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Mitochondrial DNA D-loop sequences suggest a Southeast Asian and Indian origin of Zimbabwean village chickens

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Summary

This study sought to assess mitochondrial DNA (mtDNA) diversity and phylogeographic structure of chickens from five agro-ecological zones of Zimbabwe. Furthermore, chickens from Zimbabwe were compared with populations from other geographical regions (Malawi, Sudan and Germany) and other management systems (broiler and layer purebred lines). Finally, haplotypes of these animals were aligned to chicken sequences, taken from GenBank, that reflected populations of presumed centres of domestication. A 455-bp fragment of the mtDNA D-loop region was sequenced in 283 chickens of 14 populations. Thirty-two variable sites that defined 34 haplotypes were observed. In Zimbabwean chickens, diversity within ecotypes accounted for 96.8% of the variation, indicating little differentiation between ecotypes. The 34 haplotypes clustered into three clades that corresponded to (i) Zimbabwean and Malawian chickens, (ii) broiler and layer purebred lines and Northwest European chickens, and (iii) a mixture of chickens from Zimbabwe, Sudan, Northwest Europe and the purebred lines. Diversity among clades explained more than 80% of the total variation. Results indicated the existence of two distinct maternal lineages evenly distributed among the five Zimbabwean chicken ecotypes. For one of these lineages, chickens from Zimbabwe and Malawi shared major haplotypes with chicken populations that have a Southeast Asian background. The second maternal lineage, probably from the Indian subcontinent, was common to the five Zimbabwean chicken ecotypes, Sudanese and Northwest European chickens as well as purebred broiler and layer chicken lines. A third maternal lineage excluded Zimbabwean and other African chickens and clustered with haplotypes presumably originating from South China.

Keywords maternal lineages, mitochondrial DNA, Zimbabwean chickens.

Introduction

Village chickens in Zimbabwe are distributed over a wide geographical range. They are reared by communal farmers across the country under extensive production systems. Within households, different age groups are raised as one flock. The communal ownership of the scavenging feed resources results in mixing of flocks from different households within communities. Although on average every household owns a cock, mixing of chickens results in sharing of cocks among neighbouring flocks. As a result, contiguous villages within districts would more or less constitute one breeding flock. On the other hand, because of the large geographic area of Zimbabwe and the environmental differences, genetic variation is expected among indigenous chickens from contrasting agro-ecological zones. Muchadeyi *et al.* (2007) found that although Zimbabwean chickens were distinguishable from Malawian and Sudanese chickens as well as from broiler and layer purebred lines based on microsatellite data, these ecotypes had no population substructure and made up one diverse population spread over a wide geographic range within the country. This lack of population substructuring might be because of either continuous gene flow among ecotypes or their sharing of many ancestral lineages. It is also possible that there was initially a single and diverse population that expanded into all of the agro-ecological zones.

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A number of studies have investigated the origin and dispersal of domestic chickens. Using mtDNA sequence data, Akishinonomiya *et al.* (1996) suggested that existing domestic chickens originated from *Gallus gallus gallus* in Thailand and adjacent regions. Recent studies by Liu *et al.* (2006) and Oka *et al.* (2007) gave rise to the assumption that multiple successive domestication events occurred in Southeast Asia, South China and Indian subcontinent. The relationship of these presumed ancestral chicken populations to the existing African chickens has not yet been investigated. Very little is known about the history and origin of local chickens found in Zimbabwe and other African countries. According to Crawford (1990), domesticated chickens were found in Mozambique by 1600. It has been suggested that early trade between India and East Coast Africa brought these chickens to Africa. Marle-Köster & Casey (2001) also suggested that Indian and European traders introduced domestic chickens into South Africa. Some archaeological studies have indicated that chickens were introduced into Africa via the East Africa–Southeast Asia trade links (Macdonald 1992).

The mitochondrial genome is maternally inherited and the hypervariable D-loop region of mtDNA sequence can be used to detect ancient population structures. The analysis of mtDNA haplotypes found in Zimbabwean chicken ecotypes and their comparison with other chicken populations will shed light on the history of these genetic resources. The objectives of this study were (i) to assess the population structure of the Zimbabwean chicken ecotypes at the mtDNA level and to compare this with other African chicken populations, European local breeds and purebred broiler and layer lines, and (ii) to determine the degree to which Zimbabwean ecotypes share maternal haplotypes with chicken populations from different geographical regions and production systems as well as the presumed centres of domestication and thus (iii) reveal maternal lineages of origin.

Materials and methods

Chicken populations

A total of 283 chickens were sampled from five eco-zones in Zimbabwe (Eco-I to Eco-V), from Malawi, Sudan and local Northwest Europe and from broiler and layer purebred commercial and experimental lines.

Zimbabwean ecotypes

DNA samples were collected from local chickens (ecotypes) distributed across five districts representing the agro-ecological zones of Zimbabwe. The five districts used for this study, Risitu, Hurungwe, Gutu, Gokwe-South and Beitbridge in agro-ecological zones I through to V (Eco-I to Eco-V) respectively, differed in climate and altitudes. Details about the five agro-ecological zones are given elsewhere (Muchadeyi *et al.* 2007). From each district, 20 chickens were sampled except for Eco-III, where 19 chickens were sampled. One chicken was selected per household, and at most 10 households were used per village. The number of villages sampled per district ranged from two to five. Geographical distances between agro-ecological zones ranged from 300 km (Eco-III and Eco-V) to 800 km (Eco-II and Eco-V). Details on chicken blood collection and DNA isolation were described previously (Muchadeyi *et al.* 2007).

Reference populations

Twenty chickens were sampled from each of the commercial broiler dam (BRD_A) and sire (BRS_A) lines, from two brown egg layer lines (BL_A and BL_C) and from an experimental white egg layer line (LS_S). Another 21 chickens were sampled from a commercial white egg layer line (WL_A). These purebred lines were selected from the AVIANDIV project,[2] a former European research cooperation on chicken biodiversity. In addition, 19 scavenging chickens sampled within a 50-km radius in Lilongwe, Malawi (MAL) and 20 Baladi chickens from Sudan (SUD) were included in this study. The Malawian and Sudanese chickens were reared under extensive production systems similar to those of Zimbabwean ecotypes. Twenty-four chickens of the Northwest European type were randomly sampled from fancy breeders in Germany. This set of chickens encompassed the breeds Bergische Kräher ($n = 4$), Krüper ($n = 4$), Lakenfelder ($n = 4$), Ostfriesische Möwen ($n = 4$), Thüringer Barthühner ($n = 4$) and Westfälische Totleger ($n = 4$).

mtDNA amplification and sequencing

Primers mtGlu-F (5′-GGCTTGAAAAGCCATTGTTG-3′) and mtGlu-R (5′-CCCCAAAAAGAGAAGGAACC-3′) were used to amplify a fragment of 455 bp from the D-loop region of the chicken mitochondrial genome. These primers are located at bases 16739–16775 (forward primer) and 649–668 (reverse primer) of the complete mtDNA sequence of domestic chickens (X52392, Desjardins & Morais 1990). The M13-F (5′-GTAAAACGACGGCCAG-3′) and M13-R (5′-CAGGAAACAGCTATGAC-3′) sequences were linked to the 5′ end of each of these D-loop primers. These universal sequences were used as target sites for sequencing primers after generating PCR products. PCR amplifications were based on the HotStart Taq Master Mix (Qiagen). The PCR products were purified using the ExoSAP-IT Purification Kit (USB Corp.) and sequenced with fluorescently labelled primers complementary to the universal M13-F and M13-R sequences respectively. Forward and reverse sequences were

[2]AVIANDIV EC Contract No. BIO4-CT98-0342 (1998–2000); Weigend, S. (Coordinator), M.A.M. Groenen, M. Tixier-Boichard, A. Vignal, J. Hillel, K. Wimmers, T. Burke, and A. Mäki-Tanila (http://w3.tzv.fal.de/aviandiv).

obtained using the Thermo Sequenase Cycle Sequencing Kit (USB Corp.). Sequencing products were visualized on a 6% polyacrylamide gel on a LICOR DNA sequencer. The forward and reverse DNA sequences were aligned using the ALIGNIR software program (LICOR Inc.).

Sequence variation and haplotype diversity

The position and number of variable sites as well as corresponding haplotypes were computed with MEGA version 3.1 (Kumar *et al.* 2004). The number of unique haplotypes and their distribution in the samples were computed with TCS software (Clement *et al.* 2000).

Within-population diversity

The 283 individual sequences were grouped according to their original population. Haplotype diversity (*h*), which is the probability that two haplotypes sampled within a population are different (Nei 1973), was calculated using ARLEQUIN software (Excoffier *et al.* 2006).

Determination of population substructuring

Analysis of molecular variance (AMOVA) was computed with the algorithms suggested by Excoffier *et al.* (1992), as implemented in the ARLEQUIN software. Molecular variance components were estimated among and within (i) all the 14 populations, (ii) the five Zimbabwean ecotypes, (iii) the African populations including one population from Malawi, one population from Sudan and the five Zimbabwean ecotypes, and (iv) the six purebred lines respectively.

Network analysis of haplotypes

To determine the relationships of haplotypes and link the populations under study to the presumed centres of domestication, median joining networks were constructed following the algorithms of Bandelt *et al.* (1995) and using the NETWORK 4.1 software package (http://www.fluxus-engineering.com/sharenet.htm). For each of the observed clades, the total number of individuals from the 14 populations under study, the haplotype diversity and the level of divergence from other clades were computed with ARLEQUIN. In addition, the network analysis included seven haplotypes representing the seven main clades (Clades A–G) in Japanese chicken populations (Oka *et al.* 2007) and nine haplotypes representing the nine clades (Clades A–I) in the Chinese and Eurasian region (Liu *et al.* 2006). The list of haplotypes used and the corresponding GenBank accession numbers are provided in Table S1. The haplotypes from GenBank were aligned to haplotypes observed in this study using the ALIGNIR software program. Extra nucleotide bases in the GenBank sequences that were outside the 455-bp region sequenced in our study were excluded from analysis. Various networks were constructed by using different epsilon values ranging from zero to 20 as well as by applying different weights according to the information content of the mutated positions. There were no considerable differences among the different networks except a slight increase in the network connections where clades joined. The median network presented used an epsilon value of 15 and weights that ranged from 2 for most frequent mutating sites (occurring 11 times) to 22 for mutated sites that occurred only once.

Results

Sequence variation and haplotype distribution

In total, 32 variable sites that defined 34 haplotypes were observed in the Zimbabwean chicken ecotypes, Malawian, Sudanese and Northwest European chickens and six purebred lines. All variable sites were due to substitution mutations, 94% of which were transitions (Fig. S1).

A major haplotype (C3) occurred at a frequency of 25% over all populations and was widely distributed in four Zimbabwean ecotypes (Eco-I to Eco-IV), in three of the purebred lines (LS_S, BL_A and BRD_A), in 54% of the Northwest European chickens and in 80% of the Sudanese chickens (Table 1). The second major haplotype (A1), which occurred at a frequency of 21.9% across all populations, was common to all Zimbabwean ecotypes (found in 52% of the Zimbabwean chickens) and was found in 90% of the Malawian chickens. Other frequent haplotypes included C8, which was found in a total of 25 individual chickens from the white egg layer line LS_S ($n = 11$), broiler lines BRS_A ($n = 7$) and BRD_A ($n = 6$) and Northwest European lines ($n = 1$). Haplotype B4 occurred at an overall frequency of 7.4% and was found in the two broiler lines ($n = 19$) and Northwest European chickens ($n = 2$). Haplotype B1 occurred at an overall frequency of 6.3% and was observed only in the white egg layer line (WL_A, $n = 18$).

Within-population diversity

All 14 populations were polymorphic, with the number of haplotypes ranging from two (LS_S line) to seven (Eco-IV) (Table 2). Haplotype diversity varied from 0.29 to 0.78 and was low in the chickens from Malawi and Sudan and in the white egg layers. In contrast, haplotype diversity was high and averaged 0.65 in the Zimbabwean ecotypes. Higher haplotype diversity estimates were found in the two brown egg layer lines (0.72–0.78) and in the broiler dam line (0.78).

Population structure

Across all the populations studied, variation among populations was 46.58% of the total variation while the remaining 53.42% was due to the diversity within populations (Table 3). Considering only the Zimbabwean chicken

Table 1 Distribution of mtDNA D-loop haplotypes in five Zimbabwean chicken ecotypes, Malawian and Sudanese chickens, six purebred lines and local Northwest European chickens.

Haplotype	Eco-I	Eco-II	Eco-III	Eco-IV	Eco-V	MAL	SUD	LS_S	WL_A	BL_A	BL_C	BRS_A	BRD_A	NWE	Total
A1	12	9	11	6	8	16									62
A2	1			1											2
A3	1														1
A4	1														1
A5			1		2	2									5
A6				1											1
A7						1									1
B1									18						18
B2									1						1
B3										6	6				12
B4												12	7	2	21
B5											1				1
B6											1				1
B7													1		1
C1	3	1			8									2	14
C2	1	1	2		2				1	8				4	19
C3	1	8	5	9			16	9		5			4	13	70
C4		1													1
C5				1											1
C6							3								3
C7							1								1
C8								11				7	6	1	25
C9									1						1
C10										1					1
C11												1			1
C12											7				7
C13											1				1
C14											4				4
C15													1		1
C16													1		1
C17														1	2
C18														1	1
C19				1											1
C20				1											1
Total	20	20	19	20	20	19	20	20	21	20	20	20	20	24	283

Eco-I to Eco-V, the five Zimbabwean ecotypes; MAL, Malawi; SUD, Sudan; LS_S, experimental white egg layer line; WL_A, commercial white egg layer line A; BL_A, commercial brown egg layer line A; BL_C, commercial brown egg layer line C; BRS_A, commercial broiler sire line A; BRD_A, commercial broiler dam line A; NWE, local Northwest European chicken breeds.

populations, a high level of diversity within ecotypes was found that accounted for 96.79% of the total variation, and only 3.21% of the variation in the Zimbabwean chicken populations was among ecotypes. In contrast, 39.02% of the total variation in the group of purebred lines was due to the diversity among lines. The total variance was greater for the six purebred lines than for the African group and was least for the Zimbabwean chickens. Large and significant ($P < 0.001$) F_{ST} values were observed for the purebred lines and the African chickens.

Network analysis of haplotypes

The 34 haplotypes observed in the Zimbabwean ecotypes, Malawian, Sudanese and Northwest European chickens, and in the six purebred lines clustered into three main clades (Fig. 1). Clade A centred on haplotype A1 and was made up of haplotypes from chicken populations of Zimbabwe and Malawi. Distances between haplotypes in this clade ranged from one to five mutations. Including sequences from GenBank in the analysis, haplotypes in this clade clustered with haplotype C1 from Clade C of Oka *et al.* (2007) and haplotype D1 (Clade D) of Liu *et al.* (2006). Clade B split into two subclades, B1 and B2. Subclade B1 centred around haplotype B1 and consisted of individuals mainly from white and brown egg layers. Subclade B2 consisted of one haplotype, B4, and included broiler dam and sire lines (BRS_A and BRD_A) and a small fraction of Northwest European breeds. Haplotype B1 and B4 were separated by five mutations. Haplotype B3 from Subclade

Table 2 Number of polymorphic sites, number of mtDNA D-loop haplotypes and haplotype diversity of chicken populations from five Zimbabwean ecotypes, Malawi, Sudan, Northwest Europe, six purebred lines and the observed mtDNA D-loop clades.

Population	*N*	No. of polymorphic sites	No. of haplotypes	Haplotype diversity (±SE)
African				
Eco-I	20	12	7	0.64 ± 0.12
Eco-II	20	9	5	0.66 ± 0.07
Eco-III	19	9	4	0.61 ± 0.10
Eco-IV	20	13	7	0.73 ± 0.08
Eco-V	20	10	4	0.69 ± 0.06
MAL	19	2	3	0.29 ± 0.13
SUD	20	2	3	0.35 ± 0.12
NWE	24	5	6	0.64 ± 0.10
Purebreds				
LS_S	20	1	2	0.52 ± 0.04
WL_A	21	13	4	0.27 ± 0.12
BL_A	20	9	4	0.72 ± 0.05
BL_C	20	11	6	0.78 ± 0.06
BRS_A	20	4	3	0.54 ± 0.08
BRD_A	20	13	6	0.78 ± 0.06
mtDNA clades				
Clade A	73	8	7	0.28 ± 0.07
Clade B	55	11	7	0.71 ± 0.03
Clade C	154	15	19	0.75 ± 0.03

Eco-I to Eco-V, the five Zimbabwean ecotypes; MAL, Malawi; SUD, Sudan; NWE, local Northwest European chicken breeds; LS_S, experimental white egg layer line; WL_A, commercial white egg layer line A; BL_A, commercial brown egg layer line A; BL_C, commercial brown egg layer line C; BRS_A, commercial broiler sire line A; BRD_A, commercial broiler dam line A.

Table 3 Partition of mtDNA D-loop variance within and among different population categories and the level of population substructuring (F_{ST}).

	Components of variance (% variation)			
Level of analysis	Within population	Among populations	Total	F_{ST}
Five Zimbabwean ecotypes	2.03 (96.79)	0.07 (3.21)	2.11	0.03^{NS}
Seven African populations	1.51 (70.46)	0.63 (29.54)	2.14	0.30***
Six purebred lines	1.89 (60.98)	1.21 (39.02)	3.12	0.39***
All 14 populations	1.69 (53.42)	1.47 (46.58)	3.16	0.47***
Clades (haplogroups)	0.67 (15.35)	3.70 (84.65)	4.37	0.85***

NS, not significant.
***F_{ST} significantly >0 ($P < 0.001$).

B1 was the same as the partial sequence of haplotype B1 from Clade B of Oka *et al.* (2007) and haplotype A1 from Clade A of Liu *et al.* (2006). Haplotype B4 from Subclade B2 resembled the partial sequence of haplotype E1 (Clade E) from Oka *et al.* (2007) and haplotype B1 (Clade B) from Liu *et al.* (2006). Clade C was made up of haplotype C3 at the centre surrounded by haplotypes from a wide geographic range (Zimbabwe, Sudan and all purebred lines and Northwest European chickens). The distance between haplotypes ranged from one to four mutations. Haplotype C3 resembled the partial sequence of haplotype A3 (Clade A) from Oka *et al.* (2007) and haplotype E1 from Clade E of Liu *et al.* (2006).

All five Zimbabwean ecotypes were equally represented in Clades A and C. The total number of haplotypes and the haplotype diversity values (h) of different clades are given in Table 2. A single haplotype (haplotype C20) could not be assigned to either Clade B or C and was not used in further analyses. Haplotype diversity was very low in Clade A compared with Clades B and C. Within-clade diversity accounted for 15.35% of the total variation, and the remaining 84.65% was due to variation among clades (Table 3).

Discussion

All 14 populations were polymorphic for the mtDNA D-loop region and had 34 haplotypes (Table 2). Compared with the white egg layers, Malawian and Sudanese chickens, the Zimbabwean ecotypes exhibited higher genetic variation (Table 2). The AMOVA results indicated that there was no substructuring of the Zimbabwean population. This is in agreement with our previous report using microsatellites (Muchadeyi *et al.* 2007), where genetic differentiation among local chickens of distinct eco-zones in Zimbabwe could not be demonstrated at the autosomal level. The purebred chicken lines, on the other hand, exhibited higher among-population variation because of substructuring into separate populations. A similar partitioning of within- and among-line diversity was observed at the autosomal level (Muchadeyi *et al.* 2007).

Despite the absence of among-ecotype substructures, the network analysis showed clearly distinct maternal lineages that existed and were evenly distributed in the five eco-regions of Zimbabwe. Overall, the 34 haplotypes observed in this study clustered into three divergent clades (>80% among-clade diversity).

Clade A was unique to Zimbabwe and Malawi and was not found in purebred commercial and experimental lines or in Northwest European local chickens. 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). Haplotypes from Clade A of our study also clustered 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). Oka *et al.* (2007) suggested that this clade has its roots in Southeast Asia. Liu *et al.* (2006), on the other hand, suggested that their Clade D was a product of recent domestication events in Southwest China and/or surrounding regions (Vietnam, Burma, Thailand and India).

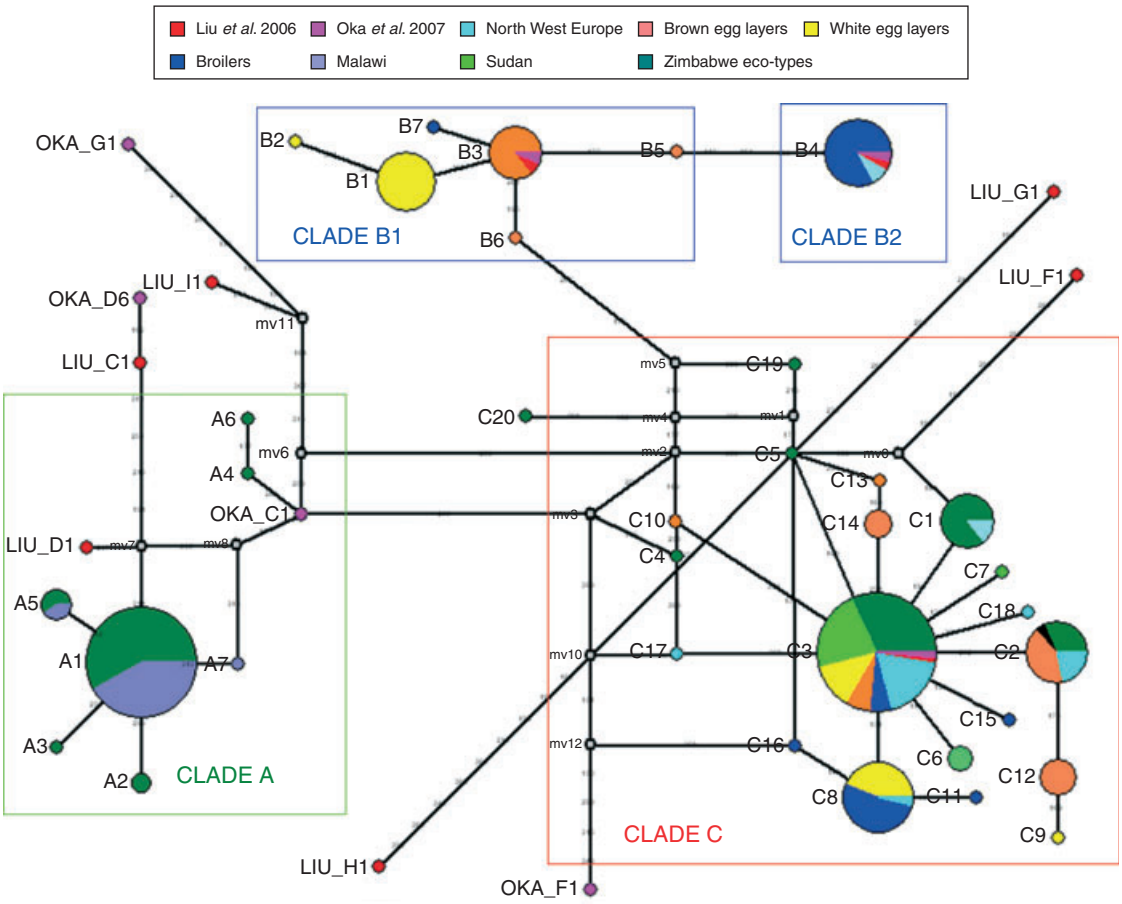

Figure 1 Median network profile of the mtDNA D-loop haplotypes observed in the current study as well as those from Liu *et al.* (2006) and Oka *et al.* (2007). The circle size corresponds to haplotype frequency, and the numbers on the line correspond to mutational positions connecting haplotypes. Empty circles are median vectors used in connecting indirectly related haplotypes.

The second haplogroup (Clade C) was common to Zimbabwean, Sudanese, Northwest European chickens and six purebred lines. This group is the same as 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. It is also similar to Clade E from Liu *et al.* (2006), which included chickens mainly from Europe, the Middle East and India. The maternal lineages associated with this clade could have originated from the Indian subcontinent (Liu *et al.* 2006). 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. 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. These populations include Zimbabwean and other African chickens, purebred commercials and experimental lines as well as Northwest European native breeds.

A third haplogroup (Clade B) was the most common in purebred broiler and layer lines and some Northwest European native chickens. This clade was not found in Zimbabwean chickens or in other African chickens. The two subclusters of these clades (Clade B1 and Clade B2) resembled Clades B and E respectively of Oka *et al.* (2007). Oka's Clade B was found in most of the Ko-Shamo fighting cocks and in commercial Rhode Island Red and White Leghorn chickens, while Oka's Clade E was observed in Shamo and Indonesian fighting cocks. Oka's Clade E sequences resembled those observed in Shamo from China and Myanmar and in several other Chinese native chicken populations. The two subclusters also resembled Clades A and B of Liu *et al.* (2006), which were mainly distributed in South China and Japan. Although distant from each other (five mutations apart), the two subclades (B1 and B2) are related and could be interpreted as being a single clade. Diversity within these subclades was low, particularly for subclade B2, which was made up of a single haplotype (B4). Treatment of these subclades as a single cluster resulted in elevated within-clade diversity (Table 2) because of the large distance between haplotypes B1 and B4. Oka *et al.* (2007) suggested that their Clade B, which is our subclade B1, was derived from Clade E, which corresponded to Clade B2 in our study. According to Liu *et al.* (2006), their Clades A and B, which corresponded to Clades B and E in Oka *et al.*

(2007), and our Clades B1 and B2 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). Oka *et al.* (2007), on the other hand, suggested that these Japanese populations may have originated in Southeast Asia.

Results from this study, like those of previous studies, indicate that there are at least three distinct maternal lineages from which current domestic chicken populations were derived. The five Zimbabwean ecotypes and the purebred lines each have a unique lineage (Clade A for Zimbabwean ecotypes and Clade B for purebred populations) plus one common maternal lineage among them (Clade C). In contrast, the Malawian and Sudanese populations form single clusters. However, there is a possibility that the limited sampling area of the Malawian and Sudanese chickens excluded other maternal lineages that might exist in these chicken populations. Results indicate that the chickens from the five agro-ecological zones of Zimbabwe came from Southeast Asia and the Indian subcontinent. Other than the single haplotype C20 that is positioned between Clade C and B, it seems that the Zimbabwean and the other African chicken populations studied had no direct link to Clade B, whose maternal lineages seem to have originated from Yunnan and/or the surrounding areas (Liu *et al.* 2006) or from Southeast Asia (Oka *et al.* 2007).

Although the five Zimbabwean eco-types, the Malawi and Sudanese chickens and the six purebreds formed distinct population clusters based on microsatellite data (Muchadeyi *et al.* 2007), these 13 populations shared the major mtDNA haplogroups. Unlike autosomal genetic markers, which are more sensitive to genetic drift because of their different mode of inheritance, mtDNA genetic structures tend to be maintained despite genetic isolation or population interbreeding. The genetic differentiation of the Zimbabwean and reference populations revealed by microsatellite analysis can therefore be explained by assuming recent genetic isolation and restricted gene flow among populations that shared some ancestral maternal lineages.

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Supporting information

Additional supporting information may be found in the online version of this article.

Figure S1 Variable sites for the 34 haplotypes observed in chickens of five Zimbabwe eco-types; native chickens from Malawi, Sudan and Northwest Europe; and six purebred lines. Dots indicate nucleotide positions identical to those of haplotype A1. Numbers at the top refer to variable sites and correspond to the nucleotide positions of haplotype A1 (AM746024).

Table S1 Haplotype names and accession numbers.

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