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CONBIAND Network: Following the genetic contributions of the Iberian pigs in the American Creole breeds using microsatellites

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Abstract. The CONBIAND network is a consortium integrated by researchers from 19 countries of America and Europe involved in biodiversity, sustainability and conservation biology. Within this network, a working group for pigs has been constituted in order to clarify the pig colonization history. Creole pigs were originated from animals brought to America by Spanish and Portuguese explorers more than 500 years ago. They are the result of genetic drift, artificial and natural selection and migration. The main goal of this project is to characterise some pig Creole breeds and to establish their genetic relationships with Spanish breeds. Several international breeds have been added in order to determine their influence on the Creoles. We have analyzed 9 Creole breeds with 24 microsatellites. Genetic distances and genetic structure were analysed in order to establish the genetic relationships between the Creoles and the Spanish pig breeds. The results derived from this project will have a great social impact, as may lead to the official registration of those populations, which are not yet officially recognized as breeds due to a lack of genetic supporting studies, but have an unquestionable productive rule in marginal areas and in a subsistence agriculture context.

Keywords. Genetic structure – Rural development – Latin American pig – Genetic distance.

Réseau CONBIAND: Recherche de traces génétiques de porcs d'Espagne chez les porcs créoles américains avec des microsatellites

Résumé. *Le réseau CONBIAND est un consortium de chercheurs de 19 pays d'Amérique et d'Europe dont les buts et objectifs sont axés sur la coopération pour le développement scientifique, social et économique de l'Amérique Latine. Au sein de ce réseau a été formé un groupe de travail spécial pour étudier la biodiversité, la durabilité et la conservation des races locales de porcs. Les porcs créoles issus d'animaux introduits en Amérique par les conquistadors espagnols et portugais il y a 500 ans, sont le résultat de la dérive génétique, de la sélection artificielle et naturelle et la migration. L'objectif de ce projet est la caractérisation de certaines races de porcs créoles et l'établissement de leurs relations génétiques avec des races espagnoles. Nous avons utilisé plusieurs races internationales en vue de déterminer leur influence sur les créoles. Nous avons analysé 9 races créoles avec 24 microsatellites. Les distances génétiques et la structure génétique ont été analysées afin d'établir les relations génétiques entre les créoles et les races porcines espagnoles. Les résultats de ce projet auront un grand impact social, car ils peuvent appuyer la reconnaissance officielle de ces populations, qui ont une importance incontestable pour la production dans les zones marginales dans un contexte d'agriculture de subsistance.*

Mots-clés. *Structure génétique – Développement rural – Porcs d'Amérique latine – Distance génétique.*

I – Introduction

The initial introduction of pigs in America occurred in the second Christopher Columbus trip, in 1493. This fact was described by Fray Bartolomé de las Casas in his “Historia de las Indias”. According this report, eight pigs were introduced in the Antilles and there they increased their population intensely in few years because the absence of illness and predators. Those animals that arrived to America with the Spanish and Portuguese explorers could be the origin of the pig populations existing today in the American continent.

Microsatellite markers have proved extremely useful for the analysis of population structure and relationships, and have been widely used for genetic characterization of several species and populations including European pig breeds (Laval *et al.* 2000; Martínez *et al.* 2000; San Cristobal *et al.* 2006; Vicente *et al.* 2008). Nevertheless, information on native breeds of Creole pigs is still scarce (Pérez *et al.* 2004; Canúl *et al.* 2005; Souza *et al.* 2010), even though they possess unique characteristics in terms of adaptation, hardiness, and quality of products. In this study we used microsatellite markers i) to evaluate the genetic relationships between the 9 American Creole breeds studied and ii) to assess with different statistical tools the integrity and degree of admixture of these native swine breeds.

II – Materials and methods

Individual blood or hair samples were collected from 1080 representative animals of the 25 populations under analysis. Specifically, the breeds studied and corresponding sample sizes are shown in Figure 1. DNA was extracted according to the method described in Walsh *et al*. (1991). A panel of 24 microsatellite markers (FAO 2004) was used: IGF1, S0002, S0005, S0026, S0068, S0090, S0101, S0155, S0178, S0215, S0225, S0226, S0227, S0228, S0355, S0386, SW24, SW72, SW240, SW632, SW857, SW911, SW936 and SW951. Microsatellite markers were amplified according the methodology used by Vicente *et al*., 2008. PCR products were separated by electrophoresis on a ABI 377XL instrument (Applied Biosystems, Foster City, CA) according to manufacturer recommendations and allele sizing was accomplished by using the internal size standard GeneScan-400HD ROX (Applied Biosystems, Warrington, UK). Results of electrophoresis were read directly and interpreted with GeneScan and Genotyper software (Applied Biosystems, Applera Europe B.V.), respectively. Genetic divergence among breeds was estimated through DA genetic distances (Nei *et al.* 1983), with the POPULATIONS software (Langella 1999). The STRUCTURE v.2.1 software (Pritchard *et al.* 2000) was used to investigate the genetic structure of the 25 populations, in order to identify population substructure and admixture. Runs of 500000 iterations after a burn-in period of 300000 iterations were performed for each K, to determine the most probable number of clusters, as inferred from the observed genotypic data. The DISTRUCT v.1.1 software (http://rosenberglab.bioinformatics.med.umich.edu/distruct.html) was used to obtain a graphical display of individual membership coefficients in each ancestral population.

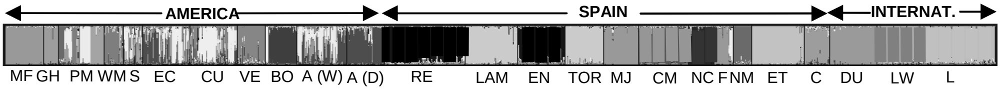
III – Results and discussion

Most American Creole pigs grouped together forming a different cluster from that the formed by the current Spanish pig breeds (Figure 1). These results are consistent with those found by Revidatti *et al*., (2010), using the same panel of microsatellites used in this study, and with those obtained by Souza *et al*., 2010, using the Illumina SNP porcine 60k chip. Creole pigs in Bolivia and U.S. breeds grouped in the same cluster that Spanish pig breeds while the Creole pig in Venezuela is not part of any defined cluster.

A population clustering assessed using Bayesian inference revealed that most American Creole populations studied show a high degree of admixture and the Pelón Mexicano from Yucatán showed a clear substructure (Fig. 2). At the other extreme, Mulefoot Hog, Guinea Hog and Wild

Mexican from USA, Criollo Venezolano, Boliviano and Argentino (Dry) showed no evidence of genetic admixture and substructure.

Fig. 1. Dendrogram constructed from DA genetic distance (Nei *et al*. 1983) among 25 pig breeds from America and Europe. Numbers are the percentage bootstrap values from 1000 replications of resampled loci.



Populations: MF: Mulefoot Hog (USA); GH: Guinea Hog(USA); PM: Pelón Mexicano (México); WM: Wild Mexico (USA); S: Criollo Salvadoreño (El Salvador); EC: Criollo Ecuatoriano (Ecuador); CU: Criollo Cubano (Cuba); VE: Criollo Venezolano (Venezuela); BO: Criollo Boliviano (Bolivia); A(W): Caacolero (Wet) (Argentina); A(D): Caracolero (Argentina); RE: Retinto, Iberian Pig (Spain); LAM: Lampiño, Iberian Pig (Spain); EN: Entrepelado, Iberian Pig (Spain); TOR: Torbiscal, Iberian Pig (Spain); MJ: Manchado de Jabugo, Iberian Pig (Spain); CM: Chato Murciano (Spain); NC; Negro Canario (Spain); F: Negro de Ibiza y Formentera (Spain); NM: Negro Mallorquín (Spain); ET: Euskal Txerria (Spain); C: Celta (Spain); DU: Duroc (International); LW: Large White (Spain); L: Landrace (Spain).

Fig. 2. Population structure of 25 pig breeds using model-based STRUCTURE program (Pritchard *et al*. 2000). Each animal is represented by a single vertical line divided into K colours, where the coloured segment shows the individual's estimated proportion of membership in that cluster. Black lines separate the populations labelled above the figure. Graphical representation of individual genotype membership coefficients (q) when K=18, the optimum K value estimated.

IV – Conclusions

The results suggest that Creole pigs constitute a differentiated group from any of the ancestral breeds. Some Creole breeds show certain level of admixture whereas other are genetically defined. The results derived from this project will have a great social impact, as may lead to the official registration of those populations, which are not yet officially recognized as breeds due to a lack of genetic supporting studies, but have an important productive rule in marginal areas and in a subsistence agriculture context.

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