BIOLOGICAL CHARACTERISTICS OF THE MULBERRY SILKWORM (BOMBYX MORI) AND ITS IMPORTANCE IN SILK PRODUCTION

Zhala Ramazanova [1], Ayaz Mammadov[1]
[1]Western Caspian University

Abstract: Bombyx mori L., 1758 (Lepidoptera: Bombycidae), is the mainstay of the silk industry and is economically highly significant. It depends on humans to complete its life cycle and has a short lifespan. Among its genes, there are many that show high homology with genes causing human diseases. At the same time, its low breeding and maintenance costs, along with minimal ethical issues and the availability of its complete genome sequence, make it a potential candidate as an alternative invertebrate model organism for life science research. In recent decades, Bombyx mori L. has been successfully used as an alternative invertebrate model organism in various scientific fields. These applications include modeling human diseases, environmental monitoring, epigenetic studies, as well as testing and discovering microbial drugs.
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***Corresponding Author:** jalaramazanova6@gmail.com

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Introduction:

The Lepidoptera order includes butterflies, skippers, and moths. These insects perform various functions in ecosystems, serving as food sources for agricultural pests, birds, bats, and other insects, and acting as nocturnal pollinators (Dar and Camal, 2021a, 2021b). Bombyx mori Linnaeus 1758, known as the silkworm, is famous for silk production and forms the foundation of the silk industry. Generally, while other lepidopteran species are polyphagous, the silkworm is a monophagous species. Its food source is exclusively the leaves of the mulberry tree, which is the only food it consumes during its larval stage.

After mating, the adult female silkworm lays eggs, which hatch into small, black, and hairy larvae. These larvae go through four molts, resulting in five different instar stages. The larvae of the fifth instar stage spin a silk cocoon, and a mature individual emerges, searches for a mate, and reproduces. After mating, the female silkworm lays eggs. When the silkworm eggs are laid, they are creamy white in color, and if they are fertile, they turn black.

History:

China, known as the "cradle of silk," has made significant progress since 1949, demonstrating incredible development in the silk industry over the last five decades and becoming the world's largest silk producer. In recent years, the silk farming of mulberry silkworms has become a key factor in ensuring sustainable employment in agriculture and strengthening economic development. The achievements in this field have been made possible through effective government policies, targeted and intensive research and development activities, and the precise implementation of these measures.

Silk farming has a 5000-year history in China. The first written information about the characteristics of silkworms in captivity was recorded in 1637 by Song Yinxing in his book Tian Gong Kai Wu. Improving the productivity of silk farming has always been considered a crucial factor, with the selection, breeding, and maintenance of better-quality silkworm species being carried out both consciously and unconsciously for a long time. However, the systematic collection and study of silkworm breeds began only in the 19th century.

The development of silk farming in Azerbaijan is based on ancient history and rich traditions. The widespread cultivation of this industry, especially in the North-Western region, particularly in Sheki, is not coincidental. Over the centuries, silk farming traditions have been preserved and developed here, playing an important role in the lives of the local population. The recognition of Sheki as a silk farming center and the widespread application of this industry in agriculture has also been confirmed by archaeological research. In the past, silk farming in Sheki (Nukha) was carried out by entrepreneurs in individual factories and workshops and developed on a limited scale. However, over time, the reputation of this valuable product – Sheki silk – spread not only within the country but also abroad, resulting in a significant increase in demand. Sheki has been one of the main silk farming centers in Azerbaijan and the entire region, a fact even reflected in the city's coat of arms. Sheki silk has always been highly valued in world markets, maintaining its reputation. The silkworms and silk threads produced here have always been considered precious. Over time, alongside the development of silk farming, a specialized class that mastered the technology and production methods of this industry has also formed.

Azerbaijan's historical location on the "Silk Road" has contributed to the formation and development of this industry, especially in Sheki. This factor also helped expand trade and economic relations with other countries. Archaeological findings prove that silk farming was already practiced during the ancient Caucasian Albania period in the temple lands. In 1985, during research in the Kudurlu village area of Sheki, valuable metal earrings resembling silkworms and a kauri-shaped cut piece were found in mound number 7.

For the first time in the field of silk farming, adaptive selection methods were applied with the aim of creating ecologically resilient hybrid lines of the mulberry silkworm. This approach not only enhanced the silkworms' ability to adapt to various ecological conditions but also enabled improvements in their biological, technological, and productivity traits. During the adaptive selection process, the most resilient and high-yielding individuals from silkworm populations were selected through natural and artificial selection methods.

As a result, the hybrid lines obtained through the application of the adaptive selection method demonstrated high adaptation capabilities, were able to develop optimally under various ecological conditions, and created a more favorable genetic foundation for silk production. This approach has opened new perspectives for the future sustainable and efficient development of silk farming.

Life Cycle of the Silkworm:

The life cycle of the silkworm begins with the female silkworm laying eggs. From the eggs, caterpillars or larvae emerge. The silkworms feed on mulberry leaves and go through the pupa stage. During the pupa stage, the silkworm attaches itself to its surroundings with a thread. Then, by waving its head, it spins a protein-based fiber into silk. Several caterpillars create a protective layer around the pupa, which is known as a cocoon. The silk thread (fiber) is obtained from the silkworm's cocoon.

The egg is the initial stage of the silkworm's life cycle. The female moth lays a single egg, about the size of a complex dot, in the summer or early autumn. The eggs remain stationary until spring, when the warmth triggers their hatching. The Bombyx mori egg is very small and has a hard structure; it is the size of a pinhead and resembles a poppy seed. The egg's shell provides protection to the developing embryo, covering it. When first laid, the egg is pale yellow in color. Within a few days, the fertile egg cell turns a bluish-gray color.

The larval stage is the vegetative phase where growth occurs. The Bombyx mori larva, commonly known as the silkworm, is a specific host of the mulberry tree. During its growth, the larva can molt four times. The stages between molts are called instars. When the silkworm hatches from the egg, it is about 1/8 inch in size and very hairy.

The new silkworms emerging from the cocoon feed exclusively on soft mulberry leaves, but as they grow, they can also eat tougher mulberry leaves. The larval stage lasts about 27 days, during which the silkworm goes through 5 instar stages.

During the pupa stage, the silkworm spins a protective cocoon. The size of the cocoon is compared to a cotton ball, and its color changes; the cocoon is spun from continuous silk threads, about 1.5 meters (approximately one mile) long. This cocoon serves as protection for the pupa. The cocoon is primarily white, but depending on the silkworm's genetics, it can also turn creamy or yellow. After the final molt inside, the cocoon transforms into a brown, chitin-covered pupa structure.

During the pupa stage, metamorphic changes occur, resulting in the silkworm transforming into a moth. If the silkworms are allowed to pass through the cocoon and mature, the silk cannot be used for commercial purposes. Therefore, after the cocoon is produced, the silkworms are killed, and the insects are immersed in boiling water to dissolve the cocoon's adhesive. The silk is then unwound and spread out over a distance of one mile to transform into thread (Figure 1).

The cocoon is the stage during which silk threads are spun around the larva to form a protective structure, serving to protect it from predators. The larva remains inside the cocoon as it transitions into the pupa stage. The color of the cocoon varies depending on the silkworm's diet, ranging from white to golden yellow. After the second larval stage is completed and the pupa turns brown, molting occurs inside the cocoon. During this period, the pupa transforms into an adult moth over approximately 2-3 weeks.

Figure 1. The pupal stage of the mulberry silkworm

The adult stage completes the life cycle of Bombyx mori. This stage is the reproductive period during which the adults mate, and females lay eggs. Moths are incapable of flying and cannot consume food as they lack functional mouthparts (Figure 2).

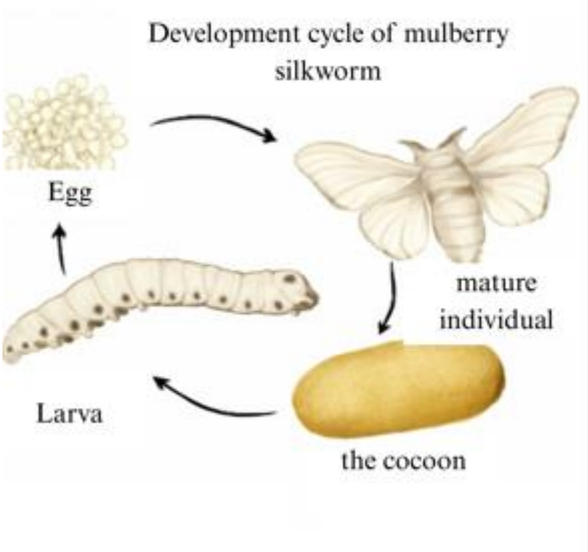

Figure 2. Development cycle of the mulberry silkworm

Feeding of the Mulberry Silkworm:

The mulberry silkworm is a delicate creature that requires great attention and care, but in return, it yields high productivity. Therefore, to achieve maximum output from the silkworms, the care and management provided to them must be of a high standard.

The most important factors that affect the growth, development, and high-quality cocoon production of the mulberry silkworm are as
. follows:

- Quality of the feed
- Quantity of the feed
- Feeding technique

From the moment the silkworm hatches, it begins searching for food, and with its weak mandibles, it finely cuts and consumes the leaves


Figure 3. Nutrition in the early years


Figure 4. Nutrition of an adult worm

A silkworm can consume an amount of leaves equivalent to its body weight in a single day. When provided with sufficient food, the

larvae develop rapidly and accumulate a large amount of fat in their bodies. This fat reserve allows them to survive without food during the pupa, moth, and egg (grana) stages.

Mulberry leaves are the only food of the mulberry silkworm. In the early days of the first instar, the larvae feed only on the soft parts of the leaves; (Figure 3) as they grow, they feed on the entire leaf, except for the veins. (Figure 4) As the larvae approach their final instar, they may even consume the petiole of the leaf and, in some cases, the mulberry tree itself.

Mature larvae rapidly and voraciously consume the leaves. Using their strong mandibles, they create oval-shaped notches on the leaves and slice them thinly. The larvae cut the leaves when they lower their heads, but cannot cut when they raise their heads.

Silk threads are distinguished by their durability, strength, and smooth texture. (Figure 5) While spinning the cocoon, the silkworm protects itself from environmental influences, and during this process, it takes on a special shape. Once the cocoon is completed, the silkworm exits this protective structure and transforms into a moth

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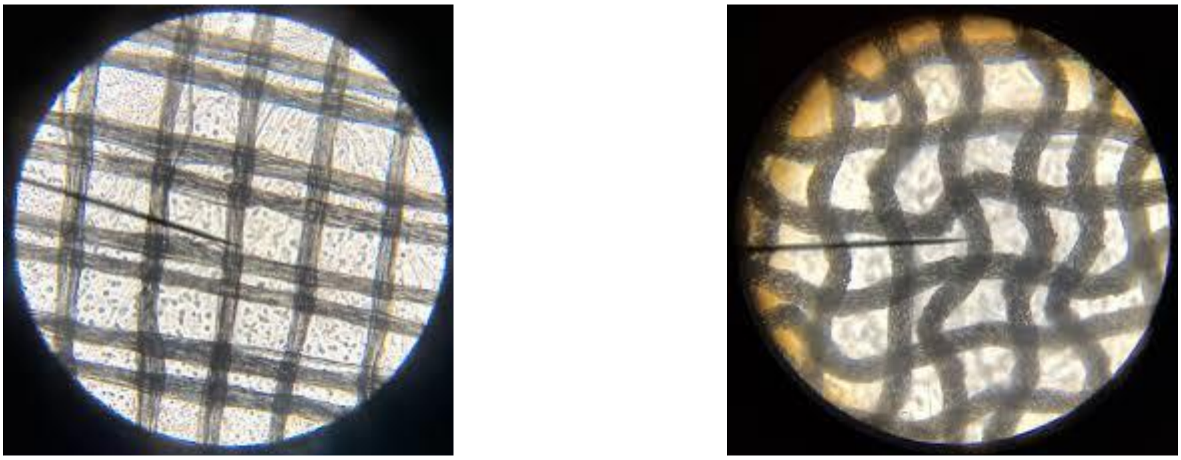

Figure 5. Appearance of silk threads under a microscope

Silk is a naturally occurring material composed of two biomacromolecules, fibroin and sericin, and is used as a high-quality textile material. In recent times, silk has been reported to have good blood compatibility [1], [2], cytocompatibility, to cause low levels of inflammation in the body, and to be biologically biodegradable. Due to these properties, silk has been studied for various applications in the biomedical field, including the production of artificial ear drums, membranes for controlled bone regeneration, bone substitute materials [10], scaffolds for tissue engineering, burn dressings, and the creation of nerve and irritation models.

Unlike synthetic polymers, silk polymers (fibroin and sericin) are naturally produced by the silkworm. Therefore, the structural characteristics and properties of silk polymers are determined during production by the silkworm. Some limitations of silk have posed obstacles to its use in biomedical fields. For example, the molecular weight (MW) of silk fibroin (SF) decreases during degumming [11] and dissolution processes. As a result, various efforts have been made to minimize the molecular degradation of SF during these processes, as the molecular weight of natural silk is determined by nature and cannot be increased. Therefore, the methods applied to produce less degraded SF play a crucial role in improving its mechanical properties.

The primary sequence of silk protein consists of hydrophobic domains made up of short side-chain amino acids, which contribute to its crystalline β-sheet structures. These structures enable the tight packing of anti-parallel chains through hydrogen bonding, ensuring that silk fibers have high mechanical strength and tensile resistance. The predominance of hydrophobic domains enhances the water resistance of silk, thereby ensuring its biological stability.

Additionally, the presence of smaller hydrophilic domains in silk fibroin interacts with the hydrophobic regions, regulating the proper assembly of the protein. This balance between hydrophilic and hydrophobic domains enhances the strength and elasticity of silk fibers, making it an ideal material for various biomedical and industrial applications. [4] These unique properties of silk make it highly suitable for use in fields ranging from the textile industry to medicine and biotechnology. Silk derived from silkworms, particularly the Bombyx mori species, and orb-weaving spiders, such as *Nephila clavipes*, have been extensively studied to understand their processing mechanisms and to explore the potential applications of these proteins as biomaterials. These silks, both natural fibers and promising materials in biomaterials engineering and nanotechnology, have attracted significant attention.

Silks produced by silkworms and orb-weaving spiders possess a range of unique characteristics. Among these features, high ecological stability, biocompatibility with living organisms, and controlled proteolytic biodegradation are of particular importance. Through proteolytic biodegradation, silk fibers gradually break down and integrate into the organism's natural metabolic pathways, making them an ideal material for medical implants and regenerative tissue engineering. [4]

Furthermore, these silks exhibit morphological flexibility and can be easily modified to create different shapes and structures. The functional groups of amino acids in silk proteins allow for specific chemical modifications, which enable the immobilization of growth factors, i.e., biomolecules that promote cell proliferation and tissue regeneration, stabilizing them on the surface of silk fibers.

In addition to all these characteristics, silk fibers possess impressive mechanical durability. Silk produced by spider silk and silkworms exhibits high tensile strength and elasticity, making them suitable for use in various fields where strong, lightweight, and durable materials are needed. [2] Therefore, silk biomaterials offer vast potential in numerous areas such as biomedicine, biotechnology, drug delivery systems, wound dressings, and tissue engineering (Table 1).

Table 1. Mechanical Properties of Biodegradable Polymer Materials

Biomaterial Source	**Modulus (GPa)**	**UTS (MPa)**	**Tensile Strain at Break (%)**
B. mori silk (with sericin)	5-12	500	19
B. mori silk (without sericin)	15-18	610-690	4-16
B. mori silk	10	740	20
N. clavipes silk	11-15	875-972	17-18
Collagen	0.0018-0.046	0.9-7.4	24-68
Crosslinked collagen	0.4-0.8	47-72	12-16
Polylactic acid	1.2-3.0	28-50	2-6

B. mori Silk Fibroin Structure:

When examining the amino acid composition of B. mori silk fibroin, it is primarily composed of glycine (Gli) (43%), alanine (Ala) (30%), and serine (Ser) (12%) (Figure 6). In heavy-chain silk fibers, there are 12 domains that form crystalline regions, and these domains intersect with non-repetitive primary sequences, leading to the formation of less organized areas within the fibers. The crystalline regions are primarily composed of Gly-X repeats, where X amino acids are represented by alanine (Ala), serine (Ser), threonine (Thr), and valine (Val). The crystalline regions typically consist of approximately 381 amino acid residues, although this number varies across different domains. For instance, the seventh domain contains 596 residues, while the twelfth domain has as few as 36 residues. This variation influences the structural and mechanical properties of silk fibers. [1]

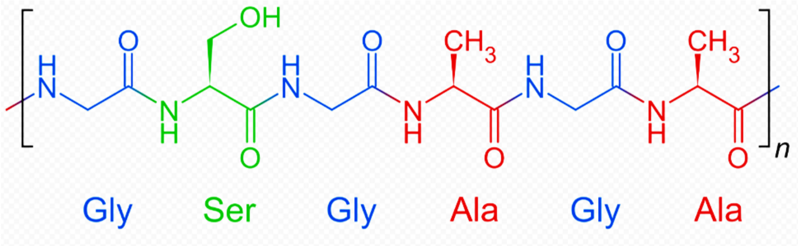

Figure 6. B. mori silk fibroin structure

Each crystalline domain is composed of smaller subdomains of hexapeptides. These subdomains include motifs such as GAGAGS, GAGAGY, GAGAGA, or GAGYGA, where G stands for glycine, A for alanine, S for serine, and Y for tyrosine. These sequences play a crucial role in the formation of the crystalline regions of silk fibers and determine their characteristics, such as strength and elasticity. [3]

Additionally, these subdomains are typically completed by tetrapeptides like GAAS or GAGS. These tetrapeptides regulate the assembly and organization of silk proteins, creating transition zones between crystalline and amorphous regions. As a result, the structural properties of silk fibers are optimized, facilitating their use in various biomaterial and industrial applications.

The biodiversity of local silkworm breeds and hybrids (*Bombyx mori* L.) in Azerbaijan was assessed for the first time using RAPD (Random Amplified Polymorphic DNA) molecular markers. In total, four RAPD primers (BGN 04, BGY 06, BGA 02, and BGW 02) and four ISSR primers (UBC 807, UBC 857, UBC 813, and UBC 827) were employed in the study. The RAPD primers generated 32 distinct bands, with fragment sizes ranging between 200 and 1200 base pairs. The number of amplicons produced per primer varied from 6 to 11, with an average polymorphism rate of 81% [13].

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