Genetic diversity of Creole hens of the Colombian southwest

Palacios, E.Y.[1,2]; Álvarez, L.[1,2@] and Muñoz, J.[2]

[1]Research Group on Animal Genetic Resources. National University of Colombia. Palmira. Colombia.
[2]Faculty of Agricultural Sciences. National University of Colombia. Palmira. Colombia.

Additional keywords

Conservation.
Microsatellite.
Genetic resource.

SUMMARY

In Colombia, the Creole hen is a socially important species due to its nutritional value, easy reproduction, adaptability and hardiness. Therefore, it was necessary to assess its diversity and genetic relationships and structure. The aim of this study was to contribute to the biodiversity knowledge in the country and to the agricultural sustainable development through the study of the genetic diversity in Creole birds. A total of 224 Creole hen samples taken from the departments of Cauca, Caldas, Chocó, Nariño, Valle and from 20 commercial lines were analysed using 17 microsatellite markers. DNA was extracted using the Salting Out method and was further amplified by the polymerase chain reaction (PCR). The statistical analysis for the evaluation of genetic diversity was performed by using ARLEQUIN program Version 3.5, GENALEX Version 6.5, Microsatellite Toolkit and FSTAT software. The population structure was determined by using STRUCTURE software, version 2.3.4. A total of 79 alleles were identified for the populations; the average number of alleles per hen was 4.65±1.66. The expected heterozygosity was higher than what was observed and varied from 0.59 in Chocó to 0.62 in Valle del Cauca. The F_{IS} value was 0.40 ($p<0.001$). The genetic difference between Creole birds and commercial-line birds estimated by the F_{ST} value was 0.10 ($p<0.001$). The highest genetic distances were found between Caldas and the rest of populations, and the lowest distances were found between Nariño and Chocó. A high genetic diversity was estimated. However, a deficit in heterozygotes was detected, indicating mating between related individuals, which may favour the expression of undesired genes and genetic diseases, compromising viability and decreasing reproduction. Two groups were found: the first was formed by birds from Chocó, Nariño and Valle del Cauca and the second by Cauca and Caldas birds.

Diversidad genética de la gallina Criolla del suroccidente colombiano

Palabras clave adicionales

Conservación.
Microsatélite.
Recurso genético.

RESUMEN

En Colombia, la gallina Criolla es socialmente importante por su aporte nutricional, fácil reproducción, adaptabilidad y rusticidad. Por lo tanto, ha sido necesario conocer cómo es su diversidad, relaciones y estructura genética. El objetivo de este estudio fue ccontribuir al conocimiento de la biodiversidad del país y al desarrollo agropecuario sostenible, mediante el estudio de la diversidad genética de aves criollas. Se analizaron 224 muestras de ADN de gallina Criolla colombiana provenientes de los departamentos del Cauca, Caldas, Chocó, Nariño y Valle del Cauca, y 20 de líneas comerciales, mediante 17 marcadores microsatélites, realizándoles extracción de ADN por el método de Salting Out y posteriormente amplificando por reacción en cadena de la polimerasa (PCR). El análisis estadístico para evaluar la diversidad genética se realizó a través de los programas ARLEQUIN v. 3.5, GENALEX v. 6.5, Microsatellite Toolkit y FSTAT. La estructura de las poblaciones se determinó por el programa STRUCTURE v. 2.3.4. Se encontraron 79 alelos para las poblaciones. El número promedio de alelos para la gallina Criolla fue de 4,65±1,66. La heterocigosidad esperada fue mayor que la observada y varió de 0,59 en Chocó a 0,62 en el Valle del Cauca. El F_{IS} fue de 0,40 ($p<0,001$). La diferencia genética entre aves criollas y líneas comerciales estimada por el F_{ST} fue 0,10 ($p<0,001$). Las mayores distancias genéticas se encontraron entre Caldas y las demás poblaciones, mientras la menor fue entre Nariño y Chocó. Se observó una alta diversidad genética, sin embargo se detectó un déficit de heterocigotos, indicando apareamientos entre individuos emparentados, que pueden favorecer la expresión de genes indeseables y enfermedades genéticas, comprometer la viabilidad y disminuir la reproducción. Se encontraron dos agrupaciones, la primera conformada por aves de Chocó, Nariño y Valle del Cauca y la segunda del Cauca y Caldas.

Información

Cronología del artículo.
Recibido/Received: 07.01.2015
Aceptado/Accepted: 18.01.2016
On-line: 16.03.2016
Correspondencia a los autores/Contact e-mail:
laalvarezf@unal.edu.co

INTRODUCTION

Creole hens are widely distributed in rural areas, where they are maintained mainly by the local inhabitants and significantly contribute to both the food supply and the economic sustainability of rural households. These birds are resistant, adapted to the rural environment, survive with few inputs and tend to adapt to the food availability fluctuations (Zaragoza, 2012). Their broodiness is one of the main advantages when compared to commercial birds (Valencia, 2009). Local birds are characterized by low or medium re-

Table I. Number of samples (N) of Creole birds, per department, and municipality (Número de muestras (N) de aves Criollas por departamento y municipio).

Department	N	Municipality
Chocó	51	Puerto Meluk, Bahia Solano, Pie de Pató
Nariño	50	Ipiales, Puerres, Potosí, Córdoba
Valle del Cauca	50	Palmira, Guacari, Buga
Cauca	51	Guapi, Toribio, Jambaló, Santander de Quilichao y Piendamó
Caldas	22	Pácora y Chinchiná.
Commercial	20	

turns and are kept in small populations. These animal populations face genetic erosion, which leads to the loss of genetic variability. This variability is important for the retention of specific features because these local species possess genes and characteristics relevant to their adaptation to specific environments and to meeting local demands (Romanov *et al.*, 1996). According to the United Nations Food and Agriculture Organization (FAO, 2007), the main threats for animal genetic resources are massive production, importation of foreign birds and indiscriminate breeding. With the decrease in peasant populations, several cultural traditions and knowledge for breeding, management and production have been lost (Valencia, 2009).

Microsatellite markers are frequently used to evaluate diversity, genetic relationships and structure due to their high abundance, ubiquity in the genome, high polymorphism degree and codominant inheritance (Tautz, 1989). To evaluate diversity in domestic birds, several studies have used microsatellites: Hillel *et al.* (2003) evaluated 52 populations of a wide range of hens; Tadano *et al.* (2008) and Dorji *et al.* (2012) evaluated local birds, including Red Jungle Fowl; Davila *et al.* (2009), Cuc *et al.* (2010), Osei-Amponsah *et al.* (2010), Méndez (2012), Nasr *et al.* (2012) and Ramadan *et al.* (2012) studied native birds; Eltanany *et al.* (2011) studied local birds and commercial species; and Tadano *et al.* (2007a) evaluated commercial lines.

The study hypothesis is as follows: there is high diversity and genetic differentiation between and within Creole hen populations in the Colombian southwest. The aim of the study was to evaluate the diversity, differentiation and genetic relationships in populations from Chocó, Valle del Cauca, Cauca, Nariño and Caldas using 17 microsatellites.

MATERIAL AND METHODS

Sampling

Two hundred twenty-four Creole hends were sampled in the departments of Caldas, Cauca, Chocó, Nariño and Valle del Cauca, of which 113 were males and 111 females. A group of 20 commercial birds was also included (Hy Line, Lohmann, Ross and Cobb 500) **(table I)**. The Creole birds were sampled according to the types of hen described by Valencia, (2008). This characterization included phenotypic features such as naked neck, feathered tarsus, and curly plumage, among others.

Molecular markers

Seventeen microsatellites were selected following FAO recommendations on the Measurement of Domestic Animal Diversity (MoDAD) program: ADL268, ADL278, MCW14, MCW34, MCW37, MCW67, MCW69, MCW78, MCW81, MCW98, MCW103, MCW183, MCW206, MCW222, MCW248, MCW29 and MCW330 (FAO, 2011).

DNA extraction and PCR amplification

DNA was extracted from 3-4 µl of blood following the Salting Out extraction protocol (Miller *et al.*, 1988). DNA quantification was determined using known concentrations of lambda bacteriophage DNA (Thermo Scientific, Waltham, Massachusetts, USA) in 0.8 % agarose gels stained with ethidium bromide (J. T. Baker, Warren, New Jersey, USA). The 17 microsatellite markers were PCR-amplified. Each PCR contained a total volume of 25 µL, from: 1X Taq buffer (Thermo Scientific, Waltham, Massachusetts, USA), 4 mM $MgCl_2$ (Thermo Scientific, Waltham, Massachusetts, USA), 0.2 mM dNTPs (Thermo Scientific, Waltham, Massachusetts, USA), 0.4 mg/mL bovine serum albumin (BSA) (Amresco, Solon, Ohio, USA), 10 ng DNA, 0.4 µM of each primer (Bioneer, Korea), and 0.03 U Taq polymerase (Thermo Scientific, Waltham, Massachusetts, USA); the final volume was adjusted with distilled water. The amplification was performed as follows: initial denaturation at 94ºC for 10 minutes, followed by 35 cycles of denaturation at 94ºC for 0.75 min, annealing at 57-64ºC depending on the microsatellite for 1 min, extension at 72ºC for 1.5 min and a final extension at 72ºC for 10 minutes. The amplified product was subsequently visualized in a 1 % agarose gel stained with ethidium bromide.

Statistical analysis

The following diversity indices were estimated: total number of alleles (TNA), mean number of alleles per locus (MNA), mean effective number of alleles (MENA), observed (Ho) and expected heterozygosity (He), Hardy-Weinberg equilibrium (HWE) and Wright's F-statistics: F_{IS}, F_{ST} and F_{IT} were calculated using Arlequin (Excoffier *et al.*, 2005) and GenAlex (Peakall and Smouse, 2006) software. The polymorphic information content (PIC) was calculated using the Microsatellite Toolkit software for Excel (Park, 2001). The allelic richness (Rt) was calculated using the FSTAT software (Goudet, 2001). An analysis of molecular variance (AMOVA) was performed with different hierarchical levels: between Creole and improved (224 Creole individuals and 20 commercial-line individuals); between communities: native (101 individuals sampled from indigenous territories of Cauca and Nariño), afro-Colombian (51 individuals from Chocó) and rural (72 individuals from Valle del Cauca and Caldas). Fixation indices were calculated, and the population structure was determined based on the F_{ST}. Likewise, the FST was calculated based on population pairs, and the Reynolds' genetic distance was also calculated (Reynolds *et al.*, 1983) using Arlequin software,

version 3.5 (Excoffier *et al.*, 2005). Population structure was determined using the cluster analysis method based on models implemented in Structure software, version 2.3.4 (Pritchard *et al.*, 2000). The clustering of individuals was tested assuming an increased number of groups (K) and using a mixture model with the correlated allelic frequencies. The procedure was implemented with 500,000 iterations after a 100,000 burn-in period for each (K). To identify the most probable (K) value, between 2 and 6 independent simulations were performed using a ΔK modal distribution (Evanno *et al.*, 2005).

RESULTS

Microsatellite marker polymorphisms

In the analysis obtained from 224 Creole and 20 commercial birds, a total of 79 alleles were detected, ranging from three alleles (ADL278 and MCW78) to 10 (MCW69), with a mean of 4.65±1.66 alleles per locus **(table II)**.

The expected heterozygosity (He) was higher than the observed heterozygosity (Ho). This last parameter ranged from 0.16±0.03 to 0.65±0.06; the lowest value was for microsatellite ADL268, and the highest value was for MCW183, with a mean of 0.35 ± 0.06. He values ranged from 0.42±0.06 to 0.77±0.02, with a mean of 0.60±0.03; the markers with the highest values were MCW69, MCW34 and MCW206.

A total of 76% of the microsatellites presented PIC values over 0.5, which were characterized as highly informative; 24% were reasonably informative; the three markers with the highest PIC values were MCW69, MCW34 and MCW183.

The F_{IS} ranged from 0.04 (MCW183) to 0.74 (ADL268) except for MCW183 was not significant (Ns) and MCW98, which was significant ($p<0.05$). The rest of the F_{IS} values were highly significant ($p<0.001$), with a mean value of 0.41±0.05, indicating an excess of homozygotes. Most microsatellites (65%) showed deviations from HWE ($p<0.05$).

Genetic diversity within populations

The estimation of genetic diversity within populations is shown in **table III**.

Table II. Genetic diversity parameters determined for 17 microsatellites in five Creole bird populations and one commercial bird population (Parámetros de diversidad genética estimados para 17 microsatélites en cinco poblaciones de aves Criollas y una población de aves comerciales).

Locus	TNA	H_e	H_o	F_{IS}	PIC	HWE
ADL268	4	0.61±0.02	0.16±0.03	0.74**	0.60	0
ADL278	3	0.61±0.01	0.21±0.08	0.70**	0.55	1
MCW14	6	0.43±0.04	0.17±0.02	0.57**	0.41	0
MCW34	6	0.71±0.04	0.45±0.05	0.38**	0.74	0
MCW37	4	0.50±0.08	0.25±0.06	0.49**	0.53	1
MCW67	4	0.60±0.03	0.18±0.04	0.67**	0.57	0
MCW69	10	0.77±0.02	0.57±0,10	0.29**	0.80	1
MCW78	3	0.61±0.01	0.21±0.08	0.56**	0.42	2
MCW81	4	0.67±0.02	0.46±0,07	0.29**	0.64	1
MCW98	5	0.57±0.03	0.50±0.05	0.12*	0.58	4
MCW103	4	0.42±0.06	0.25±0.02	0.43**	0.42	1
MCW183	6	0.69±0.04	0.65±0.06	0.04Ns	0.70	3
MCW206	4	0.70±0.02	0.30±0.03	0.57**	0.69	0
MCW222	4	0.58±0.05	0.33±0.08	0.45**	0.58	0
MCW248	4	0.53±0.03	0.37 ±0.06	0.36**	0.50	1
MCW295	4	0.42±0.07	0.30±0.07	0.30**	0.60	3
MCW330	4	0.64±0.01	0.51±0.04	0.20**	0.48	3
Mean	4.65±1.66	0.60±0.03	0.35±0.06	0.41±0.05	0.58	1.24

TNA= Total number of alleles; H_e= expected heterozygosity; H_o= observed heterozygosity; F_{IS}= Fixation index; PIC= Polymorphic information content; HWE= Hardy-Weinberg equilibrium on each locus ($p<0.05$); Ns= Non-significant; *$p<0.05$; **$p<0.001$.

The MNA for Creole populations was 4.24±1.18, while the MENA was 2.72±0.23. The mean He value (0.60±0.14) was higher than the mean Ho value (0.35±0.20) in all populations. The Ho ranged from 0.33±0.18 to 0.42±0.17, while the He ranged between 0.59±0.13 and 0.62±0.10, indicating that there is high genetic diversity in the Creole hens from the departments included in the study. The average F_{IS} between populations was very high, positive and highly significant (F_{IS}= 0.40), indicating that all five populations had heterozygote deficiency.

Genetic relationships between populations

Population structure analysis with different hierarchical levels revealed that greater genetic differentiation was obtained when comparing the Creole and commercial-line birds (F_{ST}= 0.10, $p<0.001$). The diffe-

Table III. Genetic diversity parameters within five populations of Creole birds estimated using 17 microsatellites (Parámetros de diversidad genética dentro de cinco poblaciones de aves Criollas, estimados mediante 17 microsatélites).

Population	N	MNA	MENA	AR	H_e	H_o	F_{IS}	HWE
Chocó	51	4.35±1.22	2.56	4.01	0.59±0.13	0.34±0.16	0.40**	3
Nariño	50	4.35±1.32	2.78	4.11	0.60±0.15	0.42±0.17	0.27**	5
Valle	50	4.18±0.95	2.72	3.96	0.62±0,10	0.34±0.20	0.44**	0
Cauca	51	4.29±1.26	2.74	4.05	0.60±0.13	0.33±0.18	0.43**	3
Caldas	22	4.00±1.12	2.79	3.95	0.60±0.19	0.35±0.26	0.41**	5
Mean		4.24±1.18	2.72±0.23	4.02	0.60±0.14	0.35±0.20	0.40**	3.2

N= Populations, number of individuals per population; MNA= mean number of alleles per locus; MENA= mean of effective number of alleles per locus; AR= allelic richness with a minimum value N of 18 individuals; H_e= mean expected heterozygosity; H_o= mean observed heterozygosity and their respective standard deviations; F_{IS}= endogamy coefficient within populations; HWE= Hardy-Weinberg equilibrium deviations of the number of loci estimated with 17 microsatellites in five Creole hen populations ($p< 0.05$); **$p<0.001$.

Table IV. Genetic differentiation and distances estimated for six populations. The pairwise population FST is indicated above the diagonal and Reynolds' distance below it (Estimaciones de diferenciación genética y distancias genéticas para seis poblaciones. El F_{ST} por pares de poblaciones se indica por encima de la diagonal y la distancia de Reynolds, debajo de la diagonal).

	C	N	VC	Cu	Ca	Cl
Chocó (C)	—	0.035**	0.045**	0.058**	0.117**	0.052**
Nariño (N)	0.035	—	0.039**	0.043**	0.075**	0.048**
Valle del Cauca (VC)	0.046	0.040	—	0.058**	0.083**	0.054**
Cauca (Cu)	0.060	0.044	0.059	—	0.049**	0.046**
Caldas (Ca)	0.125	0.078	0.087	0.050	—	0.095**
Commercial lines (Cl)	0.053	0.049	0.056	0.047	0.100	—

**p<0.001.

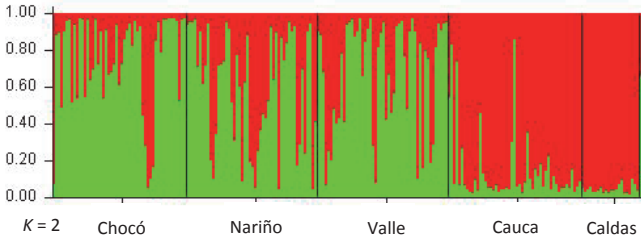

Figure 1. Genome distribution for each individual in both inferred clusters (K= 2). Each animal is represented by a vertical bar. The length of the bar colour in the vertical axis defines the membership proportion (Q), to either the first cluster (green) or the second (red) based on Bayesian analysis (structure) (Distribución del genoma de cada individuo en los dos clúster inferidos (K= 2). Cada animal está representado por una barra vertical. La longitud del color de las barras en el eje vertical define la proporción de membresía (Q) al clúster 1 (verde) o clúster 2 (rojo), basado en análisis Bayesiano (structure)).

rences were lower when Creole birds were compared (F_{ST}= 0.03; p<0.001) and when the afro-descendant, native and rural communities were compared (F_{ST}= 0.02; p<0.001).

The Reynolds' genetic distance (1983) and the F_{ST} pairwise estimations **(table IV)** revealed that the populations of Caldas/Choco were the most distant and that the Nariño/Chocó group had the lowest genetic distance. However, the commercial lines showed lower distance and genetic differences compared to the populations from Cauca and higher distance and differences compared to the population from Caldas.

Genetic structure

According to the structure results, out of the five Creole bird populations, the most probable number for K was two ancestral populations **(figure 1)**.

DISCUSSION

Microsatellite marker polymorphisms

The majority of the markers used were reliable and informative because 15 out of 17 had numbers of alleles higher or equal to four, as recommended by Nassiri *et al.*, 2007 and Nassiry *et al.*, 2009. The microsatellite panel used here was adequate for the genetic evaluation of all five Creole hen populations because, based on the PIC, all systems were determined as polymorphic. According to the classification reported by Botstein *et al.* (1980), a total of 13 out of 17 were highly informative, while four were reasonably informative. The mean PIC value (0.58) was higher compared to the value (0.46) reported by Osei-Amponsah *et al.* (2010) in two bird populations (Forest and Savannah) in Ghana. However, the value was lower than the one reported by Clementino *et al.* (2010) in ecotypes of Brazilian birds (0.73).

The mean number of alleles per locus (4.65±1.66) was higher than the numbers reported with 22 markers by Hillel *et al.* (2003) in 52 populations, Berthouly *et al.* (2008) in 14 French native and six Japanese breeds, and Leroy *et al.* (2007) in African and Asian populations. It was also higher than the number reported by Tadano *et al.* (2007b) in nine populations of Japanese native populations with a panel of 40 systems. However, Ramadan *et al.* (2012) obtained a higher average number of alleles per locus in Egyptian birds, as did Tadano *et al.* (2007a) in commercial lines and Dávila *et al.* (2009) in Spanish birds. Most markers had a He above 0.5, especially MCW69, MCW34 and MCW206, which suggests the necessity for future studies on genetic diversity in hens. The mean He was 0.60±0.03; according to Menezes (2005), an He value above 0.5 indicates high marker genetic diversity.

The mean F_{IS} value was 0.41±0.05, which was higher than the values reported by other researchers (Dorji *et al.*, 2012, Nasr *et al.*, 2012, Ramadan *et al.*, 2012, Osei-Amponsah *et al.*, 2010, Dávila *et al.*, 2009 and Tadano *et al.*, 2007b). This result is an indicator of a heterozygote deficit. Eleven out the 17 microsatellites deviated from HWE (p<0.05). Dorji *et al.* 2012 and Clementino *et al.* 2010 reported lower percentages of HWE deviation (40%) and (44%) in native birds from Bhutan and in ecotypes of Brazilian birds, respectively. The behaviors of both the FIS and the HWE can be attributed to the condition of domestic populations, where mating is not random, the proportion of males is lower compared to females, and roosters remain in the production systems for long periods of time. Moreover, most populations are very small (approximately 14 birds/producer), and subdivisions can occur between populations, including natural selection, leading to endogamy and genetic drift.

Genetic diversity within populations

The MNA obtained for all five Creole populations (4.24±1.18) is a recommended value by Barker (1994). According to Barker (1994), the mean number of alleles per locus should be higher than four to reduce the effect of the standard error when calculating the genetic distance between populations. The mean MENA (2.72±0.23) was higher than the one obtained from Balearic island races (2.47) by Mendez (2012) and was similar to the value reported by Osei-Amponsah *et al.* (2010) in Ghana local birds (2.8±1.3) and by Ramadan *et al.* (2012) in Egyptian birds (2.8±1.2).

The He value of Colombian Creole birds was high (60.0%), a much higher value than the one reported by Hillel *et al.* (2003) in 52 populations (He= 47%), which included red rooster subspecies (*Gallus gallus gallus* and *G. gallus spadiceus*), indigenous populations, and commercial and experimental lines. The He measured in this study was the same as the one for the *Gallus gallus domesticus* subspecies from the Red Jungle Fowl but was lower than the value for *Gallus gallus spadiceus* (64%). This difference indicates that the genetic diversity of Colombian Creole birds is comparable to the genetic diversity of ancestral birds.

The mean Ho value (0.35±0.20) was lower than the mean He (0.60±0.14). This was similar to the values reported by Tadano *et al.* (2007a) between Japanese native races and commercial lines, by Cuc *et al.* (2010) in Vietnamese local birds and by Dávila *et al.* (2009) in Spanish birds.

According to Simon and Buchenauer (1993), the mean F_{IS} value (0.40) indicates that the population is in danger due to the high inbreeding, and conservation processes should be implemented. The high inbreeding is most likely due to the relatively small population sizes (approximately 14 birds/producer). Additionally, bird replacement is performed with animals born within the same systems, which suffer from high degrees of relatedness and the use of low numbers of male breeders, which are replaced at an advanced age, predisposing populations to high consanguinity rates.

Genetic relationships between populations

According to Tejedor *et al.* (1999), the F_{ST} value of bird species is approximately 0.1 or even lower. As expected, the F_{ST} value was higher for the cluster between Creole and improved birds (0.10). Several researchers have reported higher values than the ones measured here (Berthouly *et al.*, 2008, Dávila *et al.*, 2009 and Zanetti *et al.*, 2011). However, Osei-Amponsah *et al.* (2010) reported a lower value (0.04).

The clustering for all five Creole populations showed a genetic differentiation of 0.03, while between communities, the value was 0.02. Both values were low but highly significant, indicating that there is genetic structure in all groups. The similarity of the genetic diversity parameters between Creole birds clustered according to department indicated low genetic differentiation between them, as confirmed by the F_{ST} values shown in **table III**.

The low genetic structure can possibly be attributed to a short evolutionary period, considering that the introduction of European hens to America has been accredited to the Spaniards. Five hundred years have passed since the birds' arrival in the 15th century, during which they have essentially adapted to the climatic, food and parasite conditions. It is assumed that the birds have had a short evolutionary period compared to the domestication process, which, according to West and Zhou (1988), occurred in the Indus Valley 5000 years ago and in western China most likely 7500 or 8000 years ago. Another reason for the low genetic structure is that the Creole populations sampled do not belong to pure cores because they show high phenotypic diversity in terms of colors, feather distribution (naked neck, feathered tarsus, ears and chins), eye and tarsus color, skeleton variants (tailless, dwarves) and comb types, among others. It is probable that the genetic flow has contributed to the similarities between birds of different departments, given the easy transport of fertile eggs and live birds and the sea connection between the departments. This connectedness allows the exchange of genes, increasing the mixture rate between populations.

Birds from Caldas had higher values for Reynold's genetic distance (1983) and the pairwise F_{ST} estimation, indicating that this population is genetically different compared to others, even commercial populations, the above can be attributed presumably to the peasant tradition of the area and to be less mixed commercial birds.

Genetic structure

The results obtained with Structure software indicated that two ancestral populations (K= 2), one formed by birds from the departments of Chocó, Nariño and Valle del Cauca, and the second formed by birds from Cauca and Caldas, maintain their own identities.

CONCLUSIONS

Creole hens from the Colombian southwestern region have high genetic diversity. However, an important heterozygote deficit was detected, indicating mating between related individuals, which may favor the expression of undesired genes and genetic diseases, compromise viability and decrease reproduction. Two clusters were identified: the first formed by birds from Chocó, Nariño and Valle del Cauca, and the second by birds from Cauca and Caldas.

BIBLIOGRAPHY

Barker, J.S. 1994. A global protocol for determining genetic distances among domestic livestock breeds. In: Proc. 5th World Congr Genet Appl Livest Prod. Dept. of Animal and Poultry Science. University of Guelph. Canadá. pp. 501-508.

Berthouly, C.; Bed'Hom, B.; Tixier-Boichard, M.; Chen, C.F.; Lee, Y.P; Laloe, D.; Legros, H.; Verrier, E. and Rognon, X. 2008. Using molecular markers and multivariate methods to study thegenetic diversity of local European and Asian chicken breeds. *Anim Genet,* 39:121-129.

Botstein, D.; White, R.L.; Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet,* 32: 314-331.

Clementino, C.S.; Barbosa, F.J.; Carvalho, A.M.; Costa-Filho, R.A.; Silva G.R.; Campelo, E.G.; Britto, F.B. and Diniz, F.M. 2010. Microsatellite DNA loci for population studies in Brazilian chicken ecotypes. *Int J Poult Sci,* 9: 1100-1106.

Cuc, N.; Simianer, H.; Eding, H.; Tieu, H.; Cuong, V.; Wollny, C.; Groeneveld, L. and Weigend, S. 2010. Assessing genetic diversity of Vietnamese local chicken breeds using microsatellites. *Anim Genet,* 41: 545-547.

Dávila, S.G.; Gil, M.G.; Resino-Talaván, P. and Campo, J.L. 2009. Evaluation of diversity between different Spanish chicken breeds, a testerline, and a White Leghorn population based on microsatellite markers. *Poult Sci,* 88: 2518-2525.

Dorji, N.; Duangjinda, M. and Phasuk, Y. 2012. Genetic characterization of Bhutanese native chickens based on an analysis of Red Junglefowl (*Gallus gallus gallus* and *Gallus gallus spadiceus*), domestic Southeast

Asian and commercial chicken lines *(Gallus gallus domesticus)*. *Genet Mol Biol,* 35: 603-609.

Eltanany, M.; Philipp, U.; Weigend, S. and Distl, O. 2011. Genetic diversity of ten Egyptian chicken strains using 29 microsatellite markers. *Anim Genet,* 42: 666-669.

Evanno, G.; Regnaut, S. and Goudet, J. 2005. Detecting the number of clusters of individuals using the software Structure: a simulation study. *Mol Ecol,* 14: 2611-2620.

Excoffier, L.; Laval, G. and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online,* 1: 47-50.

FAO. 2007. The state of the world's animal genetic resources for food and agriculture. Edited by B. Rischkowsky and D. Pilling. Rome. Italy. 511 pp.

Goudet, J. 2001. FSTAT version 1.2. A computer program to calculate F-statistics. *J Hered,* 86: 485-486.

Hillel, J.; Groenen, M.; Tixier-Boichard, M.; Korol, A.; David, L.; Kirzhner, V.; Burke, T.; Barre-dirie, A.; Crooijmans, C.; Elo, K.; Feldman, M.; Freidlin, P.; Mäki-tanila, A.; Oortwijn, M.; Thomson, P.; Vignal, A.; Wimmers, K. and Weigend, S. 2003. Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. *Genet Sel Evol,* 35: 533-557.

Leroy, G.; Kayang, B.B.; Youssao, I.A.; Yapi-Gnaoré, C.V.; Osei-Amponsah, R.; Loukou, N.E.; Fotsa, J.; Benabdeljelils, K.; Bed'hom, B.; Tixier-Boichard, M. and Rognon, X. 2012. Gene diversity, agroecological structure and introgression patterns among village chicken populations across North, West and Central Africa. *BMC Genet,* 13: 34-44.

Méndez, T.Y. 2012. Caracterización comparada de las gallinas Baleares. Tesis de doctorado. Universidad de Córdoba. España.

Menezes, M.P.C. 2005. Variabilidade e relações genéticas entre raças caprinas nativas brasileiras, ibéricas e canárias. Tese (Doutorado Integrado em Zootecnia). Universidade Federal da Paraíba. Universidade Rural de Pernambuco e Universidade Federal do Ceará. Areia-PB. Brasil.

Miller, S.A.; Dykes, D.D. and Polesky, H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res,* 16: 1215.

Nasr, E.E.; Pasha, E.M.; Esmaeil, K.S.; Nikmard, M. and Molaee, V. 2012. Genetic diversity of a native chicken breed in Iran. *J Genet,* 91: 28e-31e.

Nassiri, M.T.B.; Hamid, Z. and Tavakoli, S. 2007. The investigation of genetic variation at microsatellite loci in Mazandran native chickens. *J Poult Sci,* 6: 675-678.

Nassiry, M.R.; Javanmard, A. and Tohidi, R. 2009. Application of statistical procedures for analysis of genetic diversity in domestic animal populations. *Am J Anim Vet Sci,* 4: 136-141.

Osei-Amponsah, R.; Kayang, B.B.; Naazie, A.; Osei, Y.D.; Youssao, I.A.K.; Yapi-Gnaore, V.C.; Tixier-Boichard, M. and Rognon, X. 2010. Genetic diversity of forest and savannah chicken populations of Ghana as estimated by microsatellite markers. *Anim Sci J,* 81: 297-303.

Park, S.D.E. 2001. Trypanotolerance in west African cattle and the population genetic effects of selection. Ph.D. thesis. University of Dublin. Dublin.

Peakall, R. and Smouse, P.E. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol,* 6: 288-295.

Pritchard, J.K; Stephens, M. and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics,* 155: 945-959.

Ramadan, S.; Kayang, B.B.; Inoue, E.; Nirasawa, K.; Hayakawa, H.; Ito, S. and Inoue-Murayama, M. 2012. Evaluation of genetic diversity and conservation priorities for Egyptian chickens. *Open J Anim Sci,* 2: 183-190.

Reynolds, J.; Weir, B.S. and Cockerham, C.C. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics,* 105: 767-779.

Romanov, M.N.; Wezyk, S.; Cywa-Benko, K. and Sakhatskic, N.I. 1996. Poultry genetic resources in the countries of Eastern Europe: History and current state. *Poult Avian Biol Rev,* 7: 1-29.

Simon, D.L. and Buchenauer, D. 1993. Genetic diversity of European livestock breeds. Results of monitoring by the EAAP Working Group on Animal Genetic Resources; EAAP Animal Genetic Data Bank. Institute of Animal Breeding and Genetics. School of Veterinary Medicine. Hannover. EAAP Publication, No. 66. Wageningen Pers. Wageningen. 591 pp.

Tadano, R.; Nishibori, M.; Imamura, Y.; Matsuzaki, M.; Kinoshita, K.; Mizutani, M.; Namikawa, T. and Tsudzuki, M. 2008. High genetic divergence in miniature breeds of Japanese native chickens compared to Red Junglefowl, as revealed by microsatellite analysis. *Anim Genet,* 39: 71-78.

Tadano, R.; Nishibori, M.; Nagasaka, N. and Tsudzuki, M. 2007a. Assessing genetic diversity and population structure for commercial chicken lines based on forty microsatellite analyses. *Poult Sci,* 86: 2301-2308.

Tadano, R.; Sekino, M.; Nishibori, M. and Tsudzuki, M. 2007b. Microsatellite marker analysis for the genetic relationships among Japanese long tailed chicken breeds. *Poult Sci,* 86: 460-469.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Res,* 17: 6463-6471.

Tejedor, M.T.; Monteagudo, L.V. y Arruga, M.V. 1999. Análisis electroforético de 10 *loci* enzimáticos en perdices rojas *(Alectoris rufa)* mantenidas en cautividad [Electrophoretic analysis of 10 enzymatic loci in red-legged partridges *(Alectoris rufa)* kept in captivity]. *Arch Zootec,* 48: 175-186.

Valencia, N.F. 2009. Avances en el conocimiento de la gallina Criolla Colombiana. Memorias del X Simposio Iberoamericano Sobre Conservación y Utilización de Recursos Zoogenéticos. Universidad Nacional de Colombia. Palmira. Colombia. pp. 96-101.

West, B. and Zhou, B.X. 1988. Did chickens go north? New evidence for domestication. *J Archaeol Sci,* 15: 515-533.

Zanetti, E.; De Marchi, M.; Abbadi, M. and Cassandro, M. 2011. Variation of genetic diversity over time in local Italian chicken breeds undergoing in situ conservation. *Poult Sci,* 90: 2195-2201.

Zaragoza, M.M.L. 2012. Caracterización fenotípica, producción y uso tradicional de gallinas locales en los altos de Chiapas. Tesis de Doctorado. Colegio de Postgraduados Institución de Enseñanza e Investigación en Ciencias Agrícolas. México.