https://doi.org/10.26898/0370-8799-2023-6-11
УДК: 636.082.12
Тип статьи: оригинальная
Type of article: original

ОЦЕНКА ГЕНЕТИЧЕСКИХ РАЗЛИЧИЙ У ЖИВОТНЫХ НА ПРИМЕРЕ ПРЕДСТАВИТЕЛЕЙ РОДА *CAMELUS*

Тыщенко В.И., (✉)**Терлецкий В.П.**
Всероссийский научно-исследовательский институт генетики и разведения сельскохозяйственных животных – филиал Всероссийского института животноводства им. академика Л.К. Эрнста
Санкт-Петербург, п. Тярлево, Россия
(✉)e-mail: valeriter@mail.ru

Представлены данные о генетической изменчивости геномной ДНК двух видов верблюдов (дромедар и бактриан). Отмечено, что указанные виды имеют большое значение в ряде южных стран – используются как сельскохозяйственные, тягловые, верховые и спортивные животные. В настоящее время изучению верблюдов уделяется большое внимание с целью выявления их генетических особенностей, которые можно использовать в селекционной работе. Одним из методов исследования является мультилокусный анализ с применением меченых олигонуклеотидных зондов. Последние избирательно гибридизуются в отдельных участках геномной ДНК, приводя к формированию специфических генетических профилей, характерных для каждой особи. Мечение зонда дезоксигенином позволяет детектировать результаты гибридизации на фильтре. После проведения реакции молекулярной гибридизации зонда с геномной ДНК верблюдов было выявлено от 3 до 15 фрагментов ДНК, при этом картина гибридизации сильно отличалась у дромедаров и бактрианов, что свидетельствует о значительной генетической разнице в организации их геномов. Коэффициент сходства особей внутри популяции у бактрианов был существенно выше, чем у дромедаров (0,48 против 0,39), коэффициент межвидового сходства по этому параметру составил всего 0,13. Расчет генетического расстояния между популяциями дал довольно высокое значение – 0,305, что намного выше, чем ранее полученные данные по крупному рогатому скоту (от 0,05 до 0,10). Внутрипопуляционное генетическое разнообразие оценивали по критерию средней гетерозиготности. Расчеты показали большее генетическое разнообразие в популяции дромедаров (H = 0,72), что косвенно подтверждалось и более низким значением коэффициента сходства в этой группе животных.

Ключевые слова: бактриан, дромедар, генетическое разнообразие, олигонуклеотидный зонд

ASSESSMENT OF GENETIC DIFFERENCES IN ANIMALS AS EXEMPLIFIED BY REPRESENTATIVES OF THE GENUS *CAMELUS*

Tyshchenko V.I., (✉)**Terletskiy V.P.**
Russian Research Institute of Farm Animal Genetics and Breeding - Branch of the L.K. Ernst Federal Research Center for Animal Husbandry
Tyarlevo, Saint Petersburg, Russia
(✉)e-mail: valeriter@mail.ru

Data on the genetic variability of genomic DNA from two species of camels (Dromedary and Bactrian) are presented. It is noted that these animal species are of great importance in a number of southern countries, they are used as farm animals (milk, meat, wool), as draft, riding and sports animals. At present, much attention is paid to the study of camels in order to identify their genetic characteristics that can be used in breeding work. One of the research methods is multilocus analysis using labeled oligonucleotide probes. The latter selectively hybridize in separate regions of genomic

DNA, leading to the appearance of specific genetic profiles characteristic of each individual. Labeling the probe with digoxigenin makes it possible to detect the results of hybridization on the filter. After the reaction of molecular hybridization of the probe with genomic DNA of camels, from 3 to 15 DNA fragments were detected, while the pattern of hybridization was very different in Dromedaries and Bactrians, which indicates a significant genetic difference in the organization of genomes. The coefficient of similarity of individuals in Bactrians was significantly higher than in Dromedaries (0.48 versus 0.39); interspecific similarity coefficient in this parameter was only 0.13. The calculation of the genetic distance between populations gave a rather high value of 0.305, which is significantly higher than the previously obtained data on cattle (from 0.05 to 0.10). Intrapopulation genetic diversity was assessed by the criterion of average heterozygosity. Calculations showed greater genetic diversity in the dromedary population (H = 0.72), which was indirectly confirmed by a lower similarity coefficient in this group of animals.

Keywords: Bactrian camel, Dromedary camel, genetic diversity, oligonucleotide probe

Для цитирования: *Тыщенко В.И., Терлецкий В.П.* Оценка генетических различий у животных на примере представителей рода *Camelus* // Сибирский вестник сельскохозяйственной науки. 2023. Т. 53. № 6. С. 92–97. https://doi.org/10.26898/0370-8799-2023-6-11

For citation: Tyshchenko V.I., Terletskiy V.P. Assessment of genetic differences in animals as exemplified by representatives of the genus *Camelus*. *Sibirskii vestnik sel'skokhozyaistvennoi nauki = Siberian Herald of Agricultural Science*, 2023, vol. 53, no. 6, pp. 92–97. https://doi.org/10.26898/0370-8799-2023-6-11

Конфликт интересов
Авторы заявляют об отсутствии конфликта интересов.
Conflict of interest
The authors declare no conflict of interest.

Благодарность
Работа выполнено в рамках государственного задания (тема № 0445-2021-0010). Авторы выражают благодарность сотрудникам кафедры акушерства, хирургии и биотехнологии воспроизводства животных Казахского национального аграрного исследовательского университета (Республика Казахстан), в особенности заведующему кафедрой Е.С. Усенбекову, за предоставление биоматериала животных.
Acknowledgements
The work was accomplished with the support of the state task 0445-2021-0010. The authors express their gratitude to the staff of the Department of Obstetrics, Surgery and Biotechnology of Animal Reproduction of KazNARU (Republic of Kazakhstan), in particular to the Head of the Department Ussenbekov E.S., for taking biomaterial from animals.

INTRODUCTION

Currently, it is believed that there are three types of camels in nature - the single-humped (Dromedary), the double-humped (Bactrian), and the wild camel. The first two types are widely used in many southern countries, especially in Arab nations, for agricultural production (milk, meat, wool) and have significant social importance for the population [1, 2]. Shubat, a fermented milk beverage with many valuable properties, is made from camel milk. Unlike kumis, shubat is thicker and has a white color. Despite their scant diet, camels' milk productivity can reach 2000 liters per season [3]. Global interest in camel milk and its products is growing[1].

In modern animal husbandry, the achievements of molecular genetics are actively applied. Genomic selection, in particular, has been adopted in many countries. To date, phenotypic breed standards for camels have yet to be established [4]. This fact underscores the importance of implementing genetic approaches in studying these animals to lay the groundwork for further genomic selection. Research is being conducted on the influence of polymorphic variants of individual genes on various economically useful traits for application in breeding. Such genes include kappa-casein, diacylglycerol acyltransferase 1 (DGAT1), lactoglobulin, myostatin, etc[2]. For example, it has been established that

[1] *Rahman N., Xiaohong C., Meiqin F., Mingsheng D.* Characterization of the dominant microflora in naturally fermented camel milk shubat // World Journal of Microbiology and Biotechnology. 2009. Vol. 25. P. 1941–1946.

[2] *Pauciullo A., Giambra I.J., Iannuzzi L., Erhardt G.* The β-casein in camels: molecular characterization of the CSN2 gene, promoter analysis and genetic variability // Gene. 2014. Vol. 547. N 1. P. 159–168.

the *CSN2* gene of kappa-casein in camels is the most polymorphic in the entire family of casein genes, having 91 variants [5]. In some cases, associations between genetic polymorphism in individual genes and economically useful traits are identified. Such works exist for variants of the kappa-casein gene and the *FGF5* gene, associated with the formation of hair length in camels. A single missense mutation (C > T substitution) led to a statistically significant change in hair length [6].

Special attention in animal husbandry is given to studying population genetic parameters to refine the history of breed creation and populations, reconstruct extinct breeds, determine the direction of current breeding work, and genetic diversity in populations for use in genome conservation programs [7, 8]. In some instances, clear genetic distinctiveness of camel populations is found depending on the country of breeding. As noted by M.A. Homas et al. [9], a multilocus approach revealed the differentiation of camel populations in Saudi Arabia compared to animals from other countries.

Genomic DNA is studied by various methods, including sequencing (whole-genome or specific regions) [10], using polymorphism in microsatellite DNA[3] [11], and chip technologies for screening the genome at many loci simultaneously (SNPs). DNA level polymorphism is well-studied, as revealed by point mutations in various genes. Much more useful for studying DNA sequence polymorphism at the population level are hypervariable regions of the genome, characterized by the presence of different allelic variants (high frequency of occurrence) in different individuals in the population and a significant mutation rate (see footnote 3).

Studies are conducted on polymorphism in mitochondrial DNA. In the Indian camel population, a high level of diversity of mitochondrial genome regions was found, exceeding the indicators of other populations [12].

The existence of different camel species raised questions about their genetic closeness. This issue can be resolved by genetic methods. It is known that the two-humped camel (Bactrian) and one-humped camel (Dromedary) are classified as different species, despite their ability to interbreed. Therefore, some researchers consider them representatives of one species but different breeds.

The purpose of the research is a comparative assessment of the genetic diversity of two camel species.

The tasks are:

1) collection of the biomaterial (blood) from camels of both species;

2) extraction of high-molecular-weight genomic DNA from available samples;

3) conducting a multilocus genetic analysis to calculate the basic population genetic parameters characterizing the experimental animal samples;

4) evaluation of the obtained results.

MATERIAL AND METHODS

The objects of the study are single-humped and double-humped camels (18 individuals in each group), bred at the "Daulet-Beket" farm, located in the Ilisky district of the Almaty region of the Republic of Kazakhstan. DNA was extracted from the venous blood of animals using standard methods, including the precipitation of the leukocyte fraction, cell lysis with detergent (sodium dodecyl sulfate), and phenolic deproteinization. DNA precipitation was performed with ethanol. The precipitate was washed again in 70% ethanol, dried, and dissolved in 400 µl of TE buffer (10 mM Tris + 1 mM EDTA, pH 8.0). The quantity and quality of DNA were assessed using a NanoDrop2000 spectrophotometer.

A labeled oligonucleotide (GTG)5 containing a digoxigenin mark was used as a molecular probe. Genomic DNA was cleaved with *Hae*III restriction endonuclease, electrophoresis was performed in tris-acetate buffer, and DNA fragments separated by size were transferred to a nylon filter. After fixing the DNA on the filter, it was placed in a tray for molecular hybridization. The DNA probe complementarily bound to corresponding sections of genomic DNA on

[3]*Kiseleva T.Yu., Kantanen J., Vorobyev N.I., Podoba B.E., Terletsky V.P.* Imbalance in the linkage disequilibrium of microsatellite loci in six local populations of cattle // Genetics, 2014, Vol. 50, No. 4, pp. 406-414.

the filter. After washing off the unincorporated label, a solution for immunohistochemical detection of digoxigenin was added to the tray. Developed DNA fragments with a label appearing as dark bands were visualized, and the number of common and differing bands (pairwise on all electrophoretic tracks) was counted. Population genetic parameters (heterozygosity, genetic distance, allele frequencies) were calculated using the GelStats computer program.

RESULTS AND DISCUSSION

During the experiment, after the molecular hybridization reaction, 5 to 12 DNA fragments were detected on the filter, the number and distribution of which are characteristic of each individual (see the figure). Tracks 2–11 and 13–20 are the results of analyzing Dromedary camels; 22–31 and 33–40 are Bactrian camels. On tracks 1, 12, 21, 32, and 41, a DNA fragment length marker is shown. The range of the marker DNA fragments lengths ranged from 500 to 23,000 base pairs of the DNA. It was found that a significantly larger number of DNA fragments were identified in the Bactrian group.

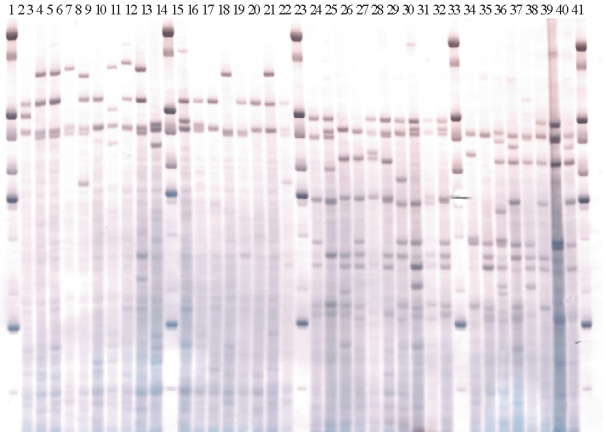
Pairwise comparison of the number of common DNA fragments between populations showed an extremely low value of the interpopulation similarity coefficient (0.13), while the intrapopulation similarity coefficient reached 0.39 for Dromedaries and 0.48 for Bactrians (see Table 1). The calculated genetic distance between the populations gave a rather high value – 0.305, which is much higher than the previously obtained indicators when comparing different breeds of cattle.

Табл. 1. Популяционно-генетические параметры исследуемых популяций верблюдов по данным ДНК-фингерпринтинга

Table 1. Population and genetic parameters of the studied camel populations according to DNA fingerprinting data

Type	*n*	Number of bands per one lane ($X \pm m$)	P	BS[1]	BS[2]	D
Dromedary camel	18	3,44 ± 0,27	$3{,}81\times10^{-2}$	0,39		
Bactrian camel	18	7,61 ± 0,34	$3{,}55\times10^{-3}$	0,48	0,13	0,305

Note. P - probability of occurrence of an identical set of all DNA fragments in the compared pairs of individuals; BS1 - intrapopulation similarity coefficient; BS2 - interpopulation similarity coefficient; D - genetic distance between populations.



ДНК-фингерпринтинг геномной ДНК верблюдов обеих групп

DNA fingerprinting of the genomic DNA of camels of two groups

The calculation of average heterozygosity showed greater genetic diversity in the Dromedary population (H = 0.72), which was indirectly confirmed by the low value of the similarity coefficient in this group of animals (see Table 2). Bactrians were characterized by greater homogeneity according to genetic parameters.

CONCLUSION

Thus, the obtained data show that the two compared camel populations are characterized by significant genetic differences. Single-humped camels have greater diversity by genetic criteria within their population. As we can see, DNA fingerprinting with a labeled DNA probe can be used to assess genetic diversity in camels.

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Табл. 2. Характеристика внутрипопуляционного генетического разнообразия верблюдов

Table 2. Characteristics of intra-population genetic diversity in camels

Type	*n*	Number of loci	Number of alleles	Number of polymorphic loci	Average heterozygosity
Dromedary camel	18	2,01	9,46	1,00	0,72
Bactrian camel	18	4,55	5,49	1,00	0,67

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Тыщенко В.И., кандидат биологических наук, старший научный сотрудник

(✉)**Терлецкий В.П.,** доктор биологических наук, главный научный сотрудник; **адрес для переписки:** Россия, 196625, г. Санкт-Петербург, п. Тярлево, Московское шоссе, 55-а; e-mail: valeriter@mail.ru

AUTHOR INFORMATION

Valentina I. Tyshchenko, Candidate of Science in Biology, Senior Researcher

(✉)**Valery P. Terletskiy,** Doctor of Science in Biology, Senior Researcher; **address:** 55-a, Moskovskoe shosse, St. Petersburg, Tyarlevo, 196625, Russia; e-mail: valeriter@mail.ru

Дата поступления статьи / Received by the editors 28.02.2023
Дата принятия к публикации / Accepted for publication 31.03.2023
Дата публикации / Published 20.07.2023