	12	293 295 297 300 302 307 311 313 **317** 320 **322** **325**
MCW0216	13	135 –165	4	9	138 141 144 146 150 **152** **156** **158** 165
MCW0222	3	205 – 240	5	4	220 223 225 227
MCW0226	15	290 – 320	-	7	290 295 297 300 303 306 308
MCW0248	1	205 – 235	6	10	216 220 222 224 227 **231** 237 **243** 245 **250**
MCW0284	4	225 – 250	2	5	228 237 239 243 245
MCW0294	Z	280 – 320	9	5	303 305 308 311 316
MCW0295	4	85 – 120	6	11	87 90 92 94 96 98 100 102 104 106 108
MCW0330	17	255 – 300	5	10	258 260 270 272 274 277 279 **283** 289 **293**

* As reported by Groenen *et al.*, 1998; Crooijmans, 2000
• Alleles in bold were observed for a specific population

The Heterozygosity or also referred to as gene diversity was estimated for all microsatellite markers and the different populations. These values are presented in Table 3.5. The heterozygosity per microsatellite within the populations ranged from as low as 4% (MCW0294 in Koekoek) to as high as 89% (MCW0295 in Ovambo). The highest H per marker was found in the Naked Neck population, where 17 from the 23 markers had H values above 60%. The mean H varied between the lowest H of 53% observed for the Koekoek population to the highest H of 64% for the Naked Neck population for all the microsatellite markers tested. The H values for the other populations were very similar. The Ovambo (62%), Botswana (61%) and Mozambique (62%) showed a higher variation, while the New Hampshire (56%), Lebowa-Venda (54%), Australorp (54%) and Zimbabwe (56%) had a relatively lower variation. Significant differences were observed only between the Naked Neck and the Koekoek populations and the Koekoek and Mozambique populations for all the microsatellite markers tested (Table 3.5)

In order to calculate the genetic distance among the populations, they had to share the same microsatellite markers. Therefore the populations were grouped, according to the loci they had in common (Table 3.6).

From Table 3.6 it can be seen that the 11 microsatellite markers of Group I were used in distance calculations for all nine populations, while for the South African populations the 11 loci of Group I and another seven markers (Group II) were included. The Australorp and Zimbabwe populations did not have sufficient samples for the four additional markers in Group III and therefore not included for calculations with the 15 markers.

Table 3.6 Grouping of native populations for microsatellite markers shared for calculation of genetic distances

Population	Group I	Group II	Group III
Koekoek	11	18	15
New Hampshire	11	18	15
Naked Neck	11	18	15
Lebowa-Venda	11	18	15
Ovambo	11	18	15
Australorp	11	18	-
Botswana	11	-	15
Mozambique	11	-	15
Zimbabwe	11	-	-

Group I : ADL0268, MCW0067, MCW0069, MCW0098, MCW0103, MCW0111, MCW0183, MCW0216, MCW0248, MCW0295, MCW0330

Group II : ADL0268, MCW0067, MCW0069, MCW0098, MCW0103, MCW0111, MCW0183, MCW0216, MCW0248, MCW0295, MCW0330 & LEI0194, MCW0034, MCW0037, MCW0078, MCW0081, MCW0284, MCW0294.

Group III: ADL0268, MCW0067, MCW0069, MCW0098, MCW0103, MCW0111, MCW0183, MCW0216, MCW0248, MCW0295, MCW0330 & ADL 278, MCW0014, MCW0078, MCW 222.

The mean F_{ST} across markers for the different groups varied from 0.179 for Group I with 11 loci to 0.195 for both Group II (18 loci) and Group III (15 loci). The F_{ST} values for the microsatellite markers across populations are shown in Addendum A 2. The genetic variability was again estimated for the three groups discussed above and results are summarized in Tables 3.7, 3.8 and 3.9. The mean sample size per locus, mean number of alleles per locus, percentage polymorphic loci and the mean heterozygosity are presented for the different loci and populations as grouped in Table 3.6. Where all the populations were uniformly analyzed with the unanimous 11 loci (Group I) the mean number of alleles per marker ranged from 4.1 to 6.4 and the H from 56% for the Koekoek to 70%

for the Naked Neck population. In this group all microsatellite markers were found to be 100% polymorphic among the populations. Among the South African populations only, the mean number of alleles per marker and the H were lower ranging from 3.9 and 50% to 5.4 and 67% in the Koekoek and the Naked Neck populations respectively. The percentage polymorphic loci were 88.9% and 94.9% for the Koekoek and New Hampshire populations respectively, because there were two markers (LEI0194 and MCW0222) that were monomorph in the Koekoek population and marker (MCW0294) in the New Hampshire population. A similar trend for the mean number of alleles per marker and H were observed for the last grouping with 15 markers.

Despite the different number of loci used for the estimation in the groups, the ranking of the populations for H remained the same. Even with only 11 loci included, Koekoek still showed the lowest and the Naked Neck the highest variation.

The R_{ST} values of the sub-populations varied between 0.014 and 0.153 for Group I with 11 loci, while larger values were observed for both Group II (0.020 to 0.529) with 18 loci and Group III (0.008 to 0.271) with 15 loci.

Genetic Distance

The genetic differences among the native populations were further evaluated by estimating the genetic distance. There are various methods for estimation of genetic distance as referred to in Chapter Two. The unbiased genetic distance (Nei, 1978) is most often used in studies of this nature and was used in estimations. These distance values were then used for constructing a phylogenetic tree.

Table 3.4 Polymorphic information content (PIC) for microsatellite markers tested in the different fowl populations

Microsatellite	Koekoek	New Hampshire	Naked Neck	Lebowa-Venda	Ovambo	Australorp	Botswana	Mozambique	Zimbabwe	Mean*	SD
ADL0112	0.55	-	0.29	0.45	0.00	0.30	-	-	0.43	0.40	0.11
ADL0268	0.47	0.69	0.69	0.64	0.73	0.78	0.67	0.55	0.41	0.62	0.12
ADL0278	0.30	0.46	0.54	0.43	0.47	-	0.50	0.55	-	0.47	0.09
MCW0014	0.00	0.40	0.37	0.23	0.35	-	0.39	0.55	-	0.38	0.10
MCW0034	0.65	0.58	0.66	0.24	0.57	0.45	-	-	0.60	0.54	0.15
MCW0037	0.46	0.30	0.66	0.29	0.63	0.44	-	-	-	0.46	0.16
MCW0067	0.48	0.63	0.71	0.74	0.63	0.59	0.58	0.55	0.56	0.61	0.08
MCW0069	0.33	0.68	0.68	0.60	0.64	0.49	0.63	0.69	0.68	0.60	0.12
MCW0078	0.31	0.24	0.42	0.38	0.34	0.27	0.67	0.52	-	0.39	0.14
MCW0081	0.67	0.38	0.57	0.54	0.52	0.40	-	-	0.57	0.38	0.10
MCW0098	0.35	0.16	0.39	0.16	0.37	0.59	0.36	0.37	0.44	0.36	0.13
MCW0103	0.37	0.52	0.56	0.44	0.45	0.13	0.18	0.42	0.27	0.37	0.15
MCW0111	0.71	0.65	0.70	0.62	0.46	0.42	0.72	0.54	0.45	0.58	0.12
MCW0183	0.42	0.50	0.50	0.31	0.61	0.67	0.69	0.71	0.73	0.57	0.15
MCW0216	0.44	0.53	0.57	0.52	0.20	0.43	0.59	0.50	0.47	0.47	0.11
MCW0222	0.00	0.48	0.57	0.51	0.61	-	0.34	0.52	-	0.51	0.09
MCW0226	0.60	-	0.68	0.61	0.47	0.56	-	-	0.48	0.57	0.08
MCW0248	0.37	0.77	0.73	0.57	0.62	0.26	0.36	0.42	0.20	0.48	0.20
MCW0284	0.37	0.23	0.29	0.22	0.47	0.41	-	-	-	0.33	0.10
MCW0294	0.038	0.00	0.37	0.58	0.57	0.26	-	-	0.37	0.36	0.20
MCW0295	0.71	0.61	0.81	0.72	0.73	0.53	0.69	0.55	0.480	0.65	0.11
MCW0330	0.65	0.55	0.59	0.68	0.79	0.73	0.66	0.65	0.61	0.66	0.07
Mean	0.46	0.50	0.57	0.47	0.54	0.47	0.54	0.54	0.48		
SD	0.17	0.17	0.15	0.17	0.15	0.17	0.17	0.09	0.14		

*Average PIC/microsatellite marker

Table 3.5 Heterozygosity values for microsatellite markers tested in different fowl populations

Microsatelite	Koekoek	New Hampshire	Naked Neck	Lebowa-Venda	Ovambo	Australorp	Botswana	Mozambique	Zimbabwe
ADL0112	0.61	-	0.37	0.51	-	0.39	-	-	0.51
ADL0268	0.53	0.77	0.77	0.70	0.78	0.83	0.75	0.62	0.49
ADL0278	0.33	0.54	0.66	0.51	0.55	-	0.59	0.65	-
LEI0194	0.00	0.65	0.81	0.50	0.76	0.71	-	-	0.52
MCW0014	0.00	0.50	0.45	0.27	0.46	-	0.50	0.65	-
MCW0034	0.72	0.66	0.73	0.29	0.68	0.49	-	-	0.70
MCW0037	0.54	0.32	0.73	0.35	0.70	0.56	-	-	-
MCW0067	0.58	0.68	0.76	0.78	0.69	0.68	0.69	0.65	0.65
MCW0069	0.36	0.74	0.73	0.67	0.71	0.58	0.70	0.76	0.74
MCW0078	0.34	0.29	0.49	0.45	0.38	0.30	0.76	0.59	-
MCW0081	0.73	0.43	0.67	0.61	0.57	0.48	-	-	0.67
MCW0098	0.43	0.17	0.52	0.17	0.50	0.80	0.49	0.51	0.51
MCW0103	0.50	0.61	0.63	0.53	0.55	0.14	0.21	0.55	0.31
MCW0111	0.76	0.70	0.75	0.70	0.54	0.47	0.79	0.60	0.57
MCW0183	0.50	0.54	0.55	0.34	0.67	0.72	0.75	0.78	0.78
MCW0216	0.53	0.61	0.65	0.60	0.23	0.54	0.68	0.61	0.59
MCW0222	0.00	0.57	0.68	0.60	0.70	-	0.37	0.62	-
MCW0226	0.67	-	0.77	0.71	0.61	0.66	-	-	0.59
MCW0248	0.50	0.81	0.77	0.65	0.70	0.31	0.44	0.47	0.22
MCW0284	0.51	0.26	0.37	0.26	0.57	0.48	-	-	-
MCW0294	0.04	0.00	0.45	0.68	0.65	0.31	-	-	0.43
MCW0295	0.76	0.70	0.85	0.77	0.77	0.62	0.76	0.62	0.58
Mean	0.53	0.56	0.64	0.54	0.62	0.54	0.61	0.62	0.56
Std Dev.	0.17	0.17	0.14	0.18	0.14	0.18	0.17	0.08	0.14

Table 3.7 Genetic variability for 11 loci for all nine populations (Group I)

Population	Mean sample size /locus	Mean number of alleles/locus	Percentage polymorphic loci	Mean H: Direct count	Mean H: Hardy-Weinberg expected
Koekoek	38.5 (2.8)	4.1 (0.5)	100.0	0.34 (0.07)	0.56 (0.04)
New Hampshire	32.5 (4.0)	5.5 (0.6)	100.0	0.41 (0.06)	0.63 (0.05)
Naked Neck	35.7 (2.6)	6.4 (0.7)	100.0	0.47 (0.05)	0.70 (0.03)
Lebowa-Venda	30.7 (2.1)	4.8 (0.4)	100.0	0.48 (0.07)	0.60 (0.06)
Ovambo	30.6 (1.6)	5.4 (0.6)	100.0	0.40 (0.05)	0.64 (0.06)
Australorp	19.6 (2.2)	4.2 (0.5)	100.0	0.51 (0.05)	0.60 (0.07)
Botswana	12.0 (0.3)	4.2 (0.5)	100.0	0.59 (0.06)	0.64 (0.06)
Mozambique	14.5 (0.6)	4.1 (0.3)	100.0	0.58 (0.06)	0.63 (0.03)
Zimbabwe	16.0 (1.1)	4.1 (0.4)	100.0	0.48 (0.07)	0.56 (0.05)

*SE of the mean

Table 3.8 Genetic variability for 18 loci in the South African populations (Group II)

Population	Mean sample size/locus	Mean number of alleles/locus	Percentage polymorphic loci	Mean H: Direct count	Mean H: Hardy-Weinberg expected
Koekoek	31.4 (3.1)	3.9 (0.4)	88.9	0.36 (0.06)	0.50 (0.05)
New Hampshire	27.3 (3.1)	4.6 (0.5)	94.4	0.37 (0.05)	0.53 (0.05)
Naked Neck	27.9 (3.0)	5.4 (0.5)	100.0	0.50 (0.04)	0.67 (0.03)
Lebowa-Venda	28.5 (1.9)	4.0 (0.4)	100.0	0.42 (0.06)	0.54 (0.05)
Ovambo	27.7 (2.2)	4.9 (0.4)	100.0	0.40 (0.05)	0.63 (0.04)
Australorp	18.9 (1.4)	3.9 (0.4)	100.0	0.46 (0.06)	0.55 (0.05)

*SE of the mean

Table 3.9 Genetic variability for 15 loci in the South African populations, Botswana and Mozambique (Group III)

Population	Mean sample size/locus	Mean number of alleles /locus	Percentage of polymorphic loci	Mean H: Direct count	Mean H: Hardy-Weinberg expected
Koekoek	33.2 (3.2)	3.7 (0.5)	86.7	0.30 (0.06)	0.46 (0.06)
New Hampshire	27.5 (3.6)	4.7 (0.6)	100.0	0.44 (0.05)	0.59 (0.04)
Naked Neck	29.1 (3.5)	5.5 (0.6)	100.0	0.53 (0.05)	0.67 (0.03)
Lebowa-Venda	27.5 (2.5)	4.3 (0.4)	100.0	0.49 (0.06)	0.57 (0.05)
Ovambo	27.3 (2.4)	4.7 (0.5)	100.0	0.41 (0.04)	0.61 (0.05)
Botswana	11.9 (0.3)	4.0 (0.4)	100.0	0.59 (0.05)	0.62 (0.05)
Mozambique	14.8 (0.5)	3.9 (0.2)	100.0	0.59 (0.04)	0.63 (0.02)

*SE of the mean

Distances were calculated according to the number of shared loci as previously described and showed in Table 3.6. Therefore three matrixes were obtained for the three groups: Group I with 11 loci, Group II with 18 loci and Group III with 15 loci (Tables 3.11, 3.12 and 3.13).

Table 3.10 **Genetic distances for all populations and 11 loci (Group I)**
Unbiased standard genetic distance (Nei, 1978): below diagonal
Standard errors: above diagonal

Population	KK	NH	NN	LV	OV	AU	BS	MS	Z
Koekoek	***	0.126	0.104	0.194	0.091	0.062	0.064	0.105	0.128
New Hampshire	**0.530**	***	0.087	0.108	0.105	0.11	0.146	0.066	0.182
Naked Neck	**0.375**	**0.250**	***	0.067	0.128	0.071	0.160	0.115	0.087
Lebowa-Venda	**0.645**	**0.241**	**0.234**	***	0.134	0.107	0.108	0.084	0.119
Ovambo	**0.241**	**0.560**	**0.387**	**0.414**	***	0.058	0.088	0.102	0.065
Australorp	**0.260**	**0.424**	**0.355**	**0.536**	**0.289**	***	0.246	0.192	0.078
Botswana	**0.342**	**0.452**	**0.555**	**0.371**	**0.378**	**0.720**	***	0.034	0.322
Mozambique	**0.351**	**0.328**	**0.424**	**0.413**	**0.331**	**0.543**	**0.093**	***	0.293
Zimbabwe	**0.437**	**0.524**	**0.414**	**0.440**	**0.383**	**0.127**	**0.994**	**0.893**	***

The smallest distance was found between the populations from Botswana and Mozambique (0.093) and the largest distance between the populations from Zimbabwe and Botswana (0.994) in Group I, where only 11 loci were included for all the populations. Among the South African populations in Group I, the distance was the smallest between the Koekoek and Ovambo (0.241) and the Lebowa-Venda and New Hampshire (0.241), while the largest distance was between the Koekoek and Lebowa-Venda populations (0.645).

Among the South African populations, where 18 loci were included for distance calculations (Group II , Table 3.11), the smallest distance was between the Naked Neck

and New Hampshire (0.218), while the largest distances were between the Koekoek and New Hampshire (0.682) and Koekoek and Lebowa-Venda (0.645). A similar trend was observed for the South African populations in Group III (Table 3.12), with the smallest distances between the New Hampshire and Naked Neck (0.180) and the Naked Neck and Lebowa-Venda (0.163). The largest distances were found between the Koekoek and Lebowa-Venda (0.649).

Table 3.11 Genetic distances for South African populations and 18 loci (Group II)
Unbiased standard genetic distance (Nei, 1978): below diagonal
Standard errors: above diagonal

Population	KK	NH	NN	LV	OV	AU
Koekoek	***	0.164	0.099	0.158	0.124	0.154
New Hampshire	**0.682**	***	0.054	0.095	0.090	0.110
Naked Neck	**0.476**	**0.218**	***	0.059	0.083	0.074
Lebowa-Venda	**0.645**	**0.270**	**0.247**	***	0.087	0.112
Ovambo	**0.465**	**0.423**	**0.284**	**0.282**	***	0.055
Australorp	**0.559**	**0.433**	**0.374**	**0.589**	**0.302**	***

Table 3.12 Genetic distances for the South African populations, Mozambique and Botswana (Group III)
Unbiased standard genetic distance (Nei, 1978): below diagonal
Standard errors: above diagonal

Population	KK	NH	NN	LV	OV	BS	MS
Koekoek	***	0.118	0.102	0.182	0.112	0.083	0.08
New Hampshire	**0.494**	***	0.062	0.069	0.09	0.118	0.087
Naked Neck	**0.434**	**0.180**	***	0.052	0.094	0.118	0.087
Lebowa-Venda	**0.649**	**0.167**	**0.163**	***	0.101	0.084	0.069
Ovambo	**0.436**	**0.397**	**0.276**	**0.308**	***	0.08	0.073
Botswana	**0.372**	**0.373**	**0.413**	**0.324**	**0.375**	***	0.033
Mozambique	**0.365**	**0.227**	**0.291**	**0.295**	**0.525**	**0.116**	***

A phenetic approach was followed for phylogenetic tree construction, as evolutionary pathways were not considered for this study. Both the neighbour-joining and UPGMA methods were applied for obtaining the trees. A tree was constructed for each of the groups shown in Table 3.6. The standard neighbour-joining trees for Groups I, II and III are presented in Figures 3.3, 3.4 and 3.5 respectively.

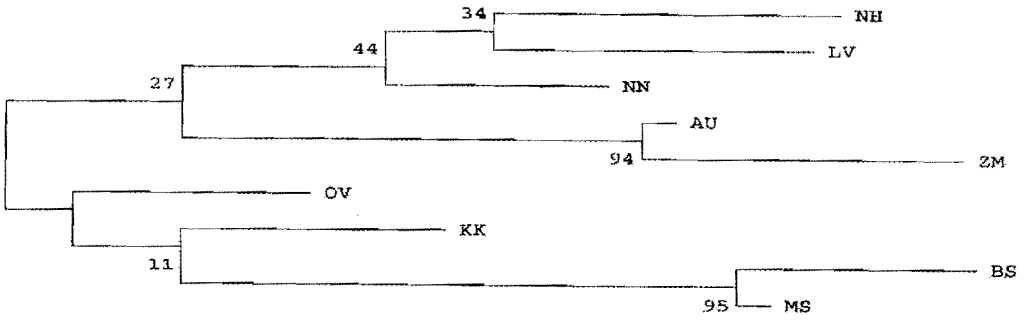

Figure 3.3 Standard neighbour-joining tree for Group I.

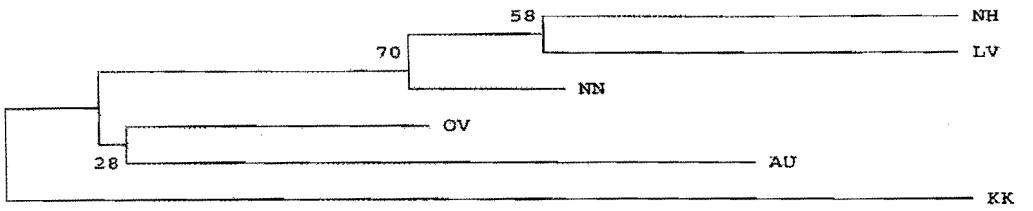

Figure 3.4 Standard neighbour-joining tree for Group II.

The New Hampshire, Lebowa-Venda and Naked Neck populations formed a cluster with a significance of 34-44% in Group I analyzed with 11 loci (Figure 3.3). This cluster remained the same for Group II and III with a higher significance of 58-70% for Group II (18 loci) and 76-46% for Group III (15 loci) (Figures 3.4 & 3.5). The Koekoek population formed a distinct branch on it's own with a high significance (100%) if

analyzed with 15 (Figure 3.5) and 18 loci (Figure 3.3). Although Koekoek still tend to form a branch on its own, when analyzed with 11 loci only, the significance for this branch was much lower. The Koekoek population was still clearly in its own group, compared to the Naked Neck, New Hampshire and Lebowa-Venda populations. The Ovambo population also tended to branch off alone with significance values that ranged from 28% (Figure 3.4) to 100% (Figure 3.3). When all populations were considered, the Ovambo showed a closer relationship with the Koekoek population. However, if only the South African populations were considered, there was a closer relationship with the Australorp, but with a very low significance. The Australorp formed a branch closer to the Zimbabwean population (94%) when all the populations were analyzed at 11 loci (Figure 3.3). The populations from Mozambique and Botswana were only included in the analyses of 11 loci (Group I) and 15 loci (Group III) and in both cases they grouped together with a relatively high significance of 95% and 59% respectively.

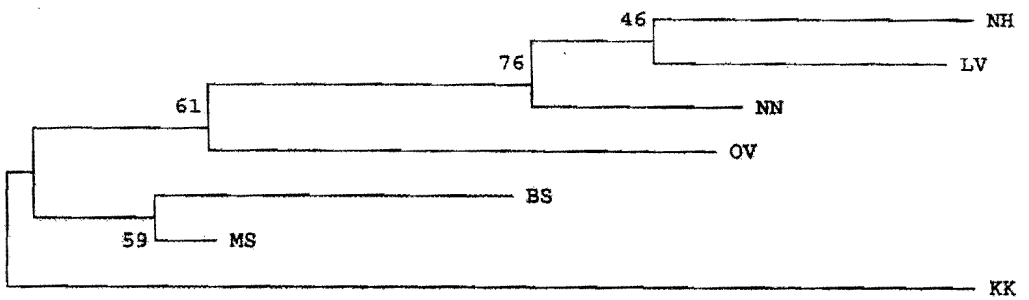

Figure 3.5 Standard neighbour-joining tree for Group III.

The trees obtained using the UPGMA method were similar to the Standard NJ trees. The topography of these trees remained the same, but with higher significance levels. Figure 3.6 shows the UPGMA tree for all the populations (Group1).

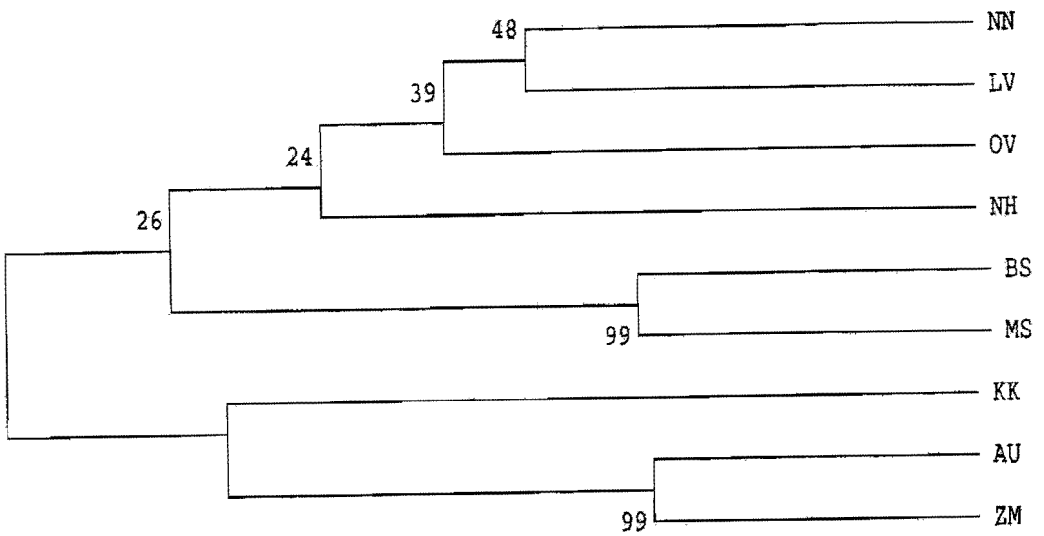

Figure 3.6 UPGMA-tree for Group I.

3.4 Discussion

Microsatellite markers

With the exception of three loci, all microsatellite markers tested were found to be highly polymorphic. In the Koekoek, markers LEI0194 and MCW0014 were monomorph, while MCW0294 was monomorph in the New Hampshire. When the characteristics of these loci were compared with the same loci reported by the Wageningen Animal and Genetics Group (Groenen *et al.*, 1998; Crooijmans, 2000), there was a tendency for a higher number of alleles per locus for the populations in this study. There were fourteen different alleles for MCW0069 in the native populations and only six in the WAU

reference population. In this study, four and seven different alleles were observed for microsatellite markers MCW0098 and MCW0103 and only two alleles in the WAU reference population. It should however be noted that the Wageningen reference population originated from a single cross between Cornish-based breeding lines. More alleles similar to this study have been found when tested in a variety of breeds (Groenen, [personal communication], 2000). Similar variation were also found with 21 microsatellite markers (including the markers applied in this study), when tested in 43 diverse European chicken populations (Schmid *et al.*, 2000).

Except for two markers, the alleles observed were all in the expected range. Microsatellite markers MCW0183 and MCW0248 had one and four more alleles not within the expected range (Table 3.3). Microsatellite markers tested in commercial broiler and layer lines indicated that the average number of alleles per loci may vary between 3.6 to 5.9 for broilers and 2.0-3.1 for layers (Groen *et al.*, 1994; Crooijmans, 1996b). In a summary of microsatellite markers for chicken, the different number of alleles per marker was between one and nine when tested in a limited number of lines (Crooijmans *et al.*, 1996b). In two other studies, which included inbred chicken lines and hybrid and native lines, microsatellite markers were also found to be highly polymorphic, with number of alleles per marker ranging from 4 to13 and 2 to11 respectively (Vanhala *et al.*, 1998; Ponsuksili *et al.*, 1996).

Eight of the twenty-three markers showed a deviation of Hardy –Weinberg equilibrium. Four of the markers tended to deviate consistently in all the populations, except for the Mozambique, Botswana and Australorp populations. Natural selection for a certain genotype may have played a role causing an increase in certain homozygous genotypes in these populations. Reasons for deviations are usually associated with the presence of null alleles, natural selection for a certain genotype that may increase the homozygotes and or the Wahlund effect, where there is a deficiency in the homozygous genotypes (Hartl, 1988). None of the deviations showed a reduction of homozygous genotypes, so the Wahlund effect is unlikely, and null alleles would only become visible in segregating

families, which cannot be concluded from this study. A possible explanation for the four markers, which deviated in a single population only, could be inaccurate genotyping, with poor gel conditions, and some heterozygotes may have been ignored.

PIC and Heterozygosity values

Both the PIC (polymorphic information content) and Heterozygosity (H) were calculated for all the microsatellite markers tested in the nine populations as the PIC values provide information on the polymorphism of the markers and the H on genetic variability within the population. The PIC takes the number and frequency of the alleles into account per marker at a specific locus, while the H indicates the number of heterozygous animals in the population (Botstein *et al.*, 1980; Buchanan *et al.*, 1994).

PIC values found in this study varied from 0.33 to 0.66 for the different microsatellite markers (Table 3.5). These values are in a similar range as PIC values reported for chickens ranging from 0.25 to 0.83 (Ponsuksili *et al.*, 1996). PIC values for all markers per population showed a much smaller variation of 0.46 to 0.57. The same trend was reported for sheep (0.51 – 0.71) and cattle (0.39 – 0.53) (Buchanan *et al.*, 1994). PIC is more valuable for indication of marker polymorphism than for variability in the populations.

Heterozygosity values were the highest for the Naked Neck population (64%) and the lowest for Koekoek (53%), Australorp (54%), Lebowa-Venda (54%) and New Hampshire (54%). The low variability for the Koekkoek and Australorp populations is in agreement with their origin and selection as distinct dualpurpose breeds over the years. The relatively low value for the Lebowa-Venda is unexpected as this is one of the populations considered being "native". A heterozygosity value closer to the Ovambo population may have been expected for the Lebowa-Venda and is it necessary to consider the origin of the Lebowa-Venda population for a possible explanation. These birds were associated with a specific geographical area. They have a distinct colour pattern and communities in this area probably preferred the pattern and selected on phenotype,

maintaining them as a "group". The relatively high variability in the Naked Neck could be attributed to the major gene (*Nana)*, which phenotypically, results in a chicken with a neck without feathers (homozygous) or a little tuft of feathers (heterozygous). Although the Naked Neck population is distinguished on phenotype as a Naked Neck, it may have been crossed with any type of fowl and therefore show a high degree of genetic variation.

Heterozygosity values were also estimated for the three groups (Table 3.6), where each group was analyzed with a different number of markers (Group I –11 loci, Group II –15 loci and Group III –18 loci). The number of loci used in the estimation of variation in the groups did influence the values. Although the ranking of the populations in terms of high and low variation remained the same, the H values decreased (e.g. in the New Hampshire, Lebowa-Venda and Australorp) when more markers were included in the calculations (Table 3.8, 3.9. and 3.10). There was a 6% and 10% difference in H for the Koekoek, Lebowa-Venda and New Hampshire populations respectively when analyzed with 18 markers. The Koekoek population also had lower values when calculations were done with 15 markers versus 18 markers. For all the other populations, differences were relatively small (1-3%). Not only the number of loci, but indeed also the polymorphic nature of the microsatellite marker and the number of animals tested, influence the estimated genetic variability. When comparing the H values based on all twenty two markers tested (Table 3.6) versus the three groups as discussed, H values are in a similar range with the lowest variation in the Koekoek and the highest in the Naked Neck populations.

Reports for H in native fowl are limited. Ponsuksili *et al.* (1996) reported H values for the Dandarawi (33.5%) and Fayomi (35.1%) from Egypt, the Kadaknath (62.9%) from India and the Nunakan (50%) from Indonesia. These are however all laboratory lines and it is not specified if they were subjected to selection. Genetic variability for commercial broiler and layer lines range from 28 to 44% heterozygosity (Groen *et al.*, 1994). Expected H for broiler hybrids were found between 37.8% and 67.1% (Vanhala *et al.*, 1998).

When the fowl populations in this study and other studies are compared to populations used in similar studies for cattle and sheep, a comparable range of genetic variation is evident, if measured by means of heterozygosity. It seems however, that there are fewer distinct alleles per locus in fowl than in cattle and sheep. In the present study, between 3.9 and 6.4 alleles per marker were observed, which is comparable to values by Groen *et al.* (1994), Ponsuksili *et al.* (1996) and Takahashi *et al.* (1998). Two studies with European cattle breeds, reported 79 different alleles among five microsatellite markers (average of 15 alleles per marker) with a maximum of 27 alleles per marker (Arranz *et al.*, 1996b). A study with Taurine and Zebu cattle in Africa found 168 unique alleles for 20 loci, while H varied between 44% and 65% (MacHugh *et al.*, 1997). Eight to seventeen different alleles per locus were observed in six sheep breeds (Buchanan *et al.*, 1994).

Although the average number of alleles per locus in fowl is not that high, there still seem to be a relatively high genetic variability among populations. In the native populations genetic variability is higher than in broiler and layer lines. This is expected as the broilers and layers were subjected to intensive selection, inbreeding and crossbreeding. The native populations tested in this study showed a relatively high genetic variability. These populations were not subjected to selection for specific traits in the past, which is also evident in their poor production performance.

In all except the population from Zimbabwe, seemingly unique alleles (alleles particular to a population) were observed. Seven such alleles were observed for the Naked Neck and New Hampshire populations, while only one in the Lebowa-Venda population. These unique alleles should be further investigated for application in identification of unknown native populations.

The different populations seemed to have diverged from each other, according to the F_{ST} and R_{ST} values calculated. F_{ST} values were calculated across the microsatellite markers

only, and indicated differences with mean values of 0.175 (Group I) and 0.195 (Group II). This is an indication of genetic divergence for fowl, as it takes only the marker information into account within the total population, while the R_{ST} values indicate the differentiation of sub-populations with reference to the total population studied. The differentiation into sub-populations was more distinct, with more microsatellite markers included in the analysis. Larger R_{ST}- values were observed for Group II (18 loci) and Group III (15 loci), than for Group I with only 11 loci.

The most prominent differentiation was found for the Koekoek and Austrolorp populations, which confirm their genetic history as breeds in South Africa. Although divergence is indicated among the other native populations of South Africa (New Hampshire, Naked Neck, Lebowa-Venda and Ovambo), it was not as distinct and could be attributed to the fact that they have not been subjected to formal selection.

Genetic distance and relatedness

Genetic distance is an indication of the degree of relatedness between populations. In this study genetic distances were estimated as summarized in Table 3.7, according to the population and the number of microsatellite markers included in the calculations. The New Hampshire and Naked Neck seem to be the most related among all the populations studied with distances ranging from 0.180 (Group III – 15 loci) to 0.218 (Group II –18 loci) and 0.250 (Group I –11 loci) (Tables 3.10, 3.11 & 3.12). Lebowa-Venda also showed a close relationship with both the New Hampshire and Naked-Neck populations, while it was the most distant from the Koekoek population. The populations from Mozambique and Botswana were included in the analyses for Groups I and III and in both had small distance values (0.093 and 0.116) indicating a close relationship.

Figures 3.3, 3.4 and 3.5 illustrate the relatedness for Groups I, II and III respectively. The NJ-method has been shown to be useful for obtaining correct tree topology in other studies including native fowl, commercial poultry and cattle (Takahashi *et al.*, 1998;

Vanhala *et al.*, 1998; MacHugh *et al.*, 1997). In this study the NJ- and UPGMA-methods were applied.

Despite the different number of loci included, the tree topology for the 3 groups remained the same. The Koekoek population, which was included in all three groups, formed a distinct branch in both the Groups II and III with a high significance. In Group I, the Koekoek tended to show relatedness with the Ovambo populations, but with a low significance. The main branch is however highly significant, distinguishing the Koekoek from the other native populations (Naked Neck, Lebowa-Venda and New Hampshire). This relationship of the Koekoek population compared to the other populations could be confirmed with its history and selection as a breed over the years. The same tree topology was obtained using the NJ- and UPGMA-method, when analyzing the different groups. In Figures 3.3 and 3.6 the relationships among the populations for group 1 are shown for the NJ - and UPGMA tree respectively.

A second prominent cluster in all three groups was found for the Naked Neck, Lebowa-Venda and New Hampshire populations. As previously mentioned, the Naked Neck population has the potential of having relations with any other fowl in the specific area or flock, as their only distinguishing characteristic is the absence of feathers on the neck. As the New Hampshire is also often used as a dualpurpose breed in various poultry farming situations, it could explain the close relation between the Naked Neck and New Hampshire. The clustering of the Lebowa-Venda population with the Naked Neck and New Hampshire is quite unexpected. The Lebowa-Venda does not resemble the other two populations in phenotype and showed a lower genetic variability as expected for a "native" breed. According to the distance measures and tree topology, these birds do share alleles and are closer related than previously thought. Both Naked Neck and New Hampshire birds are commonly found in the rural areas of the Northern Province, which includes the former Lebowa and Venda regions, associated with this population. It is therefore quite possible that genetic links among these populations exist, as no formal selection was practiced and birds are seldom confined to chicken runs or houses.

The seeming relationship between the Zimbabwe and the Australorp populations may be explained by the fact that the Australorp was often included in poultry upgrading programs in African countries and therefore hybridized with native fowls found in rural areas (Safalaoh *et al.*, 1996). The close relationship between the populations of Mozambique and Botswana is also an indication of the distribution of dualpurpose chicken breeds that were used in poultry upgrading programs in which the research institutes usually assist. The number of samples of the Zimbabwe population, as well as the populations from Mozambique and Botswana, were relatively small and represented a population kept at the specific institution. One would prefer to test larger groups of these fowl representing their original distribution in these countries to gain more conclusive evidence on their genetic make-up.

To conclude, the application of microsatellite markers allowed the demonstration of a significant degree of polymorphism and was found useful for genetic characterization of native fowl populations from the "Fowls for Africa" program. It was demonstrated that a high degree of genetic variation still exists among the populations. Unique alleles that might assist in breed identification were also identified. Genetic distances indicate that the New Hampshire, Naked Neck and Lebowa-Venda could be related, while the Koekoek and Ovambo seem to be two unrelated groups.

CHAPTER FOUR

Phenotypic characterisation of native fowl in South Africa

4.1 Introduction

Native fowl are mostly associated with the developing world. This "developing world" is often seen as "a world of problems" ranging from famine, poverty, and over-population to economic and political problems (Alan & Thomas, 1992). In cases of famine or drought the groups of people that suffer most are the rural labourers, farmers and pastoralists (Crow, 1992). These groups are dependant on various farming activities for their livelihood and survival, but also at the same time are most vulnerable to the forces of nature, economics of scale and agricultural policies. Over the past two decades new approaches were introduced in trying to solve some of the problems of these rural farmers in the developing world, including the introduction and evaluation of indigenous stock found in these countries.

Since the nineties more studies have also been recognizing the contribution of indigenous and native poultry stock to household food security (Mukerjee, 1990). In the Philippines and Burma up to 57% of the total poultry population consist of indigenous chickens and are kept mainly in back-yard systems (Aini, 1990). It is estimated that in South Asian countries, 50% of egg production are from native fowls (Banarjee & Sharma, 1998). Indigenous fowl have an economic value in contributing to protein for the household and production of manure that can be used in vegetable gardens. Their feeding and housing requirements are low and therefore the ideal specie for integrated farming systems (Aini, 1990).

Studies on native fowl regarding their production traits are limited. Definitions of production systems also vary between countries, which make comparisons in terms of production difficult. Reports also refer to native, indigenous or village fowl and it is not always clear to what extent these birds have been subjected to selection or cross breeding

and or upgrading. A brief summary of production traits for native fowl reported in literature is provided in the following Table 4.1

Table 4.1 Production traits of native chickens

Type of chicken	Production system	Live weight (kg) & age in weeks	Egg production (n weeks)	Egg weight (g)	Reference
Malayan village fowl	Free range	0.838 kg 20 weeks	60 50 weeks	42.5g	Ramlah, 1996
	Intensive	1.36 kg	133	46g	
Korean native Ogol fowl	-	1.39 kg ♂ 16 weeks 1.09 kg ♀	100 50 weeks	49g	Nahm, 1997
Village fowl Mali	Free Range	.600 kg ♂ 15 weeks .500 kg ♀	35-50 50 weeks	34.4g	Wilson *et al.*, 1987
Malawi local chicken (MLC)	Extensive	-	50-60	42.6g	Safalaoh *et al.*, 1996
Black Australorp cross MLC			100-120	49.7g	
MLC	Intensive	2.1 kg 20 weeks	232	-	
BA x MLC		2.2 kg	248 50 weeks	-	
Thai native chicken	Free range	2.2 kg 18 – 23 weeks	45-90 50 weeks	-	Catalogue of the Native Poultry of South-East Asia 1991
Country chicken of Taiwan	Intensive	2.2 kg 16 weeks	174 53 weeks	57.8g	

Native fowl vary in body size, from relatively small birds to larger birds with "long" legs. Their plumage is very colourful and feathers can also sometimes be found on the legs. Major genes such as the Naked Neck (*Nana*), Frizzle feathers (*Fz*) and Dwarf (*Dw*) are often found in the native fowl populations (Horst, 1991). In general the production of native fowls are poor in comparison to commercial stocks. Poor nutrition, housing and a

lack of breeding principles, as well as cultural attitudes are the major constraints for successful rural poultry production in the South Pacific Region (Ajuyah, 1999). In Africa poultry are kept in semi-intensive systems (backyard system) with flock sizes ranging from 50-200 birds. Output is low due to low input and disease and poor nutrition limits production (Kitalyi, 1999).

Chickens are synonymous with many South African rural and peri-urban households. Primarily rural households keep them for household food consumption. Because they are left to scavenge, mostly native fowl and some dualpurpose breeds are being kept. Since these chickens are seldom recognised as contributing to household income, they are not included in census data and until recently have not been considered in research programs. In Africa as in many parts of the developing world, it is the women and children that take care of the chickens, which do not have the same status as other livestock species like cattle, which are associated with wealth and status (Kitalyi, 1999). Very little is known of their production potential and also of their contribution to food security.

The socio-economic changes in South Africa, since 1994, have emphasized the need to restructure rural agriculture in order to direct attention towards small-scale farming and household food security. The Poultry Supply Unit of the Animal Improvement Institute at the Agricultural Research Council (Irene), established a program, "Fowls for Africa" to conserve and promote native fowl populations found in South Africa. The aim of this chapter is to describe the populations, according to phenotype (production traits), which were genetically evaluated in Chapter Three.

4.2 Materials and Methods

Native fowl populations

The native fowl populations for phenotypic description are representatives of the "Fowls for Africa" program. Birds from the Koekoek, New Hampshire, Lebowa-Venda,

Ovambo and Naked Neck populations were purchased from the Poultry Supply Unit at the ARC at Irene. The origin of these populations was described in chapter two. Commercial hybrid lines namely the Cobb broiler and Amberlink layer lines were included as benchmarks for meat and egg production respectively.

Growth and carcass traits

Trials were carried out at the Hatfield Research Farm of the University of Pretoria, to provide base-line data for growth and egg production, for the different populations. Groups of 160 Koekoek, 160 New Hampshire, 113 Naked Neck, 120 Lebowa-Venda and 105 Ovambo chickens, were tested for growth over a period of 77 days (11 weeks). A commercial broiler line, the Cobb, was included as a benchmark. There were three replicates for Naked Neck, Lebowa-Venda and Ovambo, consisting of between 35 and 40 chickens per replicate, while Koekoek and New Hampshire had four replicates with 40 chickens per replicate. They were kept in individual pens in an environmentally controlled house. Males and females were not separated. They were reared on a commercial broiler starter (22% CP, 12.5 MJ ME/kg DM), from 0 to 14 days, followed by a broiler finisher (18% CP, 13 MJ ME/kg DM) from 15 days to the end of the growth period (respectively 42 days for the Cobb broilers and 77 days for the other five populations). A vaccination programme, recommended for small farming enterprises by the Faculty of Veterinary Science at the University of Pretoria, was followed. Body weight and feed intake per pen were recorded on a weekly basis.

At the end of the growth trial ten birds of each population were randomly selected, weighed and slaughtered. The birds were electrically stunned and killed by manual exsanguination. Feathers, viscera and heads were removed and carcasses stored at -40°C until dissected. Live weight and carcass weight, with and without viscera and heads were recorded. Carcasses were thawed before dissection and the feet and abdominal fat were removed. The weights of the *M. pectoralis* and *M. supracoracoid,* as well as the appendicular and axial skeleton were recorded

The dissected carcasses, which included the muscle, bone, fat, skin and feet were grinded with a mincer to obtain a homogenous sample for subsequent fatty acid analysis (Webb *et al.*, 1994). Methyl esters were prepared. Lipids were extracted in duplicate by means of a modification of the chloroform: methanol (2:1 v/v) method (AOAC, 1975). Methyl esters of the fatty acid component of the neutral triglycerides were prepared according to the NaOH/methanol method (Slover & Lanza, 1979).

Evaluation for egg production

After completion of the growth trial of 77 days, the surplus males were discarded, while the females were raised up to 18 weeks of age. Twenty-four females of each of the native populations were randomly selected for estimation of egg production potential. The Amberlink (commercial layer line) was included as a benchmark. A natural ventilated house, equipped with a battery system was used, where the hens were kept in separate cages in order to record individual egg production. Hens were fed *ad lib.* a commercial layers mash (15% CP, 11.3 MJME/kg DM). Egg production per day per hen, as well as egg weight was recorded over a period of 50 weeks. Initial live weight was recorded when placed in the battery at 20 weeks of age and final weight at the end of the production trial (70 weeks of age). Birds were removed when they started to moult and stopped laying, which coincided with the end of the production period.

Statistical analyses

A General Linear Model (GLM) procedure of SAS (1992) was applied for analyzing the following traits:

Growth:

- Average chicken weight (ACW)
- Average feed intake per chicken (AFIC)
- Feed conversion ratio (FCR)

Carcass:

- Dressed carcass mass (DCM)
- Percentage muscle (% MS)

- Percentage breast muscle (% BMS)
- Percentage fat (% FT)
- Percentage % skin (% SK)
- Percentage bone (% BN)
- Dry matter (DM)
- Ash (A)
- Crude protein (CP)
- Crude fat (CFT)

Egg production:

- Total number of eggs at peak, 40 weeks and 51 weeks (end of production)
- Weight of eggs

4.3 Results

Growth (Live weight gain)

The growth of the native populations, were studied in terms of their weight gain over a period of 11 weeks. Weight at day-old, final weight, total feed intake and feed conversion ratio for the different populations, were summarised in Table 4.2. Significant differences were observed for economic traits namely the ACW ($P<0.0001$), AFIC ($P<0.0005$) and FCR ($P<0.0001$) among the six populations measured at 21, 42, 63 and 77 days (Table 3.4). New Hampshire showed the highest weight gain, with an average final weight of 1.21 kg, followed by Ovambo, which had the best gain of 1.18 kg of the native populations. Lebowa-Venda had the poorest weight gain and differed ($P<0.001$) from the other populations (Figures 1 & 2). Feed efficiency was poor for all populations, with feed conversion ratio, varying between 3.32 for the Koekoek to 4.06 for Naked Neck.

Table 4.2. Initial and final weights, cumulative feed intake and feed conversion for the different fowl populations

	Koekoek	New Hampshire	Naked Neck	Lebowa-Venda	Ovambo	Cobb
No of chickens	160	160	113	120	105	150
Initial weight in (g): day-old	35.4	30.0	35.5	33.5	27.0	0.40
Final weight in (g): 11 weeks	1114	1213	1062	.937	1183	2.00*
Total feed intake (g)	3680	3680	3720	3390	3610	4100
Feed conversion ratio	3.3	3.03	3.5	3.6	3.0	2.0

*Cobb was slaughtered at 6 weeks of age

Carcass composition

Carcass composition was evaluated according to dressed carcass weight, percentage muscle, breast muscle, bone, fat and skin and results are shown in Table 4.4. Cobb (broiler line) differed ($P<0.05$) from the native populations in terms of dressed carcass weight, muscle, breast muscle and fat percentage (Table 4.3). For the native populations Ovambo had the highest DCM (939.8 g), followed by New Hampshire (907 g). The percentage muscle was approximately 55% for all the native populations, except for Ovambo and New Hampshire, which only had a muscle percentage of 51%. Naked Neck had the highest percentage breast muscle (18.03% ± .5) compared to the other native populations.

Chemical analyses indicating dry matter, ash, crude protein and crude fat percentages are presented in Table 4.5. Significant differences ($P<0.05$) in CP and CFT were observed, where the lowest CP was found in Cobb and the highest in the LV. The native populations had significantly lower CFT in comparison with the commercial broiler (Table 4.4). The Lebowa-Venda chickens had the lowest fat percentage (0.42% ± .04), while Ovambo had the highest fat (2.5%) for the native populations.

Table 4.3 Least square means for growth(weight gain) traits for the different fowl populations

Line and number of chickens	Trait*	Day 1	Day 21	Day 42	Day 63	Day 77
Koekoek (160)	ACW	0.035^{a}	0.108^{ac}	0.347^{a}	0.718^{a}	1.114^{a}
	AFIC	-	0.140^{ab}	0.325^{a}	0.494^{ac}	0.435^{ac}
	FCR	-	2.58^{a}	3.708^{a}	3.307^{a}	3.323^{a}
New Hampshire (160)	ACW	0.030^{b}	0.119^{b}	0.391^{a}	0.792^{b}	**1.213^{b}**
	AFIC	-	0.168^{a}	0.288^{b}	0.462^{ab}	0.459^{a}
	FCR	-	2.76^{a}	3.010^{b}	3.091^{b}	3.831^{b}
Naked Neck (113)	ACW	0.035^{a}	0.113^{ab}	0.359^{a}	0.713^{a}	1.062^{a}
	AFIC	-	0.132^{b}	0.268^{b}	0.503^{ac}	0.491^{b}
	FCR	-	2.54^{a}	3.673^{a}	3.937^{c}	4.068^{b}
Lebowa-Venda (120)	ACW	0.033^{c}	0.102^{c}	0.304^{a}	0.618^{c}	0.936^{c}
	AFIC	-	0.173^{a}	0.277^{b}	0.426^{b}	0.422^{c}
	FCR	-	3.60^{b}	3.715^{a}	3.704^{c}	3.407^{a}
Ovambo (105)	ACW	0.027^{d}	0.109^{ac}	0.365^{a}	0.781^{b}	1.183^{b}
	AFIC	-	0.114^{b}	0.284^{b}	0.508^{c}	0.453^{ac}
	FCR	-	2.26^{a}	2.979^{b}	3.462^{a}	3.876^{b}
Cobb**(150)	ACW	0.040^{c}	0.298^{d}	1.386^{b}	-	-
	AFIC	-	0.433^{c}	0.986^{c}	-	-
	FCR	-	1.83^{c}	1.859^{c}	-	-
Standard error	ACW	0.0007	0.003	0.04	0.02	0.02
	AFIC	-	.01	.02	.02	.01
	FCR	-	.1	.2	.09	.1

*ACW : Average chicken mass (kg)

AFIC : Average feed intake per chicken (g)

FCR : Feed conversion ratio (kg feed/kg gain)

**Control : Commercial broilers slaughtered at 6 weeks of age

^{abcde} : Means within a column for the same trait with different superscripts differ (≤0.1)

Table 4.4 Least square means and standard errors for carcass traits for the different fowl populations

Population	Dressed carcass weight (g)	% Muscle	% Breast muscle	% Fat	% Skin	% Bone
Koekoek	$831.6^{abc} \pm 35.9$	$54.9^{ac} \pm 0.81$	$17.0^{ac} \pm 0.5$	$1.23^{ac} \pm 0.47$	$10.6^{a} \pm 0.5$	$33.2^{a} \pm 0.9$
New Hampshire	$907.0^{be} \pm 35.9$	$51.7^{ab} \pm 0.81$	$15.9^{ab} \pm 0.52$	$4.34^{b} \pm 047$	$12.8^{b} \pm 0.52$	$31.1^{a} \pm 0.9$
Naked Neck	$795.6^{cd} \pm 35.9$	$54.9^{c} \pm 0.8$	$18.0^{c} \pm 0.5$	$1.8^{a} \pm 0.4$	$11.7^{ab} \pm 0.5$	$31.5^{a} \pm 0.9$
Lebowa-Venda	$703.9^{d} \pm 35.9$	$53.0^{b} \pm 0.8$	$15.2^{d} \pm 0.5$	$0.4^{c} \pm 0.4$	$10.3^{a} \pm 0.5$	$36.2^{b} \pm 0.9$
Ovambo	$939.8^{e} \pm 37.5$	$51.^{9ab} \pm 0.8$	$15.9^{ab} \pm 0.5$	$2.5^{a} \pm 0.4$	$12.1^{cb} \pm 0.5$	$33.5^{a} \pm 0.9$
Cobb	$1404.7^{f} \pm 37.5$	$55.2^{d} \pm 0.8$	$20.4^{d} \pm 0.8$	$6.5^{d} \pm 0.4$	$14.2^{bd} \pm 0.5$	$24.1^{c} \pm 0.9$

*Dressed carcass weight: head and viscera removed.
Variables with different superscripts differ significantly (P<0.05).

Table 4.5 Least square means and standard errors for chemical analyses of carcasses of the different fowl populations

Population	Dry matter %	Ash %	Crude protein %	Crude fat %
Koekoek	$35.4^{ab} \pm 2.2$	$3.9^{ab} \pm 0.36$	$46.1^{a} \pm 1.2$	$28.5^{a} \pm 1.6$
New Hampshire	$35.9^{ab} \pm 1.93$	$3.78^{a} \pm .32$	$42.2^{bc} \pm 1.2$	$36.9^{bc} \pm 1.6$
Naked Neck	$35.9^{ab} \pm 2.0$	$3.9^{ab} \pm 0.34$	$45.^{2ab} \pm 1.2$	$34.9^{b} \pm 1.6$
Lebowa-Venda	$31.4^{a} \pm 1.9$	$4.7^{b} \pm 0.31$	$49.6^{d} \pm 1.1$	$28.8^{a} \pm 1.6$
Ovambo	$38.5^{b} \pm 2.2$	$2.7^{c} \pm 0.36$	$44.8^{ab} \pm 1.5$	$36.0^{bc} \pm 2.1$
Cobb	$34.4^{ab} \pm 1.7$	$2.4^{c} \pm 0.28$	$39.9^{c} \pm 1.1$	$40.6^{c} \pm 1.5$

Variables with different superscripts differ significantly (P<0.05)

Significant differences were found in the proportions of polyunsaturated fatty acids and particular, palmitoleic acid (16:1), linoleic (18:2) and linolenic acid (18:3). The highest proportion of 16:1 was observed in the Ovambo, New Hampshire and Naked Neck chickens. The proportions of linoleic and linolenic acid were the highest in Ovambo Koekoek and Lebowa-Venda (Table 4.5).

Table 4.6 Differences in fatty acid composition for the different fowl populations

Population	Fatty acids %						
	14:0	16:0	16:1	18:0	18:1	18:3	20:1
Koekoek	1.05	24.58^{abc}	7.92^{a}	8.23	45.28^{ab}	12.18^{bc}	1.54
New Hampshire	0.85	25.83^{bc}	9.85^{c}	7.74	44.27^{ab}	10.12^{ab}	1.92
Naked Neck	1.15	25.10^{bc}	8.19^{ab}	7.82	42.74^{a}	12.87^{c}	2.34
Lebowa-Venda	1.29	22.17^{a}	7.98^{a}	6.99	45.06^{ab}	14.44^{c}	2.05
Ovambo	0.92	23.71^{ab}	9.23^{bc}	6.07	46.68^{b}	12.72^{c}	1.33
Cobb	0.92	26.62^{c}	8.78^{abc}	8.37	43.11^{a}	9.58^{a}	2.42
P-value	<. 68	<0.023	<0.01	<0.318	<0.048	<0.01	<0.6
F-value	.63	.3.992	.4.80	1.32	3.15	4.88	0.76

Variables with different superscripts differ significantly (see P-value in table)

Egg production

Egg production was measured as the average number of eggs laid and total weight of eggs produced during production of 51 weeks. The average number of eggs laid per week, varied from as low as 2.1 for the Ovambo hens to as high as 4 and 6.1 for the Koekoek and Amberlink hens. Among the native populations, Koekoek had the best egg production of 204 eggs, while Ovambo produced only 91 eggs. The commercial hen line (Amberlink) had, as expected, the highest egg production of 311 eggs. Egg production was found to differ significantly ($P<0.001$) among the different populations (Table 4.6). Koekoek and Lebowa-Venda laid the heaviest eggs, excluding Amberlink, with an average weight of 52.14 g and 50.92 g respectively during the production. The Ovambo hens on average laid the smallest eggs weighing only 43.8 g (Table 4.6).

Table 4.7 Egg production traits for the different fowl populations

Line	Total eggs /hen	Minimum weight (g)	Maximum weight (g)	Mean egg weight (g); SE	Average hen weight hens (g)	#Productivity ratio
Koekoek	204	10.4	70.6	52.1 ± .09	2100	6.1
New Hampshire	189	13.2	69.9	48.0 ± .102	1997	5.4
Naked Neck	139	13.7	69.0	49.8 ± .120	1650	4.7
Lebowa-Venda	122	26.0	79.6	50.9 ± .120	1900	3.8
*Ovambo	91	20.6	66.1	43.8 ± .18	1900	2.5
Amberlink	311	19.7	82.5	58.7 ± .068	2250	9.9

*Based on 43 weeks of production.
Calculated as number of eggs x mean weight/average hen weight [75]

Koekoek and Lebowa-Venda produced light brown eggs and New Hampshire produced brown eggs. The egg colour of Naked Neck and Ovambo was a creamy (off-white) to light brown colour and varied among the birds.

4.4 Discussion

The evaluation of production traits of the native populations from the "Fowls for Africa" program was performed under commercial conditions. It is important to emphasize that the aim was not a comparison with commercial lines, but rather to asses these native

populations, under standardized conditions to obtain a guideline of their "optimum" potential for a phenotypic description. It was assumed that a commercial production system, in a controlled environment, a balanced ration and disease control might serve as an equal environment to test for differences in growth, carcass and egg production traits among these populations, as it is very difficult to obtain accurate field data from low-input systems. The discussion will therefore emphasise differences among the native populations of the "Fowls for Africa" project and be compared with other indigenous fowl found in the developing countries of the world, which are kept for household food production.

Growth (weight gain)

Growth parameters, measured in terms of weight gain, feed intake and feed efficiency, as well as egg production (number of eggs laid and egg weight), showed significant differences among the native populations. As expected, the performance of all the native populations was very poor when compared to the commercial broiler and layer lines included as benchmarks in the study.

Results obtained in this study showed that the New Hampshire chickens had the highest average weight during the growth trial. However due to the origin of the New Hampshire, it cannot be regarded as "native", although often kept by rural households. When only comparing the native populations, the Ovambo chickens had the best growth performance with a final weight of 1.18 kg, followed by Koekoek (1.1 kg) and Naked Neck (1.06 kg), while Lebowa-Venda had a significantly lower final weight of only 0.936 kg. These results are comparable with weights reported by Honeyborne & Joubert (1998) for these fowls (Ovambo: 1.6 kg, Koekoek: 1.24 kg, Naked Neck: 1.16 kg) and Lebowa-Venda: 0.987 kg), at 12 weeks of age. Average weights for the South African native populations also compare well with weights at 16 weeks of age, reported for the Korean native fowl, a dualpurpose breed with an average mature weight of 1.390 kg for males and 1.090 kg for females (Nahm, 1997). Reports also indicate that the Malawi local chicken reached a body weight of 0.615 kg at 8 weeks of age and 2.1 kg at 20 weeks of age (Safalaoh *et al.*, 1996), which is comparable with the South African populations.

Weights at 10 weeks of age for Malaysian village fowl tested under intensive systems (mixed sexes), varied between 0.670 kg and 0.753 kg with final weights of only 1.1 kg at 15 weeks of age (Ramlah, 1996).

The total feed intake was very similar for all the populations (3.61 – 3.72 kg), except for the Lebowa-Venda chickens, which had a lower intake of 3.39 kg. Feed efficiency ratios varied from 3.0 to 3.6 for the Ovambo and the Lebowa-Venda populations respectively (Table 4.2). Feed efficiency ratios reported for the Malawi local chicken and the cross with the Black Australorp were 6.9 and 6.5, which are much poorer than for the South African fowl. A fair comparison is not always possible, as test and feeding conditions are not always clearly indicated by authors.

The native fowl seem to differ distinctly in feeding behaviour from the Cobb broiler. The native chickens had difficulty feeding from the feeding troughs during the first week and feed had to be provided on flat cardboard. The native birds also spent much more time feeding around the feeding troughs as well as scavenging in the pen. This behaviour might have had an influence on their performance. These results were obtained from an intensive system with adequate nutrition, housing and disease control. A poorer performance could be expected if they were left to scavenge, although the type of feed while scavenging in combination with their genetic potential to utilise poor feed should also be considered.

Carcass composition

Carcass traits summarized in Table 4.3, indicated that Ovambo had the highest dressed carcass mass of 0.939 g. Chambers *et al.* (1981) compared the modern type broiler with chickens representing broiler lines of 1958 and 1972. The dressed carcass weight reported for males at 47 days of age was 717 g (1958), 967 g (1972) and 1088 g for the modern broiler. The weights found for the native populations in this study compared with the weights for the 1958 and 1972 broilers. Literature reports on carcass characteristics of native populations are limited. Safalaoh *et al.* (1996) reported a breast muscle percentage of 14.81% for the Malawi local chicken, which is lower than the

native populations studied, where percentage breast muscle varied between 18% for the Naked Neck and 15.2% for Lebowa-Venda. This is lower than the percentages of breast muscle reported by Chambers *et al.* (1981) for the broilers of 1958 (25.2%) males and 1972 (23.9%), respectively. Leeson & Summers (1980) reported a percentage of breast muscle of 31.3% for modern broilers slaughtered at 35 days of age. Wall & Anthony (1995) reported no significant differences in total breast weight (bone included) between Giant Jungle Fowl and broilers, but if deboned, the Jungle Fowl had less breast muscle.

The different native populations tended to have a higher percentage of bone (31.5% - 36.2%) than the control line (24.1%) (Table 4.3). The higher percentage of bone in the native fowl populations may be associated with their adaptation to flight and scavenging.

The chemical analyses indicated that there are significant differences between the Cobb and native fowls, for crude protein and crude fat (Table 4.4). A higher crude protein and lower crude fat were observed for the native populations, while the reverse was found for the Cobb broilers. The lower body fat in the native fowl can be associated with their slower growth and slower rate of maturing, while the commercial broiler was selected for fast growth resulting in reaching maturity at a younger age. If only the native populations were taken into consideration (New Hampshire excluded), the Ovambo hens, showed the fastest growth among the native fowls with a relatively high crude fat content in the body. The Ovambo also reached sexual maturity (first egg laid) at least 6 weeks before the Koekoek, Naked Neck and Lebowa-Venda hens. The higher body fat and faster growth of the Ovambo populations indicate an early maturing type. The Lebowa-Venda hens exhibited slower growth, had the lowest percentage of crude fat and were also slow to mature.

The proportions of fatty acids such as linoleic and linolenic acid, are influenced by the diet, as well as the feeding of dietary fat (Hrdinka *et al.*, 1996). Differences (see Table 4) were observed among the populations. Although, they all received the same diet, differences found are probably due to eating behaviour. The Ovambo, New Hampshire

and Naked Neck birds tend to scratch more, while eating and could pick up particles more selectively than the broilers that are more "passive" eaters.

Although no differences were observed for stearic acid (18:0) and arachidonic acid (20:0), the percentage of palmitic acid (16:0) differed between the chicken populations (Table 4.5). Breed influences on the fatty acid composition of Dorper and SA Mutton Merino wethers have been reported that indicate to possible genetic differences Webb & Casey (1995). The native fowl populations in this study, were not subjected to selection other than to survive, therefore differences could be due to a genetic adaptation in metabolism, but further study will be required for confirmation.

Egg production

In the evaluation of egg production, differences were also observed among the populations. The commercial layers (Amberlink) commenced production at 20 weeks of age as normally expected from a commercial line, while the Ovambo hens already produced their first eggs at 16 weeks of age. The other native populations and New Hampshire only started laying between 17 to 22 weeks of age. An average age at first egg or age of sexual maturity of 24 weeks was reported for Lebowa-Venda and Naked Neck hens, which is comparable to the results found in this study (Joubert, 1996). Available literature indicated that native fowls were found to reach sexual maturity between 23 weeks (Nigerian local chicken) and 24 weeks of age (Korean native fowl) (Horst, 1997; Nahm, 1997).

The Ovambo hens not only reached sexual maturity at 16 weeks of age, they also reached peak production 8 weeks after being placed in the battery system, which was at least three weeks before the other hens. The Koekoek population for example reached peak egg production at 12 weeks. Although the Ovambo hens started production ahead of the other hens, egg production had decreased to zero by 43 weeks. The initial weights of the hens varied from 1.60 kg (Naked Neck, Lebowa-Venda) to 1.84 kg (Ovambo, Koekoek), when placed in the battery, while final weights varied from 1.78 (Naked Neck) to 2.34 (Koekoek). Although the Lebowa-Venda hens exhibited poor growth up to 11 weeks, the

hens eventually compensated and ended being large and robust birds with an average weight of 2.17 kg.

Reports by Horst (1991) on the number off eggs produced by native fowl in the tropics, varied depending on the production systems applied. He found that fowls produced between 18-100 eggs under extensive conditions, while some birds produced between 25-150 eggs under intensive systems. Malawi local chickens were found to produce between 50-95 eggs per hen per year, depending on the feeding regime followed (Safalaoh *et al.*, 1996). In this study the Koekoek, Naked Neck and Lebowa-Venda hens produced 203, 151 and 122 eggs respectively, which is similar to egg production reported for natives fowl from Egypt, the Fayoumi (141) and Dandarawi (128). Except for the Ovambo hens, the other South African hens had a higher egg production than reported in literature. This could be ascribed to optimal feeding and housing conditions.

A relatively high variation was found in the weight of the eggs for the fowls in this study. The Koekoek (52.1 g) and Lebowa-Venda (50.9 g) hens had the highest average weights, while the lowest egg weight was found for Ovambo (43.8 g) (Table 4.6). Nahm (1997) reported egg weights of 49 g at 44 weeks of age for the Korean native fowl. Egg weights for the Malaysian fowls varied between 39.7 g and 46 g (Ramlah, 1996). Egg weights reported for different local chicken populations in the tropics were as low as 38 g and as high as 50 g (Horst, 1991). The fowl tested in this study were found to be quite similar to other native fowls for egg weight.

The growth potential of all the native populations were very poor and it will not be economic to keep and feed males for meat production. The populations in this study seem to be more suited for egg production, even when the relatively low production figures are taken into consideration. When total egg production and egg weights (Table 5) are taken into account, these populations have a potential of producing between 4.2 kg (Ovambo) and 10.6 kg (Koekoek) of edible protein for the household. The culled hens may also eventually be sold or slaughtered for meat. The fact that these chickens were tested under commercial conditions, especially in terms of nutrition, it can be assumed

that these results are an indication of their best performance. Under scavenger or free-range conditions, with less food and poor disease control, poorer performance would be expected.

An interesting, but important feeding behaviour pattern was observed for the young chickens. The native day-old chickens were slow to start feeding, while Cobb and New Hampshire naturally fed vigorously from day one. The Koekoek, Naked Neck, Lebowa-Venda and Ovambo chickens huddled together and searched out the darkest corner of the pens, despite of ultra-violet light provided for warmth. The first three days the starter mash had to be placed onto cardboard, as they would not feed from the trays, but scavenged around them. Behavioural studies have shown that unselected village chickens had better scavenging abilities, when compared to crossbred chickens. Crossbred chickens tended to restrict their scavenging area close to the household (Gunaratne, 1999).

The Ovambo hens did not adapt well to the battery system. They showed signs of stress when feeding and cleaning took place. The Ovambo hens also became broody, which influenced their production negatively. On the other hand broodiness may be regarded as a positive trait when evaluated under extensive systems and considered economical to increase the flock for household production.

The behavioural patterns of the native fowl seem to be adapted to extensive free ranging conditions and an evaluation under such conditions could provide valuable information for selection purposes.

Households in some countries prefer native chickens. For example, in Taiwan, where traditional cooking methods require a well-muscled carcass that will not separate from the bone in the cooking process, these birds are favoured above the commercial broilers (Catalogue of the Native Poultry of South-East Asia, 1991). It is also believed that the African populations in general also prefer free-range village chickens, because of a firmer carcass when cooked and tastier meat. Further study on the acceptability and taste of the

meat would of course be required to test and confirm this belief. It could however provide valuable information for application of these fowl in small farming systems for niche marketing and household food consumption.

Despite the expansion of the commercial poultry industry in the developing world, the native chicken, which is kept under scavenging, free range conditions in rural and peri-urban areas, is still believed to play an important role in household food security (Scherf, 1995). It is therefore important to note that the First Electronic Conference on Family Poultry (7 December 1998 – 5 March 1999) indicated a growing interest in improvement of the nutrition, selection, breeding and disease control of the village chicken.

Although most authors recognize the importance of the conservation of the native chicken as a genetic resource, there is a definite need to select for native or village chicken with improved growth and egg production, adapted to extensive systems (Horst 1997; Ramlah 1996; Banarjee & Sharma, 1998). The ability to adapt and thrive under adverse conditions may in some cases be of greater economic importance than higher performance that requires high economic inputs for environmentally controlled houses.

In Malaysia, under tropical conditions, commercial lines with combinations of major genes such as the Naked Neck gene, Frizzle feather gene and Dwarf gene were tested and found that they had a higher egg production than the commercial types (Horst, 1991). Similar results were found for an evaluation of the production performance of genotypes that included Naked Neck, Frizzle and Dwarf genes in Mozambique. Significantly higher egg production was found for genotypes with both Naked Neck and Frizzle genes in comparison to normal feathered birds (Garcês *et al.*, 2001). Upgrading, using improved male stock and the introduction of major genes in breeding programs could be considered to improve production, provided that the natural abilities to scavenge and adapt to adverse climatic conditions are considered in the selection process.

Genetic characterization, as described and discussed in Chapter Three, revealed a relatively high genetic variation among the native populations. This phenomenon is often

associated with populations not subjected to formal selection. Genetic distances indicated that there are distinct differences among the populations. Significant differences among the populations were also found for the for production traits. When comparing genetic and phenotypic results, the Koekoek population (lowest genetic variation in this study) had the highest egg production and differed significantly from the other populations. The Koekoek population was found also to be unrelated to the other populations (Figures 3.3 – 3.5). The Ovambo population, which had the highest weight gain also showed less association with the other native birds, the New Hampshire, Naked Neck and Lebowa-Venda populations. Although the Lebowa-Venda population share genes, according to phylogenetic analyses with the Naked Neck and New Hampshire, it tended to be phenotypically more different. It is probably due to no selection for production over the years. The Naked Neck population exhibited the higher genetic variation and a close relationship with the New Hampshire fowls. These two populations also tended to be more similar for phenotypic traits (weight gain).

Both the genetic and phenotypic results indicate that the populations in this study could be distinguished as different populations, which is important for conservation of these groups, as well as selection for application in household food production.

CHAPTER FIVE

Critical review and recommendations

In general the Red Jungle Fowl (*Gallus Gallus*) is accepted as the common ancestor of the domesticated chicken. The original purpose of domestication was for cultural and religious purposes. From the centres of domestication (present Pakistan, Turkistan and Iran), fowls were distributed to most parts of the world, where they were incorporated into different cultures and environments, which influenced the development of specific regional types. It was only during the 20th Century that interest in fowls was directed towards commercial production of eggs and meat. A large variety of breeds existed at that stage as a result of the "hen craze of the 19th Century", and these breeds formed the basis for selection of improved lines for either meat or egg production. There can be no doubt on the success of selection for improved production of broilers and layers; the broiler of 1950 took 77 days to reach slaughter weight versus the modern broiler of the 21st Century, that is ready for slaughter at 43 days with an average weight of 2 kg and a feed conversion ratio of less than 2:1. Over the years the breeding and selection of commercial poultry have become concentrated in a few major companies in the northern hemisphere. It is inevitable that the intensive selection, including inbreeding and crossbreeding, may lead to a decrease in genetic variation within poultry breeds and the extinction of the breed itself.

Since the late eighties concerns have been raised by the scientific community regarding the conservation of genetic resources. This led to the establishment of a Global Management Program of Farm Animal Genetic Resources by the FAO during 1992, which includes the Domestic Animal Diversity Information System (DAD-IS) for describing all farm animals in existence. Information on South African farm animals is also submitted to DAD-IS, including the native or local breeds recognized by the Rare Breeds International (RBI) and Farm Animal Conservation Trust (FACT).

In South Africa, as in many other countries, commercial production of livestock has been of main interest and native or indigenous breeds of fowl, sheep and goats were often disregarded. Production performance was much lower than the highly selected commercial breeds and advancements in technology and research favoured commercialisation of livestock, including poultry. The demand for food by a growing population, resulted in focussing on intensive production and no real need for promotion of breeds and/or low-input systems.

In South Africa the changes in the political and socio-economic scene over the past years, also resulted in changes in agricultural policies. New policies now also recognize the need for small-scale farming and low-input systems, therefore it is required of animal scientists to investigate indigenous or native breeds that may contribute to improvement of rural food production. A program "Fowls for Africa" was initiated by the Poultry Supply Unit of the Animal Improvement Institute of the ARC at Irene during 1994, with the aim to conserve the population as a genetic resource, but also for promotion of the fowls for household food production. As no research has been done on these populations, it was decided to evaluate the native fowl populations of the "Fowls for Africa" with particular reference to their genetic and phenotypic production traits.

The native fowl populations were characterized genetically using microsatellite markers as described in Chapter Three. Microsatellite markers were decided upon for this genetic analysis, as numerous microsatellites have been mapped on the chicken genome. Microsatellites have been used successfully in biodiversity studies in various farm animals and other species. The techniques for microsatellite analyses are well described and these markers have a high reproducibility. The genetic variability measured as average heterozygosity over all the markers were found to be relatively high ranging from 54% to 64%. Although this high variability could be expected from populations that have not been subjected to formal selection, a low heterozygosity is sometimes found in very small non-selected populations due to inbreeding and drift (although not likely in these populations). The Koekoek population, which has been recognized as a breed for some years, exhibited the lowest heterozygosity in this study among the "Fowl for

Africa" populations. According to phylogenetic analyses, the populations are distinguished by three significant major groupings, grouping the Naked Neck, New Hampshire and Lebowa-Venda populations together, and Ovambo and Koekoek populations on their own. The genetic variability and relatedness supports the phenotypic results for the production traits and the native populations studied could be described as different populations for the Koekoek and Ovambo populations. The genetic relatedness among the New Hampshire, Naked Neck and Lebowa-Venda was less defined. The Naked Neck and Lebowa-Venda populations seemed to be genetically more similar, while phenotypic differences for egg production and weight gain were found to be significant.

The study was the first attempt to characterize the native fowl populations of the "Fowls for Africa" in terms of genotype and phenotype. The information obtained from this study must however now be put into perspective of conservation (preservation) and/or utilization of these fowl populations. Animal scientists differ in their approaches: The concern of the conservationist (preservationist) is to conserve the breed, identify and prevent the erosion of the population as a genetic resource, while the utilizationist has interest only in the genetic usefulness of the population (Mason & Crawford, 1993). Very often research on conservation and improvement of native stock is a controversial matter with many arguments for and against the economics for such an endeavour. Although the two approaches of conservation and utilization are rather conflicting matters, it could be argued that both approaches should be followed for the native fowl in South Africa, as we not only have a responsibility for keeping the native fowls as a genetic resource for the future, but also for immediate, practical application for household food security.

There are various reasons for conserving genetic variation in native farm animal populations (Gandini & Oldenbroek, 1999), which also apply to native fowl populations. Firstly, from the viewpoint of the conservationist, the native fowl populations should be conserved as a genetic resource against future disasters; commercial chicken stocks are always in danger of severe potential erosion by infectious diseases. Secondly, native

fowls could be a source of unique alleles and contribute in the search for genes associated with health and quality traits. The high genetic variation in these populations may assist in detection of markers. The third reason for conservation is in the interest of both the conservationist and utilizationist, in preserving native fowl resources for their adaptation to harsh environments and higher survival rate under low-input systems. Fourthly, native fowls should be conserved for their socio-economic value and role in household food security. Native fowls may even contribute to organic food production and find their way into niche markets.

In addition to the reasons described above for conservation, native fowls in South Africa also have a cultural role and one would find native fowl in most rural households. Despite the development of a successful commercial poultry industry, a place has been reserved for the "chicken" in the rural household for cultural, social and food purposes. Although it is difficult to quantify, there will always be a need for fowl in many rural households, and native stocks can contribute to household security if conserved as a genetic resource, but also be applied productively in low-input systems.

Regarding conservation of the native fowls of "Fowls for Africa" it is recommended that breeding populations are kept as individual populations or breeds, especially for the Koekoek, Ovambo and Lebowa-Venda populations. The Naked Neck population should also form an individual population for utilization of the Naked Neck gene. It will be important to keep the minimum breeding animals as required by the FAO for conservation populations. To ensure that the genetic basis of the populations are not compromised over time, DNA should be stored and genetic analyses performed on a regular basis. It might be required to collect more native fowls from rural areas, not included when establishing the original population, to ensure the conservation of biodiversity in the native fowl populations of South Africa.

In addition to conservation of biodiversity, unique genetic characteristics, for example single genes (Naked Neck, Frizzle, and dwarf-gene) should be recorded for the

populations and any other distinct genetic traits that may distinguish the populations from each other.

The phenotypic information obtained from this study was from trials conducted under controlled conditions. The behavioural patterns and poor adaptation to the commercial housing facilities indicated that it is probably not the ideal approach for studying these native birds. They still exhibit characteristics such as broodiness, and the need to roost and scavenge. The more appropriate approach for assessing their production potential would be under low-input systems, because this is where these birds are mostly found and expected to survive and produce.

It is clear from the phenotypic performance traits, that production is poor in comparison to commercial stocks. For wider application of "Fowls for Africa" one could consider the selection of an "improved" line for a low-input system. This project should of course be parallel to the conservation population, which implicates two groups of fowl for each population, one for conservation and the other for selection for improved production. A dualpurpose fowl for meat and egg production would be ideal. It is however important that inherent adaptive traits of these birds should not be compromised when selecting for improved production. In this study the Koekoek population presented itself as a reasonable egg producer, but it was under ideal conditions. Under low-input systems the native breeds (Naked Neck, Ovambo, and Lebowa-Venda) might exhibit a comparatively better performance. The ability to scavenge, broodiness, as well as adaptation to high temperatures, should be considered before selecting a line and/or crossbreeding of the populations for an improved "native fowl". Although Naked Neck genotypes have been shown to be better adapted to high temperatures (Horst, 1991), they are not always acceptable to all ethnic groups. Phenotypic traits such as colour and feathers that might influence the acceptability to the consumer or cultural rites or beliefs, should also be considered in the selection process.

The conservation of biodiversity unfortunately involves relatively high input costs, with very limited economic outputs for the present. The "Fowls for Africa" has the potential

with promotion and distribution of native fowls or as improved "native fowl" to contribute to the maintenance of the project. It will however, to a large extent, always be the responsibility of government institutions to support research centre to maintain native fowl conservation populations.

The native fowl has been neglected to such an extent throughout the world that it now requires conservation more than any other domesticated specie (Crawford, 1990). The laws of nature will always dictate the necessity for genetic variation. It would be short-sighted to ignore the need for conservation of genetic variation for the future. In the quest for selection for the "best" and "maximum production" only, one could easily lose on what we have set out to achieve.

As early as 1966, Lerner and Donald stressed the need for conservation as follows (Crawford, 1990):
"It may soon become one of the implied responsibilities of any organization or institution, which control the genetic destiny of a whole species to maintain a reserve of variation for further improvements and for unforeseen shifts in the environment or in demand. Indeed, it may be said, that each generation has an obligation to see that genetic variation, like soil fertility, is not handed on to it's successors in an exhausted state".

Native fowl has survived throughout southern Africa and should be conserved and promoted in this context. In the developing world, interests and norms may differ and we should not fail to appreciate the role of native fowl in South Africa in their contribution to the livelihood of our rural communities.

Addendum A 1

Allele frequencies estimated for all populations tested

Allele frequencies in 9 populations

Locus	Population								
	KK	NH	NN	LV	OV	AU	BS	MS	ZM
adl 268									
N	17	13	14	34	36	18	13	14	14
A	.059	.000	.000	.000	.000	.000	.000	.000	.000
B	.000	.154	.000	.044	.069	.000	.000	.036	.000
C	.000	.000	.250	.441	.167	.194	.000	.000	.679
D	.000	.308	.321	.250	.111	.111	.308	.571	.000
E	.000	.154	.107	.221	.319	.278	.192	.000	.071
F	.647	.346	.286	.044	.292	.222	.346	.250	.000
G	.235	.038	.036	.000	.042	.000	.154	.107	.000
H	.059	.000	.000	.000	.000	.083	.000	.000	.000
I	.000	.000	.000	.000	.000	.000	.000	.036	.000
J	.000	.000	.000	.000	.000	.111	.000	.000	.250
MCW 67									
N	37	36	31	39	38	22	12	16	18
A	.000	.000	.048	.000	.000	.000	.000	.000	.000
B	.000	.028	.048	.000	.000	.000	.000	.000	.000
C	.000	.000	.000	.000	.013	.000	.000	.000	.000
D	.000	.000	.016	.000	.000	.000	.000	.000	.000
E	.419	.042	.242	.141	.474	.227	.000	.000	.306
F	.500	.125	.403	.218	.289	.318	.292	.500	.167
G	.027	.069	.161	.128	.105	.000	.292	.250	.000
H	.054	.500	.016	.359	.053	.432	.000	.000	.500
I	.000	.236	.032	.128	.026	.000	.417	.250	.000
J	.000	.000	.032	.026	.013	.023	.000	.000	.028
K	.000	.000	.000	.000	.026	.000	.000	.000	.000
MCW 69									
N	50	47	44	34	27	25	13	16	20
A	.000	.021	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.011	.000	.000	.000	.000	.000	.000
C	.000	.000	.011	.000	.000	.000	.000	.000	.000
D	.130	.011	.409	.456	.426	.340	.000	.000	.375
E	.060	.085	.148	.353	.222	.000	.308	.250	.000
F	.020	.298	.125	.074	.000	.000	.000	.375	.075
G	.790	.363	.273	.059	.093	.560	.462	.219	.325
H	.000	.000	.011	.000	.259	.020	.038	.125	.000
I	.000	.000	.000	.000	.000	.020	.000	.000	.075
J	.000	.000	.000	.000	.000	.000	.077	.031	.000
K	.000	.000	.000	.000	.000	.000	.038	.000	.025
L	.000	.213	.011	.000	.000	.060	.000	.000	.125
M	.000	.011	.000	.029	.000	.000	.000	.000	.000
N	.000	.000	.000	.029	.000	.000	.077	.000	.000

Locus	Population								
	KK	NH	NN	LV	OV	AU	BS	MS	ZM
MCW 98									
N	46	22	41	27	23	3	13	16	14
A	.022	.000	.000	.000	.000	.333	.000	.000	.071
B	.272	.909	.488	.907	.435	.333	.615	.469	.679
C	.707	.068	.500	.056	.565	.333	.385	.531	.214
D	.000	.023	.012	.037	.000	.000	.000	.000	.036
MCW 103									
N	36	36	37	18	30	27	13	16	17
A	.000	.028	.000	.000	.000	.000	.000	.000	.000
B	.000	.000	.000	.000	.033	.000	.000	.000	.000
C	.000	.403	.514	.028	.017	.074	.000	.031	.147
D	.000	.000	.027	.000	.000	.000	.000	.000	.029
E	.556	.472	.297	.278	.567	.926	.115	.500	.824
F	.444	.097	.162	.639	.367	.000	.885	.469	.000
G	.000	.000	.000	.056	.017	.000	.000	.000	.000
MCW 111									
N	47	46	42	18	31	25	11	14	17
A	.032	.000	.000	.000	.000	.000	.000	.000	.000
B	.021	.000	.000	.000	.000	.000	.000	.000	.000
C	.000	.043	.000	.000	.000	.000	.000	.000	.000
D	.234	.011	.274	.028	.016	.180	.000	.000	.441
E	.330	.217	.238	.389	.597	.120	.182	.071	.500
F	.287	.489	.345	.361	.323	.700	.364	.607	.059
G	.064	.120	.060	.194	.065	.000	.227	.107	.000
H	.000	.000	.012	.000	.000	.000	.000	.036	.000
I	.011	.000	.000	.000	.000	.000	.091	.000	.000
J	.000	.033	.000	.000	.000	.000	.000	.000	.000
K	.000	.000	.000	.000	.000	.000	.136	.000	.000
L	.021	.087	.071	.000	.000	.000	.000	.000	.000
M	.000	.000	.000	.028	.000	.000	.000	.000	.000
MCW 183									
N	35	21	41	31	38	22	11	15	17
A	.000	.000	.000	.000	.013	.000	.000	.000	.029
B	.300	.095	.110	.113	.053	.477	.455	.300	.412
C	.000	.024	.012	.000	.000	.045	.091	.067	.118
D	.000	.000	.012	.000	.013	.000	.000	.000	.000
E	.000	.667	.646	.806	.092	.159	.091	.333	.176
F	.057	.024	.024	.032	.500	.159	.045	.167	.088
G	.000	.071	.012	.000	.013	.000	.000	.000	.000
H	.643	.119	.171	.049	.276	.091	.227	.133	.118
I	.000	.000	.012	.000	.000	.000	.000	.000	.000
J	.000	.000	.000	.000	.039	.068	.000	.000	.059
K	.000	.000	.000	.000	.000	.000	.045	.000	.000
L	.000	.000	.000	.000	.000	.000	.045	.000	.000

Locus	Population								
	KK	NH	NN	LV	OV	AU	BS	MS	ZM
MCW 216									
N	47	46	40	38	28	26	10	9	11
A	.021	.000	.000	.000	.000	.000	.050	.000	.000
B	.000	.174	.450	.329	.018	.000	.250	.000	.091
C	.340	.522	.363	.526	.107	.423	.200	.333	.545
D	.596	.304	.162	.145	.875	.538	.500	.556	.364
E	.021	.000	.000	.000	.000	.019	.000	.000	.000
F	.000	.000	.000	.000	.000	.000	.000	.056	.000
G	.000	.000	.000	.000	.000	.019	.000	.000	.000
H	.000	.000	.025	.000	.000	.000	.000	.000	.000
I	.021	.000	.000	.000	.000	.000	.000	.056	.000
MCW 248									
N	37	46	39	33	23	19	11	14	21
A	.554	.261	.372	.333	.326	.816	.000	.000	.881
B	.446	.239	.128	.470	.413	.184	.727	.714	.095
C	.000	.033	.244	.167	.196	.000	.000	.143	.000
D	.000	.054	.141	.030	.022	.000	.227	.107	.024
E	.000	.022	.013	.000	.043	.000	.045	.036	.000
F	.000	.054	.000	.000	.000	.000	.000	.000	.000
G	.000	.259	.064	.000	.000	.000	.000	.000	.000
H	.000	.022	.000	.000	.000	.000	.000	.000	.000
I	.000	.054	.038	.000	.000	.000	.000	.000	.000
J	.000	.011	.000	.000	.000	.000	.000	.000	.000
MCW 295									
N	41	13	28	35	33	18	13	14	9
A	.000	.000	.018	.000	.000	.000	.000	.000	.000
B	.354	.000	.125	.029	.212	.361	.000	.000	.611
C	.220	.000	.143	.386	.061	.028	.038	.000	.056
D	.232	.308	.036	.000	.061	.000	.385	.571	.000
E	.085	.423	.036	.086	.015	.000	.115	.071	.000
F	.110	.000	.304	.014	.106	.500	.000	.000	.278
G	.000	.000	.018	.071	.000	.111	.000	.000	.000
H	.000	.231	.089	.229	.242	.000	.308	.250	.056
I	.000	.038	.143	.171	.061	.000	.077	.071	.000
J	.000	.000	.089	.014	.000	.000	.000	.000	.000
K	.000	.000	.000	.000	.000	.000	.077	.036	.000
MCW 330									
N	31	31	36	31	30	11	12	16	18
A	.000	.000	.139	.000	.050	.045	.000	.000	.139
B	.000	.016	.000	.000	.017	.000	.125	.125	.000
C	.081	.000	.000	.097	.250	.318	.000	.000	.528
D	.016	.016	.028	.048	.150	.091	.292	.188	.000
E	.000	.000	.000	.000	.000	.045	.000	.000	.028
F	.323	.516	.500	.355	.200	.318	.000	.000	.139
G	.387	.306	.292	.177	.133	.000	.208	.406	.000
H	.000	.000	.014	.000	.000	.000	.000	.000	.000
I	.194	.145	.028	.323	.200	.136	.375	.281	.167
J	.000	.000	.000	.000	.000	.045	.000	.000	.000

Allele frequencies from additional microsatellite markers for group

Locus	Population						
	KK	NH	NN	LV	OV	BS	MS
adl 268							
N	17	13	14	34	36	13	14
A	.059	.000	.000	.000	.000	.000	.000
B	.000	.154	.000	.044	.069	.000	.036
C	.000	.000	.250	.441	.167	.000	.000
D	.000	.308	.321	.250	.111	.308	.571
E	.000	.154	.107	.221	.319	.192	.000
F	.647	.346	.286	.044	.292	.346	.250
G	.235	.038	.036	.000	.042	.154	.107
H	.059	.000	.000	.000	.000	.000	.000
I	.000	.000	.000	.000	.000	.000	.036
adl 278							
N	16	14	9	13	11	13	16
A	.094	.143	.167	.077	.182	.577	.250
B	.813	.643	.444	.654	.182	.269	.000
C	.094	.214	.389	.269	.636	.154	.464
MCW 14							
N	16	14	9	13	12	13	14
A	1.000	.321	.222	.154	.333	.308	.357
B	.000	.000	.000	.000	.000	.000	.179
C	.000	.036	.056	.000	.000	.038	.000
D	.000	.643	.722	.846	.667	.654	.464
MCW 78							
N	26	15	14	35	38	9	16
A	.000	.000	.036	.000	.000	.000	.000
B	.019	.000	.321	.257	.118	.167	.156
C	.115	.833	.429	.700	.776	.222	.594
D	.808	.167	.214	.029	.105	.222	.219
E	.019	.000	.000	.000	.000	.000	.000
F	.038	.000	.000	.014	.000	.389	.031
MCW 222							
N	16	13	11	13	12	12	16
A	.000	.000	.000	.192	.167	.083	.031
B	1.000	.615	.455	.577	.292	.792	.500
C	.000	.154	.273	.231	.458	.042	.375
D	.000	.231	.273	.000	.083	.083	.094

Allele frequencies from additional microsatellite markers for group

Locus	Population					
	KK	**NH**	**NN**	**LV**	**OV**	**AU**
adl 104						
N	17	13	14	34	36	18
18	.059	.000	.000	.000	.000	.000
B	.000	.154	.000	.044	.069	.000
C	.000	.000	.250	.441	.167	.194
D	.000	.308	.321	.250	.111	.111
E	.000	.154	.107	.221	.319	.278
F	.647	.346	.286	.044	.292	.222
G	.235	.038	.036	.000	.042	.000
H	.059	.000	.000	.000	.000	.083
I	.000	.000	.000	.000	.000	.000
J	.000	.000	.000	.000	.000	.111
Lei 194						
N	3	22	9	23	19	16
A	.000	.000	.000	.000	.000	.125
B	.000	.000	.000	.000	.000	.313
C	.000	.000	.278	.000	.316	.438
D	.000	.068	.000	.000	.000	.063
E	.000	.000	.056	.413	.237	.000
F	1.000	.409	.278	.587	.211	.063
G	.000	.045	.167	.000	.000	.000
H	.000	.045	.000	.000	.000	.000
I	.000	.432	.222	.000	.237	.000
MCW 34						
N	22	9	19	18	8	20
A	.250	.222	.079	.167	.313	.700
B	.455	.000	.263	.000	.000	.000
C	.227	.000	.000	.000	.000	.000
D	.000	.111	.000	.000	.125	.025
E	.000	.000	.237	.833	.500	.000
F	.023	.056	.000	.000	.063	.100
G	.000	.556	.395	.000	.000	.000
H	.000	.056	.000	.000	.000	.050
I	.045	.000	.026	.000	.000	.125
MCW 37						
N	36	33	31	39	39	20
A	.000	.061	.000	.000	.013	.000
B	.028	.000	.016	.000	.000	.000
C	.056	.076	.339	.000	.423	.450
D	.611	.818	.306	.782	.295	.500
E	.306	.045	.274	.205	.205	.050
F	.000	.000	.032	.013	.000	.000
G	.000	.000	.032	.000	.051	.000
H	.000	.000	.000	.000	.013	.000

Allele frequencies in 6 populations

Locus	Population					
	KK	**NH**	**NN**	**LV**	**OV**	**AU**
MCW 78						
N	26	15	14	35	38	18
A	.000	.000	.036	.000	.000	.000
B	.019	.000	.321	.257	.118	.000
C	.115	.833	.429	.700	.776	.833
D	.808	.167	.214	.029	.105	.111
E	.019	.000	.000	.000	.000	.056
F	.038	.000	.000	.014	.000	.000
MCW 81						
N	16	25	14	26	25	21
A	.000	.100	.000	.250	.040	.048
B	.000	.000	.393	.212	.200	.000
C	.000	.740	.393	.538	.620	.286
D	.438	.160	.000	.000	.040	.000
E	.031	.000	.000	.000	.000	.000
F	.031	.000	.000	.000	.000	.000
G	.250	.000	.000	.000	.000	.000
H	.156	.000	.214	.000	.100	.667
I	.094	.000	.000	.000	.000	.000
MCW 284						
N	13	11	11	17	8	10
A	.000	.000	.000	.000	.125	.000
B	.577	.864	.773	.853	.625	.200
C	.000	.000	.000	.000	.000	.100
D	.000	.091	.000	.000	.000	.000
294	.423	.045	.227	.147	.250	.700
MCW 294						
N	25	19	12	17	24	19
A	.000	.000	.000	.000	.021	.000
B	.980	.000	.250	.265	.167	.000
C	.020	.000	.042	.353	.438	.184
D	.000	.000	.000	.000	.000	.000
E	.000	1.000	.708	.382	.375	.816

Addendum A 2

Microsatellite marker	Group I F_{ST}	Group II F_{ST}	Group III F_{ST}
ADL 268	0.172	0.117	0.130
MCW 067	0.152	0.114	0.145
MCW 069	0.165	0.177	0.176
MCW 098	0.193	0.258	0.211
MCW 103	0.284	0.221	0.205
MCW 111	0.128	0.086	0.082
MCW 183	0.205	0.264	0.231
MCW 216	0.146	0.185	0.165
MCW 248	0.229	0.109	0.141
MCW 295	0.187	0.145	0.137
MCW 330	0.133	0.073	0.105
LEI 194	-	0.262	-
MCW 034	-	0.293	-
MCW 037	-	0.144	-
MCW 078	-	0.279	0.238
MCW 081	-	0.235	-
MCW 284	-	0.208	-
MCW 294	-	0.419	-
ADL 278	-	-	0.153
MCW 014	-	-	0.261
MCW 222	-	-	0.150
Mean	**0.179**	**0.195**	**0.195**

CONCLUSION

In this study the genotypic and phenotypic properties of the native fowl populations of the "Fowls for Africa" program were investigated and successfully characterized.

A set of microsatellite markers were tested and found to be highly polymorphic and appropriate for genetic characterization of the native fowl populations. A total of 23 markers were tested and the variation measured in terms of heterozygosity and genetic distance. It was found that a higher number of markers influenced the variation estimations, as well as the polymorphic nature of the markers. Between 11 and 18 markers were included in the analyses for distance and phylogenetic tree construction. Topology of trees remained the same, but significance values for the clusters tended to increase as more markers were included.

Results indicate that the genetic variation in the native fowl populations is relatively high. The Koekoek and Australorp had the lowest variation, which is in agreement with their history as breeds in South Africa. The Naked Neck population showed the highest variation and a close relationship with both the New Hampshire and Lebowa-Venda populations. A relatively high variation was observed for the Ovambo's and they seem to be unrelated to the other native populations such as the Naked Neck and Lebowa-Venda. This three genetically distinct groups identified using microsatellite markers correlated with the phenotypic traits, described in Chapter 4.

Growth and egg production of these populations tend to be poor in comparison to commercial birds. For the phenotypic traits studied among these native populations, they seem to be more suited for egg production than meat.

In conclusion, the genetic variation found in this study indicates that there are differences among the populations that should be preserved. This may be possible through the continuation of the "Fowls for Africa" program. Genetic differences and the relationships can also be applied in combination with phenotypic traits for selection of an improved native bird for household food production in South Africa.

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