Genetic diversity in the Ramo Grande cattle breed assessed by pedigree information and microsatellite markers

A.L. Pavão[*], C. Ginja[†], L.T. Gama[‡].

[*] Direcção Regional da Agricultura e Desenvolvimento Rural, Angra do Heroísmo, Açores, Portugal
[†] Centro de Biologia Ambiental, Faculdade de Ciências – Universidade de Lisboa, Lisboa, Portugal
[‡] Faculdade de Medicina Veterinária – Universidade de Lisboa, Lisboa, Portugal

ABSTRACT: Demographic analyses of Ramo Grande included Herdbook information on 2020 cows, 423 bulls and their offspring, collected between 1982 and 2013. On average, 10.9 calves were registered per bull and 2.6 per cow. Mean age of parents was 4.0 for bulls and 5.49 for cows, with average generation interval of 4.71 years. The reference population (1057 calves born in 2011-2013), had mean inbreeding of 4.1%, effective population size of 28 and one sire contributed with 10% of the gene pool. Analyses with 25 microsatellite loci showed high levels of genetic diversity (expected heterozygosity of 0.71, mean number of 7.2 alleles/locus). The estimated deficit in heterozygosity was 3%. Bayesian analysis indicated admixture with the exotic Holstein-Friesian in some animals. Measures should be taken to strengthen the conservation program of the Ramo Grande breed, to maintain genetic diversity and to avoid further dilution from crossbreeding.
Keywords: Cattle; Genetic diversity; Inbreeding

Introduction

Ramo Grande (RG) cattle was developed in the Region of Azores – Portugal, starting in the 15th century, when Portuguese colonizers from various regions of the mainland began to populate these islands, bringing with them cattle from their regions of origin. The characteristics of this cattle changed over time, as a consequence of their adaptation to local environment, the dispersion expected from being spread in six different islands and the possible influence of exotic germplasm which entered the Azores through the years.

The breed was considered nearly extinct in the late 20th century, but efforts were undertaken to rescue it, starting with a small number of animals that were still left. Currently, there are about 1250 active cows and 60 bulls enrolled in the Herdbook, spread over six islands. The Herdbook is open to registration of animals which comply with the breed standard, and an organized breeding program is being set-up, starting with the genetic characterization, based on molecular and demographic indicators.

The aim of this study was to assess the genetic diversity and breed structure of RG cattle, using both pedigree data and microsatellite marker information, to gather baseline information essential to design an appropriate conservation strategy.

Materials and Methods

Pedigree information. Herdbook records corresponding to animals born between 1982 and 2013 were used. Pedigree completeness was assessed by the number of ancestors known and by calculating an equivalent number of complete generations known, while inbreeding and relationships were computed from the relationship matrix. The genetic contribution of founders, ancestors and herds to the reference population (calves born in the period 2011-2013) was calculated as in Boichard et al. (1997). Effective population size was estimated from both the rate of inbreeding per generation (computed from the annual rate of inbreeding and generation interval) and from the individual rate of inbreeding (Gutierrez et al., 2009). All computations were carried-out with the ENDOG software (Gutierrez and Goyache, 2005).

Genetic markers. A set of 25 microsatellites was selected from the panel recommended by FAO (2011) for diversity studies. Blood and hair samples were obtained from representative animals of the RG breed (sampled in 2004 and 2013), and of 12 Portuguese autochthonous breeds (Alentejana, Arouquesa, Barrosã, Brava de Lide, Cachena, Garvonesa, Marinhoa, Maronesa, Mertolenga,

Minhota, Mirandesa and Preta) and 3 exotic breeds of worldwide expansion (Charolais, Holstein-Friesian and Limousine). Sample sizes per breed ranged from 38 to 70. DNA was extracted following routine protocols, primers were labeled with fluorescent markers and multiplex PCR reactions were carried-out. Microsatellite loci were combined according to annealing temperatures and expected fragment sizes. PCR products were separated and analyzed by capillary electrophoresis. The software Genetix v4.05 (Belkhir et al., 2004) was used to compute the number of alleles and expected and observed heterozygosities. FSTAT (Goudet, 1995) was used to estimate allelic richness and F-statistics. Deviations from Hardy-Weinberg expectations were analyzed with Genepop (Rousset, 2008). Breed relationships and structure were assessed, respectively, by computing the D_A distance of Nei and by the Bayesian approach implemented by the STRUCTURE software package (Pritchard et al., 2000).

Table 1. Summary statistics from pedigree analyses of the reference population[a]

Parameter	Value
Equivalent number of complete generations	3.13 ± 0.84
Average inbreeding (%)	4.07 ± 7.32
Inbred animals (%)	40.6
Average inbreeding of inbred animals (%)	10.04 ± 8.49
Maximum inbreeding (%)	37.5
Average relationship for all animals/animals in same herd (%)	2.1 / 35.8
Annual rate of inbreeding (%)	0.357 ± 0.023
Rate of inbreeding per generation, ΔF_g (%)	1.682
Effective population size from ΔF_g (Ne_g) / from ΔF_i (Ne_i) [b]	29.7 / 27.1
Mean generation interval (yr)	4.71 ± 2.66
Number of founders/ancestors/founder herds represented	534 / 517 / 185
Number of founders/ancestors/founder herds supplying 50% of genetic diversity	32 / 19 / 7
Effective number of founders (f_e)/ancestors (f_a)/herds	84 / 40 / 13
Effective number of founder genomes (f_g)	26

[a] Calves born in 2011-2013 (n=1057);
[b] Effective population size computed from individual rate of inbreeding.

Table 2. Indicators of genetic diversity in Ramo Grande sampled in 2004 and 2013, compared with the mean results observed in 12 native and 3 exotic cattle breeds based on analyses of 25microsatellite loci.

Parameter	Ramo Grande (2004)	Ramo Grande (2013)	Portuguese native breeds	Exotic breeds
Observed heterozygosity	0.696 ± 0.087	0.690 ± 0.117	0.671 ± 0.053	0.711 ± 0.023
Expected heterozygosity	$0.727 \pm 0,084$	0.711 ± 0.104	0.689 ± 0.038	0.716 ± 0.011
Mean number of alleles	$7.60 \pm 2,22$	7.16 ± 1.91	6.70 ± 0.91	6.81 ± 0.08
Allelic richness	$7.47 \pm 2,12$	6.83 ± 1.73	6.41 ± 0.77	6.56 ± 0.04
Private alleles	0	2	1.33 ± 1.50	2.67 ± 2.89
F_{is}	0.042	0.031	0.028	0.006
Loci not in HW equilibrium	5	3	3.92	2.00

Results and Discussion

RG cattle are spread among six islands, but nearly one-half are in the island of São Jorge. The RG Herdbook has information on 6224 purebred and 5115 crossbred calves with birth records, produced by 2020 cows and 423 bulls in 572 herds. Registrations increased steadily until 2008 and declined afterwards. RG herds tend to be rather small (about 2.6 purebred calves registered/herd/year) and most of the cows are currently used in crossbreeding. On average, the number of registered offspring per parent is 10.9 ± 26.1 for bulls and 2.6 ± 1.9 for cows. The mean age of parents was 4.0 for bulls and 5.49 for cows, and the average generation interval was 4.71 (Table 1). Pedigree depth has increased over the years, such that in the reference population nearly all calves have parents known and 2/3 have great-grandparents known, with a mean number of equivalent generations known of 3.1. Inbreeding has increased steadily, at a rate of about 0.36%/year and 1.68%/generation. In the reference population, the mean level of inbreeding is 4.1% and 41% of the animals are inbred. The realized effective population size is about 28, which is far below the recommended minimum threshold for maintenance of genetic diversity. The average relationship among all animals was about 2%, but for animals in the same herd it was 36%, due to the existence of small and closed herds. About one-half of the current genetic diversity in RG is contributed by 32 founders and 19 ancestors, with the more influential sire contributing about 10% of the gene pool. Overall, the effective number of ancestors and founder genomes was 40 and 26, respectively.

The RG presents high levels of genetic diversity, with an expected heterozygosity of 0.71 and a mean number of alleles/locus of 7.2, which are similar to the levels observed in other Portuguese and exotic breeds (Table 2). Differences between the RG samples collected in 2004 and 2013 were minor in all the genetic parameters evaluated. The estimated deficit in heterozygosity (F_{is}), which may reflect inbreeding or breed substructure, was 4.2% and 3.1% for the RG samples collected in those years, respectively. The levels of genetic diversity were similar across islands, but the F_{is} estimates differed considerably, with the highest level observed in São Jorge (which is the island with the largest census), while the estimated kinship per island ranged from 0.26 to 0.37. The genetic relationships of RG with other Portuguese autochthonous and the exotic cattle breeds are represented in Figure 1. Clearly, the RG has some proximity with the Holstein-Friesian, which is by far the predominant breed in the Azores, and some admixture has probably taken place. The Bayesian analyses done with STRUCTURE confirmed the heterogeneous influwing

signs of admixture with other breeds. This corresponds, in most cases, to the influence of Holstein-Friesian, but some evidence was also detected of a common ancestry with some native breeds.

The genetic distances between RG populations in the various islands where it is raised are small, when evaluated with either pedigree or molecular information.

Taken together, our results indicate that, in spite of some pedigree bottlenecks, the RG shows good levels of genetic diversity, possibly as a result of the open nature of the Herdbook. The overall levels of inbreeding, assessed either by pedigree information or analyses with microsatellite markers, are moderate, even though the mean relationship within herds is extremely high. Genetic analyses indicate the proximity of RG with Holstein-Friesian, and allowed the detection of recent admixture with exotic breeds in some RG animals, suggesting that measures should be taken to exclude these animals from the Herdbook.

Conclusion

Ramo Grande cattle are spread in a large number of small herds in six different islands. The large within-herd mean relationship and low effective population size warns that inbreeding must be kept under control, by avoiding matings among close relatives and rotating animals among herds. Molecular markers indicate high levels of genetic diversity, but also signs of admixture with exotic breeds (especially Holstein-Friesian) in some Ramo Grande animals. The conservation program of Ramo Grande should be strengthened to avoid further dilution from crossbreeding.

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Figure 1. *Neighbour-Net* **dendrogram obtained from** D_A **genetic distances among breeds.**