

Morphometric and genetic variation in 8 breeds of Ethiopian camels (*Camelus dromedarius*)^{1,2}

Yoseph W. Legesse,^{*,†,3} Christopher D. Dunn,^{‡,3,4} Matthew R. Mauldin,^{§,#}
Nicté Ordonez-Garza,[‡] Gage R. Rowden,[‡] Yoseph Mekasha Gebre,[†] Mohammed Y. Kurtu,^{*,†}
Seid Mohammed Ali,[†] Wondmagegne D. Whibesilassie,^{*,†} Michael Ballou,[¶] Melaku Tefera,^{**}
Gad Perry,^{††} and Robert D. Bradley^{‡,‡‡}

*Department of Animal Production, College of Agriculture and Environmental Sciences, Haramaya University, Ethiopia; †Department of Animal and Range Sciences, College of Dryland Agriculture, Jigjiga University, Ethiopia; ‡Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409; §Poxvirus and Rabies Branch, Centers for Disease Control and Prevention Atlanta, GA 30333; #Oak Ridge Institute for Science and Education (ORISE) CDC Fellowship Program, Oakridge, TN 37831; ¶Department of Animal Production, School of Animal and Range Sciences, College of Agriculture and Environmental Sciences, Haramaya University, Ethiopia; ¶Department of Natural Resource Management, Texas Tech University, Lubbock 79409; **Faculty of Veterinary Medicine, Lilonge University of Agriculture and Natural Resources, Malawi; ††Division of International Research and Development, Office of International Affairs, Texas Tech University, Lubbock, TX 79409; and ‡‡Museum of Texas Tech University, Lubbock, TX 79409

ABSTRACT: Dromedary camels (*Camelus dromedarius*) are a domesticated and closely guarded economic staple of indigenous people located throughout Ethiopian territorial states. Seventeen morphometric variables were examined to determine intraspecific variation among 8 pastoralist-designated breeds of camels. Additionally, DNA sequences from mitochondrial cytochrome-*b* gene and genotyping of 6 nuclear microsatellite loci were examined to assess genetic diversity and phylogenetic relationship of Ethiopian camels. Examination of 525 individuals revealed significant morphometric

differentiation in Afar as compared with the remaining 7 breeds. Analysis of cytochrome-*b* sequences failed to recover monophyletic groups associated with pastoralist-recognized breeds. Analysis of 6 microsatellite loci from 104 individuals depicted no resolution of distinct genetic lineages in accordance to geographical or designated breeds. Overall, separation of 2 ecotypes based on the morphometric data was supported; however, genetic analysis of cytochrome-*b* and microsatellite data failed to support any unique genetic lineage or statistically significant population structure.

Key words: breeds, *Camelus dromedarius*, morphometrics, systematics

© The Author(s) 2018. Published by Oxford University Press on behalf of the American Society of Animal Science. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

J. Anim. Sci. 2018.96:4925–4934
doi: 10.1093/jas/sky351

¹We would like to thank the U.S. Embassy in Addis Ababa, Ethiopia, Jigjiga University, and Texas Tech University for funding this research. Thanks are also extended to the Ethiopian Institute of Biodiversity and multiple Ethiopian pastoralists for access to private land and for their cooperation, which were necessary for the study. We would also like to thank E. K. Roberts, J. Q. Francis, L. L. Lindsey, T. J. Soniat, M. E. Wagley, and M. R. Nunez-Tabares for assistance in laboratory work and reviewing previous versions of this manuscript. Thanks to N. Platt and M. Haynie for

assisting data analyses and to H. Garner and K. MacDonald for assisting depositing tissues into Natural Science Research Laboratory at the Museum of Texas Tech University.

²The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

³Equally contributing first authors.

⁴Corresponding author: Christopher.dunn@ttu.edu

Received April 4, 2018.

Accepted August 28, 2018.

INTRODUCTION

The 1-humped camel (*Camelus dromedarius*), referred to as the Arabian or dromedary camel, was domesticated approximately 4,000 yr ago in the southern Arabian Peninsula, possibly in present day Yemen and Oman (Schwartz and Dioli, 1992; Wilson, 1998). Its introduction into east Africa is thought to have occurred through the Horn of Africa, via the Suez canal, approximately 3,500 yr ago (Tefera and Getachew, 2012). According to the Food and Agriculture Organization of the United Nations (FAO), eastern Africa is known as the heartland of camel production; out of 25.89 million camels worldwide, 7.0 million, 4.25 million, and 2.40 million camels are found in Somalia, Sudan, and Ethiopia, respectively (FAO, 2011). These animals are integral, vital socioeconomic cornerstones and support the survival of millions of people in the semidry and arid zones of Asia and Africa (Epstein, 1971). The ability to survive and reproduce under prolonged water shortage, poor quality feed, and high heat load (Schroter et al., 1989; Dahlborn et al., 1992) is due to their unique morphological, behavioral, and physiological adaptations (Wilson, 1998; Ouajd and Kamel, 2009).

Knowledge about existing morphological, physiological (production profile), and genetic diversity of any animal resource is an essential prerequisite to establish effective utilization and conservation programs. The Domestic Animal Diversity Information System (DAD-IS) database and FAO recognize 97 camel breeds worldwide, based on distribution and specific characteristics (<http://www.fao.org/dad-is/>). Likewise, in eastern Africa, the classification is generally based on the ethnic groups and geographical distribution of the pastoral communities that own them (Tefera and Gebreah, 2001). The FAO officially recognizes 5 breeds in Ethiopia (Somali/Ogaden, Ethiopian Dromedary, Afar, Anfi, and Borena); however, pastoralists of the region recognize additional breeds (<http://www.fao.org/dad-is/>).

Camels are adapted to the lowlands of Ethiopia, contributing greatly to the food security and economic states of pastoralists (Eyasu, 2007; Mehari et al., 2007). They provide milk and meat of high nutritional and medicinal value (Yagil, 2004; Mehari et al., 2007; Al-Haj and Al-Kanhal, 2010) as well as transportation. They also serve as a means of investment and social prestige (Farah et al., 2007; Gwida et al., 2012). The regional states Somali, Afar, and Oromia cover 61 to 65% of the total Ethiopian land area and possess almost the

entire camel population of the country (Abebe, 2001). In the face of climate change, camels are expected to become the most important domestic livestock in terms of rural food security of northern Africa (Salem et al., 2011).

Despite the socioeconomic importance at the household and national levels, the camel represents one of the least researched domestic animal species in Ethiopia (Mehari et al., 2007; Sirak, 2012). Consequently, the country does not have a national camel breeding program, registry, or functional camel development strategy. Information regarding existing diversity and potential productivity is incomplete for Ethiopian camels.

Camel breeds are officially recognized by phenotypic and genetic characterization methods (FAO, 2011). Despite observable phenotypic diversity and reported productivity variations, there is no record of genetic characterization for Ethiopian camels. Therefore, the goals of this study were to investigate morphometric and genetic variation so that to better understand the diversity of camel breeds in eastern Ethiopia.

MATERIALS AND METHODS

Samples for this study were collected from 19 localities located in pastoral and livestock areas of Somali, Afar, and Oromia regional states of Ethiopia. Pastoralists, belonging to different communities, recognize their camels as specific breeds; however, as these locally recognized breeds are not confirmed by genetic data, they will henceforth be referred to as ecotypes to avoid confusion. Number and distribution of samples, assigned ecotypes, and number of samples used in separate analyses are available in Table 1 and Fig. 1. Five hundred thirty-five individuals belonging to the 8 ecotypes were subjected to morphological characterization; all data were collected from living individuals.

Between April and May of 2014, qualitative and quantitative characteristics (e.g., herd size and history of specific camels) were recorded for individuals of the 8 ecotypes following FAO (2011) phenotypic standards. Structured questionnaires were administered to camel owners and general information was gathered on pedigree, husbandry, classifications, productivity, and tolerance of camels. Morphologic data also were collected on general characteristics and descriptions of camels such as body color, hair length, pelage, tail length, ear size, orientation and hump size, and udder size. After morphologic characteristics were taken, ear tissue samples were collected from unrelated animals

Table 1. Number of individuals used for exomorphic and molecular studies in 8 ecotypes

Ecotype	Sampled tissue	Microsatellite study	Exomorphic study	Cyt-b study	Distribution
Jigjiga	34	13	71	3	Jigjiga zone, ESRS ¹
Issa	30	13	71	3	Sitti zone, ESRS
Hoor	34	13	71	2	Jarar, Korahe, and Shebelle zones, ESRS
Ayden	33	13	70	6	Jarar, Korahe, and Shebelle zones ESRS
Liben	32	13	66	7	Liben zone, ESRS
Borena	32	13	69	3	Borana zone, ORSE ²
Kerreyu	27	13	54	5	East Shoa, ORSE
Afar	30	13	63	3	Zone 3, ARSE ³
	252	104	535	32	Ethiopia

¹ESRS = Ethiopian Somali Regional State.

²ORSE = Oromia Regional State of Ethiopia.

³ARSE = Afar Regional State of Ethiopia.

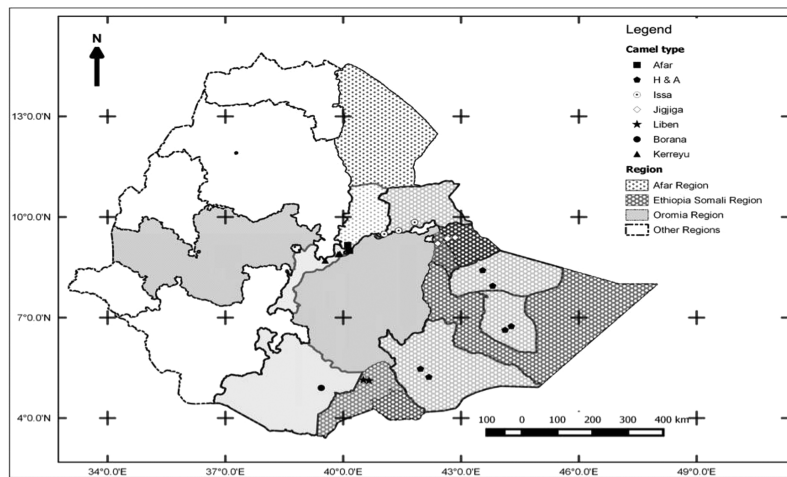


Figure 1. Distribution of localities and ecotypes that were collected for this study. Circles represent collection localities within each of the 3 regions.

of both sexes. An approximately 2 cm² area was shaved with sterile surgical blades, disinfected with 70% alcohol, and then cut with a sterile blade and collected into 50-mL screw-capped sample bottles containing 10 mL of lysis buffer (Longmire et al., 1997) and shipped at ambient temperature to Texas Tech University for analysis.

Morphologic Analysis

Populations were compared within the 8 aforementioned camel ecotypes. The morphologic analyses used 17 exomorphic variables from 525 individuals; 3 variables (hind leg hoof circumference, foreleg hoof circumference, and Weight) and 10 individuals were omitted as a result of missing data (Table 2). Recorded values were log-transformed to account for sexual dimorphism and individual outliers within each population. Standard multivariate statistics including principle component analysis (PCA) and discriminant function analysis (DFA) were performed to obtain PCA

eigenvalues, PCA percent variation, and DFA loadings (Table 3) for each character in every ecotype using the statistical packages in R (R Core Team, 2014).

R scripts for DFA and PCA were analyzed using R studio (Team, 2014) with graphical package using the morphological data. Principle component analysis was used to summarize the variation and to determine differences among the 8 ecotypes; DFA was performed to find the combination of exomorphic characters that better differentiate the ecotypes. Loadings of variables of the 2 discriminate axes were calculated to determine which individual variables contributed to the most of the variation.

Genetic Analysis

Ear tissues were heat treated to 75°C to ensure no pathogen was present in transport, which may have resulted in the low yield of genomic DNA. Genomic DNA was extracted from 0.1 g ear tissue

Table 2. Description of the 17 exomorphic characteristics and abbreviations

Abbreviation	Description
Face length (FL)	Distance from midpoint of ears to mouth tip
Distance between eyes (DBE)	Distance between the most medial part of each eye
Neck length (NL)	Distance from lower part of mandible to sternum
Height at hump (HAH)	Height from bottom of front foot to highest point of withers
Length of forelimb (LOF)	Distance from surface of the ground level to front of sternum
Depth of chest (DOC)	Distance from wither to sternum
Width of chest (WOC)	Distance from left to right upper leg
Weight	Weight in kilograms of each individual
Barrel girth (BG)	Measurement of distance around abdomen over highest part of hump
Hump circumference (HC)	Perimeter of hump from a point at anterior end of hump to a point at posterior end
Hump length (HL)	Length from bottom to tip of the hump
Body length (BL)	Horizontal distance from point of shoulder to pin bone
Width of hip distance (WOH)	Distance from left to right point of hip
Length of hind limb (LOHL)	Distance from bottom of leg to pin hip bone
Tail length (TL)	Maximum distance from tail base to tip of tail
Hind leg hoof circumference (HLHC)	Circumference of right hind leg hoof around widest part
Foreleg hoof circumference (FLHC)	Circumference of right foreleg hoof around widest part
Ear length (EL)	Length of external ear from base of skull to tip of ear
Height at shoulder (SH)	Height (vertical) from the bottom of the front foot to the highest point of the withers
Heart or chest girth (HG)	Circumference of the body immediately behind the shoulder blades in a vertical plane, perpendicular to the long axis of the body as quantified

Table 3. Principal components and discriminant functions, loadings and percentage of variants explained by PCA and DFA analyses on 17 exomorphic variables of Ethiopian dromedary camels

Variables	PCA				DFA	
	Loadings		PCA eigenvalues	PCA percent variation	Loadings	
	PC1	PC2			DF1	DF2
Face length	0.1177	-0.1102	0.2299	59.48503227	0.4555	0.1906
Distance between eyes	0.2350	-0.2796	0.0569	14.72796452	1.4139	-0.1947
Ear length	0.2200	-0.8335	0.0364	9.42071884	1.0826	2.2696
Neck length	0.1043	-0.0727	0.0247	6.38081374	0.2156	-0.1365
Height at shoulder	0.0712	0.0034	0.0107	2.77758491	-0.1447	0.3308
Length of hind limb	0.0631	-0.0593	0.0064	1.66901725	-0.4932	-0.1664
Depth of chest	0.2682	0.1517	0.0059	1.5205097	0.4450	-1.5173
Width of chest	0.3924	0.2055	0.0039	1.01215999	1.0030	-0.2650
Heart or chest girth	0.1094	0.0684	0.0028	0.72268549	-0.2242	-0.2886
Barrel girth	0.1484	0.1383	0.0024	0.60867413	-0.0887	0.0012
Hump circumference	0.4481	0.2932	0.0019	0.50064712	0.7363	-0.7786
Hump length	0.5114	-0.0199	0.0015	0.38036484	-0.0781	0.5090
Height at hump	0.1003	0.0274	0.0010	0.27029641	-0.4138	0.4637
Body length	0.1300	0.1576	0.0008	0.21416278	0.3266	-0.2217
Width of hip	0.3236	-0.0573	0.0006	0.15191386	0.0602	-0.1100
Length of hind limb	0.0502	-0.0441	0.0003	0.08769881	0.2750	-0.0072
Tail length	0.0785	0.0009	0.0003	0.06975536	-0.4559	0.1568

DFA = discriminant function analysis; PCA = principle component analysis.

using a DNeasy Blood and Tissue kit (QIAGEN, Inc., Valencia, CA). Spectrophotometer readings indicated that on average DNA samples were in the 15 to 16 ng/ μ L range. The low DNA concentration may have subsequently resulted in the inability to amplify specific loci in some individuals. Of the 252 individuals from which ear clips were sampled, 104

animals (13 per ecotype) were randomly selected for genetic characterization using microsatellite markers (Table 1). Nineteen sampling localities (Fig. 1) were distributed in the 3 separate study regions of Ethiopia (Afar, Oromia, and Somali).

Thirty-one individuals were randomly selected (Table 1) for sequencing their mitochondrial

cytochrome-*b* (*cyt-b*) gene. Using PCR, the entire *cyt-b* gene (1,140 bp, primers LGL766/765) was amplified using methods following Mehari (2007). Popart version 1.7 (<http://popart.otago.ac.nz>) was used to create a graphical representation of the TCS haplotype network. Examination of the number of *cyt-b* haplotypes within populations was used to see if the haplotypes were conserved. Alignment of sequences was done by eye in MEGA 6 (Tamura et al., 2013), and TCS (Clement et al., 2000) was used to create a TCS network.

Bayesian (Ronquist and Huelsenbeck, 2003) and likelihood characteristics, node support, and branch lengths for the *cyt-b* gene were estimated using MrBayes 3.1.2. Based on the previously published phylogenetic relationships (Montgelard, 1997), 4 sequences from GenBank, *Camelus bacterinus* (AY126625.1), *Llama glama* (AY535253.1), and *Vicugna pacos* (JF489132.1) were used as out-groups with *C. dromedarius* (AY126629.1) used as a reference sequence. Phylogenies were rooted using *C. bacterinus*, *L. glama*, and *V. pacos* and an analysis was conducted with the following options: 8 Markov chains, 10 million generations, and sample frequency of 1,000 with a burn-in of 10,000. A GTR+G model of evolution was selected by MrModeltest 2.3 (Nylander, 2004) as best fitting the data.

Seven microsatellite primer pairs (Table 4) were used, as they were shown to be highly polymorphic and among the most informative of the primers published for camel genetics to date (Nolte et al., 2005). The thermal profile includes an initial denaturation at 95°C for 10 min followed by 35 cycles each of 45 s of denaturation at 94°C, a 90-s annealing step from 55°C to 64°C (based on primer melting temperature; Table 4), and 60 s of elongation at 72°C with a final extension for 10 min at 72°C.

An Applied Biosystems 3130 Avant Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) was used for analysis of microsatellite amplicons. Reactions included 0.5 µL of 500HD RX size standard (Applied Biosystems Inc.), 11 µL of Hi-Di Formamide (Applied Biosystems Inc.), and 3.5 µL (50 ng/µL or more) of PCR product. Genemapper version 4.0 (Applied Biosystems Inc.) was used to score microsatellite allele size. GeneAIEx version 6.501 was used to determine the Ethiopian ecotype relationships with the microsatellite data.

Structure version 2.3.4 (Pritchard et al., 2000) was used to estimate population genetic structure among sampled ecotypes using multilocus genotype data. Structure options were as follows: burn-in length = 500,000, Monte Carlo Markov chain repetitions = 5,000,000, $K = 14$ (K represents range

Table 4. Characterization of 7 microsatellite loci in Ethiopian camels, primer sequences, number of alleles, polymorphic informative content (PIC) value, and observed (H_o) and expected (H_e) heterozygosity

Locus ¹	Label/T _a	Alleles, no.	H_o	H_e	PIC
VOLP03 F: AGA CGG TTG GGA AGG TGG TA R: CGA CAG CAA GGC ACA GGA	6-FAM/64	11	0.613	0.528	0.324
VOLP10 F: CTT TCT CCT TTC CTC CCT ACT R: CGT CCA CTT CCT TCA TTT C	6-FAM/55	9	0.709	0.657	0.845
VOLP67 F: TTA GAG GGT CTA TCC AGT TTC R: TGG ACC TAA AAG AGT GGA G	6-FAM/53	21	0.455	0.640	0.883
YWLL 08 F: ATC AAG TTT GAG GTG CTT TCC R: CCA TGG CAT TGT GTT GAA GAC	6-FAM/55	7	0.396	0.538	0.648
LCA56 F: ATG GTG TTT ACA GGG CGT TG R: GCA TTA CTG AAA AGC CCA GC	HEX/55	3	0.234	0.285	0.324
LCA63 F: TTA CCC AGT CCT TCG TGG G R: GGA ACC TCG TGG TTA TGG AA	HEX/58	11	0.249	0.689	0.768
LCA77 F: TGT TGA CTA GAG CCT TTT CTT CTT T R: GGG CAA GAG AGA CTG ACT GG	HEX/55	1	–	–	–

¹F = forward; R = reverse.

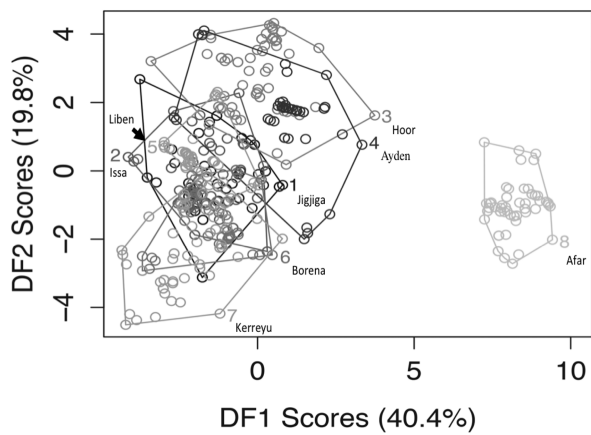


Figure 2. Graphical representation of DF1 and DF2 function analysis, based on size and shape of vectors.

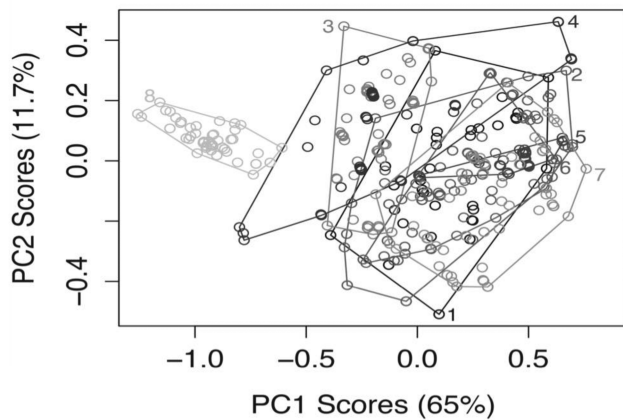


Figure 3. Plot of components 1 and 2 obtained from the PCA analysis of exomorphic characteristics of Ethiopian camels. Values in parentheses give the amount of variation explained by each principle component. PCA = principle component analysis.

of potential clusters or populations examined), with 10 iterations at each K value. Examination of K from 1 to 14 was used to identify any potential substructure within the ecotypes. This test was replicated 4 times using the following variables: prior (A), no prior (B), admixture (1), and no admixture (2) [A1, A2, B1, and B2].

Structure Harvester (Earl et al., 2012) was then used to determine the most appropriate value of K from 4 iterations of structure output. The program GeneA1Ex version 6.501 (Peakall, 2006) was used to estimate allele frequencies as well as observed and expected heterozygosity (H_o and H_e). Polymorphic information content (PIC) was analyzed using Cervus version 2.0 (Marshall et al., 1998). One locus (LCA77) was omitted from the microsatellite analysis due to its monomorphic nature. The remaining 6 loci were considered diagnostically informative and used for the remainder of the project.

RESULTS

Three exomorphic characters hump circumference (HC), hump length (HL), and ear length (EL) were the most diagnostic variables separating the 8 ecotypes. Means, standard deviations, and ranges of all measurements are provided in Table 5. The highest percentage of variation was explained by the first 2 components in the PCA (PC1 59.5% and PC2 14.7%) based on 17 exomorphic characteristics for 525 individuals. The bivariate plot of PC1 and PC2 showed a clear separation in size of the small ecotype (Afar) and samples from the remaining 7

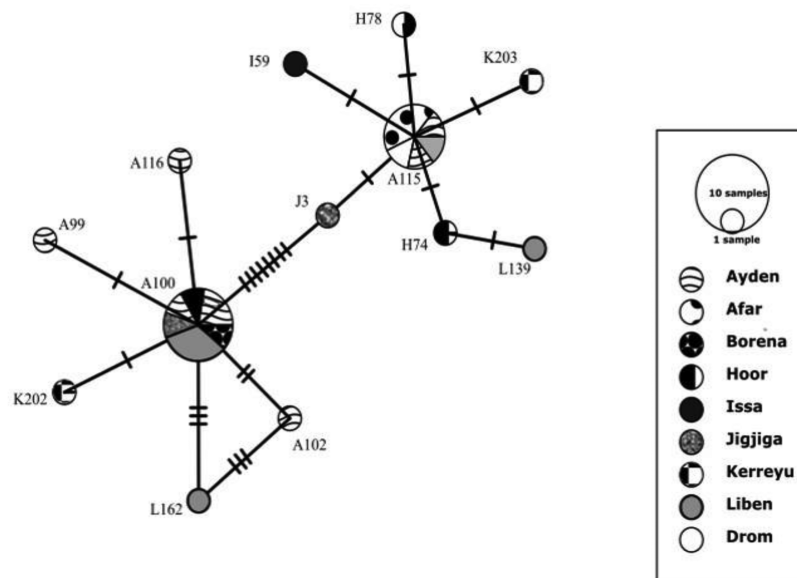


Figure 4. Haplotype network of the 33 *cyt-b* sequences for the 8 Ethiopian ecotypes.

ecotypes (Figure 2). The remaining 7 ecotypes were larger, substantially overlapped with one another, and were segregated from Afar.

Discriminant functions (DF) and loadings of variables on each conical axis are summarized in Table 3. Variables with the highest loadings and the first conical axis were related to overall size. Within the DFA, the variables distance between eyes, EL, WOC, and HC separated the ecotypes based on

size. These measurements displayed mainly positive scores on DF1 with the percentage of variation in DF1 being 37%, and DF2 being 28.2% with clear distinction of Afar from all other ecotypes (Fig. 3).

Both DFA and PCA shared 2 variables: EL and HC. These variables were averaged for the 7 ecotypes (EL: 12.1 and HC: 148.9) and Afar (EL: 11.5 and HC: 81.6), showing high disparity between Afar and the remaining ecotypes. Geographical

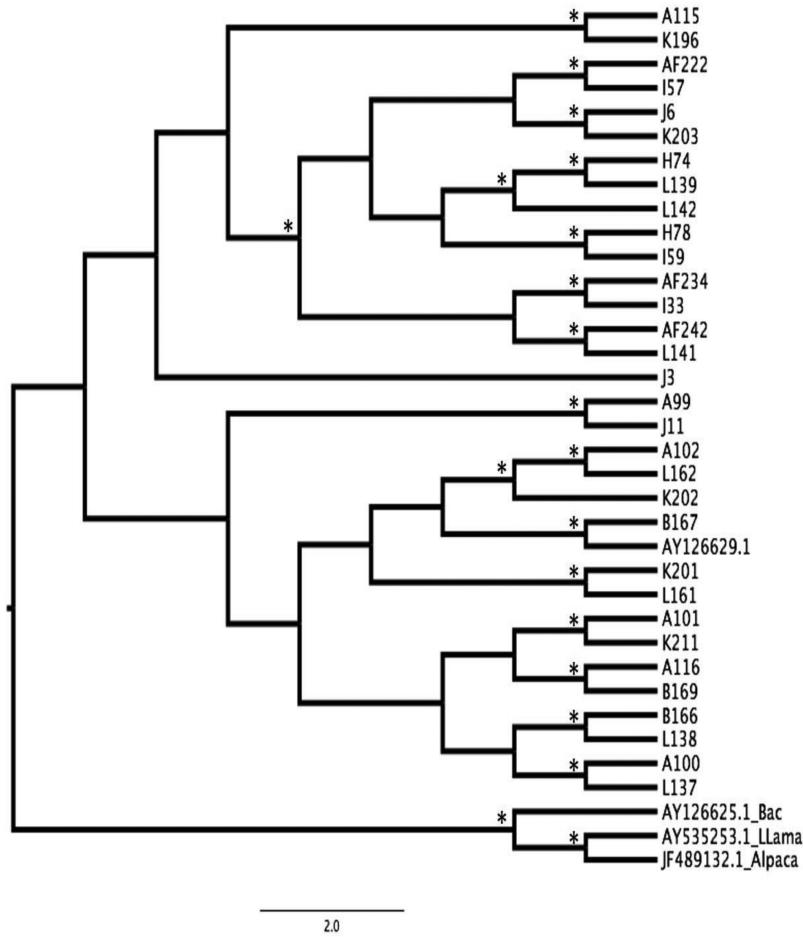


Figure 5. Bayesian cladogram with posterior probability indicated by * for 95% or greater depicting no discernable geographic pattern. Ecotypes are abbreviated to A (Ayden), AF (Afar), I (Issa), H (Hoor), K (Kerreyu), J (Jigjiga), L (Liben), and B (Borana).

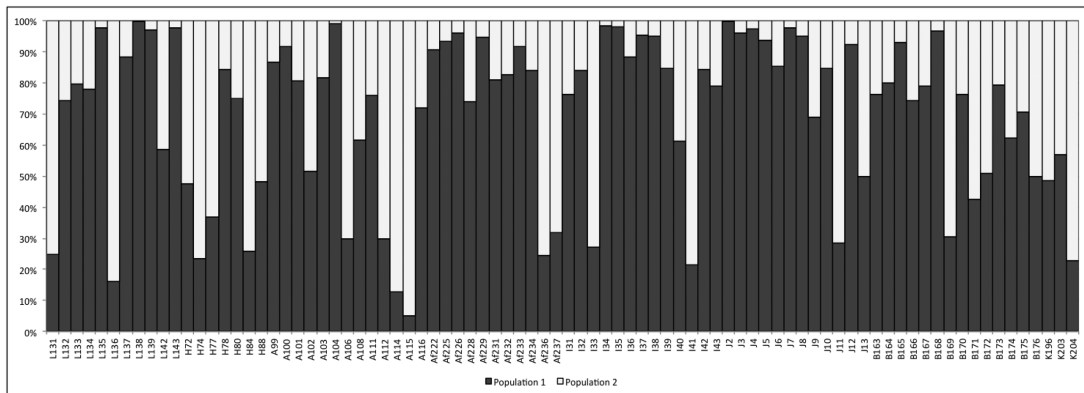


Figure 6. Structure results for the genotype information for all individuals included in this study. Specimen labels are shown below the genotype bar graph. Shading denotes the estimated proportion of the specimen's nuclear genome attributed to each cluster.

Table 5. Standard deviation, maximum length, minimum length, average between both male and female, average for female, and average for males using 17 exomorphic variables for 535 individuals

Variables	Male (<i>n</i> = 99)	Female (<i>n</i> = 425)	Male and female (<i>n</i> = 525)	Maximum length	Minimum length	SD
Face length	56.41	56.38	56.38	62	47	3.15
Distance between eyes	27.25	27.21	27.22	30	18	2.69
Ear length	13.38	13.36	13.36	16	5	1.37
Neck length	106.72	106.67	106.67	119	90	7.35
Height at shoulder	195.73	195.71	195.71	220	170	9.77
Length of hind limb	148.36	148.34	148.34	171	128	7.64
Depth of chest	75.64	75.58	75.58	96	50	10.59
Width of chest	63.54	63.46	63.46	90	34	12.29
Heart or chest girth	207.05	207.01	207.01	241	175	14.46
Barrel girth	244.9	244.89	244.89	291	115	25.42
Hump circumference	142.56	142.3	142.3	270	75	30.78
Hump length	32.62	32.51	32.51	60	15	8.66
Height at hump	210.94	210.85	210.85	253	184	12.54
Body length	148.7	148.7	148.7	181	115	14.35
Width of hip	42.48	42.45	42.45	61	30	6.75
Length of hind limb	155.88	155.88	155.88	178	137	7.40
Tail length	59.41	59.42	59.42	71	40	5.39

information was not included when analyzing the individuals because the driving force for separation of ecotypes is thought to be the isolation of pastoralists rather than the movement of domesticated camels.

Cyt-b sequences (*n* = 31) were added to reference sequences from GenBank (*n* = 4). Not all of the 31 *cyt-b* sequences were complete (1,143 bp); the first 22 bp were not amplified for these individuals, reducing the total sequence length to 1,121 bp. Excluding outgroup individuals, nucleotide composition analyzed by MEGA 6 (Tamura et al., 2013) for the individuals in this study was A = 29%, C = 27.7%, T = 28.4%, and G = 14.9%. Of the 1,121 nucleotides, 25 were variable, 1,097 were conserved, and 9 were considered phylogenetically informative. Using Popart version 1.7 minimum spanning network analysis no unique haplotype for the 8 ecotypes was recovered (Leigh and Bryant, 2015) (Fig. 4). In addition, overlays of haplotypes into the distribution of localities did not support isolation of populations by distance.

Bayesian inference analysis produced a topology (Fig. 5) that failed to recover supported monophyletic groups among samples of Ethiopian ecotypes. In Fig. 5, supported nodes were predominantly terminal and without apparent structure with regard to ecotype or geographic origin. Genetic distance between clades I and II was 1.0%,

genetic distance within clade I was 0.7% and within clade II was 0.3%.

In the microsatellite analysis, Structure Harvester determined the most appropriate value of *K* to be 2 across all runs. Two individuals from 2 different ecotypes (Jigjiga and Liben) had complete association (posterior probability of 1.0) to cluster 1. Only 2 individuals from the ecotype Ayden showed high association to cluster 2 with a posterior probability of 0.898. No individual was completely unique to cluster 2 (Fig. 6).

DISCUSSION

The morphological analysis failed to recover either the 5 defined breeds or the 8 ecotypes and instead identified only 2 significant groups: 1) Afar and 2) the remainder of the ecotypes examined in this study. Afar and Borena are the only FAO-reported breeds included in the study. The 7 remaining clustered ecotypes contain the Borena ecotype separated from the Afar ecotype. These 2 groups appear to be split based on overall size, with one group including the smallest camel ecotype (Afar) and the 7 averaged-sized ecotypes making up the other.

As previously noted, the camels of Ethiopia are separated into 5 recognized breeds (Afar, Borena, Ethiopian Dromedary this sounds including all camels in Ethiopia and Somali/Ogaden based on

their production profile and phenotypic characteristics (FAO, 2011). The distribution of these recognized breeds includes the lowland areas of the county, which overlap with the study area. When comparing *C. dromedarius* in Ethiopia, which is best suited for the semiarid dry climate of Africa, to the genetic study of camels worldwide, it was expected that low genetic diversity between the breeds/ecotypes would be present. No data of this study were comparable to previous studies (Mburu et al., 2003; Ji et al., 2009; Mishra et al., 2009; Mahmoud et al., 2012; Nouairia et al., 2015).

Although these groups are clearly differentiated morphologically, genetic differentiation was not supported. Results of the Bayesian analysis of the *cyt-b* gene fail to recover monophyletic relationships between the ecotypes. In tandem, the microsatellite analysis resulted in a $K = 2$, identifying a potential split in the genetic lineages. However, the programs limitations need to be accounted for in that it cannot determine a $K = 1$ as a result of the methodology. With the high amounts of admixture from both populations in the structure results, it should be assumed $K = 1$ is the most plausible estimate of number of identifiable clusters.

The Structure results, when plotted with the geographical locations of the sampling localities, did not show a definite pattern. There seems to be a pattern of no genetic structure among the samples; however, the genotyping data were too limited. In general, individuals with high posterior probability to cluster 1 (Jiggiga, Issa, and Afar) were geographically located in the northern portion of the study region whereas ecotypes with high probability of belonging to cluster 2 were more southern (Borana, Ayden, and Hoor); however, 2 ecotypes (Kerreyu and Liben) did not follow this trend.

CONCLUSIONS

Given the currently available data, there is insufficient molecular evidence to recognize the 8 ecotypes as genetically distinct groups; however, the morphological data are capable of separating the Afar ecotype (technically a recognized breed) from the other ecotypes. It is likely that the differences in production profiles and other phenotypic characteristics reported by pastoralists are the result of intense artificial selection for these traits, as previously noted in cattle (Hayes et al., 2009). If these traits are controlled by a few loci, the remainder of the genome may have experienced little selective pressure. It is possible that with a more thorough sampling scheme (both genomic and organismal

in scale), some level of genetic differentiation may be detected. A more detailed investigation of the molecular variability of pastoralist-recognized ecotypes, and the phenotypic expression of that variation, has the potential to increase understanding of domesticated animal breeds. More research is needed to address the intraspecies relationships of the Ethiopian camel.

LITERATURE CITED

- Abebe, F. 2001. Prevalence and intensity of ectoparasites infestation in Issa camels, Eastern Ethiopia. DVM thesis, FVM, AAU, DebreZeit, Ethiopia:6–26.
- Abebe, D., A. Cullis, A. Catley, Y. Aklilu, G. Mekonnen, and Y. Ghebrehirstos. 2008. Impact of a commercial destocking relief intervention in Moyale district, southern Ethiopia. *Disasters* 32:167–189. doi:10.1111/j.1467-7717.2007.01034.x
- Al Kanhal, H. A. 2010. Compositional, technological and nutritional aspects of dromedary camel milk. *Int. Dairy J.* 20:811–821.
- Ben Salem, H., A. Nefzaoui, and H. Abdouli. 1994. Palatability of shrubs and fodder trees measured on sheep and camels. *Cah. Opt. Medit.* 4:35–48.
- Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657–1659.
- Dahlborn, K., S. Benlamlih, R. Zine-Filali, A. Gueroulali, J. Hossaini-Hilali, and M. Oukessou. 1992. Food deprivation and refeeding in the camel (*Camelus dromedarius*). *Am. J. Physiol.* 262(6 Pt 2):R1000–R1005. doi:10.1152/ajpregu.1992.262.6.R1000
- Earl, D. A. 2012. Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. *Conserv. Genet. Resour.* 4:359–361.
- Epstein, C. J., and D. L. Daentl. 1971. Precursor pools and RNA synthesis in preimplantation mouse embryos. *Dev. Biol.* 26: 517–524.
- Eyasu, E. 2007. Threats of Climate Change on Pastoral Production under Restricted Herd mobility: A Case Study in Borena. A study conducted for SoS Sahel.
- FAO. 2011. Reproduction in camels. FAO Animal Production and Health Paper 82.
- Farah, Z., M. Mollet, M. Younan, and R. Dahir. 2007. Camel dairy in Somalia: limiting factors and development potential. *Livest. Sci.* 110:187–191.
- Gwida, M., A. El-Gohary, F. Melzer, I. Khan, U. Rösler, and H. Neubauer. 2012. Brucellosis in camels. *Res. Vet. Sci.* 92:351–355. doi:10.1016/j.rvsc.2011.05.002
- Hayes, B. J., A. J. Chamberlain, S. Maceachern, K. Savin, H. McPartlan, I. MacLeod, L. Sethuraman, and M. E. Goddard. 2009. A genome map of divergent artificial selection between *Bos taurus* dairy cattle and *Bos taurus* beef cattle. *Anim. Genet.* 40:176–184. doi:10.1111/j.1365-2052.2008.01815.x
- Ji, R., P. Cui, F. Ding, J. Geng, H. Gao, H. Zhang, J. Yu, S. Hu, and H. Meng. 2009. Monophyletic origin of domestic Bactrian camel (*Camelus bactrianus*) and its evolutionary relationship with the extant wild camel (*Camelus bactrianus ferus*). *Anim. Genet.* 40:377–382. doi:10.1111/j.1365-2052.2008.01848.x

- Leigh, J. W., and D. Bryant. 2015. popart: full-feature software for haplotype network construction. *Methods Ecol. Evol.* 6:1110–1116.
- Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections. Museum of Texas Tech University, Lubbock, TX.
- Mahmoud, A. H., M. A. Alshaikh, R. S. Aljumaah, and O. B. Mohammed. 2012. Genetic variability of camel (*Camelus dromedarius*) populations in Saudi Arabia based on microsatellites analysis. *Afr. J. Biotechnol.* 11:11173–11180.
- Marshall, T. C., J. Slate, L. E. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Mol. Ecol.* 7:639–655.
- Mburu, D. N., J. W. Ochieng, S. G. Kuria, H. Jianlin, B. Kaufmann, J. E. Rege, and O. Hanotte. 2003. Genetic diversity and relationships of indigenous Kenyan camel (*Camelus dromedarius*) populations: implications for their classification. *Anim. Genet.* 34:26–32.
- Mehari, Y., Z. Mekuriaw, and G. Gebru. 2007. Potentials of camel production in Babilie and Kebribeyah woredas of the Jijiga Zone, Somali Region, Ethiopia. *Livest. Res. Rural Dev.* 19:58.
- Mishra, B., M. S. Tantia, S. T. Bharanikumar, B. P. Mishra, and R. K. Vijh. 2009. Genetic relationship among camels of India based on microsatellite data. *Indian J. Anim. Sci.* 79:399–405.
- Montgelard, C., F. M. Catzeflis, and E. Douzery. 1997. Phylogenetic relationships of artiodactyls and cetaceans as deduced from the comparison of cytochrome b and 12S rRNA mitochondrial sequences. *Mol. Biol. Evol.* 14:550–559. doi:10.1093/oxfordjournals.molbev.a025792
- Nouairia, G., S. Kdidi, R. B. Salah, H. Hammadi, T. Khorchani, and M. H. Yahyaoui. 2015. Assessing genetic diversity of three Tunisian dromedary camel (*Camelus dromedarius*) subpopulations using microsatellite markers. *Emir. J. Food Agric.* 27:362–366.
- Nolte, M., Kotze, A., Van der Bank, F. H., and Grobler, J. P. 2005. Microsatellite markers reveal low genetic differentiation among southern African *Camelus dromedarius* populations. *S. Afr. J. Anim. Sci.* 35: 152–161.
- Nylander, J. A., Ronquist, F., Huelsenbeck, J. P., and Nieves-Aldrey, J. 2004. Bayesian phylogenetic analysis of combined data. *Syst. Biol.* 53: 47–67.
- Ouajd, S., and B. Kamel. 2009. Physiological particularities of dromedary (*Camelus dromedarius*) and experimental implications. *Scand. J. Lab. Anim. Sci.* 36:19–29.
- Peakall, R. O. D., and Smouse, P. E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes.* 6: 288–295.
- Peakall, R., and P. E. Smouse. 2012. Genalex 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28:2537–2539. doi:10.1093/bioinformatics/bts460
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- R Team. 2014. RStudio: integrated development for R. RStudio, Inc., Boston, MA. <http://www.RStudio.com/ide> (Accessed).
- Ronquist, F., and Huelsenbeck, J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics.* 19: 1572–1574.
- Schroter, R. C., D. Robertshaw, and R. Z. Filali. 1989. Brain cooling and respiratory heat exchange in camels during rest and exercise. *Respir. Physiol.* 78:95–105.
- Schwartz, H. J., M. Dioli, R. Stimmelmayer, and M. G. H. Walsh. 1992. The one-humped camel (*Camelus dromedarius*) in eastern Africa. A pictorial guide to diseases, health care and management. Verlag Josef Margraf. 11–15.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–2729. doi:10.1093/molbev/mst197
- Tefera, M., and G. Abebe. 2013. Camel in Ethiopia. Joint FAO and Ethiopian Veterinarians Association Edition.
- Tefera, M., and F. Gebreah. 2001. A study on the productivity and diseases of camels in eastern Ethiopia. *Trop. Anim. Health Prod.* 33:265–274.
- Wilson, R. 1998. Camels: the tropical agriculturalist. London, Basingstoke, UK: Macmillan Education/Wageningen; The Netherlands: CTA; 115–116 (Tropical Agriculturalist Series).
- Yagil, R. 2004. Camel milk and autoimmune diseases: historical medicine.