Domestication of South African chicken genetic resources

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Introduction

Domestic animals are recognized as an important part of biodiversity and more conservation efforts to save rare breeds are made. Genetic relationship and the domestication of South African chickens is a point of interest, as the origin of the breeds is not exactly known. According to van Marle-Koster and Casey (2001), domestic chickens were introduced into Southern Africa by early traders during the 1600's from India, Europe and sub-Saharan Africa. Some archaeological studies have indicated that chickens were introduced into Africa via the East Africa–Southeast Asia trade links (Macdonald 1992). Crawford (1990) reported that domesticated chickens were found in Mozambique by 1600. Using mtDNA sequence data, Akishinonomiya *et al.* (1996) reported that existing domestic chickens originated from Gallus gallus gallus in Thailand and adjacent regions, while recent studies by Liu *et al.* (2006), Oka *et al.* (2007) and Muchadeyi *et al.* (2008) suggested that domestication events occurred in Southeast Asia, South China and Indian subcontinent. The objective of this study is to determine the genetic relationship and the geographical origin of the South African conserved and field chicken populations using mtDNA sequence.

Material and methods

Four chicken populations, Venda (VD_C) and Ovambo (OV_C) Naked Neck (NN_C) and Potchefstroom Koekoek (PK_C) conservation flocks (n=89) from the Animal Production Institute in Irene and two field populations, Venda (VD_F) and Ovambo (OV_F) field populations (n=22) were used in this study. Blood samples were collected from the wing vein onto FTA@Micro Card (Whatman Bio Science, UK). DNA extraction was carried out following a standard Phenol/Chloroform extraction protocol (Sambrook and Russell, 2001). In addition, seven sequences in Japanese chicken populations (Oka *et al.* 2007) and nine sequences in the Chinese and Eurasian region (Liu *et al.* 2006) from Genebank was used as reference set in this study.

mtDNA amplification of 460bp from the D-loop region of the chicken mitochondrial genome was performed using primers located at 16739-16775bp forward primer (mtGlu-F 5'-GGCTTGAAAAGCCATTGTTG-3') and 649-668bp reverse primer (mtGlu-R 5'-CCCCAAAAAGAGAAGGAACC-3') of the complete mtDNA sequence of domestic chickens (X52392, Desjardins and Morais, 1990). The M13-F 5'-GTAAAACGACGGCCAG-3' and M13-R 5'-CAGGAAACAGCTATGAC-3' universal primers were linked to the 5'end of each of these D-loop primers. Polymerase chain reaction (PCR) was based on the HotStart Taq Master Mix (Qiagen). Sequencing was carried out using an automated DNA sequencer (CEQ 8800 Genetic Analysis System, Beckman Coulter) and computer software (CEQ 8800 Beckman Coulter). The forward and reverse DNA sequences were aligned using the ALIGNIR software program (LICOR Inc.).

The number of polymorphic sites, position and the corresponding haplotypes were calculated using MEGA version 3.1 (Kumar *et al.,* 2004). The number of unique haplotypes and their distribution in the samples were calculated using the TCS software (Clement *et al*., 2000). Haplotype diversity was calculated using ARLEQUIN software v. 3.1 (Excoffier *et al*. 2006). Median joining networks were constructed to determine the relationships of haplotypes, following the algorithms of Bandelt et al. (1995) using NETWORK 4.1 software (www.fluxus-engineering.com/sharenet.html). The partitioning of sequence variation in different groups of populations was computed using molecular variance (AMOVA) between and within populations applying the algorithms suggested by Excoffier *et al.* (1992) using ARLEQUIN software v. 3.1.

Results and discussion

Sequence analysis of 460bp revealed 48 polymorphic sites that define 18 haplotypes observed in the South African chicken population. A major haplotype Liu_A1 occurred at a frequency of 19.7% over all populations and was widely distributed in all observed populations. The second major haplotypes Liu_E1 and E2, which occurred at a frequency of 16.5% across all populations, was found in 88 % of the South African conserved chickens. All South African conserved and field populations observed were found to be polymorphic with the number of haplotypes ranging from three for VD_C to eight for OV_F. The lowest haplotype diversity of 0.54±0.08 was observed in VD_C chickens, while the highest value of 0.88±0.05 was observed in OV_F chickens (Table 1). Between diversity of six South African conserved and field chicken populations attributed to 12.34% of the total genetic variation while within diversity attributed to 87.66% of the total variation (Table 2).

Table 1: Number of polymorphic sites, number of mtDNA D-loop haplotypes and haplotype diversity of six South African conserved and field populations

Population	Sample size	No. of polymorphic sites	No of haplotypes	Haplotype divesity
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

Table 2: mtDNA D-loop variance within and between six South African conserved and field populations

Source of variation	Sum of squares	Variance components	Percentage variation
Between populations	40.234	0.31909	12.34189
Within populations	237.965	2.26633	87.65811
Total	278.198	2.58542	

Median network profile of the mtDNA D-loop haplotypes observed in the South African conserved and field populations and reference set consisting of Liu et al. (2006) and Oka *et al.* (2007) clustered into five main clades presented in Figure 1. Clade A harboured on haplotype Liu_A1 and was made up of haplotypes from South African conserved and one

field chicken populations. Haplotype A1 from Clade A of Liu *et al.* (2006), were mainly distributed in South China and Japan. According to Liu *et al.* (2006), their Clades A which corresponded to Clades B in Oka *et al.* (2007), and Clade A of the current study had similar geographical distributions and a close phylogenetic relationship, which indicated that both lineages originated from the same ancestral population. Based on the high proportion of unique haplotypes in Yunnan, it was suggested that both lineages could have originated in Yunnan and the surrounding regions (Liu *et al.* 2006). 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.

Clade B harboured around haplotype Lui_B1 and consisted mainly of individuals from South African field chicken populations. Haplotypes from Clade B was the same as the haplotype E1 (Clade E) from Oka *et al.* (2007) and haplotype B1 (Clade B) from Liu *et al.* (2006). 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. Clade D was made up of haplotype D1 from Clade D of Liu *et al.* (2006) and C1 of Oka *et al.* (2007). Haplotype D2 from Clade D is surrounded by haplotypes mainly from conservation flocks and two haplotypes from field populations. This haplogroup clustered with haplotypes from Clade C of Oka *et al.* (2007), which was made up of Tosa-Jidori and related native Japanese breeds and some Indonesian native chickens (Oka *et al.* 2007). Oka *et al.* (2007) suggested that this clade has its roots in Southeast Asia. Haplotypes from Clade D of our study also cluster with Clade D of Liu *et al.* (2006), which is common in jungle fowls 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 Southwest China and/or surrounding regions (Vietnam, Burma, Thailand and India). These clades also resembles Clade A of Muchadeyi *et al.,* (2008), which was unique to Zimbabwe and Malawi and was not found in purebred commercial and experimental lines or in Northwest European local chickens.

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 to the Indian subcontinent before spreading to other regions. Clade E of the current study is 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 a number of geographic regions were derived from this clade. This clade also resembled the second haplogroup from Clade C of Muchadeyi *et al.* (2008), which was common to Zimbabwean, Sudanese, Northwest European chickens and six purebred lines. 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 of Yunnan Province origin fowls.

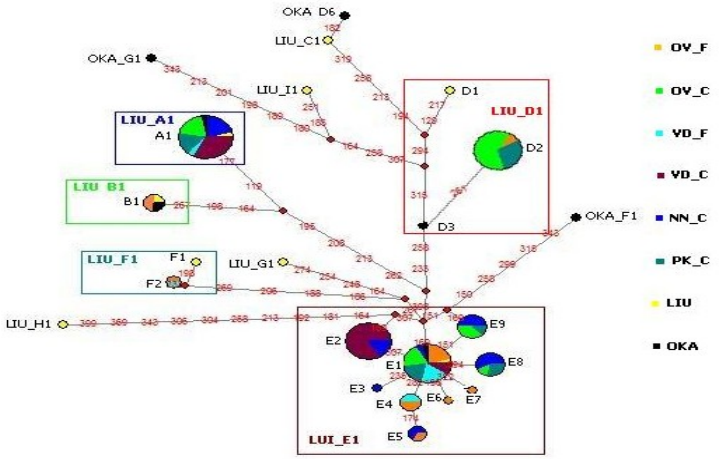

Figure 1: Median network profile of the mtDNA D-loop haplotypes observed in the six South African conserved and field populations as well as those from Liu *et al.* (2006) and Oka *et al.* (2007).

Conclusion

All six South African conserved and field populations were equally represented in Clades E, while Clade A, B, D and F were mainly made up from conservation flocks and few haplotypes from field populations. Results from this study confirm that a wide range of populations currently distributed in a number of geographic regions were derived from clade E, which suggest that the major maternal lineages contributed to the South African domestic chickens could be from Yunnan, China, Southeast Asia and Indian subcontinent.

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