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Studies on the haemocytes of mulberry silkworm *Bombyx mori* L in the region of District Amethi, Uttar Pradesh, India

[1] Saleem Ahamad, [2] Rajneesh Tripathi, [3] Indu Singh, [4] Avinash Kumar Malviya

[1, 2] Jagdeesh Prasad Jhabarmal Tiberawala University, Jhunjhunu, Rajasthan, India.
[3, 4] Kamala Nehru Institute of Physical and Social Science, Sultanpur, Uttar Pradesh, India.

Abstract
The silkworm, *Bombyx mori* L. is a delicate and sensitive lepidopteron insect, which has been domesticated for silk production. Due to continuous domestication, silkworm becomes susceptible to various diseases. The insect immune response consists of two tightly interconnected components, the cellular and the humoral responses. The cellular response is mediated by haemocytes and involves responses such as phagocytosis, encapsulation, and clotting. During the course of infection the cellular defense mechanism in silkworm is mediated by different types of haemocytes. Haemocytes are found circulating freely in the haemolymph or adhering to internal organs such as the fat body or the digestive tract of the insects. Five types of haemocytes were found in the haemolymph of *Bombyx mori* L viz., prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs) and oenocytes (ONs). The total haemocyte counts and the differential haemocyte counts vary in the different life stages. Haemocyte numbers in the hemolymph of any particular insect may vary depending on various factors, such as disease and meteorological factors, including altitude. The results show significant variations of different haemocyte in the various larval forms of silkworm. The first instar nymph contained only PRs. Second and third instar nymph had PRs and PLs. Fourth instar nymph contained PRs, PLs and GRs. These investigations may be very useful in planning rearing strategies for commercial species of Silk moths.

Keywords: Haemocytes, Mulberry silkworm, *Bombyx mori*, Amethi, Uttar Pradesh, India

1. Introduction
Silkworm *Bombyx mori* is an economical insect. Due to the centuries of domestication, it has lost its natural resistance and susceptible to different types of diseases, which leads cocoon crop loss. The major diseases affecting the silkworm are pebrine, flacherie, grasserie and muscardine, which is causing around 27-35% cocoon crop loss. Among these diseases muscardine is one of the contagious diseases. It occurs mainly in the rainy and winter season, which is the favorable season for silkworm rearing and in total crop loss around 10-40% crop loss, is occurring due to muscardine. Haemolymph is dynamic and lifesaving fluid which was closely related to all the metabolic activities of the organism. The progress of the fungal pathogen Beauveria bassiana can be monitored by studying the degree of variation in several functions, haemocyte population dynamics and biochemical constituents in the haemolymph, as the fungal pathogen confines to haemolymph till the silkworm larvae approach death. Knowledge on the haematological, biochemical, nitrogenous waste products and enzymatic changes, due to infection by the fungal pathogen in the larvae of *Bombyx mori* will be useful for evolving suitable prophylactic and control measures against the disease to enhance qualitative and quantitative cocoon yield parameters.

1.1 Mulberry silkworms
Mulberry silkworms can be categorized into three different but connected groups or types. The major groups of silkworms fall under the univoltine ('uni-'=one, 'voltine'=brood frequency) and bivoltine categories. The univoltine breed is generally linked with the geographical area within greater Europe. The eggs of this type hibernate during winter due to the cold climate, and cross-fertilize only by spring, generating silk only once annually. The second type is called bivoltine and is normally found in China, Japan, and Korea
The breeding process of this type takes place twice annually, a feat made possible through the slightly warmer climates and the resulting two lifecycles. The polyvoltine type of mulberry silkworm can only be located in the tropics. The eggs are laid by female moths and hatch within nine to 12 days, so the resulting type can have up to eight separate lifecycles throughout the year.

Fig 1: Adult silkworm moth

Eggs take about 14 days to hatch into larvae, which eat continuously. They have a preference for white mulberry, having an attraction to the mulberry odorant cis-jasmone. They are notmonophagous since they can eat other species of *Morus*, as well as some other Moraceae, mostly Osage orange.

Hatchlings and second-instar larvae are called *kego* and *chawki* in India. They are covered with tiny black hairs. When the color of their heads turns darker, it indicates they are about to molt. After molting, the instar phase of the silkworms emerge white, naked, and with little horns on their backs.

After they have molted four times, their bodies become slightly yellow and the skin becomes tighter. The larvae then prepare to enter the pupal phase of their lifecycle, and enclose themselves in a cocoon made up of raw silk produced by the salivary glands. The final molt from larva to pupa takes place within the cocoon, which provides a vital layer of protection during the vulnerable, almost motionless pupal state. Many other Lepidoptera produce cocoons, but only a few—the Bombycidae, in particular the *Bombyx* genus, and the Saturniidae, in particular the *Antheraea* genus—have been exploited for fabric production.

Fig 2: Wild silkmoth *Bombyx mandarina*

If the animal is allowed to survive after spinning its cocoon and through the pupal phase of its lifecycle, it releases proteolytic enzymes to make a hole in the cocoon so it can emerge as an adult moth. These enzymes are destructive to the silk and can cause the silk fibers to break down from over a mile in length to segments of random length, which seriously reduces the value of the silk threads, but not silk cocoons used as "stuffing" available in China and elsewhere for doonas, jackets etc. To prevent this, silkworm cocoons are boiled. The heat kills the silkworms and the water makes the cocoons easier to unravel. Often, the silkworm itself is eaten.

Bombyx mandarina, the wild silkmoth, is an insect from the moth family Bombycidae. It is the closest relative of *Bombyx mori* the domesticated silkmoth or "silkworm" (properly, this refers to the caterpillars only). Unlike the domesticated relative which is unable to fly or indeed persist outside human care, the wild silkmoth is a fairly ordinary lepidopteran. Its main difference from the domesticated taxon is the more slender body with well-developed wings in males, and the dull greyish-brown color.

1.2 Phylogeny and systematic

Together, the two species constitute the genus *Bombyx*, the true or mulberry silkmoths. The origin of the domesticated silkmoth is enigmatic. It has been suggested that it is the survivor of an extinct species which diverged from the ancestors of *Bombyx mandarin* millions of years ago. However, this is based on an untenable molecular clock hypothesis that assumes that wild and domestic silkmoths evolved equally fast after their lineages diverged. Rather, the effects of artificial selection have accelerated evolution in the domestic form to a point where it is hard to trace the origin of the numerous breeds of domestic silkworms even with the most modern molecular phylogeny methods [1]. Conceivably, today's domesticated silkworms are all descended from an initial stock of *B. mandarina* collected as late as 5000 years ago. While wild silk could have been collected and used as threads, etc., since much earlier, the technology to breed and use silkworms from a domesticated stock did not exist before the late Neolithic.

However, it has been possible to trace the geographical origin of the domestic silkmoth. The wild species occurs over a considerable range from inland China to Korea and Japan, and shows much (albeit subtle) variation. The populations from the northeastern end of the range, for example, differ in karyotype from those of inland China. Domestic silkmoths are closer to the latter regarding mt DNA sequence data, and especially lack some genetic apomorphies of the northeastern *B. mandarina*. Thus, the initial domestic stock came from China.

B. mandarina is able to hybridize with *B. mori*. Both in the wild and in a domesticated environment, females release pheromones and wait for males to be attracted and fly to them. However, *B. mori* males cannot fly. Hybridisation, therefore, inevitably means breeding between domestic (*B. mori*) females and wild (*B. mandarina*) males.

Consequently, the two silkmoths have been united as subspecies of a single species; in this case the name *Bombyx mori* which was published first applies for both. However, today it is usually recognized that the domesticated silkmoth is entirely dependent on human care for its survival and thus has a level of reproductive isolation from its wild relatives.

1.3 Cocoon

The cocoon is made of a thread of raw silk from 300 to about 900 m (1,000 to 3,000 ft) long. The fibers are very fine and lustrous, about 10 μm (0.0004 in) in diameter. About 2,000 to 3,000 cocoons are required to make a pound of silk (0.4 kg). At least 70 million pounds of raw silk are produced each year, requiring nearly 10 billion pounds of cocoons.

1.4 Silkworm

1.1.1 Production

Silkworm larvae are fed with mulberry leaves, and, after the fourth moult, climb a twig placed near them and spin their silken cocoons. This process is achieved by the worm through a dense fluid secreted from its structural glands, resulting in the fiber of the cocoon. The silk is a continuous filament comprising fibroin protein, secreted from two salivary glands in the head of each larva, and a gum called sericin, which cements the filaments. The sericin is removed by placing the cocoons in hot water, which frees the silk filaments and readies them for reeling. This is known as the degumming process [6]. The immersion in hot water also kills the silkworm pupae.

Single filaments are combined to form thread, which is drawn under tension through several guides and wound onto reels. The threads may be plied to form yarn. After drying, the raw silk is packed according to quality.

1.5 Stages of production
The stages of production are as follows:

- The silk moth lays thousands of eggs.
- The silk moth eggs hatch to form larvae or caterpillars, known as silkworms.
- The larvae feed on mulberry leaves.
- Having grown and moulted several times silkworm weaves a net to hold itself
- It swings its head from side to side in a figure '8' distributing the saliva that will form silk.
- The silk solidifies when it contacts the air.
- The silkworm spins approximately one mile of filament and completely encloses itself in a cocoon in about two or three days. The amount of usable quality silk in each cocoon is small. As a result, about 2500 silkworms are required to produce a pound of raw silk [7]
- The intact cocoons are boiled, killing the silkworm pupae.
- The silk is obtained by brushing the undamaged cocoon to find the outside end of the filament.
- The silk filaments are then wound on a reel. One cocoon contains approximately 1,000 yards of silk filament. The silk at this stage is known as raw silk. One thread comprises up to 48 individual silk filaments.

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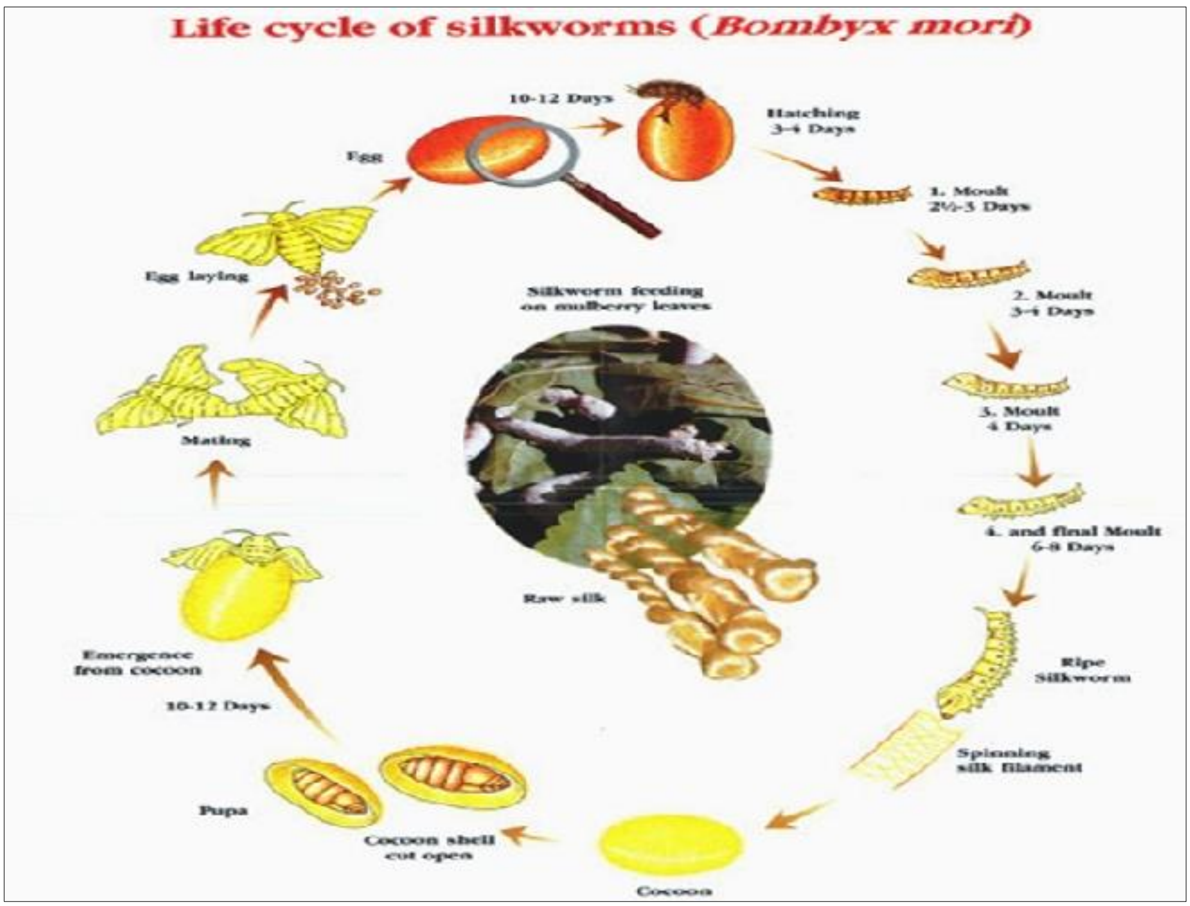

Fig 3

2. Conclusion
The insect immune response consists of two tightly interconnected components, the cellular and the humoral responses. The cellular response is mediated by haemocytes and involves responses such as phagocytosis, encapsulation, and clotting.

In most insects, there are several types of circulating hemocytes and these hemocytes have been the focus of research on cell development and differentiation. Insect hemocytes are important factors in innate immunity. They can produce many immune proteins like antibacterial peptides and PPO. In addition, hemocytes are directly involved in cellular immunity like phagocytosis, encapsulation and nodule formation. In the field of entomology, hemocytes are an attractive research arena for many scientists. Using the silkworm as a model, scientists have been extensively studying hemocyte identification, development, differentiation and cellular immune responses. Silkworm larvae have five types of circulating hemocytes that are produced by hematopoietic organs and released into the hemolymph. However, several types of circulating hemocytes also can proliferate through cell division, which contributed significantly to hematopoiesis after the larval hematopoietic organs were extirpated via a surgical procedure. Novel types of hematopoiesis in the silkworm make this insect species a valuable model to study hemocyte-related activities in the future. Now with the silkworm genome available, we anticipate being able to explore insect hemocyte immune activities at the molecular level.

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