



Multiple maternal origins of chickens: Out of the Asian jungles [☆]

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Abstract

Domestic chickens have long been important to human societies for food, religion, entertainment, and decorative uses, yet the origins and phylogeography of chickens through Eurasia remain uncertain. Here, we assessed their origins and phylogeographic history by analyzing the mitochondrial DNA hypervariable segment I (HVS-I) for 834 domestic chickens (*Gallus gallus domesticus*) across Eurasia as well as 66 wild red jungle fowls (*Gallus gallus*) from Southeast Asia and China. Phylogenetic analyses revealed nine highly divergent mtDNA clades (A–I) in which seven clades contained both the red jungle fowls and domestic chickens. There was no breed-specific clade in the chickens. The clades A, B, and E are distributed ubiquitously in Eurasia, while the other clades were restricted to South and Southeast Asia. Clade C was mainly distributed in Japan and Southeast China, while clades F and G were exclusive to Yunnan, China. The geographic distribution of clade D was closely related to the distribution of the pastime of cock fighting. Statistical tests detect population expansion within each subclade. These distinct distribution patterns and expansion signatures suggest that different clades may originate from different regions, such as Yunnan, South and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, and Thailand), and the Indian subcontinent, respectively, which support the theory of multiple origins in South and Southeast Asia.

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1. Introduction

The domestic chicken is among the most popular and widely spread domestic animal species. For thousand years, chickens have been used for food, religious activities, decorative arts, and entertainment. The chicken is the only widespread domestic species that apparently did not have origins in the Near or Middle East. The most probable wild progenitor of the domestic chicken belongs to the genus *Gallus*, however, the progenitor and the location of domestication remain controversial.

[☆] Electronic-Database Information: Accession numbers and URLs for the sequence data of mtDNA control region in this article are as follows: GenBank, <http://www.ncbi.nlm.nih.gov/web/Genbank/> under Accession Nos. AF512057–AF512060, AF512062–AF512337, AY392172–AY392407, AY642127–AY642134, and AY644966–AY665020.

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Archaeological discoveries in the Indus Valley and in Hebei Province, China, suggest that chickens were probably domesticated from the red jungle fowl (*Gallus gallus*), as early as 5400 BC (West and Zhou, 1988). Historically, there have been two hypotheses about chicken domestication: one that defends a monophyletic origin and another that defends multiple origins from several *Gallus* subspecies (Crawford, 1990, 1995). There are five possible progenitor subspecies of the red jungle fowl—*G. g. gallus* in Thailand and its adjacent regions, *G. g. spadiceus* in Burma and Yunnan Province of China, *G. g. jabouillei* in southern China and Vietnam, *G. g. murghi* in India, and *G. g. bankiva* in the Java islands (Crawford, 1990, 1995; Delacour, 1957; Howard and Moore, 1984). It is still not clear how many subspecies have contributed to the origin of chicken.

The first molecular genetic study defending a monophyletic origin of chicken was conducted by Fumihito and colleagues (1994, 1996). These authors used mitochondrial control region sequences of *Gallus* species and domestic chickens, and suggested that: (i) domestic chickens have a monophyletic origin, (ii) the continental population of the red jungle fowl subspecies (*G. g. gallus* in Southeast Asia) sufficed as the sole ancestor of all domestic chickens, and (iii) all the domestic breeds might have originated from a single domestication event that occurred in Thailand and adjacent regions (Fumihito et al., 1994, 1996). The red jungle fowl as the main progenitor of the domesticated chicken was further supported by nuclear microsatellite data from range of chicken populations (Hillel et al., 2003).

After Fumihito team studies, other scholars suggested the possibility of multiple origins of domestic chicken without providing genetic evidence (Crawford, 1990; Moiseyeva, 1998).

In addition, molecular evidence for hybridization between species in the genus *Gallus* raised the possibility that the other jungle fowl species were also progenitors of the domestic fowl (Nishibori et al., 2005), which makes this issue more complicated.

There were some limitations in Fumihito et al.'s study (1996): absence of samples from both domestic and wild subspecies of red jungle fowls from China and India, and the use of small sample sizes (e.g., *G. g. gallus*, $n=6$; *G. g. bankiva*, $n=3$; *G. g. spadiceus*, $n=3$; and *G. g. domesticus*, $n=9$; Fumihito et al., 1996). Later, Miyake sequenced more samples and the data were only reported in GenBank (Accession Nos. AB009427–AB009449, AB007718–AB007758). Among these samples, only seven individuals were from China. Our preliminary re-analyses of their 78 *G. gallus* mtDNA control region hypervariable I sequences (HVS-I) (Accession Nos. AB007719–AB007726, AB007728–AB007758; AB009427–AB009429, AB009432–AB009449, D82900–D82908, and D82916–D82923, D82925, see also Table S1) revealed at least five distinct phylogenetic clades. More recent mtDNA work on Japanese gamecocks showed four distinct phylogenetic clades (Komiyama et al., 2003). These results revealed several important new clues about the domestication of chickens:

the monophyletic origin conclusions from early studies might result from incomplete sampling of domestic chickens and red jungle fowl.

Here, we aim to demonstrate that our sampling of more wild red jungle fowl and domestic chickens from China and adjacent countries helps to discern the origin(s) of the domestic chicken in Eurasia. We assessed the details of origin and diffusion of the domestic chicken by analyzing the mtDNA HVS-I for 834 domestic chickens (*G. g. domesticus*) across Eurasia as well as 66 red jungle fowls (*G. g. gallus*) from the regions of Southeast Asia and China. Our results suggest that chickens have multiple maternal origins and that domestications occurred in at least three regions of South and Southeast Asia.

2. Materials and methods

2.1. Sampling

Blood samples of 478 individuals were collected from 31 indigenous chicken populations from small remote villages, in avoidance of sampling recent introduced individuals or crosses of the commercial lines across Eurasia with emphasis on China (see Fig. S2 and Table S1). Also samples from domestic chickens from Europe, India, Indonesia, Malaysia, and Middle East (Iran, Azerbaijan, and Turkmenistan) were also included in this study (Table S1 and Fig. S2). In addition, 38 red jungle fowl samples were also included from which: 35 were *G. g. spadiceus* (nine from Burma, 26 from Yunnan Province, China); three were *G. g. jabouillei* (from Yunnan Province, China). Finally, published data (Table S1) assembled from GenBank were also included in our analysis. Among them, 78 mtDNAs were from Chinese native chickens; 95 mtDNA sequences were from gamecocks; 31 mtDNAs were from red jungle fowls (three *G. g. bankiva*, 22 *G. g. gallus*, and six *G. g. spadiceus*); the other mtDNAs were from domestic chickens mainly from Japan and insular Southeast Asia.

2.2. DNA amplification and sequencing

Genomic DNA was extracted by standard phenol/chloroform methods. The HVS-I sequence was amplified and sequenced using primers L16750 (5'-AGGACTACGGCTTGAAAAGC-3'; Fumihito et al., 1994) and H522 (5'-ATGTGCCTGACCGAGGAACCAG-3'; Fu et al., 2001). The numbers in the primer names indicate the homologous positions of 3' end of the primers on the mtDNA complete sequence of *G. g. domesticus* (Desjardins and Morais, 1990). L and H refer to light and heavy strands, respectively. PCRs were performed in a 50 μ l volume [500 mM Tris-HCl (pH 8.3), 0.1% Triton X-100, 2.5 M KCl, 75 mM MgCl₂, 5 mM of each dNTP, 10 pM of each primer, and 1 U of *Taq* polymerase (S_{ABC})] following 35 cycles of 1 min at 94 °C, 1 min at 63 °C, and 1 min at 72 °C. PCR products were purified on spin columns (Watson Biotechnologies, Shanghai) and were directly sequenced for both strands by using

BinsgDye Terminator Cycle Sequence Kit (ABI Applied Biosystems) according to the manufacturer's manual.

2.3. Data analysis

The raw sequences obtained were edited and aligned using the DNASTar package (DNASTAR). At first, we constructed an unrooted neighbor-joining (NJ) tree of all the haplotypes under the Kimura 2-parameter model using MEGA 3 software (Kumar et al., 2004), then, to investigate the possible relationships among the sequences of each major clade in the NJ tree, median-joining networks were constructed using the program Network 3.1 (<http://www.fluxus-engineering.com/sharenet.htm>). The nucleotide diversity (π) was computed for the major clades ($N > 30$) using Arlequin 2.0 (Schneider et al., 2000). Finally, to detect signatures of population expansion a Fu's F_s test (Fu, 1997) was also applied (using Arlequin 2.0).

3. Results

We obtained 542 mtDNA HVS-I sequences from domestic chickens (see Table 1 for locations) and 38 red jungle fowls from China and Myanmar. A total of 90 variable sites were identified. No insertion/deletions (indels) were detected in our sequences. The published sequences did harbor several indels compared with our data; these indels were discarded in the following analyses. Two reported sequences (Accession Nos. AY588607 and AY465967) were not included as we suspect possible sequencing errors. Taken together, our and the published data, 103 variable sites and 169 haplotypes were found in total 834 domestic chickens and 66 red jungle fowls (Fig. S1).

Seven haplotypes were shared by both the domestic chickens and the red jungle fowls. Some haplotypes were shared between different subspecies, such as haplotype B1 shared by two *G. g. gallus* individuals, one *G. g. spadiceus* individual. Also one sample from *G. g. spadiceus* had the same sequence (haplotype D15) as a *G. g. jabouillei* individual. Two domestic chicken samples from India and one chicken sample from Fujian Province, China, shared haplotype D1 with 10 *G. g. spadiceus* individuals.

3.1. Phylogeny of the haplotypes and network profiles of the major clades

The unrooted NJ tree of the 169 haplotypes (Fig. 1) reveals nine divergent clades (A–I). Among them, clade H contained only samples of red jungle fowls, while clade C was only composed of domestic chickens. Each of the remaining seven clades comprised samples both from the red jungle fowls and the domestic chickens. A finer analysis showed that the potential roots of each of the nine clades differed from each other by at least five mutations (see Fig. 2A).

In each of the six clades (A, B, C, E, F, and G), there was a dominant haplotype, which had a relatively wider geographic distribution, with a number of domestic chickens

sharing that haplotype ranging from 30 to 121 (Fig. 2 and Table S1). However, clade D was much more variable: the number of samples sharing a certain haplotype was no more than 11, and 17 haplotypes were observed only in one sample.

In general, 94.84% of the domestic chicken samples were present in clades A, B, C, E, F, and G, while more than half of the red jungle fowls (65.15%) were distributed in clades B and D. The two dominant haplotypes (B10 and D1) of red jungle fowl in clades B and D were found in more than 10 samples. Clade A harbored two red jungle fowl samples, *G. g. spadiceus* and *G. g. jabouillei*, both from Yunnan, China. Clade B contained 21 red jungle fowls, 18 of them presented in Yunnan and consisted of two haplotypes B10 and B11, whereas the remain three samples (one from Laos and two from unknown region) shared haplotype B1. Clade F had 11 red jungle fowl samples while clade G only had one. In total, 33.33% (22/66) of the red jungle fowl samples, which covered three subspecies of *Gallus gallus* (*G. g. gallus*, *G. g. spadiceus*, and *G. g. jabouillei*) considered in this study and shared 10 haplotypes, were fallen into clade D.

The distances between each of the haplotypes and the potential root in clades A and C were all within a 3-mutation distance. The largest distance between the haplotypes and the root in clade B was 2 mutations, and reached 4 mutations in clades E and F. Clades D and G showed even larger distances within the clade (Fig. 2). It should be noticed that all the haplotypes that shared by or restricted to the red jungle fowls in clades A, B, E, and F differed from the potential root in each clade by no more than 4-mutation distance, which was within the mutation distance observed between the domestic chicken and the potential wild progenitor *G. g. gallus* (see Fig. 2).

3.2. Geographic distribution of the clades

Contracting to that in some other domestic animals such as goat (Chen et al., 2005), regional distribution of the clades was observed, which indicates some geographic structuring in chicken populations. Though, clades A, B, and E were in general the most widely distributed clades, the first two clades were mainly distributed in South China and Japan. However, clade E dominates in Europe (71.38%), the Middle East (91%), and India (55.56%). Curiously, clade C was mainly distributed in Guangxi and Guangdong Provinces of China as well as in Japan but was absent in South Asia. With the exception of two samples (from Sichuan and Henan Provinces, respectively), clades F and G were exclusive of Yunnan Province fowls. Clade D was composed of red jungle fowl and domestic chicken samples mainly from India and Indonesia, as well as from Japanese and Chinese gamecocks. Finally, clade I only contained three samples from which two of them (one domestic chicken and one red jungle fowl) were from Vietnam.

As summarized in Table 1, Yunnan Province of China contained all the seven clades that harbored most of the domestic chicken samples. In the two widely distributed

Table 1
Geographical distribution of the major clades in domestic chickens

Clade		Yunnan Province (<i>N</i> = 301)	Provinces adjacent to Yunnan ^a (<i>N</i> = 114)	Other provinces of China ^b (<i>N</i> = 180)	India (<i>N</i> = 27)	Indonesia (<i>N</i> = 12)	Continental Southeast Asia (<i>N</i> = 5)	Japan (<i>N</i> = 116)	Europe (<i>N</i> = 58)	Middle East (<i>N</i> = 16)	Total No. (<i>N</i> = 829)
Clade A	Individual ^c (%)	73 (23.92)	64 (56.14)	48 (26.67)	0	0	1 (20.00)	32 (27.59)	4 (6.90)	2 (12.50)	224
	Haplotype ^d	11 (5)	11 (5)	14 (7)	0	0	1 (0)	12 (8)	3 (1)	2 (0)	
Clade B	Individual (%)	81 (26.91)	18 (15.79)	71 (39.44)	1 (3.70)	3 (25.00)	3 (60.00)	9 (7.76)	1 (1.72)	2 (12.50)	189
	Haplotype	12 (8)	4 (2)	9 (6)	1 (0)	1 (0)	3 (0)	1 (0)	1 (0)	1 (0)	
Clade C	Individual (%)	3 (0.997)	12 (10.53)	30 (16.67)	0	0	0	42 (36.21)	0	0	87
	Haplotype	1 (0)	4 (1)	6 (2)	0	0	0	14 (12)	0	0	
Clade D	Individual (%)	3 (0.997)	0	9 (5.00)	11 (40.74)	5 (41.67)	0	13 (11.21)	0	0	41
	Haplotype	2 (2)	0	4 (2)	6 (4)	5 (3)	0	7 (5)	0	0	
Clade E	Individual (%)	10 (3.32)	16 (14.04)	21 (14.05)	15 (55.56)	4 (33.33)	0	20 (17.24)	53 (91.38)	12 (75.00)	151
	Haplotype	4 (1)	3 (0)	8 (2)	6 (5)	1 (0)	0	6 (1)	6 (2)	2 (0)	
Clade F	Individual (%)	64 (21.26)	1 (0.88)	0	0	0	0	0	0	0	65
	Haplotype	11 (11)	1 (0)	0	0	0	0	0	0	0	
Clade G	Individual (%)	67 (23.10)	3 (2.80)	1 (0.56)	0	0	0	0	0	0	71
	Haplotype	23(21)	2 (0)	1 (0)	0	0	0	0	0	0	
Clade I	Individual (%)	0	0	0	0	1 (20.00)	0	0	0	0	1

Note. The number of haplotypes and unique haplotypes for the major phylogenetic clades in different regions were counted on the basis of Supplementary Tables 1 and 2. Haplotypes are defined by substitutions only, disregarding indels.

^a Provinces adjacent to Yunnan include Guangxi, Guizhou, Tibetan, and Sichuan Provinces.

^b Other provinces of China include Guangdong, Jiangxi, Henan, Hubei, Hunan, Zhejiang, Jiangsu, Xinjiang, Liaoning, Shandong, Fujian, and Beijing.

^c Number of samples and its proportion in the clade (in parentheses).

^d Number of haplotypes and unique haplotypes (in parentheses).

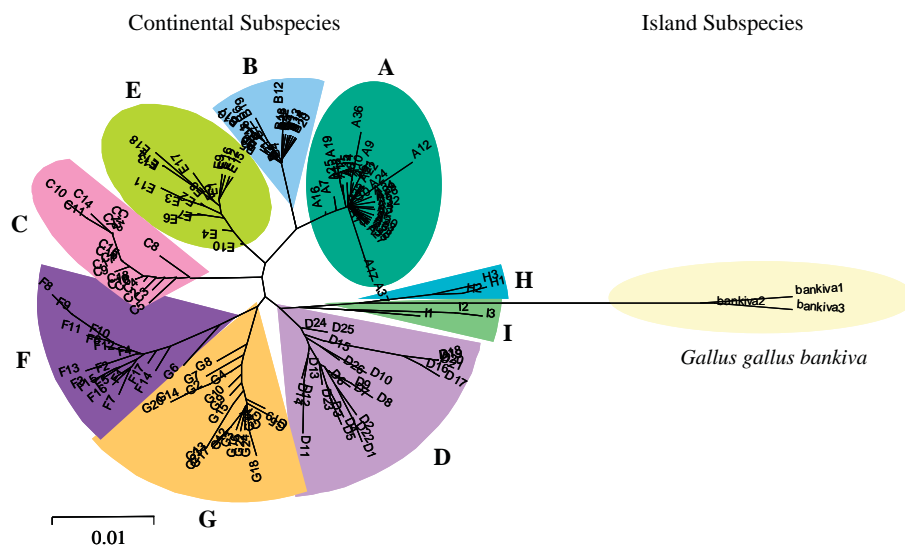


Fig. 1. Unrooted neighbor-joining (NJ) tree of 169 haplotypes in 834 domestic chickens (*G. g. domesticus*) and 66 red jungle fowls (*G. gallus*). The highly divergent mtDNA clades were marked with A–I.

clades A and B, the proportion of unique haplotypes in Yunnan was relatively higher compared with other places (excluding Japan). Most of the European and Middle East sequences fall in clade E. Most of Indian samples clustered in clades D and E and harbored high proportion of private haplotypes (4/6 in clade D and 4/5 in clade E). Most of the unique haplotypes in clade C (72.2%) were exclusive of Japanese chicken (Fig. 2D), with the gamecocks from Okinawa, Japan, forming a unique subclade in clade D (Fig. 2E). It should be mentioned that domestic chicken and red jungle fowls from Indonesia were mainly presented in clade D, and the largest distance between them was only two steps. In addition, they shared haplotypes D6 and D13. Such apparent geographic structure of the clades may reflect different origin of those clades (see Section 4 for detail).

3.3. Genetic diversity and expansion test

We only estimated the nucleotide diversity for each main clade with sample size larger than 30. As shown in Table 2, the nucleotide diversities among the clades varied substantially (0.00184–0.00404). Generally, clades A, B, and E (which were widely distributed) had lower nucleotide diversity; clade D (which contained a large number of red jungle fowls) had the highest nucleotide diversity. The *F_s* tests (Fu, 1997) of the seven major clades that harbored the domestic chicken samples were statistically significant ($P < 0.05$) and were consistent with their (roughly) star-like network pro-

files (Fig. 2), thus suggested possible population expansion in the past.

4. Discussion

4.1. Domestication of the red jungle fowl subspecies

Under the premise that the domestication of chickens occurred within South and Southeastern Asia, our data revealing matrilineal lineages from these regions may help to understand the diffusion of this specie out off this center of domestication. In this respect, our data confirm that the domestication of chickens, like all the mammalian livestock species, seems to have been replicated in several places and enrolled several divergent lineages of the wild ancestor (Beja-Pereira et al., 2004; Bruford et al., 2003; Larson et al., 2005).

On the whole, the analyzed data fit into two main clades (see Fig. 1): one formed by the continental red jungle fowl subspecies and all domestic chicken samples, which we named the continental clade, and another exclusively constituted by *G. g. bankiva* samples from Java that we named the island clade. The mean distances between these two main clusters were larger than the distances among the subclades (A–I) within continental clade (Figs. 1 and 2). Nonetheless, in a more fine scale analysis, the continental subspecies, *G. g. spadiceus* and *G. g. jabouillei*, were mainly observed in clades A, B, and F, whereas samples from *G. g. gallus* were mostly observed in clades D, H, and I. On one

Fig. 2. Network profiles of the major clades. The links are labeled by the nucleotide positions to designate transitions; transversions are further specified by adding suffixes A, G, C, and T; recurrent mutations are underlined. The order of the mutations on a branch is arbitrary. Circle areas are proportional to haplotype frequencies. (A) Overall schematic profile of the major clades. (B–H) Networks of the respective major clades. The locations of the samples are demonstrated by different colors. The mark “*” refers to the potential root. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

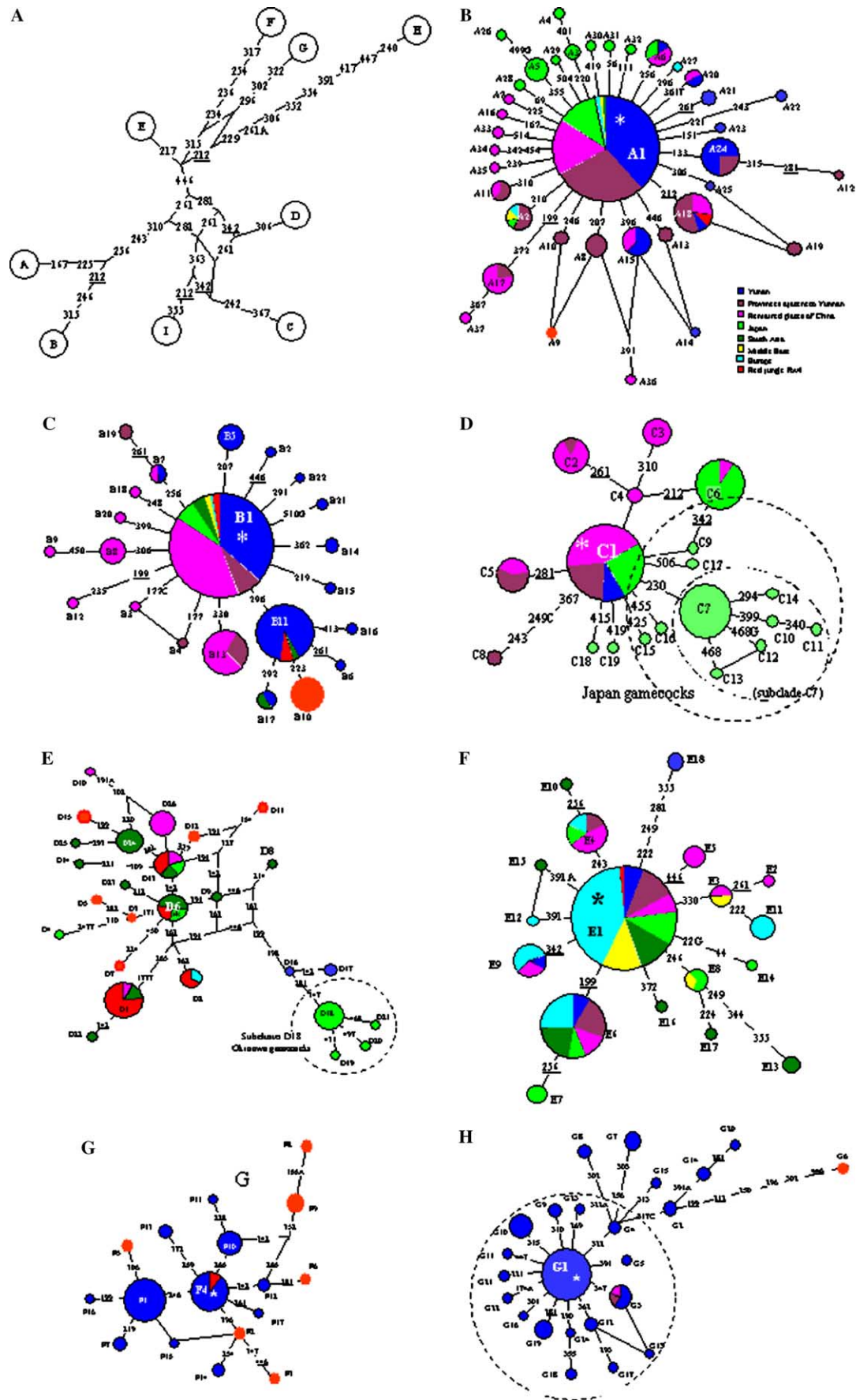


Table 2
Nucleotide diversity and Fu's F_s test of the major clades in domestic chickens and red jungle fowls

Clade	No.	% of red jungle fowls	With red jungle fowls		Excluding red jungle fowls	
			π^a	F_s^b	π	F_s
A	227	0.88	0.00263 ± 0.00021	−27.998*	0.00259 ± 0.00021	−28.117*
B	211	9.95	0.00231 ± 0.00018	−20.005*	0.00184 ± 0.00017	−21.896*
C	87	0.00	0.00401 ± 0.00033	−10.953*	0.00401 ± 0.00033	−10.953*
D	64	34.38	0.01080 ± 0.00059	−10.229*	0.01085 ± 0.00066	−6.496*
E	153	0.65	0.00274 ± 0.00032	−12.684*	0.00276 ± 0.00032	−12.636*
F	76	14.47	0.00406 ± 0.00044	−9.690*	0.00303 ± 0.00035	−4.886*
G	73	1.37	0.00444 ± 0.00061	−20.278*	0.00404 ± 0.00049	−20.230*

^a The estimation was restricted to 400 bp fragment (relative to position 1–397 in the reference sequence; all the sequences have three C insertions in the C stretch at region 48–51 in the reference sequence (Desjardins and Morais, 1990)) due to the fact that some of the published data were short compared with our own data. Clades with sample size less than 30 were not considered.

^b Fu's F_s test (Fu, 1997).

* Fu's F_s statistic reached significant level ($P < 0.05$).

hand, these results indicate the existence of substantial divergence within subspecies, which somehow conflicts with the current taxonomic classification of those subspecies. In fact, this was also noticed by Fumihito and colleagues, who challenge the taxonomic validity of the subspecies *G. g. gallus* (Fumihito et al., 1996). On the other hand, these results also suggest that most or all the continental subspecies or populations analyzed here were enrolled in the genesis of the modern domestic chicken.

4.2. mtDNA landscape patterning

Although we initially sampled the chicken according to breed classifications, we failed to identify breed-specific matrilineal clades in our study. This pattern can be explained by transportability of chickens that travel across the world carried by humans during migration or across the trade routes, throughout the past history. Also, many haplotypes were shared between domestic chicken and wild jungle fowls. Importantly, intensive interbreeding has occurred in the past, and hybridization between different breeds and sometimes even with wild jungle fowls was widely used in cultivation. The distribution patterns of clade E, together with high proportion of unique haplotypes in India (5/6), render us to suggest that this lineage might have its roots in the Indian subcontinent. The fact that clades A and B have a similar geographical distribution and a close phylogenetic relationship may indicate that both lineages originated from the same ancestral population. In addition, the high proportion of unique haplotypes in Yunnan suggests that both lineages may originate in Yunnan and/or surrounding areas.

Extensive gene flow among breeds and different regions could also lead to this pattern, but this would run into conflict with the restricted distribution of other clades. For instance, clades F and G were restricted to Yunnan, China, and clade I is mainly present in Vietnam. This combined with the estimated expansion event based on the F_s test (see Table 2) and the roughly star-like network profiles of clades F and G (Figs. 1 and 2) allows us to speculate that these two clades originated from Yunnan Province, China or in

adjacent places. Compared with other provinces in China, Yunnan Province was extensively sampled in this study, both for domestic chicken and red jungle fowl samples. However, the haplotypes belonging to clade C were seldom found in Yunnan Province. On the other hand, this clade was widely distributed in other parts of China, especially in Guangdong and Guangxi Provinces, where *G. g. jabouillei* inhabits.

Although Japanese chickens displayed the highest nucleotide diversity (0.0244 ± 0.0123) for the clade C (Fig. 2D), the absence of red jungle fowl samples in clade C favors that this clade originated from South China. A recent domestication of clade D or gene flow from domestic into the wild red jungle fowl population are two possible explanations for the fact that clade D mainly contained of red jungle fowl and gamecocks. These distinct patterns combined with archaeological records as well as with the geographic distribution of *G. gallus* are consistent with clades C and D originating relatively recently, perhaps in South and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, Thailand, and India).

Different from the diffusion of other domestic animals, the chicken as cultural usages, especially the sport of cockfighting, had substantial influence in the domestication and the dispersal of the chickens throughout the world. For example, the Japanese domestic chickens might have been derived from the Shamo traditional fighting cocks (Komiyama et al., 2004). The clade D, which contained gamecocks from China, Japan, and Madagascar, represents an analogous mirror image (at least partially) of the dispersal scenario that associated with human culture of cockfighting.

Thus, our results given above conflict with Fumihito et al.'s theory of a single domestication event (Fumihito et al., 1994, 1996).

In summary, our results suggest that: (1) despite the gene flow caused by the countless human migrations and trade relations throughout the history, only clades A, B, and E are widely distributed; (2) the most widely distributed haplotypes only represent a small portion of the intra-clade diversity (Table 1 and Fig. 2), and (3) different clades may originate from different regions, such as Yunnan, South

and Southwest China and/or surrounding areas (i.e., Vietnam, Burma, and Thailand), and the Indian subcontinent, respectively, which support the theory of multiple origins in South and Southeast Asia.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ympcv.2005.09.014](https://doi.org/10.1016/j.ympcv.2005.09.014).

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