A dedicated SNP panel for evaluating genetic diversity in a composite cattle breed.

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ABSTRACT: A custom 60K SNP panel, extracted from Bovine HD SNP chip was used to evaluate genotypic frequency changes in Braford (BF, a composite breed) when compared to progenitor breeds: Hereford (HF), Brahman (BR), and Nelore (NE). Samples from both the U. S. and Brazil were used. The new panel differentiated each breed and provided an estimation of the BF genetic composition for samples from both countries. Both Bayesian and principal component analysis indicated formation of new BF genotypes and a cluster when compared to NE, HF, and BR. Monitoring and assessing the SNP composition of this cluster may reveal important markers that lend themselves to being used to select for various traits. In addition the new SNP panel may increase the level of resolution for understanding and managing the genetic diversity of this and other composite breeds.

Keywords: Molecular marker panel; Composite cattle breeds; Genetic resources

Introduction

New livestock breeds are continually being developed to take advantage of heterosis, combining ability and complementarity of the progenitor breeds. Breeders have used this process of molding new livestock breeds since before Roman times (Wood and Orel, 2001). Much work has occurred in forming and evaluating composite breeds using *B. taurus* and *B. indicus* breeds, and many have become globally important.

Genetic comparisons between composite populations to their founding breeds have been performed (Bovine Hapmap Consortium, 2009), nevertheless the main conclusion has been that the populations were admixed. It has been recognized that *B. indicus* breeds require different SNP panels than *B. taurus* breeds to effectively identify SNPs associated with traits of interest (Matukumalli et al., 2009). But it has not been established that the 770K HD chip reduces bias toward *B. indicus;* however the 770K HD chip may present opportunities to develop smaller panels tailored to *B. taurus* and *B. indicus* evaluation. A preliminary study in Brazil using a relatively small SNP panel suggested the Braford cattle had lost substantial amounts of their *B. indicus*

component (~25% below expectation). In addition, the previous work only used Nelore when both Nelore and Brahman have been used in developing Braford.

The objective of this study was to evaluate the genetic composition of U.S. and Brazilian Braford using a customized SNP panel and comparing the composite to the progenitor breeds (Hereford, Brahman and Nelore) to determine if the *B. indicus* composition has changed. Additionally we explored how patterns of genetic diversity are changing in the development of a composite breed.

Materials and Methods

Breeds and Animals. Brazilian and U.S. samples from Braford (n=80), Nelore (n=47), Brahman (n=36) and Hereford (n=78) were collected and genotyped using the 770K SNP Illumina Bovine HD Bead Chip. Additionally, Braford birth years (1986 to 2010) and number of generations the Braford had met the targeted 3/8-5/8 combination were also used. All but 4 Braford were confirmed by pedigree records as meeting the breed standard of 3/8 zebu – 5/8 Hereford combination.

Data Analysis. The strategy used to select SNPs for analysis was to choose within each progenitor breed SNPs unique or in high frequency (therefore with greater homozgosity) for that breed (Figure 1). The SNPs selected in each breed were combined to form a new panel used in the analysis. The software packages SVS7 (Golden Helix Inc), Plink (Purcell et al, 2007) and STRUCTURE (Pritchard et al., 2000) were used to evaluate genetic diversity differences between the four breeds. STRUCUTRE runs used a burn-in of 10,000 iterations followed by 100,000 MCMC iterations to derive proportional assignments across clusters for the four breeds.

Results and Discussion

The initial STRUCTURE run at K=2 the expected partition between *B. indicus* and *B. taurus;* grouping Nelore and Brahman together in one cluster, Hereford in the second cluster and Braford split at theoretical expectations between cluster 1 and
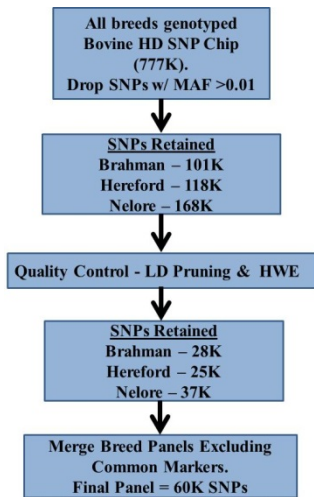
Figure 1. Procedure used to develop SNP panel for analysis.
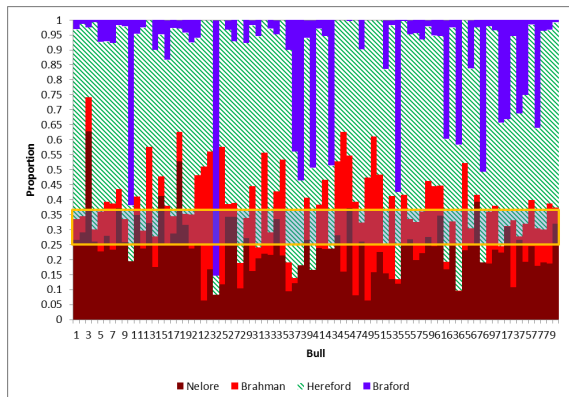
Figure 2. STRUCTURE results using K = 4. Within the gold box represents targeted proportion of *B. taurus* and *B. indicus* by breed associations.

2. When K = 3 the Brahman and Braford were split between the new cluster (30% and 17%, respectively) and the preexisting clusters where they had been placed in K = 2 (Table 1). Splitting the *B. Indicus* cluster was anticipated as Brahman was developed from 4 different zebu breeds and only sharing the foundation breed Ongole with Nelore (Mason, 1996). When K = 4 the Braford was the only breed with an average assignment of 10% in the new cluster. This subdivision of Braford appears to have been taken from the third cluster in the K = 3 analysis. Figure 2 provides the proportion of membership across the 4 clusters for Braford. Among the Braford, 18% had a

proportional assignment greater than 0.25 to the new cluster.

Table 1. Proportion of membership of pre-defined populations to three clusters.

| Breed	Cluster 1	Cluster 2	Cluster 3
Braford	0.17	0.59	0.25
Nelore	0.00	0.00	1.00
Brahman	0.30	0.00	0.69
Hereford	0.00	1.00	0.00

To further explore the K=4 finding a principal coordinate analysis was performed (Figure 3). The three progenitor breeds were distinct from each other and Braford. As expected principal coordinate 1(20% variation) separated *B. taurus* and *B. indicus* and principal coordinate 2 (2.1% variation) separated Brahman and Nelore. But it is the 3rd principal coordinate (2.0% variation) we find interesting. Figure 3 shows Braford separating from the parental breeds along the third axis and appearing to confirm the STRUCTURE findings when K=4. We think it unlikely that the additional partition represents the other breeds used to develop the Brahman since no proportional assignment for Brahman was placed in K = 4 (as was the case for K =3).
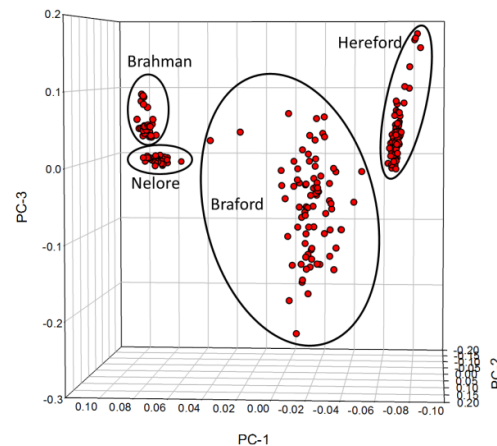
Figure 3. First 3 principal components accounting for 24% (PC-1 = 20.0%, PC-2 = 2.1%, PC-3 = 2.0%) of SNP variation and the distribution of individuals within each breed.

To better understand the composition of the Braford in K=4 the proportional assignment per animal over time was plotted (Figure 4) and showed that the proportional assignment to cluster 4 increased. It is noted that all animals with a proportion of greater than 0.2 are from Brazil. Evaluating the number generations since reaching the

3/8 – 5/8 composition for the tested animals could aid in explaining the assignment to cluster 4.
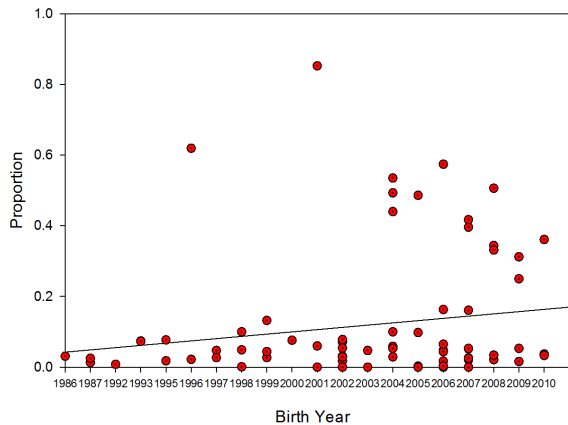
Figure 4. Individual animal assignment to cluster 4 by birth year.

Several aspects of the results suggest the new panel is functional, for example clear distinctions between *B. taurus, B. inidicus* and composite were obtained. As expected, given the criteria used in panel construction, the Braford had a higher level of heterozygosity (Figure 5) than the foundation breeds. But the reduced difference between Hereford and Braford was likely due to greater genetic similarities between these two breeds as demonstrated by a low Fst (0.06) vs Braford vs Brahman and Nelore with Fst of 0.16 to 0.19, respectively.
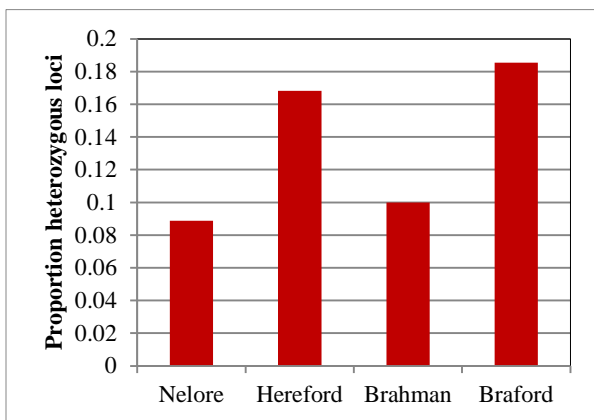
Figure 5. The proportion of heterozygous loci per breed.

As to the original question concerning Braford exhibiting a reduced proportional assignment to *B. indicus* we find no evidence that such a change is occurring. We do know this original report used a small SNP panel and therefore suspect some bias was introduced in its construction. We speculate that cluster 4 and the third principal component are detecting the formation of a "new" breed cluster for Braford, and that over time as selection and genetic drift continue to separate Braford from progenitor populations it will become more distinct. Therefore, in managing Braford genetic diversity the proportional assignments, along with other genetic variation measures, for the "new" cluster can be monitored and assessed in determining if the composite is moving disproportionately toward one of the progenitor clusters.

Conclusion

Using a customized 60K SNP panel developed specifically to evaluate genetic differences between *B. indicus*, *B. taurus* and composites breeds we show that U. S. and Brazilian Braford align with theoretical expectations of being a 3/8 and 5/8 composition, respectively. Interestingly a Braford cluster was detected which creates opportunities to be used in the future for conservation and breeding programs. Future monitoring and assessing the SNP composition of this cluster may reveal important markers that lend themselves to being used to select for various traits important to the variability of the composite breed. Additional exploration of this approach for developing specific function SNP panels with different composites and parental breeds could be useful in validating this approach.

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