INBREEDING DEPRESSION IN ADAPTATION PHENOTYPES OF TROPICAL BEEF CATTLE USING GENOME WIDE DATA

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SUMMARY

Inbreeding has the potential to negatively impact on animal performance. Strategies to monitor and mitigate inbreeding depression require that it can be estimated accurately. Here, we use genome-wide SNP data to produce three alternative estimates of inbreeding: proportion of heterozygous SNP, diagonal elements of the genomic relationship matrix and runs of homozygosity. We focus on Brahman (N = 2,112) *versus* Tropical Composite (N = 2,550) cattle which have been genotyped by a 74K Indicine chip and phenotyped for 10 traits of commercial importance to tropical adaptation. While the summary statistics for the Brahman and Tropical Composite populations are remarkably similar, the relationships of the inbreeding coefficients to the various phenotypes are different. In both populations, the proportion of heterozygous SNP was positively correlated with body condition score and negatively correlated with yearling weight. A possible explanation relating to the content of the Indicine SNP chip is briefly discussed.

INTRODUCTION

Inbreeding can reduce animal performance for commercially important traits. In order to mitigate inbreeding through management strategies and breeding programs, it is important to be able to estimate it accurately. Traditionally, inbreeding coefficients (F) have been computed from pedigree information. Given the recent availability of genome-wide single nucleotide polymorphism (SNP) data, it is now possible to exploit real genetic data to infer F. According to Saura *et al*. (2015) genomic F has the following three advantages: it measures homozygosity directly rather than its expectation; it can be applied to particular genomic regions such as those harbouring QTL; and it can be estimated with incomplete or even absent pedigree information.

Here, we explore three different measures of inbreeding using 71,726 SNP genotypes from Brahman (BB) and Tropical Composite (TC) cattle measured for 10 phenotypes of relevance to tropical adaptation. The three inbreeding measures we used are: proportion of heterozygous SNP (HET_F), diagonal elements of the genomic relationship matrix (GRM_F) and runs of homozygosity (ROH_F) (Keller *et al.* 2011; Saura *et al.* 2015). Sample size is relatively large (2,112 Brahman and 2,550 Tropical Composite) and genome-wide association studies have already been well characterised (Porto-Neto *et al.* 2014).

MATERIALS AND METHODS

Animals, genotypes and phenotypes. We used genetic and phenotypic resources outlined in Porto-Neto *et al.* (2014). In brief, 2,112 BB and 2,550 TC cattle with genotypes for 729,068 SNP. For the present work we extracted the 71,726 SNP corresponding to the GGP Indicus HD chip (http://www.neogeneurope.com/Genomics/pdf/Slicks/NE_GeneSeekCustomChipFlyer.pdf). The ten phenotypes are: coat type (COAT), coat colour (COLOR), condition score (COND), worm eggs (EPG), fly lesions (FLY), flight time (FT), navel/sheath score (NAVEL), temperature (TEMP), tick score (TICK) and yearling weight (YWT). In addition, we used the SNP-based estimated zebu content also described in Porto-Neto *et al.* (2014) as an additional variable to be related with the measures of inbreeding described next.

Measures of inbreeding. We explored three alternative measures of inbreeding:

1. GRM_F: Genomic inbreeding based on the diagonal elements of the genomic relationship matrix (GRM). The GRM was computed according to Van Raden (2008) and allele frequencies calculated separately for the Brahman and Tropical Composite population;
2. HET_F: Proportion of the total SNP genotypes that were heterozygous;
3. ROH_F: Proportion of the genome that consists of runs of homozygosity (ROH).

We followed computational approaches described in Saura *et al*. (2015) by which ROH was detected according to the following criteria: (1) At least 10 kb in a ROH window; (2) At most one heterozygous SNP; (3) A density of at least 1 SNP per 100 kb; (4) A maximum distance of 100 kb between two SNP in a given ROH.

Inbreeding depression. The effect of each measure of inbreeding on the phenotypes was estimated by fitting a model that contain the phenotype as dependent variable and the measures of inbreeding plus the zebu content as fixed regression covariates as well as the fixed class effects of contemporary group (combination of sex, year and location), age of dam. All analyses were performed using SAS 9.3 (SAS Inst., Cary, NC) one phenotype at a time and separately for the BB and the TC datasets.

RESULTS AND DISCUSSION

Table 1 shows summary statistics for the three measures of inbreeding in the two populations. For a given measure, the means are very similar in the two populations and they are also similar to the equivalent values reported by Pryce *et al.* (2014) with dairy cattle. In that work, the authors reported an average GRM_F of 1.134 and 1.144 for Holstein and Jersey, respectively. They also reported an average HET_F of 0.347 and 0.285 for the same two breeds of dairy cattle.

In spite of the overall similarities between the two populations for the 3 inbreeding measures (Table 1), there are remarkable differences in the way they correlate with the phenotypes and zebu content. A likely confound is the indicine chip which better characterises the genetic variation in the Brahman population than it does in the Tropical Composite. As expected, we find that a low heterozygosity is correlated with inbreeding in Brahman ($r = -0.271$; $P < 0.0001$). However, this relationship is actually reversed for the Tropical Composite cattle ($r = 0.495$; $P < 0.0001$).

Table 1. Summary statistics for the three measures of inbreeding in the two populations.

Population	Measure of Inbreeding	Mean	Standard Deviation	Minimum	Maximum
Brahman	HET_F	0.391	0.018	0.284	0.456
(N = 2,112)	GRM_F	1.021	0.039	0.931	1.691
	ROH_F	0.376	0.020	0.306	0.533
Tropical Composite	HET_F	0.384	0.029	0.234	0.481
(N = 2,550)	GRM_F	1.021	0.067	0.880	1.412
	ROH_F	0.382	0.032	0.278	0.566

In both populations, HET_F was positively correlated with COND and negatively correlated with YWT (Figure 1). The strongest correlation involving ROH_F was with YWT being negative for Brahman ($r = -0.175$; $P < 0.0001$) and positive for Tropical Composite ($r = 0.211$; $P < 0.0001$). The discrepancies between the populations may reflect spurious results from the Tropical Composite. These animals are only 27.2% Zebu on average (Porto-Neto *et al.* 2014) which may lead to artefacts arising from the reliance on the Indicine chip for assaying gene variants.

An alternative explanation is that these correlations, while statistically significantly different from zero, are rather small in magnitude and hence of little biological relevance, and their significance the result of the relatively large sample sizes. This feature is clearly illustrated by Figure 2 showing the scatter plot between ROH_F and YWT for the two populations.

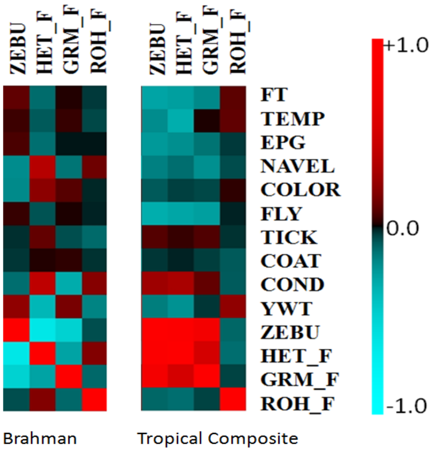

Figure 1. Heat map of the correlation matrix between zebu content and the three measures of inbreeding with each other and the ten phenotypes and for the two populations.

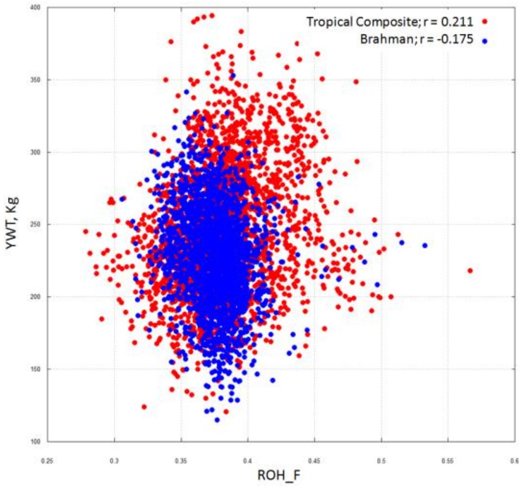

Figure 2. Scatter plot of the relationship between inbreeding based on runs of homozygocity (ROH_F) and yearling weight (YWT) for the Brahman (blue dots) and Tropical Composite (red dots).

In the Brahman dataset, the linear model used for the estimation of inbreeding depression yielded an R^2 that ranged from 18.4% for COLOR to 67.7% for YWT. Similarly, in the Tropical Composite, the R^2 ranged from 18.6% for TICK to 75.6% for YWT. Table 2 shows the estimates of inbreeding depression for each phenotype and in the two populations. In the Brahman population, significant inbreeding depression estimates were identified for FT, EPG, COLOR, COND and YWT. This contrasts with the Tropical Composite population for which only TEMP and YWT was significantly affected by either measure of inbreeding.

While HET_F and ROH_F had very similar mean and variability (Table 1), they were poorly correlated (0.171 and -0.122 in the Brahman and Tropical Composite population, respectively) which may explain the large differences observed for some of the estimates of inbreeding depression. Consistent among both populations was the effect of inbreeding depression on YWT. In the Brahman population, a 1% increase in HET_F was associated with an increase of 1.89 kg of YWT. Similarly, a 1% increase inbreeding measured by GRM_F was associated with a decrease of 1.08 kg of YWT in the Tropical Composite population.

Table 2. Estimates of inbreeding depression for the three measures of inbreeding in the ten phenotypes and two populations.

Phenotype	Brahman			Tropical Composite		
	HET_F	GRM_F	ROH_F	HET_F	GRM_F	ROH_F
FT	-101.44	-73.54	145.69*	-170.20	-14.30	-22.15
TEMP	1.01	0.20	-0.25	-2.64	0.28	1.14*
EPG	-3,097.96**	-995.83**	-329.25	-1,117.53	-440.95	-308.60
NAVEL	3.48	1.78	0.61	-2.96	-0.68	0.51
COLOR	16.25**	5.09**	-1.86	2.37	0.73	-0.13
FLY	-3.44	0.62	1.16	-2.38	-0.56	0.02
TICK	0.49	-0.80	0.30	-10.34	-0.68	2.53
COAT	2.78	0.75	-1.43	0.16	2.34	-1.32
COND	-4.61*	-2.26**	-0.60	2.23	-0.60	0.74
YWT	189.02**	38.80	4.58	-15.10	-108.42**	30.61

$^{*}P < 0.05$; $^{**}P < 0.01$

CONCLUSIONS

Traditionally, inbreeding coefficients – based on the probability that both alleles at any given locus within an individual are identical by descent – have been computed from pedigree information. However, the advent of high-density genotype data allows for the estimation of realised F which might differ even between animals that have identical pedigree.

It is worth mentioning that the pedigree-based inbreeding was of no practical use in our case because after tracing back three generations of ancestors, only four Brahman individuals were found to be inbred (all with F = 12.5%) and no Tropical Composite individuals were inbred. This is not surprising as the Beef CRC cattle were experimental cattle representing many properties, and bred with the objective to avoid, or at least minimise inbreeding.

While the average of the three inbreeding measures employed here were similar in the two populations, the Brahman population appeared to be more affected by inbreeding depression than the Tropical Composite. A comparison of the inbreeding depression estimates here with those obtained from a model that contains a single inbreeding measure (as opposed to the three of them), plus a random additive polygenic component is warranted.

Also in the future, we plan to explore different parameterizations of the GRM, and to partition ROH into two groups – 'long' and 'short,' with a view to infer past *versus* recent inbreeding as described by Saura *et al.* (2015). Characterising the gene content in the regions prioritised by differential ROH could also help identify genes of commercial relevance in the two populations. Finally, it would be worthwhile to establish what relationship exists between inbreeding and the 'missing heritability' problem, as this has still not yielded to analysis.

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