Molecular Phylogenetics and Evolution 58 (2011) 374–382

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Mitochondrial DNA reveals multiple introductions of domestic chicken in East Africa

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ARTICLE INFO

Article history:
Received 1 November 2010
Accepted 29 November 2010
Available online 5 December 2010

Keywords:
Gallus gallus
Village chicken
Control region
Indian Ocean

ABSTRACT

Chicken were possibly domesticated in South and Southeast Asia. They occur ubiquitously in East Africa where they show extensive phenotypic diversity. They appeared in the region relatively late, with the first undisputed evidence of domestic chicken in Sudan, around ~700 BC. We reveal through a detailed analysis of mitochondrial DNA D-loop sequence diversity of 512 domestic village chickens, from four East African countries (Kenya, Ethiopia, Sudan, Uganda), the presence of at least five distinct mitochondrial DNA haplogroups. Phylogeographic analyses and inclusion of reference sequences from Asia allow us to address the origin, ways of introduction and dispersion of each haplogroup. The results indicate a likely Indian subcontinent origin for the commonest haplogroup (D) and a maritime introduction for the next commonest one (A) from Southeast and/or East Asia. Recent introgression of commercial haplotypes into the gene pool of village chickens might explain the rare presence of two haplogroups (B and C) while the origin of the last haplogroup (E) remains unclear being currently observed only outside the African continent in the inland Yunnan Province of China. Our findings not only support ancient historical maritime and terrestrial contacts between Asia and East Africa, but also indicate the presence of large maternal genetic diversity in the region which could potentially support genetic improvement programmes.

1. Introduction

Village chickens (*Gallus gallus*) are ubiquitous in East Africa with a total population of 100.8 million birds (FAOSTAT, 2007). These birds fulfil various roles ranging from socio-cultural to sustaining livelihoods, suggesting a long historical presence in the region. Chicken are not a native species to Africa where no wild *Gallus sp.* is found (Delacour, 1977). A previous phylogenetic study of chicken mitochondrial DNA (mtDNA) control region suggested that the Indochinese red junglefowl subspecies *Gallus gallus gallus* was the primary maternal ancestor of all domestic fowls *G. g. domesticus* and that Southeast Asia (Thailand) was the likely center of domestication (Fumihito et al., 1996). A recent study, which analysed a larger fragment of the mtDNA control region encompassing the first hypervariable segment, in a comparatively large and diverse gene pool of domestic chickens from a wide geographic area (Europe and Asia), was the first to suggest multiple maternal geographic centers of origin for the domestic species (Liu et al., 2006). They identified nine divergent clades, (called here haplogroups), seven of which included both domestic chickens and wild red junglefowl subspecies haplotypes. Three of the nine clades involved mtDNA control region haplotypes found across Europe and Asia, whereas the other six clades contained haplotypes found exclusively in South and Southeast Asia. Both Fumihito et al. (1996) and Liu et al. (2006) studies lacked samples from the African continent.

More recently, three studies have made an attempt to address the origin of African village chickens through the analysis of partial mtDNA D-loop sequences. Muchadeyi et al. (2008) observed two distinct haplogroups in Zimbabwe village chickens which they postulated came from Southeast Asia and the Indian subcontinent. Similarly, Razafindraibe et al. (2008) observed two haplogroups in Madagascar village chicken and speculated that one was of Indonesian and the other of African continental origin or an introgression from commercial lines. At the opposite a single haplogroup thought to be of Indian origin was observed in Nigeria village chickens by Adebambo et al. (2010), while no information is yet available for the East African region.

Since historical times, Africa and Asia have been linked via maritime and terrestrial corridors. The Indian Ocean was one of the world's earliest arenas that provided a maritime corridor linking

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1055-7903/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2010.11.027

Africa and Asia. Following the monsoon wind patterns of the Indian Ocean, Asia and East Africa witnessed important seafaring and maritime exchanges across historical times. Asia and Africa were also connected via a terrestrial route that traversed the Arabian Peninsula, a region which occupies a key geographic junction, with the African landmass to the West and the Asian continent to the East (Boivin and Fuller, 2009). These terrestrial and maritime routes witnessed the long distance movement of people, livestock and crops within and between continents. Domesticates that dispersed from Asia to Africa included cattle, pigs, chicken, broomcorn millet, bananas, water yam and taro and those that moved from Africa to Asia are donkey's, sorghum, pearl millet, finger millet, cowpeas and hyacinth beans (Fuller and Boivin, 2009).

Village chickens in East Africa are found across the entire region and in all agro-ecological zones; they show large within population phenotypic diversity in plumage colour, feather morphology and pattern, skin colour, comb type etc. (Msoffe et al., 2001; Dana et al., 2010a). Raised under free-range scavenging system, village chickens contribute substantially to egg and meat production under almost a zero input system under small holder subsistence economies (Sonaiya, 1997; Kitalyi, 1998). The possible geographic origin of East African village chickens remains unknown, with the Indian subcontinent, Southeast Asia and North Africa as possible primary centers of origin and entry in agreement with our knowledge of the history of trading of the region. To investigate the origin of East African village chicken, we generated mtDNA control region sequences from 512 village chickens and analysed their phylogenetic relationships with representative Asian haplotypes from the nine haplogroups described by Liu et al. (2006). In addition, we evaluated the phylogeographic structure of the village chickens to assess their pattern of diffusion across East Africa. Our results reveal an unexpected complex pattern of introduction of domestic chicken in the region and provide new information concerning the history of trading and human contacts between East Africa and Asia.

2. Materials and methods

2.1. Sample collection and DNA extraction

Blood samples from 512 genetically unrelated village chickens were collected from 23 populations in four countries in East Africa (Table 1). All samples were from unimproved village chickens raised under free-range scavenging. Two mature birds were sampled per flock and the sampling strategy and characteristics of the sampling locations were described previously (Mwacharo et al., 2007). Genomic DNA was extracted from either whole blood using phenol–chloroform or from air dried blood preserved on FTA classic cards (Whatman Biosciences) using the manufacturers protocol. To ascertain the genetic affinities of the study populations to Asiatic and other African chickens, 30 haplotypes were downloaded from the GenBank and were included in the analysis (see Table S1). For this purpose, the central and most common haplotypes for the nine clades observed in Liu et al. (2006) were selected for the study to determine the possible origins of chicken found in East Africa within the geographic range of the wild ancestor, the red junglefowl.

2.2. PCR amplification and sequencing

The first 700 bp of the mtDNA D-loop region were amplified via PCR using primers AV1F2 (5′-AGGACTACGGCTTGAAAAGC-3′) and CR1b (5′-CCATACACGCAAACCGTCTC-3′). PCR amplifications were carried out in 25 μl reaction volumes containing 20 ng genomic DNA, 1 X PCR buffer (10 mM Tris–HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100), 2.5 mM of each dNTP, 10 pM of each primer and 1 unit of *Taq* DNA polymerase (Promega, Madison WI, USA). Thermo-cycling conditions were: 94 °C (3 min), 35 cycles of 94 °C (1 min), 58 °C (1 min) and 72 °C (2 min), and a final extension step at 72 °C (10 min). PCR products were purified using the Wizard® SV Gel and PCR Clean-Up Kit (Promega, Madison WI, USA). Purified products were sequenced directly using the BigDye® Terminator v3.1 (Applied Biosystems, USA) on an ABI prism 3100 Avant DNA analyzer. The two PCR primers and an internal primer f1-3m (5′-TGGTTCCTCGGTCAGGCACATCC-3′) were used for the sequencing reactions.

2.3. Sequence and phylogenetic analysis

For each sample, three fragments were generated. These were edited manually using BioEdit 7.0 (Hall, 1999) and subsequently joined to reconstruct a fragment of 700 bp. The fragments were aligned using Clustal X 1.83 (Thompson et al., 1997) against a reference sequence (GenBank accession number X52392; Desjardins and Morais, 1990). Subsequent analyses were restricted to the first 397 bp incorporating the first hypervariable segment (HVS1). We set to determine the number of haplogroups present in East Africa village chickens by constructing a Median-Joining (MJ) network (Bandelt et al., 1999) using NETWORK 4.5 (fluxus-engineering.com). This analysis was augmented by constructing a phylogenetic tree involving the haplotypes observed in East Africa using the Neighbour-Joining (NJ) algorithm as implemented in MEGA 4.0 (Tamura et al., 2007) following 1000 bootstrap replications. To portray the affinity of East Africa haplotypes to those observed in Asia and Africa, a MJ network incorporating the 30 haplotypes downloaded from the GenBank (see Table S1) was also constructed. The nomenclature of the haplogroups observed in this study compared to the study of Liu et al. (2006), Silva et al. (2008) and Muchadeyi et al. (2008) are shown in Table S2.

2.4. Population genetic variability and structure

Genetic variation (nucleotide diversity, haplotype diversity and nucleotide differences) for each population were calculated using DnaSP 5.10 (Librado and Rozas, 2009). Population genetic structure was assessed by nested analysis of molecular variance (AMOVA). The groupings used for AMOVA were as follows: (i) the overall dataset assuming no groups; (ii) between the haplogroups observed on the MJ network; (iii) between chicken populations found in different countries (country specific groups); (iv) between populations for haplogroup D only and (v) between populations for haplogroup A only. Phi (Φ) statistics representing haplotype correlations at various levels of hierarchical clusters Φ_{CT}, Φ_{SC}, and Φ_{ST} (Excoffier et al., 2005) were calculated. Significance testing was performed using 10,100 coalescent simulations in Arlequin 3.1 (Excoffier et al., 2005).

A Mantel test was used to assess the non-random association between genetic differentiation (F_{ST}) and geographic distances (km) between populations using the IBDWS v3.05 software (http://ibdws.sdsu.edu). Population pairwise F_{ST} values were calculated using Arlequin 3.1 (Excoffier et al., 2005). Geographic distances between populations were calculated using the MapCrow Travel Distance Calculator between central towns within the sampling locations based on geographical coordinates (http://www.mapcrow.info/). For the analysis all negative F_{ST} values were set to zero. This analysis was limited to haplogroup D with the widest geographic range in the region. Several comparative analyses were performed across all countries and between all countries.