# ARACNE

## ADVOCATING THE ROLE OF SILK ART AND CULTURAL HERITAGE AT NATIONAL AND EUROPEAN SCALE

Funded by the European Union

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## Deliverable 1.6

### Silkworm race selection rearing report

Version 1.0

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Dissemination Level

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| ☒ | **PU:** | Public |
| ☐ | **SEN:** | Sensitive |
| ☐ | **PP:** | Restricted to other programme participants (including the Commission Services) |
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| ☐ | **CO:** | Confidential, only for members of the consortium (including the Commission Services) |

## Abstract

Deliverable 1.6 is intended to provide an overview of the work done in the first year of the ARACNE project on the rearing and popularisation of local European silkworm races by several partners within their country. Deliverable 1.6 is a continuation of Deliverable 1.5 and provides evidence for each of the selected local (European) silkworm races and, most importantly, the steps that needs to be taken to achieve a level of self-sufficiency and self-reliance for the European countries that seek to revive their sericultural tradition by using local silkworm races and not relying on imports of foreign silkworm eggs that are, as a general rule, not suited to the European climatic conditions. The document also presents a model methodology of maintaining and improving a local silkworm race and the steps taken to popularise its use by local farmers.

# Partners involved in the document

| Participant n. | Participant organisation name | Short name | Check if involved |
|---|---|---|---|
| 1 Coordinator | Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria | CREA | X |
| 2 | Iniziativa Cube S.r.l. | INI | |
| 3 | Lepl State Silk Museum | SSM | X |
| 4 | Nauchen Tsentar Po Bubarstvo Vratsa | SCS | X |
| 5 | Piraeus Bank Group Cultural Foundation | PIOP | |
| 6 | Univerza V Mariboru | UM | X |
| 7 | Ethniko Kai Kapodistriako Panepistimio Athinon | NKUA | X |
| 8 | Instituto Murciano de Investigacion y Desarrollo Agrario y Medioambiental (IMIDA) | IMIDA | X |
| 9 | D'orica S.r.l. Società Benefit | DOR | |
| 10 | Chemins De La Soie - Des Cevennes aux Alpujarras | ASSOIE | |
| 11 | Sericyne | SER | |
| 12 | Universita degli Studi di Padova | UNIPD | |
| 13 | Council Of Europe - Conseil de L'europe | COE | |
| 14 | Mouseio Technis Metaxiou | ASMS | X |

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# 1. Description of the project

ARACNE project focuses on the cultural heritage of the European silk production and its preservation, protection and valorisation; it aims at reinvigorating traditional skills through the adaptive reuse of the common cultural and artistic legacy and at shaping a silk-linked European cultural identity.

The production and the past and present development of the silk sector can be again the common basis for a future European Silk Route intended as a cultural itinerary across Europe. To create a wide and well-connected network that, starting from the historical path followed by Marco Polo in his travels to East, even includes the routes of production and commercialization of silk in Europe in the following centuries, we aim to:

- Bring back silk production in vogue by reconstructing a resilient and innovative silk ecosystem that retraces the concerned European countries and promotes traditions, architecture, and both tangible and intangible heritages. The consolidation of a European Silk Route will encourage links and shared activities among European cities and regions to strengthen the preservation and protection of their culture and promote innovations in production and trade;
- Contribute to improve skills and competitiveness of silk-related European Cultural and Creative Industries through the renewal, co-development and the implementation of human-centered and place-specific silk-based cultural products, processes and service innovations, leveraging on digital applications and cutting-edge technologies, to foster the transition to more sustainable business models, and promote economic and social growth, and strengthen the reputation of European countries abroad.

## 1.1 ARACNE specific objectives

The overarching goal of ARACNE is to create a wide and well-connected Silk Innovation Ecosystem that, starting from the historical path followed by Marco Polo in his travels to East, also includes the routes of production and commercialization of silk in Europe in the following centuries. An innovation ecosystem is an interconnected network of quadruple helix stakeholders, including academia, industry and different levels of the public sector and civil society. This multi-level approach applies a systemic and bottom-up approach to creating research, innovation and knowledge. Silk Innovation Ecosystem includes every stakeholder and innovator in the cultural silk value chain even if not participating directly in the project activities. The production and, more in general, the past and present development of the silk sector in the ARACNE Consortium countries represent the common thread for the future "European Silk Route" as a cultural itinerary across Europe, to boost the European values in relation to the silk arts and CH for the benefit, prosperity, peace of our societies. To this aim, the project will explore the CCIs' capacities to create a cultural and artistic niche market where silk produced within EU boundaries will be valued as a distinct immaterial asset; on the other hand, the ambition is to contribute to stop the loss of technical, traditional and cultural know-how and skills that accompanied the decline of this fiber production and that

is detrimental exactly to those CCIs which might be active in fashion, art, design and product communication. In fact, the so-called "Silk Road" is generally associated to its Asian origin; however, its European ramifications were fundamental for the development of Europe as we know it today. More in general, the silk production (silkworm rearing, mulberry cultivation, silk reeling), originated from Asia but subsequently spread to Europe and developed strongly in the Mediterranean and Balkan regions. Bringing back silk production in vogue by reconstructing a resilient and innovative Silk Route that retraces the European countries and enhances traditions, architecture, tangible, and intangible heritage will demonstrate that silk, as a cultural legacy, can contribute to develop the European economy and enrich our society. In this context, ARACNE covers several sectors linked to content creation, conservation, exploitation, management, fruition, diffusion related to the silk historical, artistic and environmental resources and assets. The ambition of ARACNE will be reached through a set of specific, measurable, achievable, realistic and time-constrained (SMART) specific objectives:

**Objective 1:** Enhancement of knowledge and memory for the renaissance of a European Silk Innovation Ecosystem;

**Objective 2:** Co-creation of human-centred and place specific creative silk-based solutions leveraging on digital and cutting-edge technologies;

**Objective 3:** Implementation of innovative strategies and business, governance and financing models for the involved CCIs organisations and SMEs, building on previous research;

**Objective 4:** Support the establishment of a cultural European Silk Route, based on the tangible and intangible silk cultural heritage and landscapes;

**Objective 5:** Raise awareness of ARACNE results and impacts among different stakeholders of the territories and CCIs of the silk sector and raise the expectation for the constitution of a European Silk Route in support to the European silk CH and silk CCIs;

**Objective 6:** Enhance the European cultural identity and strengthen European competitiveness for a more resilient post-crisis society;

**Objective 7:** Contribution to the European Green Deal, the New European Bauhaus and the Sustainable Development Goals.

## 2. Introduction

In the following sections of this document the reader will be presented with specific details about the aims, the methodology followed and the results we have got so far regarding the rearing of local silkworm races within Europe. This report focuses on the work done on maintaining and improving the local (European) silkworm races that are scheduled, within the framework of the ARACNE project, to be used, popularised and introduced to European silkworm farmers. The work done by NKUA, CREA, SCS and SSM on maintaining, improving and distributing the local European silkworm races is presented.

Attention is paid in describing in plain language the activities, however, there are certain details and methodologies that require the reader to have a relatively good knowledge of genetics. Even at such instances, extensive use of scientific details is avoided so as to make the text easy to follow and understand.

## 2.1 Objective of the deliverable

This deliverable is an update of Deliverable 1.5 that was submitted on Month 1 of the ARACNE project. The objective of Deliverable 1.6 is to provide an update on the work outlined in Deliverable 1.5 and further provide details on the work carried out so far and the expected outcomes.

## 2.2 Document structure

The document is structured as follows: The following section (Section 3) describes the work that has been carried out by the ARACNE partners involved in introducing a flagship local silkworm race for each country and popularising its use. The texts will be devoted to presenting the work done by the ARACNE partners in maintaining and improving local silkworm races, as an examples on what can be done to popularise the use of European silkworm races by the European silkworm rearing farmers. After presenting a complete overview of the activities, the scope (section 3.1), timeline (section 3.2), criteria and resources (section 3.3), risks (section 3.5) and communication plan (section 3.6) for this activity are presented.

# 3. Local (European) silkworm races

This section of Deliverable 1.6 will present a description of the work done on the silkworm races selected for each country that participates in the ARACNE project, as outlines in Deliverable 1.5. Deliverable 1.5 was submitted on Months 1 of the ARACNE project and Deliverable 1.6 presents the progress done under the aims and scope of Deliverable 1.5. It must be stressed that the aims and scope of Deliverable 1.5 reach beyond the timeframe of the ARACNE project in an attempt for the project to leave a lasting impact on European sericulture.

Below, the reader will be presented with a brief introduction of the local race that has been selected for each participating country, the improvements carried out on each race (if applicable) and the dissemination work and plans within the country's context for each silkworm race.

It is important to stress here that this deliverable was on purpose delayed because it had to contain the work carried out during the spring rearing season of 2024.

## 3.1 Scope of using European silkworm races

One very important element in linking the Europe identity with its historical past is to preserve and even re-introduce traditions and tangible elements of the past and engage modern Europeans with their historical past. In the case of sericulture, one such tangible element is the local (European) silkworm races that have been side-lined by the massive imports of foreign hybrids that, as robust and productive as they may be, offer to European silkworm farmers no link or connection with their traditions and their identity. Far from diminishing the local agrobiodiversity, the use of imported silkworm hybrids proved to be no solution to the decline of sericulture in Europe as witnessed in the second half of the 20th century. Although once held as a solution for improving silk production in Europe, these imported silkworm hybrids caused the decline in popularity of the local (European) silkworm races and in the case of Greece such local silkworm races were lost and do not exist anymore. Agrobiodiversity is the concerted effort to preserve local diversity of plants and animals that are well-adapted to local climatic conditions but are threatened by the massive industrialisation of crop production which entails the use of hybrid plants and animals that are not necessarily fit for crop production in geographical areas that are introduced. Preserving agrobiodiversity is an effort undertaken by several countries in an attempt to prevent the loss of genetic diversity of plants and animals and it is also an effort that is supported by the Food and Agriculture Organisation of the United Nations (see: https://www.fao.org/giahs/en/). European sericulture cannot afford not to be part of such efforts if we do not want to lament in several decades from now the absolute dependence on foreign imports in this agricultural sector.

The aim of the activities presented in Deliverable 1.6 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 we attempt to re-introduce a sense of identity and link modern practices with local traditions. We are fully aware that this will be a slow and uneasy process that will require actions by related stakeholders, but it is the only way of maintaining traditions, local agrobiodiversity and local identities in locations within Europe that are strongly associated with silk production and silkworm rearing.

## 3.2 Timeline of maintaining and improving a European silkworm race

The text in this section provides an overview of the work done by NKUA in Greece with the local silkworm race called Baghdad as an example of the timeline for maintaining and improving a European silkworm race.

Eggs of the Baghdad silkworm race were imported from CREA on May 2020 on the basis of a Material Transfer Agreement and since then they have been maintained and improved at the Laboratory of Zoology, Department of Biology, National and Kapodistrian University of Athens (NKUA). At the time of writing of this deliverable the 11th generation of the race has been reared and the characteristics of the animals are continuously improved by conducting a selective breeding for increased cocoon shell weight and large, oblong size of cocoon shell

(see Figure 1). These characteristics of the Baghdad race are not expected to improve much more in the future and so the selective breeding will shift from 2025 towards producing environmentally resilient animals.

Furthermore, a selective breeding regime has been conducted since 2021 to improve the quantitative characters of the produced silk thread by this race. Under this regime, interbreeding with the $F_2$ animals of a highly productive Japanese hybrid (see Figure 1) has been conducted followed by backcross breeding of the produced pure line of animals (see Figure 1) and further interbreeding which resulted in the production of a pure line (called i-Baghdad (i.e., improved-Baghdad – see Figure 1) with greatly improved cocoon shell qualitative characters.

Interbreeding and backcross breeding are extensively used as animal breeding techniques that aim to improve certain quantitative characters of animals while maintaining and stabilising other desired characters. In the case of silkworms, such quantitative characters are regulated by complex networks of genes[1-4]. In addition, the crossbreeding of silkworm races with other races or $F_2$ offspring of other crosses has been a general feature in silkworm breeding[3,5] and some of the most productive silkworm pure lines have their ancestry, at least partially[3], from European silkworm races.

## 3.3 Criteria of preservation, selection and improvement of a European silkworm race

In this section the criteria that define the methods of preservation selection and improvement of a local European silkworm race will be described using as an exemplar silkworm race the Baghdad race that is known to have been extensively reared in Greece in the first half of the 20$^{th}$ century.

One of the distinct characters of this race is the presence of non-glued eggs. Another characteristic is its production of white and quite large cocoons that have more or less an oblong or sometimes an irregular long shape without any constriction in the middle. Breeding of the race is done by careful selection of the breeding pairs (female and male) with emphasis on production of a fraction of non-glued eggs by the bred females. Besides producing large quantities of silkworm eggs for distribution to interested silkworm rearing farmers, the maintenance of the characters of the race should be strictly followed by selecting for robust moths that exhibit excellent anatomical features. For improvement of quantitative characters such as the cocoon shell weight, a careful selection regime is followed which consists of identifying the sex of each animal and measuring the cocoon shell weight that is produced by each animal. This work can enlist the use of 400-500 animals at a time each measured individually. If all morphological and selection characters are present, then 5-10 pairs of animals are selected with emphasis given on the mating between the best female and the best male. The eggs laid by this pair and the second-best pair are then used in the subsequent generation. The eggs from a single pair should never be used for fear of the introduction of an unwarranted mutation and this is why the eggs laid by two pairs should be at least used in silkworm breeding regimes. The selection and improvement of quantitative characters is repeated in the following generation and so on and so forth. The

quantitative characters soon reach a plateau and can no longer be improved because the genetic background of the animals is limited due to inbreeding. In this case, and if further improvements are required in the quantitative characters, the genetic pool has to be replenished by interbreeding with another, distantly related race in which case the genetic phenomenon of heterosis occurs (see Figure 1).

The criteria described above and the methods that are described above may vary from country to country and different methods are used by different scientists. Therefore, what is described above cannot be used as a general rule of silkworm breeding. In the literature[5] several schemes have been described and used to good effect for each.

## 3.4 Risks associated with preserving European silkworm races within each country

The text in this section provides an overview of risks associated with preserving European silkworm races taking as an example the work done by NKUA in Greece with the local silkworm race called Baghdad.

There are several risks associated with the preservation of genetic resources such as insects and these risks are even higher when in the case of the silkworm. Insects in general are susceptible to a variety of pathogens and environmental pollutants. Silkworms are also susceptible to *Nosema bombycis*, a microsporidian that is responsible for the occurrence of the pebrine disease. The presence of this parasite has to be checked in the silkworm eggs produced and this makes mandatory the distribution of eggs that are contain no traces of this parasite. As a rule of thumb, local silkworm races should be maintained by various stakeholder within each country so that in the case of an accident, the genetic resource will not be entirely lost. Moreover, local silkworm races should be maintained in from several broods of the same race so that in case of an accident a substitute can be used. All the above suggest that the best way of maintaining local silkworm races is to allocate their use and reproduction by local silkworm farmers and impose a regime of regular checks for the presence of the microsporidian parasite. This is essentially what was done in the early 20$^{th}$ century where several silkworm breeders were breeding and preserving the local (European) silkworm races and were selling their disease-free eggs for a profit to local silkworm farmers.

## 3.5 Communication Plans for the popularisation of European silkworm races

There can be several approaches that can be implemented in our attempt to popularise the use of local (European) silkworm races. One such approach can be the introduction of such silkworm races to schools as parts of school activities to engage and educate students about the values of maintaining their local agrobiodiversity and as educational material in various biology classes. Another approach is through the mass media and social media when the availability of such European silkworm races can be advertised and interested individuals will be given stocks of eggs from these races together with brief instructions on their preservation. A third approach is through introduced legislation where local silkworm races

will be preserved by farmers' collectives and their breeding will be subsidised or otherwise promoted. A fourth approach can be through networks of interested silkworm farmers or new silkworm farmers that are interested in conducting silkworm rearing and they can be sponsored through their use of local silkworm races instead of using silkworm hybrids. All these approaches will be discussed within the framework of the ARACNE project between the stakeholders and the involved partners and this will be an on-going process that reaches beyond the extent of the ARACNE project. It is important to maintain focus on popularising the local (European) silkworm races beyond the timeframe of the ARACNE project if we want the project to have a lasting impact.

# 4. List of silkworm races selected for each country

## 4.1 Silkworm race for Greece: Baghdad

In this section the work done on maintaining and improving the indigenous silkworm race "Baghdad" for Greece will be described. The silkworm race Baghdad has a long history of existence in Greece and in Europe in general[6-9]. This race has one distinct characteristic which makes it very easily identifiable in historical texts even if the race is not clearly described: the majority of female moths lack the gene necessary for the production of glue protein that allows the eggs that are laid to stick to a surface. The gene responsible for this phenotype is called *Ng* (Non-Glue) and female moths that carry such mutation can be easily segregated to generate a pure line of silkworms that produce non-glued eggs. However, the population is usually mixed for practical reasons. The race Baghdad is first reported by E. Cornalia[9] who reports that a person called Michele Balsamo-Crivelli has brought silkworms to Italy from Persia and Bursa and these silkworms produced non-glued eggs. Michele Balsamo-Crivelli was a member of the Crivelli family of Milanese aristocrats and from historical texts can be determined that he brought those silkworms to Italy between 1830-1840. It was a very common practice in the early 19$^{th}$ century to import and introduce new silkworm races from various locations in Europe because silkworm egg trade was a very lucrative business. It can thus be determined that the Baghdad race was probably developed sometime in the early 19$^{th}$ century in the geographical region of the Middle East and took the name Baghdad in reference of a very well-known city. Since then, the Baghdad race appears in many sericulture textbook written in Greek[7,8], Italian or French and has also been introduced in Japan in the early 20$^{th}$ century where it is still maintained. One variant of this race was very popular in the 20$^{th}$ century in Soviet Union and has since been imported in China. The Baghdad race was very popular in Northern Greece up until the 1950s[8] and its derivative races may be the Bursa white, Turkey white and even some Persian races[6,7].

### 4.1.1. Maintaining and improving the Baghdad silkworm race

The breeding and improvement scheme described in the following Figure 1 is an example of the rate with which certain quantitative characters of silk production can be improved in silkworms if careful and directed selection schemes are observed. Although the work on

improving the Baghdad silkworm race started before the start of the ARACNE project, the project provided the opportunity for further intensive work on improving the quantitative characters of the race. In the future, attention will be paid in improving the resilience of this race to adverse climatic conditions while maintaining the attained quantitative character.

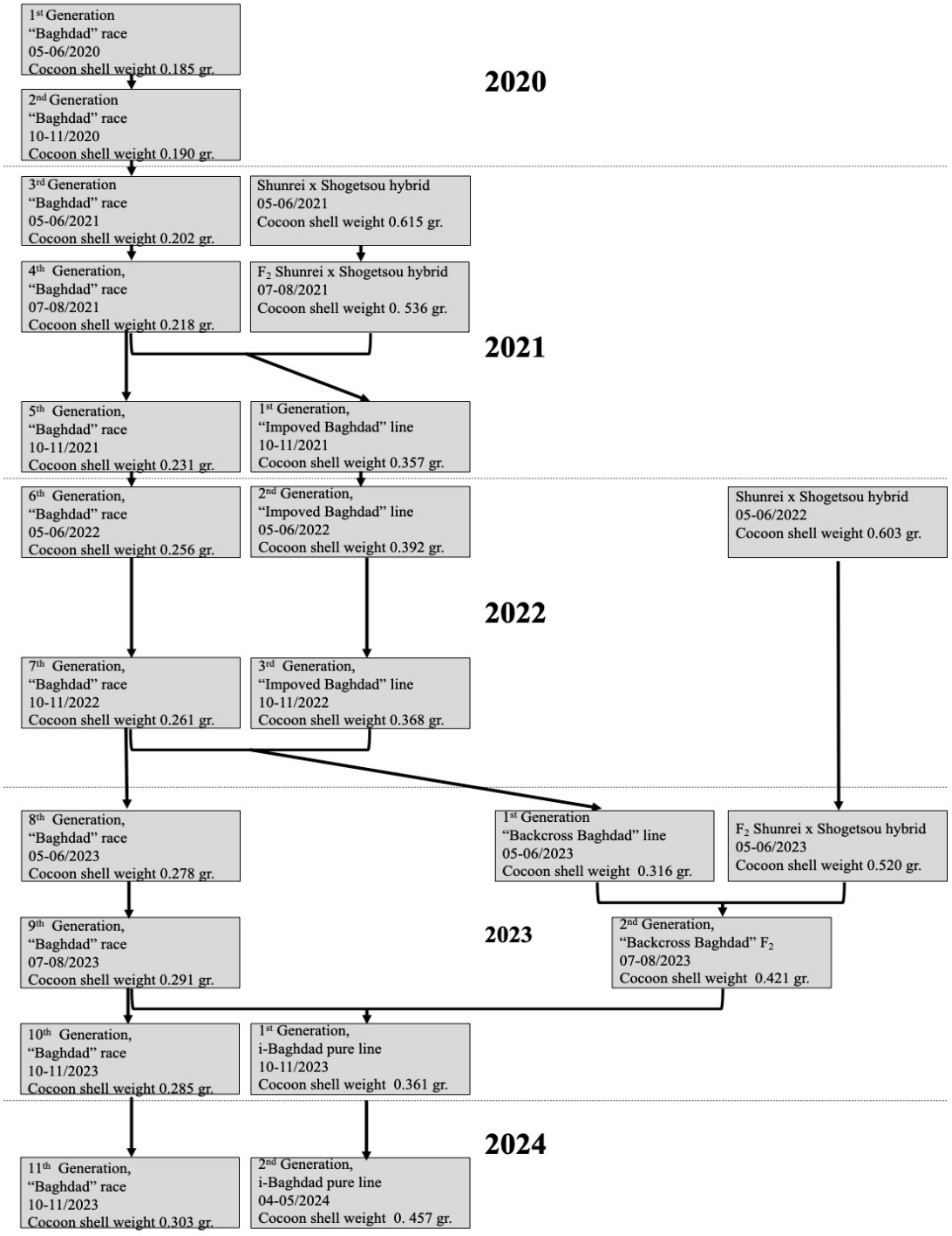


**Figure 1** - Timeline of the generation of the i-Baghdad pure line

This pure line is derived from the "Baghdad" race and will continue to be bred and improved by NKUA. The animals are selected based on the cocoon shape and larval body colouration. The values of the cocoon shell weight indicate the average cocoon shell weight of the best 5 pairs that were maintained for breeding.

### 4.1.2. Dissemination of the Baghdad race within Greece

The steps taken to disseminate and popularise the use of the Baghdad race within Greece are as follows:

1) Since Spring 2023 an agreement was reached with the Vocational School of Sericulture and Silk Production that was established in Soufli in 2022 for its students to use and be educated on the rearing of the Baghdad race. Under this scheme, boxes of eggs of the Baghdad race will be sent by NKUA to the Vocational School in Soufli where students will carry out the incubation of the eggs and the rearing of the silkworms. Students will also learn about the production of silkworm eggs and the handling of such eggs. The silkworm eggs of the Baghdad race that will be produced by the students will be returned in October of each year to NKUA for preservation and cold storage during winter and then these eggs will be sent back to the Vocational School for the students to rear them in the following year. This scheme was put into place in Spring 2023 and in Spring 2024 and will continue in perpetuity as a form of collaboration between these two entities.
2) Boxes of silkworm eggs of the Baghdad race were given to Mouseio Technis Metaxiou (ASM) which is a partner of the ARACNE project to be maintained and displayed as an exhibit for the visitors to ASM. Visitors are introduced to the characters of this local race and are encouraged to interact with the silkworms and learn more about the traditional use of this race in silk production in Soufli, Greece.
3) Boxes of silkworm eggs were given to a new silkworm farmer in Soufli who produces cocoon that are used in cosmetics applications. Under this agreement, NKUA will be providing eggs of the i-Baghdad pure line to this farmer who is interested in obtaining large and solid cocoon for their intended purposes. This novel approach will be promoted and used as an example for other farmers that want to engage in silkworm rearing.
4) Eggs of the Baghdad silkworm race were given to the Agricultural University of Athens for distribution to various secondary education schools or other individuals who are interested in rearing silkworms. Such activities should be further encouraged and promoted so as to educate young students in silkworm rearing, insect development and entomology in general.

## 4.2 Silkworm race for Italy: Bianco Italia

The Bianco Italia race is native to Italy. It is derived from the segregation of a yellow peanut-shaped cocoon race, possibly Brianzola/Brianza. It was chosen as representative of the Italian races also because it was originally endowed with a good silk production compared to other indigenous accessions. In fact, the length of the silk thread in 1960 was about 752 m, not comparable with the polyhybrid strains, but good for a race (a population of individuals

reared in a homogeneous area). The selection strategy developed in Italy will be different from that planned for Greece. In fact, Italy has its own silkworm genetic resources for the production of hybrid eggs, so the recovery of Bianco Italia has the potential to be used for cultural and tourist purposes, possibly for the production of specific products with traditional values. The production of Bianco Italia cocoons will therefore co-exist with, but not replace, the production of polyhybrid cocoons.

### 4.2.1. Maintaining and improving the Bianco Italia silkworm race

Unfortunately, over the years, there was a lack of selection pressure caused by conservation in CREA's gene bank without any specific and dedicated programme of genetic improvement, apart for morphological control. Gene banks, in fact, preserve many accessions and only those dedicated to practical uses can be constantly placed under selection pressure. The others are preserved as source of genes, but without the chance of working on these. Ancient races are in this group: the length of the silk thread of Bianco Italia cocoon decreased from 752 m (as above mentioned) to 494 m in 1991. The number of cocoons per litre increased from 500-550 in 1960 to 940 per litre in 1992, underlining the decrease in cocoon size and volume. It should be stressed that in Italy, for a long period (from 1990 to 2011-12), there was a problem with an insecticide (fenoxycarb) carried by the wind from orchards to mulberry fields in northern Italy. This pollution even affected the conservation rearing in a sublethal way, as the less productive races or individuals were much more resistant to this active ingredient than the productive and heavier larvae. The effect of this juvenile hormone mimic has therefore been counteracting efforts to increase silk/cocoon ratios and cocoon weight along the years. The first action will be to restore the former productivity of the race by broadening the genetic basis of selection by increasing the number of individuals reared and using combined selection criteria (filters) to select individuals for reproduction. The methods refer to the contemporary selection of correlated and unrelated production traits: 1) cocoon weight, 2) cocoon shell weight, 3) silk ratio, but also 4) survival rate. The actual production parameters related to individual cocoon average production are quite unsatisfactory and deserve improvement (average cocoon weight for the female silkworm: 1.421 g, while for the male: 1.175 g; average shell weight, respectively for female and male: 0.180 and 0.189; silk shell ratio in female and male: 13% and 16%). According to the fact that selection started with the season of the beginning of the project (May-June 2023), the first year, the first generation was used to multiply and homogenise the parental lines, while in 2024, during the rearing season, the selection strategy began. The plan is to carry out two generations of selection on mulberry leaf per year and as much as possible on artificial diet, to increase the number of generations of selection within the end of the project by using different feed sources.

### 4.2.2. Dissemination of the Bianco Italia race within Italy

Due to the very limited extension of the selection process (currently two generations), it was decided to postpone the popularisation of this race to a later date, as planned in WP4.

## 4.3. Silkworm race for Slovenia: Brianza

The silkworm race selected for Slovenia is called Brianza. The cocoon of this race is flesh yellow on the outside and yellow in the inner layer, which indicates that it contains a large amount of carotenoid pigments[4,10-12]. It originates from the Brianza region of Lombardy in Italy. Archival research by the Slovenian partner (University of Maribor) revealed that the Styrian Sericulture Association was founded in 1840[13,14] and began to supply Lower Styria (present-day Slovenia) with sericulture facilities in the towns of Plevno, Celje, Maribor, Ptuj and Radgona. One of the activities was the supply of silkworm races called "Little Brianza" (Slovenian Brianza), "Great Udineser", possibly a name for the silkworm race "Friulana", and the "Common Race" without further identification[15]. The idea of using the Bianco Italia as the flagship of sericulture in Italy and the race from which it derives (Brianza) for Slovenia, has the scope to underline the fact that the two regions were very closely related in the past, although different (one population with two different races). For a tourist travelling from Slovenia to Italy, it could be attracting that the two countries are characterised by sericulture practices using races with different cocoon colours.

### 4.3.1. Maintaining and improving the Brianza silkworm race

The Brianza race exhibits the same problem as the Bianco Italia with regard to the lack of specific selection and the problem of the afore-mentioned pesticide. Even in this case the length of the cocoon thread worsened along the years, being 834 m in 1960, 634 m in 1991. The actual production parameters related to individual cocoon average production are quite unsatisfactory and deserve improvement (average cocoon weight for the female silkworm: 0.997 g, while for the male: 0.829 g; average shell weight, respectively for female and male: 0.097 and 0.101; silk shell ratio in female and male: 10% and 12%). First of all, the Brianza race was used to be reared in the summer season (from August to September), therefore the right hatching period was expected before beginning selection and multiplication strategy. According to the design of an appropriate abbreviation of the diapause the race was successfully reproduced for the spring season 2024. The colour of cocoon was not perfectly homogenous; therefore, it was decided to select for this genetic trait at first (see Fig. 2).

**Figure 2** - The cocoon shell colour of the Brianza race. *The cocoon shell colour of the Brianza race is not a homogeneous yellow and therefore cocoons of similar colouration were placed together in mating frames to copulate and lay their eggs that will then be selected for a uniform cocoon shell colour at first.*

### 4.3.2. Dissemination of the Brianza race within Slovenia

Due to the very limited extension of the selection process (currently two generations), it was decided to postpone the popularisation of this race to a later date, as planned in WP4. This year the three sub-populations, from which separate coloured Brianza races will be obtained, will be multiplied; these can be distributed to Slovenian farmers or for educational aims in Slovenia.

## 4.4. Silkworm race for France: Var

The silkworm race that was selected for France is called Var. The name Var refers to the department of Var in south-eastern France. This race has been mentioned in several manuscripts dating back to the 19$^{th}$ century as an indigenous race.

The Var silkworm race is currently maintained at the Laboratory of Sericulture (CREA) in Padova, Italy and was transferred by INRA to Italy when the silkworm germ-bank in Lyon (France) was closed. The cocoon is coloured in a beautiful dark golden colour and has an elliptical shape.

### 4.4.1. Maintaining and improving the Var silkworm race

This race has not undergone any program of genetic improvement at CREA but was only preserved until the beginning of the ARACNE project. It is a very inbreed race, very sensitive to Nuclear Polyhedrosis Virus, therefore in this first year of project we tried to enlarge its population and to evaluate if it is apt to be reared on artificial diet to speed up its selection.

However, the results on the diet have not been satisfactory until now. The actual production parameters related to individual cocoon average production are quite unsatisfactory and deserve improvement in a similar way as described above for the Bianco Italia and the Brianza races. In fact, the average cocoon weight for the female silkworm: 1.163 g, while for the male: 1.015 g; average shell weight, respectively for female and male: 0.133 and 0.135; silk shell ratio in female and male: 12% and 13%.

### 4.4.2. Dissemination of the Var race within France

Due to the very limited extension of the selection process (currently two generations), we decided to postpone the popularisation of this race to a later date, as planned in WP4.

## 4.5. Silkworm race for Spain: Sierra Morena

The silkworm race that was selected for Spain is called Sierra Morena. The name Sierra Morena refers to the mountain range in Southern Spain. This silkworm race was given by Prof. Jose Cenis Anadon of the Spanish partner (IMIDA) to the Laboratory of Sericulture (CREA) in Padova, Italy in 2009 and is still maintained by CREA under the name “Baco Moro”. This race is still maintained in the region of Murcia by IMIDA. This race is popularly reared in Spain, where the silkworm larvae are reared as hobby by several individuals. Due to its origin, is a very inbreed race, that adapts very well on CREA’s artificial diet, for which it has been selected.

### 4.5.1. Maintaining and improving the Sierra Morena silkworm race

This race has not undergone any program of genetic improvement at CREA, for quantitative characters; it has only been adapted to CREA’s artificial diet. In the same way as the race Var, it is a very inbreed race, very sensitive to Nuclear Polyhedrosis Virus, therefore in this first year of the ARACNE project we tried to enlarge its population. The actual production parameters related to individual cocoon average production are quite unsatisfactory and deserve improvement as for the races selected for Italy, Slovenia or France. In fact, the average cocoon weight for the female silkworm: 1.544 g, while for the male: 1.162 g; average shell weight, respectively for female and male: 0.166 and 0.131; silk shell ratio in female and male: 11% and 11%.

### 4.5.2. Dissemination of the Sierra Morena race within Spain

Due to the very limited extension of the selection process (currently two generations), we decided to postpone the popularisation of this race to a later date, as planned in WP4.

## 4.6. Silkworm race for Bulgaria: Yellow Local

The race Yellow Local is believed to have emerged around 16th – 17th century by the so called “people’s selection”. At that time, silkworm farmers, after the cocoon production, left

some selected cocoons for race maintenance. It was called “selected for damazlak”. Those cocoons were handled so that the emerged moths could copulate and lay the eggs for the following year spring rearing.

The Yellow local race was reared in North Bulgaria and in Southwest Bulgaria. The Yellow local race is characterized by green silkworm egg serosa colour, larvae with zebra markings, yellow cocoon colour and elongated cocoon shape with slight constriction. The race Yellow local was grown mostly as pure breed until the 1960s and after that it was gradually side-lined by white cocoon hybrids. Presently the race is maintained at the SCS-Vratsa germplasm.

### 4.6.1. Maintaining and improving the Yellow Local silkworm race

In 2022 from the race Yellow Local 42 batches of eggs were obtained. After inspection for pebrine disease and the selection of egg batch in the end of March 2023, 20 batches having the typical aspect for the race egg colour and the highest number of eggs per batch, were put into incubation on 25th April 2023. On the first day of incubation the eggs were disinfected by 2 % formalin solution and after that washed carefully. Each batch was isolated to be selected for higher hatchability later on. During the egg incubation stage, the temperature was 25 – 27°C and 65 – 80 % RH were maintained. The silkworm larvae mass hatching occurred on 4th May, on the 10th day after the beginning of incubation. Out of the 20 batches, put into incubation, only the larvae from 10 batches manifested the highest hatchability were selected, mixed and left for further rearing. During the 1st and 2nd instars the larvae were reared at 27°C and covered with polyethylene to prevent the leaf moisture loss. The feeding was twice per day with tender young, sliced leaves. After the 2nd moult 12 replicates, each one having 200 larvae were counted for rearing until cocoon spinning. During the 3rd instar 26°C and 65 – 70 % RH were maintained, and the larvae were fed with whole shoots twice per day without any polyethylene covering. During 4th and 5th instars 24 – 25°C and 50 – 65 % RH were maintained, and the larvae were fed two times per day by whole shoots.

**Figure 3** - Bulgarian Yellow Local race silkworm larvae during the 5th instar

The larvae were mounted on 31st May, performing a 576 hrs total larval duration while the 5th instar duration was 168 h. The cocoon spinning was on collapsible plastic frames (see Figure 3).

The cocoons were harvested on the 8th day after mounting and cleaned from the floss. The following character values were detected: hatchability, 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 Table 1.

| Breed | Hatchability (%) | Cocoon yield by one box of silkworm eggs, kg | Pupation rate, % | Fresh cocoon weight, mg | Silk shell weight, mg | Silk shell percentage % |
|---|---|---|---|---|---|---|
| Yellow local | 97.65 | 26.77*** | 81.83*** | 1908 | 336*** | 17.61*** |
| Super 1 (control) | 98.23* | 32.34** | 87.66 | 2105* | 448 | 21.28 |
| Hesa 2 (control) | 96.79 | 28.65 | 86.34 | 1918 | 421 | 21.95 |

**Table 1** - Breeding characters in the race Yellow Local[1]

It is evident from the data obtained that Yellow Local silkworm race has high hatchability. The silk shell weight and percentage in the Yellow Local race were considerably lower than in the breeds Super 1 and Hesa 2.

After the cocoon selection a random sample of 30 1st grade cocoons was taken for making a silk reeling test. The results obtained are shown in Table 2.

| Breed | Filament length, m | Silk filament weight, mg | Reelabilty, % | Raw silk percentage, % |
|---|---|---|---|---|
| Yellow local | 965*** | 276*** | 88.32*** | 36.33* |
| Super 1 | 1213 | 376 | 90.30 | 38.67 |
| Hesa 2 (control) | 1260 | 365 | 90.70 | 39.65 |

**Table 2** - Silk filament technological characters in the race Yellow Local[2]

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

[2] The data were compared statistically to the breed Hesa 2 *P < 0.05; **P < 0.01; ***P < 0.001

Considering the above figures, we came to the conclusion that the Yellow Local silkworm race would perform better in F1 crosses with some Bulgarian breeds, having higher tolerance to adverse rearing conditions and higher silk shell percentage. For this purpose, the following F1 hybrids between Yellow Local race and the breeds Iva 1 (Japanese type, white cocoon), VB1 (Japanese type, white cocoon), HB2 (Chinese type, white cocoon) and Nova 2 (Chinese type, white cocoon) were made for further testing in 2024: Iva1 x Yellow Local, VB1 x Yellow local, HB2 x Yellow local and Nova2 x Yellow Local.

After a selection 200 cocoons from the Yellow Local race, having alive pupae, the typical cocoon colour and shape of the race and with firm silk shell, without any defects were taken, cut and separated by sex. Each cocoon was weighed in order to determine its fresh cocoon weight, silk shell weight and shell percentage. After that 50 female and 50 male cocoons were selected with the highest cocoon weight, silk shell weight and higher shell ratio. The cocoons selected were placed in copulation frames and 42 batches of eggs were produced for the race maintenance.

The rest of the cocoons with alive pupae produced in amount of 2.700 kg were used for egg production. After mating 792 batches were produced. The number of batches, obtained from 1 kg of seed cocoons was 293 which is a quite excellent result. In October, the mother moths were examined for pebrine disease. In the 42 batches for the race maintenance no evidence of pebrine was found. The silkworm eggs, directed for rearing at the field level were detached, then washed by tap water, after that treated by two salt solutions (1090 and 1070) for bad quality eggs separation and finally disinfected by 2 % formalin solution, washed and dried. Finally, 192 g of silkworm eggs from the Yellow local race were obtained. The number of eggs, obtained by 1 kg of seed cocoons was 71 g, which is considered as a very good yield. Those eggs are directed for rearing by Bulgarian farmers, families, universities, schools etc.

In 2023 from the race Yellow Local 42 batches for reproduction were obtained. After the mother moth microscope examination for pebrine disease control and the selection of egg laying in the end of March 20 egg laying having the typical for the race egg colour and the highest number of eggs per laying were put into incubation on 23$^{rd}$ April 2024. On the first day of incubation the eggs were disinfected by 2 % formalin solution and after that washed carefully. Each egg laying was isolated in a separate casserole dish in order to make a selection for higher hatchability later on. During the egg incubation the temperature was 25 – 27$^{o}$C and 65 – 80 % RH were maintained. The silkworm larvae mass hatching occurred on 3$^{rd}$ May, on the 11$^{th}$ day after the beginning of incubation. Out of 20 egg laying, put into incubation, only the larvae from 10 laying, manifested the highest hatchability were selected, mixed and left for further rearing. During the 1$^{st}$ and 2$^{nd}$ instars the larvae were reared at 27$^{o}$C and covered with polyethylene in order to prevent the leaf moisture lose. The feeding was once per day with tender young, sliced leaves. After the 2$^{nd}$ moult 16 replicates, each one having 200 larvae were counted for rearing until the cocoon spinning. During the 3$^{rd}$ instar 26$^{o}$C and 65 – 70 % RH were maintained, and the larvae were fed with whole shoots once per day without any polyethylene covering. During 4$^{th}$ and 5$^{th}$ instars 24 – 25$^{o}$C and 50 – 65 % RH were maintained, and the larvae were fed one times per day by whole shoots. On

30th May the Yellow Local race larvae were on the 5th day of the 5th instar. Until this moment the larvae manifested a normal and uniform growth without detection of any diseases.

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 created in 2023 F1 hybrids VB1 x Yellow Local and HB2 x Yellow Local were also hatched on 3rd May 2024 and reared by the same methodology as the pure Yellow Local and VB1 and HB2 breeds. After the 2nd moult from each hybrid two replicates, consisted of 200 larvae were counted and reared at the standard regime. At the same time other two replicates, consisted of 300 larvae each from each hybrid were counted and reared at adverse rearing conditions during the 4th and 5th instar. From the pure races Yellow Local, VB1 and HB2 also after the 2nd moult from each breed two replicates, consisted of 200 larvae were counted and reared at the standard regime and two replicates, consisted of 300 larvae each from each breed were counted and reared at adverse rearing conditions during the 4th and 5th instar.

| Mode of silkworm rearing | Temperature, ° C | Relative air humidity, % | Feeding space | Feeding amount | Ventilation |
|---|---|---|---|---|---|
| Provocative regime | 29 - 30 | 80 – 90 % | 11 м$^2$ for 1 box of silkworms | 1 feeding daily | Thickly closed windows and door |
| Standard technology | 23 - 25 | 55 – 70 % | 22 м$^2$ for 1 box of silkworms | 1 feeding daily | Two windows open, the door – open if necessary |

**Table 3** - The adverse silkworm rearing conditions for Yellow Local

Both F1 hybrids performed larvae with zebra markings and yellow colour of the haemolymph, which means that the two qualitative characters expression was completely dominated by the Yellow Local race.

The larvae of all the pure breeds and F1 hybrids studied at provocative regime started the cocoon spinning on 29th May. The larvae of the F1 hybrids started the cocoon spinning at standard rearing technology on 30th May. The larvae of the Yellow Local, VB1 and HB2 pure races, reared at standard regime were at the 5th day of 5th instar on 30th May 2024.

### 4.6.2. Dissemination of the Yellow Local race within Bulgaria

As mentioned above 192 g of silkworm eggs from the Yellow Local race were obtained in 2023.The following measures for the race popularization have been taken:

1) 7 groups of students from middle schools in Sofia with a total number of children 266 visited the SCS-Vratsa in October and November 2023 where the children had a meeting with the research staff, a lecture about the sericulture, ARACNE project and the old traditional Bulgarian silkworm race Yellow Local was presented to them, they visited the permanent exhibition "The history of sericulture in Bulgaria", germplasm collection, old and modern silkworm rearing houses. Especially for the students we hatched silkworm eggs and reared larvae to demonstrate to them silkworm larval rearing, cocoon spinning and moth emergence.
2) Silkworm eggs from the Yellow local race were given for spring rearing in 2024 to University of Forestry, Sofia, Agricultural University, Plovdiv, 2 schools and 2 farmers.
3) On 21st May 2024 one group of students from middle schools in Sofia with a total number of 45 children visited the SCS-Vratsa where the children had a meeting with the research staff, a lecture about the sericulture, ARACNE project and the old traditional Bulgarian silkworm race Yellow Local was presented to them, they visited the permanent exhibition "The history of sericulture in Bulgaria". They visited the silkworm rearing houses and were especially introduced with the 3rd instar larvae of the Yellow Local race maintained at the Centre.

## 4.7. Silkworm race for Georgia: Georgian White, Telavuri, Kakheti Green and Kutaisi Orange

Sericulture is the oldest agricultural branch in Georgia, having successfully gone through several historical upheavals and it is considered one of the best examples of national cultural heritage domains in Georgia. Until now, the beginning of sericulture has been associated with the name of King Vakhtang Gorgasali (the 5th century AD) but based on recent archaeological findings of organic materials found in a 4th century BC burial at Pichvnari where silk fibres were discovered, it is suggested that silk in Georgia was present as early as the 4th century BC, i.e. 2 centuries before the existence of Georgian Silk Road and 9 centuries earlier than it was considered to this day. The silkworm races in Georgia can be divided into two categories: races of folk selection and races of scientific selection. In the 19th century, after the spread of pebrine disease in Europe, several silkworm races survived in Georgia by folk selection, but at the same time they have lost their commercial value. These races are still preserved in a collection of silkworm races as selection material due to their genetic background. This group includes the following races: Georgian white cocooned, Kakhuri white (later named Telavuri), Kakhuri Green, Kutaisi Orange (N. Shavrov, 1888). Due to their high viability, these races are particularly resistant to hot and moderate climate conditions. They became the basis for the selection of the following improved races: Tbilniish-1, Tbilniish -2, Kolkhida, Imeruli, Kakhuri, Kartli, Tbilniish -3 and their hybrids (S. Ghvinepadze, 1953). This report covers characterization of the 4 folk-selected ancient Georgian races. Furthermore, to the present Georgia preserves 28 more races + 12 modern races, 44 Georgian races in total that are reproduced every year to prepare eggs for the next generation.

### 4.7.1. Maintaining and improving the Georgian silkworm races: Georgian White, Telavuri, Kakheti Green and Kutaisi Orange

The silkworm race Georgian white was obtained through folk selection and has been preserved as part of the silkworm races collection since 1900. This race's characteristics are as follows: Eggs –grey, of average size. Egg quantity per gram - 1620-1670. Worm quantity per gram of eggs - 2000-2150. Worm –mounting, length of feeding – 28-29 days. Cocoon – white, oval. Average weight of one cocoon- up to 1.8-1.9 gram. Average weight of shell - 320-340 mg, cocoon shell percentage - 18- 19%. The variety Georgian white is characterised by high viability - 99.0%, an average yield of the cocoon from 1 g of eggs of 3.0 kg. Raw and air-dried cocoons with high technological indicators: percentage of dry/fresh cocoon - 40.5%, yield of raw yarn - 37.2%, cocoon yarn length - 1000-1200 m.

**Figure 4** - The Georgian white silkworm race

The silkworm race Telavuri was obtained through folk selection and has been preserved as part of the silkworm races collection since 1900. This race's characteristics are as follows: Eggs–grey, of average size. Egg quantity per gram - 1600-1660. Worm quantity per gram of eggs - 2000-2100. Worm – mounting, length of feeding – 27-28 days. Cocoon – white, oval. Average weight per cocoon - up to 1.8-1.9 g. Average weight of cocoon shell - 330-350 mg, cocoon shell ratio – 18-19%; The variety is characterized by high viability - 99.0%, the average yield of the cocoon from 1 g of eggs – 3.7 kg. Raw and air-dried cocoons with high technological indicators: percentage of dry/fresh cocoon 38.5%, yield of raw yarn - 40.2%, cocoon yarn length - 1100 m.

**Figure 5** – The Telavuri silkworm race of Georgia

The silkworm race Kakheti Green was obtained through folk selection and has been part of the silkworm races collection since 1900. This race's characteristics are as follows: Eggs-grey, of average size. Egg quantity per gram - 1640-1660. Worm quantity per gram of eggs - 2000-2150. Worm –mounting, length of feeding – 26-27 days. Cocoon – greenish colour, elongated cylindrical shape. Average weight per cocoon - up to 1.5-1.7 g. Average weight of cocoon shell - 300-310 mg, silk percentage -15-16%. This race is characterised by high viability - 99.0%, the average yield of the cocoon from 1 g of eggs – 3.0 kg. Raw and air-dried cocoons with high technological indicators: percentage of dry/fresh cocoon - 30.5%, yield of raw yarn - 37.2%, cocoon yarn length – 1000-1100 m.

**Figure 6** - The Kakheti Green silkworm race of Georgia

The silkworm race Kutaisi Orange was obtained through folk selection has been preserved as part of the silkworm races collection since 1900. This race's characteristics are as follows: Eggs-–grey, of average size. Eggs quantity per gram - 1600-1620. Worm quantity per gram of eggs - 2000-2100. Worm – is white with yellowish legs, length of feeding – 26-27 days. Cocoon – orange colour, elongated cylindrical shape. Average weight of cocoon- up to 1,6-1,7 g. Average weight of cocoon shell - 300-310 mg, silk percentage -16-17%. This race is characterized by high viability - 99.0%, the average yield of the cocoon from 1 g of eggs – 3.0 kg. Raw and air-dried cocoons with high technological indicators: percentage of dry/fresh cocoon - 34.5%, yield of raw yarn - 35.2%, cocoon yarn length – 1000-1100 m.

**Figure 7** - The Kutaisi Orange silkworm race of Georgia

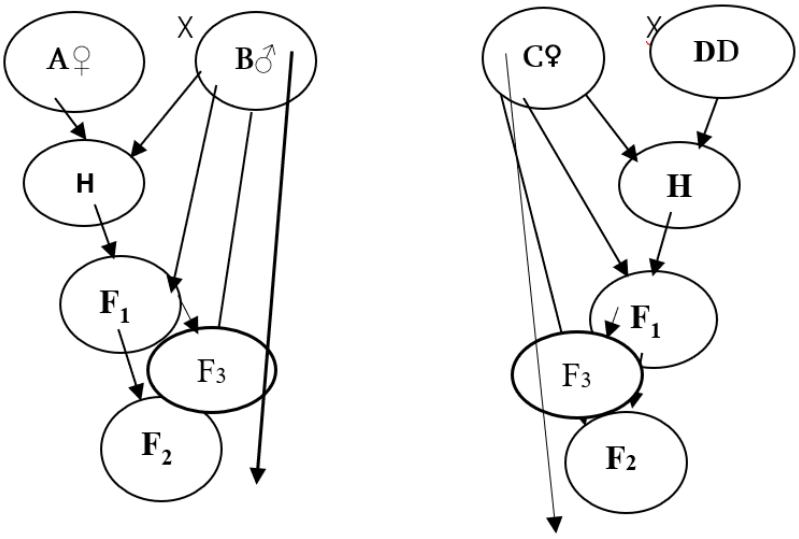


**Figure 8** - A scheme of silkworm race improvement

In Figure 8, A and C are the races that need improvement. B and D are the races that provide improvement. As a result of feeding and mating of selected improved races with the races that need improvement a hybrid is created. In the 3rd or 4th generation, as a result of mating female from the races that needs improvement and male from the created simple hybrid, an $F_1$ generation and then by selection an $F_2$ generation is created. Further selection results in $F_3$ generation, with improved characters that further need to be fixed. Due to the fact that we are dealing with very old races (140-150 years old) with decreased productivity indexes the process of improvement is expected to be slow. With this in mind, for the improvement of the race: Georgian white we have selected the Georgian race Tbilisuri. For the improvement of the race: Telavuri we have selected the Georgian race Iveria. For the improvement of the race: Kakheti Green we have selected the Georgian race Tergi Green. For the improvement of the race Kutaisi Orange we have selected another orange race, Khankou.

### 4.7.2. Dissemination of the Georgian silkworm races: Georgian White, Telavuri, Kakheti Green and Kutaisi Orange within Georgia

On May 8th[3], 2024 we distributed silkworms of Telavuri, Georgian white, Kakheti Green or Kutaisi Orange races in Kakheti region, in 4 villages of Akhmeta municipality to 11 farmers and Zemo Alvani public school.

[3] Sericulture Laboratory of the Georgian Agriculture Research Centre, which conducts preservation and improvement of all the silkworm races still preserved today and developed in Georgia, joined the ARACNE project in April 2024 and the silkworm rearing started from May the 8th.

# 5. Conclusions

The activities described in this Deliverable 1.6 are an example on how resources can be utilised to produce substantial progress in bringing local (European) silkworm race on par with commercially available silkworm hybrids. The activities described are part of an on-going process to popularise local (European) silkworm races and sustainability, enrich agrobiodiversity and link sericulture in Europe with its historical past.

# References


1 Sun, W. *et al.* Phylogeny and evolutionary history of the silkworm. *Sci China Life Sci* **55**, 483-496, doi:10.1007/s11427-012-4334-7 (2012).

2 Kawamoto, M. *et al.* High-quality genome assembly of the silkworm, Bombyx mori. *Insect Biochem Mol Biol* **107**, 53-62, doi:10.1016/j.ibmb.2019.02.002 (2019).

3 Waizumi, R. *et al.* Highly accurate genome assembly of an improved high-yielding silkworm strain, Nichi01. *G3 Genes|Genomes|Genetics*, doi:10.1093/g3journal/jkad044 (2023).

4 Lu, W. *et al.* FibH Gene Complete Sequences (FibHome) Revealed Silkworm Pedigree. *Insects* **14**, doi:10.3390/insects14030244 (2023).

5 Hiratsuka, E. *Silkworm Breeding*. (CRC Press, 1999).

6 Clugnet, L. *Géographie de la soie: étude géographique et statistique sur la production et le commerce de la soie en cocon*. (Georg, 1877).

7 Φιλιππίδης, Σ. *Μελεται επί του μεταξοσκώληκος*. 320 (Κεφαλιδης, Ν. Γ., 1890).

8 Παπαναούμ, Γ. *Σηροτροφία*. (Εκδ.: Υπουργείο Γεωργίας, Γενική Διεύθυνση Γεωργίας, 1950).

9 Cornalia, E. *Monografia del bombice del Gelso (Bombix mori linn.)*. (Tipografia di Giuseppe Bernardoni di Gio, 1856).

10 Ma, M., Hussain, M., Dong, S. & Zhou, W. Characterization of the pigment in naturally yellow-colored domestic silk. *Dyes and Pigments* **124**, 6-11, doi:https://doi.org/10.1016/j.dyepig.2015.08.003 (2016).

11 Sakudoh, T. *et al.* Carotenoid silk coloration is controlled by a carotenoid-binding protein, a product of the Yellow blood gene. *Proc Natl Acad Sci U S A* **104**, 8941-8946, doi:10.1073/pnas.0702860104 (2007).

12 Tsuchida, K. & Sakudoh, T. Recent progress in molecular genetic studies on the carotenoid transport system using cocoon-color mutants of the silkworm. *Arch Biochem Biophys* **572**, 151-157, doi:10.1016/j.abb.2014.12.029 (2015).

13 Joanneum, J. 1-20 (1840).

14 Hlubek, F. X. *Ein treues Bild Herzogthumes Steiermark. Steiermärkische Landwirtschaftsgeselschaft*. 201 (1860).

15 Reiter, A. Historische Entdeckung im Eggenberg und Wetzelsdorf. . *Blätter für Heimatkunde* **70**, 53-56 (1996).