Molecular Biology Reports (2019) 46:1307–1311
https://doi.org/10.1007/s11033-018-4445-8

SHORT COMMUNICATION

Current genetic diversity in eight local Chinese sheep populations

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Received: 25 August 2018 / Accepted: 17 October 2018 / Published online: 17 December 2018

Abstract

China has numerous local domestic sheep breeds. In this study, the genetic diversity of eight sheep populations was estimated using 17 microsatellites. Knowledge of such diversity provides novel insight into the degree of breed protection needed and the prediction of hybrid advantage. In total, 17 microsatellites were genotyped in 186 individuals from eight populations. The mean number of alleles (± SD) ranged from 3.71 ± 1.36 in Zhaotong sheep to 11.94 ± 3.58 in small-tailed Han sheep. The observed heterozygote frequency (± SD) within a population ranged from 0.482 ± 0.025 in Zhaotong sheep to 0.664 ± 0.023 in Tibetan sheep. In addition, using pairwise difference (F_{ST}) analysis, the highest within-population diversity was observed in Tibetan sheep (πX = 12.8098) and small-tailed Han (πX = 12.67873), and the lowest diversity was observed in Zhaotong sheep (πX = 7.90337). The results for genetic divergence between populations indicated that the populations were significantly different ($P < 0.05$) based on the average number of pairwise differences between populations (πXY) and the corrected average pairwise differences. Both phylogenetic networks and structure analysis showed that these eight populations were separated into three clusters in accordance with their geographical habitat, except Tibetan and Hu sheep. In short, we genotyped eight local Chinese sheep populations using 17 microsatellites, and the results indicated that their current genetic diversity is decreasing and that new conservation strategies are needed. In addition, significant genetic differences between populations could be used in cross breeding.

Keywords Diversity · Local sheep · Microsatellite · China

Introduction

Domestic sheep are an important farm animal species worldwide, both economically and culturally. In particular, some commercial lines have been introduced to improve the industrial development and genetic improvement of native breeds [3]. Recently, a number of studies have estimated the diversity in local sheep populations around the world with different genetic variants (e.g., [2, 4, 11]).

Here, to promote the development of local sheep breeding, a local academic organization, the Western–Northern Sheep Breeding Alliance, was established through the efforts of three units (Southwest University, Yunnan Animal Science and Veterinary Institute, and the Chinese Academy of Agricultural Sciences). The expected goal of the alliance is to further improve the economic value of some local Chinese breeds, including their growth rate, number of lambs, and milk production, using hybrid breeding theory.

Thus, at present, to promote the development of local sheep breeding, it is particularly urgent that the genetic diversity of breeds targeted for improvement be evaluated. In

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-018-4445-8) contains supplementary material, which is available to authorized users.

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particular, knowledge of the evolution of divergence between these breeds is helpful to further guarantee a stable and substantial hybrid advantage.

In this study we examined eight indigenous Chinese sheep populations, which represent different important economic advantages and different ecological distributions. We estimated their genetic diversity and population divergence using microsatellite markers. The aim is to assess the status of conservation and whether it is suitable for cross breeding in the future.

Materials and methods

Animals and DNA extraction

The experimental conditions used in this study were approved by the Committee on the Ethics of Animal Experiments of Southwest University [No. (2007) 3] and the Animal Protection Law of China.

Genomic DNA from 186 individual blood samples (Table 1) was extracted using a standard phenol–chloroform protocol [15], 1% agarose gel electrophoresis was used to check DNA quality, and the quantity of extracted DNA was assessed with a DTX microplate reader (Beckman Coulter, US).

Genotyping

A total of 186 individuals encompassing eight local breeds were genotyped at the 17 microsatellite markers recommended by the IGS-FAO [8]; these markers are shown in Supplemental material I. Genotyping was performed on a Genetic Analyzer 3130xl (Applied Biosystems, US); a detailed description of the genotyping can be found in E et al. [5].

Statistical analysis

Conventional genetic diversity parameters, including observed heterozygosity (*Ho*), expected heterozygosity (H_E), mean number of alleles (N_A), and polymorphism information content (PIC), were estimated with the Microsatellite Toolkit [12]. Deviations of markers from the Hardy–Weinberg equilibrium (HWE) within populations were identified using GENEPOP 3.4 software [13]. The inbreeding coefficient was estimated and Fisher's exact test with a Bonferroni correction was performed using FSTAT 2.9.3.2 [10]. Pairwise differences between populations (F_{ST}) and loci under selection were identified using Arlequin software version 3.5.1.3 [7].

Phylogenetic network (neighbor-joining) was built using PHYLIP software [9] with a Reynolds genetic distance [14].

Population structure was assessed using STRUCTURE 2.3.3 with an admixture model from $K=2$–8 in 100 runs. Assignment clusters were made with a burn-in of 50,000 and 100,000 Markov Chain Monte Carlo (MCMC) iterations. The best K value was estimated using the STRUCTURE_Harvester [6].

Results and discussion

In total, 319 alleles were identified in eight local sheep breeds across 17 microsatellites. Across populations, an average of 18.8 alleles of each marker was observed, ranging from 11 in OarFCB48 to 30 in OarFCB304. The mean observed (H_O) and expected heterozygosity (H_E) of each marker across the breeds was 0.595 (from 0.252 (ILSTS005) to 0.735 (CSRD247)) and 0.706 (from 0.498 (ILSTS005) to 0.792 (INRA063)), respectively. The *PIC* across markers was 0.654 and ranged from 0.451 (ILSTS005) to 0.792 (INRA063) across populations (Supplemental material II). This value reveals that most of the markers are highly polymorphic in the studied populations and represent their genetic diversity well.

Table 1 Original distribution and sample information for the eight sheep populations

Name	Group	Code	SZ	N	E	Native location	Main economic use
Tibetan sheep	Native	ZAS	25	29°46′48.56″	94°22′21.49″	Ling Zhi, Tibetan	Meat and wool
ZhaoTong sheep	Native	ZTS	24	27°20′17.65″	103°42′59.00″	Zhao Tong, YunNan, China	Meat
Hu sheep	Native	HUS	23	31°18′50.01″	120°36′33.48″	Hangzhou, ZheJiang, China	Meat and wool
Small-tailed Han	Native	XWS	26	35°15′23.44″	115°27′3.60″	Heze, ShanDong, China	Meat and wool
Mongolian sheep	Native	MGS	23	49°16′16.81″	120°01′44.86″	Hailaer, Inner Mongolian, China	Meat and wool
Sishui fur sheep	Native	SIS	23	35°39′53.27	117°14′46.64″	Sishui, ShanDong, China	Mink
Duolang sheep	Native	DLS	20	38°54′18.17″	77°39′0.64″	Maigaiti, Xinjiang, China	Meat
Shiping grey sheep	Native	SPS	22	23°42′29.69″	102°29′41.40″	Shiping, Yunnan, China	Meat and mink

SZ sample size, *N* north latitude, *E* east longitude, and *Code* short name of breed

Across markers, the H_E within a breed ranged from 0.495 ± 0.039 in ZTS to 0.823 ± 0.017 in XWS. The H_O ranged from 0.482 ± 0.025 in ZTS to 0.664 ± 0.023 in ZAS, and the N_A ranged from 3.71 ± 1.36 in ZTS to 11.94 ± 3.58 in XWS (Table 2). Regarding H_O, compared to results from previous research, our results showed reduced genetic diversity in some groups, such as MGS (e.g. [16]), but increased genetic diversity in other breeds, such as ZTS (e.g. [1]). It indicated that were dynamic changes in genetic diversity in different populations.

Based on the Hardy–Weinberg equilibrium (HWE) analysis, all markers in SPS were at HWE. Only one marker deviated from HWE (dHWE) in SIS, and ZTS and five other populations exhibited 4–9 markers that dHWE. The F_{IS} within populations ranged from 0.027 in ZTS to 0.241 in XWS. In most (75%) of these populations the P-value for F_{IS} was significant at the adjusted nominal level (5%). According to the HWE and F_{IS} results, inbreeding did not occur in SPS and ZTS; however, the other six populations face potential risk and are a reminder that the government should pay attention to the protection of genetic diversity of these populations and optimize current protection measures.

In the pairwise difference (F_{ST}) analysis (Fig. 1a), the highest within-population diversity was observed in ZAS ($\pi X = 12.8098$) and XWS ($\pi X = 12.67873$), and the lowest was observed in ZTS ($\pi X = 7.90337$). The results of genetic divergence between populations indicated that the populations were significantly different ($P < 0.05$) based on the average number of pairwise differences between populations (πXY) and corrected average pairwise differences [$\pi XY - (\pi X + \pi Y)/2$]. These results also indicate that high genetic divergence is available, which can be used for cross breeding.

The results from the phylogenetic network (Fig. 1b) showed that the eight populations were separated into three clusters. First, cluster I included MGS and DLS, which were collected from Inner Mongolia and Xinjiang, respectively. People in these two areas have a nomadic culture, and geography is approaching. Therefore, the exchange of gene flow in domesticated animals between the two places has been high historically due to human culture and human migration. Second, cluster II included SPS, ZTS, and HUS. It is not hard to understand the phylogenetic relationship between SPS and ZTS which were both collected from Yunnan, China. Here, HUS is a common breed in ZheJiang, China which is a large geographical area in the southwest. However, due to the high occurrence of factory farming, a strengthening in artificial breeding was a possible result, leading to inbreeding within this population. This was also revealed from F_{IS} and dHWD in this study. Thus, this phylogenetic relation between HUS and the two southwest populations was inconsistent with their geographic location. Third, cluster III contains XWS, SIS, and ZAS. XWS and SIS were

Table 2 Genetic diversity estimation and pairwise differences of eight sheep populations

Population	Genetic diversity parameter assessment						Pairwise differences analysis							
	H_O (± SD)	H_E (± SD)	N_A (± SD)	F_{IS}	P-value	dHWE	MGS	DLS	HUS	SIS	SPS	ZAS	XWS	ZTS
MGS	0.639 ± 0.024	0.749 ± 0.025	7.00 ± 1.94	0.15	0.0003#	4	11.95749	13.13967*	12.95983*	13.65548*	12.55287*	13.58043*	13.48495*	12.94475*
DLS	0.605 ± 0.027	0.727 ± 0.032	6.88 ± 2.29	0.173	0.0003#	4	1.33593*	11.65	12.84293*	13.48261*	12.63523*	13.807*	13.49038*	13.29896*
HUS	0.578 ± 0.025	0.755 ± 0.017	7.94 ± 1.89	0.238	0.0003#	6	1.14147*	1.17832*	11.67923	12.77363*	11.57362*	13.2513*	13.19691*	11.90082*
SIS	0.645 ± 0.024	0.741 ± 0.022	7.82 ± 1.74	0.133	0.0003#	1	1.65741*	1.63829*	0.91469*	12.03865	12.30682*	13.04348*	12.82023*	13.02808*
SPS	0.523 ± 0.027	0.577 ± 0.053	4.18 ± 1.51	0.096	0.0075	0	2.42349*	2.65959*	1.58337*	2.13686*	8.30127	12.27227*	12.70586*	10.0857*
ZAS	0.664 ± 0.023	0.779 ± 0.032	10.24 ± 3.44	0.151	0.0003#	6	1.19679*	1.5771*	1.00679*	0.61926*	1.71674*	12.8098	13.17115*	13.03708*
XWS	0.628 ± 0.023	0.823 ± 0.017	11.94 ± 3.58	0.241	0.0003#	9	1.16684*	1.32602*	1.01793*	0.46154*	2.21586*	0.42689*	12.67873	13.20913*
ZTS	0.482 ± 0.025	0.495 ± 0.039	3.71 ± 1.36	0.027	0.2487	1	3.01432*	3.52227*	2.10952*	3.05707*	1.98338*	2.6805*	2.91808*	7.90337

Pa is the number of private alleles, dHWE is the number of populations that deviated ($P < 0.01$) from Hardy–Weinberg equilibrium, and '#' indicates that the adjusted nominal level (5%) for one table is 0.0006 based on 1680 randomizations of P-values for F_{IS}. About pairwise differences analysis as (1) above diagonal: average number of pairwise differences between populations (πXY); (2) diagonal elements: average number of pairwise differences within populations (πX); and (3) below diagonal: corrected average pairwise differences [$\pi XY - (\pi X + \pi Y)$]

The "*" indicates a significant difference between populations ($P < 0.05$)

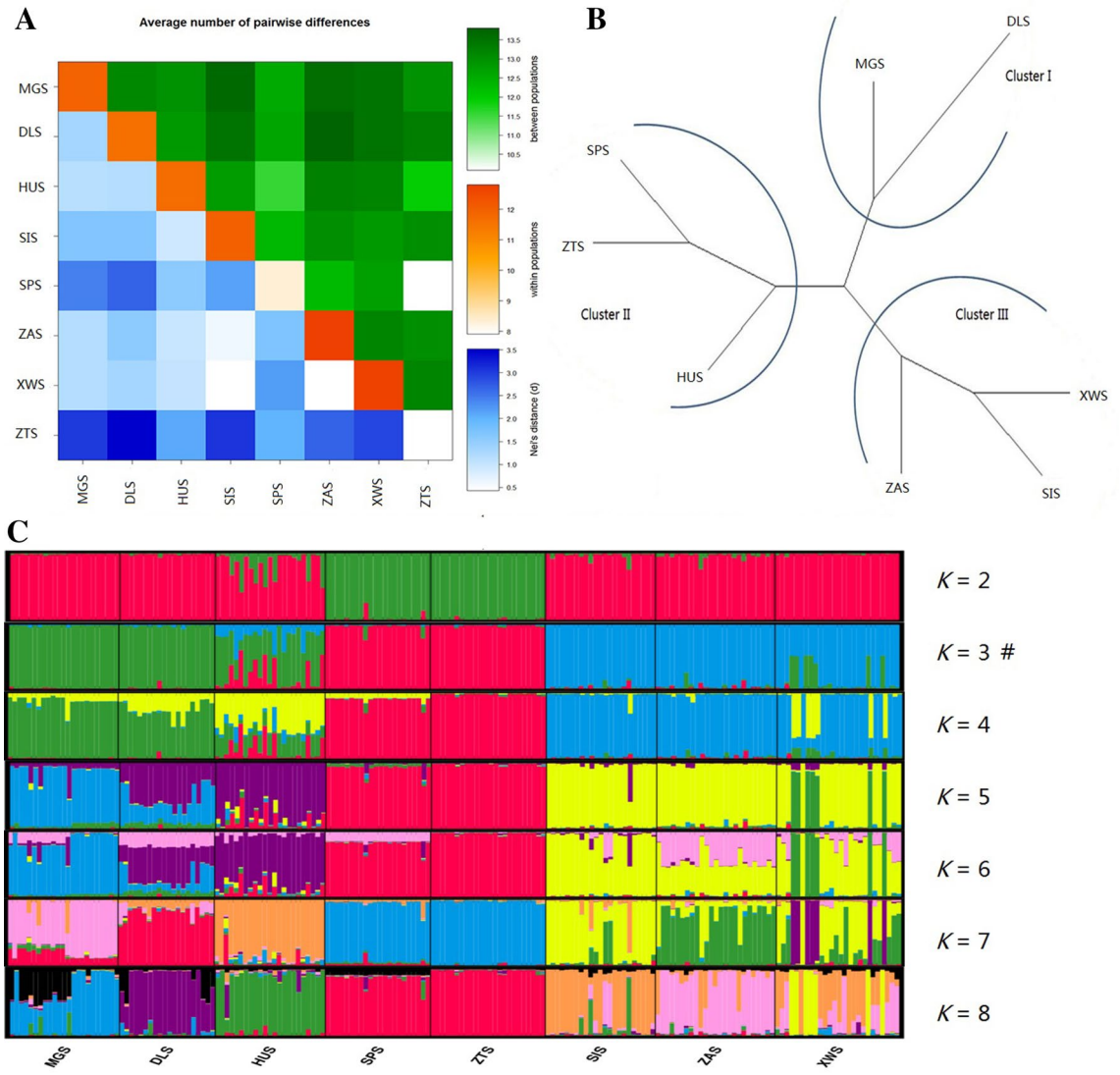

Fig. 1 Phylogenetic population structure of eight local Chinese sheep breeds. **a** Pairwise differences analysis between eight sheep populations. **b** Phylogenetic network of eight sheep populations by Reynold's genetic distance. **c** Cluster diagrams of eight sheep populations obtained using STRUCTURE

collected from Shandong, China; their phylogenetic relationship was identical to their distribution. In contrast ZAS was a free grazing breed from Tibet, therefore the smaller sample size may not accurately reflect the entire genetic diversity and population classification. In addition, STRUCTURE software was used for clustering individuals into $2 \leq K \leq 8$. The most credible K value was 3 by $\Delta K = m|L''(K)|/s|L(K)|$, and the population pattern revealed was consistent with the previously mentioned pattern (Fig. 1c). This implies that the population structure classification of this study is not affected by genetic algorithms.

In short, we genotyped eight local Chinese sheep populations using 17 microsatellites. Our results indicated that the current genetic diversity in most of these populations is going down and that their conservation strategy needs to change. In addition, from this study we observed that high genetic divergence between populations still exists. We should continue to speed up cross-breeding and take advantage of hybridization to improve production before there is an irreversible decline in genetic diversity in these populations.

Acknowledgements This work was supported by National Natural Science Foundation of China (No. 31172195), Fundamental Research Funds for the Central Universities (XDJK2018B014), People's Livelihood Special Innovation Projects of CQ CSTC (cstc2015shmszx80005).

Author contributions Y-HM and Y-FH conceived and designed the experiments. Q-HH and G-XE performed the lab work. G-XE analyzed the data and wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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