No evidence for a recent genetic bottleneck in the endangered Sheko cattle breed (African *Bos taurus*) revealed by microsatellite analysis

Hailu Dadi[1,2], Joram Mwacharo[1,3], Markos Tibbo[4], Yukimizu Takahashi[1], Koh Nomura[1], Hirofumi Hanada[1] & Takashi Amano[1]

[1] *Laboratory of Animal Genetics and Breeding, Department of Animal Science, Tokyo University of Agriculture, 1737 Funako Atsugi City, Kanagawa 243-0034, Japan*

[2]*Adami Tulu Agricultural Research Center, PO Box 35, Baatu, Ethiopia*

[3]*Kiboko Research Centre, Kenya Agricultural Research Institute, PO Box 12, Makindu 90138, Kenya*

[4]*International Center for Agricultural Research in the Dry Areas (ICARDA), PO Box 5466, Tel Hadya, Aleppo, Syria*

Sheko is African taurine cattle, valued for its milk yield, adaptation to humid tsetse infested environment and trypanotolerance. We used 30 microsatellite markers in analyzing 30 DNA samples. We found high genetic diversity and no genetic bottlenecks in endangered Sheko cattle. Sheko cattle have not undergone recent genetic bottlenecks, in spite of drastic reduction in its overall demographic population size. The results were supported by three statistical methods: (i) detection of heterozygosity excess (ii) a mode-shift indicator of allele distribution pattern (iii) the ratio of the number of alleles to the range of allele size, *M*-ratio

test. This breed reflects historical and cultural identity of local communities and represents a unique component of the global domestic animal biodiversity that deserve priority for conservation.

There is a general consensus that 70% of the livestock existing today are found in developing countries where the risk of genetic extinction is high[1]. Indeed, it is in these developing regions that various genetically distinct and best adapted animals are found. A historical dimension to the development of adaptive traits is that the longer a population remains exposed to extreme conditions the greater is the possibility for specific adaptive traits to evolve. For instance, in Africa, hot and humid forest regions where trypanosomosis predominates, several genetically adapted indigenous breeds such as N'Dama[2] and Sheko cattle[3] have developed.

Sheko breed has been recognized as one of Africa's "Big Five" vintage cows having great potential to form the genetic backbone for future survival[4]. This breed represents the last remnants of Africa's original *Bos taurus* cattle which were probably the first to be domesticated in eastern Africa. The breed is restricted to the humid Sheko and Bench districts in Southwest Ethiopia where they are maintained by a small number of local farmers. In recent times however, with a census population size of only 2400 individuals, Sheko cattle have been designated as being endangered[4,5]. The main reasons attributed to the reduction in population size are indiscriminate crossbreeding and replacement mainly with thoracic-humped zebu cattle.

To maintain genetic diversity and avoid further loss of important animal genetic resources, assessment and quantification of existing levels of genetic diversity is of high priority[6-9]. Several studies that have evaluated the genetic diversity and relationships in different breeds of African cattle using microsatellite markers have been reported in

literature[10,11]. However, there is a paucity of information regarding the effective population size (i.e. bottlenecks), within-population genetic diversity and uniqueness of the Sheko breed. Such information is vital in facilitating informed decision making for conservation and sustainable utilization of this endangered cattle breed. We have therefore evaluated the levels of genetic diversity and whether the recent reductions in population size observed in Sheko cattle could have had any negative impact on its genetic architecture.

Bottlenecks detection

Results of bottlenecks detection using Wilcoxon signed-rank and sign tests under SMM and TPM are presented in Table 1. In bottlenecked populations, the observed gene diversity exceeds the expected equilibrium gene diversity under the assumption of mutation-drift equilibrium[12]. The null hypothesis tested for heterozygosity excess using Wilcoxon sign-rank test provided ($P > 0.968$) and ($P < 0.011$) probabilities under the Step-wise mutation (SMM) and Two-Phase mutation (TPM), respectively. The null hypothesis is accepted under the SMM model only, implying that the Sheko breed has not experienced any recent genetic bottlenecks. The estimated values of the heterozygosity excess and their probabilities in sign test were 17.7 ($P > 0.061$) for the SMM and 17.81 ($P > 0.083$) for the TPM. The null hypothesis of mutation-drift equilibrium was also accepted based on the sign test under both mutation models.

Population census numbers can diminish and expand without any effect on their genetic architecture. Change in population census numbers is a demographic bottleneck and does not affect genetic variation within or among populations[13]. However, a drastic reduction in both census numbers and effective population size is a genetic bottleneck, shaping genetic variation within or among populations[14]. Bottlenecks can increase demographic stochasticity, rate of inbreeding and loss of genetic variation, thereby increasing the probability of population extinction[13,15,16]. In this study, however,

probabilities derived from Wilcoxon sign-rank and sign tests revealed no evidence for genetic bottleneck in Sheko cattle.

Additionally, the mode-shift indicator test was employed to detect genetic bottleneck[12]. The assumption behind the test is that a population under mutation-drift equilibrium is expected to have a larger proportion of alleles with low frequencies. The allele frequency distribution as revealed by this test was L-shaped, indicating a larger proportion of low frequency allele classes in Sheko breed. The mode-shift indicator test revealed a normal L-shaped distribution pattern of allele frequencies (Fig. 1); indicating the presence of a larger proportion of low frequency (< 0.1) allele classes. This result lends further support to the lack of a recent genetic bottleneck in the Sheko breed. The absence of bottleneck with the *M*-ratio test was robust; its signature could not be detected under conservative parameter values. The *M*-ratio for Sheko cattle population was 0.7457, which was not significant at the 5% level of significance. The *M*-ratio approach did not support the possibility of a genetic bottleneck in Sheko cattle. The 0.7457 mean *M* value obtained in this study was above 0.68 which Garza and Williamson[17] suggested that for data set based on ≥ 7 microsatellite loci, values of $M \leq 0.68$ are indicative of a recent population bottleneck. This result and the above tests reinforce the lack of a recent genetic bottleneck in Sheko breed.

Genetic diversity

Allele size range, observed and effective number of alleles, Shannon's information index, expected and observed heterozygosities are presented in Table 2. All 30 microsatellite loci were polymorphic with the number of observed alleles ranging between 4 at *ETH3* and *ETH152* to 12 at *MM12*. Effective number of alleles on the other hand ranged from 1.93 at *CSRM60* to 6.844 at *TGLA122*, with a mean of 3.97 ±

1.31 alleles. The average estimate of Shannon's information index as a measure of gene diversity was 1.53 ± 0.32. The mean expected and observed heterozygosities were 0.72 ± 0.09 and 0.647 ± 0.12 respectively.

Genetic analysis of the 30 microsatellite loci showed that the Sheko breed maintains a substantial level of genetic diversity. The mean number of alleles detected at the population level was 6.93, a value that falls within the range of mean number of alleles (MNA) reported in various other African cattle breeds (MNA = 6.4 – 7.0[10], 5.1 – 7.9[11]). Levels of heterozygosities were also comparable to results reported in these literatures. Reduction in population size among Sheko cattle seems not to have had a negative impact on its genetic diversity.

Implications for Conservation

Sheko cattle represent the only remnants of the original African *Bos taurus* cattle found in eastern Africa. This breed may possess unique genetic traits that may be useful in confronting new tropical diseases and unpredictable changes in environment conditions in the future. Characters related to disease resistance and adaptation to extreme environments could prove fundamental to food security for the present and future human generations. Sheko exhibit superior trypanotolerance than other indigenous cattle populations found in Ethiopia[3], implicating the genetic potential of this breed to perform cost-effectively in humid tsetse infested habitats where thoracic humped cattle may not survive in the absence of veterinary intervention. Based on our previous report on the same data set[18], Sheko breed is characterized by high levels of genetic diversity and several unique alleles (*CSRM60*: 91bp; *MM12*: 137bp, 139bp; *BM2113*: 122bp; *MB1824*: 185bp; *ILSTS006*: 299bp), which may be vital for future breed development. Another strong inference is that this population has not experienced any recent genetic

bottlenecks in spite of demographic population contraction in recent years. This means that any alleles that are unique to Sheko cattle have not been lost.

The majority of this trypanotolerant cattle breed (Sheko) has been diluted through indiscriminate crossbreeding with thoracic humped cattle. However, morphological characters in combination with genetic markers can be employed to identify the most pure individuals to be the targets for conservation, breed development and research. For conservation purposes, the most economically feasible method will be *in-situ* preservation of live animals within their production environments[7]. Such a strategy enables Sheko cattle to continue adapting to their native habitats and production environments, while offering options for genetic improvement. *Ex-situ* conservation is proposed as a last resort or should supplement *in-situ* conservation if technical and economic feasibility permit its implementation.

Methods

Sampling and microsatellite markers. Sampling location and microsatellite markers used in this analysis are well described in our previous report[18]. Thirty most likely pure Sheko individuals were carefully sampled from the breeding tract of Sheko cattle in Southwestern Ethiopia. Since records are not usually kept by the herd owners to select the most pure Sheko cattle, sampling was done on the basis of morphological characteristics and observation at field. In addition, both herd owners and district experts were also consulted to ensure that sampled individuals are representative of the Sheko breed.

Testing for possible bottlenecks. We used the same molecular data reported earlier[18]. Possible recent genetic bottlenecks were tested in Sheko breed using three methods. The

first method employed was the Wilcoxon signed-rank and sign tests[19]. The calculations were based on 1000 simulations performed under Stepwise (SMM) and Two-Phase (TPM) mutations models of microsatellite evolution. The second approach was the mode-shift indicator test of allele distribution pattern[12]. When a population passes through a recent genetic bottleneck, it tends to lose rare alleles and consequently inflates the frequencies of the common alleles[12]. BOTTLENECK version 1.2.02[19] was used to detect genetic bottlenecks. The third method used to detect possible reduction in population size was *M*-ratio test[17]. The ratio of the number of alleles (*k*) at a locus to the range of allele sizes for the same locus (*r*) is termed as *M*-ratio[17]. When a population is reduced in size, genetic drift is intensified and rare alleles are quickly lost. Consequently, the *M*-ratio will be smaller than in equilibrium population. The program simulates an equilibrium distribution of *M* in a constant size population assuming values for three parameters[17]: $\theta = 4N_e\mu$ (where N_e = effective population size, μ = mutation rate of 5 x 10^{-4}), Δ_g (mean size of non one-step mutations) and p_s (the percent of one-step mutations). The model was specified using parameter values $\theta = 10$; $\Delta_g = 3.5$ and $p_s = 90\%$. Using the conventional criteria, there is evidence of significant reduction in population size if less than 5% of the replicates are below the observed values. The analysis was carried out using the M_P_VAL programs[17].

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Author information: The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to Hailu Dadi (edenhailu@yahoo.com) or (m.tibbo@cgiar.org).

Table 1 Genetic bottleneck detection using Wilcoxon signed-rank and sign tests under Step-wise (SMM) and Two-Phase (TPM) mutations models of microsatellite evolution

Test	Model	
	SMM	TPM
Wilcoxon signed-rank test:		
Probability of Heterozygosity excess	$P > 0.968$	$P < 0.011$*
Sign test:		
Number of loci with heterozygosity excess		
Observed	13.00	22.00
Expected (probability)	17.70	17.81
Probability	$P > 0.061$	$P > 0.083$

*Null hypothesis rejected.

Table 2 Summary of statistics of genetic variation showing allele size range, observed (N_A) and effective (N_E) number of alleles, Shannon's information index (I), expected (H_E) and observed (H_O) heterozygosities analysed in Sheko breed

Locus	Allele size range	N_A	N_E	I	H_E	Observed heterozygosity
CSRM60	91-111	6	1.931	1.012	0.491	0.433
HEL13	180-194	6	3.175	1.344	0.696	0.733
HEL1	95-103	5	4.216	1.506	0.779	0.600
INRA063	177-187	5	3.130	1.337	0.695	0.533
MM12	103-143	12	6.060	2.053	0.849	0.832
INRA023	199-217	7	4.489	1.601	0.794	0.567
INRA005	131-141	6	2.466	1.193	0.607	0.466
ILSTS005	176-188	5	3.622	1.368	0.734	0.833
HAUT24	106-130	6	2.987	1.413	0.679	0.571
BM1818	254-270	8	5.523	1.830	0.833	0.833
INRA037	110-126	8	4.604	1.779	0.797	0.733
ETH152	191-199	4	2.965	1.142	0.675	0.633
HEL5	143-157	6	3.241	1.375	0.706	0.586
TGLA227	71-91	10	3.991	1.689	0.765	0.600
HAUT27	140-148	5	3.255	1.311	0.703	0.766
SPS115	246-256	5	3.384	1.346	0.716	0.733
TGLA126	109-117	5	3.222	1.344	0.704	0.586
INRA032	160-208	7	4.072	1.594	0.770	0.633
INRA035	96-118	6	2.208	1.031	0.560	0.366
CSSM66	173-195	9	4.878	1.869	0.812	0.600
ETH225	137-157	9	5.143	1.823	0.822	0.633
TGLA122	132-166	10	6.844	2.058	0.866	0.733
BM2113	122-142	7	4.036	1.629	0.766	0.733
ETH10	211-225	7	4.688	1.719	0.799	0.867
BM1824	173-187	5	2.514	1.173	0.613	0.566
HEL9	143-159	9	6.040	1.942	0.851	0.733
ETH3	107-117	4	2.518	1.097	0.615	0.500
ILSTS006	277-299	9	4.455	1.708	0.783	0.700
ETH185	218-232	6	3.586	1.423	0.735	0.633
TGLA53	151-179	11	6.642	2.106	0.867	0.667

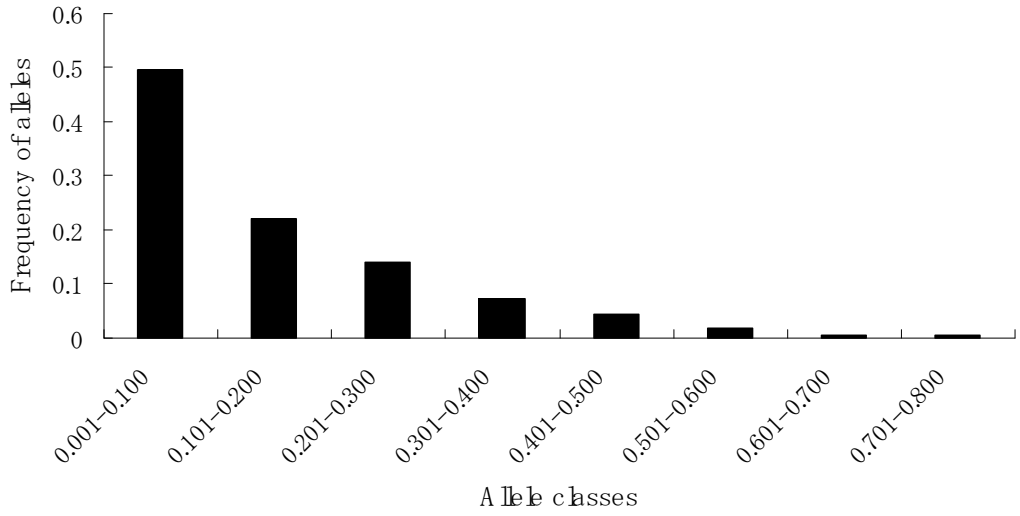

Figure 1 Normal L-shaped mode-shift graph depicting distribution of allele frequencies in Sheko cattle