

Review

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Review

The old European Silkworm Breeds, Reared in the Early 20th Century in Bulgaria and the Possibilities for Using Some of Them in the Modern Sericulture

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Simple Summary

This paper gives information about the history of Bulgarian sericulture and the characteristics of silkworm races adopted during the end of 19th and first half of 20th century in Bulgaria. It was concluded that the old silkworm breeds, reared in Bulgaria during 20th – 30th years of the 20th century were generally characterized with not very uniform larval color and markings, cocoon color and shape, comparatively high cocoon weight, but lower silk shell percentage and filament length as well as they were comparatively tolerant to NPV disease. Data about the performance of the old Bulgarian silkworm breed Yellow local in 2023 – 2024 were presented and discussed. It was detected that the Yellow local strain manifested lower values of the main productive characters such as cocoon weight, silk shell weight and percentage, silk filament length and weight, reelability and raw silk percentage, compared with the modern commercial Bulgarian white cocoon breeds. It was concluded that in order to commercialize the Yellow local strain in nowadays it needs improvement by the methods of genetics and breeding. A review of the literature about main silkworm qualitative character inheritance was made. Possible research work in improvement of the old silkworm races was recommended.

Abstract

Since the beginning of a local egg production by Pasteur's method in 1895 mostly two local silkworm breeds have been reared in Bulgaria, namely Yellow local and White Baghdad. The origin of Yellow local breed is supposed to be from the locally spread in North and South-West Bulgaria race in 16th – 19th century while the White Baghdad originated from Asia Minor. The Yellow local race was reared in whole North and in South West Bulgaria and was characterized with yellow cocoons with elongated shape with slight constriction. The White Baghdad race was reared in South East Bulgaria and consisted of 3 types, namely Edirne type, Improved Bulgarian type and Bulgarian type. White Baghdad race cocoon color varied from snow-white to light green, but the prevailing color was white. The cocoon shape was elongate with constriction. It was detected that the old silkworm breeds, reared in Bulgaria during 20th – 30th years of the 20th century were generally characterized with not very uniform larval color and markings, cocoon color and shape, comparatively high cocoon weight, but lower silk shell percentage and filament length as well as they were comparatively tolerant to NPV disease. Due to the long maintenance of those races in Bulgaria they were well adapted to the local food and climatic conditions. Presently the Yellow local strain displays a high hatchability and survivability, shorter 5th instar duration and a comparatively good reproduction capacity. On the other hand, the Yellow local strain manifests lower values of the main productive character values such as cocoon weight, silk shell weight and percentage, silk filament length and weight, reelability and raw silk percentage characters, compared with the commercial Bulgarian white cocoon breeds. Therefore, in order to commercialize the Yellow local strain in nowadays it needs improvement by the methods of genetics and breeding. A review of the literature about main silkworm qualitative

character inheritance was made. Possible research work in improvement of the old silkworm races was recommended.

Keywords: sericulture; silkworm; *Bombyx mori* L.; breeds; old; breeding; improvement

1. Introduction

A very important element in connecting European identity with its historical past is the preservation and even reintroduction of traditions and tangible elements and engaging contemporary Europeans with their historical past. In the case of sericulture, one such tangible element is the indigenous (European) breeds of silkworms, which offer European cocoon producers a link to their traditions and identity. The conservation of agrobiodiversity is an effort undertaken by a number of countries in an attempt to prevent the loss of genetic diversity of plants and animals, including the silkworm. (Bindroo and Moorthy [45]; Bajwa et al. [43]; Chandrakanth et al. [47]; Chikkaswamy and Paramanik [48]; Devi et al. [49]; Jingade et al. [52]; Kumari et al. [59]; Moorthy et al. [67]; Nezhad et al. [71]; Seong et al. [81]. By providing the opportunity to raise such indigenous silkworm breeds, modern practices could be linked to local traditions. It is clear that this would be a slow and difficult process that would require complex actions, but it is the only way to maintain the traditions, local agrobiodiversity and local identity of the regions in Europe that are closely linked to silk production and silkworm rearing. It is known that most of the old local European breeds of silkworms, such as Yellow Local in Bulgaria, Brianza in Italy, Var in France, Sierra Morena in Spain, Kakhetian Green and Kutaisi Orange in Georgia, etc. are characterized by colored cocoons [13–25]. At the same time, after the 1950s, almost all countries with developed sericulture switched to mass breeding of white-cocoon hybrids. In addition to the fact that this led to a decrease in local biodiversity, the use of imported silkworm hybrids proved unsuccessful in preventing the decline of sericulture in Europe, as observed in the second half of the 20th century. Although they were once considered a solution to improve silk production in Europe, these imported white-cocoon hybrid silkworms practically caused local and other breeds with colored cocoons not to be targeted selection for vitality and productivity, but only to be maintained as a gene pool. The lack of targeted breeding and improvement work has led to relatively low values of the main quantitative traits shaping the productivity and quality of silk in breeds with colored cocoons compared to white cocoon breeds used for industrial hybridization. In order to improve the main quantitative traits in breeds with colored cocoons, it is necessary to cross them with highly productive white cocoon breeds and subsequent selection in order to consolidate the valuable economic traits in the offspring, but while preserving the qualitative traits typical of these breeds, such as the color of the larvae, color and shape of the cocoon. In this respect it is important to study the old European breeds, qualitative and quantitative traits performance during the first half of 20th century in Bulgaria, when those breeds were widely used in the practice and were the basis of sericultural industries at that time.

2. The Sericulture Industry, Local Silkworm Breeds and Egg Production During the End of 19th and First Half of 20th Century in Bulgaria

The sericulture reached the Balkan peninsula in 7th – 8th century AD [2,6,27,30,33,36]. It is considered that the sericulture in Bulgaria was adopted at the times of first Bulgarian empire, in the end of 10th century AD. [8,19]

It was reported that in 13th and 14th century AD, the times of second Bulgarian empire there were some villages and single households whose main incomes were from sericulture and silk production [3,9,11,27,33]. The Spanish traveler from 12th century AD, Tudelski wrote that the sericulture at the Balkan peninsula was so well developed that the cities were full of silk [31–33].

During the Russian – Turkish for liberation of Bulgaria war in 1877 – 1878 many mulberry trees were cut. [24] After this war there was a mass migration of the Turkish nationality inhabitants to

Turkey and the labor force decreased. Besides the Turkish left many fertile lands which the local Bulgarians started to use for cereals production, thus the sericulture was almost forgotten (1, 26, 36). So, in 1880 due to the above reasons, and also the pebrine disease spreading the sericulture almost disappeared in newly liberated Bulgaria [10,37].

After 1890 an economic crisis happened in Bulgaria leading that most of the population got poorer. In this situation the sericulture provided good cash incomes, thus the cocoon production increased very rapidly. For example, in 1896 about 3 million mulberry saplings were produced and planted in Bulgaria [20,25]. Besides, many mulberry saplings were imported from Turkey as well [25,30]. In 1892 the first silk reeling factory, having 1000 ends was established in South Bulgaria [2,16]. It was equipped by comparatively modern for that time Italian multiends reeling machines, Batalia system [16].

The first statistical data about fresh cocoon production in Bulgaria are from 1886 when 291 tons of fresh cocoons were produced [8].

In 1896 in Vratsa the Sericulture Experiment Station was established [17,19,25,30,34].

For a period of 12 years from 1890 to 1902 the fresh cocoon production in Bulgaria was increased about 10 times and reached about 1000 tons annually [26]. This period of sericulture flourishing continued to the Balkan war, the inter-allied wars and 1st World war. The wars led to a sharp decline of cocoon production. However soon after the wars due to the increase search for silk at the market the cocoon price increased as well [25,36]. In 1929 the fresh cocoon production in Bulgaria was 2400 tons [25]. An analysis of the Bulgarian export trade in 1928 showed that the dry cocoons occupied 4th place by value among the Bulgarian goods exported [2,19]. During the period 1925 – 1929 the number of sericulture households was 70 – 80000 [19].

After 1929 suddenly a sharp decrease of silk price at the international market occurred which reflected to the cocoon price, at that time most of the cocoons were exported to Milan and Marseille (25). As a result, the annual fresh cocoon production dropped from 2300 tons in 1930 to only 1100 tons in 1931. [16,25]

Until 1930 the Bulgarian traders were satisfied to export dry cocoons due to their good prices. With the decreased cocoon prices and difficult finding markets for them some traders tried to process the cocoons inside Bulgaria and as a result for only several years dozens of silk reeling mills were established which started to reel all the cocoons produced in Bulgaria [36]. At the same time some silk weaving mills were also established. In order to stimulate the local silk industry development, the government determined protective duties for the imported silk goods [11,25]. Such silk weaving mills were opened in Ruse, Vratsa, Plovdiv, Sofia and Karlovo cities. For a comparatively short period of time during 30^{ths} of 20th century a considerable silk industry was established in Bulgaria. This industry included 1500 silk reeling mills with a capacity of 3000 t fresh cocoon processing annually, 480 mechanical silk weaving looms with an annual capacity of about 1400000 m silk fabrics, silk twisting machines with 10000 spindles and 60 tons of twisted silk annual capacity, 15 silk sock knitting machines with 100000 pairs of socks annual capacity [16,17,25,36,38,39,42].

In 1883 the first silkworm eggs, produced by Pasteur's method were imported in Bulgaria [11,17]. The local egg production by the Pasteur's method started in 1895 in Bulgaria when 2100 boxes (12 g) were produced [26]. In parallel with the locally produced eggs some silkworm eggs had also been imported from France, Italy and Turkey until 1930 [25]. The biggest local silkworm egg production was reached in 1926 when 214000 boxes (12 g) were produced [30]. After 1931 the local egg production was stabilized for a long period of time at around 125000 boxes (12 g) annually [28]. A part of the silkworm eggs locally produced was exported to Iran, Romania, Poland, Yugoslavia, Czechoslovakia, USSA, India etc. [35], what is a good proof about the high egg quality produced. Since 1896 the Sericulture Experiment Station in Vratsa has organized many training courses for silkworm egg producers [16,25,40]. At the same time some egg producers graduated training courses in the sericulture stations in Bursa, Montpellier, Padua, Ascoli, Piceno, Tbilisi etc. [34,35]. Until 1947 the local egg production was in the hands of 60 – 85 local private producers, working mainly in the regions of Vratsa, Teteven, Bjala Slatina, Botevgrad, Harmanli, Svilengrad, Ivailovgrad, Haskovo,

Plovdiv and Asenovgrad cities [30,34,41]. In 1947 the “Cooperative of Bulgarian silkworm egg producers” was created and in 1950 the whole egg production was taken by the Textile fibers state enterprise [34,35]. The egg production was centralized in 4 big factories, situated in Vratsa, Berkovitsa, Plovdiv and Harmanli cities [28,29]. In 1996 the egg production factory in Vratsa with the Sericulture Experiment Station were moved to the Agricultural academy and the rest three factories remained in Sirma JSC, then after its privatization in 1997 the egg production factories were closed.

Since the beginning of a local egg production by Pasteur’s method in 1895 mostly two local silkworm breeds have been reared in Bulgaria, namely Yellow local and White Baghdad. The origin of Yellow local breed is supposed to be from the locally spread in North and South-West Bulgaria race in 16th – 19th century [7]. The White Baghdad originated from Asia Minor [15].

The Yellow local race was reared in whole North and in South West Bulgaria. The Yellow local breed was characterized with yellow cocoon color and cocoons with elongated shape with slight constriction [14,18].

The White Baghdad race was reared in South East Bulgaria and consisted of 3 types, namely Edirne type, Improved Bulgarian type and Bulgarian type [21]. White Baghdad race cocoon color varied from snow-white to light green color, but the prevailing color was white [22]. The cocoon shape was elongate with constriction [23]. The local silkworm races were grown mostly as pure breeds until the 60s of 20th century and after that gradually they were shifted by white cocoon hybrids [12,28]. The Yellow local race is still preserved at the Scientific Center on Sericulture, Vratsa. At the Center is also maintained a breed called “White Bulgarian” which it is supposed to originate from one of White Baghdad types [4,5].

3. Characteristics of the Silkworm Breeds Reared and Tested in Bulgaria During the First Half of 20th Century

Dushev [18] studied in 1931 – 1932 at the Sericulture Experiment Station in Vratsa the larval color and markings in the Italian silkworm breeds Perudjia, Majela, Abruco, Askoli, Grand Saso, Umbrija, Bione, and Brianca, in the French breeds Var and Yellow Alpin, and in the Bulgarian local silkworm breeds Yellow local and White Baghdad. From the description of the different breeds and types, it could be seen that the color of their skin was white, zebra, gray-brown, dark brown, gray-reddish brown, chestnut and velvet. It should be noted, however, that the predominant color among all breeds and types was white. It constituted 66.40%. It was followed by the zebra color with 20.78%, gray-brown – 8.4%, dark chestnut – 3.36%, chestnut – 1.48%, gray-reddish – 0.84% and velvet – 0.84%. By virtue of their mask and the crescents that occur on 2nd, 5th and 8th vertebrae, the larvae were divided into three categories: with a stronger, medium and weak intensity of their color. Especially in the local Bulgarian breeds the larval color and markings observed were as follows:

Yellow local breed: 1. White, no mask and no moons. 2. White, medium mask, weak moons. 3. White, strong mask, strong moons. 4. Zebras, medium mask, no moons. 5. Zebras, strong mask, strong moons. 6. Gray-brown, no moons, strong mask. 7. Gray-brown zebras, no moons, strong mask. 8. Gray-brown, zebras, with moons, and strong mask.

White Baghdad breed: 1. White, no mask and no moons. 2. White, weak mask, weak moons. 3. White, medium mask, weak moons. 4. White, stronger mask, weak moons. 5. White, strong mask, strong moons. 6. Zebras with pigment, no moons and medium mask. 7. Zebras, with a strong mask, without moons. 8. Zebras, with a strong mask, weak moons and with pigment. 9. Zebras, with a strong mask, strong moons. 10. Zebras, with pigment, with a strong mask and strong moons. 11. Gray-brown, light mask, around the false legs light pink spots with weak moons. 12. Gray-brown with a darker mask, around the false legs light pink spots with stronger moons. 13. Gray-brown, darker mask, around the legs brick spots, with weak moons. 14. Gray-brown, darker mask, around the legs brick spots, with strong moons. 15. Gray-brown, zebras, strong mask, strong moons. 16. Gray-brown, zebra-like, strong mask, strong moons, brown-red spots around the legs.

It is evident from those data that the local silkworm breeds reared at that time in Bulgaria performed a wide diversity according to the larval color and markings.

According to Dushev [14] in order to meet the country's needs for yellow cocoon silkworm breeds, in 1932 the Bulgarian Agricultural Bank delivered 7,000 ounces of silkworm eggs of pure Yellow cocoon Italian breed from Italy for distribution to the sericulturists in Northern Bulgaria. Simultaneously with this delivery, the bank also purchased the Yellow local cocoon breed eggs, produced by the local egg producers, which in 1932 amounted to 3,340 ounces. The silkworm eggs purchased by the bank, both foreign and local, were stored in the winter storage of the SES - Vratsa, which was tasked, with the help of the district agronomists, with organizing their distribution among the sericulturists in the country who raised the larvae of the yellow cocoon breed. The way in which the distribution of the eggs was organized allowed the station to carry out this distribution according to a strictly defined plan, with the eggs of Italian origin being sent to some regions and that of local origin to others. In this way, it was possible to avoid the possibility of mixing the cocoons that would be obtained from the two groups of breeds and to see on a larger scale what quality cocoons are obtained from the yellow cocoon Italian breed and what from the Local yellow cocoon one. The following studies were carried out on the cocoons and the silk obtained from them: 1) Observations on the color, shape and structure of the cocoons. 2) The dimensions of the cocoons. 3) Raw silk renditta of the cocoons. 4) Length of the silk thread in a cocoon. 5) The silk titer. 6) Elasticity and strength of the silk thread. In terms of cocoon color and shape, there was no marked difference between the cocoons of the Yellow Italian and the Yellow local breeds. However, a larger difference emerged between the nature of cocoon grains of these two types: the cocoons of the Yellow Italian breed had a finer structure than those of the Yellow local breed.

Table 1. Yellow cocoon breeds cocoon size, renditta and silk filament length.

№	Breed	Cocoon size				Renditta, kg		Silk filament length, m	
		I grade		II grade		I grade	II grade	I grade	II grade
		Length, cm	Width, cm	Length, cm	Width, cm				
1	Yellow Italian	3.61	1.69	3.51	1.71	3.251	3.439	713.6	583
2	Yellow local	3.55	1.71	3.57	1.69	3.192	3.609	657	666.5

In terms of size and yield, the cocoons of the Yellow Italian and Yellow local breeds did not differ much from each other. In terms of titer, the silk of the Yellow Italian breed (of I and II grade cocoons) was higher than the Yellow local breed. In terms of elasticity and strength, the silk of the Yellow Italian breed, 1st grade, stand higher, however, right next to it with a slight difference, stand the silk of the Yellow local breed. Considering the differences obtained in the study of the different qualities, as well as the method of this study, as a general conclusion was that in terms of cocoons and silk, there was no any significant difference between the Yellow Italian and Yellow local breeds. A stronger or weaker superiority on the part of the Yellow Italian breed or the Local one was due more to the care in larval feeding than to the qualities of the breed itself.

Table 2. Yellow cocoon breeds raw silk titer, elasticity and strength.

№	Breed	Titer, denier						Elasticity						Strength					
		I grade			II grade			I grade.			II grade			I grade.			II grade		
		Max	Aver	Min.	Max	Aver	Min	Max	Aver	Min	Max	Aver	Min	Max	Aver	Min	Max	Aver	
1	Yellow w Italia n	11	16.6	13.49	10.4	16.4	13.18	63	240	171.7	38	258	147.5	32	62	47.8	26	53	40.7
2	Yellow w local	10	15	12.47	8	15.4	12.25	64	242	167.5 5	34	235	139.0 5	30	55	43.9	25	52	40.07

The studies of Dushev [21] on the White local Baghdad breed revealed that the variety in color, which was found among the larvae of Bulgarian white Baghdad breed, testified that the white Baghdad breed raised in Bulgaria country was not a pure breed, but a mixture of different breeds (White Bursen, White Edirne). The French white Baghdad breed also studied, thanks perhaps to a more systematic selection work on the part of the egg producers, represents a much greater degree of uniformity in this respect than Bulgarian white Baghdad breed. The local white Baghdad breed resisted the grasseria (NPV) disease more than the Yellow breed, the Golden Chinese and the crossbreeds, however, this breed had no immunity to the disease. Compared to the Yellow Italian and the Golden Chinese breeds, the moths of the white Baghdad breed lived almost twice as long as the Italian breed and 3 times as long as the Chinese.

The cocoons of Bulgarian white Baghdad breed did not have one established typical white color, as this may be the case for the white Chinese race. The white color of the Bulgarian white Baghdad breed was broken down into 5-8 different types of colors, which varied from snow-white to reseda. In the cocoons, the predominant white color was snow-white with a milky tint, which was also the highest percentage among the cocoons. The percentage of snow-white color reached only 10% in Bulgarian Baghdad cocoons, while in the French Baghdad, it was almost 50%.

As for the color of the white breeds from France, it should be noted that especially for the white breed, the color of the cocoons was distinguished by its predominant snow-white color. Almost all groups of this type had a markedly uniform color and attracted attention with the uniformity of their color.

The yellow local breed had the following two main colors: pale pink color with a yellow tint and pale pink with an orange tint. It was noted that in color, the cocoons of all groups showed a significant homogeneity. All this indicated a comparative purity of the yellow breed, which was reared in Bulgaria.

By shape, the cocoons of Bulgarian white Baghdad breed were divided into 3 categories: weakly attached in the middle, medium and strongly attached. In the French cocoons of the white Baghdad breed, there were only two shapes: weakly attached and medium attached ones. Strongly attached ones were absent. The largest percentage (45%) in Bulgarian white Baghdad cocoons were medium attached, followed by strongly attached ones (20%). The predominant structure of Bulgarian white Baghdad breed was medium-grained. However, the coarse-grained structure prevailed over the fine-grained. The average volume of the cocoons of Bulgarian white Baghdad breed was 10 cubic cm. The male cocoons of Bulgarian white Baghdad breed had an average length of 4 cm., and the female cocoons 4.44 cm. The length of the male and female cocoons of the French white Baghdad breed was smaller than that of Bulgarian white Baghdad cocoons. The average width of Bulgarian white Baghdad male cocoons was 1.93 cm, and of the female cocoons 2.09 cm. In terms of width, the cocoons of the French white Baghdad breed were almost the same as Bulgarian white Baghdad. Both in male and female cocoons of Bulgarian white Baghdad breed, a certain asymmetry was noticeable, which was expressed in the fact that one side (the right) of the cocoon was wider than the left. In male

cocoons, the difference between the right and left width was on average 0.095 cm. and in female cocoons 0.10 cm. The average weight of male cocoons of Bulgarian white Baghdad breed was 2.378 grams, the maximum – 3.210 grams, the average weight of the silk shell in the same cocoons was 0.390 grams. The average weight of female cocoons of the white Baghdad breed ranged from 3.076 – 3.326 grams, and the silk shells were 0.463 grams. The percentage of double cocoons in Bulgarian white Baghdad breed ranged from 1.6 – 3, in under-wrapped ones from 0.6 – 1.37, and in satin ones from 0.01 – 0.04. The average renditta from the local white Baghdad breed was 3.3591 kg, and of females 3.6088 kg in industrial cocoon reeling. In the same cocoons in single cocoon reeling, the average renditta of male cocoons was 3.0015 kg, and of females 3.2516 kg. The percentage of frizon to cocoons in Bulgarian white Baghdad breed was 9.03. The average length of the silk thread in male cocoons of Bulgarian white Baghdad breed was 808.64 m., the maximum – 1153 m. and the minimum 600 m., in female cocoons the average length was 891 m., and the maximum 1500 m. The average length of the thread from Bulgarian white Baghdad breed exceeded the length of the silk thread from the French white Baghdad breed by 62 m.

It may be concluded that the old silkworm breeds, reared in Bulgaria during 20th – 30th years of the 20th century were generally characterized with not very uniform larval color and markings, cocoon color and shape, comparatively high cocoon weight, but lower silk shell percentage and filament length as well as they were comparatively tolerant to NPV disease. Due to the long maintenance of those races in Bulgaria they were well adapted to the local food and climatic conditions.

4. Possibilities for Using Some of the Old European Breeds Reared in Bulgaria in The Modern Sericulture

Present Status of Old European Breeds in Bulgaria

The local strain Yellow local was grown mostly as pure breed until the 60s of 20th century [35,36] and after that gradually it was shifted by white cocoon hybrids. Presently among of the two old silkworm breeds White Baghdad and Yellow local, only the strain Yellow local is maintained at the Scientific Center on Sericulture (SCS) - Vratsa germplasm.

The following character values were detected in 2023 and 2024 [4,5]: hatchability, total larval period and 5th instar duration, pupation rate, fresh cocoon weight, silk shell weight, silk shell ratio, fresh cocoon yield by one box of silkworm eggs, silk filament length and weight, reelability and raw silk percentage. As controls for comparison two popular Bulgarian white cocoon breeds, namely Super 1 (Japanese type) and Hesa 2 (Chinese type), parents of the widely adopted Bulgarian F1 hybrid Super 1 x Hesa 2 and the reciprocal cross were used. The data obtained are presented in Tables 3 and 4.

Table 3. Breeding characters in the strain Yellow local average for 2023 and 2024.

Breed	Hatchability (%)	Larval period duration, h	5 th instar duration, h	Cocoon yield by one box of silkworm eggs, kg	Pupation rate, %	Fresh cocoon weight, mg	Silk shell weight, mg	Silk shell percentage %
Yellow local	98.09	698	147***	29.69	90.92*	1782**	318***	17.85***
Super 1 (control)	98.06	718*	166	33.20***	92.10***	1954** *	407	20.83**

Hesa 2 (control)	97.13	700	170	30.37	89.80	1842	395	21.44
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*The data were processed statistically towards the breed Hesa 2. *P < 0.05; **P < 0.01; ***P < 0.001.

It is evident from the data obtained that Yellow local silkworm strain performed a high hatchability. The total larval period duration was not much different, compared with the controls, but the 5th instar duration was significantly shorter in the Yellow local strain. The pupation rate was higher than the Hesa 2 breed and lower than Super one breed. The fresh cocoon yield by one box of eggs in Yellow local breed was not significantly different, compared with the Hesa 2 race and a lower value of this character was detected, compared with the race Super 1. As for the fresh cocoon weight in Yellow local strain, it was significantly lower than in the two controls. The silk shell weight and percentage in the breed Yellow local performed considerably lower than in the breeds Super 1 and Hesa 2.

It may be concluded that the cocoon weight, silk shell weight and percentage values of the old strain Yellow local are lower than those parameters in the white cocoon breeds, used as controls.

Table 4. Silk filament technological characters in the strain Yellow local average for 2023 and 2024.

Breed	Filament length, m	Silk filament weight, mg	Reelability, %	Raw silk percentage, %
Yellow local	925***	272***	87.78***	36.33***
Super 1 (control)	1168*	354	90.05	38.67
Hesa 2 (control)	1225	350	90.53	39.65

*The data were processed statistically towards the breed Hesa 2. *P < 0.05; **P < 0.01; ***P < 0.001.

It is evident from table 4 that the Yellow local silkworm strain manifested considerably lower values of the silk filament length and weight, reelability and raw silk percentage characters, compared with the two Bulgarian white cocoon breeds, used as controls.

It may be concluded that the Yellow local strain displays a high hatchability and survivability at optimal rearing conditions, shorter 5th instar duration and a comparatively good reproduction capacity. On the other hand, the Yellow local strain manifests lower values of the main productive character values such as cocoon weight, silk shell weight and percentage, silk filament length and weight, reelability and raw silk percentage characters, compared with the two Bulgarian white cocoon breeds, used as controls. Therefore, in order to commercialize the Yellow local strain in nowadays it needs improvement by the methods of genetics and breeding.

The silkworm breed improvement is achieved mainly by crossing with races, having the desired characteristics and/or manifesting a high heterosis expression [53,55–57]. According to a number of scientists [54,60,61,70,73,77], the industrial crossbreeding between two or more initial forms (breeds, lines) with different geographical and genetic origin is one of the most effective methods for creating high heterosis silkworm hybrids and their use in the production. According to these authors, the superiority in F₁ hybrids over the average parental value of their initial forms in the main productive traits is in the range of 10 – 30%. The most effective in practice and hybrid-creation is the crossbreeding of inter-race populations of different geographic and genetic origin, and also between univoltine and bivoltine with poly-voltine races [74,82,84].

In order to improve the main quantitative traits in some old breeds with colored cocoons, it is necessary to cross them with highly productive white cocoon breeds and subsequent selection in order to consolidate the valuable economic traits in the offspring, but while preserving the qualitative traits typical for these breeds, such as larval markings, cocoon color and shape. In this regard, it is of particular importance to know and study the inheritance of these qualitative traits in hybridization between breeds with colored and white cocoons. According to Tazima [85], Lea [62] and Lim [63] the

most common markings of the larvae is “lack of color” and is most often considered the main allele that has mutated and as a result other types have appeared. The larva with the genotype p/p is always without any markings, while the larva with the genotype +p/+p has the following three patterns: an “eye spot” (mask) on the dorsal thoracic part, a “half moon” and “star-shaped spots”, located on the dorsal part of the second and fifth abdominal segments, respectively. The +p allele comes in four different varieties: p1, p2, p3, p4, which determine in ascending order the intensity of the markings of the larvae. All these groups are multiple alleles located on the second chromosome, locus 0.0 and always determine the trait of markings of the larvae. Songyuan et al. [83] have identified the ‘mamo’ gene as a novel regulator of pigmentation in the silkworm *Bombyx mori*, a function that had not previously been suspected based on extensive work in *Drosophila*. The evidence supporting a role for Bm-mamo in pigmentation is compelling, including high-resolution linkage mapping of two mutant strains, expression profiling, and reproduction of mutant phenotypes with state-of-the-art RNAi and CRISPR assays.

The color of the cocoon of *Bombyx mandarina*, known as the wild silkworm, is homogeneously brownish-yellow. The cocoons of the domesticated silkworm, *Bombyx mori* L., are impressively diverse in terms of their color and can be divided into three categories according to the type and content of pigments: I - yellow-red cocoons, colored by carotenoids, and also golden, pink, rusty, etc. II - green cocoons, colored by flavonoids, having a pale green to deep green color and III - white cocoons with traces or no pigment (Sakudoh et al. [78]; Daimon et al. [50]; Sakudoh et al. [79]; Sakudoh et al. [80]). The absorption and modification of pigments from mulberry leaves in *B. mori* L. is the key to the formation of colored cocoons. This process is influenced by the genotype of the silkworm and the content of carotenoids in the midgut, hemolymph and silk gland [88]. These factors influence the determination of the color of the cocoons (Sakudoh et al. [79]). Murakami and Ohtsuki [69] in hybridization between the Cambodia breed, which has a yellow-green color of the cocoon, and the white-cocoon breed Japanese 106 found that in the F1 all cocoons had a yellow-green color. According to Doira [51] and Lim [63] the genes responsible for the yellow color of the cocoon are known, namely C – golden yellow, Cd – light yellow, Ci – yellow inner layer of the silk shell, dy – tangerine yellow hemolymph, Yc – yellow inner layer, Yf – yellow fluorescent, Yr – yellow brown. According to Tazima [85] and Banno et al. [44] the genes I and Is, known as yellow inhibitors, suppress the action of the Y gene and cause the white color of the cocoon. Kovalev and Sheveleva [58] reported that larvae with yellow hemolymph are capable of spinning both yellow and white cocoons, while larvae with colorless hemolymph spin only white cocoons. The Y gene (yellow hemolymph) controls carotenoid uptake in the intestinal mucosa and silk gland. Mutant larvae homozygous for the recessive Y allele inadequately absorb carotenoids from mulberry leaves, resulting in colorless hemolymph and white cocoons. At least six genes that control carotenoid-based cocoon color function only when co-existing with the dominant Y allele, suggesting that the Y gene plays a central role in cocoon color (Sakudoh et al., [78]). In *Bombyx mori* L., mutation of the carotenoid-binding protein (CBP) (Y locus) and/or absence of the *cameo2* gene (C locus) has been shown to result in white cocoon formation (Sakudoh et al., [78,79]; Banno et al., [44]). According to Lea [62], the genes responsible for green cocoon color are Ga, Gb, and Gc. Wilai et al. [90] crossed female moths of the Thai polyvoltine breed with males of the wild silkworm *Bombyx mandarina* and found that in the first generation the cocoon color was light green, like that of the wild silkworm. Mirhosseini et al. [66] conducted a study with five local silkworm breeds in Iran, including Baghdad, Khorasan Orange, Guilan Orange, Khorasan, crossed with two white-cocoon breeds 107 and 110 and found that the colored cocoon trait dominated over the white cocoon trait in F1. The breakdown obtained in the F2 generation was 3 parts colored and 1 part white cocoons. In the first backcross (BC1), the breakdown was 1 part colored and 1 part white cocoons. The intensity of the cocoon color in F2 and BC1 had significant differences, therefore it is assumed that the genes responsible for cocoon color in these breeds are represented as multi-alleles in the Iranian local breeds of silkworms with colored cocoons. Two main types of pigments are responsible for the color of the cocoon, namely, the ether-soluble yellowish carotenoids and the water-soluble green flavonoids (Tsuchida et al. [86];

Bhosale et al. [46]). Xu et al. [87] analyzed metabolic changes in pigments in the silkworm. The results showed that flavonoids accumulated in the midgut of 5th instar larvae, as well as carotenoids accumulated in the silk gland. It was found that the color of the cocoon is closely related to the content of both types of pigments in the silk gland. The pigments are absorbed from mulberry leaves in the midgut and then transported through the hemolymph to the silk gland to produce a cumulative effect. Yasuhiro et. Al. [89] determined in the wild silkworm *Antheraea yamamai*, which spins cocoons with green – yellow color some cocoons having less yellowish color, called emerald green (EG). The authors studied the inheritance of the EG mutation by crossing individuals that produced EG cocoons with those that produced normally colored cocoons (NG). It was found that the F1 offspring from the reciprocal cross between EG and NG individuals produced predominantly NG cocoons. It was concluded that the EG mutation is controlled by a single recessive gene locus.

Lin Zhua and Yu-Qing Zhang [64] conducted studies on the larval tissues and colored cocoons of silkworms, *Bombyx mori* L. (Lepidoptera: Bombycidae), which were fed with leaves of a cultivated mulberry, cultivar Husang 32. It was found that mulberry leaves mainly contained four types of pigments: lutein (30.86%), β -carotene (26.3%), chlorophyll a (24.62%), and chlorophyll b (18.21%). The silk glands, hemolymph, and cocoon shells of six yellow cocoon breeds were examined. The results showed that there were generally two types of carotenoids (lutein and β -carotene) in the silk gland and cocoon shell, little violaxanthin was found in the silk gland, and the pigment found in the hemolymph was mainly lutein.

The shape of the cocoon depends on the movements of the larva during its spinning, which in turn is under neurophysiological control [68]. Murakami and Ohtsuki [69] have found that the spindle-shaped shape of the cocoon is usually associated with a wavy structure of the silk shell. At the same time, the spindle-shaped shape of the cocoon is undesirable from the point of view of silk reeling because it leads to lower reelability [58]. According to Mano [65] and Ravindra Singh et al. [75,76] the evenness of the cocoon shape contributes to the production of a silk thread with a uniform thickness. According to Lea [62] it is difficult to determine a specific gene for a specific cocoon shape because its variation is like a quantitative trait, namely it is probably determined by many genes. Kovalev and Sheveleva [58] found that when crossing white-cocoon monobivoltine breeds of Japanese and Chinese type, the cocoon shape in F1 is always inherited intermediately as a quantitative trait, and in F2 a breakdown with many transitional forms from elongated with an intercept to an oval shape of the cocoon is observed. Murakami and Ohtsuki [69] when crossing the bivoltine breed C 108, characterized by an oval shape of the cocoon, with the Cambodia breed, which has a spindle-shaped cocoon, found that in F1 all cocoons were of the shape of C 108, namely oval, while in F2, 3 parts of cocoons with an oval shape and one part with a spindle-shaped shape were broken. According to Babu et. al. [72] less variation in the shape of the cocoon was found in F1 hybrids between polyvoltine and bivoltine breeds with oval cocoons compared to F1 hybrids between polyvoltine breeds and bivoltine with elongated intercepted cocoon shape. In this study, bivoltine hybrids showed less variation in cocoon shape compared to hybrids between polyvoltine and bivoltine breeds. In bivoltine hybrids, the cross between the mother with an oval cocoon shape x the father with an elongated intercepted cocoon shape showed less variation compared to the cross between the mother with an elongated intercepted cocoon shape x the father with an oval cocoon shape.

5. Possible Research Work in old European Silkworm Breeds Improvement

As mentioned above in Bulgaria the Yellow local strain was an object of a purposive selection until the 60s of 20th century and after that it was just maintained as genetic material. It led to keeping the quantitative character values near to the original ones, but without any further improvement. For the first half of 20th century the Yellow local strain was sufficiently viable and productive. However later on new commercial breeds and F1 hybrids were created, characterized with higher silk shell weight and percentage.

Considering the results obtained in our studies with the Yellow local strain, we came to the conclusion that in order to commercialize the breed in nowadays it needs improvement by the methods of genetics and breeding. For the purpose we developed the following breeding plan:

- Testing the Yellow local silkworm strain performance under unfavorable rearing conditions during the fourth and fifth instars.

This study aims to detect the Yellow local strain sturdiness in order to take decision whether the strain needs also of viability improvement.

The adverse 4th and 5th instars larvae silkworm rearing conditions are as follows:

Mode of silkworm rearing	temperature, °C	Relative air humidity, %	Feeding space	Feeding amount	Ventilation
Adverse rearing conditions	29 - 30	80 – 90 %	11 m ² for 1 box of silkworms	1 feeding daily	Thickly closed windows and door
Standard technology	23 - 25	55 – 70 %	22 m ² for 1 box of silkworms	2 feedings daily	Two windows open, the door – open if necessary

- Study on qualitative and quantitative characters inheritance in hybridization between the Yellow local strain and the white cocoon breeds VB1 and HB2 under standard and adverse rearing conditions.

This study aims to develop a breeding methodology for improvement of Yellow local strain by crossing with white cocoon breeds and at the same time keeping the strain typical qualitative characters such as larvae with zebra markings and yellow cocoon color.

The breed VB1 is of Japanese type, having larvae with normal markings and spinning white cocoons with elongated shape and slight constriction. The breed HB2 is of Chinese type, characterized with plain larvae and white oval cocoons. Both the breeds possess comparatively high tolerance to rearing at adverse conditions and a moderate productivity. The following generations will be studied: P1, P2, F1, F2, F3, BC.

- Study on F1 crosses between the Yellow local strain and some white cocoon highly productive breeds.

This study aims to test the F1 hybrids in order to use the Yellow local as one of parents of F1 hybrids which demonstrate higher productivity and by the same time having zebra larval marking and yellow cocoon color. The following two F1 hybrids will be tested: Super 1 x Yellow local and Hesa2 x Yellow local. Depending on the results obtained some F2, F3 and BC generations will also be further produced and investigated, aiming at possible creation of pure lines with the Yellow local quantitative characteristics, but with higher cocoon and shell weight and raw silk percentage.

6. ARACNE Project

ARACNE project which started in March 2023 has a duration of 36 months and involves 11 partners and 3 associated partners from 7 European union (EU) and non-EU countries. (<https://aracneproject.eu/>)

The project, whose name is inspired by the weaver transformed into a spider by the goddess Athena in Greek mythology, aims to exploit silk as the common element of the pan-European culture

and history. ARACNE has the ambition to contribute to the creation of a broad and connected innovation ecosystem related to silk in Europe, including the industrial sector, and intended as a tool for expressing cultural and landscape heritage, thus, connecting culture, tradition, and new industrial production within an ideal network of exchanges and visions.

The aim of the activities concerning the old European silkworm breeds is to gradually re-introduce the local silkworm races to individuals and stakeholders who are interested in rearing them either for educational purposes, cocoon production purposes, artistic purposes or any other purpose that such stakeholders find suitable. By connecting with local silkworm farmers and giving them the opportunity to rear such local silkworm races the project attempts to re-introduce a sense of identity and link modern practices with local traditions.

7. Conclusions

It may be concluded that the old silkworm breeds Yellow local and White Baghdad, reared in Bulgaria during 20th – 30th years of the 20th century were generally characterized with not very uniform larval color and markings, cocoon color and shape, comparatively high cocoon weight, but lower silk shell percentage and filament length as well as they were comparatively tolerant to NPV disease. Due to the long maintenance of those races in Bulgaria they were well adapted to the local food and climatic conditions.

Presently the Yellow local strain displays a high hatchability and survivability, comparatively shorter 5th instar duration and a good reproduction capacity. On the other hand, the Yellow local strain manifests lower values of the main productive characters such as cocoon weight, silk shell weight and percentage, silk filament length and weight, reelability and raw silk percentage, compared with the modern commercial Bulgarian white cocoon breeds. Therefore, in order to commercialize the Yellow local strain in nowadays it needs improvement of the silk yielding traits while preserving its qualitative characteristics by using the methods of genetics and breeding.

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