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Usefulness of running animal models in absence of pedigrees: Estimation of genetic parameters for gastrointestinal parasite resistance traits in Djallonké sheep of Burkina Faso

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ABSTRACT

The main aim of the current study was to exemplify the usefulness of pedigree-free animal models in scenarios in which available information is limited. Up to 271 Djallonké sheep individuals of Burkina Faso were typed for 29 microsatellites. Performance records were obtained from a trial designed to assess environmental factors affecting gastrointestinal parasite resistance. Packed cell volume (PCV), log-transformed fecal egg count (lnFEC) and FAffa Malan CHArt (FAMACHA) eye scores were analyzed fitting univariate and multivariate animal models. Molecular information was used to: a) construct an artificial pedigree maximizing the correlation between the molecular coancestry matrix and the coancestry of the artificial genealogies (P* Matrix); and b) computing the Ritland's estimator of between-individuals molecular coancestry (R Matrix). All animal models fitted included as fixed effects the contemporary group, day of assessment after deworming and body weight as a linear covariate. As random effects, models included the additive genetic effect and the permanent environment associated to individual. Multivariate models further included the covariances between permanent environmental and additive genetic effects. Univariate models had difficulties to separate between additive genetic and permanent environmental effects. The P* Matrix gave higher estimates of additive genetic variance than the R Matrix. Independently of the Matrix used, multivariate models gave more consistent estimates of heritability (h^2) and permanent environmental effect: estimates of h^2 for lnFEC varied from 0.063 ± 0.037 to 0.173 ± 0.076; estimates of h^2 for FAMACHA scores ranged from 0.206 ± 0.070 to 0. 343 ± 0.111; and c) estimates of h^2 for PCV ranged from 0.073 ± 0.045 to 0.142 ± 0.084. Genetic correlations estimated for the pair lnFEC-FAMACHA using multivariate models were significant in all cases varying from 0.548 ± 0.247 to 0.785 ± 0.242. Pedigree-free animal models may be advantageous in developing countries at the beginning of selection programmes or to improve the outcome of trials designed to assess performance of local breeds for economically interesting traits.

1. Introduction

Gastro-intestinal parasitic infestations cause heavy economic losses to sheep industry mainly due to increased mortality, decrease in weight gains and costs of anthelminthic therapy (Nieuwhof and Bishop, 2005). They are of particular importance for tropical production systems in which the costs of the disease are estimated as 35% to 50% of turnover (Bishop, 2012).

Since the 1990s, sheep breeding schemes in developed countries have paid major attention to resistance to nematode infections using fecal egg count (FEC) as the indicator trait for resistance. Most estimates of heritability reported for these traits varied from moderate (Safari et al., 2005; Assenza et al., 2014) to low (Gutiérrez-Gil et al., 2010; Goldberg et al., 2012). Furthermore, molecular genetics has

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allowed significant advances on the knowledge of the genomic areas associated to the trait (Gutiérrez-Gil et al., 2009; Periasamy et al., 2014; Atlija et al., 2016). However, unfortunately, this scenario does not fit to most developing countries.

The use of molecular relatedness matrices has been shown to be a suitable alternative to the genealogical relationship matrix for the estimation of genetic parameters and breeding values via animal models (Rodríguez-Ramilo et al., 2007; Frentiu et al., 2008; Berenos et al., 2014). Even if available databases are small, this approach would allow to use breeding values for economically important traits. The use of small datasets is not the only concern of pedigree-free animal models. Limitations of this approach include the non-independence of between-individuals relatedness estimates when the number of markers is lower than the number of individuals. This makes difficult to obtain a positive-definite relatedness matrix necessary to apply an animal model to performance recoding data. However, some strategies have been proposed to solve this concern (Frentiu et al., 2008).

The current study analyses records of 271 Djallonké sheep. Phenotypes were obtained from a trial designed to assess gastrointestinal parasite resistance in Djallonké individuals managed under natural parasite challenge (Traoré et al., 2017). Individuals were typed for a set of microsatellites and genetic parameters were estimated via the construction of either artificial pedigrees or a between-individuals molecular coancestry matrix. Finally, the usefulness of such approaches in the beginning of a breeding program in developing countries is discussed.

2. Materials and methods

2.1. Data

The Djallonké sheep of Burkina Faso have been previously described morphologically (Traoré et al., 2008) and genetically (Álvarez et al., 2009, 2012). Most data used in the current analysis were obtained from a recent trial designed to assess environmental factors affecting gastrointestinal parasite resistance in Djallonké sheep (Traoré et al., 2017). This field trial was carried out in the surroundings of Mangodara (southern Sudan-Guinea Savannah region of Burkina Faso; Comoé province) during the rainy season 2014. Analyses were restricted to those individuals with performance records and blood sample available for DNA extraction. After editing, analyses involved 252 individuals (81 males and 171 females), belonging to 33 different households. Nineteen (9 males and 10 females) four-months old Djallonké sheep individuals sampled from 7 different households of Dédougou (South Western Burkina Faso; Mouhoun province), were simultaneously submitted to the protocol applied in the Mangodara trial in the facilities of the Kamboinsé station of the INERA near Ouagadougou (central Sudan-Sahel Savannah region of Burkina Faso). Altogether, 271 individuals aging 2- (128), 3- (62), and 4-months old (81) with performance data and DNA sample were used for analyses.

Briefly, body weight, packed cell volume (PCV), fecal egg count (FEC) and FAffa Malan CHArt (FAMACHA©) eye scores (Bath and Van Wyk, 2009) were obtained according to the methods recently described in Traoré et al. (2017). PCV and FAMACHA scores characterize the degree of anemia resulting from parasitization of *Haemonchus contortus* (Traoré et al., 2017): the higher the FEC values, the higher the FAMACHA scores and the lower the PCV. Following Traoré et al. (2017), FEC scores were log-transformed as lnFEC = ln(FEC + 25). The individuals acquired in Dédougou were dewormed with levamisol after one week of adaptation to the INERA facilities of Kamboinsé. The individuals assessed in Mangodara were dewormed in presence of their owners. Individuals were assessed at 28 and 35 days after deworming. Before and after deworming, individuals were exposed to natural infection with gastrointestinal nematodes.

Total DNA was obtained from blood samples following standard procedures (Sambrook et al., 1989). Twenty-nine microsatellites, previously used in diversity analyses in small ruminants of Burkina Faso (Álvarez et al., 2009; Traoré et al., 2009, 2012) were analyzed for all samples. Genotyping was performed on an Automatic Sequencer ABI 310 (Applied Biosystems, Barcelona).

2.2. Genetic analyses based on microsatellites

For descriptive purposes, the multilocus genotypes were analyzed using the program MolKin (Gutiérrez et al., 2005). The following parameters were computed: observed heterozygosity, expected heterozygosity and F_{IS}. Statistical confidence on the parameters computed was assessed via bootstrapping using 1000 replicates.

An artificial pedigree (P*) of 10 discrete generations based on the between-individuals molecular coancestry matrix was inferred using the program MOLCOAN (Fernández and Toro, 2006; Cervantes et al., 2011). This program is based on a simulated annealing algorithm that maximizes the correlation between the coancestry molecular matrix, given the data, and the genealogical coancestry matrix built from the fictitious created pedigree. The annealing algorithm was fitted as follows: a) up to 2000 iterations with 5000 solutions tested in each iteration; b) 0.01 as the initial probability on which simulated annealing accepts a worse solution to avoid local minima; c) a rate of decrease of this initial probability of 0.975 per iteration; and d) the maximum number of parents managed by the simulation algorithm (125 males and 125 females) were allowed to act as parents per generation. For descriptive purposes, the artificial pedigree constructed was analyzed using the program ENDOG v4.8 (Gutiérrez and Goyache, 2005). For each individual with data, the "artificial" inbreeding (F_i^*) and individual increase in inbreeding (ΔF_i^*) coefficients (Gutiérrez et al., 2008, 2009) were computed.

Following the suggestion by Rodríguez-Ramilo et al. (2007), the between-individuals molecular coancestry matrix was computed using the estimator $\hat{f}_{R,ij}$ proposed by Ritland (1996) because it corrects for its actual variance. The Ritland's (1996) estimator of molecular coancestry (i.e. the probability that two alleles taken at random, one from each individual, are identical by state) was computed using the program PolyRelatedness v1.6 (Huang et al., 2016) as $\hat{f}_{R,ij} = \frac{\sum_k^L \sum_l^{n_k} \frac{f_{M,ijkl} - p_{kl}^2}{p_{kl}}}{\sum_k^L (n_k - 1)}$, where $f_{M,ijkl}$ is the between-individuals (i and j) molecular coancestry for each allele l at each locus k, L is the number of loci, n the number of alleles at the locus k, p_{kl} is the frequency of allele l at locus k in the base population (here the current population allele frequencies), and $(n_k - 1)$ is the weighting factor for the locus k.

Between-individuals genetic relationships assessed using molecular markers may be biased if data are affected by hidden genetic structure. This can affect performance of animal models based on molecular information as well: if individuals sharing genetic background are not randomly distributed among levels of the fixed effects included in the model fitted, estimates of genetic parameters could be biased. The existence of genetic structure in the analyzed dataset (K) was ascertained using the program STRUCTURE (Pritchard et al., 2000). STRUCTURE was run, under the admixture model, considering correlated allele frequencies. K was set to vary between 1 and 10, and 10 simulations with different starting points for each K-value were run. All runs used burn-in periods of 100,000 iterations and data collection periods of 1,000,000 iterations. The most likely K-value in the data set was identified according to Evanno et al. (2005) using the STRUCTURE HARVESTER v.0.6.8 website (Earl and vonHoldt, 2012). Identification of the most likely K is carried out computing the increase of likelihood (L) across K-values and runs as $\Delta K = \text{mean}(|L''(K)|)/\text{sd}(L(K))$. Using the Proc Freq of SAS/STAT™ v9.2 (SAS Institute, Cary, NC, USA) a Chi-squared analysis was performed to check if the individuals belonging to each cluster identified were randomly distributed among contemporary groups.