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On the origins and genetic diversity of South American chickens: one step closer

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Summary

Local chicken populations are a major source of food in the rural areas of South America. However, very little is known about their genetic composition and diversity. Here, we analyzed five populations from South America to investigate their maternal genetic origin and diversity, hoping to mitigate the lack of information on local chicken populations from this region. We also included three populations of chicken from the Iberian Peninsula and one from Easter Island, which are potential sources of the first chickens introduced in South America. The obtained sequencing data from South American chickens indicate the presence of four haplogroups (A, B, E and D) that can be further subdivided into nine subhaplogroups. Of these, four (B1, D1a, E1a(b), E1b) were absent from local Iberian Peninsula chickens and one (D1a) was present only on Easter Island. The presence of the subhaplogroups A1a(b) and E1a(b) in South America, previously only observed in Eastern Asia, and the significant population differentiation between Iberian Peninsula and South American populations, suggest a second maternal source of the extant genetic pool in South American chickens.

Keywords *Gallus gallus*, gene pool, local breeds, mtDNA

The domestic chicken, *Gallus gallus,* is one of the most important livestock species worldwide. Since domestication around 6000 BC (West & Zhou 1988), chickens have been diffusing throughout the world. Although many scholars suggest that chickens were introduced to South America by the European (Portuguese or Spanish) colonizers after 1500 AD (Seligmann 1987; Storey *et al.* 2011), multiple lines of evidence support pre-Columbus human contact between South America and Polynesia (Ladefoged *et al.* 2005; Horrocks & Wozniak 2008). Thus, some scholars have suggested that chickens were first introduced to South America by the Polynesians via the Pacific Ocean (Seligmann 1987; Pearce & Pearce 2010). A pillar of this hypothesis comes from historical information reporting that, upon his arrival in South America, the Spanish conquer Gonzalo Pizarro reported that chickens were already part of the Incan culture (for further details see e.g. Storey *et al.* 2007).

Despite this historical evidence on the introduction of chickens to South America, the genetic makeup of contemporary local populations is not well understood. Similar to those in other locations throughout the world, many of the locally adapted populations in South America are starting to disappear due to crossbreeding with commercial strains. This is particularly alarming, as commercial strains do not perform well due to the specificity of the environment and the fact that farmers do not have the ability to provide food sources other than what the animals can obtain by scavenging. Thus, most well adapted local breeds are at risk of disappearing before being properly studied.

Here we used mtDNA D-loop (control region) sequencing data to examine the origin, genetic relationships and diversity of extant native South American chickens. In

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total, 154 blood or tissue samples were collected from indigenous chickens in five South American countries: 11 from Bolivia, 12 from Colombia, 74 from Ecuador, 18 from Peru and 39 from Chile (including 14 samples from Easter Island). Furthermore, 72 additional samples representing the three extant Iberian indigenous chicken breeds (Preta Lusitânica, Pedrês Portuguesa and Amarela) were also used to investigate their genetic relationships with South American chickens (Table S1).

An 837-bp fragment of the mtDNA D-loop, located between nucleotide positions 16 760 and 797 of the reference mtDNA sequence NC_007235 (Nishibori *et al.* 2005), was PCR amplified and sequenced (see Appendix S1 for details). The raw sequences were edited and aligned using DNASTAR v.7.1 software (DNASTAR, Inc.). All sequences generated in this study were deposited in GenBank under accession nos. KU508830–KU50897, KU527564-–KU527636 and KX513777–KX513799. Furthermore, a set of 58 previously published sequences obtained from South American chickens—17 from Brazil (KF800715–KF800731), 40 from Chile (Gongora *et al.* 2008) and two ancient DNA sequences from Bolivia and Peru (Table S1; Storey *et al.* 2012) were added to the dataset and aligned using the muscle algorithm implemented in the MEGA 6.5 software package (Tamura *et al.* 2013).

The phylogeny of mtDNA lineages tend to change with the addition of more data. This makes the comparison of results from different studies difficult, as the nomenclature applied to the mtDNA sequences can vary according to different papers. In chicken, the reference work in terms of nomenclature was produced by Liu *et al.* (2006) and revised by Miao *et al.* (2013). The hierarchical phylogenetic relationships of the chicken mtDNA sequences found thoughout the world is organized in eight distinct clades (haplogroups A, B, C, D, E, F, G, H, I), which are composed of several sub-haplogroups (e.g. C1, C2). Finally, each sub-haplogroup is also composed of several haplotypes (e.g. E1a, E1b).

Most European chickens are thought to have descended from the Indian domestication center (Liu *et al.* 2006; Lyimo *et al.* 2015). In contrast, Polynesian chickens that may have been carried to South America originated in the Southeast Asia domestication center (Storey *et al.* 2011; Langford *et al.* 2013; Miao *et al.* 2013; Thomson *et al.* 2014). Therefore, we constructed a phylogenetic network with the main haplotypes present in Iberian Peninsula chickens (our dataset) and another using the main haplotypes found in Southeast Asia (sequences retrieved from GenBank). In addition, intra-population diversity measures, including nucleotide diversity (π), haplotype number (H) and haplotype diversity (Hd) (Nei 1987), were obtained using DNASP v.5.1 software (Librado & Rozas 2009). Population differentiation between South America and Iberian chickens was calculated using three different estimators: the corrected average pairwise number of differences, pairwise Φ_{ST} distance and an exact test of population differentiation implemented in ARLEQUIN v.3.5.2 (Excoffier & Lischer 2010). All sequences were classified and named using MITOTOOLPY software (Peng *et al.* 2015), which provides a classification of the mtDNA chicken sequences according the criteria proposed by Liu *et al.* (2006). Median-joining networks were used to explore evolutionary relationships between haplotypes following the algorithm designed by Bandelt *et al.* (1995) and implemented in NETWORK 4.6.1.3 software (http://www.fluxus-engineering.com/). A total of 284 mtDNA D-loop sequences from South American and European (Iberian) chickens were analyzed. The observed haplotype diversity (Hd) ranged from 0.615 to 0.842, and the nucleotide diversity from 0.005 to 0.013 (Table 1). The highest value of Hd was found in samples from Peru (0.842), and the highest value of π was observed in Brazil (0.013). The lowest Hd and π values were observed in Easter Island (0.615) and the Iberian Peninsula (0.005) respectively. The Hd and π values obtained from Iberian chickens fall within those previously reported for European chicken breeds (Ceccobelli *et al.* 2015; Lyimo *et al.* 2015). However, most of the South American populations contained higher Hd and π values than did Iberian populations.

The population differentiation tests (Table 2) showed that populations from South America and the Iberian Peninsula were significantly differentiated ($P < 0.01$; Table S2). With the exception of the Chilean population, for which the exact test supports significant population differentiation ($P < 0.05$) from all other South American populations, all South American populations displayed relatively low differentiation. This suggests that all continental South American populations, except the Chilean population, share a relatively similar genetic background. Nonetheless, the genetic differentiation between Iberian and South American populations suggests deeper geographic and reproductive isolation, whereas the same analysis suggests recent genetic exchanges among South American chickens.

The sequence variant analysis divided all sequences from South America into four main distinct haplogroups (A, B, D and E) and the Iberian sequences into the three most cosmopolitan and frequent haplogroups (A, B, E) (Miao *et al.* 2013). Looking deeper, these haplotypes could be

Table 1 Genetic variability found at the studied chicken populations.

Population	*n*	S	H	HD	π
Brazil	16	16	6	0.783 ± 0.007	0.013 ± 0.001
Iberian Peninsula	73	17	7	0.724 ± 0.001	0.005 ± 0.003
Peru	20	15	9	0.842 ± 0.004	0.007 ± 0.002
Chile	67	19	14	0.770 ± 0.001	0.009 ± 0.002
Easter Island	14	7	3	0.615 ± 0.010	0.010 ± 0.002
Ecuador	80	23	18	0.777 ± 0.002	0.006 ± 0.001
Bolivia	12	12	6	0.848 ± 0.074	0.007 ± 0.003
Colombia	15	1	2	0.248 ± 0.131	0.001 ± 0.000

n, sample size; S, number of segregating sites; H, number of different haplotypes; HD, haplotype diversity; π, nucleotide diversity.

Table 2 Measures of genetic differentiation for the studied chicken populations as estimated by the average number of pairwise differences (above the diagonal) and pairwise Φ_{ST} (below the diagonal) statistics.

	Iberian Peninsula	Ecuador	Chile	Brazil	Easter Island	Peru	Bolivia	Colombia
Iberian Peninsula		0.1656	0.5441	0.4386	0.2402	0.1513	2.6232	0.1994
Ecuador	0.0654**		0.3604	0.1752	0.2730	0.0844	2.6896	1.5425
Chile	0.1579**	0.1054**		0.4006	0.6567	0.4311	0.2760	8.4477
Brazil	0.1632**	0.0790*	0.1008*		0.5455	0.3358	0.3762	3.4000
Easter Island	0.1014**	0.0978*	0.1524**	0.1170*		0.2578	0.0824	2.0571
Peru	0.0665**	0.0341	0.1058*	0.0810*	0.0801*		2.7273	1.6869
Bolivia	0.0646*	0.0124	0.1018*	0.0597	0.1207	0.0262		1.6660
Colombia	0.0718	0.0336	0.1208*	0.1707	0.2199*	0.0452	0.0920*	

*$P < 0.05$.
**$P < 0.01$.

subdivided in nine sub-haplogroups, according to the classification by Liu *et al.* (2006) (Fig. 1; Table S3). In our dataset, the most frequent sub-haplogroup was E1b ($n = 94$ individuals) and the least frequent one was A1a(b) ($n = 3$ individuals). Although most of the observed haplotypes were shared between South America and the Iberian Peninsula (Fig. 2), E1a(b) was observed only in Ecuador, Bolivia and Easter Island, and A1a(b) was observed only in Ecuador, Bolivia and Brazil. A1a(b) is considered to be absent from European samples and present only in Eastern Asiatic chickens (Liu *et al.* 2006). E1a(b) was previously retrieved from an archaeological sample found in Bolivia and carbon-dated as from the 17th century (Storey *et al.* 2012). Besides this ancient sample, E1a(b) has been previously observed in only one local Chinese breed, *gushi* (Fig. 2; Miao *et al.* 2013).

Another interesting finding was the presence of sub-haplogroup D1a in the chickens from Easter Island, although at a lower frequency than observed in other Southern Pacific islands (Liu *et al.* 2006; Langford *et al.* 2013). Easter Island, also known as Rapa Nui, is located in the southeastern Pacific Ocean, midway between Polynesia and South America and is thought to have been colonized by the Polynesians (Hunt & Lipo 2006). Indeed, archaeological evidence suggests that chickens first spread to Oceania around 3000 BP (Storey *et al.* 2008), but the arrival of chickens on continental South America remains controversial. Storey *et al.* (2011) also found one individual belonging to haplogroup D in a sample from a Peruvian archeological site dated from late 15th or early 16th century, but we did not find any D sequence in the extant chickens from continental South America. However, data from a more recent study (Thomson *et al.* 2014) examining both modern and ancient mtDNA from the Pacific Islands suggested that haplogroups E and A were once common and widely distributed across Polynesian Islands, even though haplogroup D is the most frequent in extant Pacific Island chickens. In both studies it was proposed that chickens spread throughout the Pacific Islands in two distinct dispersion waves. Given this, the absence of the D haplotype in continental South America may indicate that the wave of chicken dispersion that introduced this haplotype across the Southern Pacific Islands stopped at Easter Island without reaching the South America continent.

On the other hand, (i) the moderate frequency of the haplotype E1a(b) observed in extant South American chickens, (ii) the circumscription of sub-haplogroups E1a (b) and A1a(b) to Southeast Asia as well as their absence from European chickens (including Iberia) and (iii) the finding of the haplogroup E (Storey & Matisoo-Smith 2014)

Position	167	199	210	212	217	222	225	243	246	256	261	281	296	306	310	315	330	342	391	Frequency							
NC_007235.1	T	T	C	G	T	A	C	T	T	T	T	A	C	T	C	T	C	A	C	BRA	BOL	IBE	PER	ECU	CHI	EI	COL
A1a(a)	C	.	T	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	0	1	3	2	3	2	0	0
A1a(b)	C	.	T	.	.	.	T	.	.	.	.	.	.	.	T	C	.	.	.	2	0	0	0	1	0	0	0
B1	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	0	4	0	5	5	0	0
D1a	.	.	.	.	C	.	.	C	C	C	C	G	T	C	T	C	.	G	.	0	0	0	0	0	0	4	0
E1	.	C	.	.	C	G	.	C	C	C	C	.	.	.	T	C	.	.	.	2	0	26	1	4	3	0	0
E1a(a)	.	.	.	.	.	.	.	C	C	C	C	.	.	.	T	C	.	G	.	1	2	38	14	35	32	8	9
E1a(b)	.	.	.	.	.	.	.	C	C	C	C	.	.	.	..	C	.	.	.	0	2	0	0	16	0	2	0
E1b	.	.	.	.	C	.	.	C	C	C	C	.	.	.	T	C	.	.	A	8	0	0	1	8	20	0	3
E1c	.	.	.	.	C	G	.	C	C	C	C	.	.	.	T	C	T	.	.	1	7	1	0	2	3	0	0

Figure 1 Haplotype sequence variants from South American and Iberian chickens. Haplotype nomenclature is according to Liu *et al.* 2006. Numbering of nucleotide sites and mutations are scored relative to the reference sequence (Nishibori *et al.* 2005; NCBI Reference Sequence NC007235.1). Dots (.) denote identity with the reference sequence. Numbers on the right are the observed haplotype counts for each population. The abbreviations for chicken population are as follows: BOL, Bolivia; BRA, Brazil; CHI, Chile mainland; COL, Colombia; ECU, Ecuador; EI, Easter Island; IBE, Iberian Peninsula; PER, Peru.

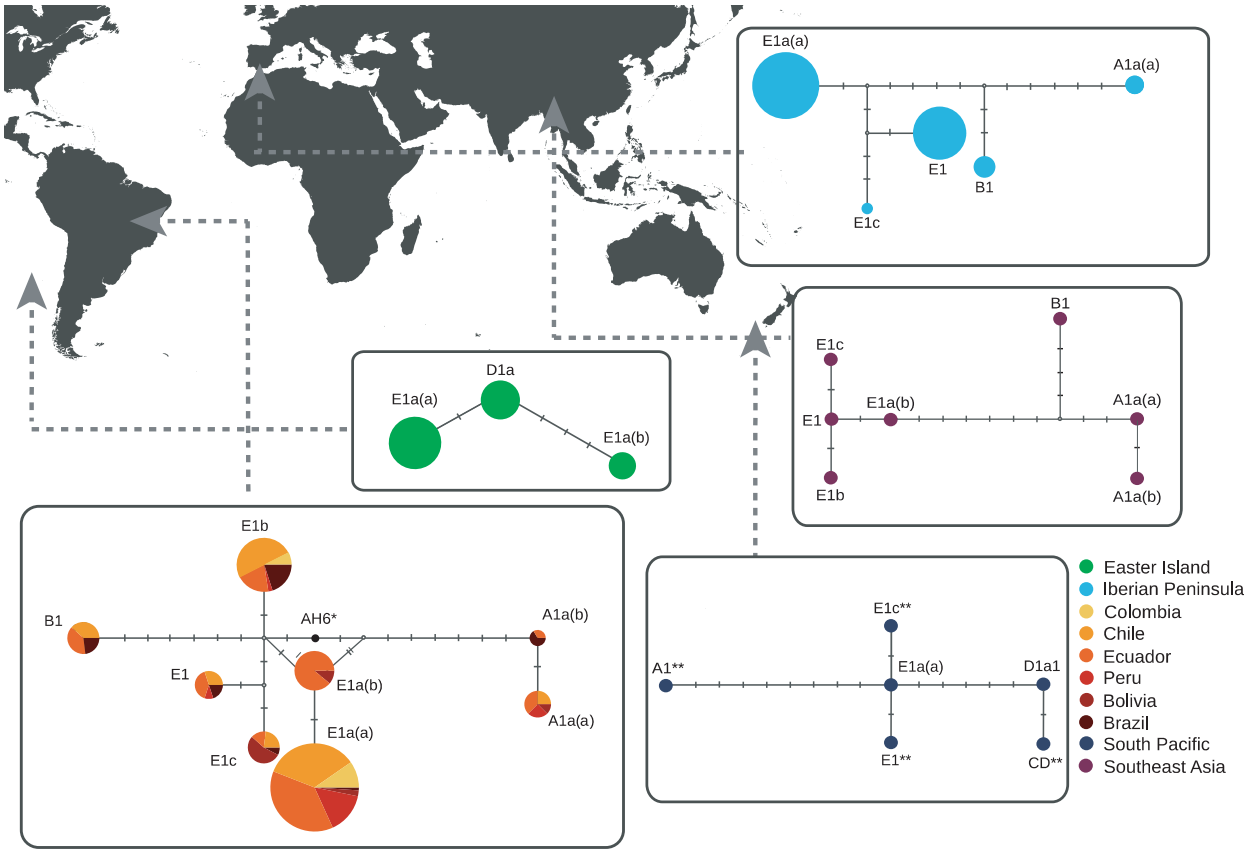

Figure 2 Median-joining network of the D-loop chicken haplotypes found in South American and Iberian chickens. The haplotype nomenclature follows Liu *et al.* (2006). The size of the circles is proportional to the frequency of each haplotype. Mutational steps between haplotypes are represented by small perpendicular lines. *Ancient DNA haplotypes found in South America (Storey *et al.* 2012). **Ancient DNA from South Pacific Islands Chicken (Thomson *et al.* 2014).

in a Chilean archeological site (El Arenal-1) radiocarbon dated to between 500 and 600 BP, together with the findings reported by Thomson *et al.* (2014) that haplogroups E and A were once widely distributed across Polynesian islands, reinforce the hypothesis that the presence of these haplotypes in continental South America may be a legacy of an early introduction of chickens to South America by Polynesians.

In summary, our genetic results provide solid evidence that the extant local chickens from continental South America originated from at least two different sources as opposed to only Europe. This demonstrates that, despite the globalization of poultry including the recent introduction of cosmopolitan commercial lineages into this region, the local chickens of South America still hold a legacy of the first chickens introduced to America.

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Conflict of interests

The authors have no conflicts of interest to declare.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Appendix S1 PCR amplification and sequencing conditions.

Table S1 Sequence information on the published GenBank sequences used in this work.

Table S2 Population Differentiation Exact Test: Non-differentiation exact *P*-values.

Table S3 Classification of the haplotypes found in South American and Iberian chicken.