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Genetic relationships between feral cattle from Chirikof Island, Alaska and other breeds

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Summary

The origin of cattle on Chirikof Island, off the coast of Alaska, is not well documented. We assessed genetic differentiation of cattle isolated on Chirikof Island from several breeds commonly used for commercial production in North America including breeds popularly believed to have contributed to the Chirikof Island population. A set of 34 microsatellite loci was used to genotype Angus, Charolais, Hereford, Highland, Limousin, Red Angus, Salers, Shorthorn, Simmental, Tarentaise and Texas Longhorn cattle sampled from North America and the Chirikof Island population. Resulting F_{ST} statistics for these loci ranged from 0.06 to 0.22 and on average, 14% of total genetic variation was between breeds. Whether population structure was modelled as a bifurcating tree or genetic network, Chirikof Island cattle appeared to be unique and strongly differentiated relative to the other breeds that were sampled. Bayesian clustering for multiple-locus assignment to genetic groups indicated low levels of admixture in the Chirikof Island population. Thus, the Chirikof Island population may be a novel genetic resource of some importance for conservation and industry.

Keywords cattle, genetic distance, genetic variation.

Introduction

Feral livestock may be sources of genetic variation with potential commercial, scientific, historical or aesthetic value (Van Vuren & Hedrick 1989). These populations may have genetic variants that are rare or absent in domesticated populations used in commerce. Sources of this variation include founder effects, random drift, mutation within the population and natural selection conferring adaptation to particular environmental conditions. The origin of Alaskan cattle on Chirikof Island is uncertain. The contemporary population of feral cattle on Chirikof Island is isolated by a treacherous sea from continental land masses and is thought to descend from many generations of feral stock (McKnight 1964). Russian trappers established a colony on nearby Kodiak Island in 1784 and cattle were subsequently raised there (Anonymous 1893; 1998; Johnson 1961), although we have found no documentation of Russian cattle on Chirikof Island. According to reports in the popular press, enterprising cattle producers have periodically added Angus, Hereford, Highland, Shorthorn and perhaps other animals to the Chirikof Island population in the 1900s (Fields 2000; d'Oro 2003; 2005).

Interest in sustainable livestock production systems may cause shifts from improved breeds to adapted breeds that are more biologically fit in low-input production systems and harsh environments (FAO 1999; Drucker *et al.* 2001). There is little substantive documentation of genetic characteristics of the cattle isolated on Chirikof Island. Thus, the primary objective of this work was to quantify genetic relationships between the cattle of Chirikof Island and contemporary North American production populations.

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Materials and methods

Blood and tissue samples were obtained from 21 cattle harvested on Chirikof Island, Alaska. Cryo-preserved samples of semen were obtained from the USDA-ARS National Animal Germplasm Repository for Angus, Charolais, Hereford, Limousin, Red Angus, Salers, Shorthorn, Tarentaise and Texas Longhorn breeds. Individuals sampled were selected from the collection using a cluster analysis based on pedigree relationship to assure a broad sample of each breed. North American breeders donated semen samples for Simmental and Highland from diverse pedigrees. All of these breeds have been imported into North America and, with the exception of Highland, have been phenotypically characterized for numerous production-related attributes (L. V. Cundiff, personal communication).

DNA was extracted from white blood cells, extended semen or meat using standard protocols (e.g. Ausubel *et al.* 1996). Samples of the Chirikof Island cattle, Angus, Charolais, Hereford, Highland, Limousin, Red Angus, Salers, Shorthorn, Simmental, Tarentaise and Texas Longhorn breeds were genotyped using a panel of 34 microsatellite markers (Table S1). These markers were identified on the basis of relative position (unlinked), fragment size (to facilitate multiplexing) and scoring ease from the genomics database at the USDA-ARS, U.S. Meat Animal Research Center (Kappes *et al.* 1997; USDA 2000). Standard PCR was performed. All microsatellites were genotyped on a Licor DNA Analyzer 4200 according to manufacturer's recommendations using primers and reaction conditions and specified in the USDA-ARS genomics database (Kappes *et al.* 1997; USDA 2000).

Statistical analyses

Measures of genotypic diversity and differentiation were analysed and displayed using several complementary approaches. Microsatellite DNA variation within breeds was quantified by average number of alleles per locus, observed heterozygosity and expected heterozygosity with the MICROSATELLITE TOOLKIT (Park 2001). Because sample sizes varied among breeds, allelic richness (i.e., the numbers of alleles standardized according to sample sizes; El Mousadik & Petit 1996; Petit *et al.* 1998) was also calculated using the F-STAT program (Goudet 1995). The GENEPOP program (Raymond & Rousset 1995) was used to test genotypes for Hardy–Weinberg equilibrium at each locus and to quantify differentiation of allele frequencies among populations by calculating F_{ST} (Weir & Cockerham 1984).

Genetic distances (unbiased genetic distance, Nei (1978) and chord distance, Cavalli-Sforza & Edwards (1967) were calculated with BIOSYS (Swofford & Selander 1981). Resulting unbiased genetic distance measures were then used to construct dendrograms by the unweighted pair-group method based on arithmetic averages (Sneath & Sokal 1973). Measures of genetic differentiation were also estimated both as an average value and as pair-wise values using AMOVA implemented in ARLEQUIN version 2.0 (Schneider *et al.* 2000). A genetic network representing the between- and within-breed variance components of genetic diversity was created using the algorithm described in Dyer & Nason (2004). The network topology obtained was rendered in a two-dimensional graph using GRAPHVIZ; software for producing a layered drawing of directed graphs (Gansner & North 2000).

A multiple-locus assignment test using the Bayesian clustering algorithm STRUCTURE (Pritchard *et al.* 2000) was used to examine underlying relationships among the breeds. Individuals were assigned to clusters based on their genotypes without *a priori* information, such as breed. The model assumes k groups, linkage equilibrium among markers and Hardy–Weinberg equilibrium within a group. The parameter k was determined by generating the posterior probability for a range of k values from 2 to 15 and assessing 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 50 000 iterations of each chain following a 100 000 iteration burn-in period. Each MCMC chain for each value of $k = 1$–10 was run 10 times. The method explicitly allows for individuals with ancestry from more than one group. These individuals are fractionally assigned to multiple groups using with a vector of ancestry proportions Q, which sums to 1.0 across the k groups. Individual assignments can vary across runs when there is a weak genetic basis for assigning an individual to a cluster. To address this variation, 100 separate MCMC chains were evaluated at the most probable value of k to examine similarity among assignments (Rosenberg *et al.* 2002).

Results and discussion

Shown in Table S1 are characterizations of the panels of microsatellite markers used in this research. The observed allelic richness indicates that, on average, only a fraction of all alleles observed were present within each population. The relative utility of each marker for discriminating among populations based on allele frequency differences is indicated by the F_{ST} statistic. On average, 14% of total genetic variation corresponded to differences between breeds and 86% arose from differences among individuals. This level of diversity among breeds is approximately 2% greater than that observed among seven European breeds of cattle by MacHugh *et al.* (1998).

Shown in Table S2 are descriptive statistics on the loci used in the study. Sample sizes for Shorthorn, Tarentaise and Texas Longhorn make precise interpretation of their relationships to other breeds difficult. Mean sample numbers per locus are slightly less than the census number of animals sampled as a result of a failed or ambiguous genotype for a

particular sample. At most loci, all populations had multiple alleles. Heterozygosity observed across all loci was consistent with heterozygosity expected based on Hardy–Weinberg equilibrium given the observed allele frequencies.

Shown in Table S3 are matrices of Cavalli-Sforza & Edwards (1967) chord genetic distances and Nei (1978) unbiased genetic distances between the sampled North American cattle populations. Generally similar conclusions were reached irrespective of the distance measure used. The average Nei's genetic distance of Chirikof Island cattle from the other breeds sampled was 0.38 (SD = 0.06) and was as great as or >78% of corresponding pair-wise distances observed between the breeds sampled from North America. This differentiation arose both due to differences in allele frequencies among populations and from the presence of private alleles unique to the various populations.

Genetic differentiation among the populations genotyped using Nei's (1978) unbiased genetic distance is illustrated in a dendrogram presented in Fig. 1. As expected, given known migration from Angus to Red Angus, these breeds were most similar to each other. The three French beef breeds (Charolais, Salers and Limousin) clustered together. Simmental and Tarentaise, continental European dual purpose breeds, clustered together and this cluster then joined the French beef breeds. This cluster of continental European breeds was then joined by Hereford and then Longhorn. The Angus-Red Angus cluster was joined by Shorthorn. All of the breeds studied that are also significantly represented in North American commercial production then formed a large cluster that was subsequently joined by Highland and then the Chirikof Island population. The dendrogram based on Cavalli-Sforza & Edwards (1967) chord genetic distances was qualitatively

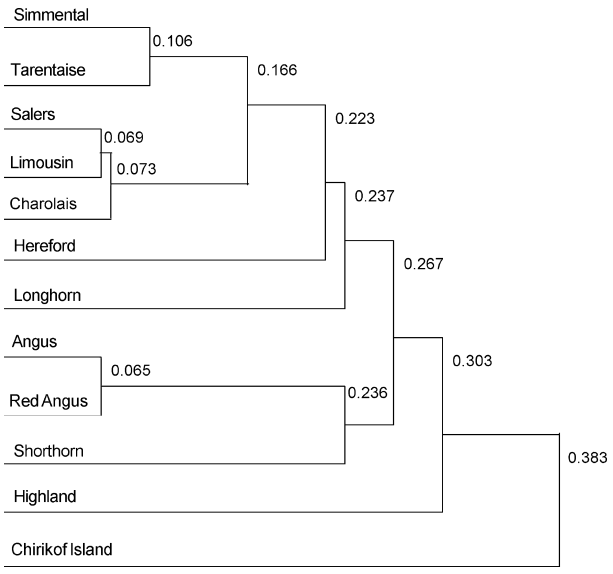

Figure 1 Dendrogram of genetic relationships among populations derived by cluster analysis using the unweighted pair group method with Nei (1978) unbiased genetic distance metric. Average genetic distances between joined clusters are indicated at each node.

similar in most respects to Fig. 1, except that the Angus and Red Angus cluster joined the large cluster composed of Hereford and the continental European breeds, and then this large cluster was joined in order by Shorthorn, Longhorn, Highland and finally the Chirikof Island population.

There were eight breeds in common in this study and earlier work of Blott *et al.* (1998) who found two major clusters of breeds. Blott *et al.* (1998) identified Limousin, Charolais, Salers and Simmental as belonging to a continental group of 12 breeds and Angus, Hereford, Shorthorn and Highland belonging to a group of 25 British and northern European breeds. Results from MacHugh *et al.* (1994; 1998) are interpreted to indicate generally similar between-breed relationships, for the four breeds in common, as found here. It is perhaps unexpected that the British breeds did not cluster together more closely in this study. However, such results are not entirely unanticipated as Wiener *et al.* (2004) also found breed pairs with strong phylogenetic support did not originate from physically close regions. It is noteworthy that genetic distances between Angus, Hereford and Highland observed here were similar to those observed by Wiener *et al.* (2004).

Use of a bifurcating tree to describe differentiation among breeds may be problematic in this study because apocryphal evidence suggests the Chirikof Island population may be an admixture. When present, such heterogeneous populations necessarily result in reticulations in tree structure. One way to handle this is to display populations as nodes within a network. Figure 2 illustrates genetic differentiation among the populations. The network uses an AMOVA approach to generate within and among variance components for all the breeds (Dyer & Nason 2004). Using this approach, between-breed variation was 12.3 ± 0.01% of the total. Graphically, the network displays differences among breeds as proportional to the distance between nodes and diversity within breeds as proportional to the diameter of the nodes. The AMOVA result suggests all 12 breeds are significantly differentiated, using a permutation test.

Using the program STRUCTURE (Pritchard *et al.* 2000), individuals were assigned to genetic clusters irrespective of breeds. The analysis identified four underlying genetic clusters (Fig. 3). STRUCTURE assigns individuals to clusters but individuals may be assigned to different clusters in multiple runs. By using a similarity coefficient among multiple runs of STRUCTURE, we estimated the stability of the assignments. The similarity coefficient (Rosenberg *et al.* 2002) compared the cluster assignments between two STRUCTURE runs and results in a value between 0 and 1 based on individual assignment coefficients. The mean similarity coefficient among 100 runs was 0.80 ± 0.03, which indicates strong stability in the genetic assignment of genotypes to each cluster. The difference between the full network (Fig. 2) and the reduced network (Fig. 3) shows the collapsing of: Angus, Red Angus and Shorthorn into one cluster; Charolais, Limousin, Salers, Simmental, Tarentaise and Texas

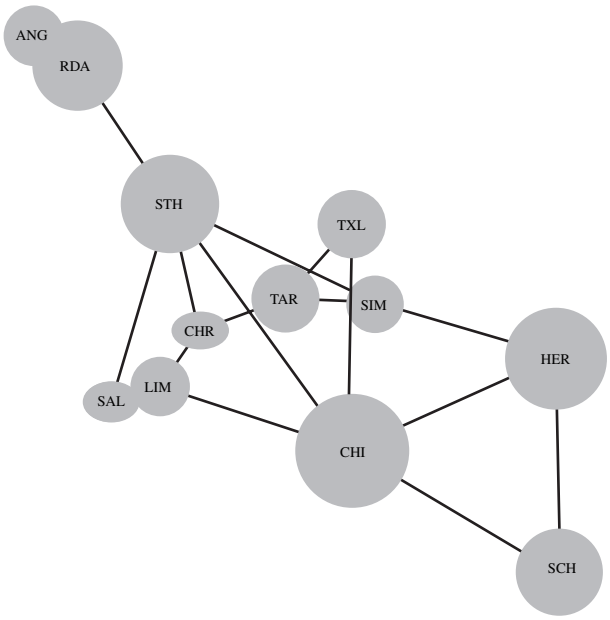

Figure 2 Network diagram of genetic relationships among breeds derived from between- and within-breed variance components of genetic diversity. Breeds are defined by nodes in the network where node diameters are proportional to the within-breed genetic variances and edges connecting the nodes are proportional to the between-breed genetic variances (ANG, Angus; CHI, Chirikof Island; CHR, Charolais; HER, Hereford; SCH, Highland; Lim, Limousin; RDA, Red Angus; SAL, Salers; STH, Shorthorn; SIM, Simmental; Tar, Tarentaise and TXL, Texas Longhorn).

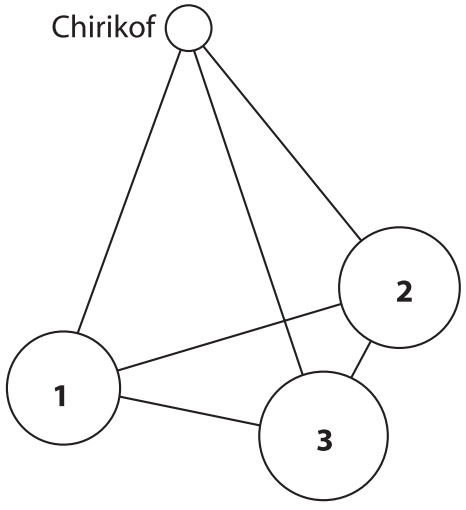

Figure 3 Network diagram illustrating most likely ($k = 4$) genetic clusters underlying the populations (1 = Angus, Red Angus, Shorthorn; 2 = Charolais, Limousin, Salers, Simmental, Tarentaise and Texas Longhorn; 3 = Hereford and Highland) evaluated.

Longhorn into a second; and Hereford and Highland into a third, while the Chirikof Island cattle remained distinct. Moreover, genotypes of the Chirikof Island cattle were completely correlated in their assignments underscoring the strong differentiation of this population.

Present analyses of genetic relationships did not definitively identify the origins of cattle on Chirikof Island. Irrespective of the statistical approach used, they appear to represent a distinct gene pool relative to the breeds that were sampled. If the Chirikof Island cattle were a recently admixed population, they might also be expected to have a greater number of alleles and higher level of heterozygosity than the presumably less admixed breeds, but the level of heterozygosity of the Chirikof Island cattle is comparable with, or less than, that observed in most breeds (Table S2). However, inbreeding due to a relatively small effective population size accompanied by random genetic drift provides a plausible explanation for the observed numbers of alleles and level of heterozygosity. Clearly, the Chirikof Island population is unique and differentiated from contemporary commercial beef germplasm widely available in North America. Thus, the Chirikof Island population represents a novel genetic resource that may be of importance for conservation and industry.

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Supplementary Material

The following supplementary material is available for this article online fromhttp://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01559.x

Table S1 Location, number of alleles observed (*N*), allelic richness (El Mousadik & Petit 1996; Petit *et al.* 1998) and F_{ST} (Weir & Cockerham 1984) for microsatellite markers used to genotype samples of breeds from the US and the Chirikof Island population.

Table S2 Characterization of sample size, polymorphism and heterozygosity for North American populations evaluated using 34 microsatellite loci.

Table S3 Matrix of Cavalli-Sforza & Edwards (1967) chord distance (below diagonal) and Nei (1978) genetic distance (above diagonal) between sampled populations based on 34 microsatellite markers.