Mitochondrial DNA-based analysis of genetic variation and relatedness among Sri Lankan indigenous chickens and the Ceylon junglefowl (*Gallus lafayetti*)

P. Silva*,†, X. Guan*, O. Ho-Shing*,‡, J. Jones§, J. Xu*, D. Hui*, D. Notter* and E. Smith*

*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA 24061, USA. †Department of Animal Science, University of Peradeniya, Peradeniya 20400, Sri Lanka. ‡Davidson College, Davidson, NC 28035, USA. §Department of Fisheries and Wildlife Science, Virginia Tech, Blacksburg, VA 24061, USA

Summary

Indigenous chickens (IC) in developing countries provide a useful resource to detect novel genes in mitochondrial and nuclear genomes. Here, we investigated the level of genetic diversity in IC from five distinct regions of Sri Lanka using a PCR-based resequencing method. In addition, we investigated the relatedness of IC to different species of junglefowls including Ceylon (CJF; *Gallus lafayetti*), a subspecies that is endemic to Sri Lanka, green (*Gallus varius*), grey (*Gallus sonneratii*) and red (*Gallus gallus*) junglefowls. A total of 140 birds including eight CJF were used to screen the control region of the mitochondrial DNA sequence for single nucleotide polymorphisms (SNPs) and other variants. We detected and validated 44 SNPs, which formed 42 haplotypes and six haplogroups in IC. The SNPs observed in the CJF were distinct and the D-loop appeared to be missing a 62-bp segment found in IC and the red junglefowl. Among the six haplogroups of IC, only one was region-specific. Estimates of haplotype and nucleotide diversities ranged from 0.901 to 0.965 and from 0.011 to 0.013 respectively, and genetic divergence was generally low. Further, variation among individuals within regions accounted for 92% of the total molecular variation among birds. The Sri Lankan IC were more closely related to red and grey junglefowls than to CJF, indicating multiple origins. The molecular information on genetic diversity revealed in our study may be useful in developing genetic improvement and conservation strategies to better utilize indigenous Sri Lankan chicken resources.

Keywords genetic diversity, indigenous chicken, Sri Lanka.

Introduction

Diversity among farm animals within and among countries is of major interest to the scientific community because it is a significant resource for livestock development and for responding to changing needs and production requirements. With the increasing world population, there is concern that the growing demands for animal products are eroding these genetic resources, especially in developing countries where most of the diversity is found. In recognition of this concern, many efforts have begun to characterize animals in developing countries to provide a

Address for correspondence

E. Smith, Department of Animal and Poultry Sciences, Virginia Tech, Litton Reaves 2250, Blacksburg, VA 24061, USA.
E-mail: esmith@vt.edu

foundation for developing sustainable genetic improvement approaches. Chief among these efforts is the programme by the Food and Agricultural Organization (FAO) of the United Nations to develop a Global Strategy for the Management of Farm Animal Genetic Resources or FAnGR (Gibson *et al.* 2006; http://www.fao.org/ag/cgrfa/AnGR.htm).

Some efforts to understand diversity in native or indigenous chickens (IC) in developing countries have been described. Using microsatellites and DNA pools, Hillel *et al.* (2003) evaluated variation in 52 chicken populations that included selected and unselected chicken stocks. Although estimates varied, it was observed that unselected populations were more diverse. In another global diversity assessment in IC and commercial chickens, Granevitze *et al.* (2007) reported that populations from Asian countries that included China and Vietnam had high microsatellite-based heterozygosities that reflected their management histories. Mwacharo *et al.* (2007) also recently used microsatellites to

assess the genetic diversity among African IC from Ethiopia, Kenya, Sudan and Uganda. The populations appeared to cluster by region as could be expected from geographic (or reproductive) isolation. A similar analysis of relatedness within and among Chinese IC using microsatellites was reported by Qu *et al.* (2006). The indigenous breeds were also highly diverse and clustered by geographic regions.

Efforts to understand genetic diversity in commercial and non-commercial chickens have also involved characterization of variation in the mitochondrial genome. Although relatively few, the mitochondrial DNA (mtDNA)-based studies have also provided insight into the maternal origins of chickens (Fumihito *et al.* 1994; Niu *et al.* 2002; Liu *et al.* 2006). In a diversity study involving the D-loop of the mitochondrion, Oka *et al.* (2007) evaluated genetic variation within and among Japanese IC and also assessed their routes of introduction into Japan. Using haplogroups, they showed that IC in Japan have multiple origins that include both game and non-game chickens. Given the consensus on the Asian origins of domestic chickens (Fumihito *et al.* 1994, 1996), characterization of IC in Asian countries thus continues to be of interest.

Sri Lankan IC are geographically isolated from the rest of the Indian sub-continent. This isolation may be responsible for the Ceylon junglefowl (CJF, *Gallus lafayetti*) being endemic to Sri Lanka (Ceylon). Like with other *Gallus* species, the relationship between CJF and the domestic chicken as well as with other Galliformes remains of interest to scientists. There is a lack of consensus, for example, on whether the domestic chicken has a mono- or polyphyletic origin that could include the CJF. Recently, Kriegs *et al.* (2007) used annotated retro-posed element activity to find the evolutionary evidence of Galliformes. Using the fixation pattern of the transposed elements in different Galliformes, they revealed that CJF and red junglefowl (RJF) hold very close taxonomic
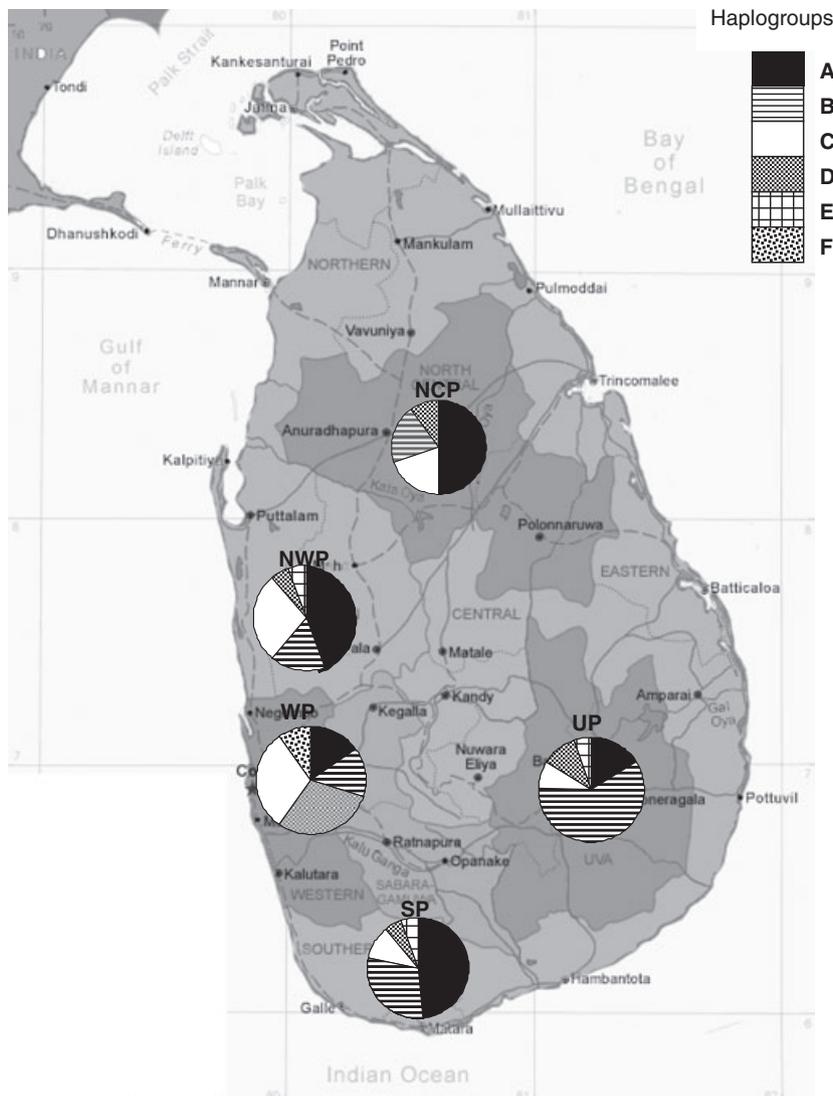

Figure 1 Sampling sites in Sri Lanka. NCP, NWP, WP, UP and SP represent North Central Province, North Western Province, Western Province, Uva Province and Southern Province respectively. The regional frequency distribution of the haplogroups is shown by each pie chart. The number of birds sampled from each region is presented in Table 2. Adapted from a scanned map held at the University of Texas Libraries.

positions in the phylogenetic tree of Galliformes. However, the contribution of CJF in the evolution of the domestic chicken has not yet been fully revealed. As a crossroads of ancient sea trade routes that connect Asia and the western world, Sri Lanka has been enriched with a variety of animal germplasms including chicken, which eventually developed into a distinct indigenous population. Diversity and relatedness among Sri Lankan IC have been little

examined. Using randomly amplified polymorphic DNA analysis, Silva & Rajapaksha (2005) reported that the Sri Lankan IC, when considered as a relatively homogenous group, were more closely related to a commercial chicken breed, the Rhode Island Red, than to CJF. To determine if chickens within Sri Lanka are a homogenous group and to further evaluate the relatedness of the IC with CJF, chickens from five different geographical locations were

Table 1 Mitochondrial D-loop haplotype frequencies and distribution in Sri Lankan indigenous chicken.

| Haplotype | Accession no. | Number | | | | | Frequency[1] |
		NCP	NWP	WP	UP	SP	
SLvtHap1	EU199906	5	2	1	4	2	0.1061
SLvtHap2	EU199907	1	–	–	–	–	0.0076
SLvtHap3	EU199908	1	–	–	–	–	0.0076
SLvtHap4	EU199909	–	–	–	–	2	0.0152
SLvtHap5	EU199910	–	1	–	–	–	0.0076
SLvtHap6	EU199911	–	–	–	–	1	0.0076
SLvtHap7	EU199912	–	–	–	–	1	0.0076
SLvtHap8	EU199913	1	–	–	1	–	0.0152
SLvtHap9	EU199914	–	–	–	–	1	0.0076
SLvtHap10	EU199915	–	1	–	–	–	0.0076
SLvtHap11	EU199916	–	2	–	–	–	0.0152
SLvtHap12	EU199917	–	–	–	–	1	0.0076
SLvtHap13	EU199918	1	2	1	–	1	0.0379
SLvtHap14	EU199919	1	–	–	–	–	0.0076
SLvtHap15	EU199920	–	–	–	–	1	0.0076
SLvtHap16	EU199921	–	–	1	–	2	0.0227
SLvtHap17	EU199922	–	–	–	–	1	0.0076
SLvtHap18	EU199923	–	–	–	–	2	0.0152
SLvtHap19	EU199924	–	–	–	1	–	0.0076
SLvtHap20	EU199925	–	–	–	–	2	0.0152
SLvtHap21	EU199926	–	–	–	–	1	0.0076
SLvtHap22	EU199927	–	–	–	1	–	0.0076
SLvtHap23	EU199928	–	–	–	1	–	0.0076
SLvtHap24	EU199929	–	1	1	10	5	0.1288
SLvtHap25	EU199930	–	–	–	–	1	0.0076
SLvtHap26	EU199931	1	2	1	3	1	0.0606
SLvtHap27	EU199932	–	–	–	1	–	0.0076
SLvtHap28	EU199933	1	–	–	–	–	0.0076
SLvtHap29	EU199934	1	–	–	–	–	0.0076
SLvtHap30	EU199935	1	–	1	4	4	0.0758
SLvtHap31	EU199936	–	–	–	2	–	0.0152
SLvtHap32	EU199937	3	4	5	3	2	0.1288
SLvtHap33	EU199938	1	1	1	–	1	0.0303
SLvtHap34	EU199939	–	–	–	–	1	0.0076
SLvtHap35	EU199940	1	–	2	2	2	0.0530
SLvtHap36	EU199941	1	1	4	2	–	0.0606
SLvtHap37	EU199942	–	1	–	–	–	0.0076
SLvtHap38	EU199943	–	–	–	–	1	0.0076
SLvtHap39	EU199944	–	–	–	–	1	0.0076
SLvtHap40	EU199945	–	–	–	1	–	0.0076
SLvtHap41	EU199946	–	–	–	1	–	0.0076
SLvtHap42	EU199947	–	–	2	–	–	0.0152

NCP, North Central Province; NWP, North Western Province; WP, Western Province; UP, Uva Province; SP, Southern Province.
[1]The cumulative frequency of the indigenous chicken population.

evaluated for genetic diversity in the mitochondrial D-loop. The data may provide some useful information in the ongoing debate about whether chickens have a single origin (Fumihito *et al.*, 1996; Hillel *et al.* 2003) or multiple origins (Liu *et al.* 2006; Oka *et al.* 2007).

Materials and methods

Samples

Blood samples were collected on FTA cards (Whatman, Inc.) from a total of 132 IC in five different geographical regions including the North Central (NCP), North Western (NWP), Southern (SP), Uva (UP) and Western (WP) Provinces (Fig. 1). Within each region, samples were collected from several birds from multiple households in different villages. To minimize the chances that the birds used from each village were related, a single bird was used from each household. The households within each village from which each bird was used were approximately 0.5–1 mile apart. Blood was also collected on FTA cards from nine birds identified as CJF at the national zoo (three birds) and from the wild in central Sri Lanka (six birds).

Molecular analysis

Extraction of DNA from the FTA cards was according to the recommended protocol of the manufacturer (Whatman, Inc.). The genomic DNA was used for PCR as previously described by Guan *et al.* (2007) with a minor modification. Although the forward primer, as described by Guan *et al.* (2007), was 5′-AGGACTACGGCTTGAAAAGC-3′, the reverse primer, 5′-GCGATCACGGACTAAAGAGG-3′, was developed for the present work using the GenBank sequence with accession number NC_001323. The amplicons of the expected size (613 bp) were processed and sequenced, and the sequences were analysed using the approach of Guan *et al.* (2007).

Population genetic analyses

The sequences were initially analysed using CLUSTALW (Higgins *et al.* 1994) as previously described (Guan *et al.* 2007). Using the DNASP software (version 4.10.9, Rozas *et al.* 2003), the following statistics were estimated from the sequence comparisons: haplotype diversity (h), nucleotide diversity (π), genetic differentiation (F_{ST}) and mismatch distribution based on pairwise differences among all haplotypes (Rogers & Harpending 1992). Parsimony network analysis of IC was performed using TCS software version 1.21 (Clement *et al.* 2000). To further evaluate the partitioning of sequence variation in the five regions, analysis of molecular variance or AMOVA among the IC from the five regions was evaluated using ARLEQUIN (version 3.01, Excoffier 2006). The *D*-statistics (Tajima 1989) were used to

estimate whether the D-loop data in the IC, though relatively small in size, were consistent with the expectation of neutrality.

Phylogenetic analyses

Genetic relatedness among birds from the five regions as well as between the IC and CJF, green (GrJF; *Gallus varius*), grey (GyJF; *Gallus sonneratii*) and red (*Gallus gallus*) junglefowls was assessed using PAUP* version 4.0 (Swofford 2002). Publicly available sequences in GenBank for the junglefowls other than CJF were used in the analyses. Phylogenetic trees were constructed using the neighbour-joining (NJ) method. One thousand bootstrap replicates were used to assess confidence in the grouping (Felsenstein & Kishino 1993).

Results and discussion

A total of 613 and 675 bp of the mitochondrial D-loop sequence from IC and CJF respectively were involved in the analyses. The difference in sequence length, considering that the amplicons sequenced were from the same primer pair, is due to an insertion of 62 bp in the CJF (Fig. S1) at position 356 of the GenBank reference *Gallus gallus* mtDNA sequence (NC_001323). This insertion in the CJF has previously been reported, based on the sequence from a single bird, to be about 61 bp and to also occur in the grey junglefowl (GyJF, Nishibori *et al.* 2005). The sequences flanking this insertion showed on average a percentage similarity of 81.5% with the IC D-loop sequence. One of the CJF samples obtained from the zoo, CJF141, lacked this insertion. In addition, the sequence of CJF141 also shared 99.5% sequence identity with the IC D-loop sequence, as shown in Fig. S1. Therefore, CJF141 was removed from further analyses because we believe it is most likely an IC.

Table 2 Sampling sites, sample size (n), haplotype distribution (f) and haplotype (h) and nucleotide diversities (π), with standard deviations in parentheses, in the indigenous chickens (IC) of Sri Lanka and Ceylon junglefowl (CJF) based on mitochondrial D-loop sequence comparisons.

Sampling sites	n	f	h	π
NCP	20	14	0.932 (0.044)	0.013 (0.001)
NWP	18	11	0.935 (0.038)	0.013 (0.001)
WP	20	11	0.905 (0.044)	0.013 (0.001)
UP	37	15	0.901 (0.033)	0.011 (0.001)
SP	37	23	0.965 (0.015)	0.012 (0.001)
CJF	8	6	0.929 (0.084)	0.012 (0.002)
Total (IC & CJF)	140	48	0.947 (0.008)	0.017 (0.001)
Total (only IC)	132	42	0.947 (0.009)	0.013 (0.000)

NCP, North Central Province; NWP, North Western Province; WP, Western Province; UP, Uva Province; SP, Southern Province.

Table 3 Inter-region haplotype (above diagonal) and nucleotide divergence (below diagonal).

	NCP	SP	UP	NWP	WP	CJF
NCP		−0.007	0.107*	−0.025	0.070	0.923
SP	0.012		0.066*	0.014	0.130*	0.926
UP	0.013	0.012		0.123*	0.183*	0.932
NWP	0.013	0.013	0.013		0.049*	0.921
WP	0.014	0.014	0.014	0.014		0.923
CJF	0.062	0.061	0.060	0.061	0.063	

NCP, North Central Province; NWP, North Western Province; WP, Western Province; UP, Uva Province; SP, Southern Province; CJF, Ceylon junglefowl.

*Significantly different at $P < 0.05$.

Within the IC, a total of 42 haplotypes were detected from 44 polymorphic sites (Table 1). The sequences of all haplotypes have been submitted to GenBank and assigned accession numbers (Table 1). The haplotypes ranged in frequency from <1% to 12%. Only three haplotypes, SLvtHap1, 26 and 32, were observed in all the five regions of Sri Lanka and 31 were detected in only one region. Sixty-one percent of the haplotypes were unique to the SP region (Fig. 1). Six haplogroups (A–F) based on shared single nucleotide polymorphisms (SNPs) as shown in Table S1 were identified from the 42 haplotypes. All but two, haplogroups E and F, were found in all the regions sampled (Fig. 1). The haplogroups ranged in frequency from 0.02 to 0.33. In the CJF, 21 SNPs formed six haplogroups (Table S2).

The diversity indices for IC ranged from 0.901 to 0.965 and from 0.011 to 0.13 for h and π respectively (Table 2). Pairwise genetic (F_{ST}) and nucleotide divergence (d_{xy}) estimates were significant for most of the comparisons (Table 3). Nucleotide divergence between CJF and each of the IC populations was also significant with F_{ST} ranging from 0.921 to 0.932 ($P < 0.05$). Both estimates of F_{ST} and d_{xy} between the CJF and IC populations were several-fold higher than those between IC populations. The negative F_{ST} values indicate negligible variation between the regions compared. Within the IC, birds from SP and WP were most divergent according to the estimates of inter-population
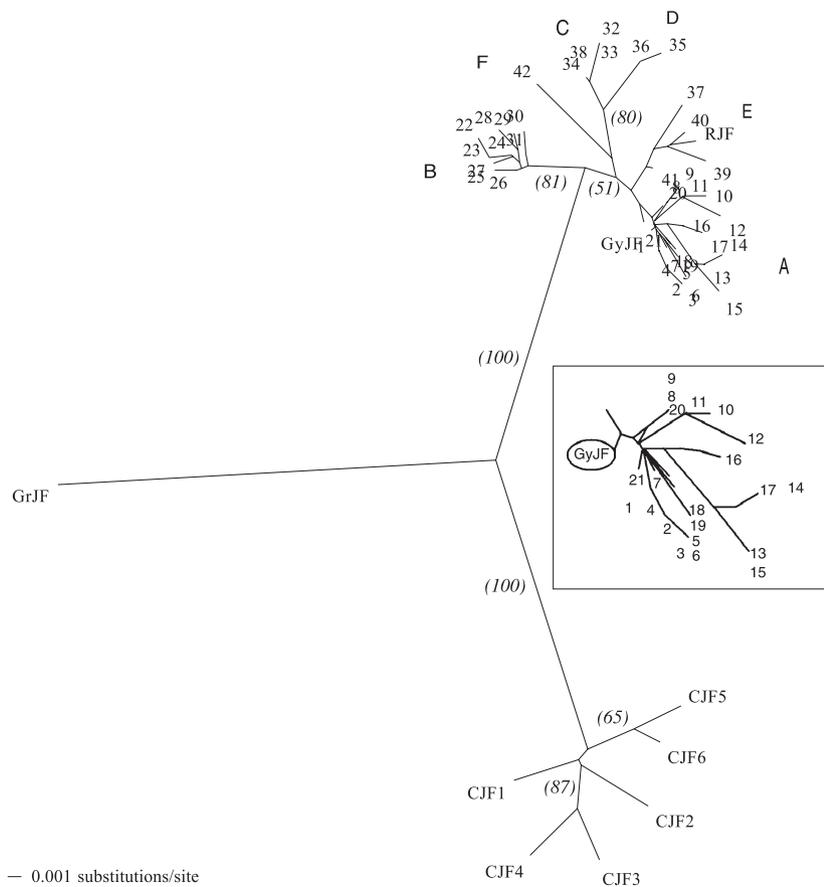

Figure 2 An unrooted neighbour-joining tree relating the mitochondrial D-loop haplotypes observed in the indigenous chickens of Sri Lanka (haplotypes 1–42) and the Ceylon (haplotypes CJF1–CJF6), red (RJF, GI71658078), grey (GyJF, GI71040179) and green junglefowls (GrJF, GI71040235). Letters A–E refer to haplogroups, with D being that of the GenBank reference sequence (NC_001323). Numbers in parentheses represent bootstrap values from 1000 replicates. The inset gives the enlargement of haplogroup A.
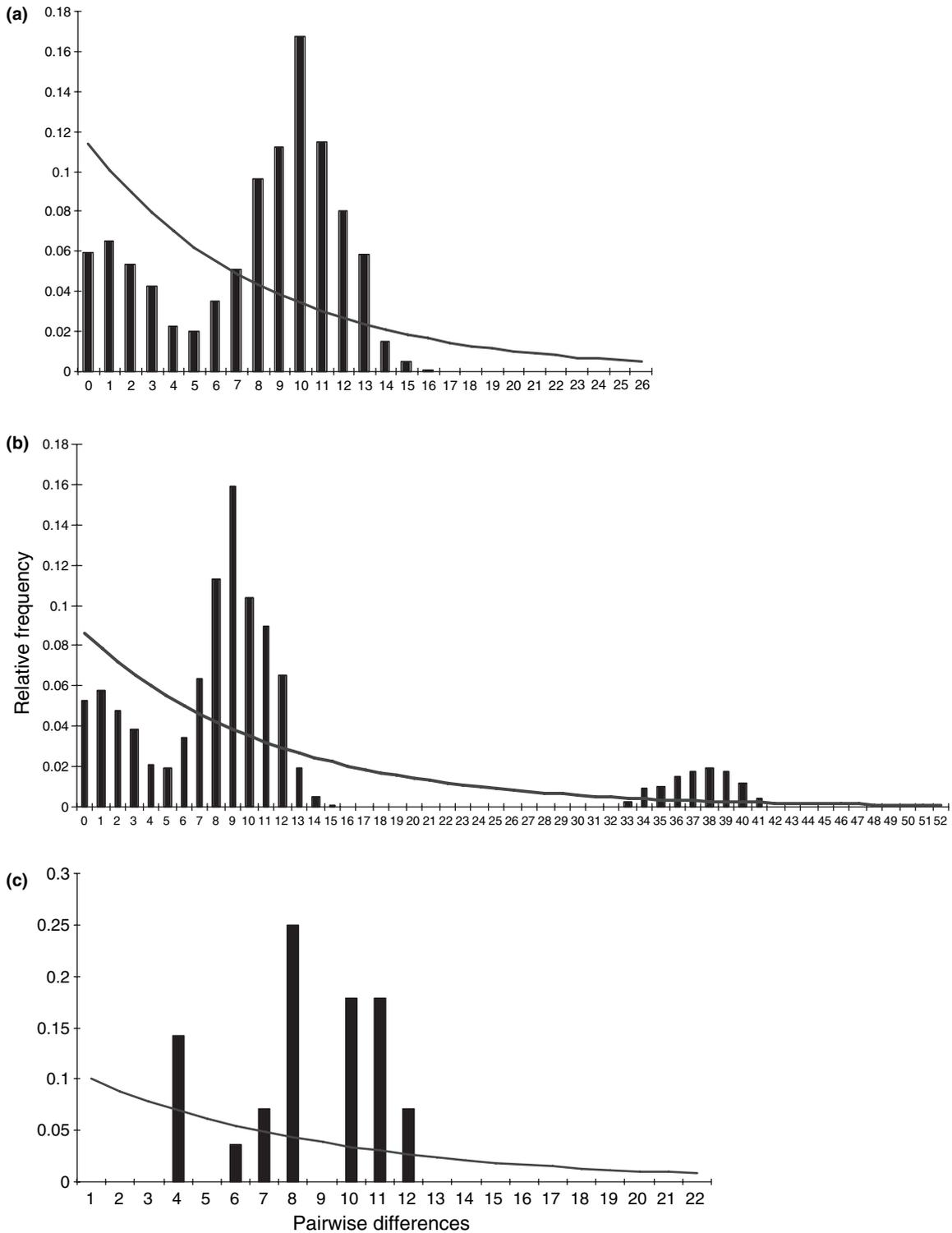
Figure 3 A frequency distribution of the number of sequence differences observed in pairwise comparisons of 613 bp of the mitochondrial D-loop of indigenous chickens (IC) from Sri Lanka and Ceylon junglefowl (CJF). (a) Comparisons within the IC. (b) Comparisons between IC and CJF. (c) Comparisons within the CJF. The within-indigenous chickens comparison lags behind both the intra-CJF derivation and the inter-CJF and IC derivation.

nucleotide divergence. Further, the analysis of molecular variation revealed significantly high (92%) within-region variation ($P < 0.05$).

The consensus of an unrooted NJ tree shows three distinct clusters: (i) RJF, GrJF and IC, (ii) the GrJF and (iii) the CJF (Fig. 2). Within the RJF, GrJF and IC cluster, the RJF appears
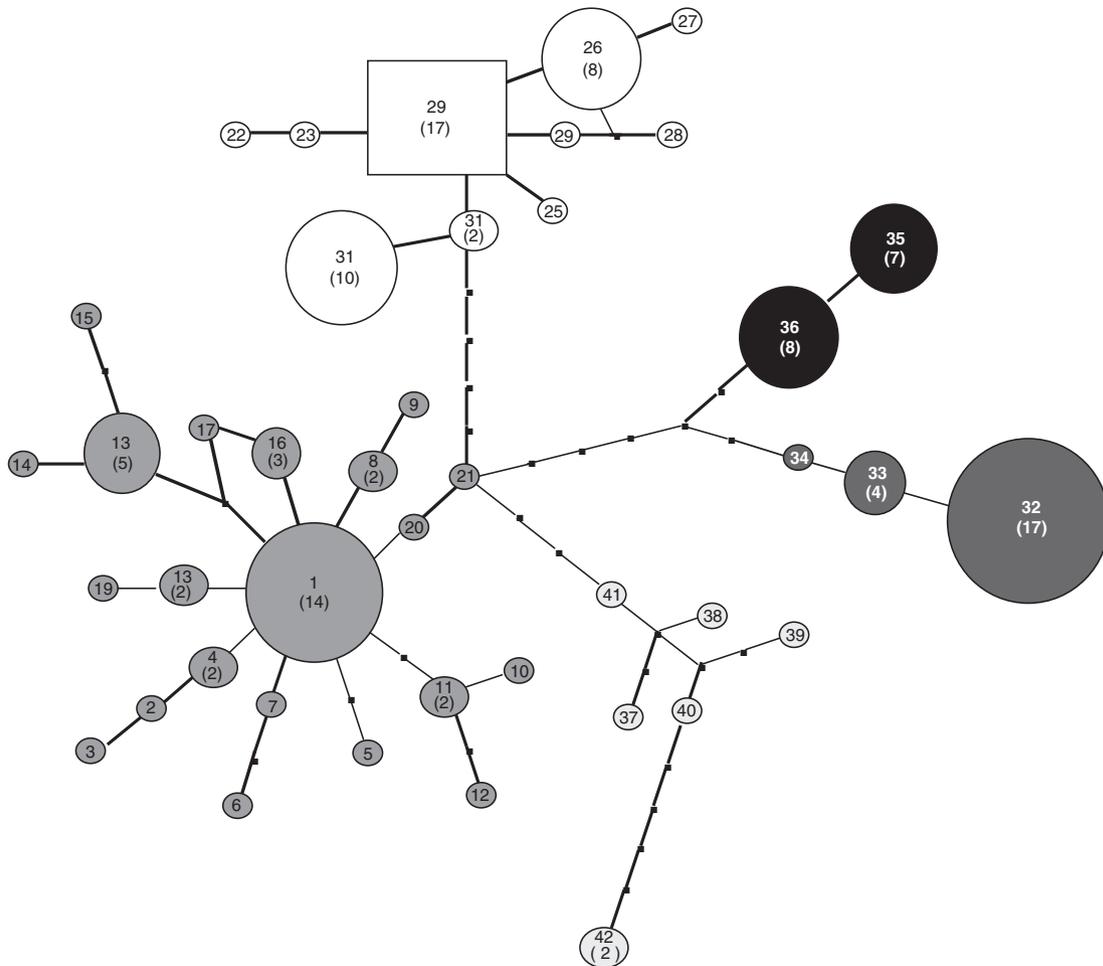
Figure 4 Parsimony network (minimum spanning tree) of the 613-bp partial D-loop sequences from indigenous chickens in Sri Lanka. Circles represent individual haplotypes as described in Table 1. A line connecting two circles, independent of length, indicates a single base pair difference between the two haplotypes. The rectangle indicates the root haplotype based on 95% probability. Filled dots on the lines represent intermediate haplotypes (theoretical) not found in the present analysis. The size of each circle is proportional to the frequency of the haplotype. The numbers in the circles indicate the haplotypes (Table 1) and the numbers in parentheses correspond to the number of individuals with that haplotype (when number >1). Different shading separates the six haplogroups, A to F.

to be most closely related to haplogroup E and the GrJF to haplogroup A.

In both CJF and IC, Tajima's D-statistics for neutrality (data not presented) were not significantly different from zero ($P > 0.10$). The average pairwise nucleotide differences were 10.63, 7.80 and 7.90 between IC and CJF, within IC and within CJF respectively. The distribution of observed mismatches of pairwise differences for the IC and CJF populations is given in Fig. 3. The IC population deviated from expected values and demonstrated a bimodal pattern of distribution (raggedness $r = 0.0129$, calculated with parameters $\theta_{final} = \infty$, $\theta_{initial} = 2.878$ and $\tau = 4.920$). The combined mismatch distribution analysis of IC + CJF (based on the parameters $\theta_{final} = \infty$, $\theta_{initial} = 9.432$ and $\tau = 1.204$) showed two major peaks (raggedness $r = 0.0126$) at around one and nine differences and a smaller peak around 38. The mismatch analysis among birds within each of the five geographic regions sampled also showed a bimodal pattern (data not shown).

The parsimony network analysis of IC haplotypes revealed five distinct groups of haplotypes with, on average, a 7–8 bp difference (Fig. 4). The five groups corresponded to five of the six haplogroups identified in this study, and are shown in Table S1 and Fig. 1. The root sequence (95% probability) is one of the most frequent haplotypes and it was included in haplogroup B. Haplogroup A was genetically diverse compared with other groups. The data further indicate that haplogroups C, D and E have diverged from haplogroup B and that haplogroup F, which is region-specific, diverged from E. Haplogroups D and C appear to be more closely related. The network analysis also appears to support the wide geographic distribution in Sri Lanka of

haplogroups A–D that was observed from the mismatch data.

The diversity estimates observed in our study were higher than the diversity indices reported for Japanese IC (0.0016; Oka *et al.* 2007), for Chinese IC (0.045; Niu *et al.* 2002) and for Indian IC (0.66; Pirany *et al.* 2007). The high genetic diversities revealed among Sri Lankan IC could be attributed to the evolutionary history of the population. The country's geographic location on the ancient trade routes, which connected East Asia to Europe and the Middle East, enabled the exchange of agriculturally important genetic materials including chicken (Chandrasiri 2002; McClellan & Dorn 2006).

Because the CJF is endemic to Sri Lanka, the current comparison with the IC provides the first direct evidence that it is not the progenitor of the native domesticated chickens in the country. Our data show that the IC are closely related to both RJF and GrJF; this appears to support reports of multiple origins of domestic chickens (Nishibori *et al.* 2005; Liu *et al.* 2006; Oka *et al.* 2007). This, however, is inconsistent with earlier reports of a monophyletic origin of domestic chickens (Fumihito *et al.* 1994, 1996).

Given the free movement of birds and farming communities across Sri Lanka, it is not surprising that the current work detected no genetic subdivision of IC. However, the relatively large number of unique haplotypes in different geographical regions may be because of the breeding pattern practised in rural areas where the birds are raised in a free range and there is limited or no exchange of breeding birds among farms (Gunaratne *et al.* 1993). This tends to minimize inbreeding, thus causing the relatively high level of haplotype diversity observed.

In summary, the current analyses indicate that in Sri Lanka, the IC have a relatively high level of genetic diversity that is consistent across a wide geographic area. The random geographic distribution of haplogroups indicates an extensive resource both for conservation and/or genetic improvement by breeding. This could be especially useful if divergent haplotypes and/or haplogroups are crossed to take advantage of heterosis. Our data, though based on only a few junglefowls and thus requiring further study, appear to indicate that the Sri Lankan IC may have originated from either RJF or GrJF and not from the CJF that is endemic to the country.

Acknowledgements

References

Chandrasiri A.D.N. (2002) *Country Report on the State of Animal Genetic Resources (Sri Lanka)*. The Food and Agriculture Organization of the United Nations, Rome.

Clement M., Pasoda D. & Crandall K. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9, 1657–60.

Excoffier L. (2006) ARLEQUIN *Ver. 3.01*. Computational and Molecular Population Genetics Lab, Zoological Institute, University of Berne, Berne.

Felsenstein J. & Kishino H. (1993) Is there something wrong with the bootstrap on phylogenies? A reply to Hillis and Bull. *Systematic Biology* 42, 193–200.

Fumihito A., Miyake T., Sumi S., Takada M., Ohno S. & Kondo N. (1994) One subspecies of the red jungle fowl (*Gallus gallus gallus*) suffices as the matriarchic ancestor of all domestic breeds. *Proceedings of the National Academy of Sciences of the United States of America* 91, 12505–9.

Fumihito A., Miyake T., Takada M., Shingu R., Endo T., Gojobori T., Kondo N. & Ohno S. (1996) Monophyletic origin and unique dispersal patterns of domestic fowls. *Proceedings of the National Academy of Sciences of the United States of America* 93, 6792–5.

Gibson J., Gamage S., Hanotte O., Iniguez L., Maillard J.C., Rischkowsky B., Semambo D. & Toll J. (2006) *Option and Strategies for the Conservation of Farm Animal Genetic Resources: Report of an International Workshop (7–10 November 2005, France)*. CGIAR System-wide Genetic Resources Programme (SGRO)/Biodiversity International, Rome, Italy.

Granevitze Z., Hillel J., Chen G.H., Cuc N.T.K., Feldman M., Eding H. & Weigend S. (2007) Genetic diversity within chicken populations from different continents and management histories. *Animal Genetics* 38, 576–83.

Guan X., Geng T., Silva P. & Smith E.J. (2007) Mitochondrial DNA sequence and haplotype variation analysis in the chicken (*Gallus gallus*). *Journal of Heredity* 98, 723–6.

Gunaratne S.P., Chandrasiri A.D.N., Hemaltha W.A.P.M. & Roberts J.A. (1993) The feed resource base for scavenging village chicken in Sri Lanka. *Tropical Animal Health and Production* 26, 249–57.

Higgins D., Thompson J., Gibson J.D., Higgins D.G. & Gobson T.J. (1994) CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–80.

Hillel J., Groenen M.A., Tixier-Boichard M. *et al.* (2003) Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. *Genetics Selection Evolution* 35, 533–57.

Kriegs J.O., Matzke A., Churakov G., Kuritzin A., Mayr G., Brosius J. & Schmitz J. (2007) Waves of genomic hitchhikers shed light on the evolution of gamebirds (Aves: Galliformes). *BMC Evolutionary Biology* 7, 190.

Liu Y., Wu G., Yao Y., Miao Y., Luikart G., Baig M., Beja-Pereira A., Ding Z., Palanichamy M.G. & Zhang Y. (2006) Multiple maternal origins of chickens: out of the Asian Jungles. *Molecular Phylogenetics and Evolution* 38, 12–9.

McClellan J.E. & Dorn H. (2006) *Science and Technology in World History: An Introduction*, 2nd edn. JHU Press, Baltimore, MD, USA.

Mwacharo J.M., Nomra K., Hanada H., Jianlin H., Hanotte O. & Amano T. (2007) Genetic relationships among Kenyan and other East African indigenous chickens. *Animal Genetics* **38**, 485–90.

Nishibori M., Shimogiri T., Hayashi T. & Yasue H. (2005) Molecular evidence for hybridization of species in the genus *Gallus* except for *Gallus varius*. *Animal Genetics* **36**, 367–75.

Niu D., Fu Y., Luo J., Ruan H., Yu X., Chen G. & Zhang Y. (2002) The origin and genetic diversity of Chinese indigenous chicken breeds. *Biochemical Genetics* **40**, 163–74.

Oka T., Ino Y., Nomura K. *et al.* (2007) Analysis of mtDNA sequences shows Japanese native chickens have multiple origins. *Animal Genetics* **38**, 287–93.

Pirany N., Romanov M.N., Ganpule S.P., Dewagowda G. & Prasad D.T. (2007) Microsatellite analysis of genetic diversity in Indian chicken populations. *Journal of Poultry Science* **44**, 19–28.

Qu L., Li X., Chen K., Yang H., Zhang L., Wu G., Hou Z., Xu G. & Yang N. (2006) Evaluation of genetic diversity in Chinese indigenous chicken breeds using microsatellite markers. *Science in China Series C: Life Sciences* **49**, 332–41.

Rogers A.R. & Harpending H. (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* **9**, 552–69.

Rozas J., Sánchez-Delbarrio J.C., Messeguer X. & Rozas R. (2003) DNASP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496–7.

Silva L.P. & Rajapaksha W.R.K.J.S. (2005) Preliminary investigation of genetic characterization of native and endemic fowl types of Sri Lanka. In: *Application of Gene-Based Technologies for Improving Animal Production and Health in Developing Countries* (Ed. by H.P.S. Makkar & G.J. Viljoen), pp. 593–604. Springer Publishers, Dordrecht, The Netherlands.

Swofford D.L. (2002) PAUP*: *Phylogenetic Analysis Using Parsimony (and Other Methods) 4.0*. Sinauer Associates, Sunderland, MA.

Tajima F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**, 585–95.

Supporting information

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

Figure S1 The alignment of mitochondrial D-loop sequences of Sri Lankan indigenous chicken (IC), nine Ceylon junglefowl and the GenBank sequence of the domestic chicken (NC_001323).

Table S1 Haplotypes and substitution sites in the D-loop region (between 1 and 613 bp) of mtDNA of indigenous chicken.

Table S2 Haplotypes and substitution sites in the D-loop region (between 1 and 675 bp) of mtDNA of Ceylon junglefowl.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting information supplied by the authors.