Diversity and origin of South African chickens

B. J. Mtileni,*† F. C. Muchadeyi,† A. Maiwashe,* M. Chimonyo,† E. Groeneveld,§ S. Weigend,§ and K. Dzama†

*Agricultural Research Council, Animal Production Institute, Private Bag X2, Irene, 0062, South Africa; §Department of Animal Science, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa; †Discipline of Animal and Poultry Science, University of KwaZulu-Natal, Private Bag X01, Scottsville, 3209, Pietermaritzburg, South Africa; and ‡Institute of Farm Animal Genetics, Friedrich-Loeffler-Institut, Höltystr.10, 31535 Neustadt-Mariensee, Germany

ABSTRACT The objectives of this study were to analyze the genetic diversity and structure of South African conserved and field chicken populations and to investigate the maternal lineages of these chicken populations. Four South African conserved chicken populations (n = 89), namely, Venda (VD_C), Ovambo, Naked Neck, and Potchefstroom Koekoek from the Animal Production Institute of the Agricultural Research Council, and 2 field populations, the Venda and Ovambo (OV_F), from which the Ovambo and the Venda conservation flocks were assumed to have been sampled, were genotyped for 460 bp of the mitochondrial DNA (mtDNA) D-loop sequence. Haplotypes of these chickens were aligned to 7 Japanese and 9 Chinese and Eurasian chicken mtDNA D-loop sequences taken from GenBank and reflecting populations from presumed centers of domestication. Sequence analysis revealed 48 polymorphic sites that defined 13 haplotypes in the South African chicken populations. All 6 South African conserved and field chicken populations observed were found to be polymorphic, with the number of haplotypes ranging from 3 for VD_C to 8 for OV_F. The lowest haplotype diversity, 0.54 ± 0.08, was observed in VD_C chickens, whereas the highest value, 0.88 ± 0.05, was observed in OV_F chickens. Genetic diversity between the 4 South African conserved and 2 field chicken populations constituted 12.34% of the total genetic variation, whereas within-population diversity constituted 87.66% of the total variation. The median network analysis of the mtDNA D-loop haplotypes observed in the South African conserved and field populations and the reference set resulted in 5 main clades. All 6 South African chickens were equally represented in the major clade, E, which is presumed to be of Indian subcontinent maternal origin and may have its roots in Southeast Asia. The results showed multiple maternal lineages of South African chickens. Conservation flocks and field chicken populations shared the major haplotypes A, D and E, which were presumed to be of Chinese, Southeast Asian, and Indian subcontinental origin.

Key words: conservation flock, field population, genetic diversity, maternal lineage, mitochondrial deoxyribonucleic acid

©2011 Poultry Science Association Inc.
Received March 25, 2011.
Accepted June 24, 2011.
Corresponding author: jmtileni@arc.agric.za

INTRODUCTION

South African domestic chickens are important bird genetic resources, and more conservation efforts are being made to save these unique genotypes. Survey findings on South African chickens have illustrated that village chickens play very important socioeconomic roles in poor rural communities in that they can convert available feed around a house or village into highly nutritious, well-appreciated products (Mtileni et al., 2009). The animal protein consumed in rural areas frequently comes from village chicken meat and eggs. Chickens also have roles in traditional ceremonies and other customs as gift payments (Mtileni et al., 2009). These highly valuable genetic resources should be conserved for their adaptive features, traits of scientific and economic interest, cultural-historical value, strong links to regional traditions, and the ability to generate income for poor rural communities. Recent findings by Mtileni et al. (2011) using microsatellites shows that South African chicken populations added diversity to the existing diversity of both other African chickens as well as to purebred commercial lines, further justifying efforts to conserve these valuable genetic resources.

Several hypotheses have been proposed regarding the maternal lineages of South African chickens. On the basis of microsatellite work, South African chickens could be a product of multiple domestication events,
leading to a high level of genetic diversity. Altogether, South African chickens could be unique lineages from the purebred lines (Mtileni et al., 2011). Muchadeyi et al. (2008) revealed that chickens from Zimbabwe were probably domesticated from Southeast Asia and the Indian subcontinent. Chickens from Malawi and Sudan were also found to have Southeast Asian and Chinese maternal origins (Muchadeyi et al., 2008), whereas chickens from Nigeria are thought to be from the Indian subcontinent (Adebambo et al., 2010). It is evident that South African chickens share the same maternal lineages.

Mitochondrial DNA sequencing could be a useful tool for studying the evolution of closely related species and maternal origins. Chicken mitochondrial DNA (mtDNA) sequence polymorphism has been used to examine genetic relationships within and among breeds, and also to address questions of chicken domestication (Liu et al., 2006; Oka et al., 2007; Muchadeyi et al., 2008; Adebambo et al., 2010; Berthouly-Salazar et al., 2010; Revay et al., 2010). Mitochondrial DNA has a strictly maternal inheritance, which means mtDNA haplotypes should be shared by all individuals within a maternal family line. Compared with our previous research using microsatellites on South African chickens (Mtileni et al., 2011), mtDNA haplotypes would be conserved in a population because of the absence of recombination. As a result, insight into the ancient genetic structure of these South African chickens that could have been destroyed at the microsatellite level because of recombination would be revealed. For example, if the conserved and field populations were from the same center of domestication, they could share a haplotype but appear different at the microsatellite level. The objectives of this study were to analyze the genetic diversity and structure of South African conserved and field chicken populations and to investigate the maternal lineages of these chicken populations.

**MATERIALS AND METHODS**

**Sampling of Chickens**

Four chicken populations, Venda (VD_C), Ovambo (OV_C), Naked Neck (NN_C), and Potchefstroom Koekoek (PK_C), from conservation flocks (n = 89) from the Animal Production Institute of the Agricultural Research Council were used in this study. Two field populations from which the OV_C and VD_C conservation flocks were assumed to have been sampled, the Venda (VD_F) and Ovambo (OV_F) field populations (n = 22), were also included in the study. The field populations were sampled from several villages in the Vhembe and Mopani Districts of Limpopo Province (VD_F chickens) and in the Kgalagadi and Namaqua Districts of Northern Cape Province of South Africa along the border post of Namibia (OV_F chickens). For each district, 2 to 5 villages were selected. The distance between villages within a district ranged from 20 to 40 km, the distance between districts within a province ranged from 100 to 500 km, and the distance between provinces was more than 1,000 km. One chicken was sampled per household. The actual numbers sampled per population are shown in Table 1.

Blood samples were collected from the wing vein onto FTA Micro Cards (Whatman Bio Science, Brentford, UK). Deoxyribonucleic acid isolation was carried out following a standard phenol-chloroform extraction protocol (Sambrook and Russell, 2001). In addition, 7 sequences from Japanese chicken populations (Oka et al., 2007) and 9 sequences from chickens in the Chinese and Eurasian region (Liu et al., 2006) were used as a reference set in this study.

**Amplification and Sequencing**

Mitochondrial DNA amplification of 460 bp from the D-loop region of the chicken mitochondrial genome was performed by using primers located at the 16,739- to 16,775-bp forward primer (mtGlu-F 5′-GGCTTGAAAGCCATTGTGTTG-3′) and 649- to 668-bp reverse primer (mtGlu-R 5′-CCCCAAAAGAGAAGGAACC-3′) of the complete mtDNA sequence of domestic chickens (X52392; Desjardins and Morais, 1990). The M13-F 5′-GTAAAACGACGGCCAG-3′ and M13-R 5′-CAGGAAAACAGCTATGAC-3′ universal primers were linked to the 5′ end of each of these D-loop primers. Polymerase chain reaction was based on a HotStar Taq Master Mix (Qiagen, Valencia, CA). The PCR products were purified using an ExoSAP-IT

| Table 1. Number of polymorphic sites, number of mitochondrial DNA D-loop haplotypes, and haplotype diversity of 6 South African conserved and field populations |
|-----------------|-----------------|-----------------|-----------------|
| Population1 | Sample size | No. of polymorphic sites | No. of haplotypes | Haplotype diversity |
| OV_F | 14 | 19 | 8 | 0.8824 ± 0.0523 |
| OV_C | 25 | 13 | 5 | 0.6900 ± 0.0798 |
| VD_F | 8 | 15 | 4 | 0.7500 ± 0.1391 |
| VD_C | 26 | 9 | 3 | 0.5415 ± 0.0750 |
| NN_C | 20 | 12 | 5 | 0.8162 ± 0.0455 |
| PK_C | 18 | 13 | 5 | 0.7908 ± 0.0518 |

1Conservation flocks: OV_C = Ovambo; VD_C = Venda; NN_C = Naked Neck; PK_C = Potchefstroom Koekoek. Field populations: OV_F = Ovambo; VD_F = Venda.
Sequence Variation and Haplotype Diversity

The number of polymorphic sites, position, and corresponding haplotypes were calculated using MEGA version 3.1 software (Kumar et al., 2004). The number of unique haplotypes and their distribution in the samples were calculated using TCS software (Clement et al., 2000). Genetic diversity within the conservation flocks (mtDNA haplotypes, mtDNA gene diversity) was compared with that of field populations. Haplotype diversity of the 6 South African conserved and field chicken populations was calculated by using ARLEQUIN software version 3.1 (Excoffier et al., 2006).

Among- and Within-Population Diversity

The partitioning of sequence variation in different groups of populations was computed using molecular variance between and within all 6 South African conserved and field populations by applying the algorithms suggested by Excoffier et al. (1992), using ARLEQUIN software version 3.1.

Network Analysis of Haplotypes

Median-joining networks were constructed to determine the evolutionary relationships of haplotypes following the algorithms of Bandelt et al. (1995), using NETWORK 4.1 software (http://www.fluxus-engineering.com/sharenet.htm). In addition, the network analysis included 9 haplotypes representing the 9 clades (clades A to I) in the Chinese and Eurasian region (Liu et al., 2006) and 7 haplotypes representing the 7 clades (clades A to G) in Japanese chicken populations (Oka et al., 2007). Haplotypes from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) were aligned with the haplotypes observed in this study. Extra nucleotide bases in the GenBank sequences that were outside the 460-bp region sequenced in the current study were excluded from analysis.

RESULTS

Sequence Polymorphism and Haplotype Distribution

Sequence analysis of 460 bp revealed 48 polymorphic sites that defined 13 haplotypes in the South African chicken population. The distribution of the mtDNA D-loop haplotypes in the South African conserved and field populations is shown in Table 2. A major haplotype, A1, occurred at a frequency of 19.7% across all the populations and was widely distributed in all observed populations. The second major haplotypes, E1 and E2, which occurred at a frequency of 16.5% across all populations, together were found in 88% of the South African conserved chicken populations.

Haplotype Diversity

The number of polymorphic sites, number of mtDNA D-loop haplotypes, and haplotype diversity of the 6 South African conserved and field populations are presented in Table 2. All South African conserved and field populations observed were found to be polymorphic, with the number of haplotypes ranging from 3 for VD_C to 8 for OV_F. The lowest haplotype diversity, 0.54 ± 0.08, was observed in VD_C chickens, whereas the highest value, 0.88 ± 0.05, was observed in OV_F chickens. The field populations exhibited higher genetic diversity than the conservation flocks.

Between- and Within-Population Diversities

Mitochondrial DNA D-loop variances within and between 6 South African conserved and field populations are presented in Table 3. Genetic diversity between the 4 South African conserved and 2 field chicken populations constituted 12.34% of the total genetic variation, whereas within-population diversity constituted 87.66% of the total variation.

Network Analysis of Haplotypes

The median network analysis of the mtDNA D-loop haplotypes observed in the South African conserved and field populations and the reference set consisting of data from Liu et al. (2006) and Oka et al. (2007) clustered into the 5 main clades presented in Figure 1. The South African conserved and field chicken populations shared some major mtDNA haplogroups (A, D, and E), whereas haplogroups B and F were exclusively for field populations. Haplotype A1, harbored on clade A, was made up of haplotypes from all South African conserved and 1 VD_F field chicken population. This haplotype (A1) resembled haplogroup A1 from clade A of Liu et al. (2006) and from haplotype B of Oka et al. (2007). Only 2 individuals of OV_F field chicken populations carried haplotype B1. Haplotypes from clade

Network Analysis of Haplotypes

The median network analysis of the mtDNA D-loop haplotypes observed in the South African conserved and field populations and the reference set consisting of data from Liu et al. (2006) and Oka et al. (2007) clustered into the 5 main clades presented in Figure 1. The South African conserved and field chicken populations shared some major mtDNA haplogroups (A, D, and E), whereas haplogroups B and F were exclusively for field populations. Haplotype A1, harbored on clade A, was made up of haplotypes from all South African conserved and 1 VD_F field chicken population. This haplotype (A1) resembled haplogroup A1 from clade A of Liu et al. (2006) and from haplotype B of Oka et al. (2007). Only 2 individuals of OV_F field chicken populations carried haplotype B1. Haplotypes from clade
B were the same as those from haplotype E1 (clade E) from Oka et al. (2007) and haplotype B1 (clade B) from Liu et al. (2006). Haplotype D2, from clade D, was surrounded by haplotypes mainly from the conservation flocks and only 2 individuals of OV_F field chicken populations. This haplogroup clustered with haplotypes from clade C of Oka et al. (2007), clade D of Liu et al. (2006), and clade A of Muchadeyi et al., (2008). All 6 South African conserved and field populations were equally represented in clade E. This clade (clade E) resembled the partial sequence of haplotype A3 from clade A of Oka et al. (2007), clade D of Liu et al. (2006), and from clade E from Liu et al. (2006), and from the second haplogroup from clade C of Muchadeyi et al. (2008). The haplotype F2 in clade F found in field populations resembled the sequence of haplotype F1 from clade F of Liu et al. (2006).

**DISCUSSION**

The relatively lower haplotype diversity in the VD_C and OV_C conservation flocks than in the respective VD_F and OV_F field populations indicated that each conserved population represented a limited sample of the gene pool, whereas the field populations had accumulated a specific and rich gene pool, highlighting the interest in and the need for conservation of these populations. Similarly, Muchadeyi et al. (2008) reported greater genetic variation in other free-ranging chickens in Zimbabwean ecotypes when compared with white egg layers and Malawian and Sudanese chicken populations. This is in agreement with our previous report using microsatellites, in which the observed within-population diversity measures indicated that the village chicken populations were more diverse than the conservation flocks (Mtileni et al. 2011).

The 13 haplotypes observed in this study belonged to the 5 haplogroups previously found by Liu et al. (2006), namely, A, B, D, E, and F (Figure 1). Four of the haplogroups found by Liu et al. (2006) were not encountered in the South African chicken populations, namely, C, which was mainly distributed in Japan and Southeast China; G, which was exclusive to Yunan, China; H, from Indonesia, in wild junglefowl of unknown origin, and in sequences from the Indian subcontinent; and I, which has been observed in only 3 *Gallus gallus*, mainly present in those from Vietnam. In the median network analysis, the mtDNA D-loop haplotypes in the South African conserved and field chicken populations were equally represented in clade E. The South African conserved and field chicken populations shared some major mtDNA haplogroups (A, D, and E), whereas haplogroups B and F had exclusively field populations. Haplotype A1, from clade A of Liu et al. (2006), was mainly distributed in South China and Japan. According to Liu et al. (2006), their clade A, which corresponded to clade B of Oka et al. (2007), and clade A of the current study had a close phylogenetic relationship, indicating that they have the same origin as haplotypes of Clade A reported by Liu et al. (2006). On the basis of the high proportion of unique haplotypes in Yunan, Liu et al. (2006) suggested that both lineages could

---

**Table 2. Distribution of mitochondrial DNA D-loop haplotypes in the South African conserved and field populations**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>OV_C</th>
<th>VD_C</th>
<th>PK_C</th>
<th>NN_C</th>
<th>OV_F</th>
<th>VD_F</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>D2</td>
<td>13</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>E1</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>E2</td>
<td>16</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>E3</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>E4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>E5</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>E6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>E7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>E8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>E9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>26</td>
<td>18</td>
<td>20</td>
<td>14</td>
<td>8</td>
<td>111</td>
</tr>
</tbody>
</table>

Conservation flock: OV_C = Ovambo; VD_C = Venda; PK_C = Potchefstroom Koekoek; NN_C = Naked Neck. Field population: OV_F = Ovambo; VD_F = Venda.

---

**Table 3. Mitochondrial DNA D-loop variance within and between 6 South African conserved and field populations**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of squares</th>
<th>Variance component</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between populations</td>
<td>40.234</td>
<td>0.31909</td>
<td>12.34189</td>
</tr>
<tr>
<td>Within populations</td>
<td>237.965</td>
<td>2.26633</td>
<td>87.65811</td>
</tr>
<tr>
<td>Total</td>
<td>278.198</td>
<td>2.58542</td>
<td></td>
</tr>
</tbody>
</table>
have originated in Yunnan and the surrounding regions. The clade B of Oka et al. (2007) was found in most of the Ko-Shamo fighting cocks and in commercial Rhode Island Red and White Leghorn chickens.

The clade E of Oka et al. (2007) was observed in Shamo and Indonesian fighting cocks, and their sequences resembled those observed in Shamo from China and Myanmar and in several other Chinese native chicken populations. Haplogroup D2 in the current study clustered with haplotypes of clade C of Oka et al. (2007), which was made up of Tosa-Jidori and related native Japanese breeds as well as some Indonesian native chickens. Oka et al. (2007) suggested that this clade has its roots in Southeast Asia. Haplotypes from clade D in our study also clustered with clade D of Liu et al. (2006), which is common in junglefowl and gamecocks from Indonesia, India, and Japan (Liu et al. 2006). Liu et al. (2006) further suggested that their clade D was a product of recent domestication events in Southeast China and surrounding regions (Vietnam, Burma, Thailand, and India). These clades also resembled clade A of Muchadeyi et al. (2008), which is unique to Zimbabwe and Malawi and was not found in purebred commercial and experimental lines or in Northwest European local chickens. It is possible that the absence of these haplotypes in South African chicken populations could be the result of no crossbreeding with purebred commercial chicken lines. It also implies that some of the differences between South African and purebred commercial lines originate from the times of domestication. These findings are in agreement with the archeological studies by Macdonald (1992), which have indicated that chickens were introduced into Africa via East African–Southeast Asian trade links.

Clade E resembled the partial sequence of haplotype A3 from clade A of Oka et al. (2007), in which Gifu-Jidori, Shokoku, and related native Japanese breeds and commercial lines (Rhode Island Red and White Leghorn) were found. Oka et al. (2007) suggested that this clade originated in Southeast Asia and was first introduced into the Indian subcontinent before spreading to other regions. Clade E in the current study was
also similar to clade E from Liu et al. (2006), which included chickens mainly from Europe, the Middle East, and India. According to Liu et al. (2006), the maternal lineages associated with this clade could have originated from the Indian subcontinent. In either case, results from this study confirm that a wide range of populations currently distributed in several geographic regions were derived from this clade. This clade also resembles the second haplogroup from clade C of Muchadeyi et al. (2008), which was common to Zimbabwean, Sudanese, and Northwest European chickens as well as 6 purebred lines. Similarly, van Marle-Köster et al. (2008) reported that domestic chickens were introduced into Southern Africa by early traders during the 1600s from India, Europe, and sub-Saharan Africa. The haplotype F2 found in field populations resembled the sequence of haplotype F1 from clade F of Liu et al. (2006). According to Liu et al. (2006), clade F was exclusively from fowl originating in Yuman Province. In our previous report using microsatellites (Mtileni et al. 2011), the South African conserved and field chicken populations formed distinct population clusters, however these populations shared the major mtDNA haplogroups. As a result, the South African conserved and field chicken populations shared some ancestral maternal lineages, which suggests that these populations could be from the same maternal lineages. South African chickens would be conserved in a population because of the absence of recombination in mitochondrial DNA-level haplotypes compared with microsatellites.

In conclusion, the results of this study suggest that the diversity of chicken mitochondrial DNA in South African chicken populations is high and shows multiple maternal lineages. South African domestic chicken mtDNA sequences could be assigned into 5 clades and probably 3 maternal lineages. Conservation flocks and field chicken populations shared 3 major haplotypes, which were presumed to be of Chinese, Southeast Asian, and Indian subcontinental origin.

ACKNOWLEDGMENTS

The Department of Science and Technology in South Africa (Pretoria, South Africa) and the Federal Ministry of Food, Agriculture and Consumer Protection of Germany are acknowledged for financial support of this study. The project was supported by the bilateral cooperation programme in agricultural research between South Africa and the Federal Republic of Germany. We also thank Extension personnel, all the development agents for providing support, and farmers in the 2 provinces of South Africa (Limpopo and Northern Cape provinces) for their cooperation during blood collection, as well as the University of Western Cape (Cape Town, South Africa), where DNA isolation was carried out. Our appreciation goes to A. Flörke and A. Weigend for their technical assistance during mtDNA sequencing at the Institute of Farm Animal Genetics, Friedrich Loeffler Institut (Neustadt-Mariensee, Germany).

REFERENCES


