

Origins of cattle on Chirikof Island, Alaska

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Abstract

Feral livestock may harbor genetic variation of commercial, scientific, historical or esthetic value. Origins and uniqueness of feral cattle on Chirikof Island, Alaska are uncertain. The island is now part of the Alaska Maritime Wildlife Refuge and Federal wildlife managers want grazing to cease, presumably leading to demise of the cattle. Here we characterize the Chirikof Island cattle relative to extant breeds and discern their origins. Our analyses support the inference that Russian cattle arrived first on Chirikof Island, then approximately 95 years ago the first European taurine cattle were introduced to the island, and finally Hereford cattle were introduced about 40 years ago. While clearly *Bos taurus taurus*, the Chirikof Island cattle appear at least as distinct as other recognized breeds. Further, this mixture of European and East-Asian cattle is unique compared to other North American breeds and we find evidence that natural selection in the relatively harsh environment of Chirikof Island has further impacted their genetic architecture. These results provide an objective basis for decisions regarding conservation of the Chirikof Island cattle.

Keywords: Genetic diversity, Conservation biology, Phylogenetic history, Admixture

Introduction

Feral livestock, animal populations which were formerly domesticated but live currently independently of humans, may be sources of genetic variation with potential

commercial, scientific, historical or esthetic value (van Vuren and Hedrick, 1989).

These populations may have genetic variants that are rare or absent in the domesticated populations used in commerce. Sources of this variation include founder effects, random drift, and mutation followed by natural selection within the population to confer adaptation to particular environmental conditions. Assessing the extent and nature of genetic diversity aids in objective and rational decision making relative to conservation (FAO, 2004). Replacing adapted breeds with ‘improved’ counterparts also may be counterproductive in harsh environments (Drucker *et al.*, 2001).

The origin of the feral cattle population on Chirikof Island is uncertain. Cattle farming was common in Russian Siberia throughout the Russian-American period (Kotkin and Wolff, 1995). Local Siberian cattle were first imported to Alaska in the 1790s as part of an effort to establish an agricultural colony in Russian America, Alaska (Bancroft, 1886). Further, the desire for beef, milk, and butter, led to a very general importation of Siberian cattle from Petropaulovsk to every post in Alaska (Elliott, 1887). In 1798, the Russians established an outpost on Chirikof Island (Long, 1975). Thus, it is plausible that cattle of Russian origin were brought to isolated Chirikof Island in the Gulf of Alaska at this time. Cattle originating from the U.S. first arrived on Chirikof Island in the mid-1880s (Long, 1975). In 1927, 400 beef cattle were tallied on Chirikof Island (USDA, 1929). According to reports in the popular press, enterprising cattle producers have periodically added Hereford, Angus, Highland, Shorthorn and perhaps other breeds of cattle to the Chirikof Island population throughout the 1900s (Fields, 2000; D’Oro, 2003, 2005). Aside from sporadic immigration events, contemporary

cattle on Chirikof Island are isolated in the North Pacific Ocean and are thought to descend from many generations of feral stock (McKnight, 1964). These events likely contribute to the complexity of the genetic structure of the cattle on Chirikof Island.

In 1980, the island became part of the Alaska Maritime Wildlife Refuge. In the 1980s, the U.S. Fish and Wildlife Service began removing introduced species from various islands in the refuge, mainly foxes introduced by fur traders, but also cattle, but not from isolated Chirikof at that time. The government granted grazing leases for Chirikof Island in the twentieth century. The last grazing lease for Chirikof Island expired in 2000, but cattle remained. A permit to remove the cattle expired in 2003, but cattle remain. In late 2013, the U.S. Fish and Wildlife Service restated its intent “to restore these islands and finally help them fulfill their congressionally mandated destiny as a wildlife refuge” (Medeiros, 2013). Federal wildlife managers seek to remove the cattle from Chirikof Island in order to stop grazing and enhance habitat for birds (Press, 2013). It is widely presumed that this restoration will result in the extirpation of the Chirikof Island cattle.

MacNeil et al. (2007) found the Chirikof Island cattle to be genetically variable and relatively unique when compared to the Angus, Charolais, Hereford, Highland, Limousin, Red Angus, Salers, Shorthorn, Simmental, Tarentaise and Texas Longhorn breeds present in the United States during the early 2000s. Since that time, *Bos taurus* cattle breeds from a wide range of geographical origins in Eurasia, mostly its northern territory, have been characterized using microsatellites (Li *et al.*, 2005; Li and Kantanen, 2010) and Decker et al. (2014) assembled the largest population sampling of

any mammalian species at time of original publication in detailing the population structure of domesticated cattle worldwide. The objective of the present work was to more definitively quantify the origins, admixture and divergence of the Chirikof Island cattle relative to other domestic breeds of cattle.

Materials and Methods

Three breeds from eastern Russia (Kalmyk [N = 28], Kazakh White-head [N = 40], and Yakut cattle [N = 60]); eleven North American breeds from MacNeil *et al.* (2006): (Charolais [N = 17], Limousin [N = 15], Simmental [N = 19], Tarentaise [N = 9], Angus [N = 22], Hereford [N = 22], Highland [N = 18], Red Angus [N = 16], Shorthorn [N = 12], Texas Longhorn [N = 9] and Criollo [N = 30]); and cattle from Chirikof Island [N = 23] (MacNeil *et al.*, 2007) were genotyped using 15 common microsatellites (*ETH10*, *ETH3*, *INRA032*, *HEL-5*, *HEL-1*, *ETH185*, *TGLA126*, *INRA005*, *ILSTS005*, *ETH225*, *TGLA227*, *BM1818*, *ILSTS006*, *INRA037*, and *INRA063*). DNA was extracted by phenol-chloroform precipitation. Standard polymerase chain reactions (PCR) were performed and samples from North America were genotyped using a Licor DNA Analyzer 4200 (LI-COR Biosciences, Lincoln, NE). Samples from Russia were genotyped using a MegaBACE™500 DNA sequencer (Amersham Biosciences, Uppsala, Sweden). Three Simmentals were genotyped using both platforms to ensure consistency in the scoring of genotypes. Sufficient DNA remained to genotype 10 of the Chirikof Island cattle using the Illumina BovineSNP50 BeadChip (Matukumalli *et al.*, 2009). The SNP data were integrated with the data generated from the worldwide sampling of domestic cattle conducted by Decker *et al.*

(2014a, 2014b). The data set contained 43,018 SNPs after removing 25 parentage SNPs that are duplicated on the BovineSNP50 BeadChip (see <https://missouri.box.com/SNP-map-unique> for the updated PLINK map file).

The microsatellite data were analyzed using the GenAIEx (Peakall and Smouse, 2006, 2012) add-in for the Excel spreadsheet software (Microsoft Corp., Redmond, WA). Measures of genotypic diversity and differentiation were analyzed and displayed using several complementary approaches. Microsatellite DNA variation among and within breeds was quantified by analysis of molecular variance with R-statistics. Principal coordinates analysis was conducted using the genetic distance covariance matrix with data standardization.

A multiple-locus assignment was performed using the Bayesian clustering algorithm STRUCTURE (Pritchard *et al.*, 2000) to examine the underlying relationships among the breeds based on the microsatellite data. Individuals were assigned to clusters based on their genotypes without the use of any *a priori* information, such as breed. The model assumes K groups, linkage equilibrium among markers and Hardy–Weinberg equilibrium within a group. The value for the parameter K that best reflected the population structure was determined by generating the posterior probability of K for a range of K values from 2 to 15, and identifying the value most appropriate to the population structure based on the posterior likelihood. Posterior probabilities were estimated using a Markov chain, Monte Carlo (MCMC) method based on 150,000 iterations of each chain following a 50,000 iteration burn-in period. Each MCMC chain for each value of $K = 2–15$ was run 10 times. The method explicitly allows for

individuals with ancestry from more than one group. Individuals are fractionally assigned to multiple groups using a vector of ancestry proportions Q , which sums to 1.0 for each individual across the K groups.

For the SNP data, autosomal SNPs and a single pseudo-autosomal X chromosome SNP were analyzed. SNP filtering was previously described (Decker *et al.*, 2014b).

Principal component analysis (Patterson *et al.*, 2006), ancestry graphs implemented in TreeMix (Pickrell and Pritchard, 2012) and ancestry models implemented in fastSTRUCTURE (Raj *et al.*, 2014) were used to assess the relationship of Chirikof Island cattle to 134 breeds of domesticated bovids. These breeds arose from three domesticated (sub)species: *Bos javanicus*, *Bos taurus indicus* and *Bos taurus taurus*.

FastSTRUCTURE, with values of K from 1 through 40, was used to evaluate ancestry proportions for K ancestral populations. Two metrics from fastSTRUCTURE were used to assess appropriate values of K for the population structure contained in the dataset. The metric K_{ϵ}^* is the value of K which maximizes the log marginal likelihood lower-bound (LLBO). The metric $K_{0.95}^*$ is the minimum value of K that accounts for almost all of the ancestry in the dataset. We also used weighted linkage disequilibrium decay statistics as implemented in a version of ALDER v1.03 (Loh *et al.*, 2013) modified to allow for more than 23 chromosomes, to estimate the number of generations since admixture and lower-bounds of ancestry fractions from the reference populations. A linkage map based on 43,573 individuals from 632 parents, primarily defining half-sib families, and genotyped with the BovineSNP50 assay was constructed at the University of Missouri (unpublished) and used to define the genetic

distances (cM) among the SNP loci. We used TreeSelect (Bhatia *et al.*, 2011) to identify selected loci for which the increased divergence occurred on the Chirikof Island branch. TreeSelect fits a three population phylogeny and identifies loci where the divergence between the central node and the population at the tip is greater than expected due to random drift alone. We used the Hereford and Highland breeds as the two other populations in the TreeSelect analysis.

Results

Results from analyses of the microsatellite genotypes are presented in Figures 1 and 2. The analysis of molecular variance indicated that, on average, 25% of the total genetic variation corresponded to differences between breeds and 75% arose from differences among individuals within breeds. This level of diversity among breeds is approximately 11% greater than was previously observed for the Chirikof Island cattle and 11 U.S beef breeds (MacNeil *et al.*, 2007), 10 of which are also represented in this study. Thus, the incorporation of the Russian breeds into the analysis significantly added to the diversity among the examined breeds.

Analysis of the microsatellite genotypes, as illustrated from the first two principle coordinates, cast the Chirikof Island cattle as being most similar to the Kalmyk breed (Figure 1). The Chirikof Island (and Kalmyk) cattle are approximately equidistant from Hereford and Yakut, consistent with the conclusion that Chirikof Island cattle are admixed between Hereford and Yakut, or similar populations (McVean, 2009). In the multiple-locus Bayesian clustering analyses, up until $K = 5$, the Chirikof Island cattle

cluster primarily with British cattle, and then for $K \geq 6$ (marginal likelihood maximized at $K = 10$), they form their own cluster (Figure S1). When $K = 2$, several Chirikof Island, Kalmyk, and Kazakh White-head individuals have large portions of Yakut-like ancestry. It is also noteworthy that at $K = 6$ many breeds, currently recognized as being distinct populations, are indicated as mixtures of several ancestral populations.

To expand the amount of genetic information used to assess the uniqueness, diversity and degree of admixture of the Chirikof Island cattle, their genotypes at 43,018 single nucleotide polymorphism (SNP) loci were used to place them in the context of a survey of 134 breeds of domesticated cattle representing 3 (sub)species worldwide (Decker *et al.*, 2014b). It is noteworthy that, relative to the smaller microsatellite dataset, the representation of cattle from Northeastern Eurasia was sparse in the SNP dataset. The first two principal components show the Chirikof Island cattle as clearly clustering at the margin of European *Bos taurus taurus*, tending towards the East Asian Hanwoo, Wagyu, and Mongolian *Bos taurus taurus* cattle (Figure 2).

In the Bayesian clustering analysis of the SNP genotypes the marginal likelihood was maximized at $K = 20$ (Figure 3). These results suggest that Chirikof cattle are of approximately 60% ancestry similar to Hereford, with the other 40% being a mixture, including Jersey, but mostly northeast-Asian ancestry. The phylogenetic network analysis agrees with this conclusion indicating that Chirikof are 60.1% Hereford with the remaining 39.9% of their ancestry coming from breeds related to Hanwoo and Wagyu which originate geographically proximal to Siberia (Figure 4).

Finally, we used weighted linkage disequilibrium decay curves to estimate the timing and amount of admixture in the Chirikof Island cattle (Loh *et al.*, 2013). With Chirikof Island cattle as the test population, Hereford as reference population 1, and Hanwoo and Wagyu combined as reference population 2, we find significant evidence that Chirikof Island cattle are admixed from relatives of these reference populations ($Z = 9.83$, $P = 8.7\text{e-}23$). However, the decay rates (number of generations since admixture) were not consistent from this analysis. In a single reference population analysis using Hereford for which the minimum distance between SNPs was 2.2 cM in the University of Missouri linkage map (due to high background linkage disequilibrium), we estimated that the admixture occurred 8.15 ± 1.59 ($Z = 5.12$) generations ago. The lower bound for the extent of Hereford ancestry in Chirikof Island cattle was estimated to be 21.0 ± 1.4 percent. Conversely, with Wagyu as the single reference for which ALDER v1.03 (Loh *et al.*, 2013) set the minimum distance between SNPs at 0.9 cM, ALDER estimated that the admixture occurred 19.59 ± 7.21 generations ago. Chirikof Island cattle have at least 5.1 ± 0.8 percent East Asian cattle ancestry. These incongruences can be easily reconciled. Assuming a generation interval of 5 years, we can infer that Russian cattle arrived first on Chirikof Island, and then approximately 95 years ago the first European taurine cattle were introduced to the island, while finally Hereford cattle were introduced to the island about 40 years ago. These analyses provide a detailed description of the nature, proportion and timing of admixture of the Chirikof Island cattle.

The Chirikof Island cattle may be an economically important resource if they harbor variants which have allowed them to adapt to the harsh environment of Chirikof Island. We performed an analysis seeking to identify selected loci, but our test was underpowered (Figures S2 and S3) due to the small sample size and granular estimates of allele frequencies. Nevertheless, we identified six loci for which the change in allele frequency suggests that strong selection has acted upon these loci (Table S1). For example, the most significant SNP on chromosome 5 at 100.3 Mbp had an allele frequency of 0.355 in the central node of the phylogeny, but an allele frequency of 0.95 in the Chirikof Island cattle. Genes in the near vicinity of these six selected SNPs are involved in immune defense response, embryonic development, mammary formation and cancer.

Discussion

In summary, our analyses support the view that the Chirikof Island cattle are a composite of British (mostly Hereford) and northeast Asian cattle. This composite, while clearly *Bos taurus taurus*, appears to be at least as distinct as recognized breeds of cattle. Further, the mixture of European and East Asian cattle employed to form the Chirikof Island population is unique compared to other North American breeds. It also appears that natural selection in the relatively harsh environment of Chirikof Island has impacted their genetic architecture.

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Conflict of Interest

The authors declare no conflict of interest.

Contributions

M.D.M., J.E.D. and J.F.T conceived the study. J.F.T. and J.E.D. provided SNP genotypes. R.D.S. provided the linkage map. L.J.A. and M.D.M provided microsatellite genotypes for North American breeds and Chirikof Island cattle. M.A.C. provided tissue samples from Chirikof Island cattle. A.M. conducted historical research on Russian settlements. J.K. provided genotypes for Siberian cattle. J.E.D and M.D.M. analyzed the data. All authors contributed to interpretation of the results. J.E.D., J.F.T. and M.D.M. drafted the manuscript with input from all authors.

Data Archiving

Data will be archived at DATA DRYAD after acceptance.

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Figure 1. Plot of principle coordinates 1 and 2 which explain 40% of the variation in relationships among Angus (ANG), Charolais (CHA), Chirikof Island cattle (CIS), Criollo cattle (NMX), Hereford (HFD), Highland (HLD), Kalmyk (KAL), Kazakh White-head (KAZ), Limousin (LIM), Red Angus (RAN), Shorthorn (SHN), Simmental (SIM), Tarentaise (TAR), Texas Longhorn (TXS) and Yakut (YKT) breeds of cattle as derived from 15 microsatellite loci.

Figure 2. (a) Principal component plot incorporating Chirikof Island cattle into the analysis of worldwide patterns of ancestry, divergence, and admixture in domesticated cattle of Decker et al. (2014). (b) Expanded image of lower left quadrant containing samples from Chirikof Island cattle.

Figure 3. Bar plot showing the extent of admixture in 134 domesticated bovids and the Chirikof Island cattle at $K = 20$ (marginal likelihood maximized) as derived from BovineSNP50 genotypes.

Figure 4. Phylogenetic network of the inferred relationships between 17 cattle breeds and the Chirikof Island cattle.

Principal Coordinates (1 vs 2)







