ABSTRACT: Along with environmental adaptation, breed hybridization has contributed to the array of coat color phenotypes observed today among Ethiopian cattle populations. Breeds adapted to lowland agroecology (Ogaden and Begait) display coats that are white, gray, and a combination of white and black with different levels of spotting. In contrast, breeds adapted to high-altitude environments, such as Arado and Guraghe, mainly display coats that are solid black, red, brown, or a mixture of various colors. We compared Ethiopian cattle populations characterized by heterogeneous coat color phenotype for the MITF locus identified from the 80K indicus SNP chip. When comparing spotted and unspotted populations, we detected a significant (P < 0.01) and high level of genetic differentiation (Fst = 0.17), which indicated that the MITF locus may influence the observed coat color variations among Ethiopian cattle populations adapted to different ecological conditions. **Keywords:** Ethiopian cattle; Coat-color variation; Adaptation; MITF

**Materials and Methods**

Populations, sample collection, and genotyping. A total of 213 animals representing six Ethiopian cattle populations were sampled from diverse agroecologies. These populations included the following: Zenga [Fogera (FG), n = 40 and Arado (AR), n = 31], Large East African zebu [Begait (BG) n = 39], Small East African zebu [Guraghe (GR), n = 33 and Ogaden, (OG), n = 30], and short horn taurine [Sheko (SH), n = 40]. Sampling locations and number of animals per population and breed group are detailed in Edea et al., 2014. Nasal swab samples were collected using the Performagene LIVESTOCK nasal swab (DNA Genotek., Kanata, ON, Canada). The widest possible range of unrelated individuals was sampled based on information obtained from the owners of the animals and pedigree records. DNA was extracted from the nasal samples according to the manufacturer’s recommendations (http://www.dnagenotek.com). All samples were genotyped using the GeneSeek Genomic Profiler Indicine HD BeadChip, an Illumina Infinium® array consisting of nearly 80,000 SNPs derived mainly from Bos indicus breeds(GeneSeek, Lincoln, NE, USA), according to Illumina’s standard protocols (http://www.illumina.com).

Breed characteristics. Ethiopian cattle are highly heterogeneous for coat color phenotypes, ranging from pure or solid to highly spotted. Begait is derived from the first introductions of zebu cattle from Asia, but its long legs and cervicothoracic hump also indicate sanga background. The breed is predominantly characterized by white coats with black spots. Fogera cattle originate from the crossing of Abyssinian zebu from the central highlands and sanga (Danakil, Raya-Azebo) and are characterized by pied coats of black and white or black and grey. Coat color in Arado is mainly uniform (72%), with red coats being the most com-
mon. Most of the Guraghe breeds display red, chestnut, and roan coats. Ogaden cattle are distinguished by their uniform plain white coat color. Sheko are believed to be the last remnants of the original humpless Shorthorn (Bos taurus) cattle in eastern Africa (http://dagris.ilri.cgiar.org).

**Data analyses.** Allelic and genotypic frequencies, minor allele frequency (MAF), and deviation from Hardy–Weinberg equilibrium (HWE) for the MITF SNP (rs137845005) were estimated using PowerMarker software. To estimate the AMOVA, observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding for the SNP, we employed Arlequin software. To test whether the SNP within the MITF gene is under directional selection, we classified the study populations into plain coat color (Guraghe and Arado) and pied or spotted groups (Begait). The selection signature was tested based on FST at different significance levels as a measure of genetic differentiation for each locus between the plain and spotted populations, following the FDIST approach, using Arlequin software version 3.5. Within this framework, we ran 20,000 coalescent simulations to obtain the p-values of locus-specific FST, conditioned on the observed levels of heterozygosity for the default settings.

**Results and Discussion**

Allele and genotypic frequencies, MAF, and diversity for the MITF SNP (rs137845005) are shown in 1. The mean MAF was highly different between the mixed (0.19) and solid coat color (0.48) phenotypes, with the lowest (0.17) and the highest (0.50) MAF values observed in Begait and Guraghe and in Sheko, respectively. Similarly, the allele frequency for the SNP (rs137845005) in the MITF gene greatly differed between the spotted or mixed (A = 0.82; G = 0.18) and plain coat color (A = 0.53; G = 0.47) phenotypes. The average homozygous genotype (AA) frequency was higher in Begait, and Fogera (0.65), whereas it was only 0.23 in solid or unspotted populations. These results revealed that the A allele was favored over the G allele in spotted cattle populations compared to populations displaying pure coat color phenotypes. For example, in the spotted Begait and Fogera populations, over 70% of the animals carried the A allele. With the exception of Begait and Arado (P < 0.01), the SNP under investigation was in HWE for all populations.

AMOVA analysis indicated that genetic variation among the breeds was significant (P < 0.001) and accounted for over 16% of the total genetic variance determining the higher coat color phenotypic variation among indigenous Ethiopian cattle populations. The inference of diversity indices for the MITF locus (rs137845005) indicated that a higher level of genetic variation was found in the unspotted populations compared to the populations with spotted or mixed coat color phenotypes. For example, the expected heterozygosity ranged from 0.28 in Begait to 0.51 in Guraghe or Sheko. Likewise, the lowest and the highest observed heterozygosity were detected for the same population groups. The lower diversity in Begait, and Fogera could be due to directional selection for the A allele over the G allele. For the MITF gene locus (rs137845005), Guraghe cattle are the most inbred, followed by Arado cattle. Previous studies have reported that variability in the MITF gene accounts for the differences between spotted and unspotted phenotypes in cattle (Fontanesi et al. 2012). A low level of diversity (π) has also been detected in MITF genes in cattle (Fontanesi et al., 2012).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Begait</th>
<th>Fogera</th>
<th>Guraghe</th>
<th>Sheko</th>
<th>Arado</th>
<th>Ogaden</th>
<th>Spotted</th>
<th>Non-spotted</th>
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<tbody>
<tr>
<td>Allele frequency</td>
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<td></td>
<td></td>
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<tr>
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<td>0.80</td>
<td>0.50</td>
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<td>0.57</td>
<td>0.82</td>
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<td>G</td>
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<td>0.43</td>
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<tr>
<td>Genotype frequency</td>
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<td>AA</td>
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<td>0.43</td>
<td>0.19</td>
<td>0.48</td>
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<tr>
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<td>0.32</td>
<td>0.51</td>
<td>0.51</td>
<td>0.50</td>
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<td>He</td>
<td>0.28</td>
<td>0.35</td>
<td>0.63</td>
<td>0.60</td>
<td>0.48</td>
<td>0.60</td>
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</table>

MAF, Minor allele frequency; HE, Expected heterozygosity; Ho, Observed heterozygosity, HWE, Hardy-Weinberg equilibrium, FSt, Within-population inbreeding.
Conclusion

We compared Ethiopian cattle populations characterized by heterogeneous coat color phenotype for the MITF locus identified from the 80K indicus SNP chip. We are interested in this gene (locus) because when we compared the two population groups (spotted and unspotted), we detected significant and high genetic differentiation (\(Fst = 0.17\)). The MITF gene is widely known to be associated with coat color pattern in cattle and plays an important role in the regulation of the TYR, TRP-1, and DCT genes, which code for enzymes involved in pigment synthesis (Busca and Ballotti, 2000; Widlund and Fisher, 2003). In mammals, melanocytes cover the entire body surface and are responsible for pigmentation of the skin, hairs, and eyes (Levy et al. 2006). As the SNP(s) upstream of the gene are important in regulating the level of gene expression, along with that of other genes, the MITF locus (rs137845005) may influence the observed coat color variation among Ethiopian cattle populations.

Literature Cited