



Short Communication

Was chicken domesticated in northern China? New evidence from mitochondrial genomes

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The timing and location of chicken (*Gallus gallus domesticus*) domestication have remained controversial for over a century [1]. Several researchers have proposed China as one of the early centers for chicken domestication [2]. This is based on a few archaeological “chicken” remains discovered in China (Table S1). Recently, one investigation detected mitochondrial DNA (mtDNA) haplotypes of modern red junglefowl (*G. gallus*), that is, the main wild ancestor of domestic chicken, in ancient DNAs extracted from the ancient “chicken remains” excavated in northern China. The authors proposed an early Holocene chicken domestication in northern China [3]. However, their conclusion was subsequently questioned by

reappraisals of sequence data and archaeological evidence [4,5]. The debate on chicken domestication in northern China continues.

If the chicken was domesticated in northern China, can any genetic footprint be detected in the modern chickens? Our preliminary analyses of a published mtDNA dataset [6] reveals that sub-haplogroup C1, defined by D-loop mutation motif (212-242-243-246-256-@261-281-@306-310-315-363-367), is widely distributed in northern China (i.e. north of the Huai River-Qin Mountains line), southern China (i.e. south of the Huai River-Qin Mountains line), Korea, and Japan. However, it is rare or absent in southwest China, Southeast Asia, and South Asia. Additionally, the sub-haplogroup C1 is absent in red junglefowl samples but being restricted to domestic chickens only. These patterns suggest sub-haplogroup C1 as a potential candidate marker for exploring the demographic history of chickens in northern China.

To shed more light on the origin of sub-haplogroup C1, we survey mtDNA variations in a wide range of domestic chicken and red

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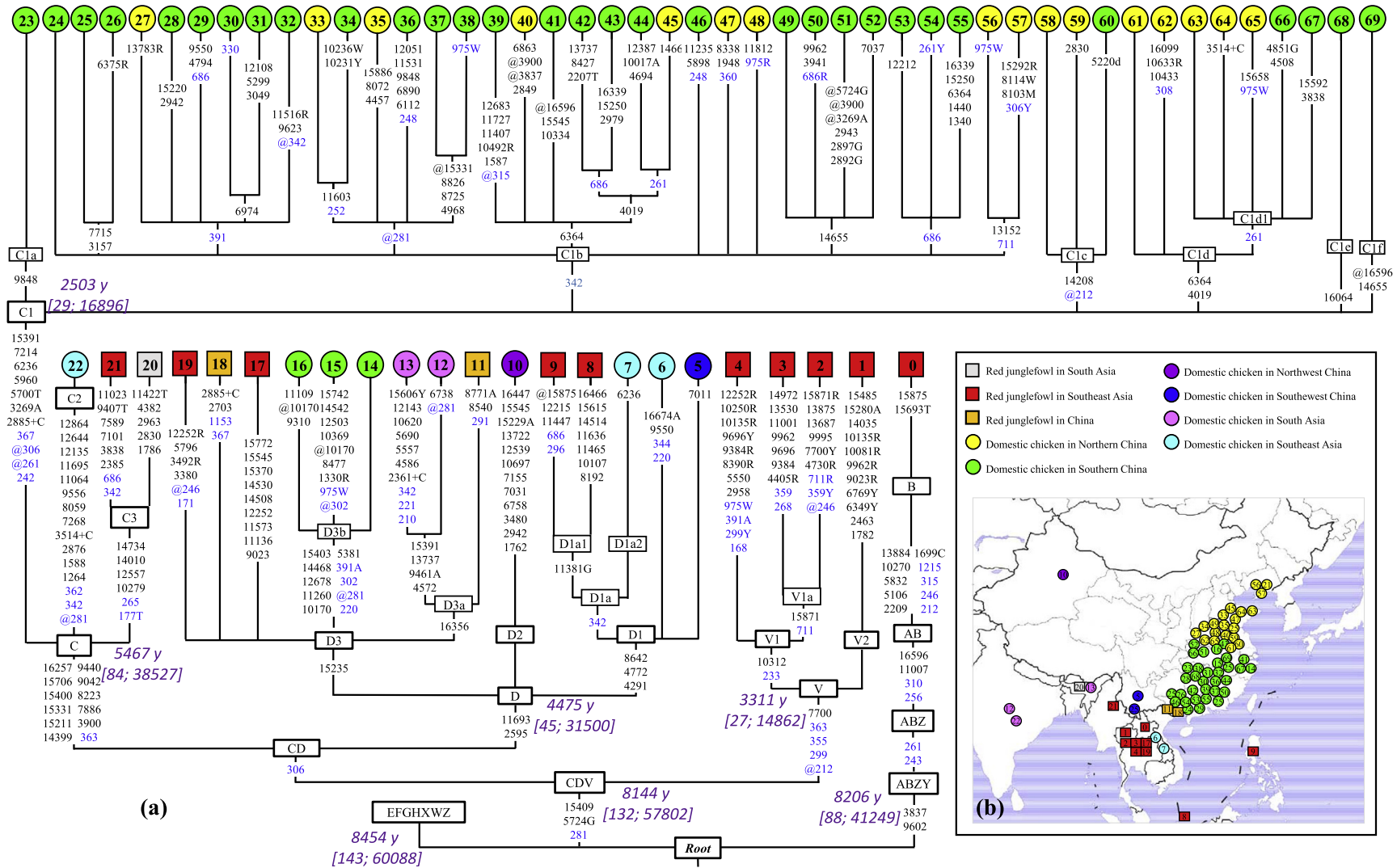


Fig. 1. Classification tree of chicken mtDNA haplogroup CDV. (a) The tree includes 76 complete sequences and illustrates sub-haplogroup affiliations (see Table S4). Some sequences shared by different haplotypes are labeled with the same number, i.e. 20, 21, and 25. Sequences 1 to 4, 15 to 19, 25, 26, 28 to 35, and 37 to 69 are newly collected, while part of 25 and others are retrieved from the GenBank. Red junglefowls are indicated with squares while domestic chickens are indicated with circles. The nucleotide positions in the sequences were scored relative to the reference sequence AP003321. Transitions are shown on the branches and transversions are further annotated by adding suffixes. Deletions and insertions are indicated by "d" and "+", respectively. Synonymous replacements are marked in blue. The prefix @ designates back mutation. "R", "W", and "Y" specify the heteroplasmic status of A/G, A/T, and C/T, respectively, at given sites. The length variations scored at np 859 (859d) and around 3941 (3946 + C, 3946 + CC, 3946 + CCC, 3945d, 3946d) are not considered. The divergence time of major haplogroups are shown for each of major nodes. (b) The map shows the geographic distribution of samples.

junglefowl populations from East and Southeast Asia. Especially, more chicken samples from northern China are incorporated. Detailed methods were described in Electronic [supplementary materials](#). Out of 6760 mtDNA D-loop sequences (1780 de novo; [Fig. S1](#) and [Table S2](#)), 1089 can be classified into mtDNA haplogroup CD ([Table S3](#)). We select 54 samples from our collection for mtDNA genome sequencing. These samples cover 24 different mtDNA D-loop haplotypes from vast regions of East Asia and Southeast Asia ([Table S4](#)). Incorporating 22 published mtDNA genomes, we reconstruct mtDNA haplogroup tree for haplogroup CD ([Table S4](#); [Fig. 1](#)). Some lineages of red junglefowls from Thailand with D-loop motif @212-243-246-256-261-281-299-@306-310-315-355-363 previously assigned into haplogroup C* ([Table S2](#)) are re-clustered as a new haplogroup V, appearing at the basal branch of haplogroup CD. Our definitions of sub-haplogroups C1-C3 and D1-D3 within haplogroups C and D, respectively, are in agreement with the previous phylogeny [6,7]. Within haplogroup C, two minor sub-haplogroups C2 and C3 are represented across in Southeast Asia and Northeast India, whereas the major sub-haplogroup C1 is distributed widely in China. To depict the phylogeographic patterns for sub-haplogroups of C1, we assign each of the 1089 D-loop sequences into haplogroups in the context of updated haplogroup tree for CDV ([Fig. 1](#)). We construct median-joining network ([Fig. S2](#)) and frequency contour map ([Fig. S3](#)). The sequences assigned to C* belong to red junglefowls living in South Asia. All above evidence points out the origin of haplogroup C in Southeast Asia.

To clarify the potential center of expansion for C1, we evaluated the genetic diversity in different geographical areas by employing all available D-loop (nps 167–446) data. The highest values of haplotype and nucleotide diversity are observed in northern China, particularly in Yellow-Huai River Basin ([Table S5](#)). Interestingly, the similar patterns are also detected in haplogroup A ([Table S6](#)). Investigation of genetic diversity and spatial frequency distribution suggests that Yellow-Huai River Basin is most likely the center of early expansion of sub-haplogroup C1. Although sub-haplogroup C1 has frequency peaks in Northwest China and Japan ([Fig. S3](#)), the indexes of genetic diversity in the two regions are lower than those in the Yellow-Huai River Basin ([Table S5](#)). The network analysis shows that lineages from Northwest China as well as Korea and Japan share the same haplotypes with those from northern and southern China, or occur as derived haplotypes ([Fig. S2](#)). Thus, geographic areas of northwest China, Korea and Japan are not considered as the potential center(s) of expansion for sub-haplogroup C1.

The large number of complete mtDNA genomes allowed us to estimate the coalescence ages of haplogroups based on a molecular rate of 3.13×10^{-7} mutations/site/year (95% confidence interval 3.75×10^{-8} – 1.12×10^{-6}) [8]. As compared with those with the relaxed clock model, the estimates under the strict clock model are preferred ([Table S8](#)). So we only consider the results of the strict clock. The age of macro-haplogroup CDV is estimated to be 8.1 thousand years ago (kya; 95% highest posterior density (HPD): 132–57,802 years), which is similar to the estimates for EFGHWX and ABZY ([Fig. 1](#), [Table S8](#)). The coalescence ages of C and C1 are dated as 5.5 kya and 2.5 kya, respectively. These indicate the dispersal starting around the middle Holocene from Southeast Asia to East Asia. The age of sub-haplogroup C1 ~2.5 kya (95% HPD: 29–16,896 years) is much recent compared to the time proposed for chicken domestication in northern China [3]. It is even more recent than the most solid archaeological evidence for chicken remains in northern China, around 3.6 kya [9]. Nevertheless, the mutation rate used in this study is based on pedigree estimates. It is generally more rapid as compared with the rates estimates based on fossil calibrations [8]. Its large 95% confidence interval also makes the 95% HPD of estimated ages range widely ([Fig. 1](#), [Table S8](#)). It is expected that using ancient mtDNA

genomes at multiple collaboration points will further improve the timescale estimates in the future.

In modern chicken samples, sub-haplogroup C1 is concentrated in game breeds ([Table S2](#)). Interestingly, the earliest cockfighting in northern China has been recorded at 517 BCE [10], which is in line with the estimated age of sub-haplogroup C1 (~2.5 kya). Our results raise the possibility that the expansion of sub-haplogroup C1 resulted from the breeding and dispersal of gamefowl in East Asia. This is supported by the parallel genetic evidence from haplogroup A. First, haplogroup A occurs in East Asian gamefowl with high frequencies, such as 66.7% in Luxi and 37.8% in Tulufan ([Table S9](#)). Second, the high genetic diversity indexes point to the Yellow-Huai River Basin of northern China as its likely center of early expansion ([Table S5–S6](#)). Third, the age of haplogroup A is dated around 2.2 kya (95% HPD: 24–15,238 years; [Table S8](#)). The similar patterns observed in both haplogroup A and sub-haplogroup C1 suggest that the two lineages may have shared the same demographic trajectory. Thus, our results present evidence that the recent demographic events (for gamefowl) played substantial roles in shaping the chicken maternal genetic pool in northern China.

In conclusion, our analyses of sub-haplogroup C1 and haplogroups A reveal the recent expansion of domestic chicken in northern China at least from a maternal perspective. The molecular dating does not support the scenario of early Holocene chicken domestication in northern China, although the time estimates with a huge 95% HPD should be treated with caution. For in-depth unraveling of the chicken domestication in northern China, more efforts in analyzing genome-wide markers as well as ancient DNA are required.

Conflict of interest

The authors declare that they have no conflict of interest.

Data accessibility

Sequences generated in this study have been deposited in the Genome Sequence Archive (<http://bigd.big.ac.cn/gsa/>; accession Nos. PRJCA000347).

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.scib.2017.12.004>.

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Xunhe Huang is an associate professor at Jiaying University. Dr. Huang's recent research is focused on genetic diversity and molecular evolution of Chinese chickens. The goal of the study is to depict the geographic distribution pattern of mtDNA haplotype and the potential breeding center of domestic chicken in China.