Genetic Diversity Analysis of Four Bactrian Camel Varieties in China

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ABSTRACT

In this study, 17 microsatellite markers were used to analyze the genetic polymorphism, genetic differentiation, gene flow and genetic distance of Alashan desert Bactrian camel, Alashan Gobi Bactrian camel, Sunite Bactrian camel and Qinghai Bactrian camel. The results indicated that the number of effective alleles of the four Bactrian camel varieties ranged from 2.7302 to 3.0524, and average theoretical heterozygosity and average polymorphic information content were 0.6283 and 0.5546, respectively. Observational heterozygosity, expected heterozygosity and polymorphic information content of Qinghai Bactrian camel were all higher than those of the other three varieties, being 0.8922, 0.6690 and 0.5813, respectively, so Qinghai Bactrian camel was of rich genetic polymorphism. Average gene flow of the microsatellite markers was 12.3188 and average Fst value was 0.0199, namely 1.99% of genetic variation derived between subpopulations and 98.01% came inside the subpopulations, revealing that the genetic differentiation degree between Bactrian camel subpopulations was low. The genetic relationship between Sunite Bactrian camel and Alashan desert Bactrian camel was close, so it was classified into the first type and that between Sunite Bactrian camel and Alashan desert Bactrian camel as the second type, but the genetic relationship of Qinghai Bactrian camel and other three Bactrian camel varieties was distant.

Bactrian camel, called “ship of the desert”, and also called camel in China, is a special variety formed through the long-time natural selection. Bactrian camels have been tamed by human beings long before, which are docile, easy to ride and suitable for carrying loads, so they are usually used as tools for riding instead of walk among the people in desert areas, and meanwhile, they can provide livestock products such as meat, milk and fur. Furthermore, they have played a significant role in the human development and desert conquering. In recent years, domestic and foreign researches regarding genetic diversity (Hedayat-Evrigh, et al., 2018) and organization structure (Ye et al., 2014a, 2014b; Wang et al., 2016) of Bactrian camels have achieved progress. Microsatellite markers have been extensively applied to genetic diversity studies of cow (Ni et al., 2018), sheep (Bai et al., 2015) and poultry (Bai et al., 2014, 2016a, 2016b, 2016c, 2017) by virtue of high abundance, good repeatability, co-dominance marker and selective neutrality. It is also widely used in plant genetic diversity research (Guo et al., 2018; Yang et al., 2013). Polyacrylamide gel electrophoresis method was used in this study to detect the polymorphism of the 17 microsatellite markers in four Bactrian camel varieties in China, expecting to provide a theoretical basis for the protection of Bactrian camel variety resources and improvement of population productivity.

Materials and methods

Blood samples (10 ml) were taken from jugular vein 40 Sunite Bactrian Camels, 40 Alashan desert Bactrian camel, 40 Alashan gobi Bactrian camel and 40 Qinghai Bactrian camel. ACD was added for anticoagulation. DNA was extracted from the blood using the whole-blood genomic DNA extraction kit (Beijing Dingguo Changsheng) method and stored at -20°C. Seventeen microsatellite markers with high polymorphism were screened (Evdotchenko et al., 2003; Sushma et al., 2014). The primers were synthesized by Shanghai Shenggong Bioengineering Technology Service Co., Ltd.

Thermal cycle for PCR comprised pre-denaturation at 94°C for 4 min, then denaturation at 94°C for 40 s, annealing at 60°C for 1 min, annealing at 72°C for 20 s, denaturation, annealing and elongation were carried out...
Table I. Population genetic diversity of four Bactrian camels.

| Population                      | Na   | Ne    | O_Hom | O_Het | E_Hom | E_Het | PIC  |
|---------------------------------|------|-------|-------|-------|-------|-------|------|
| Alashan Desert Bactrian camel   | 3.8235 | 2.8367 | 0.1309 | 0.8691 | 0.3908 | 0.6192 | 0.5461 |
| Alashan Gobi Bactrian camel     | 3.5294 | 2.7302 | 0.1403 | 0.8597 | 0.3932 | 0.6068 | 0.5308 |
| Qinghai Bactrian camel          | 3.8235 | 3.0524 | 0.1078 | 0.8922 | 0.3510 | 0.6490 | 0.5813 |
| Sunite Bactrian camel           | 3.7647 | 2.8913 | 0.1235 | 0.8765 | 0.3618 | 0.6382 | 0.5602 |

Table II. Fixed index and gene flow estimation.

| Microsatellite markers | Fis (inbreeding coefficient of total population) | Fit (inter-population differentiation coefficient) | Fst (intra-population inbreeding coefficient) | Nm (gene flow) |
|------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|----------------|
| LCA33                  | -0.3437                                       | -0.3362                                       | 0.0056                                        | 44.5645        |
| LCA37                  | -0.8362                                       | -0.8300                                       | 0.0034                                        | 73.1873        |
| LCA63                  | -0.4801                                       | -0.4738                                       | 0.0043                                        | 58.2326        |
| LCA66                  | -0.3643                                       | -0.3294                                       | 0.0256                                        | 9.5282         |
| LCA71                  | -0.7024                                       | -0.6869                                       | 0.0091                                        | 27.1327        |
| LCA82                  | -0.5725                                       | -0.5545                                       | 0.0114                                        | 21.6694        |
| LCA90                  | -0.2459                                       | -0.2114                                       | 0.0277                                        | 8.7863         |
| CMS15                  | -0.4746                                       | -0.4049                                       | 0.0473                                        | 5.0378         |
| CMS18                  | -0.5894                                       | -0.5846                                       | 0.0030                                        | 83.1109        |
| CMS36                  | 0.8525                                        | 0.8584                                        | 0.0401                                        | 5.9919         |
| CMS104                 | -0.4715                                       | -0.4208                                       | 0.0345                                        | 7.0058         |
| CVRL101                | -0.3397                                       | -0.3263                                       | 0.0100                                        | 24.7006        |
| YWLL29                 | -0.1197                                       | -0.0927                                       | 0.0241                                        | 10.1027        |
| YWLL36                 | -0.6685                                       | -0.6201                                       | 0.0290                                        | 8.3595         |
| YWLL44                 | -0.4361                                       | -0.3897                                       | 0.0323                                        | 7.4981         |
| VOLP08                 | -0.3489                                       | -0.3478                                       | 0.0009                                        | 291.4601       |
| VOLP32                 | -0.3702                                       | -0.3281                                       | 0.0307                                        | 7.8965         |
| Mean                   | -0.4093                                       | -0.3813                                       | 0.0199                                        | 12.3188        |

Table III. Genetic distance and coherence of four Bactrian camel populations.

| Populations                    | Alashan Desert Bactrian camel | Qinghai Bactrian camel | Sunite Bactrian camel | Alashan Gobi Bactrian camel |
|--------------------------------|--------------------------------|------------------------|-----------------------|-----------------------------|
| Alashan Desert Bactrian camel  | 0.9553                         | 0.9563                 | 0.9607                |                             |
| Qinghai Bactrian camel         | 0.0458                         | 0.9408                 | 0.9545                |                             |
| Sunite Bactrian camel          | 0.0446                         | 0.0610                 | 0.9695                |                             |
| Alashan Gobi Bactrian camel    | 0.0401                         | 0.0466                 | 0.0310                |                             |

Note: The upper triangle is genetic consistency and the lower triangle is Nei’s genetic distance.

for 35 cycles, then elongation at 72°C and finally the reaction was completed and cooled and preserved at 4°C.

For SSCP, 15% non denaturing polyacrylamide gels were used to detect the products. Silver nitrate dyeing method is used for dyeing, mainly through fixation, oxidation, dyeing, color rendering, photography and other links.

Popgene32 software was used to calculate numbers of effective alleles, allele frequencies and heterozygosity of microsatellites.

Results and discussion

Genetic polymorphisms of the 17 microsatellite markers in the four Bactrian camel varieties are seen in Table I. It could be seen that number of effective alleles of the four varieties ranged from 2.7302 to 3.0524.
Observational heterozygosity, expected heterozygosity and polymorphic information content of Qinghai Bactrian camel were all higher than those of the other three varieties, being 0.8922, 0.6490 and 0.5813, respectively, so Qinghai Bactrian camel was of rich genetic polymorphism.

Fixed indices and gene flows of the 17 microsatellite markers in the four Bactrian camel populations are seen in Table II according to which Fis and Fts values of microsatellites are negative except for CMS36. The average Fis and Fts values were -0.409 and -0.3813, respectively. According to Wight (1978), if the population Fst value is within 0-0.05, no differentiation exists between the subpopulations. If Fst value is within 0.05-0.15, moderate differentiation is considered. If it is between 0.15-0.25, high differentiation is manifested. Average Fst value of the microsatellite markers in this study was 0.0199, namely 1.99% of genetic variation derived between subpopulations and 98.01% came inside the subpopulations, revealing that the genetic differentiation degree between Bactrian camel subpopulations was low or even no differentiation existed. Gene flows of the microsatellite markers were large with average value of 12.3188. The gene flow values in this study were larger than the study result by Tian et al. (2012) (Nm=5.4869). It was revealed in this study that gene exchange degree between the four Bactrian camel subpopulations in different areas was high, which resulted in their low genetic differentiation.

The genetic distance and identity values between the Bactrian camel subpopulations are shown in Table III. It could be observed that Nei’s genetic distance between the subpopulations was small, ranging from 0.0310 to 0.0610, but the genetic identity was large (0.9408-0.9695). The cluster diagram of the four Bactrian camel populations is seen in Figure 1, which shows that the genetic relationship between Sunite Bactrian camel and Alashan Gobi Bactrian camel was close, so it was classified into the first type and that between Sunite Bactrian camel and Alashan desert Bactrian camel as the second type. The study by Mburu et al. (2003) indicates that 6 dromedary populations are divided into 2 isolated subpopulations. Gene exchange exists between the Bactrian resources, which is an important reason for the correlation between clustering result and the ecological area. This study indicated that genetic relationships of Sunite Bactrian camel, Alashan gobi Bactrian camel and Alashan desert Bactrian camel, which were all located in the Inner Mongolia Autonomous Region, were close due to the frequent gene exchange, and located in Gansu Province, Qinghai Bactrian camel had less frequent gene exchange with other 3 Bactrian camel varieties, so its genetic relationships with them were distant.

All camels in China are Bactrian camels which are dominant livestock resources in desert areas. There are over 280,000 camels in China, where most of them are located in the Inner Mongolia Autonomous Region, accounting for about 67% of the total number, followed by those in Xinjiang Uygur Autonomous Region, accounting for about 20%. The protection of genetic polymorphism of Bactrian camel resources in the Inner Mongolia Autonomous Region not only refers to reasonably managing and utilizing the existing resources but also can maintain a certain resource potential for the future demand.

Fig.1. Genetic clustering of four Bactrian camel varieties.

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Statement of conflict of interest
The author declares there is no conflict of interest.

References
Bai, J.Y., Pang, Y.Z., Qi, Y.X., Zhang, X.H. and Yun, Y.X. 2017. Indian J. Anim. Res., 51: 851-855.
Bai, J.Y., Pang, Y.Z., Wu, S.J., Yu, M.Q. and Zhang, X.H., 2016a. Indian J. Anim. Res., 50: 1-7.
Bai, J.Y., Pang, Y.Z., Zhang, X.H., Yun, Y.X. and Qi, Y.X. 2016b. Brazilian J. Poult. Sci., 18: 519-524. https://doi.org/10.1590/1806-9061-2015-0101
Bai, J.Y., Pang, Y.Z., Qi, Y.X., Zhang, X.H. and Yun, X.Y. 2016c. Brazilian J. Poult. Sci., 18: 27-32. https://doi.org/10.1590/1806-9061-2015-0101
Bai, J.Y., Yang, Y.B., Wang, Y.Q., Zhang, X.H. and Pang, Y.Z., 2015. Indian J. Anim. Res., 49: 585-590.
Bai, J.Y., Jia, X.P., Yang, Y.B., Zhang, X.H., Pang,Y.Z., Wang,Y.Q. and Qi, Y.X., 2014. J. Anim. Pl. Sci., 24: 965-968.
Evdotchenko, D., Han, Y., Bartenschlager, H., Preuss, S. and Geldermann, H., 2003. Mol. Ecol. Notes, 3: 431-434. https://doi.org/10.1046/j.1471-8286.2003.00477.x
Guo, L.L., Guo, D.L., Zhao, W. and Hou, X.G., 2018. J. Horticul. Sci. Biotechnol., 93: 416-424. https://doi.org/10.1080/14620316.2017.1373039
Hedayat-Evrigh, N., Miraei-Ashtiani, S.R., Shahrebabak,
M.M., Evrigh, R.K. and Pourasad, K., 2018. *J. Agric. Sci. Technol.*, 20: 1137-1148.

Mburu, D.N., Ochieng. J.W., Kuria, S.G., Jianlin, H., Kaufmann, B., Rege, J.E. and Hanotte, O. 2003. *Anim. Genet.*, 34: 26-32. https://doi.org/10.1046/j.1365-2052.2003.00937.x

Ni, W.W., Jiang, A., Zhang, J., Ei, G.X. and Huang, Y.F., 2018. *Indian J. Anim. Res.*, 52: 1543-1547.

Sushma, P., Ali, S., Banerjee, P. and Joshi, J., 2014. *Int. J. Biomed. Life Sci.*, 5: 286-296.

Tian, Y.Z., Nuerbiya, W., Wang, L.J., Wu, W.W., Xu, X.M., Zhang, Y.H., Azhi, T., Tian, K.C., 2012. *Anim. Husb. Vet. Med.*, 44: 38-43.

Wang, H.J., Ma, K.M., Liu, Z.H., Jin, D.Z. and Wei, W.Q., 2016. *J. Camel Pract. Res.*, 23: 241-246.

https://doi.org/10.5958/2277-8934.2016.00041.2

Wright, S., 1978. In: *Variability within and among natural populations*. University of Chicago Press, Chicago.

Yang, J., Dai, P.F., Zhou, T.H., Huang, Z.H., Feng, L., Su H.L., Liu, Z.L. and Zhao, G.F. 2013. *Scient. Hortic.*, 150: 1-10. https://doi.org/10.1016/j.scienta.2012.11.004

Ye, W.L., Wang, F.L., Xie, Z.H., Wang, Y.G., Lin, B. and Wang, J.L., 2014a. *J. Camel Pract. Res.*, 21: 191-198. https://doi.org/10.5958/2277-8934.2014.00033.2

Ye, W.L., Xie, Z.H., Wang, F.L., Gen, X., Dong, S. and Wang, J.L. 2014b. *J. Camel Pract. Res.*, 21: 103-109. https://doi.org/10.5958/2277-8934.2014.00020.4