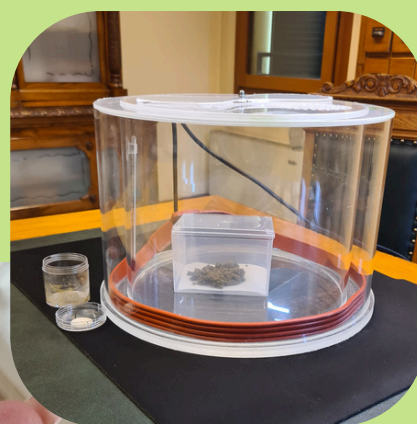


Silk knights

The silkworm, the mulberry tree,
and instructions for the educational
rearing kit



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

The ARACNE project

The ARACNE project (an acronym for 'Advocating the Role of Silk Art and Cultural Heritage at National and European scale', is named after the weaver transformed into a spider by the goddess Athena, in Greek mythology, and makes silk the common element of pan-European culture and history.

ARACNE aims to contribute to the creation of a broad and interconnected ecosystem of innovation related to silk in Europe, as an industrial sector and as an instrument of expression of cultural heritage and landscape, combining culture, tradition and new industrial production in an ideal network of exchanges and visions.

ARACNE started in 2023 and has a duration of 36 months, involves 11 partners and 3 ASSOCIATED PARTNERS from 7 European and non-European countries. With a budget of around EUR 3 millions, the project aims to achieve the following specific objectives:

Objectives:

01

- Enhancement of knowledge and memory for the renaissance of a European Silk Innovation Ecosystem

02

- Co-creation of human-centred and place specific creative silk-based solutions leveraging on digital technologies

03

- Implementation of innovative strategies, governance and financing models for the involved organisations

04

- Support the establishment of a European Silk Route, based on the tangible and intangible silk cultural heritage

05

- Raise awareness of ARACNE results, impacts and expectation for the constitution of a European Silk Route

06

- Enhance the European cultural identity and strengthen European competitiveness for a resilient society

07

- Contribution to the European Green Deal, the New European Bauhaus and the Sustainable Development Goals

The silk knights



Educational paths




The European project ARACNE has among its objectives the involvement of students in the research and dissemination of silk-related culture.

To this end, several educational different educational paths have been proposed. For primary and secondary schools an EDUCATIONAL KIT has been developed that allows the rearing of silkworms in the classroom on an artificial diet to keep the larvae under controlled temperature and humidity. The life cycle of the insect can also be carried outside the season, at times when there are no leaves on the mulberry tree, the only food of the silkworm. The kit is reusable and allows students to understand the process of insect metamorphosis and to become directly familiar with the developmental stages of the silkworm.

Educational aims

- 🔍 **Skills of observation** encourage children to closely study the development of the silkworm and to identify the mulberry plant.
- 🔍 **Development of scientific curiosity** stimulate interest in the natural sciences through exploring and sensory experiences.
- 🔍 **Show how nature and human activity are connected** teach children about the historical and economic role of the silkworm and how it is linked to the culture and history of their region.
- 🔍 **Encourage empathy towards living beings** help children to understand how to have a kind and respectful relationship with animals and plants.

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01

An amazing
insect



1.1 Physiological characteristics of *Bombyx mori*

The silkworm (*Bombyx mori*) is an insect of the order Lepidoptera and the family Bombycidae.

Scientific classification:

- Kingdom: Animalia
- Phylum: Arthropoda
- Class: Insecta
- Order: Lepidoptera
- Family: Bombycidae
- Genus: Bombyx
- Species: mori

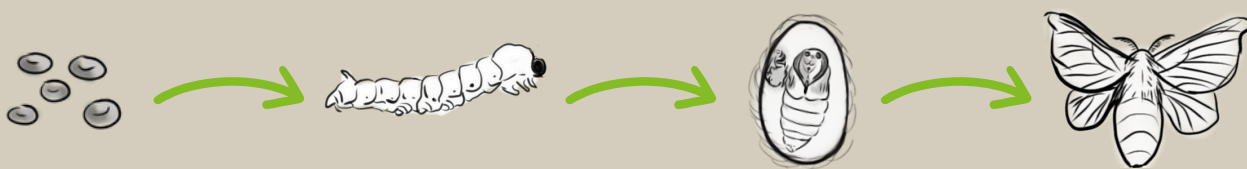
It is a **holometabolous** insect, which means that it undergoes complete metamorphosis. This means that it goes through four different stages in its life cycle, during which it completely changes its appearance and anatomy.

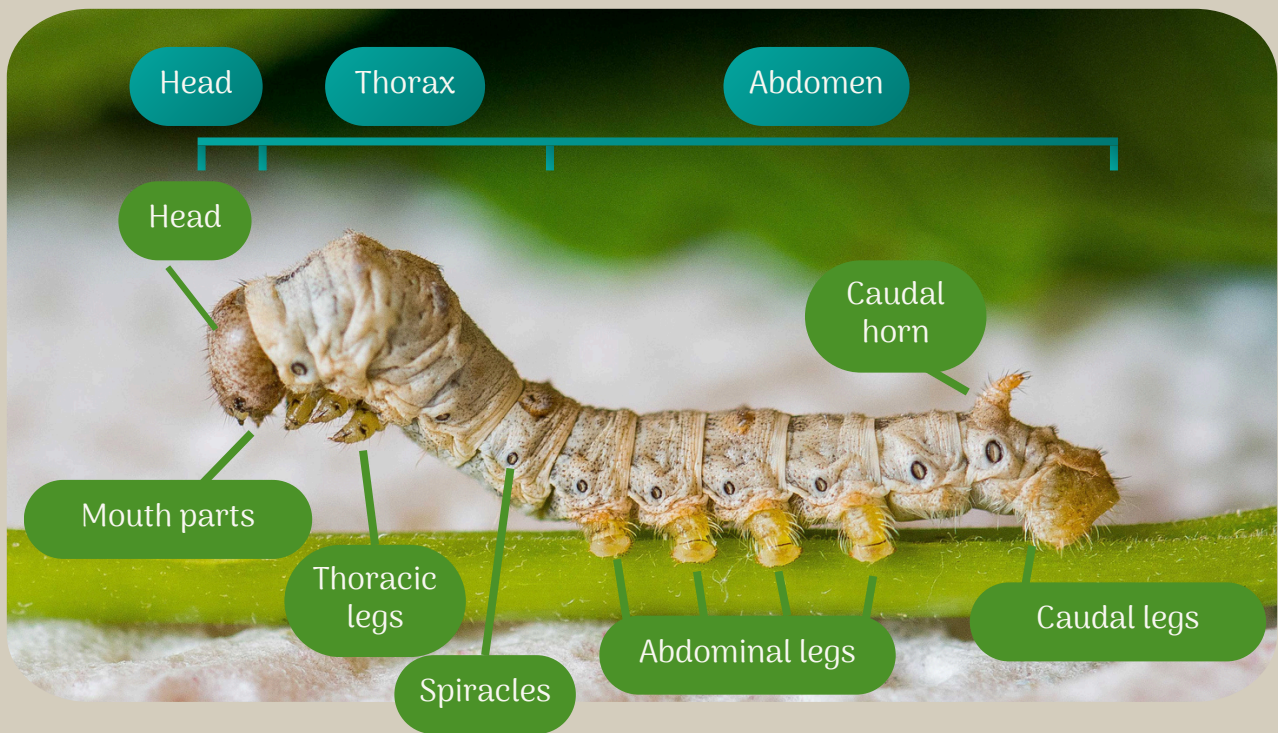
Egg
from which the silkworm hatches.

Larva (worm)
the actual silkworm, which grows and feeds on mulberry leaves.

Chrysalis or Pupa
the intermediate stage of transformation in the cocoon.

Adult (moth)
which is the mature insect ready to reproduce.

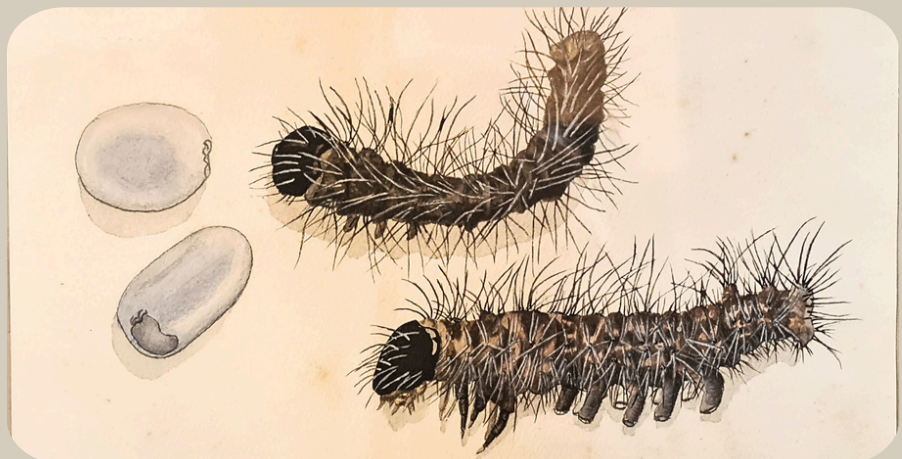




1.2 The larva

The larvae hatch from eggs about 1 mm in diameter, the colour of which is usually grey but can vary among strains. When they are laid, they are mainly yellow, then turn brown, then grey in about 72 hours. At the end of the incubation period, the egg whites (becomes lighter) because the embryo, which is about to hatch, feeds on the dark membrane, which surrounds it, and protects it (in nature) from the sun's rays.

At birth, the larva is about 2 mm long, dark and hairy.



Like other insects, the body of the larva consists of three parts: head, thorax and abdomen. The head is dark and contains the mouthparts, which allow the silkworm to feed on mulberry leaves. The thorax has a small protuberance, and many strains have dark spots resembling a mask or eyes ("eye spot"). There are three pairs of legs on the underside, which the larva mainly uses to bring the leaf to its mouth. The abdomen takes up most of the body: on the upper part we can see the segments and dark star spots, while on the lower part there are five pairs of prolegs, which allow the larva to climb. All along the body we find the spiracles, which are small openings that allow the larva to breathe.



The mature larva measures about 8-9 cm and increases its weight 8000 times since hatching. As the silkworm prepares to spin, i.e. to find an ideal place to build its cocoon, it turns yellowish and becomes slightly transparent as it stops absorbing nutrients from the leaves and expels the remains of its last meal. The silk is produced by two glands on either side of the body and excreted through an opening under the mouthparts called the spinneret.

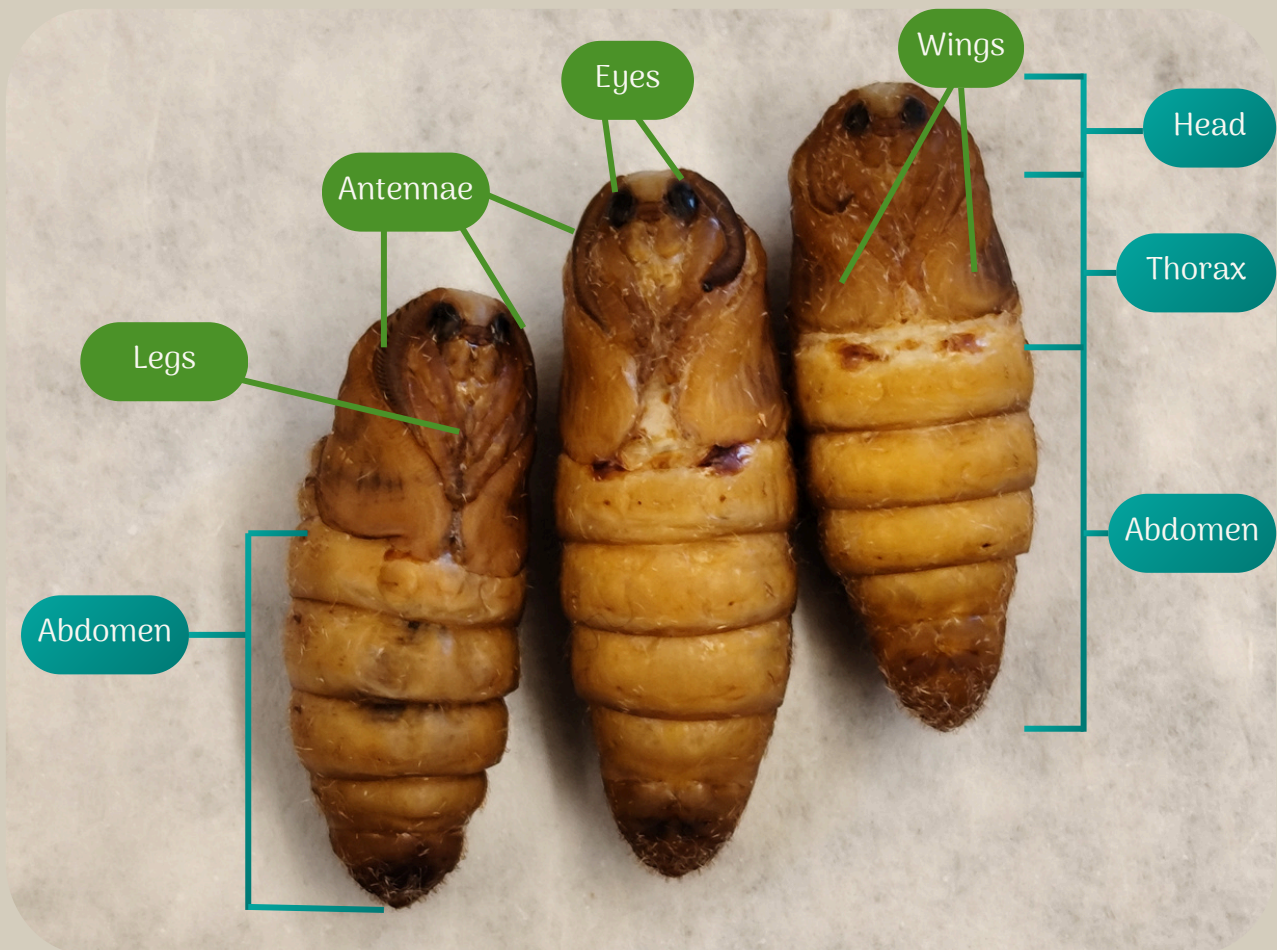


1.3 The chrysalis and the cocoon

The cocoon is completed in two to three days and is made up of a single continuous silk thread, the length of which varies depending on the strain and can be up to 1,500 metres.

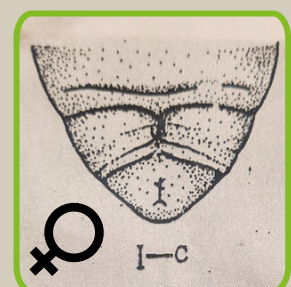
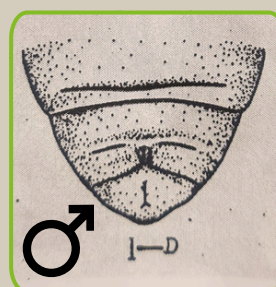
The colour also varies from strain to strain, ranging from orange to bright yellow, pink and pale green. This is because the digestive systems of the larvae of different strains allow different amounts and qualities of the pigments in the mulberry leaf to pass through to the silk glands and from there to the thread. The colour is lost in the industrial processing of cocoons, partly because it is washed away with hot water and partly because it is sensitive to light and heat.

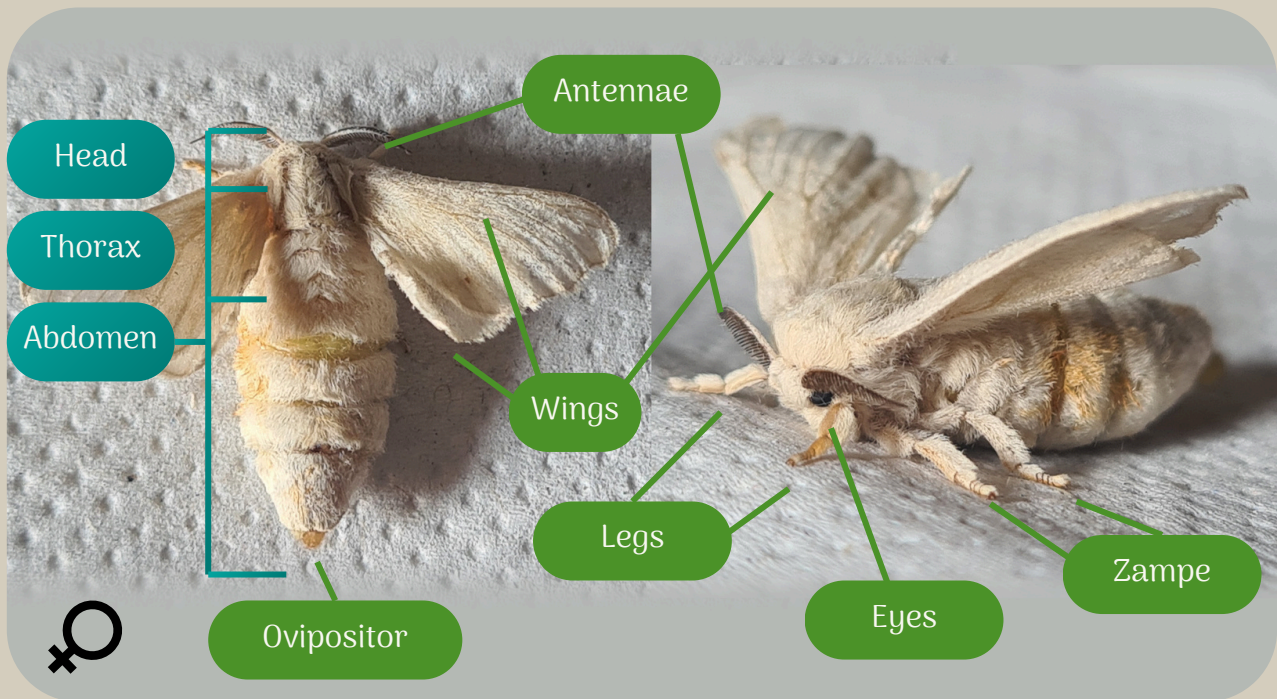




The function of cocoon is to protect the larva while it transforms into a chrysalis and then into a moth. This transformation is a process that makes the animal particularly vulnerable.

Before the start of egg production process, the man removes chrysalises the cocoon after a week and divide them into males and females. In order to identify the sex, it is necessary to carefully observe the abdomen, which has special markings that make it possible to separate the males from the females and then to control the mating in order to plan the necessary crosses to obtain more productive breeds, such as the poly-hybrid used for commercial rearing (and also used in the educational kit), obtained by crossing 4 breeds.





1.4 The moth

The adult moth is creamy white and about 5 cm long. It has two pairs of wings that do not allow it to fly. It emerges from the cocoon thanks to a secretion that dissolves the glue holding the silk threads together, allowing it to break through. Like many other lepidopterans, the silkworm moth cannot feed because it no longer has the organs needed to absorb nutrients. As a result, it lives only a few days for the sole purpose of reproduction.

The males can be distinguished from the females by the size of their abdomens, which are much larger in females because they contain the eggs.



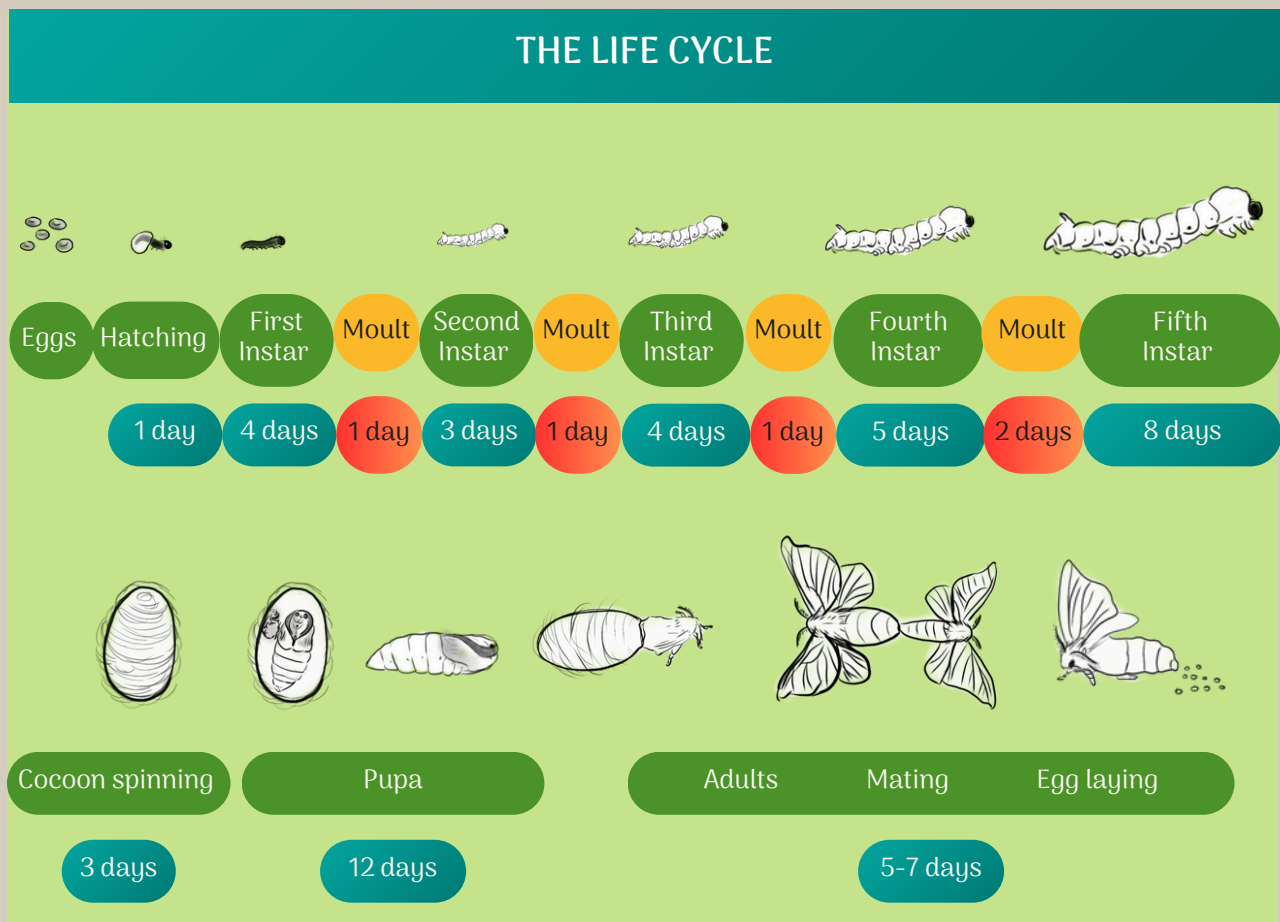
Adults mate for a few hours. At the end of the mating they separate and the female lays about 500 eggs.

1.5 The life cycle

The biological cycle of the silkworm lasts between 45 and 50 days in total, depending on environmental conditions (temperature and humidity affect the duration of each phase).

The larval phase is the longest and lasts about 24 days in total, divided into five instars, separated by four moults.

The counting of days always refers to the first feeding after birth or the moult.

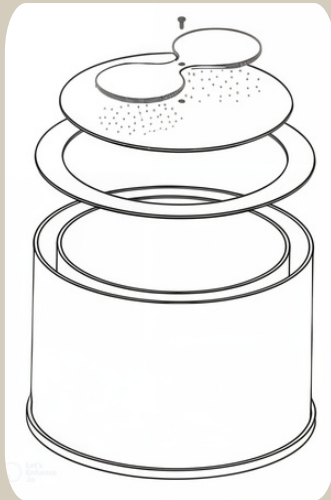


02

The educational kit: rearing in the classroom

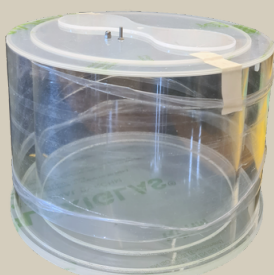


2.1 What the KIT contains



The DIDACTIC KIT consists of the following materials that must be partly assembled:

- Large plexiglass container with cavity (in which to carry out the rearing)
- Adapter ring for plexiglass lid
- Circular perforated plexiglass lid
- Plexiglass hole cover (to be mounted on the lid)
- 1 star-shaped screw (for mounting the lid)
- 1 nut (for mounting the lid)
- 1 thin brush with soft synthetic bristles (for moving newly hatched larvae)
- 1 plastic tweezers (for moving the larvae)
- 1 environmental thermometer
- Electric heater
- 1 container with silkworm eggs
- 14 containers with diet



The material is reusable after being washed
(see chap. 2.10)

2.2 Preparation: what you need

To conduct silkworm rearing, it is necessary to obtain the following materials:



- Plastic cutlery or spatula (for portioning the diet)
- 2 Small transparent plastic containers with lids (approx. 10x15 cm)
- Bottle stopper
- Oven paper
- Blotting paper (roll)
- Magnifying glass
- Straw paper grammage 100 (alternatively non-glossy parcel paper or opaque white cardboard)
- Cardboard cutouts
- Phillips screwdriver (for lid assembly)
- Microfibre cloth or non-abrasive sponge (for cleaning the kit)
- IPA cleaner Isopropyl alcohol (for kit cleaning - no ethyl or 'pink' alcohol) or alcohol-free glass cleaner
- Disposable gloves*
- Precision balance (at least one decimal)*

*Not mandatory

2.3 Good practices: Observing and monitoring

A few simple rules to follow for successful rearing:



- **CLEANLINESS:** always wash your hands thoroughly and sanitise tools after use.
A clean environment minimises the risk of the silkworm getting sick.



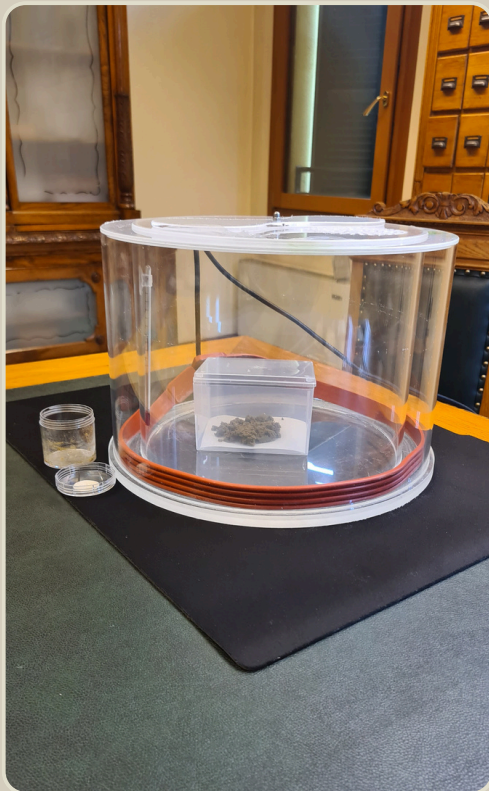
- **OBSERVATION:** as the silkworm is a living being, it is necessary to observe its behaviour and appearance. The indications on the timing of the various stages are subject to variations that can be caused by many factors (changes in temperature, excessive humidity, diseases). It is important to look closely at the eggs and larvae with a magnifying glass to identify the time of birth and of moulting.



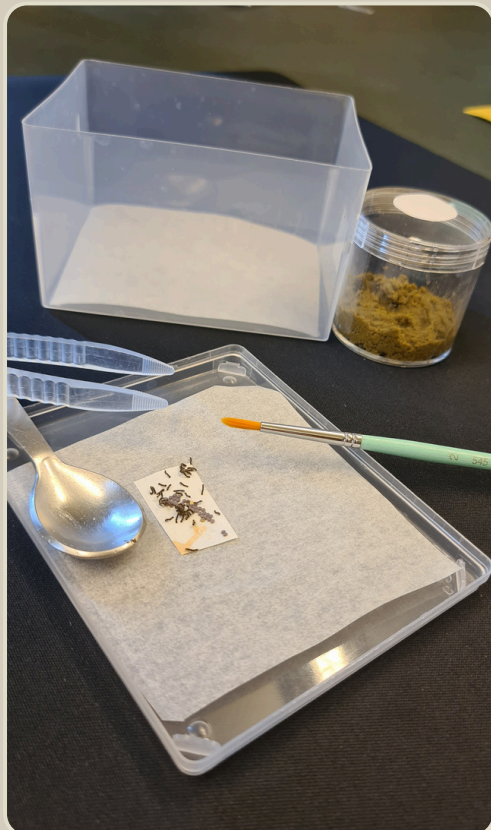
- **MONITORING:** check the growth of the larvae on a daily basis and record your observations. Control the temperature and humidity. To monitor the development of the larvae, it is important to count the days from the administration of the first meal after each it is important to count the days from the administration of the first meal.

NOTE: identify the correct period to start rearing. The activity cannot be carried out during periods of long breaks for holidays and long weekends, as it requires constant supervision. The kit can be left unattended for a maximum of 48 hours, taking care to check and possibly administer the meal for both days (weekend). If it is not possible to be present at all times, we recommend that you take the kit home with you for as long as necessary, taking great care during transport.

2.3.1 Prepare the kit to accommodate the eggs



- Remove the protective film from the plexiglass parts of the kit and clean them (see Chapter 2.10 *Cleaning the kit*). Dry the kit with paper towels.
- Fasten the cover for the ventilation holes on the screw and the supplied nut using the star-shaped screwdriver, taking care not to overtighten to prevent rotation of the component and to avoid damaging the disc.
- Place the electric heater into the cavity of the large container by inserting the wire into the groove provided.
- Place the thermometer inside the large container. Close with the cover, insert the plug of the heater and adjust the thermostat until the temperature inside stabilises at 25°C.



2.3.2 Putting the kit into operation

- Cover the base of the small transparent plastic container with a sheet of oven paper cut to size.
- Insert the bottle cap inside with a folded piece of absorbent paper soaked in water.
- Place the eggs in the small container and place its lid without closing it, leaving a gap for air to pass through.
- Place the small box in the centre of the container and close the lid, keeping the ventilation holes closed too.

2.4 Egg incubation

The packaging of the silkworm eggs is marked with the date of dispatch. The incubation period can vary considerably depending on the temperature conditions to which the eggs are exposed during their journey. Low temperatures slow down the development of the embryo.

The first larvae (or young silkworms) should appear within a time window of 10 to 15 days from the date of shipment. To ensure incubation under optimum temperature conditions it is advisable to place the eggs in the kit as soon as they are received and follow the instructions for setting up the equipment and start rearing.

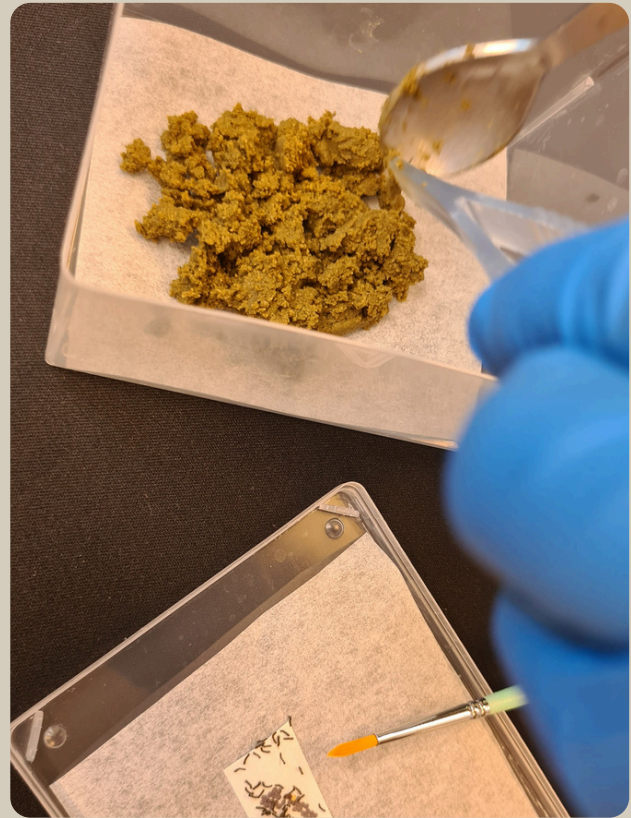
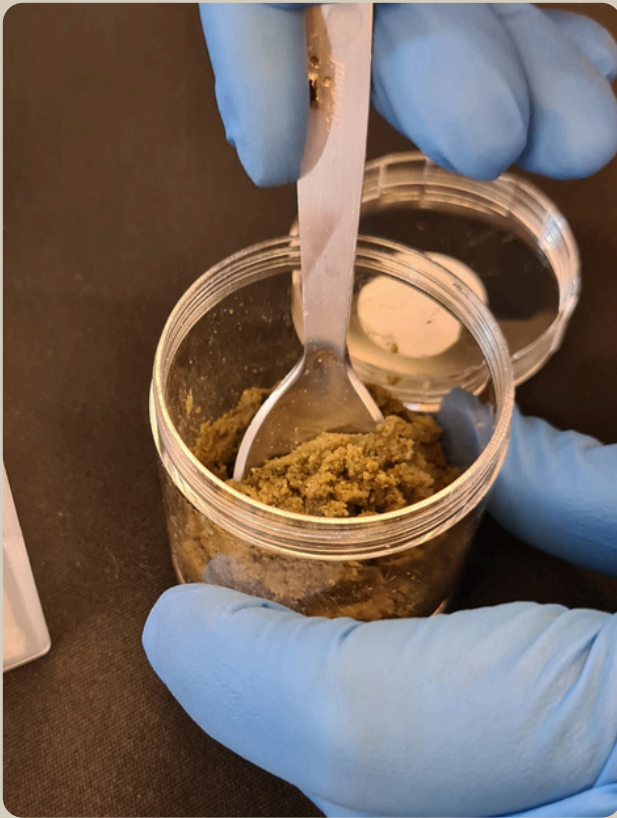




2.5 The hatching of the larvae

When the eggs begin to hatch, you will see a few larvae, small millimetres in size, covered with dark bristles. The light-coloured shells of the eggs from which larvae have hatched are clearly distinguishable from the unhatched, dark-coloured eggs.

Birth takes about 24 hours from the appearance of the first hatchlings. After one day, the newborn larvae should be separated from the unhatched eggs and counted. Empty shells and unhatched eggs should be disposed of in the organic waste.



2.6 Feeding the larvae

The educational kit includes jars of DIET to be fed to the worms. Each packet is labelled with the instar to which the food is intended. The food should be stored in the fridge like any other perishable food.

- **First instar** 1 jar of diet for the entire duration
- **Second instar** 1 jar of diet for the entire duration
- **Third instar** 1 jar of diet for the entire duration
- **Fourth instar** 2 jars of diet, one at the beginning and one at the middle of the development
- **Fifth instar** 8 small doses of diet to be given daily

From the first to the third instar the silkworm must be reared in the small transparent plastic container with the lid.

The diet is sufficient for feeding a maximum 20 larvae in the fifth instar. It is necessary to carry out the selection during the molting phases.



First instar: newly-hatched larvae



Second instar: larvae on the diet



Third instar: larvae on the diet



Fourth instar: larvae on the diet



Fifth instar: larvae on the diet



Fifth instar: larva during the spinning phase

2.6.1 1.1.1 How to give the diet

The diet should be given at **room temperature**. Take the jar out of the fridge and wait half an hour.

Chop up the diet with plastic spatula and distribute it evenly on new, clean oven paper in the rearing container (small plastic container for the first instars or large tub from the kit for the last two instars). The food should be placed in small portions very close together and not too spread* out on the bottom of the box, or will dry out quickly.

Use the brush or plastic tweezers to transfer the worms to the new diet.**

Caution: worms produce silk to attach themselves to surfaces. It may be useful to use a stick (such as a coffee stick) to gently separate the larvae from the food and tools.

The first meal should be given 24 hours after the first eggs have hatched. The larvae should be picked up with the brush and gently placed on the food.

Do not feed during the moult. It is important to stop feeding the larvae until the end of this physiological phase.

* From the first to the third instar, the diet shall not be too spread (take as example the image hereunder on the left), while in the other instars, it may be more spread (as in the image hereunder on the right).

The fine brush is used to shift the larvæ only in the first instar. **DO NOT USE the tweezers on the newly hatched silkworms



2.7 Moulting

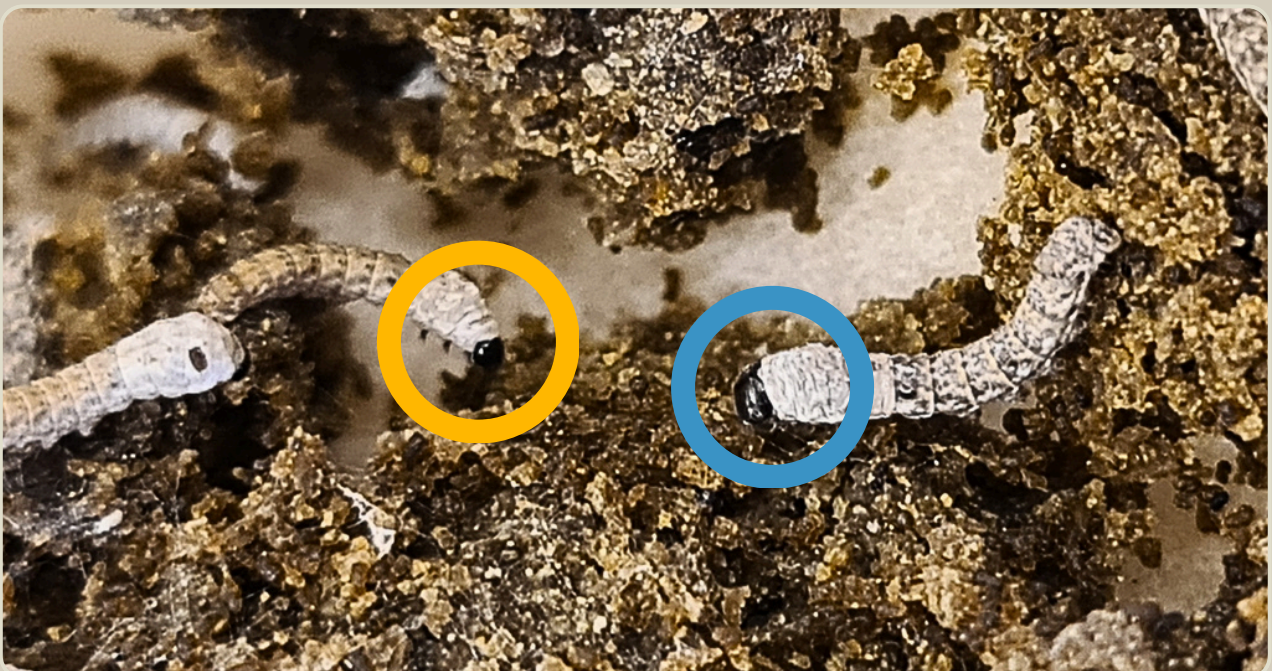
Moulting is a delicate stage in the development of larvae. Care must be taken during rearing to identify the moment when the first worms are about to moult, by detecting the signs:

- the larva stops eating
- the larva does not move and remains in a fixed position with the thorax upwards and the head downwards.

It is important **not to touch the moulting worm** in order not to compromise the process. To free itself from the exuvia (the old skin), the larva anchors itself to the surface. There is a risk, if you touch it, to damage the anchorage and compromising escape from the exuvia.

when the first worms have completed their moult you can separate them from those still moulting.

To distinguish a moulting worm from a still-moulting or not moulted worm, you must look carefully at the head (cephalic capsule).



The moulting worms have a much larger head (blue circle), almost twice the size of the non-moulted worms (yellow circle).

Use tweezers to move the moulted larvae and gently, with the help of the stick, place them into a separate plastic box without food out of the kit.

Keep the moulted worms fasting for 24 hours at a temperature of not less than 20 °C.

This step allows rearing the larvae in a synchronised manner without disrupting their growth, as unfed larvae at low temperatures 'suspend' their development temporarily.

The moulting larva, on the other hand, is characterised by the fact that it is motionless, not feeding, and by the presence of a triangle on its head, similar to a hood, which is formed by the capsule that is about to emerge and pushes the old one forward until it detaches (in the picture circled in green). The new head capsule is clear because the chitin that forms the exoskeleton is still soft and gradually becomes darker while hardening.

NOTE: If in doubt, it is preferable not to touch the larvae and only move them when the end of the moult has been determined.





After 24 hours, use tweezers to remove the remaining moulted worms and transfer them into the box without the food, along with the others.

Discard the old food and the worms that have not moulted, as they may be diseased and may not survive*.

Clean the tray, place a new sheet of greaseproof paper and distribute the new food.

Use tweezers to move the moulted larvae onto the new food and put everything back in the kit at a temperature of 25 °C.

The daily count can be resumed from the first feeding. The larvae will then all be at the same growth stage.

Caution: The moult between IV instar and V instar takes 48 hours. Continue to separate the moulted worms on both days.

*You can use late-moulting larvae as food for birds or reptiles.

2.8 Spinning phase

At the end of the fifth instar, the larva has reached maturity: it measures 8-9 cm and has increased its weight 8000 times since birth. Once they have reached full development, the worms prepare to spin.

This stage can be identified by the following signs:

- The larva stops feeding
- The larva becomes paler, yellowish and slightly transparent.
- The larva purges its gut (gut emptying) so that the excrements appear green and softer, as the worm stops absorbing nutrients (as opposed to the black and solid ones it produced during the growth phase).
- The larva tends to climb upwards in search of a place to build the cocoon.



In order to spin its cocoon, the silkworm must construct a structure that is anchored to 3 support points at the right distance from each other. Its instinct leads it to find the ideal place upwards, away from the dampness of the bottom of the box.

Therefore, before placing the spinning frames in the box, it is necessary to prepare the bed, i.e. the bottom of the box. Remove the food and clean the kit with paper towels, trying to remove faeces and food remains. Place a piece of cardboard on the bottom, some straw paper (or opaque wrapping paper) and a few sheets of blotting paper so that the entire bottom of the kit is covered. It is necessary to line the bottom of the kit to absorb excess moisture and prevent the formation of mould

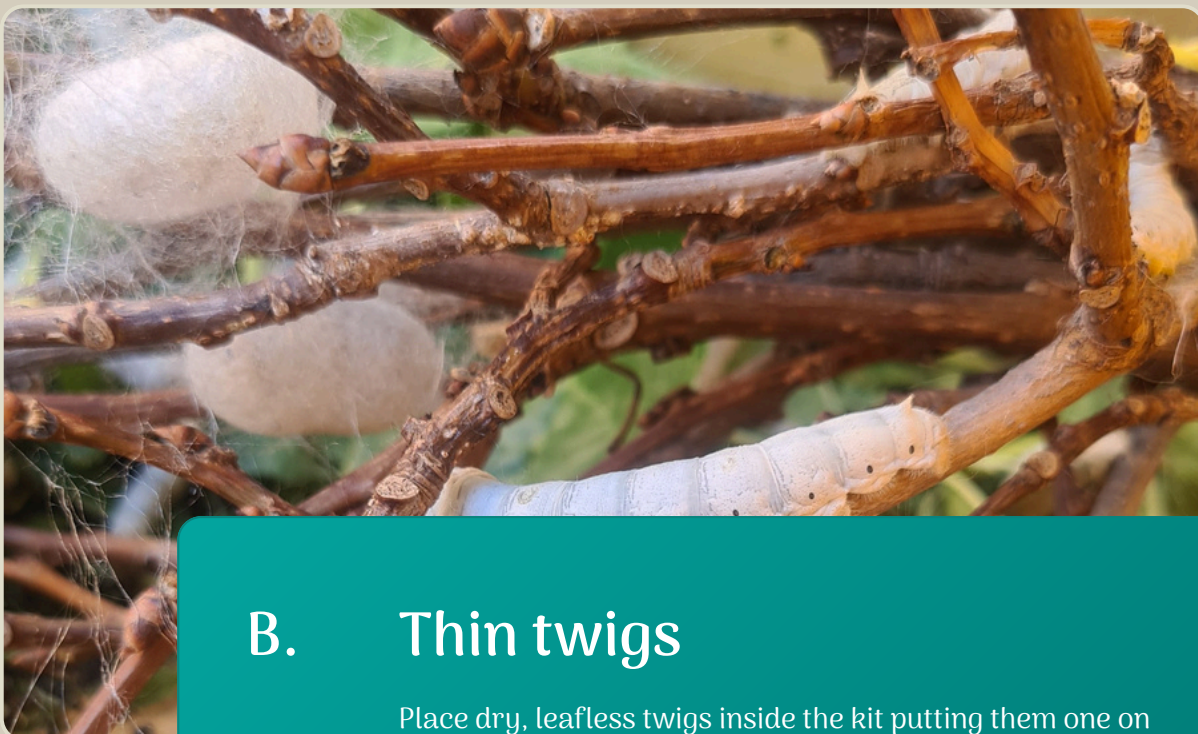


There are different ways to build the spinning frames:



A. Paper cup or thin cardboard

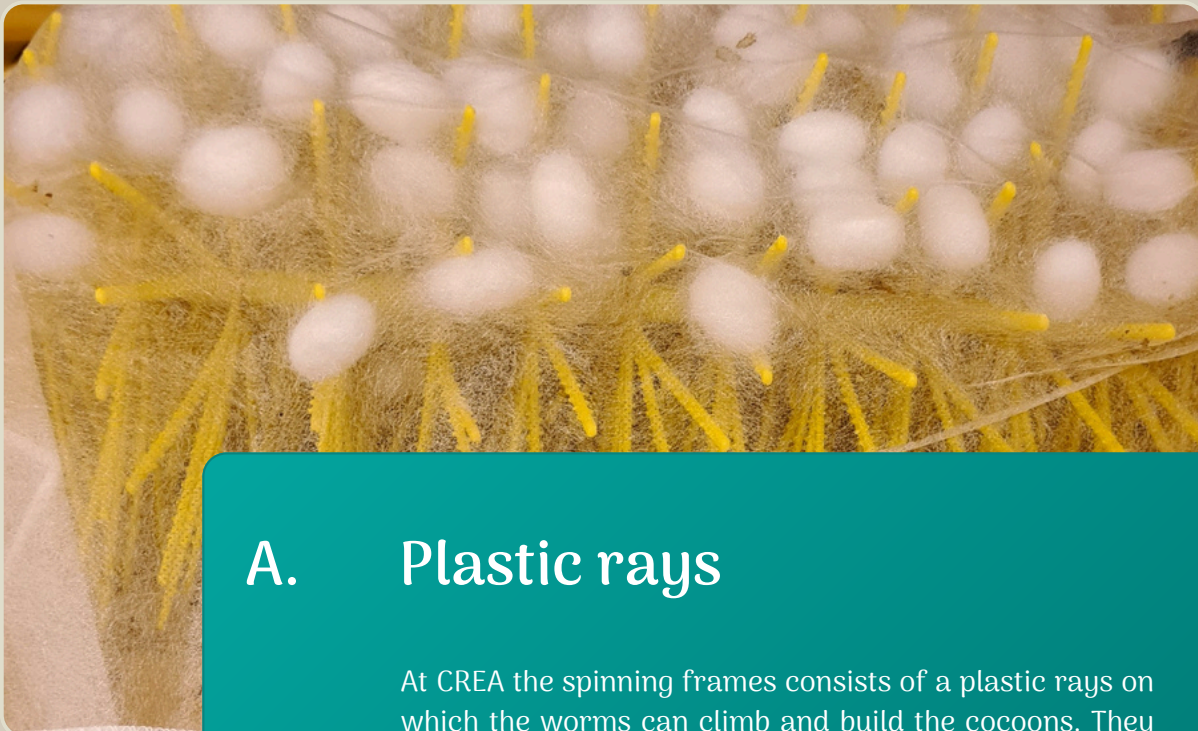
Construct a pyramid structure with small coffee paper cups from which to remove the bottom or rigid cardboard. Use the stapler and not glue or tape, to avoid intoxicating the worms. Hold the frame a few centimeters from the lid.



B. Thin twigs

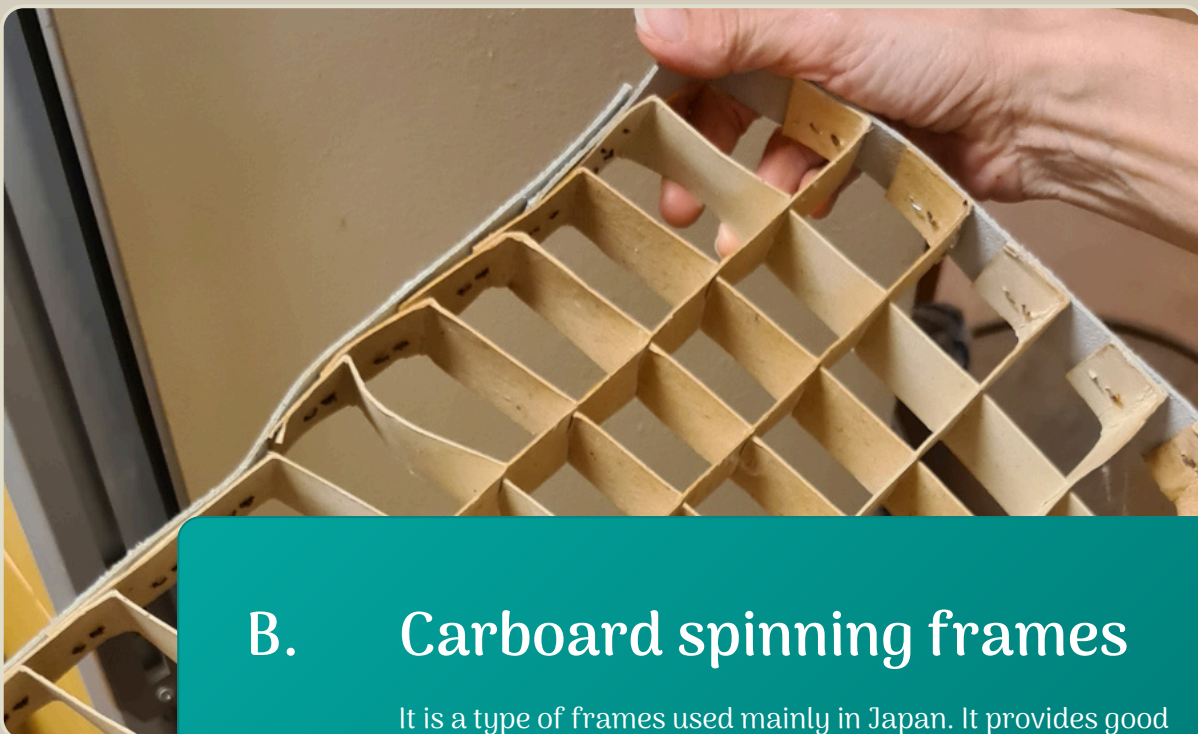
Place dry, leafless twigs inside the kit putting them one on top of the other, crossing them so as to construct a sort of net on which the worms can climb.

The spinning frames in commercial rearing for cocoon production:



A. Plastic rays

At CREA the spinning frames consists of a plastic rays on which the worms can climb and build the cocoons. They are practical tools because they are washable and reusable.



B. Carboard spinning frames

It is a type of frames used mainly in Japan. It provides good support for the larvae, but it can only be sterilised with an open flame and is labour intensive for the operator.



2.9 Cocoons and moths

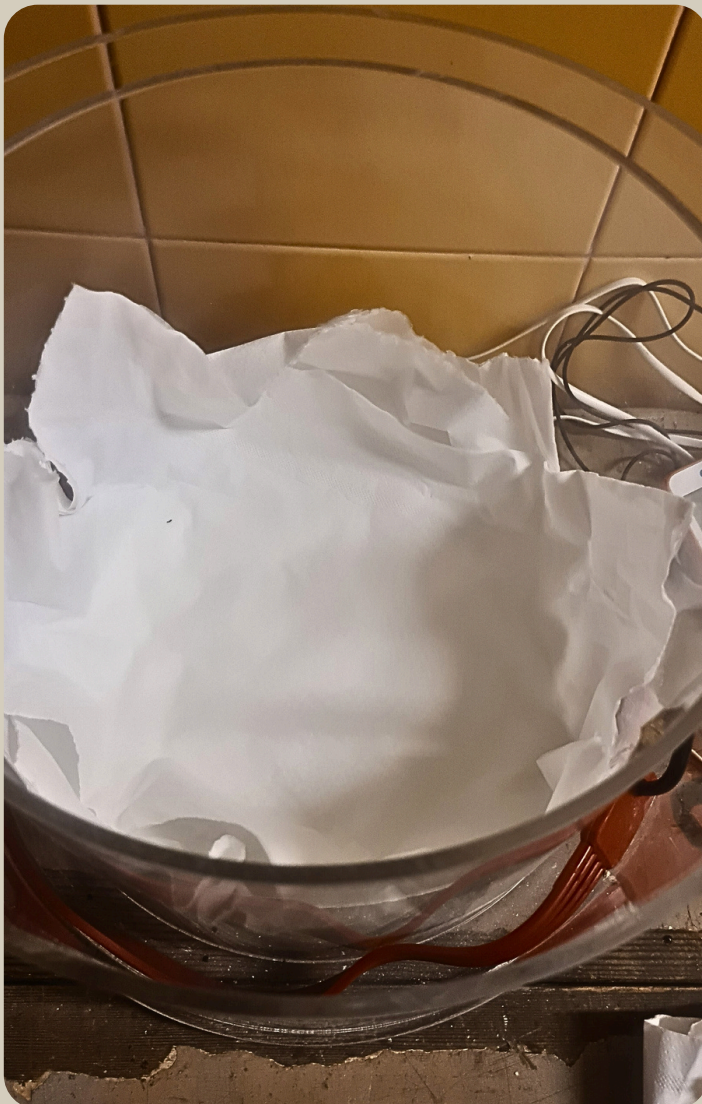
When the larvae climb to the frames, they start spinning their own cocoons. It takes about 2-3 days to complete them. After a week, it is possible to move the cocoons, as the transformation of the larva into a pupa has already occurred, and there is no risk of compromising the development of this process.

Open the kit and gently remove the spinning frames, being careful not to damage any cocoons built in contact with the walls or the lid.

Carefully detach the cocoons from the spinning frame.

Clean the cocoons from the floss, which is the first strand of silk emitted by the silkworm to prepare the structure on which to spin the cocoon.

This silk floss can also be used in certain production sectors, for example to create padding and to extract silk proteins for the cosmetic or biomedical industries.



Clean the kit well with absorbent paper and a damp cloth, making sure to dry the inside of the kit completely. Line the bottom with a folded sheet of kraft paper. Cover the base and the walls of the kit with several layers of absorbent paper up to halfway, in order to prepare the place to host the moths, which are skilled climbers.

Position the thermometer again and place the cocoons inside the kit. Close the lid while keeping the ventilation holes open.



The emergence of the adult (a term that indicates the moth's exit from the cocoon) begins about two weeks after spinning.

The adult secretes a drop of liquid through its mouth opening, dissolving the glue that holds the thread folded in the cocoon, and it creates an opening with its legs among the silk fibres.

Once emerged, the moth can expel a yellowish liquid, a residue from the transformation of its internal organs. This is why it is important to properly line the kit's container with absorbent paper.



Silk moths lack a digestive system, so they cannot feed. They live for a few days solely to reproduce and ensure the continuity of the species. Male moths immediately begin searching for a female to mate with.

At the end of the mating, the female lays eggs that stick to the surface thanks to a glue substance produced by the moths itself. This can also happen on the walls of the kit.

6.10 Kit cleaning

Once the rearing is completed, it is time to clean the kit and dispose of the organic waste in the compost bin.

DO NOT keep the eggs*, as without analysing the moths under a microscope, it is not possible to rule out contamination from diseases transmissible to the next generation; furthermore, without the correct preservation procedure, it would still be very unlikely for the larvae to hatch uniformly.

- Remove the heater and store it away.
- Unscrew the ventilation hole cover from the lid of the kit.
- Rinse the plexiglass components with warm water and gently wipe with a non-abrasive sponge or microfiber cloth to remove any eggs or stains from the walls of the tank. A thin toothbrush can be used to assist in this process.
- Clean the internal walls of the tank with a microfiber cloth and isopropyl alcohol (or a non-alcoholic glass cleaner).
- Dry the kit thoroughly to avoid limescale deposits.
- Wash all tools with soap or detergent.

CAUTION:

DO NOT use ethanol or cleaners that contain it, as it can cloud the plexiglass, compromising the kit.

*Eggs for a new rearing program can be required from the relevant entity for the territorial jurisdiction: in Italy, CREA-AA – the Sericulture Laboratory of Padua provides them free of charge for educational purposes. It is not recommended to obtain eggs from farms that cannot guarantee their healthiness.

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