GENETIC ANALYSIS AND HEREDITARY TRANSMISSION OF COLOR AT KARAKUL DE BOTOSANI SHEEP

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Abstract

The Botosani Karakul breed was created for the skin production. In this case, the improvement program aims at specific objectives for this production, and the color diversification is perhaps the most important objective of genetic improvement. In order to support the farmers in this research, important aspects associated with the improvement activity were followed. According to this conception, the research objectives referred to the genetic analysis and establishing the interval between generations, as well as the genetic transmission of colors takes place by crossing different parents as a variety of color. In order to obtain real data, the way colors are transmitted was monitored for eight successive generations of lambs resulting from the direct crossing of black individuals selected for this purpose. The analysis of the final data indicates that out of 3.403 descendants, 98.15% had black color at birth, with variations per generation from a minimum of 97.46% to a maximum of 98.78%. Following the analysis of the color transmission in the Botosani Karakul breed, it was found that the proportion of lambs that had a different color was 1.85%, of which the unwanted types represented by marks, was only 0.30%. There were lambs that had some differently pigmented areas on the body which was possible due to the activation of genes from other locus, being determined by the R0-4 and r0-4 polyallelic series, and the rr genotype is responsible for their absence. The appearance of white spots on the chest and head is due to the fact that the genotype of Botosani Karakul breed, just like the Asian Karakul, contains the Q gene while the other sheep breeds have the qq genotype.

Key words: *pelts, color heredity, Botosani Karakul Seep, genetic improvement*

INTRODUCTION

The Karakul sheep breed is spread in Rusia, Afghanistan, Iran, Namibia, South Africa, and Romania (Nel, 1991). The skins are obtained from other breeds as black and gray Turcana (Pascal, 2015), Moldavian Karakul in Republic of Moldova (Buzu, 2011), Gotland in Suedia (Näsholm, 2005, Näsholm, 2008), the islandic sheep (Eythorsdottir, 1999) and gray Shiraz in Zandi, Iran (Nanekarani et al., 2010), Romanov in ex-URSS, and Wrzosowska in Polonia (Flamant et al., 1982) are raised for high quality skins and milk. Also, Karakul sheep are available and in all Central Asian countries, except Kyrgyzstan and show a tremendous diversity which is the result of an well organized selection process during the Soviet period, in particular in Uzbekistan (Iñiguez et al.2008).

In Romania, sheep rearing for pelts production have a history of more of one century. The first data which confirm this aspect was published by Ion Ionescu de la Brad who affirmed that "*Siad Pascha brought sheep with wide tail in 1850 and quartered them in Dobrogea"* area situated in the South-Eastern part of Romania at the Black Sea shore. Other data which confirms this aspect is included in the narrations of Teodoreanu (Ursu. et al., 1991), through which is highlighted that first sheep for pelts were introduced, between 1882 and 1902, by the breeders from Botosani area (MAIA), which is situated in the North-East part of Romania, being also the birthplace of Botosani Karakul breed.

After year 1930, more studies and research had taken place which aimed the rearing of Karakul breed and the quality of pelts obtained (Buzu, 2016, p 44) however, for the first time, a hereditary analysis on a pure breed Karakul ram were done by Cardas and Nica (cited by Buzu, 2016). They studied the way in which are transmitted in off springs the characters

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which influence the quality of fibres, curls and looping.

The breed recorded a significant increase until 1989 because the pelts were requested on the domestic market, as well as on the international one, being capitalized mainly in Russia and in other countries which formed the CAER market (Manole et al., 2006). In 1989 the breed represented more than 750 thousand heads, after 1990 under the influences which took place in Romanian agriculture and by changing of capital property forms, the breed flock gradually decreased. In according with National Agency for Animal Husbandry, nowadays the Botosani Karakul breed represents 2.52% form the breed structure in Romania, representing around 220 thousand heads.

In order to reach the current level of improvement of the production characters, to the consolidation of the body conformation and constitution but also to the diversification of the colors, strict measures of selection and management of the reproduction activity were applied (Pascal, 2015). To evaluate the stage of breeding but also to check out the way of transmission of characters on which it depends the quality of Karakul de Botosani pelts, in Romania, were effectuated many research which were conducted in different period of time (Contescu et al., 1938; Nica, 1929; Niga, 1977; Pascal et al., 1994; Pascal, 2001; Pascal, 2011; Nechifor et al., 2014; Nechifor et al., 2015; Nechifor, 2017).

The main purpose of this article is to carry out an analysis regarding the genetic transmission of colors in the Botosani Karakul breed in order to identify new methods of their diversification.

MATERIAL AND METHODS

Data and pedigree information were obtained from the Breeding Station of Karakul sheep located in Botosani county, from the Nort-Eastern part of Romania. Biological material based on which was studied the color transmission was represented by black adult ewes and reproduction rams belonging to Botosani Karakul breed.

In each reproduction cycle pairs matching was realized based on the data obtained from studying of genealogical files and evaluation of transmission way for colors took place during seven lambing seasons conducted between 2009 and 2015 period. In this entire interval, the live flock utilised for reproduction was represented by a total number of 2722 adult individuals from which 38 were rams and 2684 adult ewes, and the total number of obtained lambs was 3403 individuals.

To determine the current status of breed were utilised identity data from genealogical herd book were utilized. In genetic analysis and for interpretation of color transmission way were utilized mathematical techniques which give solutions for prediction of frequency for some genetic constitutions specific to descendants. Statistical methods are used in quantitative and qualitative genetics also when we want to determine the signification of deviations from the expected results of experimental analysis.

The Statistical Package for the Social Sciences model was used for data processing because statistical analysis with SPSS allows statistical data to be treated without requiring knowledge of calculation formulas, combining statistical processing possibilities with the facilities offered by spreadsheet programs (Excel, Lotus, Quattro Pro) for condensing data into tables and their graphic representation. The research activities were performed in accordance with the European Union's Directive for animal experimentation (Directive 2010/63/EU).

RESULTS AND DISCUSSIONS

Genetic analyses of Botosani Karakul sheep breed

Until now the study of coat color inheritance in sheep proved the dominance of white color over pigmented/black coat or skin and of black over brown. Due to the current knowledge of the molecular basis of ovine coat color inheritance, there is no molecular test to distinguish coat color types in sheep although some are available for other species, such as cattle, dogs, and horses. Understanding the genetic background of variation in one of the most important phenotypic traits in livestock would help to identify new genes which have a great effect on the coat color type. Considering that coat color variation is a crucial trait for discriminating between breeds (including sheep), it is important to broaden our knowledge of the genetic background of pigmentation. The results may be used in the future to determine the genetic pattern of a breed. Until now, identified candidate genes that have a significant impact on color type in mammals mainly code for factors located in melanocytes (Koseniuk, et al. 2018**)**. The proposed candidate genes codefor the melanocortin 1 receptor (MC1R), agouti signaling protein (ASIP), tyrosinase-related protein 1 (TYRP1), microphthalmia-associated transcription factor MITF, and v-kit Hardy–Zuckerman 4 feline sarcoma viral onco-gene homologue (KIT). However, there is still no conclusive evidence of established polymorphisms for specific coat color types in sheep (Koseniuk et al. 2018). Color diversity results from the presence and biochemical activity of melanocytes, cells derived from the ectoderm. These cells are specialized in the production of melanin - pigment that protects the organisms against ultraviolet radiation (Solano, 2014). Melanin is divided into eu-melanin (black/brown, pigmented phenotype) and phenolanin (red/yellow, non-pigmented phenotype). In embryonic development, melanoblasts emerge from the neural crest, migrate to the skin over a period of time, and then develop into mature forms of melanocytes (Parichy et al., 2006).

Genetic analyses it is a more and more common method used in breeding as well as in establishing of priorities for conservation of populations which are in risk situations (Ligda et al., 2009). Also, genetic evaluation has the aim to analyze the genetic characteristics which influence the performances of sheep and to estimate genetic parameters (Lalit et al., 2016; Grosu et al., 2005).

The genetic analysis showed that Botosani Karakul breed was genetically composed, as follows: in 1951, from the total genes fund, 44.3% belonged to Turcana breed (European Zackel group), and after increasing of absorption degree the similarity with the local breed decreased in 1998 at only 8.7% (Ursu, 1998).

Interval between generations, calculated during 1950-1990, was of 4.78 years, value which is lower with 0.65 compared to other local sheep breeds. This aspect is explained by the fact that valuable reproducers were kept in activity for a longer period of time, respectively 7-8 years (Ursu, et al. 1994). Determination of interval between generations during 1990-2015 had values of 4.15 years and 4.20 years between mother and daughter and mother – son and respectively 4.25 years and 4.28 years between father and son respectively father – daughter (figure 1). This average reduction of the interval between generations at values lower than 4.3 years show that in the last period of time took place a significant improvement of precocity in the Botosani Karakul breed.

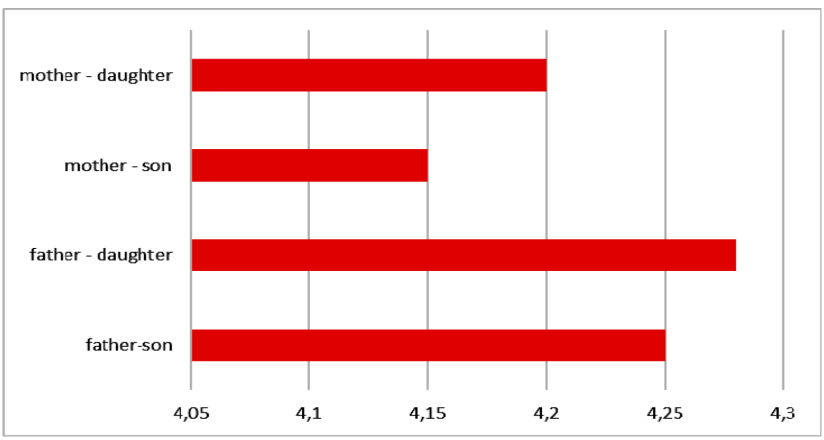

Figure 1. Interval between generations in Botosani Karakul black sheep

To maintain a certain genetic structure, but also for preventing the immigration phenomenon of genes, the import of rams was closely correlated with the reproductive isolation. Untill 1966, population was reproductively opened and presented negative values due to the migration of genes outside population. After that, by increasing of exigencies for quality appreciation and retain of male reproducers, concomitant with utilization at reproduction only of the tested rams in according with their descendance coming from your own breed, was achieved a significant decreasing of genes' migration, aspect which had positive effects on increasing of similarity degree between the individuals from inside population but also on consolidation of characters from which depend the pelts quality.

Hereditary transmission of color in Botosani Karakul black variety

Color, mainly the uniformity of basic nuance, represents a great importance because give to pelts a remarkable esthetical and commercial value even if the curls have less valuable particularities. Nuance is considered, by some researchers (Gingineishvili, 1975), as variation of a color which appear due to morphological structure of pilous cover and characteristics of fibers pigmentation at lambs. Those individuals which have different colors and nuances make possible the differentiation inside breed of some smaller taxonomic categories, respectively of some specialized types which could be used in different mating combinations, function of genetic constant and hereditary transmission (Pascal, 2015). Coat color is determined by amounts and types of melanin produced and released by melanocytes resident in the skin (Ito et al. 2000 and Ito et al. 2008).

The color diversity results from the presence and bio-chemical activity of melanocytes, the cells derived from the ectoderm. These cells are specialized in producing melanin – pigments protecting organisms from ultraviolet radiation (Solano, 2014). Melanin are divided into eu-melanin (black/brown, pigmented phenotype) and pheomelanin (red/yellow, non-pigmented phenotype). In embryonic development, melanoblasts arise from the neural crest, migrate to the skin within a certain time frame, and then develop to mature forms of melanocytes (Parichy et al., 2006). Diversity of sheep colors aroused a major interest, reason for, in many regions of the world, research aiming to discern the genetic transmission way in descendance (Contescu, 1932; Rae, 1956; Warwick et al., 1957; Berge, 1958; Berge1974; Adalsteinsson, 1970; Ryder, 1968, 1980).

For sheep breeds reared for pelts the most elaborated research regarding the in heritage of characters from which depend the quality and color of looping were coordinated by Berge 1957; Nel 1967, Greeff et al. 1993. Regarding strictly the genetic transmission of the studied colors, also the research effectuated by Gingineishvili 1975; Nel 1967; Greeff et al. 1984; Schoeman 1998; Ursu, 1988, showed the fact that this character is dependent by the presence of two gene-types, from which one is a dominant black homozygote and another one is black heterozygote, mentioning that the last one could also appear by crossbreeding of individuals which belongs to other color varieties.

For Botosani Karakul breed the most complex research conducted for knowing the transmission way of colors were initiated after year 1975, being mainly determined by the high request for colored pelts demanded by Eastern European former communist countries. This tendency lead to the approach in profile research of new targets, through which was aimed also the diversification and consolidation of varieties with other colors, respectively brown, white, gray, pink (Niga, 1977). This aspect was possible because was observed that also at Botosani Karakul apparition of colored lambs is produced when ewes heterozygote gene-type for color meats also a heterozygote gene-type for color at in the ram. Research effectuated by Ursu (1998) and Nechifor et al. (2018, 2016, 2015) confirm this aspect because enlightened the fact that apparition of colored lambs is realized when the utilized ram for reproduction was homozygote.

In the case of the current research, to verify the transmission way of colors we analysed the genealogical files to identify heterozygote Karakul black individuals, which in their hereditary substrate have also gene which determine the apparition of other colors (table 1). Those ones were utilized for guided crossings tracking the transmission way of color for eight successive generations of lambs.

Table 1. Distribution of Botosani Karakul black variety breed and percentage for color nuances

Color type	n	%
Black with intense gloss and bluish reflections	1578	57.97
Black with nuance to brown	610	22.40
Standard black	465	17.08
Black with reddish reflections	69	2.53
Total	2722	100

Evaluation of individual genealogical files revealed the fact that at a rate of 57.97% was observed, at the evaluation moment, the specific characters for pelts with basic color being black associated with an intense gloss and bluish reflections. Also, through the fact that in live flock rate of females which had at birth the black color associated with some certain reddish reflections, was of only 2.53% indicate a certain breeding degree for the character represented by color type.

To obtain accurate data, the heritage of colors was tracked during eight successive generations of lambs resulted from guided crossings of individuals with black color specially selected for this purpose. Analysis of final data showed that from the total of 3403 resulted descendants a rate of 98.15% had black color at birth, with variations per generations from a minimum of 97.46% to a maximum of 98.78% obtained in 2015 and respectively in 2014 (table 2).

Table 2. Color transmission in Botosani Karakul black variety

Year	n	Color of descendants											
		Black		Brown		Gray		White		Spotted		Other colors	
		n	%	n	%	n	%	n	%	n	%	n	%
2009	450	440	97.79	4	0.88	2	0.45	1	0.22	2	0.44	1	0.22
2010	517	508	98.25	4	0.77	3	0.58	-	-	1	0.20	1	0.20
2011	455	448	98.46	3	0.66	3	0.66	-	-	-	-	1	0.22
2012	480	473	98.54	1	0.21	3	0.63	-	-	2	0.41	1	0.21
2013	501	490	97.80	4	0.80	2	0.40	-	-	2	0.40	3	0.60
2014	488	482	98.78	4	0.82	-	-	1	0.20	-	-	1	0.20
2015	512	499	97.46	6	1.18	-	-	2	0.39	3	0.58	2	0.39
Total	3403	3340	98.15	26	0.76	13	0.38	4	0.11	10	0.30	10	0.30

In according with the research effectuated by Vasin (1928) cited by Demerec (1956) and by Mochnacs et al. (1978) color transmission at Karakul breed is controlled by a series of epistatic genes, and each of them is represented by two alleles morphs, by which one determine the presence of color and the other one the absence of color. If at other sheep breeds the knowing of color transmission way haven't the same importance, at the ones created and reared for pelts this aspect is extremely important because contribute at color diversification and utilization of pelts with better efficiency in the fashion industry.

All those colors and many others are the result of the interaction of various genes that determine the specifics of the coat color of Karakul sheep. A number of sets of nomenclature and symbols have been used to represent the various loci and their alleles that are involved. In the 1980 and 1990, a standardized set, based closely on those of the mouse and other species was developed (Lundie, 2011). Using this as the framework, the alleles of the Extension, Agouti, Brown, Spotting, Pigmented Head and Roan loci are described using fat-tailed sheep (mainly Damara, Karakul and Persian) as examples. Further discussion includes other types of "white markings", the Ticking locus and the Sur loci. Emergence by mutation of the E+ locus (c.199C>T, p.R67C) will result in obtaining skins with a reddish color or shades of brown (Fontanesi, et al. 2010)

Regarding the genetic determinism of colors, the studies effectuated by Ryder, cited by Sandu (1993) showed that also at sheep are some genes which interfere in formation of a certain pigment (eumelanin for black and pheomelanin for brown color) but also others which are responsible for pigments distribution at the level of the same fibre. Synthesizing possibility for a certain pigment type represent the effect of ***C*** gene, and by the fact that from the gene-type of those breed in missing ***c*** allele result that all sheep breeds must be colored. Moreover, by the fact that all sheep breeds are homozygote also on ***B*** gene (responsible by apparition of black color at all sheep breeds) result that all sheep must have only this color. In according with other results obtained in other research could be observed that on ***B-locus*** are founded two alleles which are responsible for expression of a black type color, with ***B*** dominant which is responsible by apparition of black lambs and ***b*** recessive type producing a dilution of black color to nuances of brown similar with chocolate or moorit (Adalsteinsson, 1980 and 1983, cited by Schoeman, 1988).

However, in sheep could be observed a large scale of colors, consequence of the fact that in their genetic structure could also be found the gene ***E*** with epistatic effect, responsible with distribution of eumelanin pigment and which produce pigment only in the absence of gene ***D*** (Ryder, 1980). The presence of gene ***E*** even when it is in a heterozygote state determine, at Karakul as well as at Turcană, apparition in descendance of black phenotype, dominant type, no matter of gene-type of ***D*** locus (responsible with color dilution), with the condition that on ***F*** locus to be founded the dominant gene which ban the synthesis of pheomelanin pigment.

In the case of analysis of color transmission way at Botosani Karakul breed was observed that the rate of lambs which had another color was 1.85%, from which the unwanted types represented by spotted, were of only 0.30%. In this case, apparition of lambs which had on the body some areas differently pigmented represent the effect of activation of genes placed on other loci, being determined by poly-allelic series R_{0-4} and r_{0-4} and their missing is responsible for gene-type ***rr***. Apparition of white spots on chest and had is due to the fact that in gene-type of Karakul de Botoşani breed, as well as at Asian Karakul, could be founded gene ***Q*** while other sheep breeds had gene-type ***qq***. Also, for apparition of some white spots with irregular shape, disposed on a black background, seems to be responsible gene-type ***ss***. About this aspect Vasin (1928) cited by Mochnacs et al. (1978) release the hypothesis that the presence and the size of some white spots placed on head and tail indicate the homozygote state of parents.

Based on the obtained results could be observed the fact that by apparition in descendance of lambs which had totally different colors from parents result that among black sheep from live flock were some which had a recessive gene which interfere in melanin pigments synthesis. Result that at Botosani Karakul activation of ***allele F*** determine the dominant black color and ***allele f*** determine apparition of brown-maroon color with a dominant type, as well as other European breeds (Ryder, 1980).

So, at analysed breed gene-type *CCBBEEDDFF* and *CCBBE-D-(dd)F-* are responsible for dominant black color, and gene-type *CCBBE-(ee)D-(dd)ff-* for apparition of brown lambs. Presence of gene ***D*** on gene-type ***ee*** dilute the color till white type *CCBBeeD-F-*(*ff*), and absence of genes ***E*** and ***D*** is at the base of obtaining of some brown colors with reddish reflections of the halili type - with gene-type *CCBBE-D-*(*dd*)*ff.*

Also, in the frame of effectuated research was observed the fact that by crossing of black parents resulted in descendance also lambs with a composed color, in the way that fibres had dark nuances at base (from intense black to nuances of light brown) and lighter to the top (from nuances of brown-reddish to yellow similar to wheat spike). This aspect confirms that alleles from ***e locus*** extend or diminish the quantity of eumelanin (black or brown pigment) and have opposite effect on pheomelanin (yellow or red pigment), considerate to be responsible for apparition of fibers which had a similar color with bronze or amber.

In according with Ryder (1980) dominant gene E^d was observed also at other breeds and have the role to control the uniform covering of layer in the presence of any agouti genes. So, at some mammal's breeds genes placed on agouti locus determine apparition of wild type color and dominant mutations have as result the onset of a pleiotropic syndrome which is characterised by excessive quantities of pigment at the level of covering fibres (Berge, 1964 cited by Ryder, 1980; Michaud et al., 1994).

However Rae (1956) cited by Ryder (1980) released the hypothesis that the absence of any ***allele e*** (responsible with yellow spreading) didn't represent a certitude that ***allele E*** really exist in sheep. Based on other research Searle (1968), show that commonly exist the possibility to confuse the series of alleles ***A*** and ***E***, because dominant colors (black and / or brown) appear also at some sheep breeds. This fact strength the idea that apparition of both colors is due to the presence of allele E^d with or without homozygote for brown. Lauvergne et al. (1977) demonstrated the presence of allele E^+ both at wild mouflon as well at domesticated sheep. Black pigmentation is also believed to be induced by dominant extension (ED) alleles, while recessive alleles cause pheomelanin synthesis. It is also noted that the activation of mutated agouti alleles is dependent on the presence of wild-type Extension alleles (E+). Dominant agouti alleles cause pheomelanin phenotypes, while recessive alleles induce black color (Bennett și Lamoreux, 2003). This last is of special interest to breeders since it is impossible to distinguish by eye a sheep with recessive alleles causing black fur color. This is the reason why black lambs are sometimes born in flocks of white sheep (Våge et al., 2003)

Having in view all those information could be concluded that specific gene-type of lambs which had on skin surface fibres with different coloring on their length were of type CCBBE-(ee) D-(dd) ffG (table 3).

Table 3. Genetic explanation of color inheritance the case of crossing between black parents belonging to Botosani Karakul breed

Color of lambs	n	Segregation rate	Genetic explanation	
			Parents	Lambs
Black	3340	0.9815	CCBBEEDDFF x CCBBEEDDFF CCBBE-D-F- x CCBBE-D-F-	CCBBEEDDFF-homozygote type CCBBE-D-(dd)F-heterozygote type
Brown	26	0.0076	CCBBE-DF- x CCBBE-DF-	CCBBE-(ee)D-(dd)ff - brown
Gray	13	0.0038	CCBBE-D-F-G x CCBBE-F-G	CCBBE-(ee)D-(dd)ffG - gray
White	4	0.0011	CCBBE-D-F x CCBBE-D-F	CCBBeeD-F-(ff) - white
Other colors	20	0.0060	CCBBE-D-F x CCBBE-D-F	CCBBE-D-(dd)ff – brown, halili

Based on the obtained results could be observed that transmission of black color at Botosani Karakul is similar with the transmission way found in other breeds. Inheritance results in Botosani Karakul lambs with other colors confirm the fact that in expression of black color (***E locus***) are involved two alleles with totally different effects. One of those alleles had a dominant type (ED) and had a double effect, in the way that determine apparition of black color but have an inhibitor effect on alleles situated on ***A locus***. The second allele coded with ***e*** or sometimes could be even ***E*** is recessive and favor's the activity of all alleles placed on ***A locus***.

GENERAL CONCLUSION

Based on the obtained results was observed that color transmission in the case of crossing of parents from the black variety of Botosani Karakul breed is similar with the transmission way found at other breeds from those morphological and productive type. However, the knowing of color transmission way in sheep from Botosani Karakul breed represents an important genetic information which could be used when we want a diversification of pelts' basic color, with important economic effects which could restart the rearing of Karakul sheep group. However, due to the fact that in the expression of pelts' quality, together with color are many characters which participate in expression of pelts' quality, to make the rearing of Karakul sheep an efficient activity must be done all the efforts for obtaining of pelts with a remarkable quality.

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