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Sheep genetic resources in Bulgaria with focus on breeds with coloured wool

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Abstract: This review describes sheep genetic resources in Bulgaria with coloured wool and evaluates the country's potential for coloured wool production. In 2018, Bulgaria counted 125,422 animals belonging to 18 native sheep breeds, six of which are in danger of extinction. Native sheep breeds in Bulgaria can be divided into three groups depending on the fleece colour of the animals: i) sheep breeds with fully pigmented fleece in all animals; ii) sheep breeds with animals with fully pigmented fleece or fully white fleece; iii) sheep breeds with spotted coloured fleece. Colouration in populations of the native Bulgarian sheep breeds can be explained by the phenotypic expression of several alleles of coat colour genes: *Extension*^{D}, *Agouti*^{a} and *Pigmented head*^{T}, while white colour is due to the presence of the dominant *Agouti*^{wt} allele.

Based on the relative share of the animals with coloured wool, the country's potential for annual coloured wool production from native breeds was estimated at a minimum of 133,791 kg of unwashed wool. Recent tendencies in lifestyle changes of the Bulgarian people are gradually leading to increased interest in naturally coloured wool products, which may benefit the conservation of endangered native breeds by promoting relevant breeding programmes.

Keywords: native sheep breeds, coloured wool, genetic loci

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Introduction

Historically, the development of the wool textile industry in Bulgaria has gone through many stages. In the first half of the 20th century, through a number of laws, all governments in Bulgaria encouraged local textile products (Savov, 1964). For example, The wool-textile industry had received a number of tax reliefs and aids in the import of machinery and raw materials (including imports of high-quality wool and yarn) for the production of textile products, which has led to an increase in the production of fine worsted white wool fabrics.

During the wars, and especially during the First World War, the wool-textile industry continued to work, with much of the raw material used being local coarse wool. When purchasing local coarse wool, the textile industry in Bulgaria preferred to buy coloured wool for the production of military fabric and blankets because there was no need to dye the wool. The main supply of this wool was from the areas where Karnobat, Copper-red Shumen, Dubenska and other native sheep breeds with coloured wool have been raised (Savov, 1964).

Following state policy since 1950, these local breeds have been crossed with rams of merino breeds to create new breeds of sheep for the production of fine merino white wool needed for the production of thin fabrics for finer woolen products. By 1983, Bulgarian production reached 33,000 tonnes of wool, a large part of which was merino white wool. After 1990, as a result of the economic and political crisis of the socialist system in Eastern European countries, the annual production of 28,000 tonnes of wool in 1990 collapsed to 6,500 tonnes by 2005 and recently to about 2,946 tonnes in 2017 (FAO, 2017). In general, this decline in production was observed in many wool-producing countries around the world including Bulgaria.

By the middle of the 20th century, the manufacturing of handmade textile products had declined and nowa-

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days, they are produced only by a small number of artisans, as well as by some people continuing folk traditions.

In recent years, efforts to protect the environment and tendencies towards a more biological and ecological lifestyle have increased consumers' interest in woolen handicrafts and textile products from naturally coloured animal fibres. There is a growing concern to add value to sheep production in view of possible decreases in agricultural premiums and growing demand for organically produced agricultural products (Chaupin, 2014). Renewed interest in local, natural resources due to concerns over the carbon footprint of long-distance transportation of goods fuels a growing movement for 'sustainable fashion' that takes into consideration the ecological impact of garment production (Chaupin, 2014). In this context, the naturally coloured wool obtained from some sheep breeds acquires a new meaning.

The colour of the wool is not included as a selection trait in any breeding programme in Bulgaria and therefore there has so far been no scientific research on this feature in the country. Until now, there were no studies about genetic polymorphisms in native sheep breeds in Bulgaria in which the coat colour was subject to genetic assessments.

The aim of this review is to describe and characterize sheep genetic resources with coloured wool in Bulgaria and to evaluate the country's potential for coloured wool production, as well as to give some explanations about genetic background of sheep colour variations.

National gene pool of native sheep breeds

According to official statistics, in Bulgaria there currently are 18 native, eight locally adapted and seven introduced sheep breeds (Table 1) and the number of sheep in the country in the last eight years was about 1.3 million. According to total population size, Bulgarian native sheep breeds can be classified in several categories of risk status using FAO criteria (FAO, 2013). Six of the native sheep breeds are 'endangered' and five breeds are 'vulnerable'. The remaining seven breeds have the risk status 'not at risk' (Table 1).

In the country, there are nine breeding organisations for native sheep breeds which perform breeding programmes approved by the Ministry of Agriculture, Food and Forests (MAFF), all of which put emphasis on preserving native sheep breeds as genetic resources and overcoming the risk of extinction by increasing their population size. The breeding programmes of almost all native sheep breeds are currently not focused on colour of wool or its yield and qualities in the context of the emerging interest in the textile industry and for other coloured wool products.

Depending on the colour of the fleece, the native sheep breeds in Bulgaria can be divided into three groups: 1) breeds with fully pigmented fleece; 2) breeds with both fully pigmented and fully white fleece and 3) breeds with spotted coloured fleece.

Table 1. Native sheep breeds in Bulgaria and their population size (including ewes and rams) and risk status according to FAO criteria in 2018. (Data source: Executive Agency on Selection and Reproduction in Animal Breeding (EASRAB; https://www.iasrj.eu/).

Breed №	Breed name	Population size	Risk status
1	Copper-red Shumen	16,382	Not at risk
2	Karnobatska	1,301	Endangered
3	Karakachanska	11,621	Not at risk
4	Dubenska	12,660	Not at risk
5	White Maritza	886	Endangered
6	Kotlenska	5,998	Vulnerable
7	Black-headed Pleven	18,452	Not at risk
8	Native Starozagorska	834	Endangered
9	Tetevenska	4,642	Vulnerable
10	Koprivshtenska	3,375	Endangered
11	Zapadnostaroplaninska	4,407	Vulnerable
12	Srednorodopska	8,716	Not at risk
13	Srednostaroplaninska	10,091	Not at risk
14	Patch-faced Maritza	8,037	Not at risk
15	Elin-Pelinska	6,599	Vulnerable
16	Replianska	5,982	Vulnerable
17	Sakarska	2,238	Endangered
18	Breznishka	3,201	Endangered
Total number of native breed sheep in 2018		125,422	

The genetic basis of coloured wool

The coat colour of sheep is often not only a visual trait in the morphological characterization of sheep breeds, but sometimes also an important trait for selection. Classic genetics, which relied on 'by eye' classification of coat colour type, was helpful in breeding schemes for many years (Cieslak *et al*, 2011). Genetic control of coat colour is performed by 11 identified loci: *Agouti* (*A*), *Albino* (*C*), *Australian Piebald* (*AsP*), *Brown* (*B*), *Extension* (*E*), *Pigmented head* (*Ph*), *Roan* (*Rn*), *Spotting* (*S*), *Sur Bukhara* (*SuB*), *Sur Surkhandarya* (*SuS*) and *Ticking* (*Ti*) (Sponenberg *et al*, 1996; Sponenberg, 1997).

The colour diversity results from the presence and biochemical activity of melanocytes, cells that are specialized in producing melanins (Koseniuk *et al*, 2018) . In sheep, as in other mammals, there are two types of melanins: eumelanin and pheomelanin. Eumelanin causes either a black or brown shade of wool. Pheomelanin results in a red, tan or fawn colour of wool (Lundie, 2011). It is widely recognized that the production of eumelanin and pheomelanin are genetically controlled by the *Extension* and *Agouti* loci (Searle, 1968). The *Agouti* locus encodes for the agouti signalling protein (ASIP; Bultman *et al*, 1992). This small paracrine signalling molecule interacts with the product of the *Extension* locus that encode for the melanocortin 1 receptor (MC1R). Many authors have identified two alleles at the *Extension* locus: the dominant black allele (E^D) is responsible for black

colour in a few coloured breeds, and the wild type allele *(E^+) is* widely distributed in most breeds in which the *Agouti* locus is most likely responsible for the majority of colour variation in wool (Searle, 1968; Sponenberg, 1997). There are assumptions that there is a third allele in the *Extension* locus but, unlike in other mammals, this is not yet well documented in sheep and its potential role in the phenotypic manifestation of wool colour is not well understood. Fontanesi *et al* (2010) studied the polymorphisms of the *MC1R* gene in nine Italian sheep breeds and identified one non-synonymous mutation (C199T) causing an amino acid substitution (R67C) in a highly conserved position in the first intracellular loop of the MC1R protein. The authors proposed that this mutation, identified only in the Valle del Belice breed, may represent the recessive *eallele* in the ovine *Extension* series, but this is not completely recognized in sheep. A different study to assess the genetic polymorphisms of the *MC1R* gene in eleven local Greek sheep breeds (Stamatis *et al*, 2017) did not find the putative *e* allele proposed by Fontanesi *et al* (2010). Yang *et al* (2013) investigated the variability in the MC1R protein and its possible association with coat colour in ten Chinese sheep breeds and also did not detect the recessive allele *e*. There is an epistatic interaction between the *Extension* and *Agouti* loci (Searle, 1968). The presence of the (E^D) allele in the homozygous (E^DE^D) or heterozygous ($E^DE^{+)}$ state leads to phenotypic manifestation of the black colour, while the *E^+allele* in the homozygous state (E^+E^+) is a prerequisite for the phenotypic manifestation of the *Agouti locus* alleles. The wild type allele E^+ is the most common *Extension* allele in most European breeds of sheep in which segregation at the *Agouti* locus accounts for the majority of colour variation (Sponenberg, 1997).

The predominant allele at the *Agouti* locus is A^{Wt}, which is dominant and produces white fleece in most wool breeds (Notter and Sponenberg, 2002). The most common alternative allele at this locus is the so-called *non-agouti* allele (A^a). This allele is recessive and in sheep of most breeds this results in a completely black colour at birth. Animals of genotype A^aA^a are generally black although the final expression of colour can be influenced by other genes and depends on modifiers at independent loci (Notter and Sponenberg, 2002). Between the A^{Wt} and A^a alleles, there are other intermediate *Agouti* alleles that cause different patterns of pigmentation. Some of them can cause reversals of pigmentation type, so that areas that are eumelanic in one pattern are pheomelanic in another and vice versa (Sponenberg, 1997).

The *Spotted* locus (*S*) is responsible for white spotting. According to the Committee on Genetic Nomenclature of Sheep and Goats (COGNOSAG) nomenclature there are two alleles in this locus (Broad *et al*, 1999). The wild type allele (S^+) results in a solid coloured animal. The spotted allele (S^s) in eumelanic animals usually produces some white markings on the crown of the head, tip of the tail and bottom of the legs. When the *S^s allele* occurs in a pheomelanic background colour it produces a piebald animal when heterozygous and mainly white animals when homozygous (Lauvergne and Raffier, 1975).

Four alleles are known at the *Pigmented Head* (*Ph*) locus: wild type (Ph^+), Persian (Ph^P), Turkish (Ph^T) and Afgan lethal (Ph^{afl}) (Lundie, 2011). Since the Ph^P and Ph^{afl}alleles cannot be found in Bulgarian sheep breeds and the *Ph^+allele* is practically impossible to distinguish from other alleles that cause the same solid-coloured animals, the most interesting allele for Bulgarian sheep breeds is Ph^T, which is used in the selection of the Patch-faced Maritza sheep breed (see below).

In some wool sheep breeds animals with brown coat colour occasionally appear. Brown colour is controlled by the *Brown* gene, for which there are two alleles (Notter and Sponenberg, 2002; Lundie, 2011). The wild type allele (B^+) is dominant and the most common allele at this *locus*, resulting in black eumelanin pigment. The *Brown* allele (B^b) is a rare recessive allele that when homozygous results in brown (chocolate shade) eumelanin pigment (Lundie, 2011). The *Brown* locus controls tyrosinase-related protein 1 (TRP-1) which has an important role within melanocytes (Jackson, 1994). Brown colour is quite common in the Northern Short-tailed group of breeds of Europe and found more rarely in other European breeds such as the Merino and Romney (Lundie, 2011).

The discussion below of native sheep breeds in Bulgaria includes some hypotheses about genetic loci and alleles affecting coat colour in sheep populations, following the recommendations of COGNOSAG.

Breeds with fully pigmented fleece

Copper-red Shumen

The Copper-red Shumen sheep is an ancient native sheep breed in Bulgaria with a population size in 2018 of 16,382 (Table 1). According to the style of the fleece there are two types: 'kabarliavi', with open fleece of semi-rough, mixed wool, and 'rudavi', with a homogeneous, thin wool (Staikova *et al*, 2015). The wool yield varies from 2.711 to 3.454 kg and the average staple length is 14.13 cm (Staikova, 2005). Adult animals of the breed are almost 100% coloured with red-brown or chocolate-coloured wool (Figure 1). The face, ears and legs are covered with short black hairs. The lambs at birth are solid black and develop the typical copper-red colour only after weaning at three months of age and later after shearing. Some individuals display lighter colour while, in others, it can be more intense and in different shades (Staikova *et al*, 2015). Animals with white colour are very rare in this breed. Based on our observations and interviews with specialists, when mating this breed with white animals, all offspring in F1 were black, which can be attributed to the phenotypic expression of the dominant E^D allele, suggesting that the colour genotype of the typical animals is E^DE^D or E^DE^+. Only 4-7% of animals

a

b

Figure 1. Copper-red Shumen sheep breed. a) ewes b) rams. (Photographs A. Vuchkov (a) and D. Dimov (b))

in the population are born black with white spots on the top of the head and tip of the tail, attributed to the phenotypic expression of the *Spotted* allele S^s. Thus, the genotype which causes the colour can be described as $E^D E^D S^+ S^s$ or $E^D E^+ S^+ S^s$. The change in colour at an early age from black to reddish-brown may be due to the action of other gene modifiers, which are not known at this stage.

Karnobatska

The Karnobat native sheep is an autochthonous sheep breed, known in the past for its delicious meat (Stefanova and Iliev, 2005) and soft pigmented wool (Hlebarov, 1933). The tail is thin, but at the base there is an enlargement of subcutaneous fat which reaches the hock joints. The length of the tail is between 24 and 26 cm. The live weight of the ewes varies from 42 to 47 kg (Iliev, 2012). Wool yield in recent years varied by about 3 to 3.6 kg in ewes, and 5 to 5.6 kg in rams Iliev (2007).

The lambs are born completely black, and subsequently the fleece lightens to different shades of brown (Staikova and Iliev, 2017). The front of the head and legs are covered with short black hairs, the body is covered with coloured wool fibres (Figure 2). Animals with almost white coat colour are rarely encountered, but these are usually removed from the population. When crossing with white sheep of other breeds in F1 mostly black animals are born, which is reason to assume that the colour of the coat is determined by the dominant E^D allele as in the Copper-red Shumen breed. The change in colour of the wool from black to reddish-brown is probably due to the action of gene modifiers, and probably a certain influence is exerted by environmental factors, such as age or sunlight.

Breeds with both fully pigmented and fully white fleece

Karakachanska

The Karakachan sheep is a typical mountain breed, small in height with a compact body, extremely mobile and very lively temperament. The fleece is open and has a hairy conformation (Figure 3).

a

b

Figure 2. Karnobat native sheep breed. a) ewe b) group of ewes. (Photograph: A. Vuchkov)

The wool is coarse, mixed with long fibres and relative low density. The wool yield is 2.7 kg, wool length 16 cm and fibre diameter is around 80 μm. Pigmented sheep are dominant in the population, and white, non-pigmented sheep are relatively rare (Barić, 1952; Hlebarov, 1942; Milić, 1954; Sedefchev and Sedefchev, 2011). Animals are typically one colour, with black head and legs. The lamb's fleece is black, changing with age to shades of grey-black or grey.

According to our observations, the pigmentation in this breed may be due to the dominant allele E^D in the *Extension* locus. The extremely rare white colour in this breed is due to the dominant allele A^{wt} in the *Agouti* locus, which is expressed in the absence of E^D in the *Extension* locus. Expression of the recessive S^s allele in the *Spotted* locus was also observed in about 10% of the animals with white spots on the top of the head and the tip of the tail.

White Maritza

The White Maritza sheep breed is a multi-purpose native breed for milk, meat and wool. This native lowland sheep breed, contrary to notions of native sheep breeds, has a good milk yield (110.57 l) and high body weight of ewes (71.71 kg) and rams (100.12 kg) (Dimov, 2011b). The size of this population is comparatively small and in 2018 was estimated at about 886 sheep including 40 rams and therefore considered endangered. The fleece looks almost closed and has staple conformation with staple length of 11.19 cm (Dimov, 2015). The average fiber diameter is 32.1 μm (Dimov and Djorbineva, 1999) and according to the Bradford system for wool classification, this means 48^{th} quality (USDA, 2018).

The typical fleece colour of Maritza sheep is white (Figure 4a), but sheep with coloured fleece (Figure 4c) can also be found. The wool around the head is not shorn and a so-called 'cowl' is formed over the

Figure 3. Karakchan sheep breed. a) flock of ewes with lambs b) black lamb (genotype E^D) c) black and white lamb d) black lamb with white spots caused by S^s allele at *Spotted* locus. (Photographs: A. Vuchkov)

Figure 4. White Maritza sheep breed with different colour genotypes. a) genotype $E^+E^+A^{Wt}A^{Wt}$, b)genotype $E^+E^+A^aA^aS^+S^s$, c) black mother genotype $E^+E^+A^aA^aS^+S^s$ and white lamb genotype $E^+E^+A^{Wt}A^a$, d) piebald ram, genotype $E^+E^+A^aA^aS^sS^s$. (Photographs: D. Dimov)

years. This cowl has decorative character and satisfies the aesthetic criteria of the shepherds. There are also piebald sheep (1% of the population, Figure 4d). There is no doubt that white colour in over 90% of the animals in this population is caused by the dominant allele (A^{wt}) at the *Agouti* locus, and the genotype of animals with white fleece can be described as $E^+E^+A^{Wt}A^{Wt}$or $E^+E^+A^{Wt}A^a$ (Figure 4). The 'black' colour of animals in this population is recessive and it is due to a *non-agouti* allele (A^a) in a homozygous state A^aA^a (Figure 4b). The lambs are born completely black or dark brown and subsequently the wool acquires a reddish-brown colour, but the front and legs remain black.

The genotype of pigmented animals in the population of this breed can be described as $E^+E^+A^aA^a$. In most cases, pigmented animals in this population have a white spot on the head and tip of the tail, which could be perceived as phenotypic expression of the S^s allele at the *Spotted* locus. Then, genotype of such pigmented animals can be described as $E^+E^+A^aA^aS^+S^s$ (Figure 4b) or $E^+E^+A^aA^aS^sS^s$ (Figure 4d).

Native Starozagorska

The native Starozagorska is a native sheep breed that has taken its name from the area where it was created (Stara Zagora). The geographic area where the breed is kept mainly covers the plains of the districts in Southern Bulgaria: Stara Zagora, Sliven, Haskovo and Yambol. This is a typical large, lowland sheep with a long, narrow, unfleeced neck and head and a convex profile. The ears are long, wide and lop-eared and the belly and legs are naked and unfleeced. The fleece is semi-open and the wool is soft and mostly white with a fibre thickness of 39.6 μm Hlebarov (1937). Typical

Native Starozagorska sheep breed are white, but they also appear pigmented (reddish-brown) with a white spot on an oblong white strip on the face called 'brezi' by native people (Figure 5). There are sheep breeders who prefer to breed coloured sheep (Djorbineva, 2008). Pigmented animals turn black and, with age, the colour of the wool acquires a reddish-brown colour, but the covering hairs on the front and legs remain intensely black. The average live weight of ewes is 64 kg and 80 to 90 kg in rams (Djorbineva, 1984). Recently, there is information about animals with higher body weight. The wool yield for the ewes is 2.8 kg, and 3.5 kg for the rams. The staple length is 9 to 10 cm.

The white colour in this breed is dominant and the genotype of animals with white coat can be either $E^{+}E^{+}A^{Wt}A^{Wt}$ or $E^{+}E^{+}A^{Wt}A^{a}$ (Figure 5a). The genotype of the coloured variety in this breed can be $E^{+}E^{+}A^{a}A^{a}S^{+}S^{s}$ (Figure 5b). At its most extreme piebald animals may be found ($E^{+}E^{+}A^{a}A^{a}S^{s}S^{s}$).

Dubenska

The Dubenska sheep breed is a native sheep breed found in the central part of the Sredna Gora region. Dubenska sheep are medium-sized and their tail is long, reaching below the hocks, sometimes to the ground. The sheep are well-fleeced; the head is fleeced up to the line of the eyes, the belly is totally fleeced, the forelimbs are fleeced up to the carpal joints, and the hind limbs are fleeced until under the hocks and, in some cases, they are completely fleeced. Until the 1930s, the predominant share of the Dubian sheep population was pigmented (brown-black, downy, reddish-brown) and white animals were rarely found (Figure 6a, b). This was related to the production of a coarse, woolen natural brown cloth called 'Shiak', which was known from the Karlovo district in the past, but is no longer in high demand. In the modern population, the white sheep are beginning to dominate and the proportion of coloured sheep is about 60%. The wool is mostly 46-48th quality in the Bradford system for wool classification (USDA, 2018).

The white colour of about 40% of the animals in the population is due to the dominant allele A^{wt} of the *Agouti* locus, but the remaining 60% of the animals are pigmented and this is due to the recessive *non-agouti* allele A^{a}. The frequency of these two alleles in the population depends on the farmer's choice for white or pigmented lambs. Keeping in mind that it is phenotypically difficult to distinguish the manifestation of the E^{D} allele in the *Extension* locus and the recessive allele A^{a} in the *Agouti* locus, it would be very interesting to perform DNA analysis to prove the presence or absence of the dominant allele E^{D} in the *Extension* locus for this breed.

Black-headed Pleven

The Black-headed Pleven is one of the most popular native sheep breeds in Bulgaria. This breed has a large population (18,452 sheep) and can mainly be found in the northern part of the country, but there are also flocks in Southern Bulgaria.

The head is long and narrow with long ears. The lambs are born black, sometimes with a white spot on the crown of the head and tip of the tail, but then the wool on the body fades to white (Figure 7a, b). The colour of the fleece is usually white (Figure 7d) but black hairs can often be found among the white fibres (Figure 7c), making the fleece look faded and grey (Ivanov, 1942). About 5% of the sheep have a fully coloured fleece. The tail is long and covered with wool that corresponds to the quality of the wool on the body. The live weight of ewes is about 55 to 70 kg, and of rams 80 to 100 kg. The wool yield in the ewes is about 2.8 to 3.5 kg and for the rams 4 to 4.5 kg. The staple length is about 12 to 16 cm and fibre thickness is 30.09 to 32.97 μm (Georgiev, 1990).

a b

Figure 5. Native Starozagorska sheep breed. a) genotype $E^{+}E^{+}A^{Wt-}$, b) genotype $E^{+}E^{+}A^{a}A^{a}S^{+}S^{s}$. (Photographs: D. Dimov)

The coat colour of the Black-headed Pleven sheep breed is very similar to that of many other breeds that have black faces and legs and white fleece (e.g. Tsigai, Suffolk breeds). It has been suggested that this is due to another gene called *Dark Brown* (Sponenberg, 1997), but this colouring is still poorly understood. All crosses of Black-headed Pleven with white wool sheep breeds look piebald or similar due to the Ph^T allele in the *Pigmented head* locus, which excludes the presence of dominant E^D allele in the *Extension* locus. Probably this lightening of the wool is due to the interaction of the *Agouti* and the *Brown* loci or to the action of other gene modifiers.

Kotlenska

The animals of the Kotlenska sheep breed are relatively small, the fleeced tail reaching the hock joints. The body, including the abdomen, is well fleeced. The fleece is mostly semi-open white or coloured with shades of grayish to black. The wool is roughly mixed with a fibre length of 14 to 18 cm. Average wool yield is 1.9 kg for ewes, and 3.25 kg for rams (Tzochev *et al*, 2017).

Tetevenska

The Tetevenska sheep breed can be found in the region of the Central Balkan Mountains. At the age of 3.5 years, the ewes of this breed reach 43.47 kg. The wool yield of the Tetevenska sheep breed is about 3 kg with a variation of 3.03 to 3.28 kg. They have a non-uniform mixed coarse or semi-rough wool and have a fibre length of 12.93 cm. White coloured fleece dominates in the population, but also coloured sheep with a different nuance of the fleece colour (black, red, grey) can be found (Genkovski, 2002). An approved breeding programme for preservation of the Tetevenska sheep breed was carried out by the approved breeding association with headquarters in the town of Troyan. According to the association's data, by 2018 the population size was 4.642 animals (ewes and rams), with a risk status of vulnerable. From the point of view of coat colour, the Tetevenska sheep breed is poorly studied.

Koprivshtenska

This native breed is at risk of extinction. Its population size at 2018 was 3,375 (Table 1). The wool of the sheep is mostly uniform, but there are also animals with non-uniform wool. The fibre thickness of uniform wool varies in a very wide range from 29.1 to 40.0 μm. The fleece is white, but in around 40% of the animals, the colour is dark brown. The weight of the fleece averages 3.45 kg and the fibre length 10 to12 cm (EASRAB, 2013).

Figure 6. Dubenska sheep breed. a) flock, b) old ewe, c) lambs, d) lamb with black fleece variety (genotype $E^+E^+A^aA^aS^+S^s$). (Photographs D. Dimov)

Figure 7. Black-headed Pleven sheep breed. a) male hogged, b) ram, c) black fibres visible in white fleece, d) fleece with only white fibres. (Photographs: A. Vuchkov)

Zapadnostaroplaninska

This is a popular native sheep breed from the western part of the country. According to its population size of 4,407 in 2018 (Table 1), the breed's risk status is endangered. Average fleece weight is about 3.0 kg with 10 to 12 cm fibre length and 31.1 to 40.0 μm fibre thickness. The fleece is usually closed and predominantly white, but about 10% of the sheep in the population have coloured fleece (EASRAB, 2013). In terms of wool and hair colour, the population is quite diverse and fully pigmented or completely white sheep can be found, but there are also piebald animals. From the point of view of coat colour, the Zapadnostaroplaninska sheep breed is poorly studied.

Srednorodopska

This breed is typical in the Rodopa Mountain in the southern part of the country, where sheep are capable of long transitions in the mountains on steep and rocky terrains. The population size is 8,716, which defines the risk status as not endangered (Table 1). The wool is rough and around 55% of the animals have coloured fleece. The wool yield is 1.75 kg (EASRAB, 2013).

Srednostaroplaninska

This native sheep breed is typical of the Central Balkan mountains. Some flocks can be found in settlements in lowlands close to the mountains. The wool is coarse mixed and the fleece is made up of three types of fibres, fine, coarse and medium. The fleece is coloured with shades of grey, fawn and red. The wool yield is 2.75 kg per ewe and 3.5 kg per ram (EASRAB, 2013).

Sakarska

This breed is a typical, rare native breed with endangered risk status named after the Sakar Mountain in Southeastern Bulgaria. Today it is widespread in other parts of Southern Bulgaria. The colour of the fleece is mostly white, but 5% of the animals in the population have pigmented fleece. The weight of the fleece is 2.9 kg and fibres are coarse (EASRAB, 2013).

Breeds with spotted coloured fleece

Patch-faced Maritza

The area in which the Patch-faced Maritza sheep breed was created and became popular is located to the west and north of the city of Plovdiv (central part of Southern Bulgaria). In recent years, the Patch-faced Maritza sheep have gained popularity and have spread to other areas including Sofia, Stara Zagora, Haskovo, Yambol, Burgas, Kardzhali, Sliven and Blagoevgrad. The elongated form of the body, the limbs and the tail are characteristic of the Patch-faced Maritza sheep breed (Figure 8a). The head and legs are long and unfleeced, also the tail is thin and long. The belly is also usually unfleeced but, in

some animals, it is poorly fleeced. The wool is uniform with a fibre thickness of 35.01 μm which, according to the Bradford classification, means 46^{th} quality (Dimov and Djorbineva, 1999; USDA, 2018). Black spots with varying sizes are found on the face, the legs and on certain areas of the body, e.g. the root of the tail, the chest and abdomen (Figure 8b, d). Coloured wool usually grows on the pigmented areas of the body, which is why animals with larger spots on the skin of the body look piebald. However, the main fleece mass has a white colour. The pigmentation on the face, shape and size of the ears, and certain features in the body, are crucial in the selection of male and female lambs for breeding (Figure 8c). The weight of the fleece after shearing is 2.8 kg, with a fibre length of 11.2 cm. Our studies show that the live weight of ewes can reach 74.47 kg (Figure 8b) and of rams up to 121.14 kg (Dimov *et al*, 2016). The level of nutrition and flock husbandry has a strong influence on the live weight of the ewes and the rams, thus other reports of different flocks recorded sheep live weight in the range of 68.21 kg to 90.82 kg (Dimov *et al*, 2016).

The breed standard of Patch-faced Maritza sheep is associated with specific pigmented noses, eye patches, ears, root of the tail and distal parts of the legs (Dimov, 2011a). This phenotype is due to the *turkish* allele located in the *Pigmented head* locus (Sponenberg, 1997; Lundie, 2011). The genetics of the *turkish* allele (Ph^T) was first reported in the Akkaraman sheep breed in Turkey (Mason, 1967). In the homozygous state, the *turkish* allele causes an intense black colour of the specific spots on the head, ears, body and legs (Figure 8a, b, c).

Occasionally, in the population of Patch-faced Maritza sheep breed there are piebald animals with smaller or larger coloured spots on the body (Figure 8d). Fully pigmented animals with a white spot on the crown of head and tip of the tail, due to the S^s allele of the *Spotted* locus, are also relatively rare.

Figure 8. Patch-faced Maritza sheep breed. a) male hogged (colour genotype $E^+E^+A^aA^a$ Ph^tPh^t), b) flock (colour genotype homozygous for turkish allele Ph^t), c) lambs (homozygous for turkish allele Ph^t), d) piebald ram. (Photographs: D. Dimov)

The *turkish* allele (Ph^T) is the main reason for the coat colour also of the Breznishka sheep breed and probably of the Elin-Pelinska sheep breed (Figure 9a). In the population of Elin-Pelinska sheep breed, the colour pattern of the head (Figure 9b) is different from that of Patch-faced Maritza, but it is probably also regulated by the *turkish* allele Ph^T.

Other native sheep breeds listed in Table 1 have a white fleece colour and do not contribute to the production of coloured wool nor are they well studied.

Production potential for natural coloured wool in Bulgaria

Considering the great genetic diversity of native sheep breeds in Bulgaria and especially those with coloured wool, we assessed the country's potential for producing coloured wool based on the average wool yield and the percentage of animals with pigmented wool in their populations EASRAB (2013). This is essential for the prospect of business strategies that could be based on the use of naturally coloured wool.

Based on the population sizes of the native sheep breeds and the relative share of the animals with coloured wool, the country's potential for coloured wool production was estimated at 133,791 kg of wool (Table 2), which can be considered the minimum amount of naturally coloured wool that can be produced in the country. In addition, some breeds also produce grey wool, caused by black fibres present in the otherwise white fleece, which adds a grey hue to the wool.

a

b

Figure 9. Elin-Pelinska sheep breed. a) Ram, b) Typical colour pattern of the head. (Photographs: D. Dimov)

Primary wool processing

According to the European Directive 1069/2009 Art. 10, greasy wool produced in sheep farms is classified as 'animal by-product' of the 3^{rd} category European Commission (2009). This circumstance has added a series of regulations and administrative requirements to handling of grease wool (Chaupin, 2014). Pursuant to the Waste Management Act in Bulgaria (WMA, 2012), the primary processing of grease wool and in particular its washing must be carried out in specialised licensed enterprises. An essential requirement for obtaining a permit in the country for wool washing production is the availability of a wastewater purification plant. At this moment, only one company in the country meets the requirements of the Waste Management Act and can lawfully carry out all wool treatments of sorting, scouring, carding and combing, focusing, however, on large batches of wool. At present, there are no suitable enterprises for the legal processing of small batches of wool produced by small producers.

The market for naturally coloured wool products

Current trends in lifestyle, focusing on protection of the environment, reducing carbon emissions, protecting traditional production systems and sustainable rural development, are gradually increasing interest in naturally coloured wool products, providing a unique opportunity for breeders of native sheep breeds to add value to the annual wool production in their farms.

Current purchase prices of greasy wool announced by the main Bulgarian wool processing company KOLHIDA-SLIVEN JSC are presented in Table 3.

Unfortunately, for many years, the low purchase prices of greasy wool led to a sceptical attitude of farmers to the wool as a product that could bring additional income. At present, there are no farmer initiatives to produce wool products from coloured wool. The market for naturally coloured wool products is heterogeneous. In the country's markets, naturally coloured wool from native breeds can be seen in handmade socks, hats, sweaters, vests (Figure 10), usually produced by artisans and not the farmers themselves. There are informal communities of artists in the country who produce various souvenirs and articles, but this market is weak and, at this stage, artworks made of naturally coloured wool are mainly sold around the major tourist centres in the country.

Table 2. Potential of native sheep breeds in Bulgaria for coloured wool production. Data source: Executive Agency on Selection and Reproduction in Animal Breeding (EASRAB; https://www.iasrj.eu/).

Breed name	Population size	Wool Yield (kg)	Coloured animals (%)	Coloured animals (total)	Potential for coloured wool production (kg)
Copper-red Shumen	16,382	2.50	100	16,382	40,955
Karnobatska	1,301	3.25	100	11,301	4,228
Karakachanska	11,621	2.75	100	11,621	31,958
Dubenska	12,660	2.75	60	7,596	20,889
White Maritza	886	3.50	8.5	75	264
Kotlenska	5,998	1.90	70	4,199	7,977
Black-headed Pleven	18,452	3.15	8	1,476	4,650
Native Starozagorska	834	3.75	2	17	63
Tetevenska	4,642	2.50	10	464	1,161
Koprivshtenska	3,375	3.45	40	1,350	4,658
Zapadnostaroplaninska	4,407	3.00	10	441	1,322
Srednorodopska	8,716	1.75	55	4,358	8,389
Srednostaroplaninska	10,091	2.75	10	1,009	2,775
Patch-faced Maritza	8,037	2.60	20	1,607	4,179
Sakarska	2,238	2.90	5	112	325
Elin-Pelinska	6,599	2.50	0	0	
Replianska	5,982	2.50	0	0	
Breznishka	3,201	2.70	0	0	
Country potential	**125,422**			**62,008**	**133,791**

Figure 10. Artisan products made from naturally coloured wool available in Bulgaria. a) handmade wool socks, b) woolen works of art and naturally coloured yarn. (Photographs: D. Dimov)

Table 3. Purchase prices of greasy wool from native sheep breeds in Bulgaria in 2019.

Colour of the wool	price (€/kg)
White	0.66
Grey	0.41
Coloured	0.31
Trim	0.12

Conclusions

The wide variety of native sheep breeds in Bulgaria, in whose populations there are animals with coloured wool, can be used to satisfy the new trends in the textile industry. In this review, we have highlighted the phenotypic expression of several genetic alleles responsible for wool colour (E^D, A^a, Ph^T) in the populations of native Bulgarian sheep breeds. The white colour in populations of several breeds is due to the presence of the dominant A^{wt} allele in the *Agouti* locus. Bulgaria's potential for the production of coloured wool was estimated at 133,791 kg, which can be considered as a minimum at this stage, and with an appropriate market demand, this quantity can be increased. Bulgarian native sheep breeds have the potential to produce different types of wool, which is a prerequisite for various applications in the textile, fashion and art industries. In the immediate future, the market for naturally coloured wool products could be enlarged by increasing public awareness for their consumption and promoting fashion trends which respect environmental protection.

Supplemental data

Supplemental file: Abstract in French.

Author contributions

The authors contributed equally to the writing of this review.

Conflict of interest statement

The authors declare that no conflict of interest exists.

References

Barić, C. (1952). Grada I eksterijer kucovlaške ovce. *Poljoprivrednaznanstvena smotra* 13. (in Croatian).

Broad, T. E., Dolling, C. H., Lauvergne, J.-J., and Millar, P. (1999). Revised COGNOSAG guidelines for gene nomenclature in ruminants 1998. *Genetics Selection Evolution* 31(3), 263–263. doi: https://doi.org/10.1186/1297-9686-31-3-263

Bultman, S. J., Michaud, E. J., and Woychik, R. P. (1992). Molecular characterization of the mouse agouti locus. *Cell* 71(7), 1195–1204. doi: https://doi.org/10.1016/s0092-8674(05)80067-4

Chaupin, M. T. (2014). Developing a market for European wool. In *Timeless coloured sheep,* ed. du Toit, D. 87-89.

Cieslak, M., Reissmann, M., Hofreiter, M., and Ludwig, A. (2011). Colours of domestication. *Biological Reviews* 86(4), 885–899. doi: https://doi.org/10.1111/j.1469-185x.2011.00177.x

Dimov, D. (2011a). Breeding program of Patch-faced Maritza sheep breed. (Intelexpert-94 Publishing house), (in Bulgarian).

Dimov, D. (2011b). Milk yield and body weight of White Maritza sheep. In Proceedings of 8th Global conference on the conservation of Animal Genetic Resources, , 113-115.

Dimov, D. (2015). White Maritza sheep - origin, state and perspectives for development (Plovdiv: Intelexpert-94 Publishing house).

Dimov, D. and Djorbineva, M. (1999). Study on thickness of wool fibres of White and Patch Faced Maritza sheep. *Scientific Works* XLIV(3), 191–194.

Dimov, D., Vuchkov, A., and Ivanov, I. (2016). Study on ewe live weight of Patch-faced Maritza sheep breed. In 2nd International Symposium for Agriculture and Food, 67-72.

Djorbineva, M. (1984). Variability of selection traits of local Stara Zagora sheep breed and possibilities for their improvement. Ph.D. thesis, Research institute of cattle breeding and sheep breeding, Agricultural Academy, Stara Zagora. (in Bulgarian).

Djorbineva, M. (2008). The Stara Zagora sheep breed and breeding association's breeding activities. *Sheep Breeding* (2), 7–13.

EASRAB (2013). Livestock breeds in the republic of Bulgaria. Catalogue 4th edition.

European Commission (2009). Regulation laying down health rules as regards animal by-products and derived products not intended for human consumption and repealing Regulation (EC) No 1774/2002 (Animal by-products Regulation). url: https://eur-lex.europa.eu/eli/reg/2009/1069/.

FAO (2013). In vivo conservation of animal genetic resources volume 14 of *FAO Animal Production and Health Guidelines*. (Rome: FAO). url: http://www.fao.org/3/i3327e/i3327e00.htm.

FAO (2017). FAOSTAT. url: http://www.fao.org/faostat/en/. accessed date: 2021-05-26

Fontanesi, F., Beretti, V., Riggio, S., Olio, D., Calascibetta, V., Russo, B., and Portolano (2010). Sequence characterization of the melanocortin 1 receptor (MC1R) gene in sheep with different coat colours and identification of the putative e allele at the ovine Extension locus. *Small Ruminant Research* 91(2-3), 200–207. doi: https://doi.org/10.1016/j.smallrumres.2010.03.015

Genkovski, D. (2002). Comparative characteristics of different breeds and varieties of sheep, reared under the conditions of the Central Balkan Mountains. Ph.D. thesis, Research Institute of Mountain Stockbreeding and Agriculture, Trojan. National Centers of Agrarian Sciences, Sofia, Bulgaria.

Georgiev, D. (1990). Genealogical structure, phenotypic and genotypic characterization of selection trait in sheep of Black-head Pleven sheep breed by the example of the flock of village Riben to PJK "G.Dimitrov". Ph.D. thesis, Agricultural academy, Sofia, Bulgaria.

Hlebarov, G. (1933). The Karnobat sheep volume 31 of *Scientific works*. (Sofia: Bulgarian Agricultural Society), 104p.

Hlebarov, G. (1937). Sheep breeds and varieties in Bulgaria. In Yearbook of Sofia University, Faculty of Aronomy and Forestry, Sofia.

Hlebarov, G. (1942). Karakachan sheep. In Yearbook of Sofia University, Faculty of Agriculture and Forestry, Sofia.

Iliev, M. (2007). Characteristics of Karnobat local sheep. In Scientific and practical conference with international participation "AGROECO 2007", 265-269.

Iliev, M. (2012). Characteristics of productivity in sheep from Karnobat local breed. *Animal Sciences* 3, 11–16.

Ivanov, P. (1942). Black-head Pleven sheep breed. Study on the exterior and productive performances (Pleven) 116p.

Jackson, I. J. (1994). Molecular and Developmental Genetics of Mouse Coat Color. *Annual Review of Genetics* 28(1), 189–217. doi: https://doi.org/10.1146/annurev.ge.28.120194.001201

Koseniuk, A., Ropka-Molik, K., Rubi, D., and Smołucha, G. (2018). Genetic background of coat colour in sheep. *Archives Animal Breeding* 61(2), 173–178. doi: https://doi.org/10.5194/aab-61-173-2018

Lauvergne, J. J. and Raffier, P. (1975). Génétique de la couleur de la toison des races ovines françaises solognote, bizet et berrichonne. *Annales de Génétique et de Sélection Animale* 7(3), 263–276. (in French). doi: https://doi.org/10.1186/1297-9686-7-3-263

Lundie, R. S. (2011). The genetics of colour in fat-tailed sheep: a review. *Tropical Animal Health and Production* 43(7), 1245–1265. doi: https://doi.org/10.1007/s11250-011-9850-0

Mason, I. L. (1967). Sheep breeds of the Mediterranean (Rome: FAO).

Milić, L. (1954). Prilog poznavanju osobina crne vlaške (karakačanske) ovce I način stočarenja s njom (Skopje: Zavod za stočarstvo NR Makedonije), (in Serbian).

Notter, D. R. and Sponenberg, D. P. (2002). Genetic control of colour in Dorper sheep and their Crosses. *Sheep & goat research journal* 17(2), 48–51.

Savov, T. (1964). Development of sheep farming in Bulgaria till 9 September 1944, ed. and others. (Sofia: Publishing House of the Bulgarian Academy of Sciences).

Searle, A. G. (1968). Comparative Genetics of Coat Colour in mammals (London, UK: Logos Press).

Sedefchev, S. and Sedefchev, A. (2011). Comparison of exterior parameters of in situ conserved Karakachan sheep with the breed type from the first half of the twentieth century. *Agrarni Nauki* 3(6), 147–153. (in Bulgarian).

Sponenberg, D. P. (1997). Genetics of colour and hair texture. In *The genetics of sheep,* ed. Piper, L. and Ruvinsky, A., (New York: CAB International), 51-86.

Sponenberg, D. P., Dolling, C. H. S., Lundie, R. S., Rae, A. L., Renieri, C., and Lauvergne, J. J. (1996). Coat colour loci (Category 1). In *Mendelian Inheritance in Sheep 1996 (Mis 1996),* ed. Lauvergne, J. J., Dolling, C. H. S., and Renieri, C., (France, Italy: COGNOSAG and University of Camerino), 13-57.

Staikova, G. (2005). Study on the value of the productive traits in sheep from the Karakachan breed and Copper-Red Shumen strain. Ph.D. thesis, Agricultural institute, Shumen, Bulgaria.

Staikova, G. and Iliev, M. (2017). Karnobat sheep breed. *Bulgarian Journal of Animal Husbandry* 3, 3–15.

Staikova, G., Stancheva, N., and Dimitrova, I. (2015). Copper-red Shumen sheep breed. *Bulgarian Journal of Animal Husbandry* 52(5), 72–80. (in Bulgarian).

Stamatis, C., Giannoulis, T., Galliopoulou, E., Billinis, C., and Mamuris, Z. (2017). Genetic analysis of melanocortin 1 receptor gene in endangered Greek sheep breeds. *Small Ruminant Research* 157, 54–56. doi: https://doi.org/10.1016/j.smallrumres.2017.09.018

Stefanova, S. and Iliev, M. (2005). Comparative evaluation of the slaughter qualities and the main tissue structures in the carcass of lambs from the Karnobat thin-wool and Karnobat local breeds. In and others, Sat. Selection and agrotechnics of field crops. Livestock. Balkan Scientific Conference, 586-592.

Tzochev, I., Slavov, R., Karadjov, H., Bonchevska, S., and Nikolova, T. (2017). Breeding program of Kotlenska sheep breed in Bulgaria.

USDA (2018). Wool Grades and Standards. url: https://www.ams.usda.gov/grades-standards/wool-grades-and-standards. accessed date: 2021-05-26

WMA (2012). Waste Management Act. url: https://www.me.government.bg/en/library/wastem-anagement-act-342-c25-m258-1.html.

Yang, G.-L., Fu, D.-L., Lang, X., Wang, Y.-T., Cheng, S.-R., Fang, S.-L., and Luo, Y.-Z. (2013). Mutations in MC1R Gene Determine Black Coat Color Phenotype in Chinese Sheep. *The Scientific World Journal* 2013, 1–8. doi: https://doi.org/10.1155/2013/675382