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Genetic Relatedness Among Wild, Domestic and Brazilian Fighting Roosters

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Fighting roosters, *Gallus gallus*, phylogenetic tree, wild roosters.

ABSTRACT

Except for the meat- and egg-type strains used in commercial poultry farms in Brazil, there are no scientific reports about the origin of birds from the genus *Gallus* that have been introduced in this country with domestication or fighting purposes. Therefore, the aim of this study was to identify the position of the Brazilian Game Bird in the phylogenetic tree of the genus *Gallus* by nucleotide sequence analysis of the mitochondrial DNA D-loop region. The results indicate that fighting roosters comprise two different clusters within the species *Gallus gallus domesticus*. One of the clusters is related to the wild ancestors, while the other one is more related to the birds raised by the poultry industry. In conclusion, Brazilian fighting roosters have originated from the red jungle fowl (*Gallus gallus*) and belong to the subspecies *Gallus gallus domesticus*.

INTRODUCTION

The genus *Gallus* is originated from Asia and comprises four species (Hutt, 1949): *Gallus varius* (Shaw.), *Gallus sonneratii* (Temminck), *Gallus lafayettei* (Lesson) and *Gallus gallus* (Linné). According to Darwin (1875), the domestic fowl originates from the species *G. gallus* (*Gallus gallus domesticus*), and molecular analyses of mitochondrial DNA have confirmed that *G. gallus* is the monophyletic origin of the domestic fowl (Fumihito *et al.*, 1996; Fumihito *et al.*, 1994).

Domestication of fowls started in Taiwan and neighbor regions in Southeastern Asia (Wood-Gush, 1959; Zeuner, 1963; Fumihito *et al.*, 1994, 1996), and it was brought to China in 6000 a.C. (West & Zhou, 1988). Evidences of hen domestication were found in 16 Neolithic archeological sites in Northeastern China, corroborating the above-mentioned findings. It is believed that domestication of fowls was related to religious aspects (Zeuner, 1963), and also to leisure (cockfighting) and adornment (use of feathers in clothes).

Studies performed by Komiyama *et al.* (2003) and Komiyama *et al.* (2004) have demonstrated the relationship between the origin of different native Japanese breeds and cultural traditions. The birds were brought to Japan through two different routes, Central China and Southeastern Asia, and originated the Shamo breed (Komiyama *et al.*, 2003). This is a traditional breed of fighting roosters that was involved in the formation of many Japanese breeds. Besides, singing roosters are another cultural tradition in Japan, and the three Japanese breeds of singing roosters have also originated from Shamo (Komiyama *et al.*, 2004), which evidences that our ancestors have first tried to domesticate the birds for cultural purposes before raising them as food sources.

Fowls were introduced in Europe through Greece, where they were used in cockfighting rings and fights by Romans, who also started to



rear these birds as food sources (Pereira, 2001). According to Moiseyeva *et al.* (2003), the many breeds and strains of hens in the world represent four evolutionary lines, classified by the authors according to the main purposes of rearing:

- a) egg-type birds,
- b) fighting birds,
- c) meat-type birds and
- d) ornamental birds.

Fighting birds would have evolved directly from wild progenitors, whereas meat-type strains would have evolved from fighting strains.

In Brazil, except for the meat- and egg-type strains used in commercial poultry farms, there are no scientific reports about the origin of the birds that belong to the genus *Gallus* that have firstly been introduced in this country by Portuguese colonists and then used for domestic rearing or fighting purposes. Almeida (2005) pointed out the interest of farmers for pure-bred galliformes, including giant roosters, dwarf chickens, long-tailed Japanese roosters and also fighting roosters. Two groups of fighting roosters have been mentioned in that study: Bankivoid and Malayoid types. Bankivoids are long-tailed birds and perch more frequently, whereas the short-tailed and high-stationed Malayoids are characterized by the terrestrial habits. Malayoids are the main type of birds in Brazilian aviaries. According to Almeida (2005), the most important breeds of fighting roosters in Brazil are:

- a) the tall and slender Shamo, originated from Taiwan and developed by the Japanese;
- b) the Thai, that has three combs, a Malayoid breed whose genes are found in virtually all crosses of fighting roosters in Brazil;
- c) Rajah Murgh, which is a variation of the Asil (or Assel) breed that has originated from India and is the largest fighting bird, and finally
- d) Combatente Brasileiro (Brazilian Game Bird), which is not yet recognized as an official breed because it is a result of many crosses that included mainly Malayoid-type birds like Shamo and Thai, and to which it looks much alike.

However, Brazilian fighters from crosses of Bankivoid and Asil roosters have been also produced. The latter are also known as Indian or Creole rooster.

The aim of this study was to identify the position of the Brazilian Game Bird in the phylogenetic tree of the genus *Gallus*.

MATERIAL AND METHODS

Samples

Blood samples were taken from the ulnar vein in nine adult fighting roosters in three aviaries in the states of Bahia, São Paulo and Rio de Janeiro, Brazil. The blood samples were transferred to polypropylene tubes containing 100% ethanol and taken to the laboratory. A sequence of approximately 440 bases of the mitochondrial DNA D-loop region was sequenced and used in the analysis of genetic relatedness to sequences of other nine species, subspecies and breeds of the genus *Gallus*. These sequences were obtained from the international database of nucleotide sequences International Nucleotide Sequence Database Collaboration (DDBJ/EMBL/GenBank), and are shown in Table 1. Chinese Quail (*Coturnix chinensis*) was used as the external group.

Amplification and Sequencing

DNA was extracted according to standard protocols. Briefly, DNA was purified with phenol and chloroform, and precipitated with ethanol (Sambrook *et al.*, 1989).

The control region of the mitochondrial DNA (D-loop) was amplified by polymerase chain reaction (PCR) using the primers H1254 (5' ATGAATTCTTGGCATCTTCAGTGCCA 3') and L16750 (5' AGGACTACGGCTTGAAAAGC 3') (Desjardins and Morais, 1990). Amplification was performed using reaction buffer (10 mM Tris-HCl pH 8.4, 50 mM KCl), 2.0 mM MgCl₂, 0.2 mM of each dNTP, 15 pmol of each primer, 2.0²U of Taq DNA polymerase (Pharmacia), approximately 70 ng of genomic DNA and water to a final volume of 25 µL. Cycling conditions included an initial denaturation step at 94°C for 10 minutes, 35 cycles (94°C for 1 min; 55°C for 2 min; 72°C for 1.5 min) and a final extension step at 72°C for 10 min. The amplification product was separated in 2% low melting agarose gels by electrophoresis. The target fragment (~ 1200 bp) was eluted and purified. This product was then submitted to a new PCR and purified using a commercial product (MiniElute PCR Purification Kit, Qiagen, Germany). Afterwards, sequencing was performed using a commercial kit (Big Dye Terminator Kit) in an automatic sequencer (ABI 3700, Applied Biosystems).

Phylogenetic analysis

The nucleotide sequences of the D-loop region of the mitochondrial DNA obtained from the 18 samples of fighting, domestic and wild roosters were aligned


Table 1 - Sequences from GeneBank used in the present study.

	Scientific name	Common name	Access no.
Wild roosters	<i>Gallus lafayettei</i>	Ceylon Jungle Fowl	D82909.1
	<i>Gallus varius</i>	Green Jungle Fowl	D82914.1
	<i>Gallus sonneratii</i>	Grey Jungle Fowl	D66892.1
	<i>Gallus gallus bankiva</i>	Red Jungle Fowl	AB009431.1
	<i>Gallus gallus spadiceus</i>	Red Jungle Fowl	AF512188.1
Domestic roosters	<i>Gallus gallus domesticus</i>	Shamo	AB007754.1
	<i>Gallus gallus domesticus</i>	White Leghorn	AB007723.1
	<i>Gallus gallus domesticus</i>	Barred Plymouth Rock	AB007719.1
External group	<i>Coturnix chinensis</i>	Chinese quail	D66888.1

using the software CLUSTALW (<http://www.ebi.ac.uk/clusterw/>). The alignment was visually examined and then edited using the software BioEdit (Hall, 1999), which resulted in sequences of 440 bases. The number of nucleotide substitutions was estimated using the Two-Parameter Method of Kimura (1980), and a phylogenetic tree was generated according to the neighbor-joining method (Saitou and Nei, 1987) using the software Mega v.3 (Kumar *et al.*, 2004). Statistical significance was evaluated by bootstrap analysis using 1000 repetitions in order to establish the confidence level of the clustering in the phylogenetic tree.

RESULTS

According to Figure 1, the phylogenetic analysis of fighting, domestic, and wild roosters showed three main clusters with high bootstrap values: Cluster A comprised wild rooster samples (*Gallus varius*, *Gallus lafayettei* and *Gallus sonneratii*); Cluster B included the wild subspecies of the genus *Gallus gallus* (*G. g. bankiva* and *G. g. spadiceus*); and Cluster C comprised the domestic roosters (*Gallus gallus domesticus* - Barred Plymouth Rock and White Leghorn), Brazilian fighting roosters (01F to 09F) and Shamo, the Japanese breed of fighting roosters (*Gallus gallus domesticus*).

DISCUSSION

The genus *Gallus* is comprised by four species: *G. gallus*, *G. varius*, *G. lafayettei* and *G. sonneratii*. The genetic relatedness among them obtained in this study (Figure 1) is in accordance to the data available in the literature. According to the present findings, *G. varius*, *G. lafayettei* and *G. sonneratii* were within the same evolutionary branch and might have originated from a common ancestor almost simultaneously along the evolutionary scale, as previously observed by Fumihito *et al.* (1996).

Cluster B comprises samples of *Gallus gallus*, but *G.*

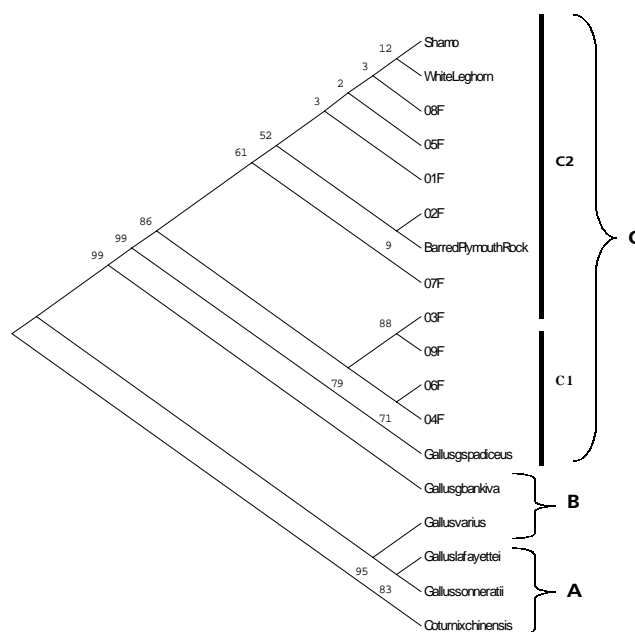


Figure 1 – Phylogenetic tree of fighting, domestic and wild roosters together with *Coturnix chinensis* as external reference. The tree was built by the neighbor-joining method using 440 bases of the D-loop region of the mtDNA. The bootstrap analysis was repeated 1000 times. The 18 samples formed three clusters: A (wild roosters), B (wild subspecies of *Gallus gallus*) and C (domestic and fighting roosters – *G. g. domesticus*). The arrows indicate the bootstrap values at the separation of clusters A and B (100), and of clusters B and C (99).

g. bankiva precedes the other subspecies. It is known that the domestic fowl originated from *Gallus gallus gallus*, in Southeastern Asia (Fumihito *et al.*, 1996). The clustering seen in the dendrogram is highly consistent, as evidenced by the high bootstrap values obtained. Values above 70 indicate high confidence of the data set. The bootstrap value was 100 in wild roosters (Cluster A) and the subspecies of *Gallus gallus* (Cluster B), while a bootstrap values of 99 was obtained for Cluster B and samples belonging to the domestic subspecies (*Gallus g. domesticus* - group C).



Cluster C showed a bootstrap value of 86 and is comprised by meat- and egg-type breeds, and fighting roosters. Two sub-clusters were identified. The first includes Brazilian fighting roosters (C1) and the second is comprised by fighting roosters and breeds used in the poultry industry (C2). All birds in Cluster C belong to the subspecies *Gallus gallus domesticus*. However, these findings suggest that the birds clustered in C1 are closer to the wild ancestors than those in C2. The results obtained for C1 are corroborated by Zeuner (1963), who hypothesized that hen domestication was driven by the use of birds for leisure (fighting) and adornment (feathers for clothes).

On the other hand, the cluster C2 includes not only fighting birds, but also those used in food production. These findings may indicate the occurrence of crossings among the breeds that are included in this cluster. The mitochondrial DNA is transferred to the progeny by the maternal strain. This suggests that fighting roosters have been eventually mated to females from other domestic breeds. The Brazilian birds in cluster C2 might represent the Brazilian Game Bird, which has resulted from crossings involving Asian and Indian breeds (Almeida, 2005). It is noteworthy that the low bootstrap values observed in cluster C2 indicate that the relative position of each sample may vary within the cluster during the rebuilding of the phylogenetic tree. Moreover, it is not possible to evidence a genetic precedence of a given breed over the others.

The present results indicate that both the fighting roosters and the other breeds used for food purposes originated from a common ancestor, the red jungle fowl (*Gallus gallus*). Currently, however, these breeds are genetically closer to each other than to the wild roosters. A study on the evolution of birds of the genus *Gallus* and the origin of the successive breeds has suggested that the different types of birds have not appeared simultaneously (Moiseyeva *et al.*, 2003). Furthermore, it has been also considered that egg-type breeds, or Mediterranean breeds, are possibly the oldest, and the fighting breeds have evolved either directly from the wild ancestors or from the egg-type breeds shortly after (Moiseyeva *et al.*, 2003). Komiyama *et al.* (2003) has identified the origin of the Japanese breed Shamo and its precedence over three variations of singing roosters. This finding shows that the cultural tradition of rooster fighting was older than the contests of singing roosters (Komiyama *et al.*, 2004).

CONCLUSIONS

In conclusion, the Brazilian Game Bird, as well as the breeds reared for food production, has originated from the red jungle fowl (*Gallus gallus*) and belong to the subspecies *Gallus gallus domesticus*.

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