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of the Institute of Plant Biology and Biotechnology

БИОЛОГИЯ ЖӘНЕ МЕДИЦИНА СЕРИЯСЫ

♦

СЕРИЯ БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ

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MOLECULAR-GENETIC MONITORING OF CAMELS OF ARVANA BREED OF ARYS-TURKESTAN POPULATION WITH THE USAGE OF DNA-TECHNOLOGY

Abstract. In this article identification and certification questions of high dairy effective genotypes of camels of Arvana breed of Arys-Turkestan population with the usage of DNA-technology are considered. Performed molecular-genetic analysis has shown, that population of camels has subdivision on breed parameter. Each subpopulation of camels of Arvana breed has a distinctive gene pool for the given population. The obtained data can be used at development of actions for preservation of their unique gene pools well adapted for local conditions. In the Arys-Turkestan zone of camel breeding («Syzdybekov A», «Usenov N» farms) are identified and passported high dairy effective genotypes of Arvana camels, with the use of DNA-technology in number of 200 heads of animals. Interpopulation distinctions between populations of camels on 7 microsatellite loci have been received on population «Usenov N» an average alleles – 6.28, heterozygosity-0.68, inbreeding – 0.0096, feature of the given population in 2 loci presence of private alleles, as is distinctive line of the given population. Distinctive feature of population «Syzdybekov A.» is presence in 3 loci of private alleles. On the given population an average quantity of alleles – 6.43, heterozygosity-0.70, inbreeding – 0.0077.

Key words: camels, DNA, primers, loci, alleles, STR-polymorphism, genetic monitoring.

Introduction. The major problem of genetic researches in animal industries is perfection of methods of genotypic estimation of animals essentially influencing on productivity of selection process. Studying of polymorphic systems of blood of animals has provided zooengineering science with a quality monitoring of an origin, an estimation of genetic features of breeds, herds and lines, definitions of level of genetic similarity between them and forecasting of heterosis effect [1].

Modern achievements in the field of molecular genetics, successes in decoding of genomes of many animals and plants, including camels (2014), have essentially expanded a base of marker-auxiliary selection and have caused an actuality of strategy and tactics development of genetic monitoring in animal industries taking into account specificity of each subindustry. Now many selection programs on improvement of breeds of animals are based on use of genetic markers that opens real possibilities for monitoring of genealogical structure, preservation of an optimum level of a genetic variety, selection of animals with the a glance of their genotypic estimations. For improvement of quality of production of animal industries of Kazakhstan and its integration on the world market application of the advanced selection-genetic methods which allow to create new highly productive breeds, types and lines of the animals adapted for this or that zone of cultivation is required. For efficiency of selection process, selection of highly productive individuals should be carried out with observance of strict genetic monitoring. In breeding animal industries, including camel breeding, the method of the genetic control of an origin of agricultural animals is very important, and genetic certification of significant genotypes is an obligatory element of zootechnical control in breeding economy. Identification of allele pool of domestic breeds of camels of a dairy direction earlier in Kazakhstan was not carried out. Thereupon genetic

researches for carrying out of optimization of structure, identification and certification of valuable genotypes, and also ordering of genetic resources in dairy camel breeding are actual.

Scientific novelty of research consists in studying and detection of the selection importance of domestic breeds of camels with use of DNA-technologies in which research of biological tests (noninvasive procedure) gives possibility of an estimation of animals at a birth, being based on 7 microsatellites of DNA. Genetic profiles of camels will be constructed and genetic value of certain loci and their alleles variants is identified.

The practical importance of work consists in DNA-analysis carrying out (in laboratory conditions of institute) for definition of breeding value of animals from economy of various categories. Results of research will allow to isolate perspective animals with certain genetic markers to use them in the further selective-breeding work for formation of highly productive herd on dairy efficiency. Created electronic genetic database on the basis of optimization of structure, identification of animals and ordering of genetic resources will be promoted to acceleration of selective-breeding work. Certification of the researched breeding animals gives the objective characteristic of each individual on genetic parametres.

The work purpose is identification of possible polymorphisms of 7 STR-markers at population of camels, using a polimerase-chain reaction method (PCR) and on this basis identification and certification of high dairy effective camels of Arvana breed of Arys-Turkestan population.

For object in view performance of following problems were solved: to lead selection of biomaterials from animals of the Arys-Turkestan zone of camel breeding, to carry out the primary molecular-genetic multiplex analysis of the received biomaterials of camels of a dairy direction on 7 loci of microsatellites, to establish genetic profiles of camels of a dairy direction, to carry out complex analysis of investigated populations of camels of a dairy direction by modern methods of population genetics, to carry out action on pawning for a long-term storage of isolated DNA of camels of a dairy direction, to develop an electronic genetic database and on its basis to carry out ordering of the received population-genetic data.

The estimation of a spectrum of a genetic variety of breeds demands studying whenever possible the big number of the isolated populations in various ecological-climatic zones. The existing contribution to a genetic variety of breeds is brought by the regional populations which gene pool, as a rule, was formed in the conditions of relative isolation and on the basis of local cattle - the carrier of own unique allele pool. Besides, on allele profiles of breeds used in an agricultural production, considerable influence render plans of selection-breeding work [2].

In cattle breeding for today the following questions are actual: studying of genetic features of breed, their phylogenesis and breed forming, similarities and distinctions, revealing of genetic anomalies at animals, their origins.

Studying of genetic structure of artificial formed populations in animal industries represents the big theoretical interest, and also allows to formulate the proved recommendations for perfection of existing breeds and breeding work with them, corresponding to actual requirements of economic practice [3].

In the characteristic of allele pool breeds and populations of agricultural animals find application genetic markers of different types. At camels, the greatest distribution have received two types - erythrocytic antigenes of blood groups and microsatellites. Genetic markers allow to judge degree of heterozygosity of animals, degree of consolidation of hereditary qualities of breeds, types, lines, about genetic distinctions between them.

For effective selection on increase of quantity of protein in milk modern genetic and biotechnological methods, in particular the molecular-genetic analysis are used, allowing to reveal polymorphism of genes of proteins of milk. It is established positive connection of a genotype and haplotype on autosomal loci with protein-dairy properties of milk of camel female [4].

Sequencing of camels' genome allows to identify genes or genomic areas of loci of quantitative signs (QTLs), which are important at selection on reproductive, feeding and meat-and-milk qualities. The mutations forming new versions of alleles, spending the basis of one gene, lead to formation of new sequence of amino acids in protein (kappa-casein). All listed phenomena unite in concept gene or point mutations. They can be harmful (BLAD, CVM), useful (kappa-casein) or neutral (blood groups, microsatellites).

Introduction of method of polymerase chain reaction (PCR) in laboratory practice became one of the most important events in clinical laboratory diagnostics in last decades. PCR method lifts diagnostics on

essentially new level - level of definition of DNA or RNA that allows carrying out straight detection of the infectious agent or a genetic mutation.

In the developed countries PCR method is used both in medicine, and in agriculture. In our republic PCR method is applied in medical institutions, and in animal industries, particularly, in cattle breeding, it is applied boundedly.

Genetic typing at DNA level can be used at diagnostics of hereditary diseases, such as the combined immunodeficiency (SCID - severe combined immunodeficiency disease), a periodic paralysis of horses (HYPP, hyperkaliemic periodic paralysis) and others.

The question, which is closely connected with identification and the control of an origin revealing of carriers of the genetic defects causing display of hereditary diseases at camels. Now the laboratory can offer identification of some of the most widespread hereditary diseases at camels. Search work on revealing of markers for revealing of genetic defects, characteristic for camels of domestic livestock is conducted also.

Monitoring of immunogenotypic indicators at animals is necessary for realization of genetic potential and reception of healthy posterity. In this connection monitoring of the maintenance of the basic classes of antibodies at camels promotes maintenance of immunity and as consequence, to preservation of health of animals and reception of viable posterity.

The international society on studying of genetics of animals offers panels of loci of microsatellites for principal views of the agricultural, domestic and cultivated animals in which are included the most informative loci used at the control of reliability of an origin. Thus, microsatellite profiles can be used as criteria of an estimation of genuineness of strain of camels [5].

Earlier scale works on identification, ordering and certification of genetic resources of domestic breeds of camels in Kazakhstan were not carried out.

Methods. Objects of researches were served biosamples (histologic tests) of camels of Turkmen Arabian Arvana breed.

The method of DNA-researches was applied on the basis of institute laboratory in department of genetics of agricultural animals. As a material biological materials of animals from two base economy (LLP «Usenov N», LLP «Syzdybekov A») are used. For DNA isolation there have been used commercial sets of leading firms of manufacturers: GenePak PCR Core; Qiagen; Lithex; DNA-technology; Diatom DNA; ExtraGene DNA Prep.

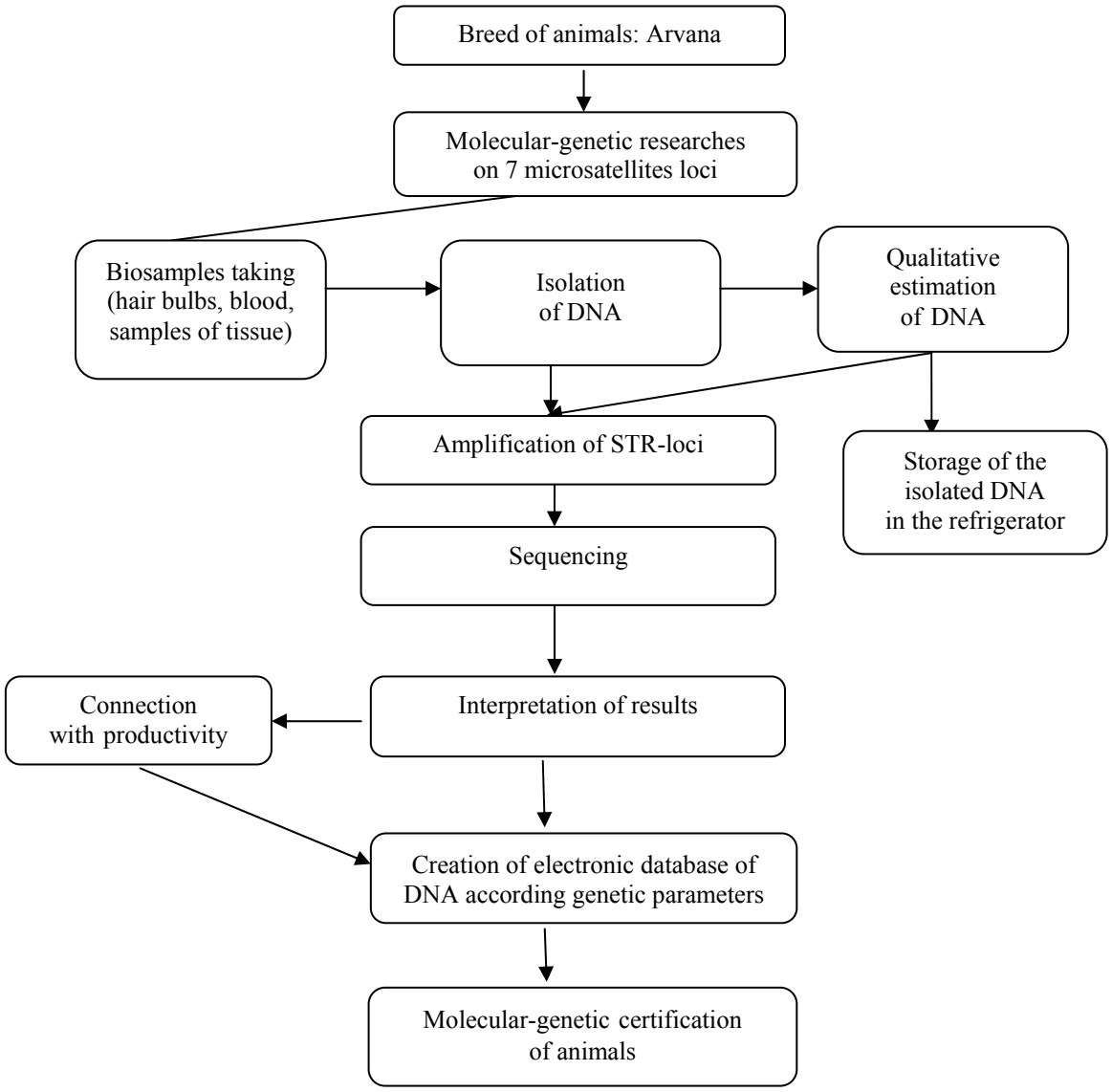
For the purpose of studying of interbreeding differentiation the genetic analysis of typing results of 200 heads of animals of domestic Arvana breed has been carried out on 7 loci of microsatellites of DNA according to the research scheme (figure).

As material for researches the samples of DNA isolated from histologic tests with use of sets Diatom DNA and ElxtraGene DNA Prep served. The isolated samples of DNA were amplificated on amplifier 2720 Thermal Cycler, on the basis of a set of primers of StockMarks firm. All works on isolation, amplifications and sequencing have been carried out according to reports of the manufacturer, adapted to the concrete complete of reagents.

Polymorphism of microsatellites of DNA defined with the help of sequenator ABI-310. The panel of typing of DNA consists of 7 microsatellites, characteristic for carrying out of genetic examination of an origin of camels. Interpretation of graphic profiles of results of samples genetic typing and definition of genotypes of camels were carried out with the recommendation of the International institute of camel breeding (International Camelid Institute) and the International society of genetics of animals (International Society for Animal Genetics) [6].

Genetic-populational analysis taking into account frequencies of occurrence of alleles of microsatellite loci, level of multiformity and heterozygosity degrees, was carried out by the standard techniques. Biometric calculations were carries out according to Statistical calculations carried out with use of a statistical package and own development with use of an algorithmic language of programming Fortran PowerStation. Databases are developed with use of package Microsoft Office Access 2007.

The final stage of amplified fragments analysis is identification of alleles and an establishment of genetic profiles of investigated samples of DNA [7]. The established genetic profile of an animal is a basis for registration of the genetic report of testing in which are specified revealed allele variants on each investigated locus.



Scheme of researches

Table 1 – Specific loci for camels genetic typing

#	Loci	Length	Primer (5'-3') straight	Primer (5'-3') reverse
11	LCA8	211-261	GCTGAACCACAATGCAAAGA	AATGCAGATGTGCCTCAGTT
22	LCA37	124-174	AAACCTAATTACCTCCCCCA	CCATGTAGTTGCAGGACACG
33	LCA56	133-171	ATGGTGTTTACAGGGCGTTG	GCATTACTGAAAAGCCCAGG
44	LCA65	159-193	TTTTTCCCCTGTGGTTGAAT	AACTCAGCTGTTGTCAGGGG
55	LCA66	216-266	GTGCAGCGTCCAAATAGTCA	CCAGCATCGTCCAGTATTCA
66	YWLL29	210-232	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG
77	YWLL44	84-136	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA

Results. Researches were carries out on biomaterials (histologic materials) animals from two base economy (LLP «Usenov N», LLP «Syzdybekov A»). The size of sample was 102 heads (2 bores-male, 50 female camels and 50 colts) of each economy.

The carried out researches of studied sample of camels have revealed presence of 47 alleles 7 loci of microsatellites, that on the average on a locus has made accordingly 6.71 for Arvana breed [8].

In microsatellite loci at studied types of animals identified 5 private alleles. Number of effective alleles (they bring the greatest contribution to calculation of degree of heterozygosity) were in all loci of microsatellites that specifies in more uniform distribution of alleles of microsatellites in population. The analysis of microsatellite profiles has allowed to differentiate animals of the given breed correctly.

Table 2 – Revealed allele variants at population of camels of Arvana breed. By a fat font private alleles are allocated

Loci	Number of alleles	Name of the alleles
LCA8	9	213, 215, **217**, 219, 227, 229, 239, 241, 243
LCA37	7	128, 134, 148, 152, 158, 160, 162
LCA56	6	139, 141, 147, 151, 153, 155
LCA65	6	161, 163, 165, 171, 173, **175**
LCA66	7	218, 220, **222**, 228, 230, 240, **242**
YWLL29	4	216, 220, 222, 226
YWLL44	8	96, 98, 108, 110, 114, 116, 120, **122**
Average number of alleles	6.71	Observed heterozygosity – 0.69, expected – 0.67, size of casual inbreeding – 0.0083

Advantage of microsatellites at detection of a pedigree belonging is caused by that first, gives in to a differentiation polymorphism level, and secondly is possible classification on private alleles.

Use of homogeneous selection is directed on fastening of signs of efficiency of parents in posterity. On the basis of it it is possible to draw a conclusion, that microsatellite profiles can be used as criteria of an estimation of degree of heterogeneity of selection of parental pairs at thoroughbred cultivation. [9]

It is necessary to notice, that the revealed laws should be extended with care to camels of other breeds, types or even herds. The data cited in the present work though are scientifically proved and experimentally proved, demand acknowledgement within the limits of more scale researches both in pedigree, and in population aspect. [10]

We also result of the analysis of a genetic variety of Arvana breed camels with application as criteria of an indicator of heterozygosity and sizes of casual inbreeding for two populations (see tables 3, 4).

Table 3 – Revealed allele variants and indicators of a genetic variety at camels of Arvana breed (population LLP «Usenov N»). By a fat font private alleles are allocated

Loci	Number of alleles	Name of the alleles
LCA8	8	213, 215, 219, 227, 229, 239, 241, 243
LCA37	7	128, 134, 148, 152, 158, 160, 162
LCA56	6	139, 141, 147, 151, 153, 155
LCA65	5	161, 163, 165, 171, 173
LCA66	6	218, 220, **222**, 228, 230, 240
YWLL29	4	216, 220, 222, 226
YWLL44	8	96, 98, 108, 110, 114, 116, 120, **122**
Average number of alleles	6.28	Observed heterozygosity – 0.68, expected – 0.72, indicator of casual inbreeding – 0.0096

Table 4 – Revealed allele variants indicators of genetic variety at camels of Arvana breed (population of LLP «Syzdybekov A»). By a fat font private alleles are allocated

Loci	Number of alleles	Name of the alleles
LCA8	9	213, 215, **217**, 219, 227, 229, 239, 241, 243
LCA37	7	128, 134, 148, 152, 158, 160, 162
LCA56	6	139, 141, 147, 151, 153, 155
LCA65	6	161, 163, 165, 171,173, **175**
LCA66	6	218, 220, 228, 230, 240, **242**
YWLL29	4	216, 220, 222,226
YWLL44	7	96, 98, 108, 110, 114, 116, 120
Average number of alleles	6.43	Observed heterozygosity – 0.70, expected – 0.71, indicator of casual inbreeding – 0.0077

As follows from the above-stated data interbreeding distinctions between populations of camels on 7 microsatellite loci have been received on population «Usenov N» an average alleles – 6.28, heterozygosity-0.68, inbreeding – 0.0096, feature of the given population that at 2 loci present private alleles, as is distinctive line of the given population. Distinctive feature of population «Syzdybekov N» is presence in 3 loci of private alleles. On the given population average alleles – 6.43, heterozygosity-0.70, inbreeding – 0.0077.

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ДНҚ ТЕХНОЛОГИЯЛАРЫН ҚОЛДАНА ОТЫРЫП, АРЫС-ТҮРКІСТАН ПОПУЛЯЦИЯСЫНДА ӨСІРІЛЕТІН АРУАНА ТҰҚЫМДЫ ТҮЙЕЛЕРГЕ МОЛЕКУЛЯРЛЫ-ГЕНЕТИКАЛЫҚ МОНИТОРИНГ ЖҮРГІЗУ

Аннотация. Мақалада ДНҚ-технологияларын қолдана отырып Арыс-Түркістан популяциясында өсірілетін аруана текті асыл тұқымды түйелерді бірегейлендіру және құжаттандыру қарастырылған. Барлық генетикалық және генеалогиляық зерттеу жергілікті шаруашылықтарда жүргізілді. Түйе малдарының зерттелген тұқымдарында толық айырмашылық табылды. Әрбір тұқымда өздеріне тән аллелдер табылды. Арыс-Түркістан аймағында өсіріліп жатқан (ш/қ «Сыздықбеков А», ш/қ «Үсенов Н») түйелерден асыл тұқымды мал тобын құру үшін, сүт өнімділігі жоғары генотипті малдарды ДНК-технологиясын қолдана отырып, 200 бас түйелерді бірегейлендіру және құжаттандыру. Әртүрлі мал тобындағы түйелердің айырмашылығы

«Үсенов Н» ш/қ малдарда аллельдердің орташа саны - 6,28, гетерозиготалығы - 0,68, туыстығы - 0,096. Бұл мал тобының ерекшелігі 2 локуста өзіне тән аллельдер кездесті. «Сыздықбеков А» ш/ш мал тобының айрықша ерекшелігі 3 локуста өзіне тән аллельдер кездесті. Бұл мал тобының орташа аллельдер саны - 6,43, гетерозиготалығы - 0,70, туыстығы- 0,0077.

Түйін сөздер: түйе, ДНҚ, праймерлер, локус, аллельдер, STR-полиморфизм, генетикалық мониторинг.

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МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ МОНИТОРИНГ ВЕРБЛЮДОВ ПОРОДЫ АРВАНА АРЫСЬ-ТУРКЕСТАНСКОЙ ПОПУЛЯЦИИ С ИСПОЛЬЗОВАНИЕМ ДНК-ТЕХНОЛОГИИ

Аннотация. В статье рассмотрены вопросы идентификации и паспортизации высокомолочных генотипов верблюдов породы арвана арысь-туркестанской популяции с использованием ДНК-технологии. Проведенный молекулярно-генетический анализ показал, что популяция верблюдов имеет подразделенность по параметру породы. Каждая субпопуляция верблюдов породы арвана имеет отличительный для данной популяции генофонд. Полученные данные могут быть использованы при разработке мероприятий по сохранению их уникальных генофондов, хорошо адаптированных к местным условиям. В Арысь-Туркестанской зоне верблюдоводства (КХ «Сыздыкбеков А.», КХ «Усенов Н.») идентифицированы и паспортизированы высокомолочные генотипы верблюдов арвана с использованием ДНК-технологии в количестве 200 голов животных. Межпопуляционные различия между популяциями верблюдов по 7 микросателлитным локусам были получены по популяции «Усенов Н» среднее число аллелей - 6,28, гетерозиготность - 0,68, инбридинг - 0,0096, особенность данной популяции в 2-х локусах присутствие приватных аллелей, что и является отличительной чертой данной популяции. Отличительной особенностью популяции «Сыздыкбеков А» является наличие в 3 локусах приватных аллелей. По данной популяции среднее число аллелей - 6,43, гетерозиготность - 0,70, инбридинг - 0,0077.

Ключевые слова: верблюды, ДНК, праймеры, локусы, аллели, STR-полиморфизм, генетический мониторинг.

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