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).

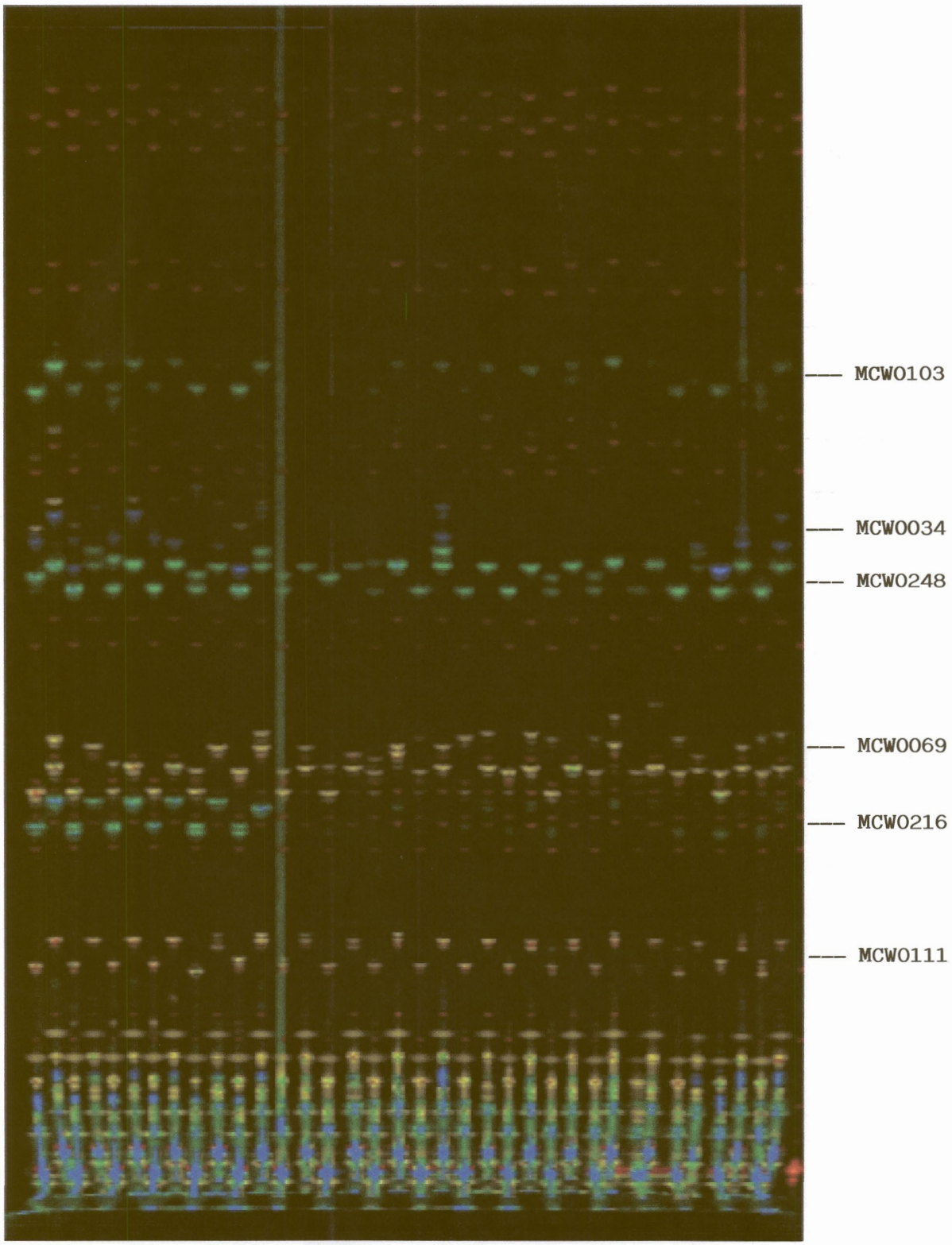

Figure 3.1 **Automated Genescan for three populations and six microsatellite markers.**

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.

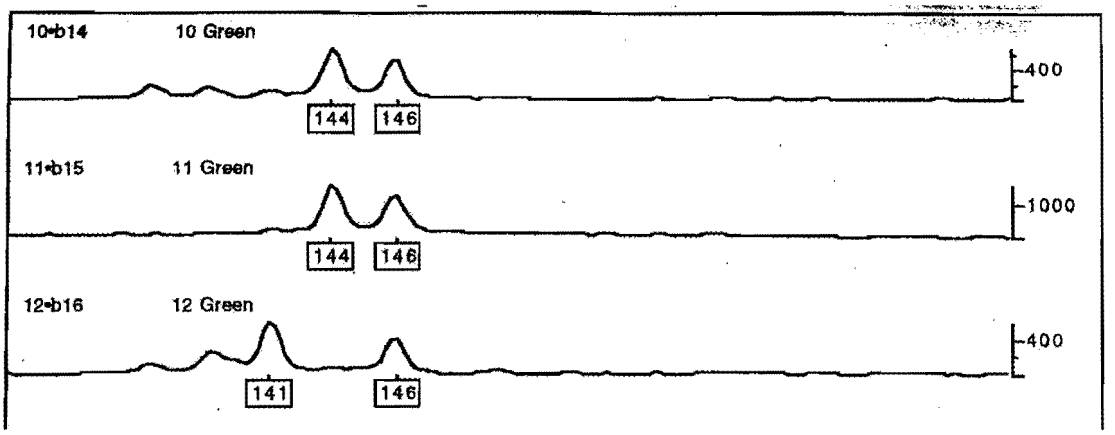

Figure 3.2 Alleles observed for three New Hampshire individuals with Genotyper analysis and microsatellite marker MCW0216.

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.

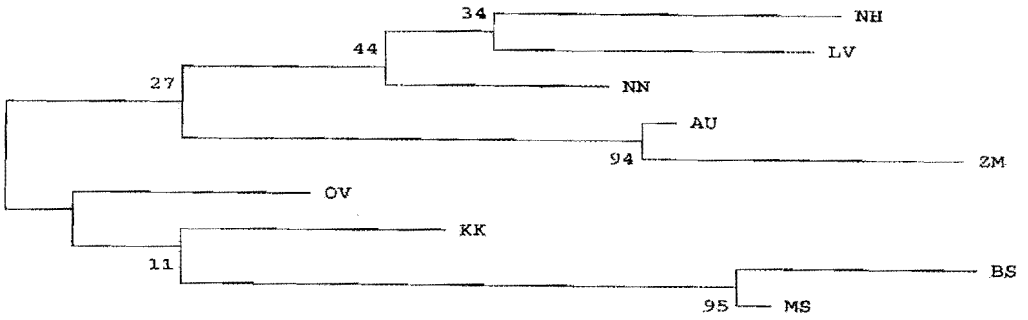

Figure 3.3 Standard neighbour-joining tree for Group I.

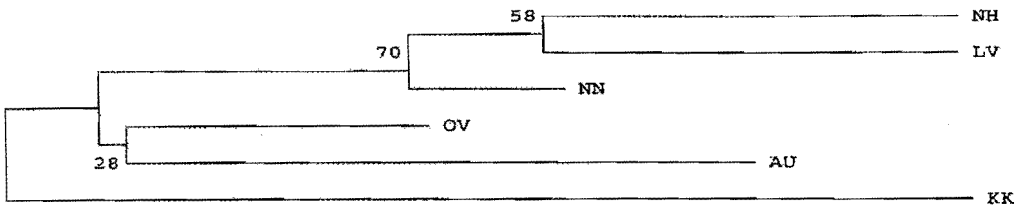

Figure 3.4 Standard neighbour-joining tree for Group II.

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.

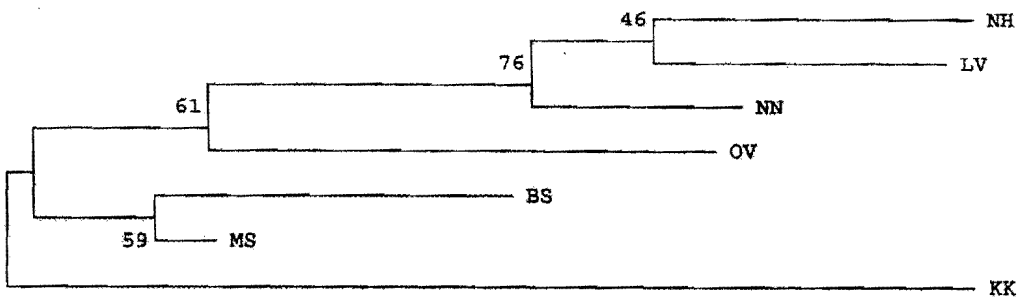

Figure 3.5 Standard neighbour-joining tree for Group III.

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).

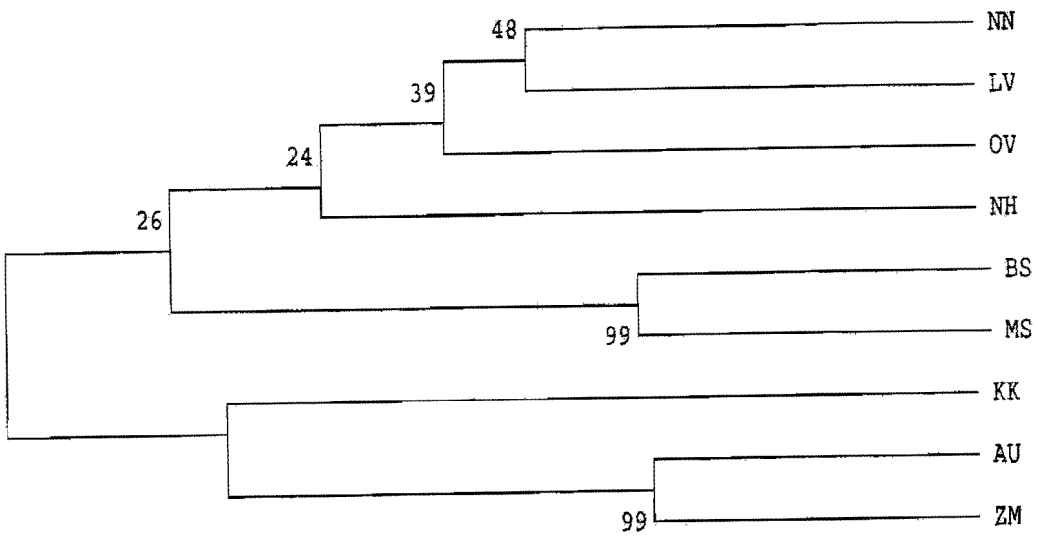

Figure 3.6 UPGMA-tree for Group I.

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.