

Break the Cocoon



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Funded by Jiangsu Preponderant Discipline
Biotechnology and Medicine Transformation

Silkworms were first domesticated in China and have benefited humans for a long time. Little “Tian Chong” has left memories and reveries over thousands of years to Chinese people. A string of tiny silver silk has erected a bridge between Chinese and western cultures. The “Silk Road” that has survived for thousands of years has brought about rapid development of Chinese and Western civilizations. Nowadays, the banner of the “Belt and Road Initiative” flutters high on the earth, marking the aspiration of Chinese people for friendship, peace, and development. To this end, the author has compiled this science work Break the Cocoon with literature and history data and illustrations, and strived to prevail Chinese culture, popularize science knowledge, and promote the development of sericulture.

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First published in 2019

978-1-78900-098-6 (online)

978-1-78900-099-3 (print)

ISCI Publishing LTD.
Kemp House
160 City Road
London EC1V 2NX, UK
www.iscipublishing.org



About the Author

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Preface

Can¹ (silkworm) consists of a “tian² (sky)” and “chong³ (worm)”. I didn’t mean to discuss the origin of the Chinese character, but Can must imply a certain culture. Well-known legends about Leizu (a legendary Chinese empress and wife of Yellow Emperor) who discovered silkworm breeding and Maiden Matou (goddess of silkworm) credited China with silk contribution to humans. Silkworm-related discoveries in Hemudu and Yangshao cultural sites undoubtedly evidenced that it is Chinese people who invented sericulture.

In ancient times, it was so difficult to crack the mystery of silkworms in cocooning and reeling silk that descendants always compared silkworm breeding on white mulberry trees and silk reeling to Four Great Inventions. Chinese culture is inclusive and Chinese people live in harmony with other nations. It is widely read that Zhang Qian who served as an envoy traveled to Western Regions⁴ and created the “Silk Road”. A “Tian Chong (silkworm)” silk bridged Chinese and western cultures and made them integrate and exchange for thousands of years. Sericulture spread in the rest of the world and benefits all people beyond measure.

In the kingdom of insects, when it comes to economic index, silkworms and bees must come first. They contribute a considerable part of clothing and food. Silkworms not only produce silk that can be luxurious clothing but also a delicacy for humans. Each link of a “white mulberry, silkworm, bombyx mori, silk moth, cocoon, silk and fabric” chain has its merits. An article entitled by *Old Materials, New Hope* in *Science* shows us the promising future of silk in medical and optical areas. Scientists have acknowledged that silkworms are model organisms of Lepidoptera, but now silkworms are being model organisms of life science. In near future, we believe that as silkworm baculovirus expression vector improves itself, a technical innovation with silkworm as bioreactor surely brings new benefits to humans. Ever since humans broke the code, Chinese sericulture researchers will volunteer to take on the mission of Chinese people to conquer barricade in silkworm genomes. This treasure, left behind by ancestors, will evidently glow in boundless shine again as the “One Belt, One Road” Initiative launched.

At the beginning of the reform and opening-up in China, I was fortunate enough to get myself tied to silkworm in the university. For over 3 decades in scientific research, I have been so addicted to “silkworms”. Witnessing the rapid development of sericulture science in China, I wanted to write a book long before, but I was busy with daily chores and only finalized the work and sent to the press at this time. And your advice or suggestions will be much appreciated.

Author



Foreword

Many readers never tire of silk or the Silk Roads. Other readers seek any book on technology and medicine. Still, it is rare to find a book that is totally new on any of these subjects. Professor Keping Chen's *Break the Cocoon*, uniquely interweaves all four subjects into a book packed with new information on each of the topics. It is, as the subtitle indicates, a belt around a newly woven road.

The Silkworm Goddess first came on the scene in China in middle of the second millennium BC, some two millennia after the oldest evidence of silk in China. By the end of the first millennium BC, the goddess was a beauty who ate hibiscus leaves that brought silver silk from her lips in a silk-making wilderness. Should there be wonder that in the fourth century BC, 7600 kilometres to the west, Aristotle was responsible for the family name *Bombyx mori* for silkworms or that cloth produced by species of this order known Lepidoptera came to the attention of Seneca the Elder, Virgil, and Piny the Elder before the end of the first century AD or that silk had become a coveted commodity in ancient Rome?

Sporting a silk garment, one may be little aware of the complicated process or many pitfalls that must be avoided from holding eggs in one's hands to transportation across mountains and deserts that brought silk to the markets of Eurasia: Cold, heat, starvation, fullness, sparsity molting, wakeup, rapidness, and slowness must be balanced in order for there to be washing, molting, plucking, rubbing, dividing, picking, mounting, warming, dismounting, hoarding, rolling, weaving, reeling, dyeing, spinning, and cutting. The process occurred in North China and South China. Only in the last stages could the patterns worn by Chinese courtiers in handscrolls from the eighth century onward, courtesans in Japanese woodblock prints, and Roman aristocracy—strings of pearls, satin with flowers, quadrifolious, flora and fauna, and geometric patterns--be achieved. Such it was that silk was gold.

The achievements of course assume that bacteria and fungus could be avoided to keep the silkworms healthy. The variants of these diseases determined the success or precariousness of the Chinese economy. Detailed illustrations of them juxtaposed alongside the silk patterns achieved since the first centuries AD impress on the reader the complex systems that challenge the perfection of Chinese silk at every stage. Here for the first time one can appreciate the biosystems that made it possible for pluckers, rollers, dyers, and spinners to have success.

Indeed, the minute glands of the silkworm on either side of a newly hatched species can be likened to a bulletproof, protective vest. Smooth as the liquid secreted by the silkworm may be, it is as tensile as steel wires. This same liquid can protect human skin, can resist certain viruses, and may be used as an antioxidant. The insoluble protein produced by the larvae of Aristotle's *bombyx mori* also offers hope for artificial skin,

combination with other chemicals to attack bacteria, cures for diseases of the digestive system, and even a curative effect for metabolism of the liver and attack against hepatitis. The protein silk can be processed for use in jellies, sauces, sweets, and as a health food. Some of these adaptations are made from waste silk. Ultimately, mulberry leaves and silkworms may be the keys to longevity. Even silkworm excrement is used in Chinese medicine. Pupa, a chrysalis, of the stage of the silkworm between larva and full development, has extraordinary high protein content.

Every aspect of the silkworm is much more than versatile. Anyone who reads this book will never look at a cocoon, silkworm, bolt of silk, or certain medicines the same way again. Another of Professor Keping Chen's books has already received recognition with East China's First Prize in Outstanding Science and Technology. This new book in English will open new fields of history, Eurasian connections, technology and medicine to its reader at a similarly high level.

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1 Tracing Life to Find Rising of Insect Kingdom

1.1 Sparks of Life

While watching programs like Animal World, we often cannot help marveling at life. Alas, life is a real miracle! There are various forms of life on this planet: roaring and awe-inspiring lions; flying and singing birds; dancing butterflies; swimming fishes and wandering beasts; and more. Indeed, life comes in various shapes and sorts. No wonder we will be dazzled by such diversity. Different animals and plants reinforce each other, yet counteract each other. They look independent from each other, but indeed relate to each other. Behind these living beings lie myriad mysteries. Uncovering such mysteries and exploring the underlying science has been what we humans have long been working at.

After ten-month pregnancy, a baby was born, crying at the long night for declaring his new life. Young parents are so joyful and cannot move eyes from their baby and think life is a real miracle. At this moment, most of us will thank delivery mothers than nature. It is natural for us to get married and give birth, but how many people will trace the origin of life?

The origin of life is an unsolved mystery from ancient times. When, where and how did life on the Earth appear? Is there life on other planets? If any, what forms do they take? Over thousands of years, scientists work day and night attempting to uncover the mystery. However, until now, it is still unclear and achievements are just a drop in the bucket.

“In the beginning God created the heavens and the Earth”, according to Holy Bible. This is creationism. As is recorded in Old Testament, God created everything in seven days. The first day, God created the light and divided day and night on the dark, formless and watery Earth. The second day, God made the sky, and separated the waters which were under the sky from the waters which were above the sky. The third day, God separated lands from oceans and made seeds and plants. The fourth day, God created the moon, the sun, and the stars. The fifth day, God created the sea life and the bird life. The sixth day, God created the animals, insects, beasts, men and women. The seventh day, God rested. During the Spring and Autumn Period in China 2,500 years ago, Lao Zi, an ancient philosopher, explained evolution of all universal things with “Taoism”. He wrote in Tao Te Ching, “One is the child of the divine law. After one come two, after two come three, after three come all things.” That is to say, living things on the Earth share the same ancestor, the ancestor is the one, which is generated by the heaven and the Earth and then begets two, three, and a living world. The nature theory of 19th century believed that life naturally evolves from non-living things. In the western world, Aristotle

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(384–322 BC, ancient sage, great philosopher, scientist, and educator in the ancient world history, epitome of Greek philosophy, student of Plato, and teacher of Alexander) was an abiogenist. Greeks think that insects were born in the soil, seeds will germinate from the soil in the new spring, insects will break the eggshells left last year, and this seems normal and correct. Egyptians think that life comes from the river Nile. In China, Book of Rites of the Western Han Dynasty (206 BC–AD 24) thus described, “rotten grass generates glowworms in summer”. Also in Lvshi Chunqiu (also known as Master Lü's Spring and Autumn Annals), there was a theory of “rotten grass turning into glowworms”. This saying can also be found in Huainanzi written by Liu An of the Han Dynasty. Growth and development of glowworms, like other insects, includes four life stages, *i.e.*, egg, larva, pupa, and adult, and the glowworm is a holometabolism insect and only lives for about half a month. There are more than 2,000 glowworms globally, with some able to give off three-color light. Scientific research showed that light given off by 37 glowworms is equivalent to light of one candle. However, heat of each glow is only one four hundred thousandth, so people call it “cold light”. As early as the Jin Dynasty of China, there was a record of collecting glowworms as light for the sake of reading. In Cheyin Biography of Book of Jin, it was said that Yin was too poor to afford lamp oil, so he caught glowworms and put them into a bag for lighting books so as to be able to read day and night. Up to now, scientists are researching other cold light sources as efficient as glowworms. Glowworm is a predaceous beetle and serves as a “bodyguard” for crops and an “analgesist”. It can anesthetize snails and oncomelania skillfully and drink their meat with after turning their meat into fluid with its tubular mouth. Nonetheless, these are not the origin of life, and they are the extension of life.



Figure 1. Charles Robert Darwin. (Source: <http://www.wikipedia.org>).

Charles Robert Darwin (1809–1882), an English naturalist and the founder of the theory of evolution, got onboard HMS Beagle to travel around the world in 1831 when he was 22 years old. Through five-year scientific survey, he collected many data on animals, plants, and geography. In 1859, Darwin published *On the Origin of Species*, which caused a sensation to the academic world. He evidenced with convincing facts that life was not created by God but evolved from simple to complex, from low to high forms in heredity, metamorphosis, fight for survival, and natural selection. In the book, the theory of “survival of the fittest in natural selection” has been widely accepted. Following the publication of *On the Origin of Species*, an unprecedented change was brought about as well as biologists saw a new hope for unveiling the thousand-year-old mystery of the origin of life. Stanley Miller, an American chemist, first demonstrated the theory of chemical evolution of the origin of life. He injected ammonia, hydrogen, water, and carbon monoxide into a sealed bottle and inserted two metal rods from two ends of the bottle. Then he powered on to simulate phenomenon of lightning. The result indicated that when “lightning” streaks, molecules are connected in different ways to generate many primary substances of life—amino acids. This experiment showed that with lightning, organic molecules on the Earth could be synthesized from inorganic molecules.

The origin and evolution of life is accompanied with that of the universe. Now people know that life is composed of different tissues, tissues consist of cells, cells of different organelles, and organelles of various macromolecules and micromolecules. Even some archaeobacteria and virus are formed by macromolecules and micromolecules. Basic elements of life, such as carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur, might have existed before the formation of life. This might be the consequence of evolution of some elements after big bang. In the vast cosmic space, chemical change does not only happen to the Earth but also to other planets. These planets are being a part of the drastic change. Today, substances similar to life have also been discovered during our scientific space investigation. Amid interstellar evolution, there must be collisions and powerful chemical reaction and these energy change and chemical reaction can transform inorganic substances into organic ones. Some unimolecules such as amino acids, purine, and pyrimidine might be formed in the interstellar dust or aggregated in nebula and transformed into macromolecules like polypeptide, and polynucleotide under a certain circumstance on the planet surface. These macromolecules eventually constitute a primitive biological system on the Earth in some transition ways of biological evolution we don't know yet, and that is, life with primitive cell structures. The biological evolution starts from now and generates countless and complicated life forms on the Earth.

Then what is the most primitive life? About 3.8 billion years ago, continents have stably formed on the Earth. Different evidences manifested that at that time liquid hydrosphere might be hot or even boiling. Some extant extremely thermophilic

archaebacteria and methanobacteria might be the closest to the most ancient life form on the Earth. The most ancient biological fossils we have found are rocks in the west of Australia that are 3.5 billion years old. These fossils look like blue-green algae and they are some primitive lives and invisible to naked eyes and are microns to tens of microns in size. In addition, scientists discovered carbon in the extant 3.85 billion-year-old rocks in Greenland. As we know, there are inorganic carbon and organic carbon as well as heavy and light carbon. Scientists speculated the source of these carbons according to the ratio of light carbon to heavy carbon. They analyzed according to isotope and inferred these carbons are organic carbon and come from organisms. In other words, the supposed time range of the origin of life is greatly narrowed which is between 4 billion years ago and 3.8 billion years ago. There is no denying that life is a result of evolution, life has developed from simple forms to complex forms, and life cannot be created.

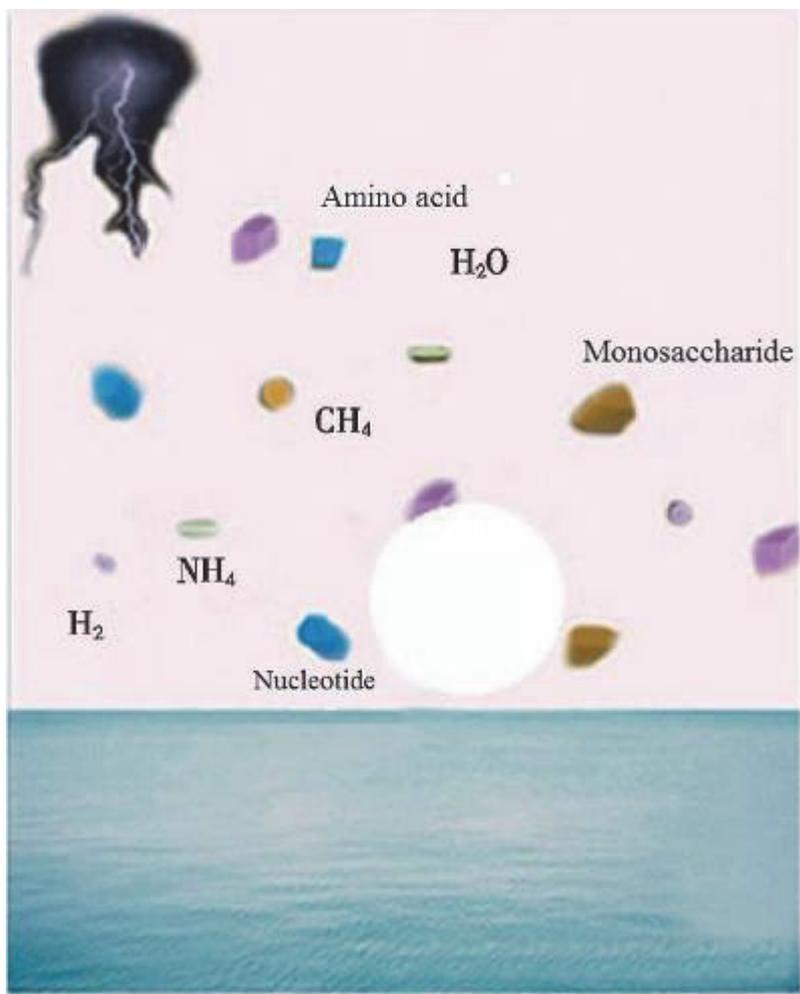
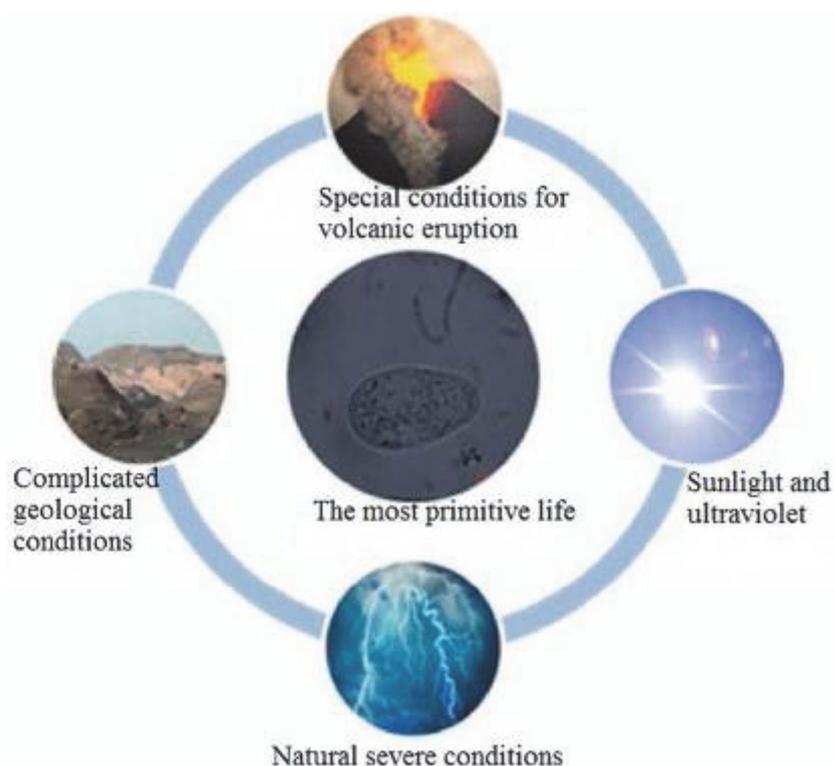


Figure 2. Speculation on primitive atmospheric compositions.

The origin of life is generally like this. Before 4 billion years, inorganic molecules on the Earth were synthesized into organic micromolecules, which were polymerized to form macromolecules. The macromolecules evolved into nuclein and active protein,

which generated a self-replication primitive cell wrapped by biomembrane. This cell might be heterotrophic or chemoautotrophic or might be analogous to modern thermophilic archaeobacteria nearby hot springs. The most primitive acellular living beings evolved to cellular prokaryote and then to eukaryotic unicellular organisms. They developed in different directions into fungi, plants, and animals respectively. Plants evolved from algae, psilophyte, pteridophyte, and gymnosperm to angiosperm. Animals evolved from flagellate to multicellular animals, from multicellular animals to chordates, and at last to higher chordates, vertebrate. Vertebral fish first evolved to amphibians and to reptiles, then to mammals and birds. A branch of mammals evolved into higher animal, humans. However, we should clearly understand that there is a long way to go before solving the mystery of the origin of life. Amid evolutions from inorganics to organics and from organic compounds to organic beings there are a lot of unsolved mysteries. All we know about the evolution from prokaryotic organisms to eukaryotic organisms is only a drop in the bucket. There was a metaphor that these inorganic matters are like a garbage heap, which includes plastics, bottles, iron, waste metal, and oil, but life is a unicell, like spare parts. After a gust of typhoon, these parts are assembled into a Benz car. The metaphor didn't get to the point. The typhoon should not be only one, but one follows another at long or short intervals. Therefore, it could be imagined that the origin of life must be tough. Maybe the blue planet we live is the only paradise for living beings in the universe. So, we need to protect our Earth and cherish life on the Earth and don't count on the second origin of life.



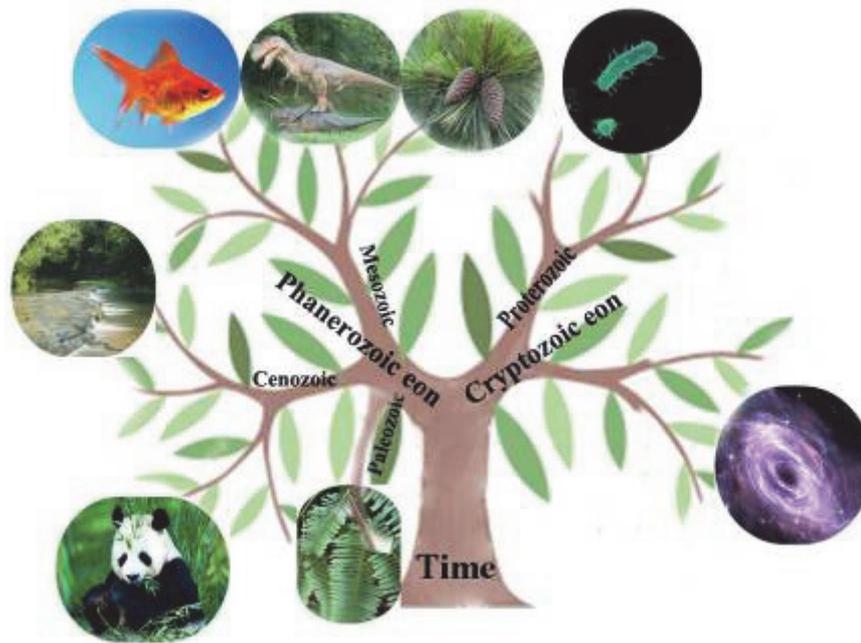


Figure 3. The origin of life.

1.2 Insect Kingdom

Now, let's put aside the origin of life and it is too big to state it clearly with limited words. What I'm interested in are insects. Talking about insects, people can easily think of colorful butterflies, honey-making bees, silk reeling and cocooning silkworms, singing cicadas, fighting crickets, glowing fireflies, cutie ladybirds, mantis with preying forelegs and bulging eyes, disgusting flies, mosquitoes, cockroaches and more. Stepping into the insect kingdom is like entering a new world. A yellowish green dragonfly looks like a fighter plane chasing little moths; ladybirds walk on brushwood leaves with great dignity and smell preys. Black ants in throngs go and come from their colony to transmit messages. Grasshoppers stand at the tip of branches, rhythmically playing songs with long feelers. In spring, summer and autumn, you can see insects everywhere. At night, you can look up at stars, amazing at the wonderful world and anticipating a promising future.

Insects are a largest family in the animal world and their "descendants" spread all over the world, without any rivals. So far, there are more than 10 million insects globally, 1.1 million of which was named. Insects are the most thriving animals. According to incomplete statistics, insects account for more than 75% of animals. Even in the whole biosphere on the Earth, including plants, fungi, bacteria, and virus, the variety of insects can still reach 53%. Humans always stand on top of a pyramid of life, and under the top is a huge family of insects. From an economic perspective, "the king is who controls the most". In view of this, insects can be called a master of the Earth. But indeed, poor insects are not as smart as humans. Today, some insects are extinguished by human-made

pesticide and some others have been put on dining tables.

So, when on earth did insects appear? This might be easier to answer as compared to the mystery of the origin of life. No humans were lucky enough to witness emergence of insects, as insects exist much earlier than humans. In view of this, insects should be “predecessors” of humans. Then how do we know the emergence time of insects? Humans are intelligent and so-called brain of life. We can infer and judge the time by archaeological investigation and fossils without seeing their emergence. A fossil is a stone, which is formed by life body or remains living in the distant past. In long geological ages, countless lives used to live on the Earth. Their remains after death or marks left after living were buried by sand or soil. Many years later, though organic matters in these life bodies were decomposed totally, the hard parts like shells, skeletons, and branches were fossilized into stone with surrounding sediments and their original forms and structure (even some tiny internal structure) still remained. Similarly, some marks left by living beings were remained. We call these fossilized life bodies or remains fossils. Based on the fossils, we can restore original looking of ancient animals and plants, describe their living conditions and environment and judge the age of formation of the stratum where fossils were buried and changes in all ages, and others. Nowadays, fossils humans found are trilobite, phytolite, shell fossils, footprint fossils, dinosaur fossils, and ichthyolite. Early Paleozoic era (570–409 million years ago) was divided into Cambrian, Ordovician, and Silurian. The Earth entered Silurian (originated from an ancient Welsh people name “silures”, which was later translated into Japanese Kanji and adopted in China) era (about 440 million years ago) is the last era of the early Paleozoic era as well as the third age of the Paleozoic era. Appearance of plants and development of Gnathostomata were the most significant biological evolution events in the Silurian. Big change happened to marine invertebrates, trilobite used to be thriving started disappearing. Eurypteris began to appear until insects turned up on the Earth in the Silurian age. Ordovician was the second age of the Paleozoic era, started 500 million years ago, and lasted 65 million years. The latest research found that in the Ordovician age, the weather on the Earth was warm, shallow seas spread widely and large areas (including most of areas in China) of the world were covered by shallow seawaters. Marine organisms were multiplied rapidly and are more thriving than Cambrian. In general, the origin of insects could trace to the Silurian age, which is very close to algae that were believed to appear 510 million years ago.

Insects in Silurian were mostly wingless insects, such as moths. Most moths are oval and fully black brown or black, multiplied 1 generation each year, distributed in southern and northern China, and mainly jeopardized *pinus massoniana*, *pinus densiflora*, *pinus armandi*, and *pinus tabuliformis*. Moths are also known as silverfish, fish moth, scolytidae or bookworm *etc.* and are a kind of old insects. In addition, silverfish is the general name for fish-moth insects. Fish-moth insects are a kind of primitive wingless insects and there are more than 100 species in the world.

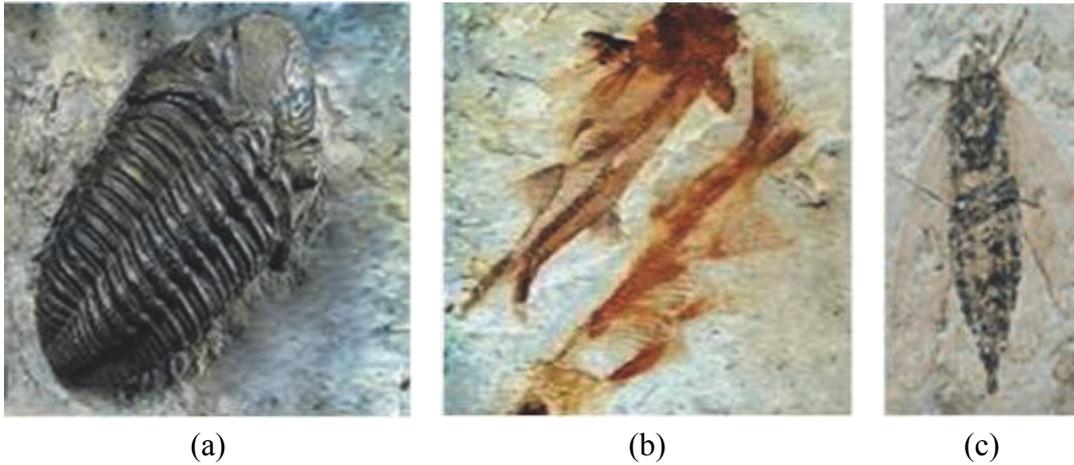


Figure 4. (a) Trilobite; (b) Lycopoda; (c) Lycopoda.



Figure 5. Silverfish (Source: <http://www.wikipedia.org>).

Devonian (400 million years ago) was the first age of the late Paleozoic era. Due to the impact of Caledonian movement and Hercynian movement (started in Devonian), many regions in Devonian elevated and broken the seawater to be land, and the ancient landform was much different from that in the early Paleozoic era. During this time, great change occurred to evolution of insects. They got onboard their flight journey and evolved to be first flyers. Modern scientific researches indicated that wings were more likely evolved from external feathering gills of aquatic insects. Ancient winged insects prevailed for a time and became a king of sky. Now we may see in films and soap operas dinosaurs roaring their time in the late Carboniferous period and conquering the sky, but this only happened after insects did. As an old saying, “the waves behind ride on the ones before”, Carboniferous started from 354 million years ago is the representative time of prosperity of vegetation. The stratum formed in this period contained abundant coal, this is where Carboniferous got its name. At this time, oxygen concentration in the atmosphere was up to 35%, much higher than now (21%). Insects had tiny air vessels all over their body, so they could inhale oxygen directly, and high-concentration oxygen

helped them grow big. Under this circumstance, huge Arthropoda came into being, such as an ephemera, a giant scorpion, a spider as big as spider plants, and giant dragonflies as big as an eagle *etc.* Ephemeroptera, aka. Ephemera, had ancient but special characters and were the most primitive winged insects on the Earth. It was reported recently that there were millions of ephemera insects gathering along the Danube in Hungary and in the basins of the Mississippi River and La Crosse River in Wisconsin and in Pennsylvania, hovering in the sky like a “black cloud” of light to moderate rain. Ancient giant dragonfly fossils have been found in America, France, England, and Russia. Nowadays, one of the ancient giant dragonfly fossils stored in Harvard University had a 72 cm-long wing and was the biggest dragonfly in the history as well as the prototype of airplanes. The most ancient abysmal scorpion lived 460–255 million years ago and was 2.5 m long. It was ferocious and used to a conqueror of a part of the sea.

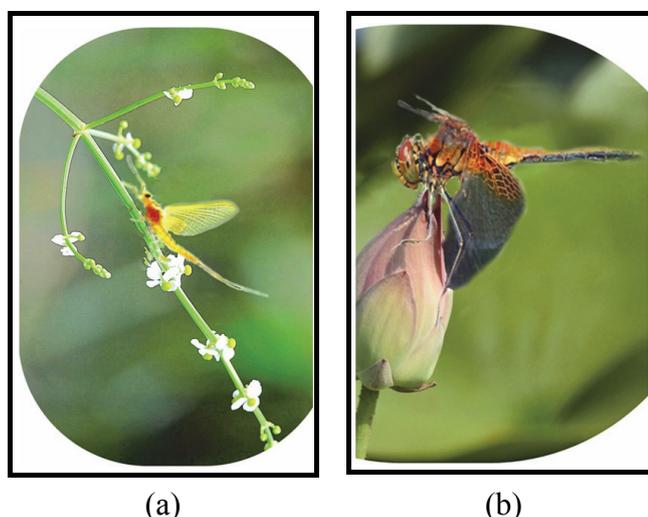


Figure 6. (a) Ephemera; (b) Ephemera.

The last period of the Paleozoic era is Permian and also an important coal-forming time. Permian started 292 million years ago and finished 250 million years ago, lasting 45 million years. Mass extinction in Permian was the most severe event throughout the whole history. As estimated, nearly 96% of species on the Earth were gone, including 90% of marine live beings and 70% of land vertebrates. Palaeodictyoptera used to fly in the sky together with ancient dragonflies all died out in this mass extinction and was the only family extirpated in the history of 400 million years. The mass extinction of Permian terminated the “kingly” position of trilobite that ruled the sea for 300 million years. The Earth took on an entirely new look. The Earth entered Mesozoic (including Triassic, Jurassic and Cretaceous) from Paleozoic. Through this “shuffling”, reptiles came on the stage after Cretaceous and dinosaurs and mammals roared to the sky and controlled the Earth. After the great devastating on the Earth, low species were replaced with and evolved into higher, more intelligent, and more adaptively ones, opening up a

road for birth of humans at the top of pyramids.

Although insects were experienced great extinction in Permian, just as the description in *On the Origin of Species* of Darwin, “survival of the fittest”, another family of insects, Polyneoptera, was thriving and indefectable ever since 300 million years ago and tended to be more reproductive after the Permian extinction. The most representative specie of life and vitality is cockroach. Cockroaches were youngsters of Blattaria and didn’t come into being until Jurassic, while cockroach-similar insects extant in Carboniferous were common ancestors of Polyneoptera and different from modern cockroaches.

Ancestors of Dictyoptera are insects similar to cockroaches. Over time, their offspring would definitely be independent. One of branches evolved into pure carnivorous mantis. Ancient Greek regarded mantis as Phrophet, because a mantis with forelegs rising up looked like a praying young lady and was called a prayer. The mantis is ferocious and aggressive, female mantis will eat male mantis and is monogenetic and it could be inferred that the mantis world was ruled by female mantis. Another army of insects occupied an ecological niche of the litter layer and plays a role of decomposer, and they are cockroaches of Blattaria. Cockroaches presented lignivorous and social tendency in the late period of evolution. In the past, only cockroaches were classified as Blattaria, however, modern classification methods told us that termites of Isoptera were recategorized into Termitidae of Blattaria and turned into social cockroaches.

During Triassic, the ocean area decreased gradually while the land area became larger and larger. Plants overgrew, insects were multiplied, species were greatly increased, and ants, flies, Lepidoptera insects turned up in succession. Nearly at this time, blooming plants were thriving, beautiful flowers sent fragrance to the nature, looking like a pretty girl and they are slim and graceful and swung in the wind. The variety of these plants will induce polyphyletic emergence of bees, hornets, and butterflies. Jurassic was the golden age of dinosaurs, and dinosaurian was overpowered other things. At that time, all kinds of dinosaurs gathered together to make a dinosaur world. During the same time, more than 1000 species of insects shuttled back and forth in forests, lakes, and swamps. Grubs, bark lice, and fleas turned up scrambling for a foothold on the Earth.

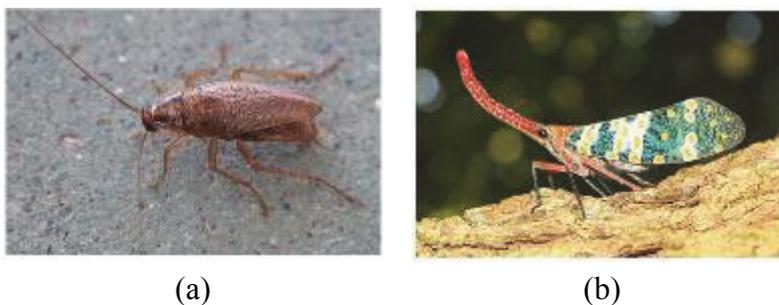


Figure 7. (a) *Blattella Germanica*; (b) *Pyrops Candelaria*.
(Source: <http://www.wikipedia.org>).

Until late Carboniferous, some holometabolous insects had appeared like a fetus in a mother. However, they didn't get into their boom until angiosperm came into being. Imaginably, this is a hard time. Some insects totally changed and evolved into a stage of complete metamorphosis. Pupa and butterfly were divided and had their own functions. Larva is simple and had nothing to pursue except eating his full. Pupa stayed on its bed or in cocoons and kept fit waiting for mating. Adults were vigorous and focused on courtship and reproduction. As adults and larva were different, they could get into different ecological niches. Butterflies and silkworms are insects of complete metamorphosis, and butterflies can dance in the sky while moths with wings cannot fly. Insects of complete metamorphosis look like marvelous creatures but they are the most successfully evolving animals. Evolution of the insects is not independent but closely related to that of other beings. There is a biological chain in the nature, living beings on the chain are dependent one another. The biological chain presented a phenomenon that everything has its conqueror and maintained the survival number and balance among species. Insects, since the first day they appeared on the Earth, are twins for plants. They deeply understood that honor one and you honor them all and vice versa. In the nature, herbivore insects and pollinating insects are catalyst for making plant diverse. In turn, diversity of plants made insects diverse and adaptive. Plants will develop various defense mechanisms for herbivore insects, such as flowers for pollinating insects. In order to effectively take food and pollinate, herbivore insects competed and evolved in throngs. Plus preying and co-evolution of parasitic insects, diversity of insects and angiosperms was so astonishing. Today, colorful plants and various insects we see are the results of natural evolution. The win-win effect that insects and plants made has been models for the nature. I think humans will eventually realize that only harmony can develop after bloody fights and bitter price.

Cretaceous was the last period of Mesozoic. This period was named as its stratum was deposited by Cretaceous and experienced 80 million years. Continental plates were separated by oceans and the Earth got warm and dry during this time. In the early Cretaceous, gymnosperm and pteridophyte on the land also dominated the plant world. Pines, cypresses, cycads, ginkgo, filicophytina, and some nodular plants were the major floras. Angiosperm came into being in the early Cretaceous, multiplied in the mid-term and maintained the dominant positions in the terrestrial plants in the late Cretaceous. Beeches, banyans, lily magnolia, maples, oaks, poplars, camphor trees, walnuts, and sycamore have shown and looked like the flora of Cenozoic. The emergence and development of angiosperm was not only a big revolution but also brought about great impact on animals. They made the Earth green. Angiosperms provided so much food for insects, birds, and mammals that they can happily live on the Earth and multiply. With the aid of evolution of angiosperms, insects also experienced an explosive evolution and took the absolute advantages globally. This is uprising of insects of complete metamorphosis of Big Four. The Big Four represented four insect families taking the

absolute advantages in the animal world named by entomologists. They are Hymenoptera (sphecomyrma), Coleopteran (beetles), Lepidoptera (butterflies and moths), and Diptera (mosquitoes and flies). Definitely, hymenoptera headed the others. The Hymenoptera insects were first separated and eventually evolved into major pollinating insects for angiosperms. Bees, and ants belonged to Pterygota and complete metamorphosis and spread all over the world, and the tropical and subtropical areas had more species. So far, there are 120,000 species of Hymenoptera insects in the world, and China has more than 2,300. Hymenoptera is the third largest family in Insecta (next to Coleopteran and Lepidoptera and got its name from its transparent wings. Coleopteran followed and established its own position. It is the most successful and diversified specie on the Earth. There are more than 350,000 species of Coleopteran (beetles), of which Curculionidae is the largest group and has 60,000 species, 10 times of mammals. Coleopteran insects have hard shells and posed a mighty image of a soldier. The posture might imitate an ancient soldier in a cruel battle.

The third is Lepidoptera and the fourth is Diptera. Lepidoptera include moths and butterflies and are insects of complete metamorphosis. So far there are about 200,000 species globally, and 800 have been reported in China. Wings of many kinds of moths in Lepidoptera are full of lines and spots formed by colorful scales and scientists named them later according to the shape, position, and color of the scales. Lepidoptera insects are the “major force” to jeopardize crops and “tax” on humans. Noticeably, however, silkworms introduced in this book, belonging to Lepidoptera though, are economically important insects for human beings. Diptera is the fourth largest family only next to Coleoptera, Lepidoptera, and Hymenoptera. There are 110,000 species that have been reported and widely spread, and China has about 7,400 species. Diptera has its advantages and disadvantages. On the one hand, some species will transmit diseases. On the other hand, genetic knowledge and development was mostly attained according to experiments on *drosophila melanogaster*. Diptera insects only have a pair of wings, and their underwings had been degenerated into a pair of club-shaped organs, which are used for balancing during flight. The wings and the balance rod of some species have been degenerated too much to fly. Diptera insects can affect human lives, so most of them are notorious. The humans killed by mosquitoes much outnumbered those killed for other reasons. Before humans got their burial culture, flies were responsible for burying the dead. Everything in the nature has both sides. Lepidoptera insects are good helpers for creating colorful flora and Diptera insects take up an important job to move the dead back to the nature. Their roles in formation and harmony of the ecological system on the Earth might be much more important than humans.

The kingdom of insects had been formed initially in Carboniferous and always flourishing and invigorating as the appearance of angiosperm. They have experienced four extinctions (one in late Devonian, one between Permian and Triassic, one between Triassic and Jurassic, and one between Cretaceous and Paleogene) in five on the Earth

and evolved. Each time of extinction would kill over 70% of life. Ancestors saw off relatives from one generation to another. The living survived from the dead, reproduced, made changes and evolved into the most successful animals on the Earth. We could believe that insects would be still the first to survive from the ruins in next extinction. In the next evolution, they can always occupy a half biosphere and the banner of the insect kingdom will be about to fly on the Earth.

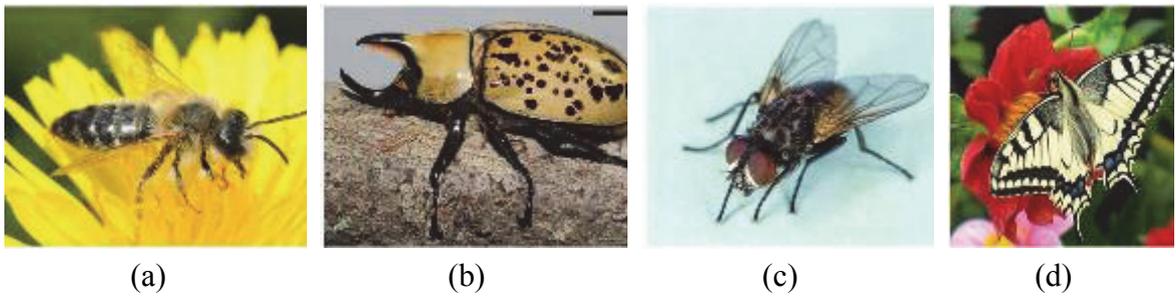


Figure 8. (a) Bee in Hymenoptera; (b) Unicorn Beetle in Coleoptera; (c) Diptera Fly; (d) Butterfly in Lepidoptera. (Source: <http://www.wikipedia.org>).

2 The Realm of Silkworms

2.1 Tales and Legends

Long long ago, humans were ignorant of the nature, including mountains, rivers, the sun, the moon, oceans, insects, and beasts. They considered all changes as a “ruler of life” and worshipped it. Humans also told many tales and legends about silkworms.

If you asked in which document Goddess of Silkworms were first narrated in China, it should be oracle inscriptions of the Shang Dynasty (16th–11th century BC). There were facts that cows, pigs, and sheep were sacrificed for worshipping Goddess of Silkworms. In Shang and Zhou Dynasties, silkworms were called deity or ancestors.

Bombyx mori, also known as silkworm, is a silk-producing insect lived on mulberry leaves and was domesticated from primitive silkworms inhabited in the mulberry forest by Chinese ancient people. Wild silkworms jeopardizing mulberry trees and it shared the same ancestor. Later on, silkworm was named as Leizu, Maiden Silkworm, Maiden Matou, Ma Mingwang, Bodhisattva Maming, Maiden Canhua, Silk Fairy, and Empress Silkworm and was the ruler of silkworms and mulberry for ancient Chinese people. China was the first country to plant mulberry trees and breed silkworms and in a very long time also the only country to reel silk and manufacture fabrics in the world. In ancient China with the simple agricultural and social economic structure of men working on the farm and women weaving, silkworms and mulberry played important roles. For this, a Tang Dynasty poet, Meng Haoran wrote lines of “open a window, seeing a threshing ground and a vegetable garden, drinking wine and talking Sang and Ma” (mulberry and flax, a metaphor for farm work). No matter aristocrats or folks expressed high respect to the silkworm god. As is recorded in ancient official sacrificial classics, “emperors and dukes must plant mulberry trees and breed silkworms in ancient China” (Ji Yi in the Book of Rites), “Emperor farmed and then bred silkworms” (Yue Ling). Before Han Dynasty, silkworms had been deified and called Ancestral Silkworm, meaning god of silkworms.

Canshen (god of silkworm) worshipped in the folk referred to Maiden Silkworm and Maiden Matou in the fairy tale of silkworms.

As is recorded in the Book of Mountains and Seas, at the place of sunrise and by the vast sea, there were three cloud-kissing hibiscus trees. As the legend said, before the sun rose, nine suns bathed in the blue sea and rose over the hibiscus trees, another one under the hibiscus trees, and the suns with water dried radiated brilliant light and brought a fresh morning for humans. Under the sunlight, a young lady wearing horsehide knelt down before one of hibiscus trees, eating leaves and giving out silver silk from the mouth. Ancient people called the wildness Silk Making Land.

Ancient people mistakenly took hibiscus as mulberry trees, which provided many sources for tellers of legends. Ancestral Silkworms were the gift from the nature. Then the “mulberry” leaves, as food for the deity, must be leaves of hibiscus trees, a deity tree planted by Jade Emperor. Definitely, agricultural books in ancient China never confused hibiscus with mulberry trees. Hibiscus is also known as Chinese rose and commonly called Red Flower in southern China as most of its flowers are red. Hibiscus trees always grow in pairwise and lean close to each other, so it was named Fusang in China (Fu in Chinese means supporting with hands). Of course, this book will not investigate if hibiscus is a mulberry tree. We want to question why a beautiful girl in the legend wore horsehide and transformed into a silkworm and how she became the goddess of silkworms.

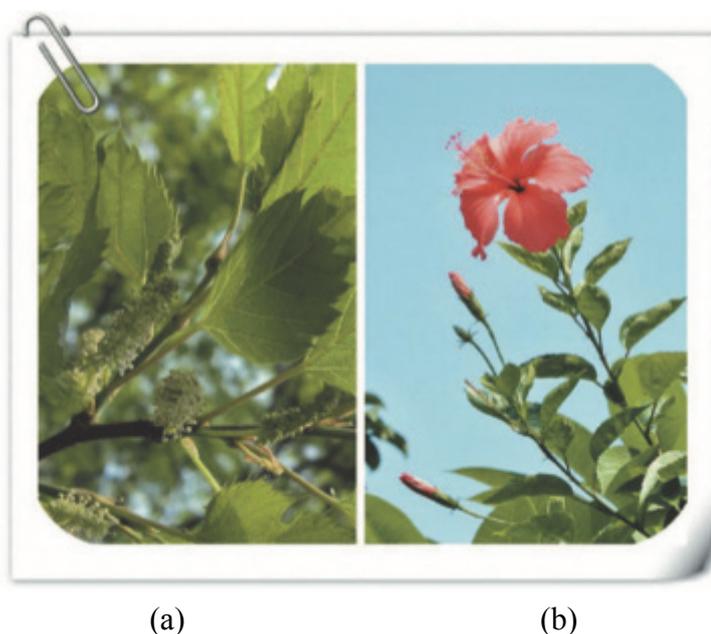


Figure 9. (a) Mulberry; (b) Hibiscus.

According to an ancient legend, a father set out for a war, with a daughter and a male horse staying at home. The daughter was accompanied with the horse day by day. One day, she missed her father so much that she talked to the horse jokingly, “if you can bring my father back, I will marry you”. The horse hearing the words made a neigh sound and ran to the battle to take her father back. The father and the daughter were happy with the reunion. However, the horse refused to eat and every time he saw the daughter, he was excited and made a loud noise. The father felt strange and asked her daughter the reason. His daughter told him the joke she made before. He was so angry and killed the horse with his sword and hung the horsehide in the yard. At a time, the daughter went into the yard while a wind blew heavily to lift the horsehide up and wrap the girl into the sky. A few days later, people saw the girl and the horsehide turned into a silkworm, eating leaves of a tree and giving out silk. People were sad about the girl, so

called the tree “sang” (mulberry, sang in Chinese is homonymic to “sang” (mourning) to memorize sacrifice of the girl. The father was heartbroken and felt sad every day. One day, the girl rode on the horse and floated down from the sky, telling her father, “the Emperor titled me as a fairy maiden and ranked me as one of nine-palace imperial concubines, I was free in the heaven and don't be sad for me”. After saying that, she flied away. This is the widely spread story of Maiden Matou among ancient Chinese people.

It is said that Yellow Emperor defeated Chiyou and composed a Song of Maple Drum for celebrating his victory. The song was powerful and energetic. Triumphant soldiers sang loud to the accompaniment of the maple drum, dancing in postures simulating subduing actions. At this moment when soldiers were celebrating joyfully, a beautiful lady in horsehide slowly landed from the sky, with yellow and white silk in hands, the yellow silk looked like gold and the white one like silver, and both were dedicated to Yellow Emperor. Yellow Emperor pulled off the horsehide and the lady immediately turned into a silkworm with a horse head and spin long and flickering silks from her mouth. When seeing this, the emperor amazed at it and was deeply moved and sent the silkworm to Leizu (family name: Xiling) as gift. Leizu adhered to the imperial edict and taught people breeding silkworms. She was one of the most distinguished goddesses, virtuous and warmhearted. In practice of silkworm breeding, she bred primitive silkworms indoors so as to prevent rain and wind and insects and birds from jeopardizing them. From then on, people followed her way, and sericulture was promoted nationwide. Hence Leizu was honored as an inventor of silkworm breeding and regarded as Ancestral Silkworm.



Figure 10. Goddess of Silkworm Maiden Matou.

2.2 Archaeological Investigation

There were many myths handed down from age to age. The earliest story would always start from the myths. A nation develops more, people will feel more puzzled at

the world and where they come from and find different answers. The first answer usually came from the myths, which marks the origin of civilization. These different answers seem some unreasonable myths for modern people. However, for people at that time, they are rational. Ancient people explained and expanded these myths in their own ways and passed them from generations to generations. They believed that this was the origin of the universe, humans, and all things in nature. The origin of sericulture may be not as mysterious and complicated as the legends, but historic study and modern archaeological excavation confirmed that after the age of fishing and hunting went into the age of family property, silkworms have been bred indoors. From Neolithic site (4887 ± 96 BC) discovered in Hemudu Village in Yuyao of Hangzhou between 1975 and 1978, bone cups with silk weaving patterns and silkworm patterns and spinning tools were unearthed. In 1985, there was silk fabric, silk yarns, and silk ribbons among silk products unearthed from Qianshanyang, Wuxing of Zhejiang. Via C14 dating and tree ring, the age was determined at 3310 ± 135 BC, namely the unearthed products were relics 5208 years ago. In 1984, many silk products used for wrapping corpses 3500 years ago were also uncovered in Yangshao cultural site in Qingtai Village of Henan Yingyang, including plain woven fabric and light-color silk. This indicated that 5500 years ago there had been many silk fabrics. Xiyin Village in Xia County, 20 km away from the northeast of Yuncheng City of Shanxi, used to be a remote place and later was famous all over the world due to an archaeological excavation by Li Ji in 1926. In 1927, Research Institute of Tsinghua published Prehistoric Relics of Xiyin Village written by Li Ji, which was the first archaeological report of Chinese scholar in modern archaeological history. As recorded in the book, “interestingly, we found a half-cut silk-like cocoon and observed it under a microscope, another half had been rotten but still glowing, and the cut part was flat and straight.” Liu Chongle, biology professor of Tsinghua, also checked several times for me, he said he could not affirm it was cocoon, but found no proof to deny it. Compared with cocoons of silkworms bred in Xiyin Village now, it was a littler smaller than the smallest one. This cocoon was buried almost at the bottom of the pit, and it cannot intrude from other places, because the earth color showed nothing marks of disturbance. Neither was it spun by wild worms, because it was cut purposefully. Mr. Li said later, “in the painted pottery site in Xiyin Village, I excavated a half-cut cocoon, I had brought it to Washington DC to check and proved it was an ancestor of silkworms. In view of this, it was an undoubted fact that silk was invented and developed in China.” This cocoon sample attracted many attentions from scholars who studied science and civilization in China from all over the world. Japanese scholar reconstructed one with silk as its photo of the half cocoon (provided by Chinese Taipei National Palace Museum) in 1968. The recombination showed that the cocoon was 1.52 cm long and 0.71 cm wide, and the cut part is 17% of the full cocoon. Accordingly, it was a mulberry cocoon, namely, a wild silkworm cocoon.

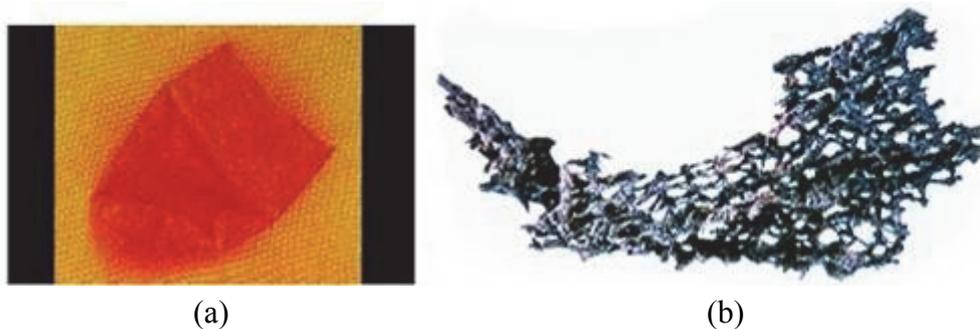


Figure 11. (a) Half-cocoon (Yangshao Culture) in Qingtai Village, Henan Province; (b) Fabric Remnants Yangshao Cultural Site, 1926, Xiyin Village, Xiaxian, Shanxi Province.

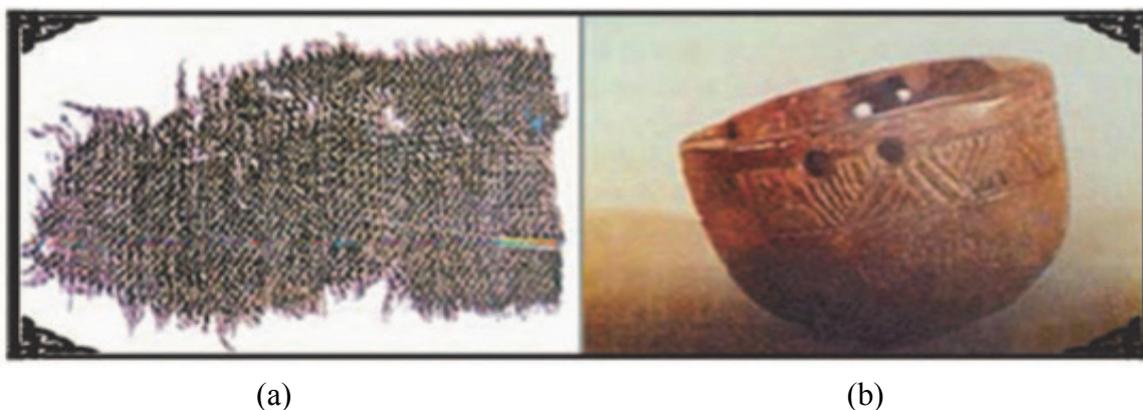


Figure 12. (a) Tough-silk sheet Qianshanyang Site (of Liangzhu culture) in Huzhou, Zhejiang Province; (b) Ivory sculpture with silkworm patterns earliest silkworm pattern unearthed from Hemudu Site in Yuyao, Zhejiang.

However, another Japanese scholar Ikeda Kenji proposed after several investigations that it was a cocoon of domesticated silkworm and was small because of low-grade evolution. Nonetheless, there were many unsolved mysteries of the half cocoon, it has been always collected in the National Palace Museum as the proof of Yangshao cultural relics and ancient silk of China.

From the perspectives of legends, historic data, archaeological relics, and the law of social development, it can be seen that eating pupa by collecting wild silkworm cocoons, keeping warm by making silk cotton with cocoon silks, and producing fishing and hunting tools with silk were prior to silk reeling and silkworm breeding, and the emergence of many silk products later also indicated that silkworms have been bred indoors. Accordingly, we could believe that at least 5,500 years ago, China had invented silkworm breeding. As early as the Neolithic age, our ancestors produced silk products

with silk and had high-level silk reeling and weaving technology. At that time, they not only had enough knowledge about silkworms but also started worshipping it. The position of China as State of Silk has been established since the Neolithic age.

2.3 Silk-Secreting Insects

On the Earth humans live, there are a variety of insects that can secrete silk. The mouths of Lepidoptera, Trichoptera, and Hymenoptera insects have infralabial gland (aka. spinning gland) with special function. When larvae grow to a certain stage, silk protein in their urosome will be secreted from the infralabial gland for nesting as a “home” of the rest of their lives. Not just insects have the external secretion gland for secreting silk-like substance, such as spiders, and isopoda. Dactylus gland of Embioptera insects, Malpighian tubule of some Coleoptera and Neuroptera insects, and anococcygeal caudate lobe of cockroach larva can also secrete silk-like substance. One of Chalcididae insects has infralabial gland connected with hindgut and can secrete silk-like substance and discharge through hindgut. There is a kind of bees that can secrete silk for covering their beehive. Wonders never cease in such a great world. There is a cat in South America that can spin silk like silkworms, and people there call it “silk spinning cat”. There is a pair of opening pits in the mouth of the silk spinning cat, where silk liquid is stored. If the pits are filled with silk liquid, the cat will stop taking food for two weeks and spin silk instead. During spinning, the cat is irritable and will keep running. If local people tied it on a rod, it will run around the rod, spinning silk on the rod. A cat spins two times of silk in a year. Silk spun by the cat is thicker and stronger than that of silkworms and can be made into tough fabric, which is popular among people. On North Sporades Islands in Greece, there are silk-spinning snakes, silk spun by the snakes is tough and acid and alkaline resistant, and resistant to seawater corrosion. Local fishermen will use it to make fishing nets. There is a silk-spinning frog in Casari River of South America, whose silk glands on two sides of its head can eject white thick liquid. This kind of thick liquid will be condensed into a silk net as meeting water and air, and the silk net can be used for catching insects, fish and insects.

Why insects spinning silk? Generally speaking, there were some reasons. The first is to keep posture, such as Papilionidae and Pieridae insects of Lepidoptera, and they will tie up themselves to other objects with silk when pupating. The second is to move, such as Lepidoptera insects, especially phalaenae larvae, and they can suspend themselves with silk while moving around the silk. The third is for protection. The most vulnerable time of insects is from pupating to eclosion. For example, at this time, silkworms will construct a thick cocoon in two days and nights to separate their bodies from the outside world and to protect pupa when they smoothly develop into moths. From the physiological prospective, larvae took much food, protein will be stored inside

sericterium (silk gland) and be decomposed into amino acid later on, and too much amino acid will poison them. There was an experiment that a tester sealed the silk gland with wax to prevent silkworms from spinning silk, and he found the silkworms were intoxicated to death as a result. This is similar to protein poisoning in humans.



Figure 13. (a) Mulberry pyralid imago; (b) Wild mulberry silkworm larva.

The most excellent silk maker of Lepidoptera insects must be “silkworms”, and of them the most famous are Bombycidae and Saturniidae. They are incomparable and unrivaled by any other silk-spinning creatures. Wild silkworms and domesticated silkworms belong to Bombycidae, and wild silkworms widely grow in Southern and Northern China. Though much smaller than domesticated silkworms, they are still ancestors of them. Now we still don’t know that how wild silkworms were born. The genetic study indicated that their chromosomes are different from those of domesticated silkworms. In fact, many species of Chinese agricultural production were domesticated later, for example, dogs are the earliest domesticated one. It might be Leizu who domesticated them and she was a great female in Ancient China. The characters of wild silkworms also varied as domesticated and selected by humans. So, the wild silkworms we see today are somewhat different from the domesticated silkworms.

Saturniidae insects are diversified. Of them, there is a kind of cocooning insects living on oak tree leaves called Chinese *Antheraea pernyi* (tusser). Most of them spread in China. Some are also seen in North Korea, South Korea, Russia, Ukraine, India, and Japan. Tusser is an insect of complete metamorphosis and will go through four stages of egg, larva, pupa, and imago and development stages with totally different physiological functions during its lifetime. It will molt four times, and each molting marks an increase of 1 instar. A tusser needs 50 days or so from eggs to 5-instar mature cocoon. A spring tusser will eat 30–50 g of leaves throughout its life. Chinese tusser got its name, as it prefers oak tree leaves. Grown tussers eat more than 80% of leaves. The spring tusser is about 14 g in weight and the autumn tusser is about 21 g. A tusser, growing into largest, is 2000–3000 times of a newly hatched larva in weight. Chinese tusser at different stages could be treasured. Cocoons can be used for reeling silk, tussah silk can be made into

tussah cloth, pupa can be food, moths and pupa are raw materials in chemical, medical, and food industries, and eggs are perfect vectors of *Trichogramma* for biological control.

Tasar silkworm mainly lives on *Terminalia catappa* leaves. Muga silkworm mainly lives on *Machilus* and *Litsea pungens* leaves.

Camphor silkworm larvae live on leaves of camphor and maple trees and Chinese chestnuts. Their silk is tough and tensile. In Southern China, some people use them to make fishing lines. *Dictyoploca japonica* Butler making reddish brown cocoons spreads in both Southern and Northern China. The body of larva is covered with white hair, so Japanese people usually call it “Hakumo Taro”. Camphor silkworm lives on camphor tree leaves and can produce high-quality silk. However, those living on leaves of maple, purpleblow maple, multiflora rose, sand pear, guava, and tanoak trees produce inferior-quality silk and mainly spread in China, Vietnam, and India. The most widely spread region is Hainan Island in China. Camphor silkworm experiences one generation in a year and enters diapause in the pupa stage. The proper temperature for imago eclosion is 16–17 °C. Camphor silkworm has 8 instars, which are 80 days in total. An adult female silkworm weighs 16g while a male silkworm weighs 10 g. Silkworm breeders usually won't let them cocoon. Instead, they will immerse adult silkworms into water until death, then tear open the silkworm urosome between the second to third leg with hands, take out two silk yarns and soak them into glacial acetic acid (concentration at 2.5%), and finally draw out the silk after 5–7 min. The silk drawn out can be 200 cm long and is smooth, transparent, tensile, and waterproof when washed. It is shadowless in water and can be the optimal fishing line. Almost 1,000 silkworms can make 500 g of silk. Except for fishing lines, camphor silk can be manufactured as high-quality suture lines for medical operation. Camphor silkworm cocoons can also be used for reeling silk, but not much. So far, only China produces camphor silk all over the world.

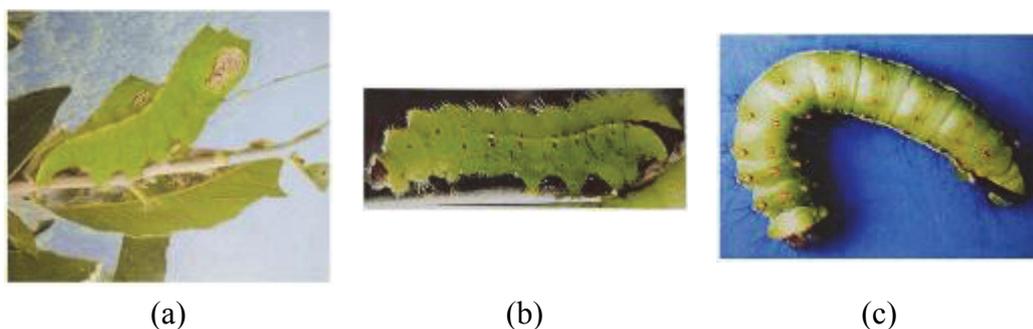


Figure 14. (a) Tusser larva; (b) Tasah larva; (c) Muga larva.

Wild silkworm is King or Queen of Silk and lives on leaves of oak trees of Fagaceae, such as Eastern Liaoning oak trees, Mongolia oak trees, and others, which mainly grow in China, North Korea, South Korea, and Japan. The wild silkworm is a univoltine insect of complete metamorphosis. Egg stage lasts 270 days or so. In this regard, wild silkworms sleep long during their whole life and could be called lazy bones in the insect

world. By the way, as to sleeping, some people in modern days will more or less have sleeping problems. They will try various ways to improve sleeping. In comparison, many animals in the nature are luckier. They spend 80% of time a day in sleeping and could be honored as Sleeping God. Koalas are the best sleepers in the world. They live in Australia and rest on eucalyptus trees and sleep 22 hours a day. Of their wakeup time, most is for eating and they are slackers in true sense. Brown bears sleep 20 hours a day, and almost 83% of their lifetime is spent during sleeping. Giant armadillo sleeps more than 18 hours a day and is nocturnal, though most of time is spent in dreams. Their sleeping quality is so enviable that people having poor sleeping quality may ask them for the secret. Let's turn back to larvae of wild silkworms. The stage of larva should experience 4 sleeping stages and 5 instars from incubation to cocooning, which are 50–60 days in total. It needs 7–8 days from cocooning to pupating and 20–30 days from pupating to eclosion. The proper temperature for egg incubation is 18 °C and the relative humidity is 75–85%. For eclosion, the proper temperature is 22–26 °C. When growing largest, a wild silkworm weighs 17–20 g, nearly 4,000 times of a newly hatched silkworm. What a big little guy! The cocoon of the wild silkworm is green, and silk can be reeled from it, is high in quality and soft and can remain green naturally without dyeing. In addition, the silk has unique luster and the fabric made from it is bright in color and attractive and could be the superior product. Wild silkworm is a precious specie in the nature given by the “heaven”. Wild silk is priceless and known as “Soft Emerald”. It can glow unique color under daylight. Some clothes can show more luxurious and elegant only ornamented with some wild silk.



Figure 15. Larva of Camphor Silkworm.

Long before, some foreign traders crossed the vast ocean at all costs to China, just like Xuanzang (famous Chinese Buddhist monk). They loved silk products and believed that these products could bring safe and happy life and good luck. Some clothes and ornaments would be sold in higher price if decorated with silk. In the past, garments ornamented with silk were only provided for royals or high-ranking officials and passed from generations to generations. Silk, once came out in China, has become a hot trend for a quite long time in the rest of the world. People were rushing to buy it as if a precious jewel fell into others hands. The production of silk also brought economic prosperity for

China. At the end of 20th century, it was said that there was a report on Japanese newspaper that all textile products in a discovered thousand-year-old tomb were weathered into pieces, only a garment on a mummy remained intact and tensile, and through investigation, the garment was proved to be made of silk 1000 years ago. The invention of silk has been the pride in Chinese textile history.



Figure 16. (a) Koalas (Sleeping God) are the best sleepers in the world; (b) Squirrels sleep 14 hours a day, but are extremely active when stay awake.

About wild silkworms, there were many stories in ancient China. It was said that at the enthronement ceremony of an emperor of Tang Dynasty, a green silkworm suddenly fell onto the ground of the palace, the emperor took it as a heaven-sent gift and granted as “Tian Can (Heaven Silkworm)”. The silk is a thin and tiny yarn and can be reeled densely on hands. It is as sharp as a knife blade that can pierce armor, as soft as water that can dance like a butterfly. In Chinese Kung Fu novels, there are many weapons that martial art masters own such as Silk and Thin Knife, and they can kill people without any trace. Although they are exaggerations of novelists, this also can demonstrate tenacity and value of the silk.



Figure 17. Wild silkworm larvae.

There is a saying that *cordyceps sinensis* (a highly valued herbal remedy in China) is good, but wild silkworms are better. Pupa as food can prevent and treat cardiovascular and cerebrovascular diseases, rickets, and iron deficiency anemia, and especially has magic curative effect on hepatitides, nephritides, and diabetes. In addition, pupa can also be used with radiotherapy and chemotherapy to prevent leukocytopenia. Fat composition in pupa includes 75% of unsaturated fatty acids, of which linoleic acid and linolenic acid are high in content and major ingredients in some medicine for treating hypertension and hepatitides. Antibacterial peptide extracted from pupa has remarkable curative effect on anemia, aplastic anemia, leukocytopenia, and Leukemia. Food pupa tastes sweet and nutured and can tonify Yang and people eating it usually will have a fair complexion.

Eri-silkworm is a multivoltine silk insect of complete metamorphosis without diapause, was first found in India and introduced into Gaoxiong, a city in Taiwan Province of China around 1938 and into Northeast, Eastern, and Southern China in 1940. It looks ugly and not adorable for children. Because of their thorns all over their bodies, so children thought it a little scary. One generation of eri-silkworm needs to experience four stages of egg, larva, pupa, and imago. The stage of egg lasts 10 days, the stage of larva is about 20 days for 4 sleeping stages and 5 instars, the stage of pupa takes 20 days, and the full generation needs 45–50 days. The egg cannot be incubated at the temperature below 16.5 °C or above 32 °C and the proper temperature is 25 °C. The proper breeding temperature is 24 °C around. The proper protection temperature for pupa is 25 °C and the relative humidity is 75–90%. Until growing into the largest, the silkworm weighs 7 g around, which is 5400 times of that of a newly hatched silkworm. Eri-silkworm larva is white, yellow or blue, has black spots or no spots, and can live in high temperature and humidity. It prefers castor leaves, cassava or ailanthus leaves. As we all know, oil can be squeezed from castor and can be sold at high price. Castor leaves can be used as herbal medicine in China having effect of dispelling wind and eliminating dampness and effect of detoxifying and diminishing swelling. Castor cannot be taken as food directly, and a little intake will lead to dizziness, tinnitus, abnormal body heat, increased heart rate (120–140 times/minute), and red eyes. Excessive intake will cause visual impairment, and nerve injury or death for the worse. Eri-silkworm cocoons cannot be used for reeling silk, however, after refining, the cocoons can be good industrial raw materials for silk spinning. The eri-silkworm silk, similar to silkworm silk, is tensile. After combed, they can produce 60.9% of filament and 35.3% of stable in 1–4 grades, and only 3.8% of loss is caused.

Chestnut silkworm is one of economic cocooning insects living on walnut and chestnut leaves, also known as lantern silkworm in China. It widely spreads in Japan and China. In the wild, larvae are incubated in the latter part of May, have 4 or 5 sleeping stages, become mature in the latter part of June, and cocoon in 50 days. The cocoon looks like a lantern and has meshes of different size. Cocooning needs 2 days and it needs 3–5 days from cocooning to pupating. The imagoes are chestnut brown, copper green, tiger yellow, or squirrel grey. Chestnut silkworms are prototropic and can be easily

separated during mating when being disturbed. So, it should be especially noted that mating should be carried out in a quiet and non-disturbed environment. Chestnut silkworm silk is high in quality, and fluorescence reflectivity of hand spun silk is up to 14.9%, this rate of plain yarn and dyed yarn is 1.10% and 9.23% higher than that of tussah hand spun silk. Dyed chestnut silkworm silk glitters under the natural light observed with naked eyes. Chestnut silkworm cocoons can be used for reeling silk and can be raw materials for silk spinning, and the silk is good in quality. The silk gland taken out from the matured silkworm urosome can be drawn long as fish lines or medical silk after soaked in acid.

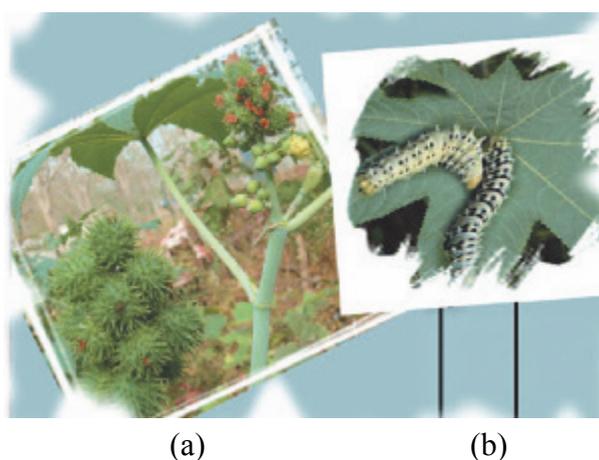


Figure 18. (a) Castor; (b) Eri-silkworm.

Chestnut silkworm silk is expensive natural fibers and can glitter special fluorescent light. It is usually used for making various anti-fake logos and high-end garments. Its price is 10 times of that of the tussah. Chestnut silkworm pupa is high-quality health care food containing high protein and low fat and contains several special components that can be used for treating different diseases.



Figure 19. (a) Chestnut tree; (b) Chestnut silkworm larva.

Ailanthus silkworm eats ailanthus leaves as well as Chinese tallow, castor, Chinese ilex, michelia, paulownia, Chinese parasol, and camphor. It is also an economic insect that can spin silk and cocoon. The ailanthus silkworms can be seen in China, Japan, and India, and are univoltine, bivoltine, and quadrivoltins. Silkworms in cold areas are usually univoltine and those in Taiwan, China are quadrivoltins. The silkworm-rearing season lasts 30–40 days. The matured silkworms make cocoons on 2 to 3 leaves. Cocoons are greyish brown and shaped like a spindle with a hole at the top end and have elongated stems. Each cocoon weighs about 3 g, a cocoon shell weighs about 0.3 g, and the cocoon shell rate is 10–12%. Generally, in rural areas, they are used for reeling raw silk, and the spun silk fabric is called ailanthus silk. The ailanthus silkworm has a greenish brown body but the head, front end of a neck, rear edge of a front chest, back of a urosome, lateral edge, and rear end are white. Each part of the urosome back has 6 pairs of white spots, with discontinuous white lines in between. The fore wings of the ailanthus silkworm are brown, the rear edge of the top angle of the fore wings are shaped like an obtuse hook. The top angle is round but protruding, pinkish purple, and has black eye-shaped spots. Over the spots are white arc. The centers of forewing and underwing have large crescent spots respectively, and each crescent spot has a dark brown upper edge and an earth yellow lower edge and has a transparent part in between. The outer side has a wide band running through the full wings. The wide band is pink in the middle, white outside, dark brown inside, and brown in base corners. The outer edge of the wide band has a curved white line. An imago could be as long as 25–30 mm and spreads the wings by 110–130 mm. Ailanthus silkworms are not adorable due to its ugly looking. However, it is strange that people love to eat fried eggs with ailanthus as they thought it was tasty, but they thought ailanthus silkworms are smelly. In fact, sheer smell has no good or bad division, and it is just about people's like or dislike. Some people may like this smell but some others don't. For example, some like smell of ink of boots, but others don't. Some like smell of petrol oil, but others don't. Even the same person may have different preference to the same odor in different time. From the biological perspective, the reasons may differ, such as evolution, heredity, and physiology.

Attacus atlas silkworm, also known as large saturniid moth or large *attacus atlas* moth, is mainly found in China, India, Japan, Burma, Vietnam, Singapore, and Indonesia. Larva lives on leaves of tens of plants, mostly tallow trees, camphor, willows, lebbek, barberry, sweet potato, green bristlegrass, apple trees, ilex, and birch. It can be seen from this that *attacus atlas* silkworm may be a foodie. *Attacus atlas* silkworm is bivoltine or trivoltine, and lives through the winter in pupa. The breeding season is from April to November each year. An imago can spread wings by 25–30 cm, is one of the largest moths and known as “King of Moth”. The wing tip is curved like a sickle, and the head, chest, and urosome are reddish brown. Both forewing and underwing have triangular transparent spots at the center, the outer edges of the spots are black, and the outer edges of the black lines are light red or purplish brown. The outer edges of the forewing and

underwing are brown and have wavy black lines. The forewing surface has an eye-shaped pattern. It needs to two nights to lay eggs, and laying once can have 200 eggs. The egg stage of each generation is 7–10 days. The larva stage lasts 6 or 7 instars and the full instar is 35 days for spring and summer silkworms and 40 days for autumn silkworms. A spring silkworm cocoon is larger than an autumn one. A cocoon is usually 8 cm long and 3 cm wide and weighs 6.5–10 g. The cocoon shell weighs 0.9–1.1 g, and the cocoon shell rate is about 10%. The cocoon of *attacus atlas* silkworm is large, but has not much silk. Nutrition from leaves taken in is mostly transferred to pupa, and boiled-off cocoons can spin silk of good quality and tenacity. Spun silk is called “watery silk”, which is as soft as water and durable.

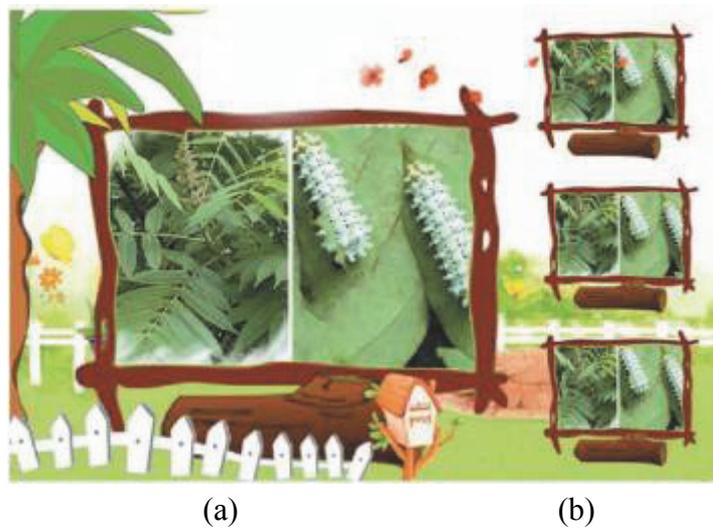


Figure 20. (a) *Ailanthus*; (b) *Ailanthus* silkworms.

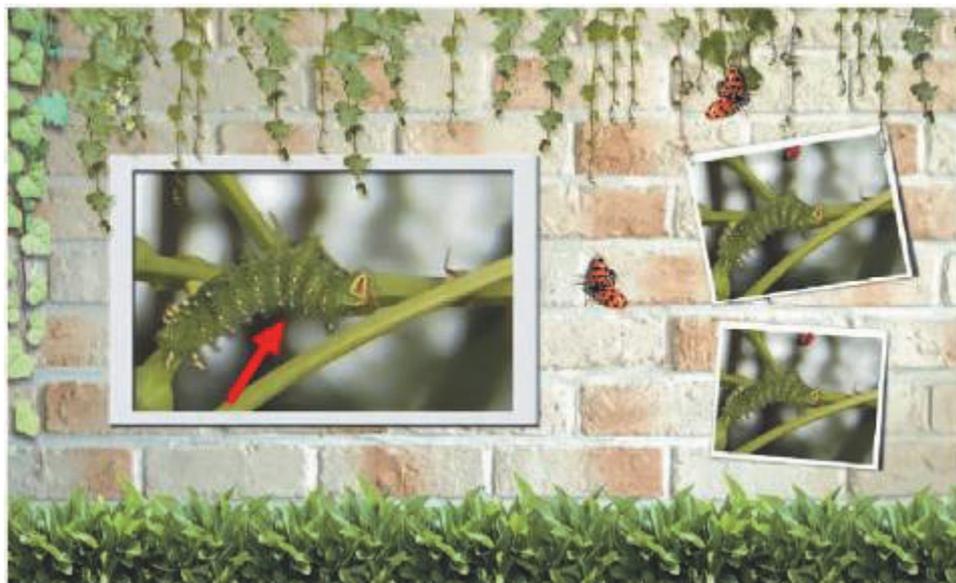


Figure 21. *Attacus atlas* silkworm.

Except silkworms, there are many other silk-spinning and cocooning insects in Lepidoptera. In Indonesia, there is a wild saturniid insect living on camphor and *Phoebe zhennan* tree leaves and making golden cocoons on leaves. In southern and central Africa, there are many silkworms (30–200) making a dark brown coconut-size cocoon together. There is also an insect in Tanzania and Cameroon making a cocoon that is 20–40 cm long and 10–15 cm in diameter and the cocoon shell weighs 13–50 g, a 4 cm-long colony grows in the cocoon shell and this kind of silk is called honeycomb silk. In addition, *Cnidocampa flauescens walker* of Limacodidae makes a hard cocoon and lives through the winter in the soil, and its cocoon is like a sparrow egg having black spots. Mulberry pyralid larva of Pyralidae can synthesize and secrete protein-like silk from the silk gland. There are 800 species of psychid reported, and more than 10 species were found in China. Psychid widely spreads and is polyphagous. Its larva spins silk as a bag. The bag looks like a cocoon and has a shell of 6–15 cm in size, and the surface of the bag is attached to a broken twig, fallen leaf or soil. The larva rests inside and will stretch the head and thorax out burdened with the bag when moving. A matured larva will hang the bag onto a plant with silk, pupating inside. After eclosion, a male psychid will fly from the lower end of the bag and look for a female couple. A female psychid will still nest in the bag after eclosion, extending the head and thorax out awaiting a male one for mating. The female psychid lays eggs in the bag or leaves fertilized eggs in the urosome. One female psychid can lay 100–200 eggs, or 3000 at the most. Psychid larvae are major pests for forests, fruit trees, and street trees. In serious cases, they will eat up all leaves and trees are full of their bags. The larvae can move to other crops after eating up all tree leaves and jeopardize fruits and seed production. Psychid is polyphagous. One of psychid species can jeopardize more than 70 species of plants, such as orange, litchi, banana, longan, chestnut, coffee trees, kapok, loquat, mango, camphor, acacia rachii, and *dalbergia hupeana*. In view of this, psychid might be a nuisance. However, from the other side, you have to admit that it is intelligent and smart. As a matter of fact, it just fights for survival and multiplying on the Earth.

Currently, with the development of modern industry, pesticides and chemical substance cause more serious pollution to the environment, and wild silk-secreting animals on the Earth are decreasing and many of them are on the brink of extinction and in urgent need of protection, study, development and utilization. I was once questioned why we urbanized and why we deepen the urbanization. Urbanization brings huge damage to the ecological environment. Population growth will inevitably cause a chain of social problems on traffic, human, and medical care. If the ecological environment is worsening, where will humans live in and develop?



Figure 22. Wise man: Psychid.

2.4 All Silkworms' Life

Bombyx mori belongs to Bombycidae. It was said that ancient Greek scholar Aristotle imagined that silk could make a sound of “Bang”, so he named mulberry silkworm with Greek “Bombos” and Mori came from the genus name of mulberry *Morus*.

Bombyx mori is an oligophagous insect and loves mulberry leaves only throughout all its life. Other plant leaves such as romaine lettuce leaves, cudrania leaves, paper mulberry leaves, elm leaves, and lettuce leaves could only be snacks. *Bombyx mori* is an insect of complete metamorphosis and its whole life includes four stages of egg, larva, pupa, and imago having totally different physiological functions. The stage of larva refers to a period from egg to cocoon at the proper temperature and lasts 22–25 days. A silkworm can eat 20–25 g of fresh mulberry leaves throughout its life and will molt 4 times. Its weight can be increased 10,000 times when it grows to the largest. The silkworm will eat a great many mulberry leaves at its last instar. In this instar, intake of mulberry leaves accounts for 85% of the total intake of all instars. Cocoons can reel silk. One cocoon is one silk yarn, which is 800–1500 m long. Silk is excellent textile fiber and

a raw material for manufacturing silk fabric.

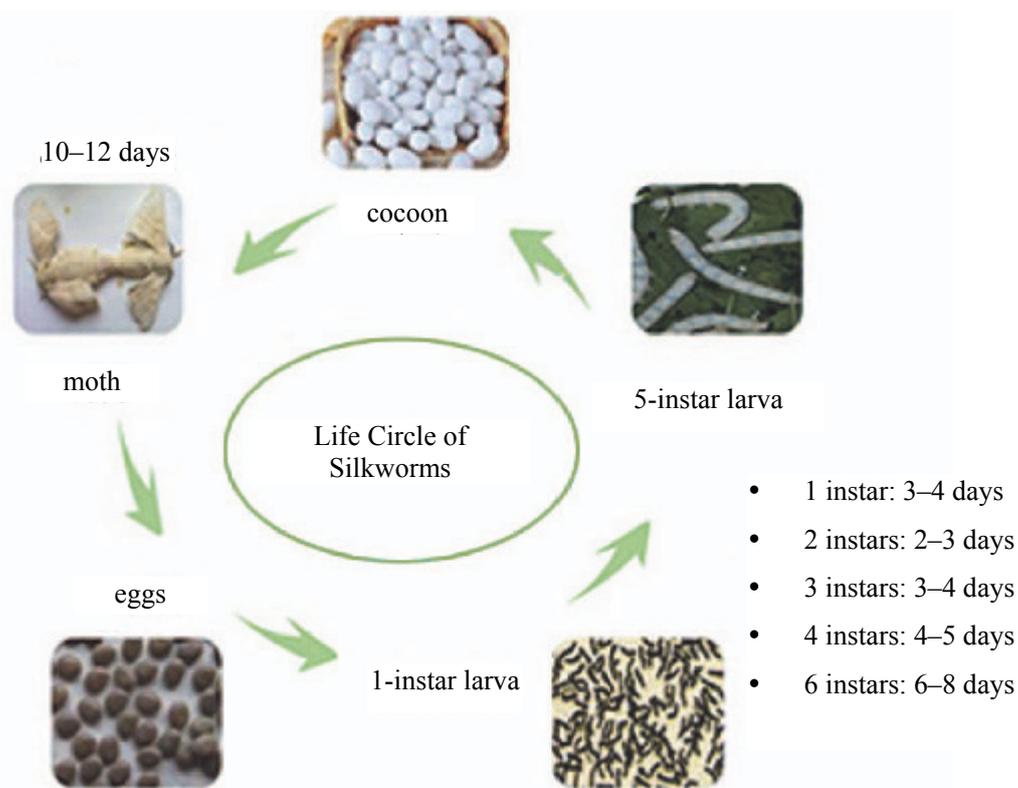


Figure 23. Complete generation of silkworm.

The reproduction modes of animals include oviparity and viviparity. Oviparous animals lay eggs to reproduce. In other words, laid eggs will be incubated in the proper environment. Most birds, reptiles, fish, and insects are oviparous. But eggs of some animals develop to be fetus inside the uterus of a parent, and this mode is called viviparity. The reproduction mode of mammals is also viviparity, such as bats, seals, ursine seals, dolphins, baboons, pangolin, meerkat, anteater, sea otters, domesticated animals, feline animals, rodent species, and primates. Generally, the viviparity survival rate is higher than that of oviparity. Although we still don't know why in the nature there are two reproduction modes, but we indeed know oviparous animals will lay many eggs, and this may be the result that oviparous animals feel threats towards their offspring from biological attacks and environmental extremes. A silkworm perpetuates their kind by laying eggs. A moth can lay 500 eggs. This is really generative. Amazingly, males and females are almost equal in number, which seems to be a general law in nature. But a mother moth will die soon after laying eggs and cannot enjoy the happiness of family. Moth eggs are flat and are 1.3 mm long, 1.1–1.2 mm wide, and 0.5–0.6 mm thick, as large as a sesame. In common cases, 1600–2300 eggs weigh 1 g. Eggs of *bombyx mori* are usually oval with one end a little tapering. Eggs just laid have bulges on the surface and are plump. In 2–3 days, as water and nutrition in eggs consume, eggs will recess

gradually at the surface center. Over time, the recess will deepen to form an “egg pit”, also called “water drain”.

If magnified, an egg looks like a blockhouse built in wars. One pointed end has an egg hole, just like a black point. *Bombyx mori* is oviparous. An egg has been formed in the urosome when a female moth broke through a cocoon. A sperm of a male moth will first enter the egg via the egg hole during mating. As we all know, hens mate first and lay eggs after that. Each mating of a cock and a hen, 1.5–8 billion sperms will enter cloaca of the hen. Most of sperms will die during their journey, only a few of them will arrive the destination waiting for eggs from an ovary. After mating, the shortest time of a hen producing a first zygote (chicken egg) is 25.33 hours, the longest is 139 hours, and the average time is 49.15 hours. Fertility rate is up to the highest in the second day of mating, and still at 82.0% in the seventh day. The shortest continuous fertilization time of the mated hen is 3 days and the longest is 21 days and the average time is 12.3 days. Both are oviparous animals, silkworms and hens are quite different in physiological behaviors. It will be found under a microscope that surrounding patterns of a silkworm egg are obviously different from other parts, which are regular in arrangement and shaped like flower petals, similar to a chrysanthemum flower. Eggs vary in size, shape, and color as different silkworm species. Chinese silkworm eggs are generally greyish green, Japanese ones are mostly greyish purple, and western Asian and European ones are greyish brown mixed with green.

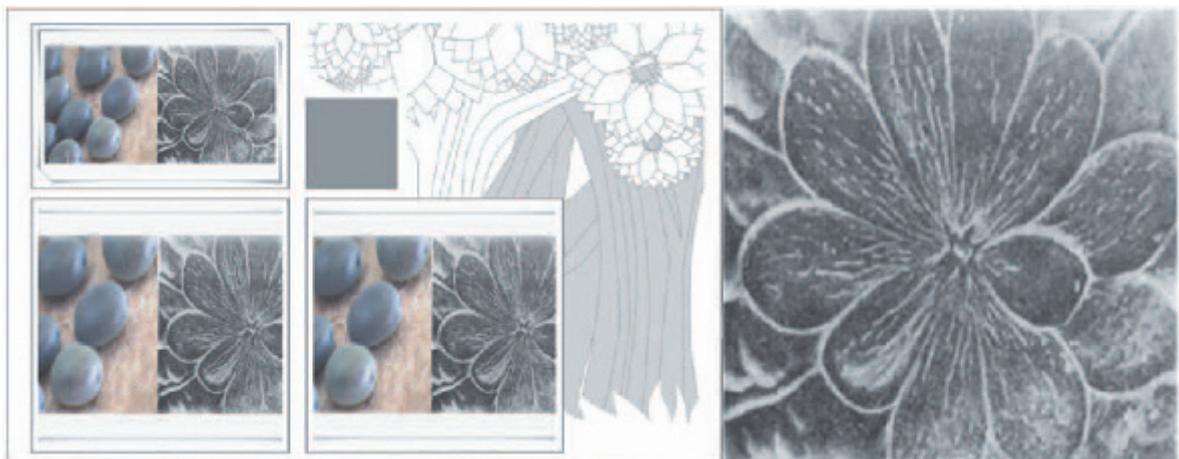


Figure 24. (a) Black spot on eggs are egg holes; (b) Chrysanthemum-like pattern under microscope.

Except short and oval, eggs may also be spindle-shape, kidney-shape, or long. They are white, brown, orange, apricot yellow, or rusty. Generally speaking, eggs just laid are light yellow and will turn red in 12 hours and eventually proper color. Eggs change like a chameleon at this time, but will enter diapause when eggs are in proper color, just like some animals start dormant. Only the coldness in winter can unlock the diapause, and

eggs will be hatched in the next spring. This may be because silkworms feel safe in the eggshells rather than finding no food in winter. Definitely, from the physiological prospective, whether eggs enter the diapause mainly depends on whether suboesophageal ganglion of female pupa can secrete diapause hormone or not. Corpora cardiac hormone has auxiliary effect on causing diapause as well. When diapause eggs enter diapause, pigment granules will form and deposit in chorion cells and presents blackish brown. Scientists observed this physiological change and invented a method to disturb diapause, and the method is to add hydrochloric acid at proper temperature and time. Thus, silkworms can be bred in any time. By the way, non-diapause eggs will not change color after being laid 2–7 days because chorion cells have no enzyme system that can generate chorion pigment. But when an embryo develops into green, eggs will change as well, that is, turn into greenish grey. This variety is usually multivoltine without diapause. Multivoltine breeds are mostly found in tropical regions of Southeast Asia. These regions are high in temperature in four seasons, and mulberry trees grow well and provide sufficient food for silkworms, creating conditions to form multivoltine breeds with local characteristics. Of multivoltine breeds, cocoons are usually small and silk is short. Silk of a cocoon is about 800m. Here, I suddenly thought of Law of Conservation of Energy demonstrated by German chemist Meyer and Britain physicist Joule. Of univoltine breeds, silk is long as well as the stage of pupa. For multivoltine breeds, silk is short as well as time and they can be bred several times in a year. Generally, both are similar, and this may be another kind of “Conservation of Energy” in the silkworm family.

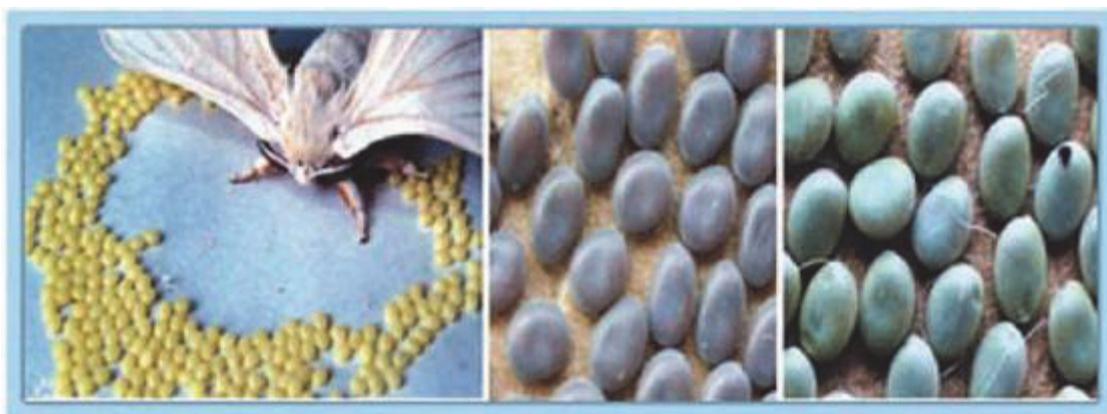


Figure 25. Color changes like chameleon.

It is not easy for a silkworm to crawl out of an eggshell. Most of us shall know, chicks, ducklings, and goslings can be hatched from eggs with their mothers' body temperature. However, silkworm eggs are not hatched by moths, because moths will say goodbye to them after laying eggs. Unfortunately, baby silkworms will never see their parents again since their birth and are cared by silkworm breeders. Then how do little silkworms break the shell? In ancient time, people put eggs in their clothes in spring, the

body temperature 37 °C was appropriate to development of eggs. In 10 days or so, silkworm would crawl out of the eggshells. In modern sericulture, people heat the environment artificially to 28 °C and set humidity (about 90%), and eggs will develop into newly hatched silkworms in 10 days. As one end of eggs will present a little green in the 9th day of incubation, professionals call it a hatching hastening process. In general, a newly hatched silkworm will crawl out of an eggshell in the 11th day of hatching, which is similar to chicks getting out, what is different is that silkworms are smaller. A newly hatched silkworm has blackish brown seta all over its body and is as small as an ant.

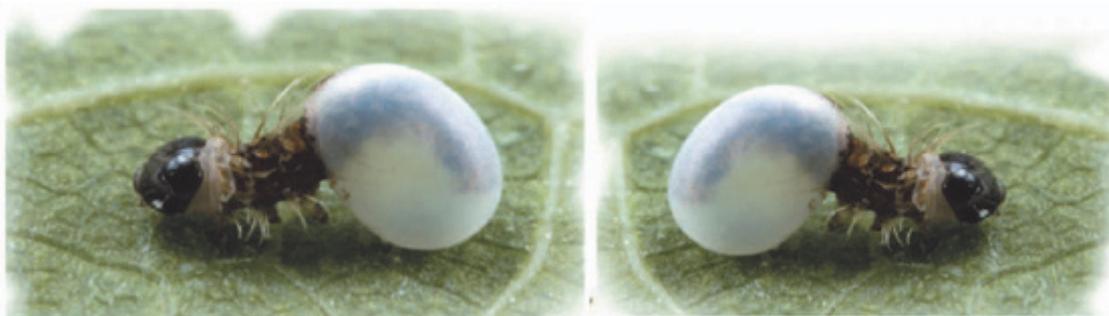


Figure 26. Newly hatched silkworms.

A newly hatched silkworm crawling out of the eggshell is called larva. It likes eating tender leaves and prefers high temperature, the proper temperature is 28 °C and 85% of humidity is good. The silkworm grows fast, the first instar only lasts 3 days, and after the first instar, the silkworm will stop eating and start molting just like changing clothes. The second instar is the shortest, about two and a half days during which the silkworm will sleep and molt again. The third instar needs more than 3 days and the silkworm will sleep and molt again and again. But at this time, molting is different from the former instars. Color of the silkworm will change a lot and turn inherent. Metaphorically, children grow up into adult. The fourth instar is a little longer, about 4 days and the fifth instar needs 7–8 days. In the fifth instar, a silkworm growing extremely weighs 10,000 times of the newly hatched silkworm. Of course, some silkworms are trimolters, that is to say, larvae have experienced only four instars. In general, skin of just-molted silkworms will be wrinkled and unsmooth. Later on, with taking in mulberry leaves, silkworms will grow up and skin is tense, their “clothes” look tidy and beautiful. This is easily understood for us. New clothes may be a little loose, for we don’t need to keep changing clothes during growth. It can be boldly inferred that silkworms may know the Economic Principles and how to save and reserve energy for spinning silk and multiplying.

There are a variety of silkworm species. Some silkworms as white as snow are usually found in China, some dark and having spots are found in Japan. European species are large in size. In addition, there are black silkworms as black as a black swan, tiger silkworms with patterns looking like tigers, and silkworms with horns, *etc.* They might

be specific reflections of biodiversity. Nowadays, silkworm study scientists collect data of sudden-changing eggs and body colors to establish a silkworm mutation gene bank. The gene bank is important and provides significant data for studying certain pathology and heredity. By the way, there is a huge mutation gene bank of fruit bats as well, we usually say that silkworms are equally important as fruit bats in view of genetics, and in a way, this indicated that silkworms contribute a lot to genetics study.



Figure 27. A silkworm just molted holds up its head high.



Figure 28. Various spots, pupa, cocoons, moths.

From the above, you may gain some knowledge about silkworms. Now I want to introduce the exterior structure of silkworms. People will be always confused about which side is the head of a silkworm when seeing it for the first time. Generally, the silkworm head is a forepart, small, oblate and dark brown. The head will turn lighter in color with instars, has dense and symmetric seta on the surface, and is the center of sense and ingestion. 3 segments of the thorax have 1 pair of thoracic legs respectively. 10 segments of the urosome have 4 pairs of prolegs and 1 pair of caudal legs. The center of the back of the 8th urosome segment has a caudal horn. The 1st thorax segment and the 1st to 8th segments have a pair of spiracles respectively for changing air with the outside world. People will ask if silkworms have eyes. The answer is yes. Silkworms indeed got ocellus (simple eye). The eye of insects has a lot of photosensory cells, which can sense change of light. Usually imagoes got ommateum (compound eye) and ommateum is the major visual organ of insects. The ommateum is composed of many small hexagonal and some circular eyes and can see image of objects, especially moving objects and can tell colors. The range of vision of the ommateum varies as insects. For example, flies can see 0.4–0.7 m, and bees can see 0.5–0.6 m. Different insects have different number of small eyes. For example, the ommateum of a cockroach is composed of 1,800 small eyes, a worker bee has 6,300 small eyes, a queen bee has 4,920 small eyes, a drone has 13,090 small eyes. A mosquito has 50 small eyes, a fly has 6,000–8,000 small eyes, some of butterflies and moths have 12,000–17,000 small eyes, and two big lantern-like eyes of a dragonfly are composed of 10,000 to 28,000 small eyes. Generally, the larger ommateum is, the more there are small eyes, the better vision is, and insects can see more clearly. Prolegs of silkworms are soft and podomere-free protrusions, with disc-shaped distal ends and blackish brown falcus on the inner flange. The shape of the falcus cannot be seen with naked eyes, but shown clear under a microscope. The falcus line up semi-circularly in two rows, their distal ends are sharp and pointed, which allows prolegs to grip. Ancestors of domesticated silkworms still live in the wild. The falcus of wild silkworms have strong gripping power. However, domesticated silkworms have been bred indoor long before, so the function of falcus has degenerated. French naturalist Lamarck expounded his evolutionary theory in his book *Philosophie Zoologique* and proposed two laws, *Use and Disuse*, and *Inheritance of Acquired Traits*. Lamarck believed both of them are the cause of heteromorphosis as well as the adaptive process. He proposed that species are changing, not changing is a relative concept, and the reason of evolution is the direct effect on organisms from the environment. Lamarck thought living beings will change their habits under the direct impact of the new environment and some frequently used organs will develop and grow while the others not used will degenerate. He proposed that adaptation is a process of biological evolution. For this, he took an example of giraffe. He said ancestors of giraffe were short and couldn't reach leaves of tall trees. If leaves of the short trees could not satisfy them, they would try to eat leaves of tall trees and stretch out their necks, and necks became longer and longer as

time passed. This phenotypic trait passed from generations to generations and giraffes evolved as we see nowadays. His theory might be flawed, because genes didn't change even if bodies changed. No matter how tall giraffes are, it would not pass to the next generation. Later, Darwin disproved his point. Despite limitations to the theory of use and disuse, there were many evidences in the natural world. Here we don't discuss whether this point makes sense or not, we focus on the fact that silkworms are susceptible to pathogenic microorganisms as their faecal pellets will scratch one another if silkworms are overcrowded. So, it is suggested that enough space should be reserved for silkworms when bred or they will "fight" each other. Here I can't help thinking that humans develop and housing is improved, people lived in the wild in early time just like wild silkworms, but now, with the development of society, people move into tall buildings from cottages, and of course, breeding conditions of silkworms shall also be improved. Big change has occurred to the silkworm breeding environment and conditions. Compared with the past, it can be said that silkworms live in "villas". We could consider it is an act of keeping pace with times.

A silkworm will be matured after eating mulberry leaves for 25 days and start spinning silk and cocooning. Cocooning takes 2–3 days. Later on, the silkworm will turn small to be a spindle-shaped pupa. The pupa looks like a plump beauty. She stops eating and stays still in the villa-like cocoon, reserving strength and waiting for breaking out of the cocoon and transforming into a moth. The pupa has a head, a chest, and a urosome. The head is small and has a compound eye and tentacles. Thoracic legs and wings grow in the chest. The round urosome has 8 metameres. Professionals can judge gender of silkworms from size of pupa and lines and brown spots on the rear surface. Generally speaking, a male pupa is small with a pointed end, and there is a spot on the rear surface while at this position of a female pupa, there is an X-shape. An imago after eclosion always throws up alkaline liquid to wet and soften the cocoon shell at the head end, tearing with thoracic legs and coming out of the cocoon. Wings are flexible and folded initially and gradually stretch out as liquid is dried. The moth looks like a butterfly, covering with white hair all over the body. As two pairs of wings are small, it cannot fly. Moth scales are similar to those on fish under a magnifier, which might be ornaments they wear. The moth head is circular, with a bulging compound eye and tentacles. Three pairs of thoracic legs and two pairs of wings grow at the chest. The urosome has no prolegs and the metamere at the end has evolved to be an external reproductive organ. The female moth is large and a slower crawler while the male one is small but fast. The moth will urinate when it first came out, then the male moth will spread wings, looking for couples, and the female one stays quiet waiting for mating of the male moth. The female moth can lay eggs after mated for 2–4 hours. One female moth can lay 400–700 eggs, most of which were laid on the day of eclosion and others would be laid in 3 days. The imago won't take in food and die several days later after they completed their mission of reproduction, laying eggs.

A male silkworm just coming out of the eggshell has 1 pair of testes. At the fifth instar, there are 1.4–2 million enpyrene spermatozoons formed. Until the middle and late stage of pupa, there are many apyrene sperms formed. The female moth has 1 pair of ovariums when hatched and beautiful oviducts. Through dissection, you will find 8 oviducts, 4 of which on each side. They look like pearl necklaces in the jewelry shop. Pupa will turn into an egg in the 9th day of pupating and move down in the oviducts. On 1–2 days before evolving into a moth, ootid nucleus breaks up for the first time and stops in the intermediate stage. After moths are mated, sperms enter eggs to stimulate ovokaryon in the intermediate stage of the first meiosis to split again until 40 min later eggs are laid. The second meiosis begins 60 min later than egg laying and terminates after 20 min. In 2 h after eggs are laid, arrhenokaryon and thelykaryon are integrated and start cleavage. About 15 hours after eggs are laid, blastoderm is formed. A part of cells of blastoderm on one side of micropyle gradually thickens to form a germ band. About 24 hours after eggs are laid, the germ band separates from the blastoderm to form an embryo. The embryo of a diapause egg has no big change in appearance during diapause. Afterwards, as temperature falls, diapause of the egg will eventually terminate. The proper temperature to terminate diapause is 5 °C, generally, diapause will be gradually terminated since the temperature is lower than 15 °C.

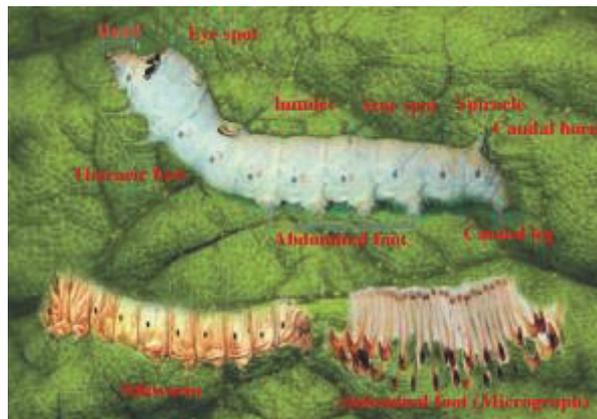


Figure 29. The body of pupa.



(a)



(b)

Figure 30. (a) Pupa—sleeping beauty; (b) Female pupa and male pupa.

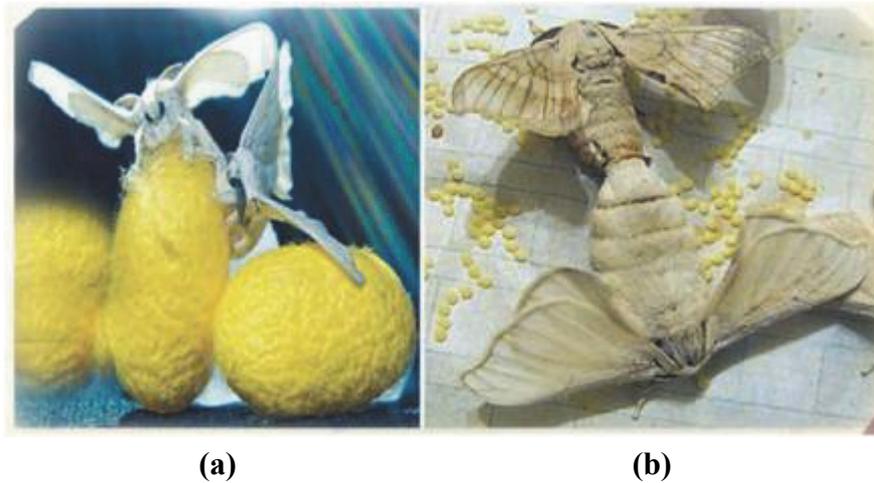


Figure 31. (a) Emergence-breaking the cocoon; (b) Mating.

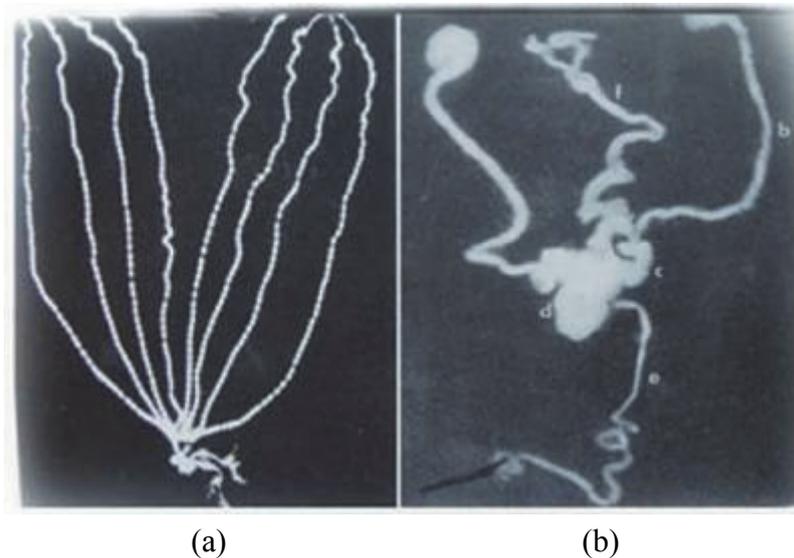


Figure 32. (a) Oviducts look like pearl necklaces; (b) Reproductive organ of male moth.

Based on the aforementioned knowledge about domesticated silkworms, now we talk about methods of breeding silkworms. The methods of breeding silkworms usually depend on physiological characters of silkworms at different stages. Silkworms at 1–3 instars are usually called young silkworms. A place for breeding young silkworms is commonly covered with a plastic film with four sides folded up so as to keep warm and hydrated. The temperature is 28 °C and humidity is about 85%. Leaves for breeding young silkworms should be cut into small fragments, because the young silkworms are too small to crawl throughout the whole leaf. It is not recommended to feed young silkworms with a whole leaf in case of bad effects on them. The leaf fragments are best to be 2 times of silkworms. Silkworms are fed 3 times a day, just like three meals of humans. The feed each

time depends on development and appetite of silkworms, and leaf leftover. If all leaves are eaten up, the feed can be increased slightly next time, or otherwise decreased. Silkworms are neat freaks, mulberry leaves left and feces should be cleaned in time. Silkworms at 4–5 instars are called grown silkworms. High temperature and humidity should be avoided for grown silkworms, a place for breeding grown silkworms should be well ventilated, and sufficient mulberry leaves should be provided. Mulberry leaves should be stored and spread on the ground of a cool, wet, and clean room. It is recommended to pick up mulberry leaves in the morning and store ration of a day. Intake of silkworms of the 4th instar accounts for 10% of that of silkworms of full instars, and intake of silkworms of the 5th instar accounts for 85%. Silkworms entering the third day of the 5th instar have a good appetite for mulberry leaves. Healthy silkworms seemingly make a raining sound and sing a lovely song when eating. In hot and dry days, mulberry leaves should be sprayed clean water first due to low water content, and this measure can evidently increase yield.



Figure 33. Breeding of young and grown silkworms.

Domesticated silkworms contribute a lot in its short life, no wonder a poet of Tang Dynasty wrote such beautiful poem, “Spring silkworms spin silk no end till their death; candles stop shedding tears only when burned up”. A process from eggs, to larva, pupa, and moth is called a generation, which is also the characteristics of complete metamorphosis. In genera, one generation finished in one year is called univoltine specie, and two generations in a year are called biovoltine breed. In Southern China and India, there are multivoltine breeds completing 3 generations in a year. The multivoltine breeds are short in life and small in size and cocoons and silk is short.

2.5 Sericulture in Ancient China

Leizu, wife of Yellow Emperor, was the earliest one breeding silkworms and spinning silk in the world history. The sericulture started from her benefited Chinese people and the rest of the world and composed brilliant chapters in Chinese culture and world history. As recorded in legends, Leizu's servants found cocoons in the wild and thought they were sort of celestial fruits and brought them back to their master. Leizu was so smart that she found drawing silk by boiling the cocoons and invented the sericulture.

According to documents, domestication of silkworms has long existed before Xia Dynasty. Women Officer Silkworm in Shang Dynasty was designated to manage silkworm breeding, which is equivalent to a silkworm technician nowadays. As recorded in oracle inscriptions, people worshipped God of Silkworm and Shang Jiawei (the ninth chief leader of Shang Tribe), showing their respect to sericulture. Breeds at that time included *Ailanthus* silkworms, Ji silkworms, chestnut silkworms, and domesticated silkworms. Domesticated silkworms were also called Lei silkworm, which might be named after Leizu who invented sericulture. Wild silkworms and domesticated silkworms were multivoltine and gradually evolved to be bivoltine and univoltine and the representative one is trimolter. In Zhou Dynasty, people were also keen on silkworm breeding. Both emperors and officials owned "silkworm breeding fields and rooms". Silkworm eggs were washed in February and hatched in lunar March 1st. Ancient people had some knowledge about egg washing, hatching, molting, pupating, cocooning, and moth and sericulture tools such as foil, silkworm basket stands, silkworm baskets, and reed mats were documented. From Western Zhou Dynasty to the Spring and Autumn Periods, people usually bred univoltine spring silkworms and summer silkworms were forbidden. Until Zhou Dynasty, sericulture methods had been matured and egg washing referred to cleaning bacteria on eggs, similar to washing and disinfecting of today's sericulture. At that time, people boiled water with *Herba Artemisiae Sieversianae* to soak eggs and hasten hatching.

In the Warring States Period, people got deeper knowledge about silkworms, knowing that silkworms had no gender (we know it is not true today) but moths had. Since then, people have invented treatment technology for living cocoons, mainly including exposing and vibrating to kill pupae. Up to now, pupae are killed by high temperature and dried cocoons can be stored and spun silk as required. *Shijing*, also the *Classic of Poetry*, compiled in the Spring and Autumn Periods, has vivid descriptions about women picking up mulberry leaves to feed silkworms. As written in *July of Odes of Bin*, "in a sunny day of spring, song birds were singing, and women lifting baskets and walking on paths and picking up tender leaves of mulberry trees." On a bronze ware (*Drawing of Mulberry Leaf Picking*) of the Warring States Period unearthed today, there was a vivid depiction of women picking up mulberry leaves. Inside 10 mu of *Odes of*

Wei in Shijing described that inside 10 mu of mulberry field, harvesters were busy in picking up mulberry leaves. Also as recorded in Emperor Lianghui of Mencius, “around houses of 5 mu planted mulberry trees, people over 50 years old can wear silk”. It could be seen that silk played a great role in daily life of people. The Spring and Autumn Annals also has a story. A prince of Jin Kingdom Chong’er exiled in other states for long due to persecution and escaped to Kingdom of Qi. Duke Huan of Qi was kind and married a kindred girl Miss Jiang to him. From then on, Chong’er was passive and indulged himself in an easy life. However, followers who ran away from Jin together with Chong’er didn’t let things go on like that. One day, they were plotting in the mulberry forest about how to make Chong’er return to Jin as early as possible. A housemaid of Miss Jiang overheard their conspiracy when picking up mulberry leaves and told Miss Jiang. Unexpectedly, Miss Jiang can’t agree more on the plot. She killed the housemaid for keeping secret and tried to help Chong’er leave Qi. Chong’er took back the throne and became Duke Wen of Jin. We can see from the story that in aristocrats’ house were planted mulberry trees, and planting mulberry trees for breeding silkworms is an important task in maids’ work. Another story said that during the Warring States Period, Cao Cao held a feast in Bronze Swallow Terrace of Ye City (Anyang in Henan province). During the feast, he ordered his royal guard to hang a coat armor on an aspen tree and asked present officers to compete in archery. The one shot the coat armor would win it as trophy. However, more than one officer shot the coat armor, so they grabbed it and torn apart. Cao Cao laughed and said, “you have no need to fight, and I will give each of you a bolt of tapestry”. The coat armor was made of Xichuan Red Tapestry and the tapestry awarding the officers was known as “Sichuan Tapestry” (made of silk). From this, we knew that even in the Warring States Period, tapestry produced in Sichuan Plain was also valued by Wei people in the Yellow River basin.

Ancient Chinese people have had much experience in breeding silkworms. A famous philosopher Xun Kuang (313–238 BC) studied the pattern of silkworm breeding and proposed in Odes to Silkworms that “cocoons can be made after molting three times”. Wild silkworms were also used in Qin and Han Dynasties. Until Southern and Northern Dynasties great improvement had made in breed selecting and preparing techniques. At that time, a method of controlling hatching of silkworms by low temperature had been invented. During breeding, people had noticed the biological effects on silkworms from mulberry leaves, fire, cold, heat, dryness, and humidity. Cocooning frames were mounted outdoor in sunny days and indoor in rainy days and included planar frames and suspending frames. Regardless of Southern or Northern China, there were two methods for treating cocoons, exposure and salt soaking. Storing cocoons preferred the salt soaking method. Sericulture in Tang Dynasty followed the methods of former dynasties. People usually bred multivoltine silkworms, most of which were trimolters and tetramolters. Egg washing was carried out in the wild before or after Grain Rain (6th solar term), which was different from tubbing of later ages. Sericulture in Song Dynasty

had been developed well. The production process included egg washing, hatching, feeding, first molting, second molting, third molting, separating, leaf pickup, wakeup, silk rubbing, mounting cocooning frames, baking foil, demounting cocooning frames, selecting cocoons, and storing cocoons. Egg washing was carried out separately by several times, the first time was in the 12th lunar month for disinfecting by freezing, and the second time was before hastening hatching in Grain Rain and used warm water to wash. Egg warming in Qingming was via body temperature and bran fire. Newly hatched silkworms were collected by goose feather brushes and mulberry leaf inducement. Leaves for feeding newly hatched silkworms should be cut into pieces, young silkworms should be fed with tender leaves and temperature should be monitored and controlled. Grown silkworms should be fed frequently with sparse leaves and sand should be removed in time. The cocooning frames were umbrella-shaped and temperature should be increased properly. Cocoon storage mostly relied on the salt mixing method. Farmers would not store cocoons long for keeping their luster. Before Song and Yuan Dynasties, wild tussers were used to produce silk, yarns, and crude silk. After that, wild silkworms had been domesticated widely in Denglai of Shandong and yield was increased greatly. During Southern Song Dynasty, wild silkworms were soaked or smoked in or by vinegar and split from the urosome to draw silk out from the vinegar. One silkworm could produce 2 m of silk. Some people consider it as the prelude to artificial fibers in modern days. Then spinning silk also succeeded. Sericulture of wild silkworms had widespread in regions of Lu (Shandong), Liaoning, Shaanxi, Yu (Henan), Guizhou, and Wan (Anhui). Tussers include different breeds of *Cudrania* silkworms, *ailanthus* silkworms, willow silkworms, maple silkworms, and others. To Ming Dynasty, a set of state-of-art technique and experience has formed for breeding wild silkworms outdoors. By the end of Ming, tussah silk of Shandong was famous at home and abroad and sericulture has developed from collecting silkworms from the nature to breeding in the wild.

Yuan Dynasty had strict control of sericulture, stressed breeding of multivoltine silkworms and appropriately controlled the number of spring and autumn silkworms. Sericulture in Yuan Dynasty could be concluded into “Ten Conditions”, “Three Colors”, “Eight Appropriateness”, “Three Degrees of Sparsity”, and “Five Factors”. The ten conditions referred to cold, heat, starvation, fullness, sparsity, density, molting, wakeup, rapidness, and slowness (leaf feeding speed). Leaf feed depended on the skin colors of silkworms. Feeding started in the white color, increased in the green color (silkworms were hungry if the skin has wrinkles), and stopped in the yellow color. Eight appropriatenesses referred to light and shade, cold and heat, wind speed, and feeding speed. Three degrees of sparsity referred to sparsity degrees of setting newly hatched silkworms, mounting boxes, and mounting cocooning frames. Five factors mainly referred to sound, odor, light, color, and hygiene that can affect growth of silkworms. These techniques were almost the same as those of modern sericulture, which reflected that the sericulture at that time has well developed. In Ming Dynasty, people emphasized

more breed selection and improvement. Then Tianlu method was employed for egg washing, and it used whitewash and bitter water to wash eggs and rule out inferior eggs. Domesticated silkworms should be one of breeds, of which the hybrid advantages were first discovered and employed. This indeed is the great cause in agricultural science. Song Yingxing, a scientist who lived in late Ming Dynasty and early Qing Dynasty, dedicated his whole life to investigating and studying the agriculture and handicraft and collecting abundant scientific data. His work and researches involved different disciplines such as natural science and humanity. Of all his masterpieces, *Tiangong Kaiwu* was honored as an “encyclopedia of China in 17th century”. In this book, he said, eggs laid after mating of early males with late females were the best. So, it can be seen that in Ming Dynasty, people had hybridized univoltine and bivoltine moths to breed silkworms that were strong and spun long silk. In Qing Dynasty, there were local silkworm breeds suitable for Jiangsu, Zhejiang, Sichuan, Hunan, Hubei, Guangdong, and Guizhou. In these regions, Yuhang, Xinchang, and Xiaoshan of Hangzhou were famous for their good breeds and later developed to be production sites of modern silkworm breeds. Technically, overhead cocooning frames were employed in the sericulture of that time instead of ground frames and silkworms would be picked out with ages. “Drying the entrance” was changed into “heating silkworms instead of cocoons”. At the beginning of feeding leaves of trimolting of silkworms, silkworms were fed with *Cudrania* leaves twice or three times for saving mulberry leaves. Silk was tenacious and glossy. If leaves were insufficient, leaves can be mixed with white flour to feed silkworms, so that silk is white and tenacious. Mulberry leaves in autumn picked up should be dried and smashed and stored in a dry place. If wet by rain, leaves can be mixed with leaf powder so that water can be absorbed, silkworms could be full easily, and leaves can be saved. These were effective methods for saving leaves and also the prototype of artificial feed invented in later generations, which embodied intelligence of ancestors. In 1898, a silkworm academy was established in Hangzhou for learning foreign silkworm breeding experience and theories, studying how to treat pebrine disease and breed a new batch of breeds with new methods such as Qingzhu, Xinyuan, Zhugui, and Hongqing. People hybridized Hongqing and Zhugui to breed Qinggui, the earliest variety of China. Qinggui accounted for a fairly large proportion in sericulture. From then on, provinces established silkworm academies and silkworm and mulberry test fields successively. Sericulture has seen better days. Silkworms in Guangdong were mostly bivoltine and multivoltine. Eight generations of silkworms can be bred in each year in Lianping, and six generations in Shunde. Eggs can be divided into golden and silver according to the skin color. The silver eggs were for hatching summer and autumn silkworms. In regions south of the Yangtze River, silkworms were called Dazao for their large size and cocoons. In Sichuan and Shandong, univoltine breeds were employed and accounted for a large proportion nationwide. Silkworm breeders in Jiahu of Zhejiang summed up the experience of “drying at the entrance”, which employed heating to dry so as to increase quality and

reelability of cocoons. People in regions south of the Yangtze River farmed fish in ponds and raised livestock, combining with mulberry planting and silkworm breeding, which promoted each other in the cyclic conditions of nature and has been the success example for the ecological cycle of modern agriculture. In the meantime, quarantine and selection were used to prevent infectious diseases such as grasserie and flacherie. All of these indicated that the sericulture at that time has formed a complete system.

Now I want to share a classic drawing of ancient sericulture science, Pictures of Farming and Weaving. The Picture like science drawings of today was drawn by artist Lou Chou of Year Shaoxing of Southern Song Dynasty. Emperors were pushing ploughs and plowshares, queens were advocating silkworm breeding, men were farming and women were weaving, which depicted a beautiful drawing of small-scale peasant economy in ancient China. There were 45 pictures of farming and weaving, including 21 pictures of farming and 24 pictures of weaving. The Pictures were highly praised by Emperor Gaozong and Queen Wu wrote inscriptions for them. The Emperor Gaozong summoned Lou Chou, showing his Pictures of Farming and Weaving in front of imperial concubines and the Pictures were praised by the court and the commonly, which triggered the first highlight of development of the Pictures of Farming and Weaving. From then on, different versions of Pictures of Farming and Weaving came into being and became a special phenomenon in Chinese history of painting, science, agriculture, and art and one of the treasures of Chinese cultural heritages. The Pictures of Farming and Weaving experienced a thousand years and circulated all over the world and now there are many imitation versions collected in the United States, Great Britain, Japan, and DPRK. According to statistics collected by Watabe Takeshi from School of Humanities of Tokai University of Japan, there were 56 versions of the Pictures of Farming and Weaving he had studied. The Pictures of Farming and Weaving are regarded as “the earliest and complete drawing of men farming and women weaving” and “first agriculture science drawing of the world”. Emperor Kangxi of Qing Dynasty saw the Pictures in his Southern tour and felt empathy towards weaving women and farming men, so he ordered an imperial painter Jiao Bingzhen to redraw the pictures based on Lou’s, and there were 23 farming pictures and 23 weaving pictures, each of which included a given poem. The Pictures of Farming and Weaving are a series of drawings for advocating farming and weaving and elaborately recording the farming and weaving events. As “the drawings are exhaustive and poems are emotionally moving” and they described farming and weaving and detailed production process vividly and elaborately, the Pictures played an extraordinary role in popularizing agricultural knowledge and farming techniques and promoting social productivity development. They themselves are extremely valued artistic treasure.

Until time of Emperor Yongzheng, he wrote poems in the Pictures of Farming and Weaving, combining artistic five-character poems and powerful handwriting and sealed with Seal of Prince Yong and Pochen Buddhist (both referred to Emperor Yongzheng).

These made the Pictures perfect in poems, calligraphy, and drawing and imperial artistic treasure. In Chinese traditional agriculture, men farming and women weaving could be self-supported. It should be a kind of blessing for the common if they had a hardworking king sensitive to people's need. I think that might be a China dream in the feudal society.

Now let's enjoy the poems written in the second part of Pictures of Farming and Weaving by Emperor Yongzheng. They are Silkworm Egg Washing, Second Molting, Third Molting, Daqi, Silk Rubbing, Silkworm Dividing, Mulberry Picking, Mounting, Warming-up, Dismounting, Cocoon Picking, Cocoon Hoarding, Silk Spinning, Silkworm Moth, Silkworm Goddess, Silk Rolling, Silk Weaving, Silk Reeling, Silk and Yarn, Dyeing, Flower Spinning, Silk Cutting, Clothes Cutting, *etc.*

Silkworm Egg Washing

When rain comes, the willows waft in the breeze;
As stream surges, the peach-petals keep afloat.
Villagers savour vernal lamb wine heartily
While girls wash silkworm eggs diligently.
Their delicate hands move the washing basin
Before the new larva hatch and wriggle down the paper.
From snow-white cocoons the silk can be obtained.
O such is part and particle of the first of Four Virtues.



Second Molting

As the blackbird begins to tweet lightly,
Silkworms lie and dimolt in the tray.
Along the road, verdant mulberries look so thick;
On the dike, emerald grasses still feel so weak.
Rearers desire the sun to shine outside the curtain,
Yet fear the cold may come here once more.
These women having so much work to do
Leave their babies uncared for completely.



Third Molting

Window curtain remains unmoved in the gentle breeze
Mulberry and cudrania trees run riot amid the vernal dew.
To care for silkworms trimolting in the tray,
Rearers light up the lamp at fifth watch.
The eldest aunt does not yet sleep on the mat and
The youngest aunt even has no time to comb her hair.
Later when cock crows over the next door,
The neighbour comes to hurry them into farming.



Daqi

This spring is not too cold or too warm,
So the mulberry trees grow very well.
In the tray there are thin mulberry leaves,
All of which were perhaps plucked early.
The depth of springtime is not known,
The life of silkworms seems never end.
O which families are these ladies from?
They are sightseeing in the verdant grasses.



Silk Rubbing

The final silk has its own right time—
Neither too old nor too delicate product rubs well.
She works hard with her parents-in-law
At rearing the silkworm's day and night.
Sparks set off a fire in the earthen pot;
Stars shimmer through the curtain.
When silk rubbing is done step by step,
She still has to look after her young children.



Silkworm Dividing

Spring swallows fly in the gentle breeze;
Silkworms enjoy the long hours of daylight.
Rearers divide up silkworms at sunrise;
Leaves fall from mulberry trees in the ravine.
Young women pluck mulberry leaves
And carry them back in the basket.
Before the gate are stretches of rustling wheat.
Alas, these yellow clouds blend into the verdant earth.



Mulberry Picking

Now that the spring is at its balmiest,
All families pluck mulberry leaves in haste.
White dew looks dense and thick;
Green forest feels cold and damp.
On high boughs, the pickers climb like monkeys;
As mulberries are thrown, young kids come to collect.
Yesterday all got back with full baskets,
But vexed women didn't give their kids any berry.



Mounting

East household urges the family to plough
And west cottage begins to soak their grain seeds.
Cuckoos are heard calling under the high moon,
Silkworms are found ripening in the late spring.
Those little insects crouch back and wriggle down
To produce the sleek, smooth silk.
Rearers put the well-cut grasses on the frames
For mounting's sake while their daughters look aside.



Warming-up

Late spring witnesses a display of flowers
Early summer sees cool rain as wheat ripens.
Reed curtain closes crab-catchers' cottages while
Pine in the pot warms silkworm breeders' houses.
Snow-white and fragrant are cocoons,
All of which come from the silk of such caterpillars.
On the village road, there are few ones' idling
When swallows return home chirping and cheeping.



Dismounting

Last month breeders washed the silkworm eggs
And this month they pick out new cocoons.
Washing starts when willows sprout new leaves;
Picking begins as willows burst into bloom.
Before village women grease their faces,
The scenery has changed so much.
The neighbour plays a melodious tune,
Making all of them feel jubilant together.



Cocoon Picking

Reapers pick out strips of fragrant snows—
Cocoons while the sun rises over the roof.
Such fleecy covers are indispensable
For making fine silk and floss silk.
Dear grannies, please select good ones;
Dear sons, please make them grow strong.
Later when the Plum Rain is over, rice seedlings
Are planted alongside surging streams.



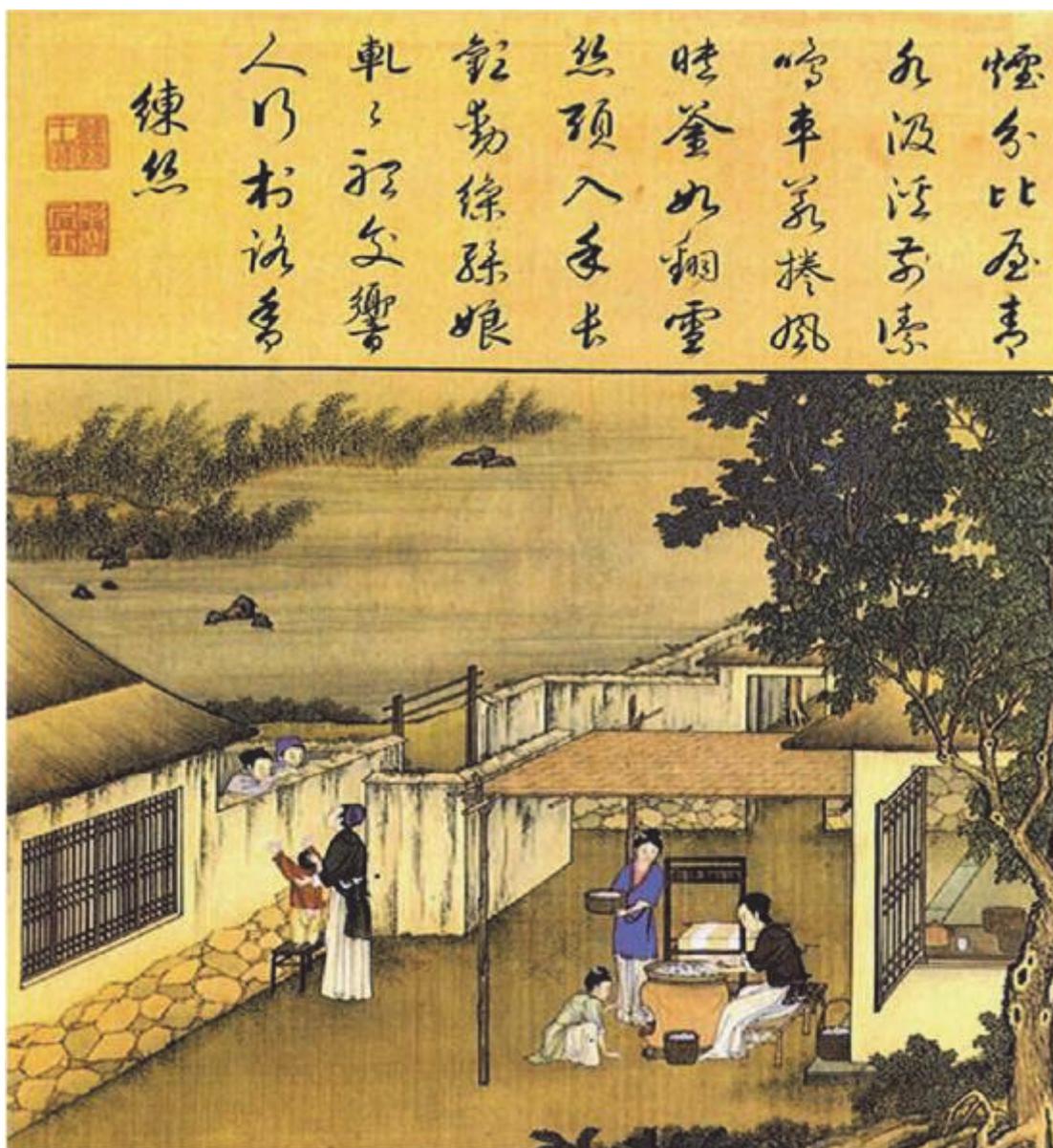
Cocoon Hoarding

Parasols and bamboos grow beside rustic houses;
Ploughs and spades are good farming tools.
Late spring sees silkworm breeding and mulberry growing.
At silkworm trays, rural women work very hard.
Although massive dismounting comes to an end,
They still have to hoard the cocoons well.
Alas, thanks to the grace of the heaven,
They harvest baskets of snowy cocoons!



Silk Spinning

Misty rural houses are dyed with a green hue;
Stream feels cleaner when women draw water.
Looms rumble like flurries of wind,
Cocoons look like flakes of snow.
The silk-head touches long by the hand;
Spinning woman makes the reeler move around.
These machines chug in a symphonic way,
Spreading a dim fragrance to passers-by.



Silkworm Moth

Women pass through the village mouth
To do their work with utmost diligence.
Moths emerge from the cocoons and begin to flutter,
Their pink wings look so sleek and smooth.
Seedling leaves have turned green
And mulberry twigs are verdant anew.
The female breeders carry moths to the waterside,
Such is the rural custom passed down to this day.



Silkworm Goddess

If there is a bumper harvest, peasants will
Clean the courtyard to worship the goddess
By offering silk in the jade utensils
And wine in the goblets and jars.
Their wives and children come over the hallway-steps;
Their patriarchs and eldest sons pay tribute to Her.
Everyone shares the same simple wish—
May peasants enjoy a better harvest next year!



Silk Rolling

O what a slender woman operating the spinner! Bramble
Hairpin and coarse clothes indicate how rustic she looks.

One after another thread of silk is used and

Many a squeak is heard as new brocade comes out.

On the wheel, there is silk rolling up and down

In the daytime, she sits so long unconsciously.

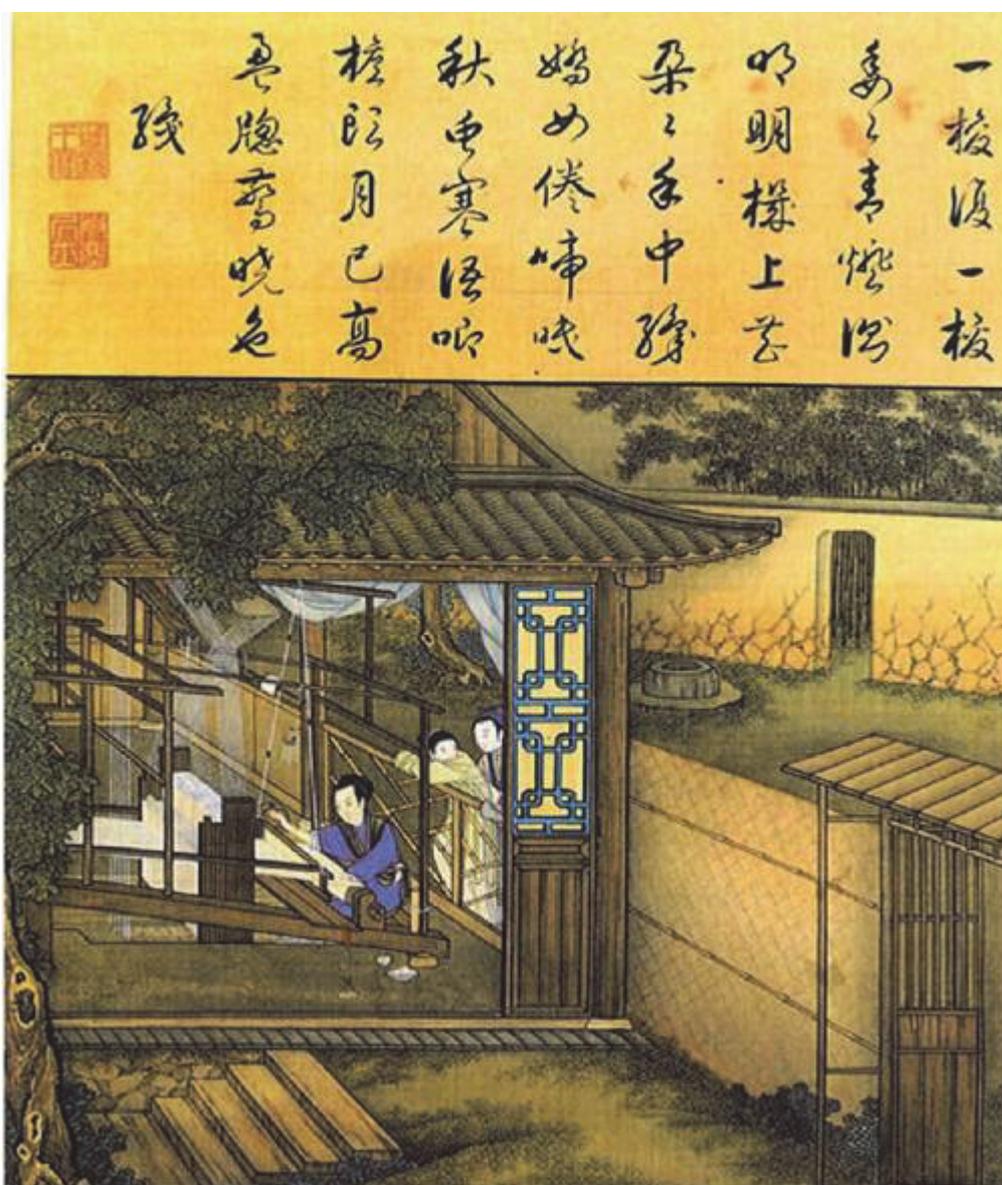
Later she washes the silk and returns across the stream

As the sun is setting against the west corner of her cottage.



Silk Weaving

One shuttle after another goes along
A downward oblique route beside the oil lamp.
So bright looks each flower on the loom
And that is a product of her handiwork.
Her dear daughter is in sound sleep
Amid the bleak chirping of autumn insects.
A moon hangs too high above the eaves,
Heralds the first ray of dawn outside the window.



Silk Reeling

Handiwork alone needs much effort and energy.
Yet rural women have to work till late night.
Under the dim lamplight, they reel the silk;
As the tool is heavy, their delicate wrists suffer.
Slowly a coldness creeps into the hair of these ladies;
As it comes to the middle of the night.
It's not our hearts that do not feel busy.
If we truly feel so, the silk would be in a mess.



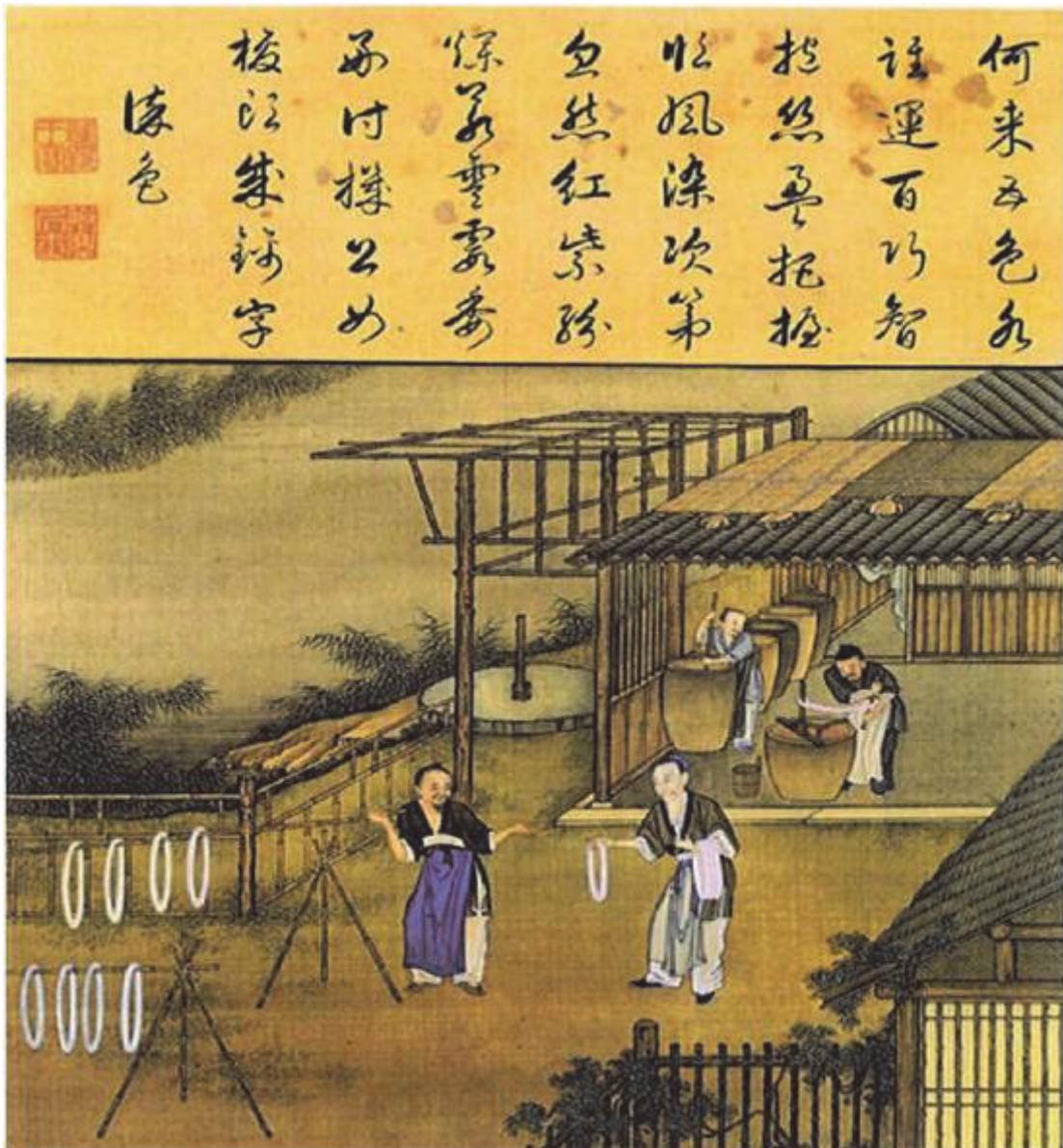
Silk and Yarn

The past silk on the reeler turns into
The present yarn on the spindle.
Women weave the yarns with
The greatest care and caution.
Just look! Only myriad threads of silk
Can make a one-zhang-long fabric.
Alas, how could those city dandies
Understand the effort behind silk and satin?



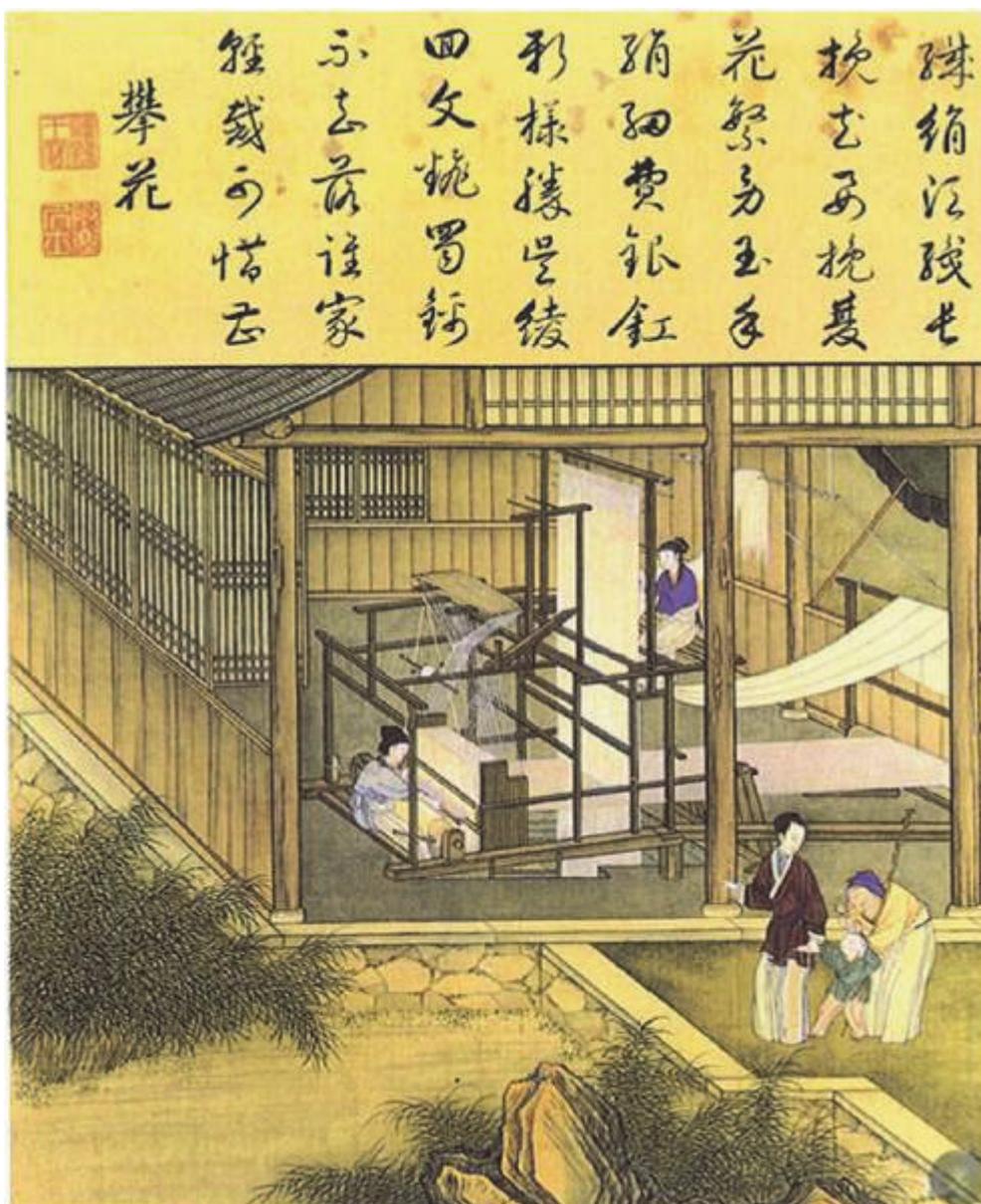
Dyeing

Where could the five-hued water be discovered?
Who could unleash the stunning ingenuity?
Mix together a handful of silk-threads
And dye one thread after another in the zephyr.
In the twinkling of an eye, red and purple
Come together as if rosy clouds were unfolding.
Just let the weaver use them to
Make flamboyant brocade with shuttles.



Flower Spinning

Fabric should be long as much as possible;
Flowers should be made in pairs indeed.
Spinning lush flowers entails the delicate hands of women.
Making gauzy silk involves a great amount of lamp oil.
The resulting new design even eclipses the satin of Wu;
The circular pattern outshines the brocade of Shu.
Who would have the great luck of getting it?
It's a pity that the silk may be cut at random.



Silk Cutting

One thousand and ten thousand silk-threads
Form a whole piece that embodies so much effort.
Breeders sigh deeply while holding the rulers
And lower their heads before cutting the silk.

The artwork shows off a bright hue of
Red peaches and emerald willows.
As long as my parents-in-law feel warm,
I would like to bear the cold and chill!



Clothes Cutting

When it comes to the ninth month.
It can't be delayed to make winter clothes.
Women engage in cutting fabrics
And measuring sizes carefully.
Scissors and rulers, in the wind, touch so cold;
Black and yellow, like clouds, look so good.
Thanks to the grace of the emperor and the heaven,
Silkworms may help peasants resist the winter.



Silk is one of basic necessities supporting human reproduction. Such a beautiful and precious spinning material reflects intelligence of ancient Chinese people. The primitive silk spinning method was to soak cocoons in hot water and draw silk with hands to reel it on a silk basket. In view of unearthed silk cultural relics of Shang Dynasty, warps and wefts had been cabled at that time, and the prototype of a cord wheel with spindle has appeared, which presented high level of the silk spinning technique. During Western Zhou Dynasty, people started making silk brocade robes with husks. Until Wei and Jin Southern and Northern Dynasties, the cord wheel has provided with a hasp for fixing, reeling, and cabling. An Unlined Yarn Robe of 49g in weight unearthed from Tomb 1 of Mawangdui could be said that it is “as light as feather and as thin as cicada’s wings”, which represented the prime time of silk production in the Warring States Period, Qin, and Han Dynasties. Silk products were rich in color and varieties, including tough silk, figured woven silk, and brocade. Appearance of brocade was also a milestone in Chinese silk history. Brocade combines excellent performance of silk with art, which makes silk an elegant material as well as a work of art. It adds cultural connotations and historic value to silk products and has profound effects. Later on, from generations to generations, Chinese silk industry has fully developed.



Figure 34. (a) Silk of the Warring States Period; (b) Unlined Yarn Robe unearthed from Tomb 1 of Mawangdui.

From Sui to Tang Dynasty, ancient Chinese articles of clothes were in full bloom. Political stability, economic boom, improvement in production and spinning technology, frequent foreign contacts, and the like all pushed development of clothes. Clothes design, color, and pattern all presented an unprecedented level. Of clothes for women in this period all over the world, Chinese clothes should be the highlighted part, caps and clothes were so gorgeous and ornaments were so attractive that people had no time to take in all. In two hundred years of Tang Dynasty, there were frock of Han clothing, round-collar robes, and Hu clothing. The silk industry in Tang Dynasty was highly developed, the spinning technique was increasingly exquisite, and silk products were

diversified, mainly including tough silk, damask, brocade, satin, yarn, and figured woven silk. Damask is a silk product with twill weave, and tough silk is plain weave fabric made of raw silk and characterized by light weight. There were many well-known silk products, including brocade with turtle Wang (king)-character patterns, brocade with strings of pearls and couple duck patterns, brocade with pig head pattern, Fangsheng brocade with phoenix pattern, brocade with horse rider pattern, brocade with iridescence jacquard pattern, brocade with bird and flower patterns, brocade with variant Buddha flower pattern, brocade with variant Buddha flower and bird pattern, brocade shoes with cloud-pattern vamp, rhombic-pattern figured woven silk, brocade with bouquet pattern, figured woven silk with strings of pearls and double dragon pattern, twisted-fabric silk with rhombic flower pattern, tough silk with overprint pattern, printing yarn with mandarin ducks, printing yarn with rider hunting pattern, transparent golden yarn, printing satin with grape patterns, brocade with iridescence pattern, silk flower, *etc.* Most silk products of Tang Dynasty were unearthed in Astana, and in addition to those mentioned above, there were colorful brocade with strings of pearls, satin with flower pattern, brocade with flower, tree, and sheep pattern, baltik silk with flower and bird patterns, Fangsheng double-layer brocade with quadrifolious pattern, and a variety of brocade with animal, flower, bird, and geometric patterns. In Tang Dynasty, weft brocade had come into being and gradually taken place of warp brocade of Han Dynasty. The weft brocade had more options of patterns and color, its fabric structure was more complicated and brighter in color, and the fabricating process was much improved. In *Taiping Guangji* (Extensive Records of the Taiping Era) compiled by Li Fang, Hu Meng, and Li Mu of Song Dynasty was recorded a kind of light satin, and a bolt of satin as long as 4 zhang (about 13.3 m) weighed 25 g. Yarn is a kind of fabric full of meshes in the surface, light yarn of Bozhou was so thin that felt like nothing and garments made of it were as light as fog. Brocade is high-end fabric with colorful flower patterns, Yizhou and Yangzhou were famous places of brocade production, and Sichuan brocade were colorful and exquisite beyond compare.



Figure 35. Costumes of Tang Dynasty.



Figure 36. Female costumes of Tang Dynasty.



Figure 37. Zhou Fang toning Zheng and drinking tea



Figure 38. Man-drawn carriage in ancient times.

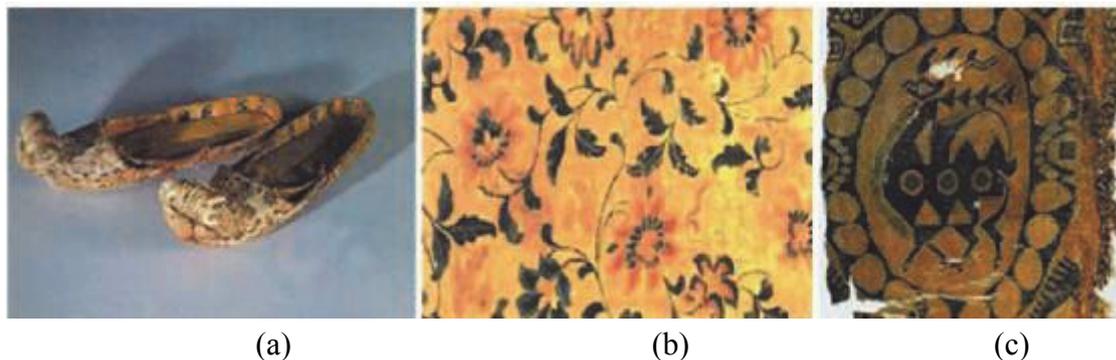


Figure 39. (a) Brocade shoes of Tang Dynasty; (b) Silk fabric; (c) Elegant silk product of Tang Dynasty.

Until Song and Yuan Dynasties, another person like Leizu turned up, and she was Huang Daopo. Different from Leizu, Huang Daopo rose from poverty and experienced hardship during her lifetime. Huang has been an inspirational example for ancient Chinese women, a skillful woman of the cotton spinning and weaving technology, and honored as an earliest ancestor of cloth by people. She dealt with cloth during her whole life, but still made great contribution to ancient Chinese silk spinning technology. Qin Rongguang of Qing Dynasty wrote Zhuzhi Lines of Play to praise her.

In Wunijing stands a shrine of Granny Huang.

She made Sanlin become a land of Standard Cloth.

Alas, she taught all plebs to make clothes.

No wonder the granny enjoys perpetual sacrifice.

At the time there was widespread folk music also written to praise her.

Granny Huang,

Granny Huang,

Teaches me to spin silk;

Teaches me to weave cloth.

O two barrels on loom and two sheets of cloth!

Huang Daopo (1245–unknown) was from Wunijing Town, Songjiang (now Huajing Town, Xuhui District). She was born in a chaotic era of wars between Song Dynasty and Yuan Dynasty. Mongolian army directed at Lin'an, a capital at the time. The country was broken apart and people lived in hardship. Huang lost her parents and relatives and was sold as a child bribe in her teenage. She undertook all the housework, chopping woods, cooking, washing clothes, sewing and mending. She was clever and deft, fond of learning and good at thinking, learned well and completely mastered skills from adults. In spite of her ingenuity and hardworking, her parents in law and husband tortured her much. Sufferings destroyed but cultivated her. After beaten by her husband and parents in law savagely, she was locked in a woodshed, not allowed to eat and sleep. She couldn't tolerate the bad treatment anymore and decided to escape. At the midnight, she managed

to escape and hid on a boat docked by Huangpu River. The boat owner felt sympathetic for her and took her to Yazhou, the south end of Hainan Island, now Ya county of Hainan. At that time, Li sheets, ornaments, and saddles produced by Li people in Ya county were well known and the cotton spinning technique was advanced. Being there, Huang was intelligent and learned the spinning technology from Li people. She combined the advantages of the Li and Han spinning technology and gradually became an outstanding spinning worker. She was popular and well known. Huang has been in Li areas for about 30 years and made good friends with Li people. However, she felt homesick much harder as she got older and older. As the time flies, by the end of 13th century, Southern Song Dynasty had been overturned for many years. To ease conflicts among different peoples, the ruler of Yuan Dynasty launched some measures to resume production and the regions south of the Yangtze River started prosperous again. Huang Daopo heard that and decided to go her old home. One day of 1295, Huang said goodbye to her second old home and left beautiful Yazhou for her home alongside the Yangtze River by boat. Ravaged by wars, Huang's old home had been changed a lot. Her parents in law and husband died long ago and friends then never saw again. Then the only hope was to teach the skills she learned before to fellows and relatives. She devoted herself to teaching and innovating the cotton spinning technique regardless of her physical weakness and loneliness. Local people liked her and respected her, she put herself out to passionately teach local people the excellent cotton making technology of Li people without anything hidden. Meanwhile, Huang combined the advanced experience of the Li's cotton making technology with the local production practice, and endeavored to invent. She completely reformed the cotton spinning tools and technology at that time and produced new rolling, beating, spinning, and weaving tools and improved the cotton spinning industry of her hometown. She also invented a three-spindle pedaling reeling wheel instead of a single-spindle hand reeling wheel in the past. The three-spindle pedaling reeling wheel had great power and can free hands to grip and pull out cotton yarns. It can spin three yarns at a time, so yarn production is fast and high in output. The three-spindle pedaling reeling wheel was the most advanced reeling wheel in the world and was a great technical innovation. On the weaving process, Huang improved the loom. She and local people invented the cotton spinning techniques together, including staggering yarns of different colors, color matching, twining yarns to form patterns, and jacquard weaving with reference to the traditional Chinese techniques and the advantages of "Yazhou quilt" woven by Li people. She successfully woven quilts, mattresses, belts, and handkerchiefs with folding twigs, group phoenixes, chess composition, and characters, and they were as colorful as drawings. "Wujingni quilt" was renowned nationwide. For this, a Yuan poet wrote a poem to praise it.

Huang Daopo is a woman example of diligence, intelligence, kindness, and selflessness. Her name and contributions will be remembered by many people. In 2010, a TV drama, the Weaver, directed by Li Guoli, drew materials from the legends of Huang Daopo.

In Ming and Qing Dynasties, due to the germination and development of capitalism, silk production and trade have changed drastically. Silk were increasingly sold as commercial goods and the foreign trade of silk developed fast. The regions around Suhu south of the Yangtze River had developed to be the most important silk production place, a batch of classic silk production cities and towns emerged, and officially operated weaving organizations also developed well. At this time, Chinese silk industry had developed into its prime time.

Ming was a feudal dynasty of centralization that overturned the ruling of Yuan Dynasty. At the beginning, the ruler took measures to emphasize agriculture and advocate frugality and developed the social economy. The production areas of silkworms and silk were reduced in Ming Dynasty, but the regional intensive production centered at the regions south of the Yangtze River had formed, including Suzhou, Hangzhou, Songjiang, Jiaying, and Huzhou, five important cities of silk. Since the middle of Ming Dynasty, people loved extravagance and luxury, and in the context of commodity economy and specialized work division, the silk industry in the regions south of the Yangtze River boomed up.

The official operated textile industry of Ming Dynasty was large in scale. Except central dyeing and weaving institutes set in Nanjing and Beijing, there were local dyeing and weaving agencies in Suzhou and Hangzhou, regions of silk production, as well as other 20 areas. They provided silk for palace and governments every year. At that time, silk was produced by agencies or subcontracted. Agencies hired people on shift to produce silk, while subcontracting was carried by non-organizational craftsman. These craftsmen had relations of dependence not so strict as Yuan Dynasty. In the 16th century, Portuguese opened up direct trade between Europe and China. Ming Dynasty banned on maritime trade with foreign countries. Foreign trade was tributary trade under the control of emperors. Silk products entered surrounding nations and regions in a manner of imperial reward. In the middle of Ming Dynasty, many raw silk and silk products were sold to Japan or Europe by way of Macao as a result that ban on the maritime trade was released.

In early Qing Dynasty, the silk industry was seriously destructed in wars. Since Emperor Kangxi, silk products gained great development. Regionally, the silk industry of Qing was intensified in areas around Taihu Lake and Pearl River Delta, and regions south of the Yangtze River had been the center of national silk industry in scale and level.

The official operated weaving system of Qing Dynasty abandoned the craftsman register institution. Raw materials were procured and the overall scale was smaller than that of Ming Dynasty. Important institutions such as Jiangning Weaving Bureau, Suzhou Weaving Bureau, and Hangzhou Weaving Bureau, which were known as “Jiangnan Bureaus”, were responsible for supplying various silk products for palaces and officials. The scale of civil silk production was expanded to some extent, and division of labor and regions tended to be more specific. A batch of silk production cities and towns appeared, products were diversified, and the domestic market was prosperous. For foreign trade, emperors laid strict ban on maritime trade in early Qing Dynasty and Emperor Kangxi

loosened the control for one time but refrained it again. Except Guangzhou for commercial intercourse, other ports were closed. Yue Custom was the only management organization for foreign trade in Guangzhou Port. Nonetheless, export of raw silk to Japan and of raw silk and textile products to European countries has had a certain scale.

In late Qing Dynasty, Chinese silk industry went downhill under double blow of exorbitant taxes and dumping of foreign silk.



Figure 40. Ming Emperor paid a visit to Sichuan.

Modern reeling mills only make rumbling and chugging sound. Ten years ago, women working in reeling mills were painstaking in hot workshops with silk flying and making things in a blur. Silk reeling is in fact to draw silk from tens of cocoons. In this process, silk will always be broken and woman workers should reconnect them once they found, so workers should have quick eyes and deft hands. There was a Japanese film, *Oh! The Nomugi Pass*, telling 100 young ladies said farewell to their family, crossed the Nomugi pass, and left for working in a reeling mill in Okaya of Nagano Prefecture. Of course, with improvement of modern silk reeling technology, silk reeling women have been much alleviated. However, I still believed that “of those who are dressed in fine silk, none has ever raised a silkworm”, described in a poem *Silkworm Women of Northern Song* poet Zhang Yu.

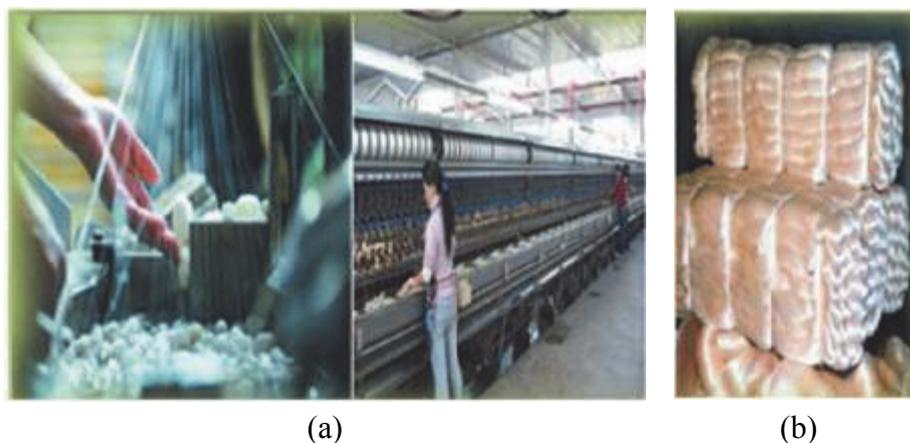


Figure 41. (a) Modern silk reeling; (b) Finished product of silk.

3 Deadly Diseases to Silkworms

Looking around, it is pleasant to see green forestry and water and a beautiful mulberry garden. Silkworm breeding ladies are shuttling back and forth in the mulberry forest, tasting mulberry fruits and picking up leaves. This is a harmonious and picturesque life given to humans by the nature. However, mulberry trees and silkworms are not always safe but being threatened by diseases and pests.

Mulberry trees, as the major food source for domesticated silkworms, are always faced with threats. Silkworms like eating mulberry leaves, but some disease and pests in the nature will never allow them to enjoy the feast alone. Mulberry trees are unlikely green if without management, fertilization, and pesticide application, and instead, they should be devastated with yellow leaves. In particular, mulberry trees are susceptible to diseases caused by microorganisms and attack from wild insects. There is a wide range of diseases and insect attacks and now we describe the major ones.



Figure 42. Green mulberry garden.

3.1 Mulberry Bacterial Diseases

Mulberry bacterial blight, also known as leaf curl, mainly jeopardizes shoots and tender leaves. Stricken leaves will be brown or rotten and curly, buds will wither and shoots will darken. Blight and leaf curl are most commonly seen. Pathogenic bacteria of the blight can attack mulberry trees from five different routes. Routes are taken differently, attack ways may differ as well as harms, and mulberry trees will present different types of symptoms. First, bacteria invade through stoma and cause dotted yellowish-brown spots and mesophyll on the periphery of leaves will turn lighter green.

Second, bacteria invade vascular tissue through the wounded petiole or leaf vein. On leaves are there polygonal spots, which are shaped like oil stain initially and turn yellowish brown later. Some of them will combine each other to make leaves yellow or fall off. Third, bacteria invade through blade tips to turn tender leaves or shoots black or rotten from the original. If in highly humid days, the affected part will flow light yellow bacterial ooze. Fourth, bacteria infect through twig epidermis, the infected part heaves to be long-strip or shuttle-shaped spots, and more on the upper part. Fifth, bacteria invade xylem or medulla from bast and leaves have brown strips. Leaf-curl stricken leaves have brown near-round spots, the rear disease part is perforated, leave rims turn brown, leaves are rotten or peripheral tissues turn lighter green. Leaf veins are infected, and leaves will curl backwards, and will fall off in severe cases. Shoots are infected, fracturing shuttle-shaped black spots will turn up, and buds will wither and turn black. Under the conditions of high temperature and humidity, the infected part will flow out light-yellow liquid, bacterial ooze. After dry, bacterial ooze can be condensed into bright pearls or mycoderm.



Figure 43. Bacterial blight symptoms.

Bacterial wilt is mainly caused by a bacterium, which jeopardizes tomatoes, eggplants, peppers, potatoes, ginger, *etc.* Mulberry trees like other trees have vascular tissues for absorbing nutrition and water in the soil. Bacteria of the wilt will destroy the vascular tissues and trees will wilt. It can be seen from the section of rhizome epidermis of an infected strain that the xylem has brown stripes. In serious cases, brown stripes will extend to the xylem of stem or root to turn it brown or black, and white bacterial ooze can flow out of the cut. The root epidermis infected for long will be rotten and shed, and the xylem will turn black and rotten.

3.2 Fungal Diseases

The fungal diseases include mulberry fruit sclerotiniosis, mulberry botrytis cinerea, mulberry rust, mulberry helicobasidium mompa, and mulberry septobasidium pedicellatum. Of them, the mulberry fruit sclerotiniosis includes ciboria shiraiana, mitrula shiraiana, and ciboria carunculoides. They are caused by fungus. Mulberry fruits used to fresh fruits beloved by people. Each spring, people will pick up mulberry fruits in the mulberry garden. These juicy fruits are also the favorite for fungus. Once fungus infected mulberry fruits, mulberry fruits would lose the original red and purple, plump, and bright states, and would turn big or small or strange in shape and color and black sclerotium were formed. Ciboria shiraiana presents symptoms, for example, infected fruits are expanded, perianth are thick and swelling and white or greyish white. Indeed, they are some ropes, which are stinky. At the center of the infected mulberry fruit, there is a black and hard sclerotium. Mitrula shiraiana is manifested in symptoms that the infected mulberry fruit is obviously small, greyish white, and hard, with dark brown spots on the surface, and inside it there are irregular black sclerotium shaped like rat feces. Symptoms of ciboria carunculoides include that infected small fruits suddenly turn big with small sclerotium inside, and are greyish black and easy to fall, and bacteria live through the winter in the soil in the form of the sclerotium. In the second year, when mulberry flower blossoms up and conditions are appropriate, bacteria will release ascospore, ascospore flows and flies with air and spreads to female flowers, and its destroy activities on mulberry fruits begin. In spring, the weather is warm and rainy and the soil is humid, which are beneficial to germination of sclerotium, many apotheciums are engendered to seriously infect the mulberry trees. Those growing in poor ventilation and light transmittance and humid low land and fructuous and old are easily heavily attacked.



Figure 44. Symptoms of mulberry fruit sclerotiniosis.

Botrytis cinerea is a commonly seen fungal disease that is hard to treat and belongs

to low-temperature and humid type diseases. It mainly jeopardizes grapes, tomatoes, eggplants, cucumbers, and mulberry trees and is caused by *Deuteromycotina* fungus, the high-incidence season is the time when growth temperature for pathogenic bacteria is 20–25 °C and humidity is always 90% and seedlings of mulberry trees are mainly harming objects. The affected nursery stock has light brown until brown or dark brown spots. On the infected part white silk-like ropes grow and extend radially, and ropes clustered together are brown sclerotium shaped like oil seeds. After small sclerotium is formed, white ropes gradually disappear, during which the affected epidermis is ulcerated and easy to strip, silky fibers are exposed, infected leaves turn yellow and withered, and the whole tree will wither to death in serious cases.

Mulberry brown blotch is a foliage disease and belongs to fungus diseases. Its pathogenic bacterium is *Deuteromycotina* fungus. Mulberry brown blotch mostly hits in the stage of tender leaves. In the beginning, the affected leaves have dark water stain in sesame size, which gradually enlarges to be circular or irregular brown spots. On the spots are white or light red or blackish brown stains, in humid environment, leaves are rotten and perforated, and in a dry state, leaves crack up in the middle. Mulberry brown blotch easily occurs in the high-temperature and humid environment. Mulberry rust, also known as golden mulberry or golden leaves, is caused by basidiomycetes and belongs to *Basidiomycotina* fungus. It mainly jeopardizes buds, spires, and shoots. Being infected, buds will distort or bend and will never germinate. Buds, stems, and flowers on the shoots will locally turn thick or bend or distort and have orange spots. After infected, leaves will have small round spots on the front and backsides, the small spots gradually heave to be green bulbs and then turn yellow and orange, epidermis bursts up and orange powdery basidiomycetes spread all over the leaves, so the leaves are called golden leaves. The mulberry flowers infected will turn irregular and swelling. The infected mulberry roots will lose luster and in the late stage, roots will be covered with orange powder.



Figure 45. Symptoms of *botrytis cinerea*.



Figure 46. Symptoms of brown blotch.

The pathogen of mulberry *Helicobasidium mompa* is basidiomycotina fungus. Bacteria attack from the tender root and spread to thicker rootlets and main roots. The infected roots are yellowish brown at the beginning and turn blackish brown. In serious cases, epidermis is ulcerated and turns black, purplish brown silky ropes can be seen on the surface of the infected roots and clustered to be rhizomorphs, and rhizomorphs crisscross each other to be a network, and the roots are covered with Eprinomectin. In the late period, a layer of purplish red hairy mycoderm is formed the basal part of the trunks and the ground. If the mulberry roots are infected, branches and leaves grow slow and thin, leaves turn yellow and those below will fall off early. Branch tips or small twigs will wither to death until the whole tree dies. There are two kinds of ropes. One is vegetative hyphae invading the epidermis, and the other is generative hyphae parasitizing to the root surface.

The pathogen of mulberry *Septobasidium pedicellatum* is basidiomycotina fungus. It will hit in the entire development period and mainly jeopardizes old branches. Circular or irregular thick film, similar to plaster, is usually formed on twigs. There are two commonly seen diseases of *Septobasidium pedicellatum*, grey *Septobasidium pedicellatum* and brown *Septobasidium pedicellatum*. At the beginning of the former, the color is dark brown, and turns rat grey and brownish black, and then cracking occurs. For the latter, the color is chestnut brown with greyish white, and the surface of mycoderm is hairy. The brown *Septobasidium pedicellatum* spends winter on branches and communicates basidiospore in May and June of the coming year. Basidiospore sometimes attaches to scale insects and infects healthy branches or trees. Both easily hit the mulberry gardens where the soil is humid and light and ventilation are in poor conditions.

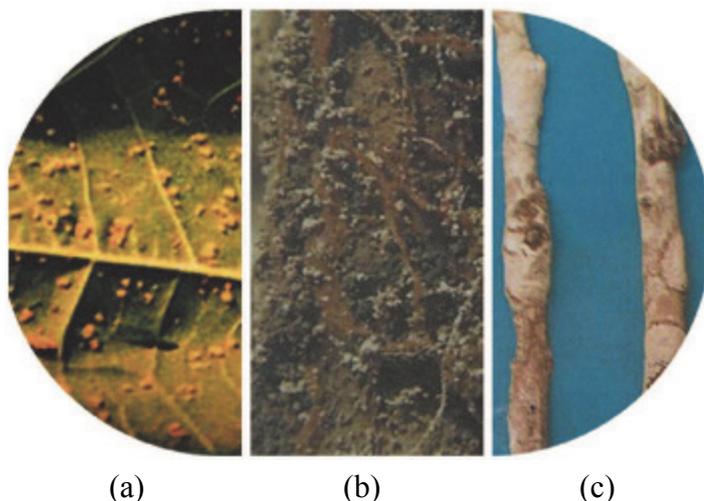


Figure 47. (a) Mulberry rust; (b) Mulberry helicobasidium mompa; (c) Mulberry septobasidium pedicellatum.

3.3 Mulberry Nematode

Mulberry nematode mainly refers to mulberry root-knot nematode. Its pathogen is root-knot nematode. Nematode mainly harms the mulberry trees from the underground, like a Tunnel War. They will greedily suck roots' nutrient substances and secrete saliva after hitting mulberry trees. This kind of saliva contains secreta that can stimulate root cells to expand, and giant cells are generated so that the roots are overgrowing to form burl-shaped things in different sizes, that is, root nodules. Root nodules are not all in one size, some as small as beans, some as large as eggs. In serious cases, root nodules are connected like a necklace and thin roots turn black and rotten. Once mulberry roots are infected by nematode, the number of fibrous roots will drastically decrease, water delivery will be hindered, which slows down the growth of the upper parts of trees as a result.

3.4 Mulberry Virus Diseases

There are two major mulberry virus diseases, mulberry latent virus (MyLV) and mulberry mosaic dwarf disease (MMDD). The pathogen of the MyLV is a kind of closterovirus and belongs to RNA virus. Magnified under an electron microscope, MyLV looks like threads. Mulberry trees infected germinate late in spring, infected leaves curl at the beginning with rims zigzagged until the end of leaves, which forming like a chicken claw. In serious cases, mulberry mesophyll will completely disappear and only veins left. Mulberry trees are infected and veins of some infected leaves are combined together to make infected leaves silky, so that this disease is also known as the silk-leaf disease.

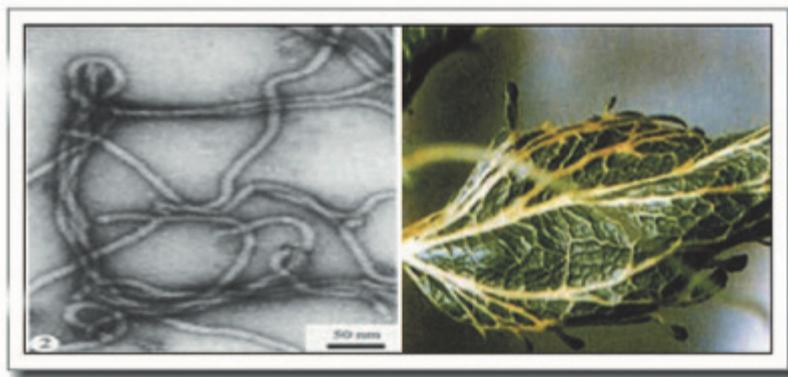


Figure 48. MyLV and its symptoms.

The pathogen of MMDD is a mycoplasma-like organism (MLO), a mycoplasma-like microorganism. The mycoplasma-like microorganism is a unicellular microorganism, smaller than bacterium but larger than virus. It has polymorphism and circular, oval or irregular shapes, is 200–300 nm in size, and has no cell walls. In case trees are slightly infected, leaves at the tip of branches grow small and thin with tiny veins, slightly curl backwards and turn yellow, and ancillary buds germinate early. In case trees are moderately infected, leaves are much smaller and curl backwards to greater extent, and turn yellow and rough, internodes are short in distance, phyllotaxy is in disorder, and trees have many small side branches and cannot bear fruits. In serious cases, leaves are small like a cat ear, ancillary buds germinate all the time, and twigs overgrow like a broom. In general, a single branch is infected and then the whole tree is infected. MMDD is usually communicated via mulberry rhombic-marked leafhoppers and *hishimonus sellatus* uhlers.

3.5 Insect Pests for Mulberry Trees

Mulberry pyralidae, commonly known as leaf roller, are a family of Lepidoptera in the superfamily Pyraloidea and harmful for mulberry trees. Pyralidae larva usually spit silk to roll leaves first when injuring trees, hiding and eating mesophyll in which and finally only veins and epidermis of mulberry leaves are left to form a transparent greyish brown film. The film breaks to form a hole, which is called “window”. Larva feces pollute leaves and impact quality of mulberry leaves, so that the leaves turn yellow and have adverse effect on silkworm breeding or causing diseases in Autumn. Eggs of pyralidae are small and 0.7 mm long and are oblate, light green and with wax on the surface. Larvae of last instar are 24 mm or so long, reddish brown in the head, light green in thorax and urosome, and dark green in the back, each thoracic segment has black hairy scales, and each scale has 1–2 rigid hair. Pupa is 11 mm long, shaped like a spindle, and yellowish brown. Longitudinal ridges heave at the center of the thorax and back, and 8 thin and long

barbs grow at the end. An imago is 10 mm long and about 20 mm if wings stretch, is dark brown, and has white palea in silk-like brightness. It has a small head with white hair on two sides, a large and round black compound eye. Tentacles are greyish white and whip-shaped. The middle of the thorax and back is in dark color. Fore and hind wings are white mixed with purple and reflective, the fore wings have 5 light dark brown strips, a white round hole is formed below the middle, a brown spot is found in the hole, and the hind wings have broad dark-brown strips along the outer rim. Enemies of pyralidae are trichogramma evanescens westwood, trichogramma dendrolimi, and cedria paradoxa. Pyralidae have greatly damage although they are small. They are not neat freaks, eating and excreting, messing around mulberry leaves and affecting the breeding quality.



Figure 49. Pathogenic symptoms of *Diaphania pyloalis* (walker).

Mulberry geometer moths are a family of Lepidoptera in the superfamily of Geometridae and also known as Noctuidea. They mainly injure *Elaeagnus angustifolia*, poplar, willow, elm, pagoda, apple, pear trees, and *salix mongolica*. Geometer moth larvae jeopardize sprouts, spires, and flower buds. In serious cases, they eat up all leaves of their host trees. The emergence period of mulberry geometer moths is early, larvae develop fast and eat much and disasters are usually caused due to their good appetite. In general cases, their host trees may be affected. What're worse, branches are withered and tree vigor fades, injuring insects are rampant to cause death of host trees in a large area. Larvae eat mulberry sprouts in early spring and only bracts are left. In spring, larvae eat into sprouts and then leaves to make them incised. Seriously, veins are left to make trees weak, and leaves are reduced. Mulberry geometer moths are one of major pests for mulberry trees in spring. Eggs are oblate and 0.8 mm long. Larvae are cylindrical and the front end is thin but the rear end is thick. Small black spots are distributed with the back, and two pairs of prolegs grow on the 6th and 10th urites. Pupae are cylindrical, 19 mm long, and purplish brown, and has rough irregular patterns. Cremaster is triangular,

cocoons are light brown, and cocoon shells are thin. Larvae are 16–20 mm long and greyish brown, and wing surfaces are distributed with black short patterns and have black wavy and inclined cross lines. Mulberry geometer moths are good disguisers, and usually disguise themselves by mimesis or color the same as branches to avoid attacks from other insects and birds.



Figure 50. Larva and imago of mulberry geometer moths.

Gypsy moths are family of Lepidoptera in the superfamily of Lymantriidae and also known as *lymantria dispar linnaeus*. They can be found in various silkworm areas. There are more than 500 varieties of host trees including mulberry, apple, pear, peach, apricot, walnut, persimmon, chestnut, elm, and poplar trees. They jeopardize trees in different ways. Larvae of the second instar are distributed, they hide in barks, branches, weeds, and stone in the daylight, and attack in the evening. Larvae nibble up leaves and all leaves on the trees can be eaten up in serious cases. Eggs are round and 0.9–1.3 mm in diameter, and are yellowish brown at the beginning and then gradually turn greyish brown. Larvae are 50–70 mm long and their head are yellowish brown. Somite has 6 nodules arranged crossly, a pair of nodules at the center of the back is bright in color, the first to fifth segments are bluish grey and the sixth to eleventh segments are purplish red, on which there are brownish black short hair. Nodules on two sides of each segment are covered with a bundle of long yellowish white and black hair, and nodules on two sides of the front thorax grow large and are covered with long black hair. The center of the back of the sixth and seventh urites has a red cylindrical poison gland (eversible gland). Young pupae are reddish brown and turn blackish brown. At the nodules of larvae, there is yellow short hair. Male and female imagoes are different in appearance. The male one is smaller than the female one and is dark brown. The head is yellowish brown and tentacles are feathery and brown, the back is greyish white, the outer rim of the fore wings are ribbon-shaped, and the other part is greyish white. There are 4–5 dark brown wavy cross lines on the wing surfaces. Male moths are flying about in the daylight like

dancing, so we call them “dancing Gypsy”. Female moths are lazy and usually reposing on branches and twigs or laying eggs in the grass, and eggs are clustered and covered with a layer of hair at the end of the urosome of female moths, which are visible. The egg cluster can withstand low temperature and overwhelming in water for a long time and has powerful stress resistance. Imagoes are phototatic.

Spilosoma urticae are a family of Lepidoptera of the genus of *spilosoma* in the superfamily of Arctiidae and mainly spread in Heilongjiang, Hebei, Liaoning, Shandong, Jiangsu, and Zhejiang provinces of China. In recent years, Shandong, Hebei, and Liaoning were seriously hit. They damage mulberry trees, corns, cotton, wheat, grains, peanuts, beans, melons, and mint *etc.* Larvae eat leaves to make them incised or holed, and in serious cases, only veins are left. Female imagoes are white, the part over labipalp is black, and the part below is white. Tentacles are ended with black. Thoracic feet have black areas and the part over femur is yellow. Eggs are light yellow, and larvae are yellowish brown and turn dark brown after the 4th instar. Larvae of the final instar are 21.5–25.8 mm long and covered with long hair, rigid hair is dark grey, and each segment of the thorax back has 8 nodules. Pupae are oval and blackish brown, the parts between segments are yellow and the surface is rough and distributed with small points, and a thin layer of cocoon is formed during pupating.



Figure 51. Larvae and imagoes of Gypsy moths.



Figure 52. Larvae and imagoes of *Spilosoma urticae*.

Mulberry caterpillars are a family of Lepidoptera in the superfamily of Noctuidae, also known as *polia illoba* butler. They spread in Heilongjiang, Inner Mongolia, Hebei, Gansu, Jiangsu, and Jiangxi and are harmful for mulberry, eggplants, spinach beet, carrots, beet, strawberry, medlar, chrysanthemum, crowndaisy chrysanthemum, kidney beans, Chinese artichoke, peas, Medicago, soybeans, red beans, green beans, and blackberry. Larvae eat leaves to make them incised or holed. In serious cases, leaves can be eaten up. They are also harmful for sprouts, flower buds, and berries. Imagoes are brown or reddish brown, urosome is brown, and the urosome end is covered with long brown hair. Eggs are hemispherical, and are light green when first laid and turn purplish brown. Newly hatched larvae are light greyish brown, with urosome purplish red and are covered with large and black scales, legs look like geometer moths, larvae are green or bluish green in the 3rd instar and show reddish brown after the 4th instar and turn reddish brown overall in the 6th instar. Pupae are dark brown, maxillary palpus can stretch to the rear rim of the 4th urite. Pupae have rough body surface, cremaster is short and thick, and the tail end is forked.



Figure 53. Larvae and imagoes of mulberry caterpillars.

Prodenia litura are a family of Lepidoptera in the superfamily of Noctuidae. They are parasitic to many plants, including 290 varieties of plants in 29 families such as mulberry, cotton, corns, sweet potatoes, Chinese yam, and lotus roots. Larvae eat tender leaves of their hosts, and some of them bite branch barks and leave little veins, twigs, and branches. Imagoes are dark brown, the thorax back has white hair, the fore wings are greyish brown and have many patterns. They have white transverse wavy lines on the inner and outer sides, visible white inclined ribbon-like patterns, so also called inclined-pattern moths in China. Eggs are oblate and are yellowish white when laid initially and turn dark grey, and are clustered and covered with yellowish brown hair. The head of a larva is blackish brown, and the thorax is colorful, from earth yellow to blackish green. The body surface is distributed with white small spots. Each segment has a pair of subtriangular semilune black spots. Pupae are cylindrical and reddish brown, and the end has a pair of short barbs.



Figure 54. Larvae and imagoes of *Prodenia litura*.

Acronicta major belongs to an order of Lepidoptera in the family of Noctuidae. Their hosts include mulberry trees, may trees, peach, plum, apricot, orange trees, and toona sinensis. Larvae damage lower epidermis and mesophyll of leaves, and only upper epidermis are left. Imagoes are chewing leaves with veins left. In serious case, all leaves can be eaten up. Imagoes are dark grey, with greyish white on the urosome. Eggs are oblate and light yellow or yellowish brown. Larvae are black and covered with yellow long and short hair and thick black needle-shaped barbs. Black short barbs grow on back nodules in cluster and look like a back line. Two sides and body side are yellow, and nodules on the body side are protruding prominently. The head, prothoracic scutum, and thoracic legs are black and bright, and there are 45–52 uniordinal crochets. Larvae have 6 instars in total and are different in color and patterns for each instar. Pupae are elliptical and brown or blackish brown. Cocoons are long and oval and silk is thick and compact and is greyish white and earth yellow.



Figure 55. *Acronicta major*.

Setora postornata, *Parasa consocia* walker, *Parasa consocia*, *Thosea sinensis* walker, and *Cnidocampa flavescens* walker are commonly seen on mulberry trees. Larvae of instars mainly jeopardize leaf back, eating mesophyll to generate transparent spots or lesion on leaves. Imagoes can eat mulberry leaves to make a large incision. In serious cases, all leaves are eaten up except stems. Larvae have poisonous hair and can make

skin rash and itching in case of touching. *Setora postornata* are polyphagous. Except mulberry trees, they are also harmful to tea, pear trees and elms. The fore wings and body of imagoes are light grey and reddish brown, the fore wings have misty black spots, and 1/3 of the basal part is reddish brown. The outer rim is straight, the central line and the submarginal rim are obscure bands, and two sides and outer rim of the central band are light bluish grey. The rear wings are greyish brown and subtriangular and have long hair. Eggs are oval and flat and are light yellowish white and semitransparent. Larvae of the final instar are long and oval, with bulging on the back and are yellowish green and green. Each segment has 2 pairs of branch-shaped barbs, growing above the back subline and supraspiracular line. The front back has a green or reddish-purple angular protrusion, which is significantly inclined forwards. The back line is greenish blue. Pupae are 15 mm long, light yellow and oval. Cocoons are oval and brown.

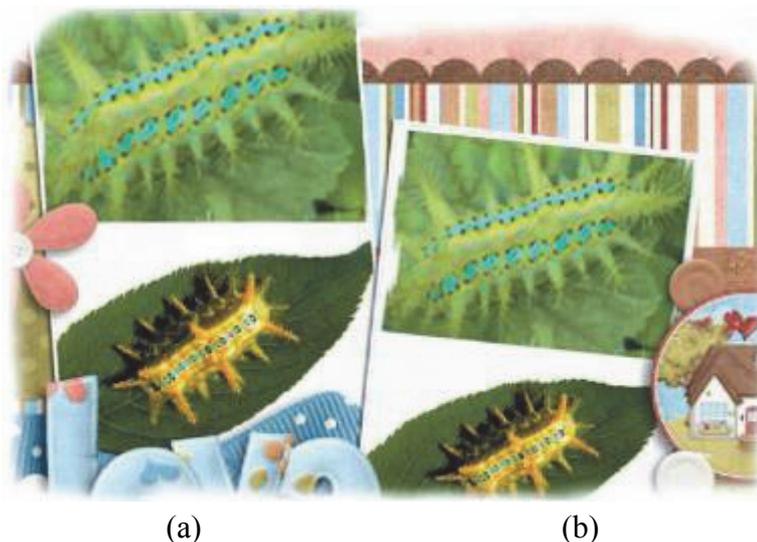


Figure 56. (a) *Parasa consocia* walker; (b) *Cnidocampa flavescens* walker.

Hyphantria cunea are a family of Lepidoptera in the superfamily of Arctiidae, also called fall webworms. There are more than 300 host plants, including mulberry, strawberry, persimmon, apple, peach, plum, *malus spectabilis*, may trees, pear, cherry, and grape trees. Larvae eat leaves and shoots, low-instar larvae bite mesophyll to make residual epidermis white film shape, and days later the epidermis is withered. A little older larva eats leaves to generate incision and holes. In serious case, all leaves can be eaten up, imagoes are white, and the compound eye is blackish brown. Larvae have black heads or red heads. In China, most larvae have black heads.



Figure 57. Larvae and imagoes of *Hyphantria cunea*.

Rondotia menciiana moore belongs to the family of Lepidoptera in the super family of Bombycidae, also called mulberry silkworm. They spread over Jiangsu, Zhejiang, Anhui, Shandong, Henan, Hebei, Shanxi, Shaanxi, Gansu, Hunan, Hubei, Jiangxi, Sichuan, Guangdong, and three provinces in Northeast China. Their hosts include mulberry, medlar, and paper mulberry trees. Larvae eat mesophyll on the back of leaves to form holes in different size. In serious case, only veins are left. Wings of imagoes are yellow. Eggs are oval and white and turn pink before hatching. Of larvae in the final instar, the head is brown, the thorax is milky white, and each segment has wrinkles. Between wrinkles there are black spots. And when larvae grow up, the black spots will disappear. Pupae are milky white and cylindrical, the compound eye and spiracle are brown. Cocoons are yellow and elliptical, and silk layers are loose.

Porthesia xanthocampa mainly refers to golden caterpillars and *porthesia similis*. Golden caterpillar is an ecological subspecies and looks nearly the same as *porthesia similis*, also called *euproctis similis fueezssly*. They are mainly parasitic on mulberry trees, apples, pears, peaches, may trees, apricots, plums, persimmons, chestnuts, *malus spectabilis*, cherries, and willow trees. Larvae newly hatched gather on the leaf back, eating mesophyll, so that transparent spots turn up on the leaves. Larvae after 3 instars will cause damage separately. They can incise leaves, and even only veins are left. For sprouts of mulberry trees, larvae eat leaves from the outside to inside, which makes leaves withered and fallen and affects breeding of spring silkworms. If contacting poisonous hair, silkworms are poisoned and black spots can be induced. In case people contact poisonous hair, dermatitis can be caused and some may lead to inflammation of lymph. Imagoes are white, eggs are greyish white and oblate, and the egg mass are elongated and covered with yellow hair. The head of larva is blackish brown, and the body is yellow. Larvae of *porthesia similis* are mostly black. Pupae are cylindrical and yellowish brown and are covered with yellowish brown hair. The urosome back has 1–3

segments, each of which has 4 nodules. Cocoons are oval and light brown and covered with a little black hair. Golden caterpillar and porthesia similis are differentiated by the larva color and the color of forewing spots of imagoes.

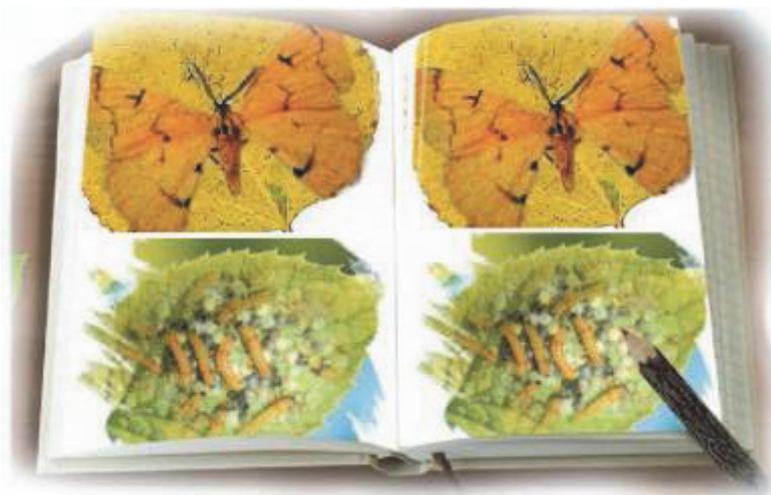


Figure 58. Larvae and imagoes of *Rondotia menciana moore*.

Cicadella viridis are a family of Homoptera in the superfamily of Cicadellidae. They can be found throughout China. There are 160 host plants in 39 families, including mulberry, tea, paddy rice, grains, sorghum, corns, wheat, vegetables, and fruits. If imagoes and nymphs suck juice on the leaf back, small angular spots turn up on the leaf surface, leaf texture is deteriorated and hardened earlier. Male imagoes are slightly smaller than female ones and are bluish green. The head is orange and has a small black spot on each side, and there are 2 compound eyes, red. There are 2 polygonal black spots between the compound eyes. Eggs are elongated and round and slightly curved with one end tipped, milky white and yellowish white. Nymphs are similar to imagoes and there are 5 instars in total.

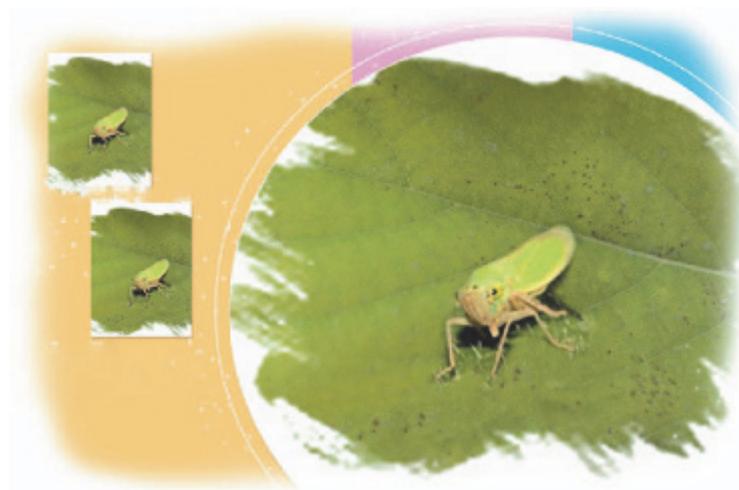


Figure 59. *Cicadella viridis*.

Mulberry whitefly is a family of Homoptera in the superfamily of aleyrodid, also known as louse, mulberry scale, and *Parabemisia myricae*. They spread over mulberry planting areas nationwide and are parasitic on mulberry, tea, red bayberry, plum, apricot, persimmon, and orange trees. When damaging hosts, nymphs suck leaf juice and cause withering of leaves and shoots. Honeydew excretes pollutes leaves and affects quality and yield. Imagoes are yellow and covered with white powder. Their heads are round and small, and the compound eye is blackish brown and kidney-shaped. Tentacle is whip-shaped and has 7 segments. Wings are milky white. Eggs are conical and milky white to light yellow. Larvae are oblate and light yellow and are covered with wax, their body has rigid hair, and the scalpellum end is blackish brown. Pupae are oblate, the compound eye is red, the back is milky white and the back center heaves up a little.



Figure 60. Mulberry whitefly.

Crisicoccus moricola tang is a family of Homoptera in the superfamily of Pseudococcidae. Mulberry leaves damaged by *crisicoccus moricola* tang are curly and wrinkled, and fruits are shriveled and fall off. In serious case, the vigor of trees weakens and the trees are susceptible to pests. Female larvae are oval, the back and the urosome are distributed with cryptae, and segmental venter has rigid rods. Eggs are long and elliptical, are light yellow when laid initially and turn light brown when hatched. Nymphs are long and elliptical, light brown, and sexually active, and covered with a little white powder. Tentacle has 8 segments. Nymph of 2 instars is reddish brown, begins taking in food and is covered with much white powder. After taking food, they start crawling.

Drosicha contrahens walker is a family of Homoptera in the superfamily of Monophlebidae, also known as mulberry bug. They spread over Liaoning, Jilin, Hebei, Henan, Shandong, Zhejiang, Jiangsu, Fujian, Guangdong, and Taiwan of China and are parasitic on mulberry, peach, grape, apple, broad bean, and white poplar trees. In spring, nymphs gather on annual mulberry twigs or near the basal part of white buds, and pierce

Break the Cocoon

barks to suck juice to make leaves yellow and small or wither. Sometimes buds will wither and turn brown and growth of spring buds will be affected. In addition, female walkers jeopardize leaves. Imago is oval and stingless and has small and short legs, and wrinkles and heaves on the back, it is reddish brown and has slightly high and yellow edge, and tentacles, mouthparts, and legs are all black, and the body is covered with white wax powder. Male imago is purplish red, and wings are black and have wavy patterns. Eggs are oval and are white when first laid and turn yellow later. Pupa are reddish brown on the back and yellow around, the mouthpart disappears, and cocoons are white and oblong.



Figure 61. *Crisicoccus moricola* tang.

Mimastra cyanura Hope is a family of Coleoptera in the superfamily of chrysomelidae, also known as almond chrysomelid. They are mostly found in Jiangsu, Zhejiang, Fujian, Jiangxi, Guangdong, Sichuan, Guizhou, and Hunan. Mulberry trees, ramie, hemp, elm trees, and Chinese parasol trees are their major hosts. Imagoes eat leaves, especially tender ones just stretching. In common cases, leaves are incised or holed. Seriously, tender leaves on twigs in the whole mulberry garden will be eaten up, and only veins are left. Feces pollute leaves below. In serious case, yield and quality of spring mulberry leaves will be affected. Larvae live on moss, vegetable leaves, and weeds. An imago is 8–12 mm long and yellow or bluish black at the end, oblong and their head is yellow. Eggs are 0.7–1 mm, spherical or elliptical and yellow. Larva of the last instar is 10 mm long, cylindrical and earth yellow, and the tail end is fat and curved. The head is black, the thorax and urosome are earth yellow, and podical plates of the prothorax and urosome are black and bright. Each segment of the thorax and urosome has 7-8 pairs of dark brown wart-shaped heaves and 3 pairs of black thoracic feet. An exarate pupa is 8 mm long and bright yellow. A wing bug is as long as 4 urites. The back is blackish brown and has many wart-shaped heaves and bristles.

Baris deplanata Roeloffs is a family of Coleoptera in the superfamily of

Curculionidae, also known as rhinoceros beetle. They are mostly found in eastern, southern, central, and southwest China and Taiwan. A larva can drill a hole like a mouse and bore into barks, eating the formation layer of mulberry trees and damaging the affected part. An imago eats winter buds and germinated buds or tender leaves in spring and affects germination rate. After mulberry trees are cut down in summer, imagoes eat normal buds and shoots below the section. In serious case, buds can be eaten up and new shoots cannot sprout. Imagoes lay eggs in holes of the basal part of twigs and resultantly twigs wither or break up. They are oblong and black and bright. Their heads are small with fistula curving down. The tentacle is in the shape of elbow and has 12 segments. Elytra are black and have 10 longitudinal spots. The hind wings are greyish yellow and membrane is semi-transparent. Eggs are oblong and milky white and turn greyish yellow before hatching. A larva of the last instar is cylindrical, apodous, soft and fat, and is milky white initially and turns light yellow after mature, and the head is brown. Pupae are spindle-shaped.



Figure 62. *Mimastra cyanura* Hope.

Xylinophorus mongolicus Faust is a family of Coleoptera in the superfamily of Curculionidae. They are mostly found in Northeast China, Inner Mongolia, and Northwest China and jeopardize mulberry trees, cotton, flax, corn, grains, beet, apple trees, betel nut palms, peaches, cherry trees, jujubes, chestnuts, walnuts, poplar, beans, and melons. Imagoes live on tender leaves, shoots, and growth points of mulberry trees. If gathering, they eat up tender buds, and shoots before getting out of soil will be always eaten up or bald. Seedlings seldom grow up and usually break up. In serious case, no

seedlings can grow on the whole land. *Xylinophorus mongolicus* Faust can heavily damage grafted seedlings that survive or tender buds of treelets about to coming out. Eggs are oblong and are milky white when first laid and turn dark black. Larvae are milky white and have no legs. Pupae are cream yellow. Imagoes are grey and covered with blackish brown scales, fistula is short and flat and thin in the middle, and tentacles are reddish brown.



Figure 63. *Xylinophorus mongolicus* Faust.

Apriona germari is a family of Coleoptera in the superfamily of cerambycidae, also known as black longicorn. They are harmful for mulberry trees, apple and pear trees, cherry-apple trees, plums, cherry, oranges, fig, and loquats. Imagoes live on tender bark and leaves, larvae drill holes and pierce the bark and xylem, eating downwards. In holes they made there are no feces, and a great many feces can be discharged outwards at certain distance. You can examine trunks of infected mulberry trees in the garden and find holes and feces on the trunks. Imagoes are blackish brown and black and covered with bluish brown or brownish yellow hair. Eggs are oblong and slightly flat but curved and are milky white at the beginning and turn light brown. Larvae are cylindrical and milky white. The head is yellowish brown and mostly retracts in the prothorax. Pupae are spindle-shaped and light yellow at the beginning and turn yellowish brown, wing buds are as long as three segments, and the tail end has verticillate bristles. As an ox does, an imago has two horns and so is also called longicorn.

Mulberry gall-midge is a family of Diptera in the superfamily of Cecidomyiidae and mostly found in Guangdong province of China. Larvae are parasitic on mulberry shoots and tender leaves. With a mouthpart, it pierces terminal buds and suck juice out, such that the terminal buds change color, bend, wither, turn black, and fall off, commonly known as “baldness”. A female imago has a tentacle of 14 segments and a large urosome and a long ovipositor. A male imago has a tentacle of 26 segments and is thin. Eggs are oblong and banana-shaped and are colorless and light yellow. Larvae are maggot-shaped and transparent and milky white. Pupae are long circular.



Figure 64. Larva and imago of mulberry gall-midge.

Eotetranychus suginamensis Yokoyama is also known as red spider, red dragon and red sand. 4 major mites jeopardizing mulberry trees include *Eotetranychus suginamensis* Yokoyama, *Tetranychus cinnabarinus*, *Panonychus citri* McGregor, and *Tetranychus kanzawai*. They usually hit on the same mulberry tree. Imagoes and nymphs of *Eotetranychus suginamensis* Yokoyama suck juice in leaf back, the damaged leaf back shows silkscreen, peeling-off husk, and semitransparent white spots and then withers. In serious case, the leaf back seems being burned and leaves are falling off. A female yokoyama is elliptical and light yellowish white. A male yokoyama is spindle-shaped, and two sides on the back have dark green spots. Eggs are spherical, colorless at the beginning and turn light yellow. A larva is light yellow and lemon and elliptical. The back line raises and is dark red. The back hair is white and grows on the nodules.



Figure 65. *Tetranychus cinnabarinus*.

Pseudodendrothrips mori is a family of Thysanoptera in the superfamily of Thripidae, also known as mulberry thrips or scorpionfly. They mainly spread in Liaoning, Henan, Hebei, Shandong, Shanxi, Anhui, Jiangsu, Zhejiang, Sichuan, Guangdong, and

Taiwan. Imagoes and nymphs suck juice from leaves, stems, and twigs with their mouthparts. Affected leaves have small white transparent pits and turn brown, hardened or distorted. In serious cases, leaves curve and fall off. Seriously hit mulberry gardens cannot supply sufficient leaves to spring and autumn silkworms, cocoons cannot harvest and “small bugs lead to great disaster”. An imago is spindle-shaped and light yellow. The fore part of the head and tentacles are greyish yellow and light yellow. The head is wide, and two compound eyes protrude from two sides of the head and are dark red. 2 pairs of wings are long but narrow and transparent and have fringe setae on the periphery. The prothorax is shorter than the head, and the basal part of the prothorax is rough but heaved and has one vein. Eggs are kidney-shaped, colorless, and transparent. Newly hatched nymphs are colorless or nearly colorless, nymphs of the second instar are light green, those of the third instar are yellowish green, and those of the fourth instar are orange. Nymphs have no wings and similar to imagoes.

Mulberry apolygus lucorum is a family of Hemiptera in the superfamily of Miridae, also known as cotton plant bug or small bug. They are parasites on trees of mulberry, tea, cotton, and flax and one of the important destructive insects in companion planting areas of mulberry and cotton or mulberry and flax. Nymphs of the first instar suck juice from buds immediately after breaking out and pierce blooming buds to suck juice as buds grow up. In this case, blackish brown spots or holes will appear, and seriously, leaves will have many holes, which distorts or break up the leaves and affects photosynthesis and growth of leaves. Imagoes of the first generation lay eggs on their hosts in the mulberry garden, nymphs are hatched from eggs of the second generation and jeopardize mulberry trees, and the later generations will keep damaging cotton and flax. Eggs are preferably laid on tender and green leaves and mainly on tender stems, petiole or leaf edges. Newly hatched nymphs also prefer tender leaves. Hibernating eggs are preferably laid on dry and withered trees, mostly on medulla of the section of dead branches. Imagoes are yellowish green or green and flat. The compound eye is reddish brown, and tentacles are light brown. The prothorax is light yellowish green and the tergum has many tiny black incisions. The forewing is green and the membrane is light brown. Legs are orange, femur is thick and each segment has small barbs and bristles. Eggs are long pocket shaped, yellowish green and have creamy operculum and recess in the middle. Newly hatched nymphs are short and thick, look like imagoes, and are green, there are many black bristles on their body surface, and a wing bud is as long as four segments of the urosome. Their major enemies are orius minutus and propylaea japonica.

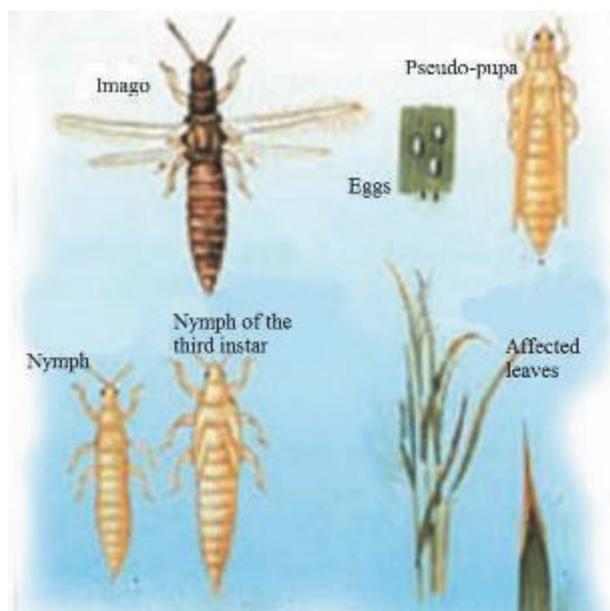


Figure 66. Pseudodendrothrips mori.

3.6 Enemies of Silkworms

Mulberry trees grow in the field and are exposed to many insects and pests. If diseases and pests leave uncontrolled, breeding of silkworms will be inevitably affected.

As for domesticated silkworms, though bred indoors, they are still susceptible to many enemies, and it could be calamitous. To be specifically, silkworms are always exposed to viral diseases, fungal infectious diseases, pebrine diseases, bacterial diseases, pesticide poisoning, and others.

I. Viral Diseases

Domesticated silkworm is an economic insect and enjoys great popularity in the insect kingdom. Breeders strictly control the room temperature and humidity and pick up mulberry leaves as the physiological requirements of silkworms. When you enter a silkworm breeding room, you may hear rustling sound, which is made by healthy silkworms of five instars. At this time, hardworking breeders might feel joyous. However, things not always go well. There are so many enemies for silkworms in their lifetime and silkworms are vulnerable. Among silkworm diseases, a rhabdovirus, people usually called NPV, is one of the major enemies for silkworms. In 1912, Germany scientists first proved that NPV is pathogenic virus of grasserie. In 1947, Bergold succeeded in separating and purifying silkworm rhabdovirus and started the rhabdovirus research. Rhabdovirus comes from invertebrates, and there are more than 600 types found so far. However, only 20 types have been studied in terms of molecular biology. Genome of rhabdovirus is a single closed circular double-chain DNA of 80–160 kb. Rhabdovirus of different species changes a lot and has about 150 genes. The genome replicates and

duplicates in cell nucleus, so this rhabdovirus is named as NPV. Virion can be wrapped by polyhedron in the cell to form a 1–5 μ m long inclusion body that is polygonal. There are two types of virus, inclusion body and extracellular budded virus. They play different roles in infection. Inclusion body transmits virus between insects, which is called horizontal infection. The extracellular budded virus transmits between individual cells. The inclusion body virus goes and comes inside silkworms and damages their health.

NPV intrudes silkworms from their mouthparts or injury. The mouthpart is the major infection way. Infected silkworms are sleepless, puffy, with segment heaving, and act manically and die with body bursting up and pus discharging. These symptoms are mainly caused by virus multiplying *in vivo* and damaging tissues. Silkworms cannot speak and tell breeders “they are infected and suffered”. Instead, they can only keep crawling to send signals for breeders. In terms of biology, virus is not a complete life body and but its existence has to rely on living body. When virus attacks silkworm, it finds its host and struggles to use the host to be outpouring nutrition source. If virus multiplies to some extent, silkworms cannot tolerate and will burst up and new virus generations will infect other silkworms. From this, it can be seen the principle of suitability and restriction of living beings in the nature. In general, it takes 3–5 days from infection to morbidity. Once hit, silkworms are not possibly treated. NPV for silkworms is equivalent to cancer for humans and has great impact on sericulture and it should be prevented.

The second is *Bombyx mori* cytoplasmic polyhedrosis (BmCPV). Its pathogen is RNA virus. Under a microscope, the virus is tetragonal or polygonal. It specifically infects silkworm midgut (MG) and stays in cytoplasm, where it gets its name and is also known as midgut grasserie. For groups, symptoms and pathological changes include variable size, slow development and irregularity, poor appetite, and serious pollution in silkworm rearing beds by a great many residual leaves. For individuals, the first symptom is the transparent upper body. Inclusions in the thorax are liquefied and transparent, and with the deterioration of disease, the whole body is semitransparent and they crawl to the edge of the rearing beds, procluminate there. The second is curling up. As the disease came on, molted silkworms turn small and weak. The third is vomiting and diarrhea. Before dying, silkworms vomit and at the beginning of morbidity, grown silkworms defecate soft and shapeless feces. In case of deterioration, feces turn green and brown and become milky white or white liquid in late stage. Through dissection, it can be seen with naked eyes that infected silkworm’s midgut is white. This is actually the result of multiplication of virus. BmCPV is infectious, which worries breeders.

Bombyx mori densovirus. Its pathogen is DNA virus. The virus is unique for it has two genomes. So far, the researchers from the author’s lab have completed analysis of the genomes and changed its classification of virus. It belongs to densovirus, so we gave it a new name BDV. Virus specifically infects a certain type of cells in the midgut. The morbidity course is long but slow, groups of silkworms are

uneven in development, and rearing beds are seriously polluted. Infected silkworms are yellow, molted silkworms curl up, turn transparent and purge. In serious case, the whole body of infected silkworms turns fully transparent, and a trachea cluster can be seen.



Figure 67. NPV and virus under microscope.



Figure 68. BmCPV and transparent body, slow development, milky white feces, and white rear midgut.



Figure 69. BDV thorax transparent silkworm.

II. Fungal Infectious Diseases

The pathogen of *Bombyx mori* damping-off disease is *Bacillus thuringiensis* damping-off varietas of Eubacteriales in the genus of bacillus. It was first separated from a dead *Ephestia kuehniella* in a flourmill in Thuringia of German and is pathogenic for insects. It was named officially in 1915. Damping-off disease is the result of silkworms eating bacillus generated by the pathogenic bacteria. If bacillus hits silkworms through wounds, it may lead to septicaemia. Insects in mulberry gardens such as *Porthesia xanthocampa* Dyer and *Diaphania pyloalis* walker are vulnerable. Infectious pests, dead silkworms, feces, and bacterial insecticide for agriculture and forest are all sources of infection of damping-off disease. When damping-off disease hits, infectious silkworms stop eating mulberry leaves, with head and thorax heaving and tail legs curling inwards, spasmodic and paralytic, and finally die as lying on side. When silkworms just died, their heads rolled up like a beak, there were hard clots at the rear end of the urosome, and they are slightly cadaveric rigidity. 1–2 days after their death, large green cadaveric ecchymoses appeared on 1–3 segments of the urosome and then turn blackish brown and expand, the tissues of the whole body started separating and black liquid flowed out. The pathogen is bad for silkworms. However, things usually have two sides, harm and benefit are relative terms. Scientists found *Bacillus thuringiensis* (Bt for short) is a group of crystal-producing bacillus containing many variants. It can produce two types of toxin, endotoxin (parasporal crystal) and exotoxin. They can stop pests from eating and let them die of starvation. Therefore, Bt can be used for manufacturing low-toxin insecticide to cope with pests of Orthoptera, Coleoptera, Diptera, and Hymenoptera, especially Lepidoptera. Researchers from Monsanto conducted field test in consecutive two years and found good effect of pest control. As pests eat parasporal crystal and spore, parasporal crystal will dissolve in the intestinal alkaline environment and release toxin that is toxic for Lepidoptera larvae. This toxin can paralyze midgut of larvae and deactivate sodium and K pump and pests are poisoned, lose appetite gradually and nonreactive to contact. They are aposeptic, vomit, diarrhetic, slow, curling-up or rolled-up. In general, they will not damage crops and die of midgut breakage and septicaemia caused by toxin entering blood after the disease comes on. At the same time, parasporal crystal or spore swollen reproduce fast in the digestive tract and speed up death of pests. Dead larvae are soft and black. It can be seen from its action mechanism that pests will only eat Bt and die after a morbidity process. In other words, it takes 48 hours to kill them, not as fast effective as chemical pesticide. However, pests affected will vomit and be purged, cannot eat and move and damage crops any longer. Beta-exotoxin is RNA polymerase competitive inhibitor. When pests eat bacillus, the beta-exotoxin inside bacillus can inhibit RNA polymerase to prevent hormone from synthesizing for growth of larvae. As a result, larvae cannot be pupated normally or will be developmental malformation.

Muscardine refers to a disease caused by parasitic fungus that muscardine-stricken silkworms become rigid after death. Muscardine includes white muscardine, green

muscardine, yellow muscardine, and aspergillosis, in addition to grey muscardine, black muscardine and grass muscardine. They are all fungal diseases. Silkworms hit by muscardine will show different symptoms. 1–2 hours later death, bodies are rigid. 1–2 days later, aerial hyphae in different colors grow at the coria, mouthpart, and spiracle and distribute the whole body at last. Generally, muscardine is named after the color of the aerial hyphae.

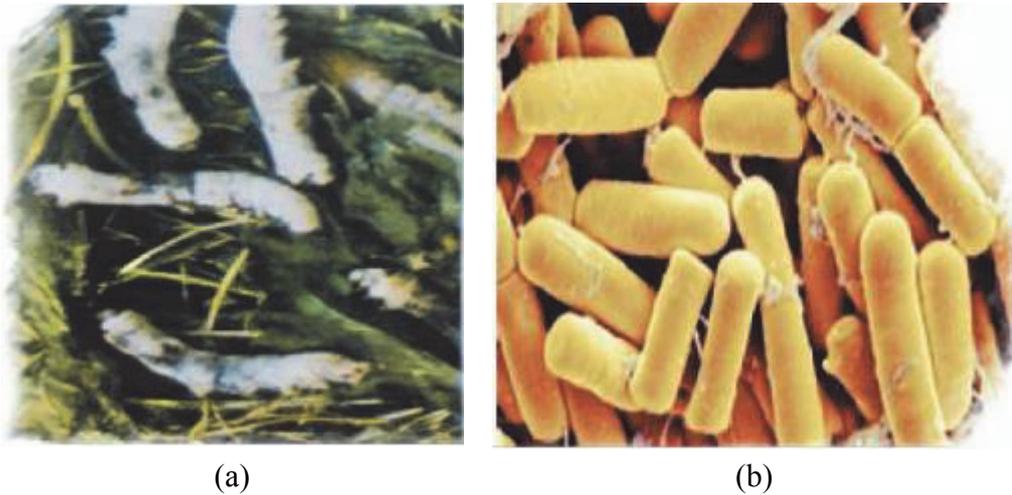


Figure 70. (a) Dead damping-off silkworms; (b) Zoom-in photo of *Bacillus Thuringiensis*.



Figure 71. Muscardine.

III. Pebrine

The first prevalence of pebrine was in 1845. It first hit France and later spread to

Italy, Spain, Syria, and Roman. Pebrine once almost destroyed sericulture in France and Italy. Up to now, none of sericulture countries in the world luckily escape attack of pebrine. The pebrine is the major threats for production of breeding silkworms. Semina-infected silkworms and silkworms infected seriously in the 1–2 instars mostly die in the rearing season. During gathering of newly hatched silkworms, 3% of sick silkworms can result 60% of morbidity of grown silkworms, what's even worse, no breeding silkworms are produced and the sericulture will suffer a serious economic loss. *Nosema bombycis* is only 0.5 μm long, equivalent to one 140th of the diameter of a hair. In one silkworm cell hundreds of *nosema bombycis* can be parasitized. *Nosema bombycis* can communicate through eggs and feces and enter the digestive tract of silkworms, tiny amoeba attacking cells on the wall of digestive tract split, multiply and spread over the whole body, and finally grow to be spore. The spore is egg-shaped and has a "polar capsule" with long polar filament. In the past, pathogen of pebrine was believed to be *nosema bombycis* in the genus of *Nosema* in the family of *Microsporidia* in *Protozoa*. The latest research result shows that *nosema bombycis* belongs to *fungi*. *Nosema bombycis* experiences three stages throughout its life, spore germination, vegetative reproduction, and spore formation. It can survive for several years naturally. Different development stages of silkworms will show different symptoms after infection. In the young stage, 2 days after newly hatched silkworms are gathered, silkworms' hair is curled up and silkworms are in dark color, thin, stunted. Seriously, they will die gradually. In the stage of grown silkworms, they are dark rusty, slow in movement, poor in appetite and stunted and are not of uniform size, blackish brown spots are densely distributed on the silkworm back or spiracular line. In serious case, silkworms are scaled half and die. In the stage of matured silkworms, most of them cannot make cocoons or are slow in spinning silk, or most cocoons they made are thin. During pupa time, pupae are dark in color and pallid with urosome flabby, and sluggish. Fat is coarse and not full, there are reddish brown spots, and blood is highly viscous. During moth time, moth wings are thin and crispy, scaly hair is rare and easy to fall, wings do not stretch fully and are easy to curl up. Moths have small thorax and few eggs, there are yellowish brown spots on two sides of the back-wing tubes on the urosome, blood is turbid, and urine is reddish brown. Eggs are not of uniform size and arranged in disorder, are overlapped and easy to fall off due to poor attachment, more eggs are nonfertilized and dead. In common case, some of eggs see no difference from normal ones.

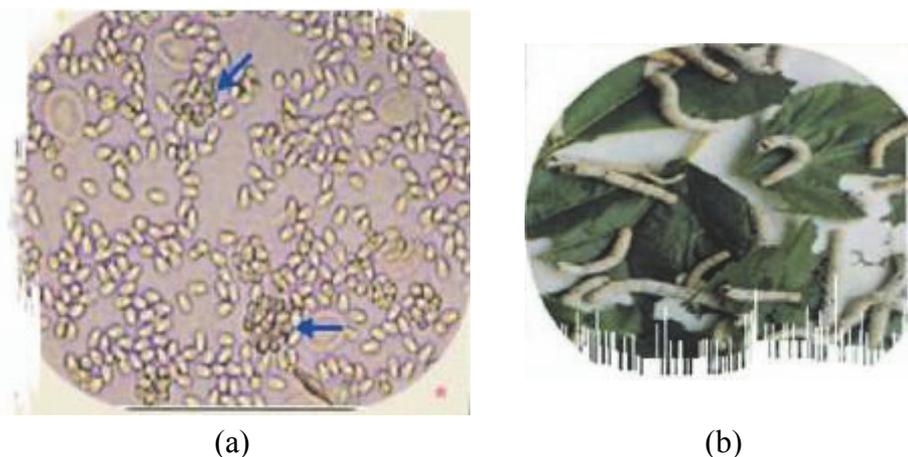


Figure 72. (a) Microsporidia; (b) Symptoms of silkworm group.

IV. Bacterial Disease

There are three types of septicaemia for silkworms, which are classified according to different pathogenic bacteria and different body features after death. They are *Bacillus septicaemia*, *Serratia marcescens bizzic*, and *Aeromonas septicaemia*. Infected silkworms will stop eating suddenly and be slow in movement, some have bulging thorax, vomit and defecate soft or toruloid feces, and sometimes they will die of spasm. Silkworms just died will be temporarily rigid and rhombic, with bulging thorax and raising head and tail, urosome arching towards the venter, prolegs inclined backwards and segments retracting. Afterwards, the body becomes flabby and stretching, the head and thorax extend out and turn soft and different color, internal organs are dissociated and liquefied, and the body will burst up with stinky liquid flowing out by slight vibration. During pupa time, at the beginning of infection, the body is rotten and turns black, and with slight vibration, black or red liquid will flow out. Infected with different septicaemia bacteria, silkworms may be rotten in different degrees and turn different colors.

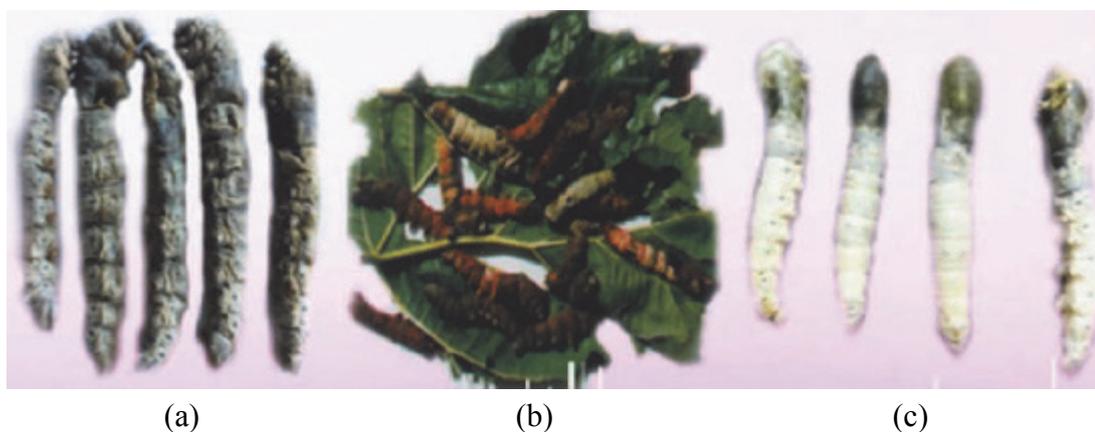


Figure 73. (a) *Bacillus septicaemia*; (b) *Serratia marcescens bizzic*; (c) *Aeromonas septicaemia*.

V. Pesticide Poisoning

People love to nickname adorable silkworms as “baby silkworm”. Now that babies need care. They are vulnerable. Some chemicals and residual pesticide on crops will lead to their poisoning and death. In addition, tobacco has great impacts on silkworms. It is harmful to humans as well as silkworms. Tobacco contains nicotine that is toxic. Although silkworms will not smoke, yet nicotine emitted from tobacco during growth will pollute mulberry leaves and even 100–150 m away from the tobacco field, mulberry leaves are likely exposed to pollution. Silkworms may be poisoned by mulberry leaves polluted by nicotine for 30–60 days. Nicotine can act on the nervous system of silkworms and paralyze their nerves after they eat mulberry leaves with toxins such as nicotine. If nicotine content per kilogram of mulberry leaves is 5 mg, silkworms eating one kilogram of mulberry leaves will suffer acute poisoning. If the content is 3 mg, silkworms continuing to be fed with these leaves will suffer chronic poisoning. Furthermore, pesticide and fluoride in the air can also poison silkworms. Poisoned silkworms are irritable, young silkworms poisoned will spin silk outside cocoons, lose appetite and crawl to the edges of rearing beds. Grown silkworms refuse to eat and crawl to the edges of rearing beds. In 2–3 days, without taking in, they stop growing and die with transparent thorax. Slightly infectious silkworms keep away from poison can still grow and spin silk making cocoons, but sometimes they spin flat silk, make abnormal cocoons or cannot make any cocoons.

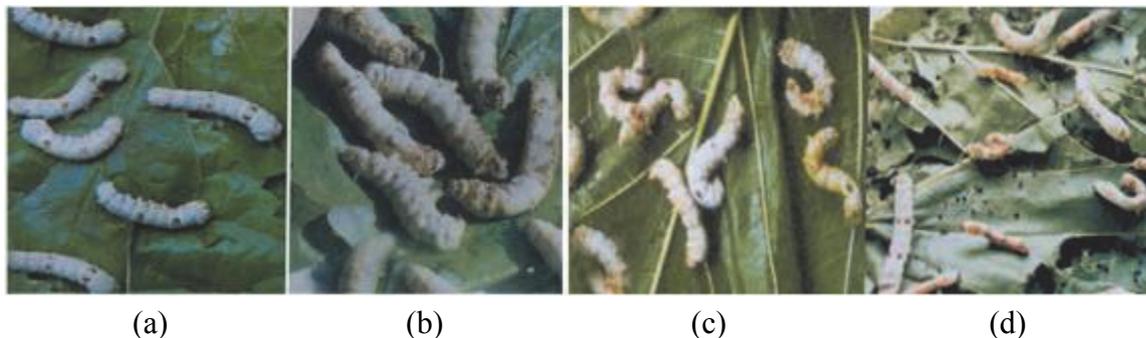


Figure 74. (a) Symptoms of tobacco poisoning; (b) Symptoms of pesticide poisoning; (c) Symptoms of fenvalerate poisoning (convulsion stage); (d) Group symptoms of fluoride poisoning.

VI. Others

Bombyx mori myiasis may confuse people how flies can cause disease? Indeed, flies can. However, flies that can cause diseases for silkworms are different from housefly and flesh fly we see in daily life. They are parasitic on a variety of insects and lay eggs, each female fly can lay 300–500 eggs, male and female flies can mate several times, and in the second day of mating, female fly can lay eggs. During laying eggs, the

female fly can get close to silkworms by tracing smell of silkworms, land on silkworms suddenly and fly away after laying 1–2 eggs. Fly eggs are mostly laid from the first to second segment of urosome and coria of the ninth to tenth segment and near the venter. A blackish brown horn-shaped speckle can be seen on the parasitic part of silkworms and a milky white fly eggshell is usually attached to the speckle. Disease-stricken silkworms are swelling or distorted towards one side. Sometimes host silkworms may turn bluish purple or reddish brown, which is easily diagnosed as septicaemia. In this case, the final decision can be made after tearing out the silkworm body and locating the fly. Host silkworms are precocious, so parasitism rate in precocious silkworms is high. Silkworm pathology scholars gave it a common name, multivoltine fly maggot.



Figure 75. Nuisance: fly.

4 Silk Road Shining through the Ages

Lu Xun had a well-known remark in his article *My Old Home*, “for actually the earth had no roads to begin with, but when many men pass one way, a road is made”. On the Earth human live, there are narrow trails beset with brambles, as well as expressways leading to everywhere. Roads may be not unusual, however people will never forget a great envoy in ancient China, Zhang Qian. He traveled several times to the Western Regions (a Han Dynasty term for the area west of Yumenguan, including what is now Xinjiang and parts of Central Asia), the route of which he walked was called Silk Road. The Silk Road, not just as ordinary as others, was a well-known trade road through a thousand years and even a friendship road of Chinese and western cultures. Nowadays, this ancient road still attracts people from all over the world. It symbolized friendship between China and foreign countries in the world civilization history. Opening-up of the Silk Road was a shining chapter in Chinese civilization, also a great pioneer undertaking of the entire human civilization. About this, there have been many records in the *Records of Grand Historian*, the *Book of Han*, and *Great Tang Records on the Western Regions*. In the end of 19th century, the east-to-west route was first coined as “Silk Road” by a Germany geologist Richthofen. Based on many years of researches, Swedish geographer Sven Hedin wrote a book entitled “*The Silk Road*”. From then on, the “Silk Road” has gained widespread acceptance. Generally speaking, the Silk Road was an ancient network of trade routes connecting the East and West and stretching from East Asia, Central Asia and Western Asia to Europe and North Africa. It played a significant role in the world history and was a traffic artery on the Eurasia and a bridge between Chinese, Indian, and Greek cultures.



Figure 76. Camel train on ancient Silk Road.

Chinese people invented sericulture, which afterwards became well known in the world just as the Four Great Inventions of China. From Shang and Zhou Dynasties to the Warring States Period, the silk weaving technology in Ancient China had developed to a

high level. The Silk Road is another contribution ancient Chinese people made to the rest of the world. It has passed long and marked special dedication of Chinese people to the world civilization. Over a thousand years, scholars tried to give this road names, like “Road of Jade”, “Road of Jewelry”, “Road of Buddhist”, or “Road of China”. However, for world people, the Silk Road is irreplaceable. The Silk Road was a way leading to the rest of the world in ancient China, a window presenting great innovations and splendid civilization for other people, and also a communication route for ancient China to integrate and exchange with western civilization and advancing world history together.

4.1 Founder of the “Silk Road”

Zhang Qian was a great explorer and envoy in the Western Han Dynasty. He was ambitious and traveled to the Western Regions. Over 13 years of journey, he left his footprints all over Mountain Tianshan, Central Asia and Western Asia and opened up the “Silk Road”.



Figure 77. Wall Painting on Zhang Qian’s Travel (early Tang Dynasty, Cave 323, imitated by Shao Hongjiang, collected by Dunhuang Academy).

Let’s roughly trace back to the historical background of Zhang Qian’s journey to the Western Regions. During competition between Chu and Han, Xiongnu ruler Modu seized the chance to expand its power and occupy a vast area of Northeast, Northern, and Western China, establishing a unified slavery regime. Modu conquered the Western Regions and levied heavy tax to all levels of governments, nibbling up territory of Han Dynasty and invading and robbing people in Central China. Emperor Wu of Han was the sixth emperor of Western Han and also a terrific politician, strategist and poet. After he acceded to the throne, he was told by a Xiongnu surrender that Da Yuezhi (Rouzhi, now

Afghanistan) moving to west China was always faced with threats from Xiongnu and conceived a revenge to Xiongnu. Later, he devised a strategy to attack Xiongnu from the “right” side together with Yuezhi.

Desert, oasis, ice cold, storm, huge cloud of sand, deep darkness, dead silence and monster roaring all terrified people. On a vast area, only shepherds cook, smoke rose up to the sky, and camel bells were ringing, resounding on the desolate. Here were the Western Regions of China, including Xinjiang, west to Congling (now Pamir Mountains), to Lake Balkhash in Central Asia. At that time, there were more than 30 regimes in the Western Regions.

In 139 BC, Zhang Qian led 100 followers, hired a Hun Tangyifu as a tour guide and left Chang’an for the Western Regions. Getting into Hexi Corridor, Zhang followed waters and campfire to hide from any suspected tracking and guard against possible assaults and sneak attack. Unfortunately, just when they hurriedly passed through the Corridor, they were caught by horse soldiers of Xiongnu. The ruler of Xiongnu would definitely not free them, as he knew what they planned to do. Zhang and Tangyifu were forced to shepherd sheep and horses separately and under custody of Huns. The ruler married a Xiongnu girl to Zhang as his wife in such a way to prevent him from escape. However, Zhang Qian safeguarded his integrity and accomplished the mission which assigned by Emperor Wu. During this time, he never faltered his determination to communicate with Da Yuezhi. He was held for 10 years long and escaped the control area of Xiongnu together with his followers in Sixth Year of Yuanguang (129 BC).

Recovered from hurry, Zhang continued to undertake the mission of “travel to the Western Regions”. This is an arduous marching. On the vast gobi desert, sand and stone were raised by storm, heat tortured people; Congling was too high to least likely climb over, snow and ice covered all things and cold wind penetrated people’s bones. Along the way, there were rare people and lacks of water, and beasts came and went. Zhang Qian braved the wind and dew and had a rough time. They ate up all rations and Tangyifu shot birds and beasts as food. During the journey, some followers died of starvation or thirst or were buried in yellow sand or ice caves. They went along the southern foot of Mountain Tianshan, passing Yanqi, Guizi and Shule, traversed desert and icing Congling (now Pamir Mountains), and finally arrived Dayuan (now Fergana). Dayuan was a rich state in Central Asia with the population of tens of thousand and 70 towns and cities. The country abounded with “horses of heaven”. The emperor of Dayuan had planned to establish diplomatic relations with Han Dynasty, warmly welcomed Zhang and sent tour guides and translators to escort them to Kangju (now in Uzbekistan and Tadzhikistan). The ruler of Kangju sent soldiers to escort them to Da Yuezhi. At the same time, Da Yuezhi had suffered a lot. The enemy state Wusun had invaded Da Yuezhi with the support of Xiongnu. People of Da Yuezhi were forced to move west from Yili River and enter Guishui areas near Aral Sea. They conquered Da Xia and built new homeland, calling it “Xiao Yuezhi”. Xiao Yuezhi carried on farming and developed into a wealthy

and strong state, and was unwilling to revenge.

Zhang Qian stayed in Daxia for one year and returned with disappointment. On his way home, Zhang took the southern way for avoiding the control area of Xiongnu. They tramped over Congling, walked on foot along the northern foot of Mountain Kunlun, and entered the living area of Qiang people by way of Shache (a county in Xinjiang), Yutian (now Hetian city in Xinjiang), Shanshan (now Ruoqiang in Xinjiang). Then Qiang people had been reduced to dependency on Xiongnu. Zhang Qian and his companies were captured again by Xiongnu horse soldiers and put into prison for another 1 year. In 126 BC, Zhang Qian and Tangyifu took the opportunity of internal disorder in Xiongnu and escaped back to Han. Emperor Wu of Han heard his report about the Western Regions and felt so satisfied to confer Zhang as Marquis Bowang and Tangyifu as Royal Servant as rewards. Zhang Qian was the first person who opened up extraterritorial roads and played an unprecedented role in development of Chinese and western traffic.

In 119 BC, Xiongnu people retreated from Hexi Corridor and moved towards the northwest. Depending on people and financial materials in the Western Regions, Xiongnu continued to be against Han. Emperor Wu of Han reassigned Zhang Qian as Colonel and 300 followers to the Western Regions with golden coins, silk and thousands of cattle and sheep. Once arriving in Wusun, Zhang tried to persuade Wusun come over and pledge allegiance to Han. But he failed because of internal disorder in Wusun. However, Zhang's deputy envoy paid visit to Dayuan, Kangju, Anxi, and Da Xia in Central Asia and strengthened relations between Han and the Western Regions. Zhang Qian returned and arrived in Chang'an in 115 BC. In the second year, Zhang died in Chang'an. In 105 BC, Han sent a silk trade team to Anxi. On the border there had been trade activities between China and the Western Regions at that time. The places where the silk trade team walked through are the well-known Silk Road today.



Figure 78. Silk Road. (Source: <http://www.wikipedia.org>).

4.2 *Western Dream of Silk*

2,000 years ago, there was another ancient country, Roman Kingdom. It was said that when Troy was attacked by Greek, Aeneas, son of Venus, and his followers escaped to ancient Italy by way of Carthage along Northern Africa. Italy was ruled by King Latium and Aeneas defeated Latium and built up a new kingdom (Alba). His offspring Romulus established a new state Roman and became the first king. Chinese silk is soft and delicate and is beyond comparison by flax and wool that Europeans usually have in luster and quality. In 1st century BC, Caesar, president of Roman Republic, went to theater in a silk robe made in China, and shining and luxurious silk attracted all eyes and western people at that time couldn't believe that there was so thin and soft and elegant fabric in the world. They thought this might be God's mind and considered that beautiful silk must come for "heaven". Cosmas, a Greek writer and poet, said, "if there is heaven in the world, how can you stop people who are bent on seeking heaven? The heaven is an ideal world, in people's world is there a thing as beautiful and mysterious as the heaven, and that is silk from Seres (ancient name of China in western world in 4th century), a country at the other end of the world. People indulge themselves in a life of physical pleasure and fear nothing to chase after silk, which is unstoppable, just as their belief in the heaven". Across the vast ocean, it was a long and arduous journey and Europeans knew nothing about "Country of Silk". Ancient Greek called China "Seres", a country of silk production. They heard through grapevine and passed on errors that described Chinese people were as magic as All-mighty Gods in temples.

About records of silk in western documentation, the first record should be in a *Naturalis Historia* by Pliny the Elder, a Roman writer before 2000 years ago. Pliny complained in his book with Taoism looking that women in semitransparent silk were seemingly naked, which was an offence against decency. He thought the obnoxious textile came from a far eastern country, Seres. He also said, "Seres people are taller than ordinary people, have red hair and blue eyes, made loud noise without language, and have long life as long as 200". They envisioned raw materials of silk were soft down growing on the trees, Seres people picked them up and soaked in the water, then they "combed white leaves to weave shining silk strands". There was a Greek geographer named Pausanius who made even more bizarre conjectures. According to him, the silk used by the Seres people to make satin materials came from a kind of bug which he called "Little Ser". These bugs had eight feet and a size about twice that of a beetle. They spit out silk like spiders weaving a web. The Seres people domesticated these bugs in the winter and summer months. The bugs' feet were bound using their own silk and were fed with millet for the first four years. On the fifth, they were fed with reed, their favorite food. The bugs over-ate until they died with their bodies filled with silk. And this is what the westerners imagined about the silkworm in the 2nd century. By the 4th century, the Greeks again envisioned another entity called "lambs wool tree" that could spill out silk.

This is what they said: “There are trees in the forest which people tender to diligently. From the trees, they brush and comb, and obtain intricate filaments that have textures that are half woolly and half with silk-like viscosity.” This fabric was made into “transparent robe” for Roman ladies. At last, researchers believed firmly that the fabric made of “lambs wool on trees” was the silk we knew well. Why wool on trees is silk? The question is related to cognitional linguistics. Because Roman people never saw silkworms and mulberry trees in daily life, they were hard to imagine how silk protein fibers spilled out by silkworms were made into fabric. They could only think of the smoothest fabric, wool. However, they seemingly knew this material is not from lambs but trees. So, they invented a new word, “lambs wool trees”, to represent a new thing, silk.

Apollonius’s *Argonauts* first published in ancient Greece, and is the only full-length narrative poem kept integrally except Homeric Hymns. The book introduced the story about ancient Greek hero Aeson’s adventure along the coast of the Black Sea. A legend said on the coast of the Black Sea, far away from Greece, Colchis (now Caucasus) produced rare treasure, Golden Fleece. At the time, many heroes stepped on an arduous journey for getting it, but no one succeeded, and most of them even had no chance to see it and died during their long journey. Aeson showed up later. He was a son of the king, and exiled as his throne was usurped by his uncle. Two decades of exile made a little prince an ambitious warrior. He held two spears in hands, wearing leopard fur with long hair falling over shoulders. What a hero is in front of us. After he went home and asked his throne back, his uncle promised him with evil intent that if he could fetch legendary “Golden Fleece” from an alien country in the Black Sea regions, he would return the throne. Attempting to take back Golden Fleece, Aeson invited some famous heroes at the time to join his adventure. Under guidance of goddess Athena, the smartest ship maker of Greece used wood non-corrosive in seawater to build a great ship, which could be equipped with 50 boatmen. After the ship was built, people named it after the ship maker, Argus, which literally means “fast ship”. It was the first ship that Greek sailed on the sea. Wood for making the ship nose was given by Athena and was take from oak trees from Dodona. “Argo” ship braved winds and waves, like Tang Monk, Xuanzang who went on a pilgrimage for Buddhist scriptures. They went through untold hardships and finally arrived in Aea of Scythia along the east coast of the Black Sea. Medea, a daughter of the king, was shot by Cupid’s arrow and fell in love with Aeson. She decided to help her lover at all costs and told Aeson secret steps to fetch Golden Fleece, helped him defeating the fire-spitting bull and Dragon Knight and lethargizing the poison dragon. Seen from afar, Aeson fetched Golden Fleece from a tall oak tree looking alike dark cloud, the rising sun dazzled eyes, and all things turned red. He took “Golden Fleece from the oak tree” and the broad “Golden Fleece” in front of him “shone golden light like fire” and shone red his cheek and forehead. It was as big and heavy as fur of a one-year-old bull or a male Mazama. On his way home, the ground of all places he passed by lighted up. He put it on his left shoulder first and let it fall from the neck to toes and rolled the rest up in the hands for fear that

people or deity he met would rob it. They hid from pursuers, and Medea paid great cost, who betrayed her father for the sake of love, helped Aeson attain Golden Fleece, and killed her young brother pursuing killing her lover. After many other sufferings, Aeson finally made his way back to Greece, his cherished homeland.



Figure 79. Colorful silk.

I didn't mean to get stuck in the stories. However, I really want to compare similarities in stores of Pliny and Apollonius. As *Naturalis Historia* recorded, Seres people had no “silk”, and they just attained wool from tree leaves and the wool could make into “transparent clothes”. Does this kind of wool on trees be speculated as silk? Or might this be the earliest records about “silk” in Europe?

Silk was so mysterious at the time that Roman people took it as a sacred thing. They were willing to buy it with any cost. Silk price in the Roman market drastically rose up. It was as high as 12 liang (50 g) of gold per pound. Much gold was used for buying silk products and clothing and Roman had a great deal of gold circulated in foreign countries. Senate found this and banned silk trade. This measure excluded ordinary people but only real noblemen could possibly buy superior silk clothing or products. Many high officials and noble lords thought silk clothes represented their position. Many beautiful noble ladies showed off Chinese silk clothes and caught eyes from gentlemen with their fine figure in silk. Until today, Roman people are still passionate with silk.

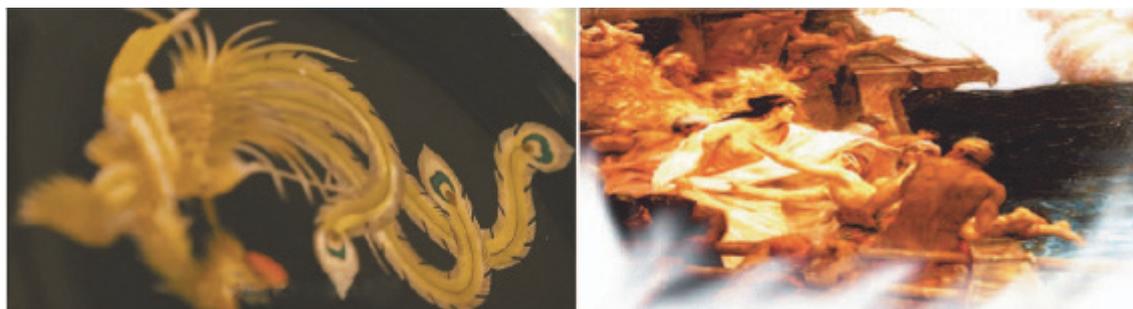


Figure 80. Golden Fleece in legends.

In 17th–18th century, tapestry manufactured by Aubusson, a French town, was popular. The only extant Aubusson tapestry, which was manufactured in the mid-term of 18th century and stolen in 1982, was 4.3 m long and 2 m wide and was evaluated at 7,000–9,000 pounds. About 1745, another silk tapestry manufactured in Aubusson was 2.33 m high and 5.16 m wide and collected in China Pavilion of the Louvre Museum. Noble women and monks on the picture were both in silk. At the time, silk produced in Europe was comparable to those of China, however, Roman people still thought only Chinese silk could be the most decent for showing their dignity.



Figure 81. We are all silk fans.

4.3 Trade War of Silk

In ancient China, Han people knew nothing about ancient Roman. They just heard about that it was powerful and extremely far away in the west and thought they were like Qin people, so Roman was called Daqin. People also called it “Sea West State” because it was west to the sea. Roman Empire at the time was a major consumer country of Chinese silk as well as a major country selling silk in the western market. Due to the geographic location, Roman people should sail across the sea to China. On the east of the sea were

Anxi and Tiaozi. Anxi was also known as Parthia and established in 247 BC. Arsacids was the first king and was translated into Chinese “Anxi” in Han, so the country was named after the king’s. Anxi had close relation to Han Dynasty and was the place which must be passed. In 97 BC, Ban Chao sent Gan Ying to Roman when Persia was ruled by Parthia. Gan Ying arrived the Persian Gulf and planned to take a boat to Red Sea and Syria. However, Persian said with bad intent that the sea is boundless, it took three months to sail across the sea if in good weather, but in bad weather with storm it needed two years, so any one who wanted to sail across the sea should stock up three years of food rations. What’s worse, people were easy to be homesick and died of nostalgia. Gan felt frustrated and decided to give up. Of course, there was one thing that Persian didn’t tell him. Actually, there was another land route to Roman instead of the water route.

Persian dealt with Eastern Rome and Tujue (Turk) with bad diplomatic means and war for seeking permanent benefits of silk trade. Many wars were waged to scramble the right of silk trade in history. Roman people were vexed about monopoly of Persian in silk trade and both parties started a feud. Until the middle of 6th century, Eastern Rome had a determined emperor Justinian the Great (from 527 to 565). He hoped to satisfy demands of Roman people for silk once and for all. In 568 AD, the Eastern Rome allied Turk. At the time, Western Turk had controlled states in the Western Regions and became a powerful chief along the Silk Road. Western Turk gained abundant silk and gold from the Northern Qi Dynasty and the Northern Zhou Dynasty and had planned to deal with Persia and Eastern Rome directly long ago. Both parties reached on agreements quickly and decided that silk was provided for Eastern Rome via Western Turk. Persian learned that, placed obstacles for them and took risks to kill Turk diplomatic corps. The murder provoked a war. With the support of Western Turk, Eastern Rome attacked Persia in 571 and the war lasted for 20 years and hung in balance. This war was the famous “Trade War of Silk”.

4.4 More About Silkworm Breeding

Silk has had long history in China. The weaving technology first appeared 5,500 years ago and silkworm breeding can date to the 3rd century BC. As early as the Spring and Autumn Period and the Warring States Period, farming and sericulture was an important national policy for prosperity of states and people. At the time silk had been exported to foreign countries. The Book of Ezekiel of the Old Testament, which was compiled in ancient Persia in the 5th century BC has mentioned that Jehovah wanted to dress Jerusalem up with the most beautiful and luxurious clothes and he talked about “silk” twice as he described the “most beautiful fabric”. At the same time, Gods of ancient Greece were all dressed with transparent and soft robes, for example, Athena Statue in Parthenon Temple. These robes were more likely Chinese silk products

imported from Persia. As for how the silk production technology was revealed, there were several versions. According to historical data, the first Chinese chief who paid a visit to western countries might be Emperor Mu of Western Zhou Dynasty in the 10th century BC. He set off from Central China, traveled towards west to northwest China, arrived in some clans of Central Asia. During his journey, silk was taken as national gift and given to the visited clans. This might be the earliest history about silk exported to the west. Until the 2nd century BC, after Emperor Wu of Han sent Zhang Qian to the Western Regions, the oasis route from Xinjiang to Western Asia was opened up, which generally referred to the Silk Road and was several centuries later than the steppe route. The silkworm breeding technology in ancient China was kept confidential for outsiders, which added some mysterious color to the process of exporting to the west. It was said that a missionary in the 6th century came to Constantinople and knew that Persian traders were avaricious and sold silk at high price. He then made suggestions to Justinian the Great and said he could resolve the problem that Eastern Rome purchased silk from Persia and other countries. Justinian the Great was happy to hear that and promised him great rewards if he did it. As a result, he made a long journey to the East, which might be either Yutian or Central China. As expected, he attained mulberry seeds and silkworm eggs from the folk. He also stole the mulberry planting and silkworm breeding technology and hid it in his bamboo mace and returned to Eastern Rome along the Caucasus Mountains. Justinian the Great and his officials were so happy to see the missionary and his things. Unexpectedly it was a flash in the pan. The missionary was in a rush for his great rewards and mistakenly placed silkworm eggs into the soil and mulberry seeds in rooms. As a result, no mulberry trees grew up and no eggs were hatched, which turned out to be a big joke in the Roman palace. Some Indian monks in Constantinople heard about the joke and told Justinian that they had been to Salinda (probably in Xinjiang today) and found that silk was spun by worms. They said it was impossible to take the worms out of that country but eggs instead. They finally brought silkworm eggs and mulberry seeds out and successfully hatched eggs and planted trees. Thus, Empire Rome had attained silk growing in the west as raw materials for weaving. The History of the Decline and Fall of the Roman Empire written by English historian Edward Gibbon also recorded the similar story. Of all stories, the most widespread legend was recorded in Great Tang Records on the Western Regions of Xuanzang. It said that there was a country called Kustana in Yutian, where no silkworms and mulberry trees. The king sent envoy to ask a neighbor country in the east for silkworm eggs and mulberry seeds but he was rejected. The neighbor country also issued strict order to ban export of silkworm eggs and mulberry seeds. Kustana could think of no way at all and so sought a marriage alliance with China. China's emperor was friendly and agreed upon the marriage alliance for safeguarding safety of the Western Regions. However, the critical problem is to plead the princess secretly to take silkworm eggs and mulberry seeds out of China. Through consideration, the king selected a group of able envoys and

maidservants and told them to complete his secret mission. He ordered them to tell the princess about the mission before she left her country and must make it done. Envoys received the mission and arrived in China. On the day of escorting, the princess hid the silkworm eggs and mulberry seeds in her hat. Guardians at the outbound gate searched all belongings except the hat. Consequently, the hidings were brought to Kustana and Kustana attained the mulberry planting and silkworm breeding technology.

Sericulture directly pushed the silk weaving technology forward and developed the one-and-only silk weaving technology, leading printing and dyeing process, and colorful patterns, and silk had become an elegant belonging for showing power of emperors and beauty of women. Since Zhang Qian traveled to the Western Regions, silk has also become the major foreign trade product of China. The Silk Road, over thousands of meters and one thousand years, was the major channel for communicating ancient Chinese people and the world as well as source of activity in economic and cultural development in China. Silkworm eggs and silkworm breeding methods in all sericulture countries in the world were directly or indirectly transmitted from China. They were transmitted to North Korea 3,000 years ago, to Japan and Vietnam 2,000 years ago, countries in Central Asia 1,600 years, Europe 1,400 years ago, and South Africa 400 years ago. China is an inventor of silk reeling and weaving and takes the absolute leading position in a long time, and this is one of the greatest contributions Chinese people made.



Figure 82. In 552, Justinian the Great of Byzantium took over maces in which silkworm eggs were hid from two monks.



Figure 83. Communications of silkworm eggs and silkworm breeding method.



Figure 84. Kimono.

4.5 Hidden Treasure on the Silk Road

Han and Tang were rich and arrogant, generous but terrifying and received tributes from neighboring countries. The arm force of Han was far-famed in Central Asia, Caspian Sea, and Lake Baikal in Russia. The splendid style of Tang had great effect on India, Arab, and East Europe areas. Until Ming and Qing Dynasties, China had dependent states. Since Tang and Song Dynasties, China had gradually relied on Maritime Silk Road. Via the Silk Road, envoys and traders came and went. In Yuan Dynasty, Marco Polo arrived Dadu, the capital of Yuan, via the Silk Road. Zheng He of Ming Dynasty reached the eastern coast and Red Sea in his voyage.

The Silk Road is 1,700 km in China from Chang'an (now Xi'an), by way of Hexi Corridor, including the former North road and Qinghai province, to Dunhuang, Jumenguan, and Yangguan to the Western Regions. At present, there are abundant sites, remains, and relics related to Chinese-western cultural communications preserved in Shaanxi, Gansu, Ningxia, Qinghai, and Xinjiang along the oasis route. They witnessed the opening-up, continuation, prosperity and recession and caught eyes from men of insight and attracted followers. The Silk Road is like a color ribbon, linking ancient Asia, Europe, and Africa together. Via the Silk Road, the Four Great Inventions, the silkworm breeding and weaving technology, colorful silk products, tea, and china could be spread all over the world and has had unmeasurable impact on the social and economic development of the rest of the world. Meanwhile, Chinese and foreign traders brought horses and grapes of Central Asia, Buddhism, music, sugar boiling methods and medicine of India, musical instruments, production of gold and silver ware, astronomy, and mathematics of Western Asia, cotton, tobacco, sweet potatoes of Americas into China through the Silk Road and upgraded and developed Chinese civilization. On the road over 7,000 km, silk, like chinaware produced in China, became a symbol of prosperity in civilization of East Asia. At the time, leaders and aristocrats of other countries considered Chinese silk clothing products in Phoenicia red and chinaware at home as an emblem of honor.

4.6 New Start of the Belt and Road

The Silk Road has a long history and will never see an end. In the past, it was a bridge of friendship and used to be destroyed by wars. Nowadays, miseries have faded and the Silk Road is regarded as a belt to link eastern civilization with western one. In recent years, UNESCO launched "Silk Road Study Plan" and called the Silk Road "Road of Dialogue", aiming to promote dialogue and exchange between east and west. For Chinese people, today's Silk Road is an opening-up road, advancing road, and a road leading to a bright future in 21st century.

In September and October 2013, Xi Jinping, president of PRC, proposed the initiative of "the Silk Road Economic Belt and the 21st-Century Maritime Silk Road" (hereinafter referred to as the Belt and Road) twice. President Xi pointed out that "the Belt and Road" was the idea and initiative of cooperation and development and the economic cooperation partnership depending on the extant multilateral mechanism between China and related countries and on the extant and effective regional cooperation platform with an aim to hold the flag of peaceful development high and seek active development with countries along the road in the name of a historical symbol the "Silk Road" and to build the community of shared interest, common destiny, and responsibility featuring political mutual trust, economic integration, and cultural inclusiveness. The

Belt and Road should devote to connectivity in Asia, Europe, and Africa and ocean areas, to establishing and strengthening connectivity and partnership of along-road countries, to build an all-round, multitier and complex connectivity network for multilateral, independent, balanced, and sustainable development of the alongside states. The connectivity project of the Belt and Road will realize cooperation in development strategies of alongside countries, dig deeper in market potential in these regions, attract capitals and consumption, create demands and jobs, increase cultural communications and mutual learning in civilization, and lead a harmonious, peaceful, and prosperous life for people getting to know, trust and respect each other well. The unprecedented idea comes from the history and takes reality into consideration, communicating China and foreign countries, following the trends of peace, development, cooperation, and win-win, carrying the dream of development and prosperity of the along-road countries and giving ancient Silk Road brand-new connotations.

5 Domesticated Silkworm as Bioreactor, A Key into Medicine Production

5.1 Make Good Use of Disadvantages

Every coin has two sides. What silkworm breeders dislike are enemies threatening silkworms. However, most of silkworm diseases are skillfully used by scientists. Through research, scientists find that NPV of silkworms is a buried treasure. They assume, silkworms are vulnerable to NPV and the disease comes on fast, so it can infer that NPV genome is highly multiplicative and fast in expression. So, if by proper recombination and addition of some GGATCC and insert of exogenous gene to be expressed into the NPV genome according to the genetic engineering principle, virus protein is expressed while the exogenous protein is expressed as well and is what we need.

As we know, silkworms are insects and can be eaten directly, and people eating them show no immune response. Therefore, the expressed exogenous protein can be purified and separated and directly taken orally. Based on the assumption above, scientists started their research. In 1999, Japanese scholars sequenced the NPV genome and then cracked it, which laid foundation for recombination of the expression vector of NPV. They found the NPV genome had 128,413 basic groups, about 140 genes. The genes act differently in the silkworms, some expressed early, some late, and some are indispensable for virus. Without them, virus is less toxic in domesticated silkworms or even will not multiply. Some others are dispensable and without them, there might be no big deal for the virus. Baculovirus is a type of insect virus of host specificity and is widely used to produce exogenous protein in insect cells or larvae. The latest study indicated that Baculovirus couldn't infect mammals, but could get into cells of mammals from different species and histologic origins and expresses exogenous genes under control of mammal promoter. This hinted that the Baculovirus could serve as vector of genetic treatment and have great potential. Entering the insect cells is realized by endocytosis. Glycoprotein gp64 on the bursa is integrated with cytomembrane, which helps virus enter the cell. The reported mammal cell sensitive to Baculovirus is hepatic cell, for example, of humans, rats, and rabbits. In addition, another finding is that the recombinant Baculovirus can express exogenous DNA in the non-hepatic cells, for example, human cervical cancer cell lines HeLa, HEK cell lines 293, CHO, BHK, and African green monkey kidney cell line CV21. So far, with Baculovirus as vector, gene transfer and expression of many cell lines from different species and histologic origins have been realized. Baculovirus has different transduction efficiency for different

mammal cells. From this, we infer that a special receptor might exist on cytomembrane of different cells. The structure and abundance of this receptor on cells of different animals may differentiate.

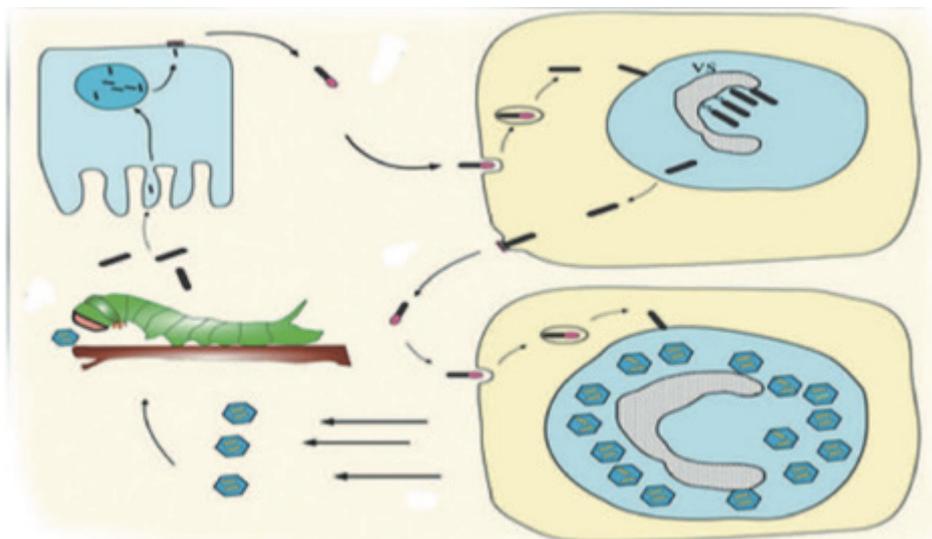


Figure 85. Reproduction and communication of polyhedrosis in silkworm cells.

5.2 Eukaryotic Expressing System

In 1983, the eukaryotic expressing system of Baculovirus developed by Japanese scientists is safe and able to process and modify expression products, express multiple exogenous genes at a time, and is suitable for expressing cytotoxic protein. It has been one of the four great expressing systems in the genetic engineering area. However, the early Baculovirus system is complicated in technology, and especially, it takes long time to sort out recombinant exogenous genes and it is hard to insert exogenous genes. Until 1993, scientists established an effective Baculovirus expressing shuttle vector system. This shuttle vector is a plasmid in a bacteria cell and can be reproduced in bacteria. But it is a virus in an insect cell and can be reproduced and expressed and constitutes a progeny virus. This breakthrough abandoned an old concept of 100 years that it must be living cells to reproduce virus genome, and all operations from bacteria to Baculovirus are completed in bacteria, recombination rate is 100% and operation is simple. Because of no background interference, plaque is not needed to purify and it only takes 8 days to have recombinant virus. That is to say, an exogenous gene is inserted into the Baculovirus genome and can be expressed very soon.

A domesticated silkworm is a natural bioreactor and is easy to breed, expressive, inexpensive, and safe. At present, there are many proteins expressed efficiently in the domesticated silkworms. The Baculovirus expressing system, different from others, has the following advantages.

(1) The recombinant protein has the complete biological function. The Baculovirus expressing system can provide a good condition for folding, matching of disulfide bond, and formation of oligomer of high-expressive exogenous protein in cells and can make the expression product resemble natural protein structurally and functionally.

(2) It can process and modify after translation. The Baculovirus expressing system has the ability to process the protein after complete translation, including glycosylation, phosphorylation, acylations, excision of signal peptide, cutting and decomposition of peptide fragment. Modified locus is identical with the natural protein in cells. The contrast experiment indicated that the glycosylation locus occurring in the insect cell is totally the same as that in the mammal cells but the modified oligosaccharides are not identical. As the difference has different impact on different interest protein, the expressing system of insects can also be an ideal model for studying the impact of glycosylation on the structure and function of the protein. This is important. Although the Colibacillus prokaryotic expressing system can express exogenous protein at high speed, yet the resultant exogenous protein is inactive or lacks of some functions due to lack of processing and modification of protein.

(3) It has high expression level. Compared with other eukaryotic expressing system, the Baculovirus expressing system is characterized by its ability to express the recombinant protein at high level. The highest about of the interest protein can reach 50% of the total protein.

(4) It can accommodate the insertion fragment of macromolecule. The Baculovirus virion can expand and envelope big gene fragments. However, it is yet to know the upper limit of the length of exogenous genes that the Baculovirus can accommodate.

(5) It can express multiple genes at the same time. The Baculovirus expressing system is able to express several genes in the same cell. It can either employ different recombinant viruses to infect cells or clone two exogenous genes on the same transfer vector. The expression product can be processed to form active heterodimer or polymer.

In addition, the Baculovirus expressing system is able to cut and has a function of locating the recombinant protein expect the ability to express genome DNA, for example, transferring nucleoprotein to nucleus, positioning nucleoprotein on membranes, and secreting protein outside cells. Baculovirus is non-infectious for vertebrates. Extant researches also show that its promoter is inactive in animal cells. Therefore, in expressing cancer genes or potential toxic protein, the Baculovirus expressing system may be superior to others.

5.3 Stage for Green Fluorescent Protein (GFP)

For better understanding of readers on the Baculovirus expressing system, we take an example. Assume that we want to express an exogenous gene, for example, a wild

green fluorescent protein (GFP) in jellyfish. Now we transfer GFP into bees or fish, and you can observe green light under a fluorescent microscope. This shows that the GFP has been expressed in bees or fish. Now we plan to transfer the enhanced gene GFP into silkworm cells or pupa. To express this exogenous protein, we carry out an experiment as follows.

First step: employ the PCR to expand the interest gene GFP. PCR is a molecular biology word. It sounds complicated, but indeed it is not. It was invented during simulating the reproduction process of DNA. The finder was a great scientist and awarded Nobel Prize. Since the interest gene GFP was achieved, the interest gene was cloned to a vector.

About “clone”, it is a popular word and also mentioned in official reports. A bacterium can be separated into two in 20 min, a grape branch is cut into 10 parts, and 10 parts may grow into 10 grape trees under certain time and conditions. Each piece of cactus cut into several pieces can take root in the soil; a stolon a strawberry tree crawls along can grow into hundreds of strawberry seedlings in a year. All of these are realized depending on organisms separating into two or a small part growing up to propagate. This is so-called vegetative propagation. Clone was originated from Greek “Klone”, meaning seedlings or tender twig or cutting is cultivated into plants in a manner of vegetative propagation or reproduction, such as cutting or grafting. Simply saying, it is copy as network language. In terms of molecular biology, it refers to a process that a gene is transferred to a vector. The method is simple and is to cut a vector with enzyme digestion and connect the interest gene onto it.



Figure 86. GFP expressed in fish.

Second step: cut off the interest gene GFP on the cloned vector with a certain enzyme. The enzyme is equivalent to scissors and can cut off the interest gene. The interest gene is connected to another vector, which is also called transposition vector. It is responsible for transferring the interest gene to the Baculovirus genome.

Third step: transfer the transposition vector with the interest gene to *Colibacillus* competent cell DH10Bac, culture with bacteria and screen with antibiotics. Any white

bacterial plague found indicated that the interest gene has been transferred to the Baculovirus genome.

Fourth step: transfer the Baculovirus (also known as recombinant virus) of the interest gene GFP to domesticated silkworm cells or infect silkworm pupae; observe under the stereo fluorescence microscope. If any infected silkworm cells or pupae show green fluorescent light, it indicated that the interest gene GFP was expressed in the silkworm cells and pupae. This process is actually to realize expression of the exogenous gene.

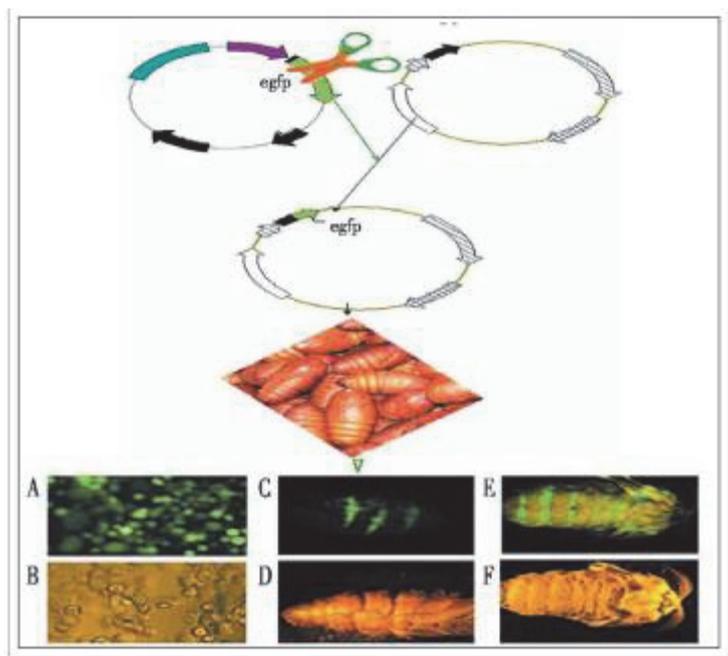


Figure 87. Clone.

Fifth step: exogenous protein should be separated and purified in case of drug use after expressed in the silkworm cells or pupae. By means of hi-tech (for example, deep freezing, cryodrying, and high-speed centrifugation), active constituents can be extracted and prepared into related dosage so as to meet needs for disease treatment and prevention and health care.

5.4 Future of Baculovirus

The Baculovirus expression system with special properties has been widely applied to fields of medicine R&D, vaccine production, and recombinant virus insecticide. According to incomplete statistics, there were 200 institutions at home and abroad carrying out related researches and the number of the expression genes is over 400. The most eye-catching project is to use the silkworms as bioreactor to product

interferon, flu vaccine, and hepatitis B vaccine for humans and auxin for pigs and fish.

The Baculovirus is safe to humans and animals and will not damage ecological balance in a large scale. Insecticide has been a hotspot in R&D of Biopesticide. However, it must restructure the wild Baculovirus, which is slow in effect, especially silkworm Baculovirus. As silkworm Baculovirus can infect a small range of insects, it as insecticide can kill limited insects. Many scientists are trying to restructure silkworm Baculovirus to expand its insecticide range. In addition, disadvantages and related technology of insect Baculovirus as the eukaryotic expressing system need to be improved. Continuous expression, better than instant expression, will not affect yield. Glycosylation is different from mammal cells. Glycosyl side chain has high content of seminose and lacks of compound oligosaccharide. In the basic study and application technology of the Baculovirus system, the genomics of Baculovirus, especially functional genomics, is still under-researched and related researches on high expression and control mechanism of late genes are still seldom fruited. It is unclear about other possible hosts of Baculovirus, and purification and multi-element expression of the expression products are underdeveloped and should be improved in the future. At present, research should be focused on the genomics, especially functional genomics of Baculovirus. On the one hand, this helps improve the Baculovirus vector. On the other hand, some genetic structures and functions adjusting exogenous protein expression can be figured out, which is conducive to effective expression and control of the exogenous gene.

The abovementioned technology is to employ Baculovirus to express exogenous genes and produce exogenous protein, which is an instant expression method. That is to say, only in domesticated silkworm cells or body can express the exogenous protein within a time. Scientists hope to permanently insert the exogenous protein into the silkworm genome so as to permanently express the exogenous protein. If the assumption works, silkworms having the exogenous genes are called transgenic silkworms.

Here I have to talk about transgene. Transgenesis is to transfer separated and modified genes into a genome. The technology to express the exogenous genes and changing biological characters is called transgenesis, also as known as “genetic engineering”, and “genetic transformation”. By transgenesis, genes of different species can be recombined through the genetic engineering, humans can directionally modify the genetic characteristics of living beings according to their wills and create new lives. Common tools and methods of transgenesis are gene guns, microinjection, electric shock, and liposome.

5.5 On Transgenesis and Clone

In 1974, an American molecular biologist Norman Cohen transferred penicillinfast

gene on staphylococcus aureus plasmid into Colibacillus, which unveiled the prelude of the application of transgenesis. Until 1982, Lilly, a US company, first used Colibacillus to produce recombinant insulin, which marks a first genetic engineering drug came into being. In 1992, the Netherlands raised a transgenic ox implanted with EPO genes, which can stimulate erythropoiesis and is good medicine for curing anemia.

In March 2000, a clone pig turned out. In the following, a controversy on if GMO food is okay to eat never stops. In the early 1990s, a first GMO food on the market, transgenic tomato, showed up in the US (the result of this project was scored in English at first), and since then GMO products



Figure 88. GMO plants and animals.

went popular. Statistically, there are 43 varieties of GMO products that have been identified by FDA. The United States produces the most GMO food products, over 60% of processed food contains transgenic ingredients, and over 90% of beans and 50% of corns and wheat are genetically modified. GMO plants include tomatoes, potatoes, and corns, and GMO animals include fish, cattle, and sheep. Though not different in taste from ordinary food, GMO plants and animals have outstanding advantages, for example, high yield, resistance to pests, virus and herbicide, better quality, and stress resistance.

In 1996, China developed herbicide resistant GMO hybrid rice for the first time, which provided a new method for solving a long-challenged problem about purity of seeds of hybrid rice. This achievement was listed at the top of China Top Ten Hi-Tech Advances voted by 500 academicians from Chinese Academy of Sciences and Chinese Academy of Engineering in 1997. In 2009, Approval List of the Second Batch of Agricultural GMO Safety Certificate was released, and two varieties of Bt GMO rice “Huahui No.1” and “Bt Shanyou63” was issued safety certificate of GMO rice by the

Ministry of Agriculture and approved being planted in Hubei province. Director of the department of agriculture and food in Greenpeace said, Greenpeace is always against that GMO rice goes commercial in China, if it does, it will be planted in a large area, and under the action of gene drift, it will be an extinction threat to wild rice. So far it has no confirmed effect of eating GMO rice on humans, so it is intolerable to put people's life at potential risks. Now the GMO rice has a different reputation among people.

In 1994 researchers from DuPont Pioneer tried to transfer encoded cysteine protein in Brazil nuts into soybeans to improve its nutrition quality. This proposal started from good, however, test on the soybean genetically modified with the encoded cysteine protein found later that people allergic to Brazil nuts are also allergic to soybeans, so Pioneer canceled this project. But rumor that GMO soybeans are allergic appeared. The "brazil nut incidence" was the only GMO food case that didn't go commercial as it is allergic.



Figure 89. GMO rice may not be so popular among people.

Pusztai Affair was considered a turn point to start controversy on safety of genetically modified crops. In the autumn of 1998, a scientist Arpad Pusztai from Scottish institute, Rowett, fed rats with genetically modified potatoes of Galanthus Nivalis Agglutinin (GNA) in his experiment and claimed that growth of body and organs of rats had stunted and the rats' immune systems were repressed. The finding immediately made a stir. Pushed by NGO for environment protection, like Greenpeace, in Europe a storm against GMO food was caused. The British Royal Society paid high attention to this affair and organized experts for peer review of the experiment. The result showed that Pusztai's experiment was incomplete and inaccurate, and the conclusion he made was unreliable.

In May 1999, entomological professor Losey from Cornell University published an article on Nature and claimed that he gave *Asclepias curassavica* leaves mixed with Bt

GMO maize pollen to monarch butterfly larvae and found that they were stunted in growth and death rate was as high as 44%. Losey believed that this result indicated Bt GMO crops also threatened non-targeted insects. Later on, EPA gathered entomological experts to study the abovementioned problem of monarch butterflies. However, the result showed that Bt GMO maize pollen had no threats on monarch butterflies. The author conducted a similar experiment before, feeding GMO rice to domesticated silkworms, and through the experiment, I didn't see any adverse reactions and changes. Of course, this was only a preliminary experiment and I didn't trace propagation of different generations.

Huazhong Agricultural University gave GMO rice for small pigs for 90 days. The result indicated that small pigs fed with GMO rice showed no difference from those eating ordinary rice in growth.

The invention of transgenesis makes a great contribution to the society and is a transformation and innovation to nature. Theoretically, a comparatively perfect balance system has been formed for living beings through a chronicle process of evolution. This system should comprise an individual one and an integral one. If exogenous genes intrude, balance will be definitely broken up, which may be bad for living beings. However, for humans, the good outweighed the bad, as humans usually modified genes according to their own wills and purposes. At the same time, we should clearly know that scientific technology is a double-edged sword, and creatures in the nature are never isolated and have formed a complete and harmonious biological chain. Will changing characters of a specie affect balance of its chain? Will exogenous genes affect other species? Or are there any effects of genetically modified food on human health? And so on. Nowadays, all kinds of reports have been published through network and media. Here I still insist that the double-edged sword should be used in right ways, just as dynamite, which can be not only used in wars for killing people but also for tunneling.

5.6 Genetic Modification in Domesticated Silkworms

As we all know, domesticated silkworms spit silk. They have a big silk gland (sericterium). The silk gland of a fifth-instar silkworm can be as heavy as 40% of silkworm weight. Each silkworm can synthesize 0.3–0.5 g of silk protein. Estimated on 80 $\mu\text{g}/\text{cell}$ each day, the protein synthesis speed is faster than that of human livers. What an amazing speed.

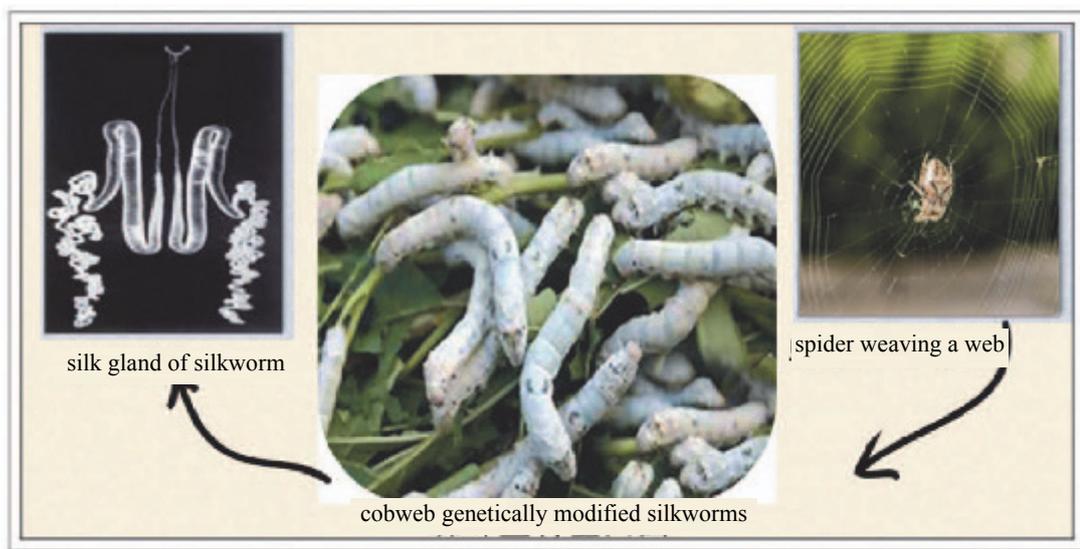


Figure 90. Cobweb genetically modified silkworms.

Scientists are trying to transfer exogenous protein genes into the silk gland of silkworms. That is to say, the silk gland of the silkworms is used as a bioreactor to product exogenous protein for keeping humans healthy. At the beginning of 2012, Donald Jarvis of University of Wyoming and his colleagues cultivated a genetically modified silkworm that can express a spider gene sequence. The genetically silkworm can spit tough fibers containing spider protein, and the fibers are flexible and ductile and can be used for medical and military purposes. This result was published on Proceedings of the National Academy of Sciences of the United States. Malcolm Fraser, a biological professor in University of Notre Dame, found that a genetic factor known as piggyBac transposons could play an important role in breeding of the genetically modified silkworm. This genetic factor can change the original genetic structure and sequence just as operation of “cut and paste” in a computer. With the aid of ZFN, researchers inserted spider protein genes into silkworms, so that the silkworms can spit super silk similar to cobweb. It is said that the super silk can be used for producing bulletproof vests. I think you may know an American movie Spiderman, who is superhero shaped by Marvel Comics. What appeals me is cobweb in the move, it may be exaggerated but magic, and its special functions were fully shown in the movie. Scientists had found the excellent performance of cobweb. In terms of mechanical strength, cobweb can be comparable to Kevlar fibers used for making bulletproof vests and light helmets. Its tensile strength is a dozen times of steel. In terms of flexibility, cobweb can be dragged to 1–1.5 times of its length without breakage, and is extremely flexible. However, spiders are never hard workers. Natural cobweb is mainly used for making a web and is quite low in production. In addition, spiders eat each other, and cannot be bred in large scale, while silkworms can well solve this problem. Just waiting, a bright future will come for silkworms.

In early 2014, National Institute of Agrobiological Sciences of Japan cultivated a

silkworm implanted with a green fluorescent protein gene. The genetically modified silk of this silkworm can be different colors under sunlight. For example, under a blue light, it will show fluorescent green under an orange filter. Further, scientists are working on transferring fluorescent protein in the nature into silkworm silk and making silkworms spin natural colorful silk. Thus, environment pollution caused by dyeing can be reduced but also clothes made of silk are healthier and environment friendly.

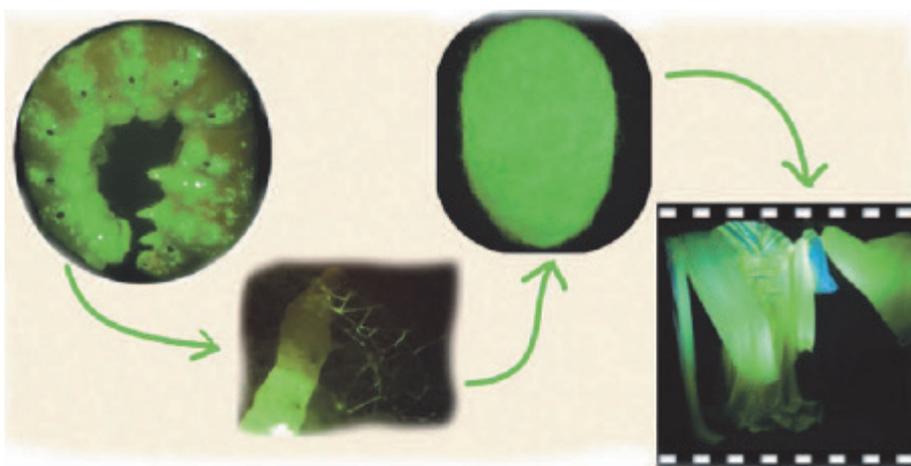


Figure 91. GMO silk made into clothing fabric.

In recent years, antibody medicine gradually turns to be novel diagnostic agent and therapeutic agent. Antibody is an immune globulin (mainly generated by leukomonocyte) generated by organism in response to stimulation of antigenicity. It can combine with heterogenous materials and play a role in preventing and treating diseases. To put it simply, antibody is also a protein, a glycoprotein with Y-shaped looking and is composed of two heavy chains (long) and two light chains (short) in pairwise. The antibody can identify a “specific” part of an exogenous substance, and this part is called “antigen”. Antigen and antibody are like a couple, if antigen compares to a wife, then antibody to a husband. When the wife enters a room, the husband will hold his wife in arms. Therefore, in brief, the antibody may kill a pathogen entering to an organism or neutralize harmful substances. As the antibody is so powerful, can the antibody genes be expressed via silkworms? The answer is yes.

Scientists assume that, if they use the silk gland as a bioreactor, set up a proper vector, connect the heavy chains and light chains of the antibody to the vector and obtain two vectors, later the two vectors are injected into silkworm eggs respectively by microinjection, the heavy chains and light chains of the antibody are inserted into a fibroin gene through recombination, and in this way, the genetically modified silkworms with the heavy chains and light chains of the antibody are bred. Thus, the genetically modified silkworms can spit protein carrying antibody heavy chains and light chains. Finally, they smashed cocoons to separate and purify, and the antibody what they need is

obtained. So far, this project has been conducted in Japan.

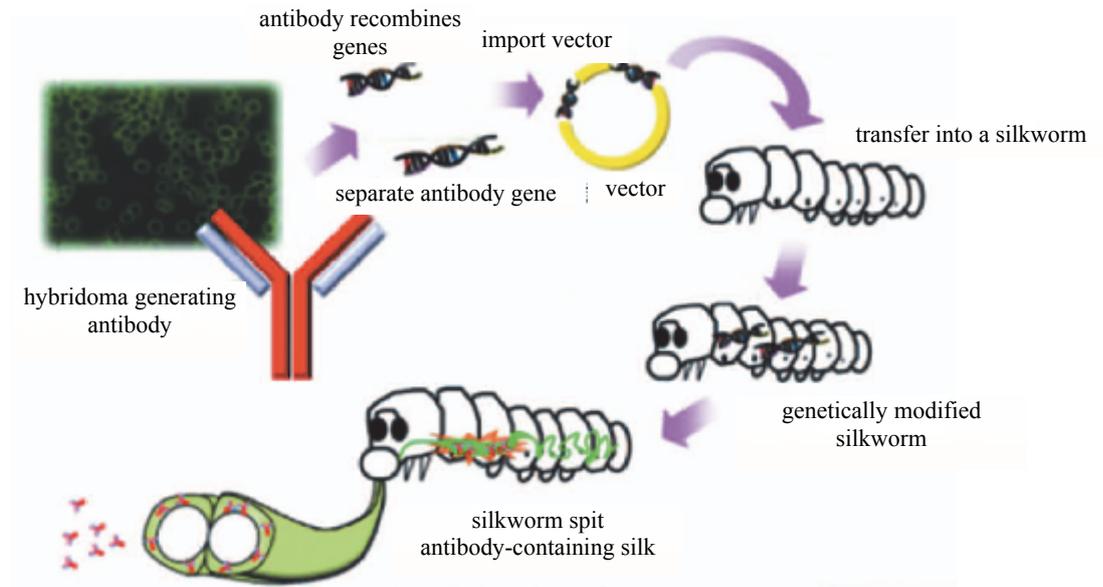


Figure 92. Antibody production process.

6 Genome Sequencing Unveiling a Secret of “Tian Chong”

6.1 Magic Four of DNA

As you sow so shall you reap, and this is experience people get from life. To be astonishing, how accurate the biological inheritance in the nature is and what things are manipulating this in organisms? It's DNA. Frederic Griffith was the first person discovering DNA is a genetic substance. British physicist Francis Crick and American biologist James Watson discovered the double-helix structure of DNA together. Crick was born in June 8, 1918 in Northampton of England and majored in physics in college. He also deeply interested in biology. He was enlightened by a book, *What is Life*, written by Erwin Schrödinger, a famous physicist. The book predicted that a new era of biological research is about to begin and pointed out that the biological problem will be definitely solved by physics and chemistry and it is more likely to discover a new physics law in biological researches. In 1951, Crick met Watson in the Cavendish Laboratory in Cambridge University, a well-known lab where James Maxwell, Ernest Rutherford and Bohr used to work here and 30 Nobel Prize winners came from. Later, they usually argued on academic problems but admired each other. They soon reached a consensus that the molecular structure of DNA is a key to unveil the secret of heredity. Once by chance, they attended an academic conference in Naples. There, Wilkins showed them a picture without letting Franklin know. Watson and Crick were excited after seeing the picture and immediately realized a fact that has been well known now, two chains backboneed with phosphoric acid are intertwined to be a double-helix structure, which is connected by hydrogen bonds. Afterwards, they proposed the double-helix structure of DNA and published it in *Nature* on May 25, 1953. The double-helix structure shows that DNA molecules can replicate themselves during cell division and clearly stated that to maintain multiplication of offspring and species stable must have the mechanism of the genetic nature and replication in cells. This finding was a milestone in biology and unveiled the prelude of an era of molecular biology, so that genetic research could upgrade to a molecular level. About 50 years later, new sciences, such as molecular genetics, molecular immunology, and cytobiology, turned up like mushrooms after rain. The secrets of life were more clearly uncovered from an angle of molecules one by one. The recombination technology of DNA opened up a new way for study and researches using biological engineering means. Crick said, “double helix is an extraordinary molecule. Humans have about 500,000 years of history, civilization began 100,000 years ago, and the United States has been built for 200 years. However, DNA and RNA have

existed for billions of years. Double helix is active in living beings and we are the first to find it on the Earth”.



Figure 93. Watson and Crick.

6.2 Deciphering “Magic Four”

The mysterious genetic material, DNA, is composed of Magic Four. It has four codons, A (Adenine), T (Thymine), G (Guanine), and C (Cytosine), which are randomly sequenced. However, humans cannot find out a good method to determine these four basic groups in DNA. If DNA is a book with genetic codes hidden, humans who even don't know the 4 basic letters making up a password will neither have a chance to decipher genetic codes and explain the mystery of life nor read the book of “Magic Four”.

The barrier was not broken through until a genius scientist Frederick Sanger turned up. Sanger was a British biochemist and won two Nobel Prizes in Chemistry in 1958 and 1980. He was the fourth person to be awarded Nobel Prize twice and the only one who won two Nobel Prizes in Chemistry. Sanger created a genetic study sequencing method of humans and was honored as “Father of Genetics”.

20 years later than the discovery of double helix, Sanger invented a chain termination method in 1975 to determine the DNA sequence. The chain termination method can measure the DNA sequence of some basic groups for a very small number of viruses, but was in vain for longer DNA. For humans, a large DNA sequence is more frustrating for them. However, hero always shows up in the critical juncture just as described in an ancient Chinese kongfu novel. Of course, some other scientists discovered a special restriction enzyme in *Colibacillus*. This enzyme is as sharp as

scissors and can cut DNA in a specific sequence into small fragments. The finding solved the first problem in Sanger's research, and even extremely large DNA molecule can be cut into small fragments prior to sequencing by the restriction enzyme. In 1978, Werner Arber, Daniel Nathans, and Hamilton Smith who discovered restriction enzyme shared a common honor of Nobel Prize in Medicine and Physiology.

6.3 Polymerase Chain Reaction (PCR) Technology

During this time, life science has been more and more popular. To sequence large genomes is around the corner and genomes of various species have been accessible. However, how to amplify DNA in vitro and make researches materials accessible is another challenge in the study of life science. The challenge is always accompanied by a solution. Later, another in-vitro amplification technique, PCR, came into being.

It is not hard to understand the principle and practice of PCR. Since the double helix structure of DNA was discovered in 1953, people have clearly understood the replication principle of DNA and that DNA will inevitably replicate during cell division. Polymerase used for replicating DNA in cells was separated successfully early in 1956. Replicating DNA in test tubes has been a common method. If DNA is a double-chain structure, it must open two chains at first during replication. At high temperature, two strands of DNA chains will be separated into one. As temperature gets down, two complementary DNA chains can restore back to two strands again. DNA molecules are resistant to high temperature, but polymerase required for replication of DNA is protein, which will inactivate at high temperature. This is also one of the reasons that researchers had no solution of amplifying DNA in vitro. In addition, it is as hard as finding a needle in a haystack to make a "primer" from a known sequence in thousands of DNA to "fish" required fragments and replicate them. This is another reason deterring biologists. To enlarge, or biologically, amplify DNA in vitro needs to extract DNA from an organism and design a primer, and the primer is usually the same as a DNA template (the double helix of DNA is formed in a manner of A-T and G-C. At last, under the action of polymerase, DNA is modified at different temperatures, synthesized by the primer, and replicated, and amplified exponentially.

Apparently, though scientists had learned the principle of DNA replication, it is hard to successfully replicate DNA in vitro. Similarly, at this time another legendary scientist solved this problem. He is Kary Mullis. Mullis invented the polymerase chain reaction, known as PCR and thoroughly solved the problem about DNA replication in vitro. In recognition of his invention, he was awarded Nobel Prize in Chemistry in 1993.

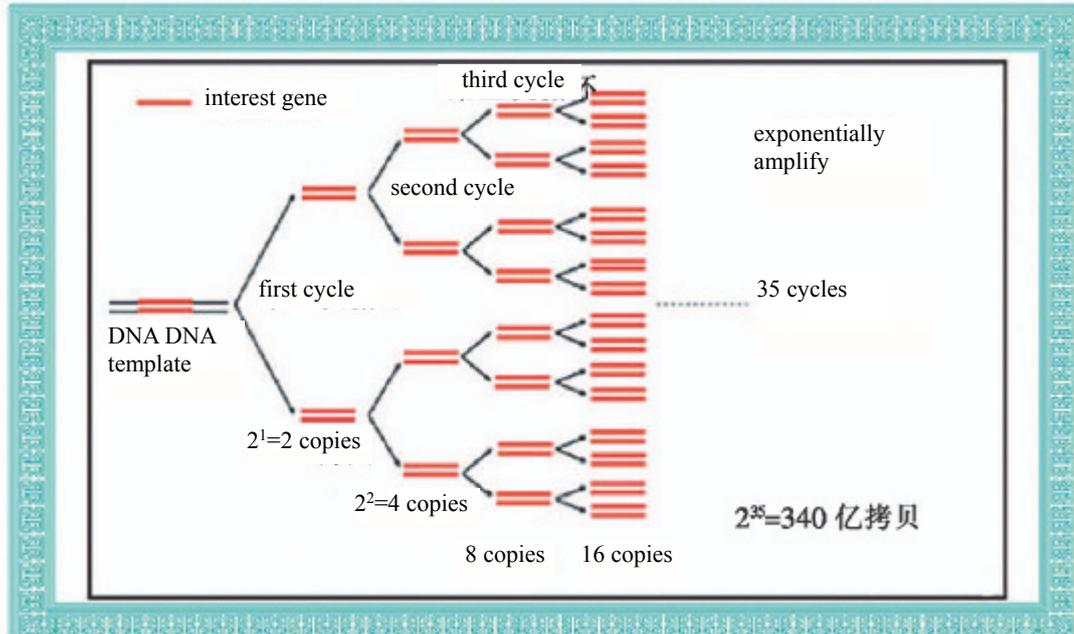


Figure 94. Sketch map of DNA amplification in vitro.



Figure 95. Kary Mullis and his work *Dancing Naked in the Mind Field*.

Talking about Mullis, there are some anecdotes. Mullis attained his doctor's degree of organic chemistry synthesis in UCB in 1972. He was unruly and romantic. When he was young, he had many strange ideas, wrote an article to explain big bang of the universe and submitted it to *Nature*. Unexpectedly, the article was published and gained him reputation. Where is the idea of PCR from?

Mullis once told the source of the PCR concept in his biography *Dancing Naked in the Mind Field*. It was a Friday night in spring of 1983, and he drove his girlfriend to a countryside hub for the weekend. On the winding road, repeated replication of DNA hit

into his mind. Of course, he was a good driver or otherwise it must be dangerous. Mullis thought it might be a simple idea at the beginning and should be proposed before. However, after searching a great many documents, he was surprised that nothing was found. Then Mullis started to practice his great idea. Since September 1983, he carried out experiments, but failed to get right results because of his shortage in molecular biology knowledge. In November 1984, technicians from the Mullis research team attained a reliable result for the first time, proving that PCR was feasible. Later his research team carried out stricter experiments and succeeded. However, Mullis' paper was rejected by Nature and Science. After several twists and turns, his paper was published on Methods in Enzymology in 1984. In the following, PCR and its applications went widely known. However, it was quite complicated to carry out in-vitro PCR at the time since Colibacillus DNA polymerase was easily modified at high temperature and new DNA polymerase should be added constantly. To develop a matured PCR technology needed high-temperature resistant DNA polymerase.

After reading Qian's thesis, Mullis proposed applying the TaqDNA polymerase to PCR. His colleagues followed steps in Qian's thesis and separated purified TaqDNA polymerase in three weeks. In June 1986, TaqDNA polymerase was first applied to PCR and achieved amazingly good results. TaqDNA polymerase simplifies the process of PCR, and its specificity and activity are better than the enzyme before, and background noise is nearly eliminated. Until this time, the PCR technology totally succeeded.

6.4 Sequencing of Human Genomes

With improvement of the DNA sequencing technology, sequencing a large genome seemed ready. After some simple microorganism genomes were sequenced, scientists were thinking of detecting 3 billion basic groups on 24 chromosomes one by one so as to crack up the "book" of human genes from God.

The human genome project (HGP) was first proposed by American scientists in 1985 and researchers from America, England, France, Germany, Japan and China took part in the project with the budget as high as 3 billion USD. The HGP, Manhattan Project, and Apollo Program were called "three science plans", and HGP was praised as the "moon project" in life science.



Figure 96. Human chromosome and genome structure.

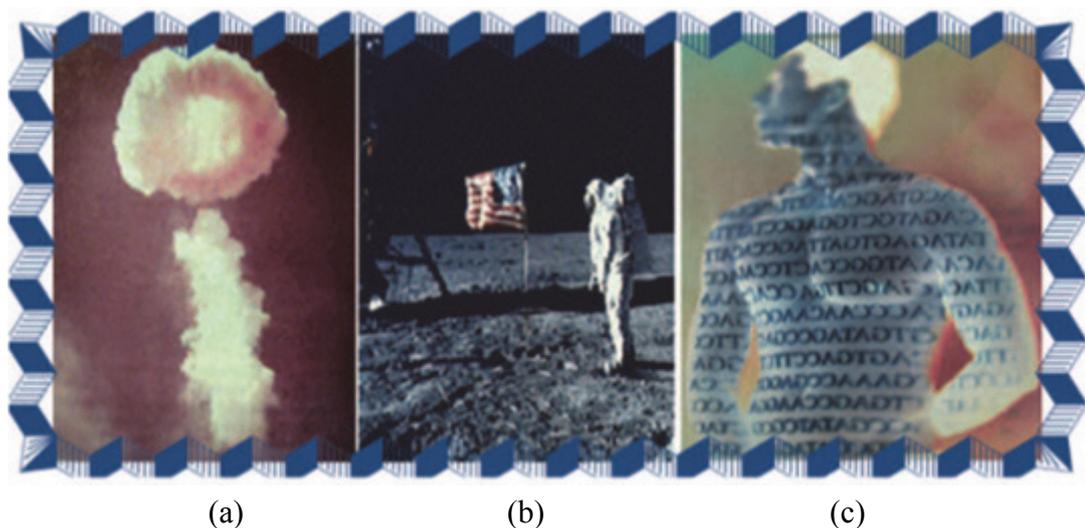


Figure 97. (a) Manhattan Project; (b) Apollo Program; (c) HGP.

In 1988, Watson set off a new journey and presided over the HGP. The HGP was initiated formally in 1990. However, in 1992 Watson was fired for opposing NIH (National Institutes of Health) to apply a patent for human genes and left the project resolutely. In 1993, 43-year-old Collins took over the position of Watson and was responsible for the HGP. Collins was the director of National Human Genome Research Center of NIH, president of Institute of Medicine, and academician of National Academy of Sciences. The work team he led had made great contribution in the discovery of cystic fibrosis, neurofibroma, and Huntington's disease and research of adult promyelocytic leukemia of special type. Collins was so called “Gene Hunter” by people. However, it was not plain sailing for him in the HGP. In the eighth year since the HGP was initiated, another scientist giant Craig Venter stood out. Venter invented a technology of rapidly identifying mRNA in cells and applied in identifying human brain genes. He discovered cDNA, also known as Expressed Sequence Tags (ESTs). In other words, DNA was obtained through reverse transcription of mRNA,

which contributed a lot to the HGP. Venter tried to apply a patent for these identified genes, but failed. He thought the chain termination method invented by Sanger of sequencing genes one by one was ineffective and proposed a “shotgun sequencing” method. The method seems not complex. It is to randomly disorder the genomes, build up a clone library, and sequence fragments respectively, then connect sequences one by one to each chromosome according to fragments and tags in correspondence and a linkage map, and complete a sequence map. In 1998, Venter raised funds to start up Celera Genomics and launched the human genome project of his own, competing with the international HGP led by Collins. Celera Genomics caught up with and overtook the proceeding of the HGP. Collis was faced with great pressure and both sides competed fiercely. The then-president of the United States Bill Clinton came forward and made peace. On June 26, 2000, a great era finally came and Bill Clinton together with presidents of six other countries announced the completion of the HGP draft.

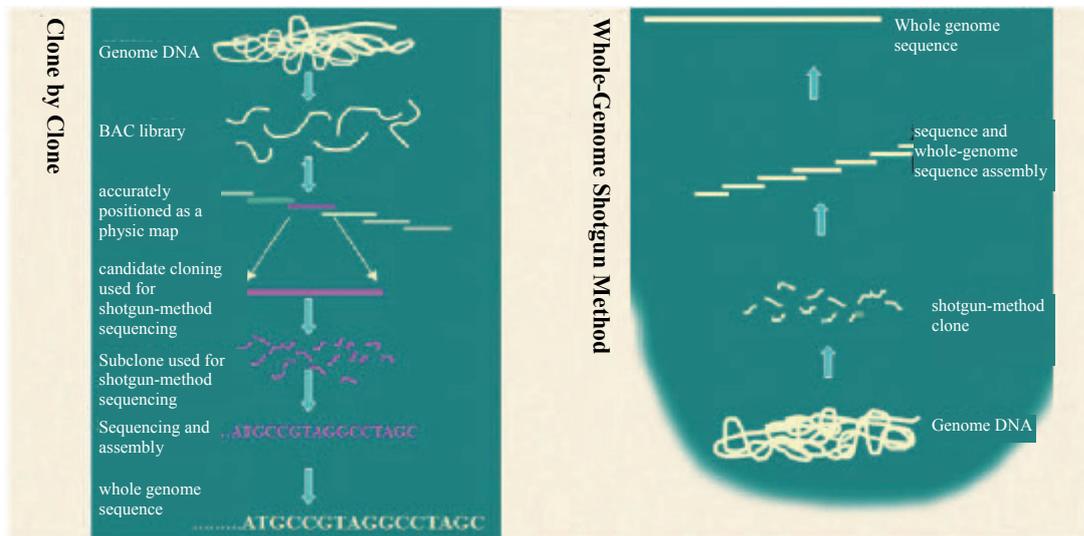


Figure 98. Two methods of genome sequencing.

6.5 “Against War” of Silkworm Genomes

Upon the completion of the HGP draft, the craze spread over the academic circle of the world, and genome sequencing of some model organisms was unveiled. Domesticated silkworms as model organisms of Lepidoptera play a leading role. In September 1995, sericulture authority Xiang Zhonghuai and professor Li Zhengang of University of Science and Technology of China proposed a concept of a silkworm genome project and began to set up a genome biomaterial system. In October 2000, Academic Annual Meeting of Chinese Society of Sericulture Sciences was held and president of the Society made a report entitled Development of Genome Biology and Silkworm Genome Project. Professor Yang Huanming, director of Beijing Genomics Institute (BGI), was invited to Key

Laboratory of Silkworm Genome Biology of Southwest Agricultural University by Academician Xiang Zhonghuai and professor Lu Cheng in November 2000. In December 2000, Xiang Zhonghuai and Yang Huanming jointly made a report entitled Proposal for Modern Silk Road of China in 21st Century on “Promoting Chinese Silkworm Genome Project” in the name of Southwest Agricultural University and BGI and submitted to the Department of Basic Research, and the Department of Rural and Social Development in Ministry of Science and Technology, the Department of Technology and Equipment of State Economic and Trade Commission, the Department of Technology Education and the Division of Development and Planning in Ministry of Agriculture, the Department of Life Sciences of National Natural Science Foundation of China (NSFC), the Biological Bureau of Chinese Academy of Sciences, the Department of Agriculture, Textile and Environment Engineering and Industrial Engineering Technology Committee of Chinese Academy of Engineering. The Proposal pointed that silk enjoyed equal popularity with art of printing, gunpowder, and compass and was a monument of cultural science. The Silk Road has been recognized in the whole world and the treasure of Chinese civilization. As the era of genomes is coming, behind the Silk Road were no longer camels and desert and it must be modern science and technology and international competitiveness of intellectual property of genes. China is a country of silk, and Chinese people are supposed to devote themselves to the competition of the Silk Road of 21st century. Sericulture is the traditional advantage industry in China. Nowadays, there are 20 million farmers, the silk industry with the annual output of 70 billion RMB and foreign trade of billions of dollars in China. The yield and export of silk are 70% and 80% of the total quota in the world respectively. Related gene patent and intellectual proper competition of silk will decide the silk development destiny. We should exert ourselves to rejuvenate the sericulture. The domesticated silkworm has diversified genetic characters and has been studied for a century long as a laboratory insect. The distinct genetic background is an important reason of determining the domesticated silkworm as “representative” in the Lepidoptera insect genome project. Most Lepidoptera insects are harmful for crops and forestry and cause billions of US dollars of direct economic loss each year. Each year about 12 billion dollars of insecticide is cost globally, and human food, health, and environment are faced with more threats. Cracking the silkworm genome is a great contribution to prevention and control of Lepidoptera insects. Crop and forest insects mostly belong to Lepidoptera and Lepidoptera insects account for 80% of agricultural insects. As Lepidoptera insects are highly similar, it only needs to analyze the genes and specific functions of a model insect and can study a new theory and technology of insect control and treatment from the weakness of insects. The domesticated silkworm is a typical representative of Lepidoptera insects and an ideal biological model. The basic study on it contributes 80% to the success of the basic study of Lepidoptera insects. By the study on physiology, pathology, development, behaviors, and genetic lethal genes of domesticated silkworms, the insect treatment technology can be developed from insects themselves, threats of insects and pests can be eliminated

thoroughly, and new breakthrough can be made on control of crop and forestry insects. The domesticated silkworm is similar to humans in the basic life system, substance metabolism, energy metabolism, and mode of inheritance. Amongst the genetic resources of domesticated silkworms there are many disease models, such as cancer model, diabetes model, and developmental malformation model. Study on these models will play a great role in cloning new genes of human disease control, studying disease mechanism, and developing new drugs and have important reference value in treating human diseases and prolonging human lives. The domesticated silkworm is a perfect bioreactor and the silk gland in the silkworm is an effective protein factory. Through genome study, the control mechanism in silk production is thoroughly analyzed, and silkworms can be used for producing high-purity medicinal protein good for humans. So far, South Korea and Japan work together and had used silkworms as bioreactor to produce hypertension and diabetes medicine and attained more than 10 patents. The Proposal accelerates the implementation of the silkworm genome project in China.

The competition on Lepidoptera insects is fierce internationally. In August 2001, 8 countries including Japan, France, and America summoned a preliminary international Lepidoptera insect genome project meeting in Lyon in France. In September 2002, Tsukuba International Research and Exchange Center of Japan summoned a first international Lepidoptera insect genome seminar, and 120 scientists from 12 countries including Japan, China, the United States, South Korea, India, Thailand, France, Greece, Czech, Australia, Newzeland, and Canada. Professors and experts from Southwest Agricultural University, Institute of Plant Physiology and Ecology, SIBS (Shanghai Institute of Biological Science), CAS, and Zhejiang University of China attended the meeting. A year later, the international insect molecular biology and genetics meeting was held in Greece. The domesticated silkworm genome attracted attention from participants and scientists reached a consensus that the domesticated silkworm can be the representative of Lepidoptera. Japan individually initiated the domesticated silkworm whole-genome sequencing in March 2003 and triggered the fierce rivalry globally. Over years of preparation, Chinese silkworm genome project was finally launched in June 2003 through discussions with the support and participation of Southwest Agricultural University, Beijing Genome Institute (BGI) of CAS, National Center for Gene Research of CAS, Institute of Sericulture of Chinese Academy of Agricultural Sciences, Zhejiang University, Suzhou University, South China Agricultural University, and Jiangzu University. Scientists from Southwest Agricultural University and BGI worked day and night and overcome difficulties and announced to the world on November 15, 2003 that the first Chinese silkworm genome framework in the world was completed the Mulberry Key Laboratory of the Department of Agriculture of Southwest Agricultural University and BGI. In only 5 months, the Mulberry Key Laboratory of the Department of Agriculture of Southwest Agricultural University finished all jobs from establishment of the gene library to sequencing and framework assembly and created a new record in the genome study. The research optimized the

genome sequencing technology and greatly lowered the cost. The coverage rate was 6 times of before, and the silkworm genome draft was completed. 16,000 complete genes and 7,000 gene fragments were attained, and it was inferred that the silkworm has more than 20,000 genes, 6,000 of which are new. The genetic information of some important functions related to development, metamorphosis, and gender control was acquired. The completion of the Chinese silkworm genome framework marked a critical winning in the international cooperation and competition of China in the international Lepidoptera insect genome project. The first silkworm genome framework first published to the rest of the world represented the cutting-edge level of China in silkworm genome research. This is the starting point and monument of the new Silk Road in 21st Century.

With the support of sufficient funds, Japan boasted about dominating the research in domesticated silkworms and claimed that 2003 was a Year One of 21st-century Silk Road from Japan. Chinese and Japanese scientists competed against each other, but Chinese scientists won this battle. The world was shocked as the domesticated silkworm genome framework drawn by Chinese scientists was released. Many foreign experts couldn't believe that it was China to realize that. After that, Japanese scientists who were fully funded and owned cutting-edge technology abandoned bias on China and organized an expert team to seek for cooperation with Chinese academicians.

In 2004, Southwest Agricultural University successfully drew out the domesticated silkworm genome framework and published the research results on Nature. At the same time, academicians from the university set up a world largest domesticated silkworm expression sequence tags database, discovered a key function gene group closely related to gender, development, metamorphosis, and hormonal regulation of the domesticated silkworms, and achieved theoretical results of great value in the structural characteristics of the genome, gene structure and evolution, and comparative genomics of the domesticated silkworms. The result had broad impacts at home and abroad and was awarded Top Ten Technology Advances of Chinese Universities of Year 2003 and listed as a Technological Achievement of 60th Anniversary of Founding of China. Chinese scientists made great contribution to the critical frontier fields such as human genomes, plant genomes, organism genomes, and genome sequencing of domesticated silkworms.

After the completion of the domesticated silkworm framework, the research team of domesticated silkworm genome from Southwest University cooperated with a team from Japan early in 2007 with a goal of drawing a fine sequence map of silkworm genome at the lowest cost. In December 2008, the research team announced the completion of the first fine map of the silkworm genome in the world. Compared the former domesticated silkworm genome framework, the fine map of the silkworm genome has the advantages of high gene coverage rate, more complete genome assembly and more accurate gene identification. Its genome sequence coverage reaches 8.48 times, and gene coverage is up to 99.6%. By deep analysis on the domesticated silkworm genome, there were 14,623 genes predicted and on this basis, the integration of the fine map and the molecular

linkage map was completed. So far 76.7% of genome fragments and 87.4% of genes were located on the chromosome of the domesticated silkworm.

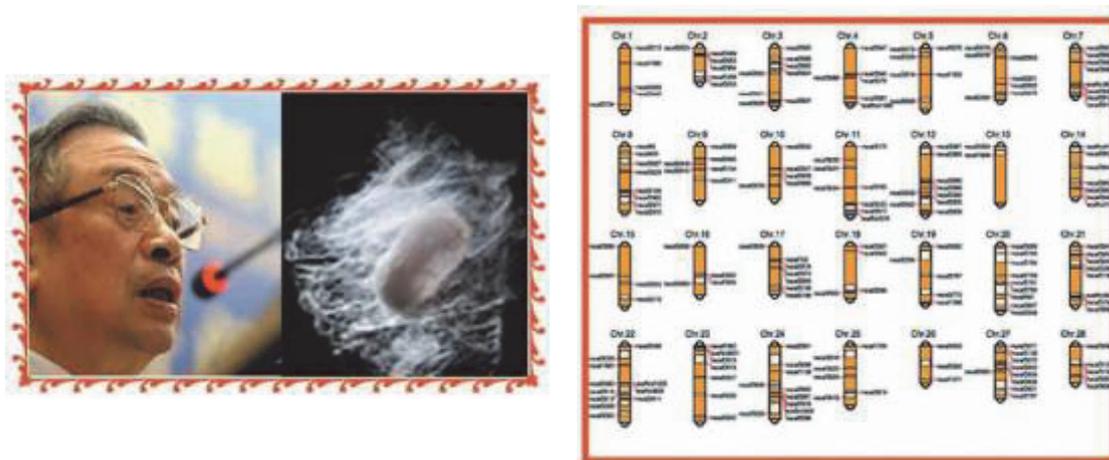


Figure 99. Xiang Zhonghuai was announcing the completion of the domesticated silkworm genome framework to the world.

6.6 Golden Age of Science of Sericulture

Once there is a way, there are people walk on. Since the announcement of the fine map, science of sericulture is thriving and scientists are vying inside. From the perspective of history of sericulture, today should be the “golden age” for it. Researchers of sericulture start their work on the functional genomes and proteome of domesticated silkworms in response to encouragement of academicians Xiang Zhonghuai. Xiang later led a team engaging in sequencing mulberry genomes. This project was carried out by Zhejiang Academy of Agricultural Sciences, Guangdong Academy of Agricultural Sciences, and Chinese Academy of Forestry and took 3 years to complete. The material for sequencing is *Morus notabilis*. In related documents the cardinal number of mulberry chromosomes was identified at 14, however, this number in *Morus notabilis* is 7, which is one of the greatest contributions to the subject of mulberry trees over a hundred years. The sequence of the genome of *Morus notabilis* is about 330 Mb. Researchers identified 123 Mb of repetitive sequences and 29,338 genes, of which 60.8% was supported by sequencing data of transcriptomics. The genome comparison between the mulberry trees and other sequenced Rosales plants shows that the evolution speed of the former is 3 times faster than the latter. Except some Rosales plants, mulberry is one of Eudicots having no genome duplication in 100 million years. Researchers discovered 5 miRNAs of mulberry trees in the haemolymph and silk gland of the silkworms. This shows the mutual effects between the plants and herbivores at the molecular level. Meanwhile, sequencing was also conducted on the destructive pathogenic fungus, *Microsporidia*. At present,

Liaoning Institute of Science of Sericulture and BGI (Shenzhen) worked together and completed the re-sequencing of whole genome of tussah through 2 years of devotion.

Huang Yongping and his team from SIBS, CAS, Meng Zhiqi and his team from Zhejiang Academy of Agricultural Sciences, and Oxitec worked in cooperation to have genetically modified silkworms, synthesize lethal protein in female silkworms, and realize that only male silkworms can survive. Xu Weihua and his team from Sun Yat-sen University published research articles on growth and development, and metamorphosis molecular basis of silkworms (insects) on the international magazines in two consecutive years of 2012 and 2013. Suzhou University, Tufts University of the United States, Beijing Institute of Technology, Third Military Medical University, Southwest University, and Fudan University also cooperated in researching manufacturing methods and performance of various new silk materials (membrane, nanomaterials, and stents) and their applications in medical tissue engineering and achieved great results. Researchers Yao Qin and Chen Keping of Jiangsu University started separation and purification of *Bombyx mori* densovirus Zhenjiang Strain (BmDENV-ZJ) and cloning of genomes since 2002 and independently completed sequencing and analysis of the virus genomes in 2005. They explained the nuclein characters, genome structure, and gene encoding strategies of this virus. In 2010, well-known virus expert Peter Tijssen suggested to International Committee on Taxonomy of Viruses (ICTV) to change the name and classification of the *Bombyx mori* densovirus according to the research results of China on the BmDENV-ZJ. Until 2012, 189 ICTV members voted to decide to establish a new superfamily of Bidnaviridae and a new family of Bidensovirus, name the domesticated silkworm as Bidensovirus and assign the Bidensovirus as a representative specie of the Bidensovirus family. The decision increases the virus classification to 94 and BmBDV is the only virus specie in the newly added virus family. This marks that China has reached the international leading level in the study of DNA Bidensovirus.

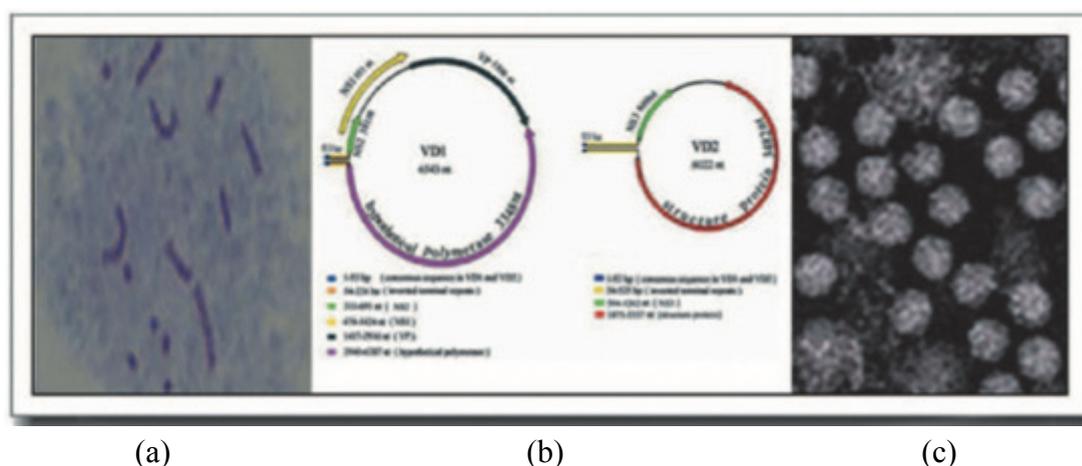


Figure 100. (a) Micrograph of Mulberry (*Morus notabilis*) chromosome; (b) BmBDV genome structure; (c) BmBDV electron micrograph.

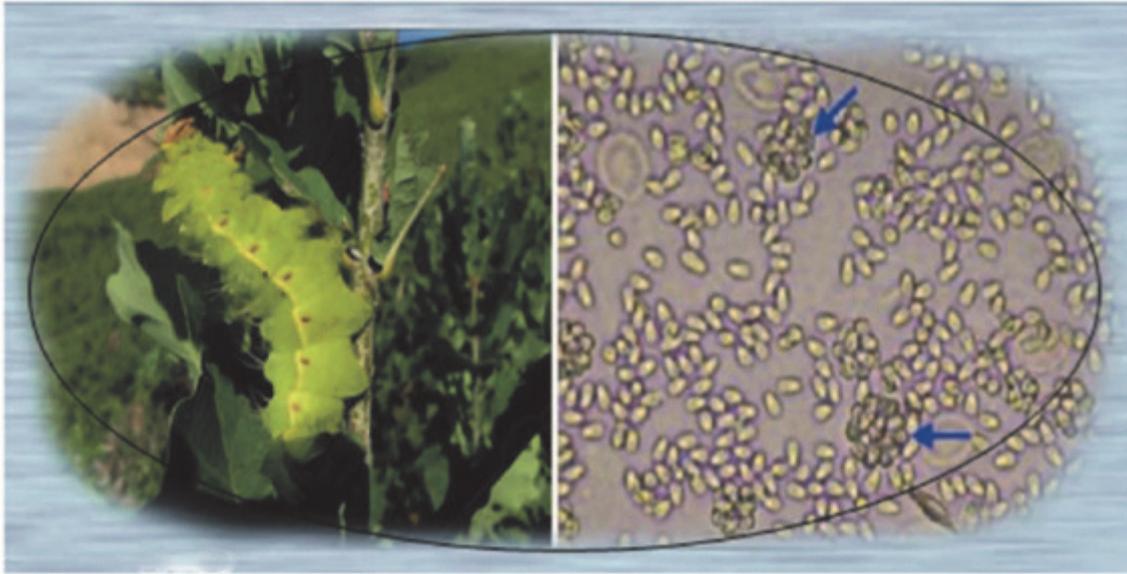


Figure 101. Tussah and micrograph of Microsporidia.

7 Model Organism, Hidden Value in Research

Humans build up a simple model with an intelligent method for uncovering the relationship between phenomenon and essence in natural science. As a matter of fact, the model is an expression form of the system, process, object or concept we study. Exploring the mystery of life and disclosing the essence of life always get started from the simplest organism. In the early study of life science, people always focus on some commonly seen organisms as materials and had no idea about the model organism. With the development of science, more and more knowledges of life emerge and are in urgent need of sorting and summing up systematically to know the whole process of life completely. Nevertheless, people have limited power and will possibly study all living beings one by one. Thus, some representative organisms are selected, which is the first impetus to induce the model organism. At the same time, in the medical field, out of ethical problems, humans cannot be test objects in experiments. So, it is inevitable to find reliable substitutes. This is the second impetus. For example, the gene expression mechanism of *Colibacillus*, cell cycle control of budding yeast, and growth and development of fruit bats are mostly applicable even for mammals and humans. Therefore, it is important to select a proper model organism for researching the life science. By reason of evolution, the basic forms of many life activities or genes are conservative in many species on the Earth. However, some species give birth to new features under natural selection and artificial selection for a long time. From the perspective of molecular level, new genes or metamorphosis genes appear, so that these species can survive and multiple to be a part of a wonderful world and provide a material foundation for study of the comparative genomics.

The model organism is an important carrier to unveil the phenomenon and essence in a simple way. As proceeding of the life science, knowledge and selection of the model organism will update. There should be basic common points as the model organism. First, it should be helpful to solve the problem of researchers and can represent a certain large group of the nature. Second, it should be harmless to humans and environment and easy to obtain and breed and multiple under the laboratory conditions. Third, it should be short in generations and have abundant filial generations and clear genetic background. Fourth, it should be easily operated in experiments, especially by genetic means and phenotypic analysis methods. Fifth, it should be used by the society directly or indirectly and the experiment results can be practiced or be used for collecting necessary information.

Upon the completion of the HGP and coming of the post era of genome study, the study strategies on the model organism have attracted a lot of concerns. This is because we are unable to test on human bodies even in a purpose of analyzing the functions of human genes and the physiological and pathological process of humans. However, the organism evolved from common ancestors and genes of important functions for life

activities are too conservative to make big changes. In other words, the structure and functions of some genes can be studied on other proper organisms. Likely, humans' physiological and pathological process can be simulated on proper organisms.

7.1 Model Organism on Stage

In gene language, humans and rats are “brothers”. A disease study model from rats to humans is becoming a “popular principle” in the post genome era. Fruit bats, rats, nematode, and zebrafish homologous to human genes are undoubtedly in place of humans in research, that is, model organisms for cracking up codes of life phenomena. Present days, the most widespread and commonly seen model organisms are saccharomycetes in fungi, Colibacillus in prokaryote, nematode in lower invertebrates, fruit bats in insects, zebrafish in Pisces, rats in mammals, and Arabidopsis thaliana in plants.

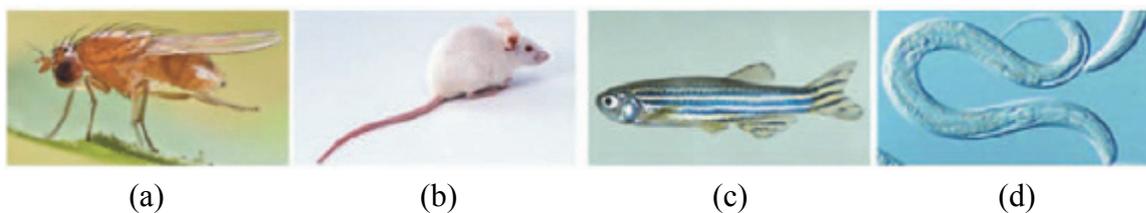


Figure 102. (a) Fruit bat; (b) Rat; (c) Zebrafish; (d) *Caenorhabditis elegans*.



Figure 103. (a) *Arabidopsis thaliana*; (b) Rice; (c) *Colibacillus*; (d) *Saccharomycetes*.

(1) Fruit bat. As early as 1908, genius geneticist Morgan put the fruit bat on the stage of genetics study. Since then, the fruit bat has played an important role on the historical stage of development of life sciences in 20th century. It is an active model organism and can be found everywhere in study of genetics, developmental gene regulation, neurologic diseases, Parkinson's diseases, Alzheimer's disease, drug addiction and alcoholism, ageing and longevity, and study between learning memory and some cognitive behaviors. It has been a hundred years long for the fruit bat being a material in genetics study and the mutant strain used in studying the relation between genes and characters, and during this time, some scientists were awarded Nobel Prize. Nowadays, tools for researching genetics have developed gradually and the fruit bat had made

indelible contribution to extant genetic knowledge.

The fruit bat spreads in the temperate and tropical regions and lives on rotten fruits. So, it can be found in orchards and markets. Except Antarctic and Arctic Poles, there are at least 1,000 species of fruit bats found so far, most of which live on rotten fruits or plants, and some live on fungi, sap, or pollen. Without food supply, the fruit bat can survive for 50 hours, but it can only live for 1 day without water. Food for imago of the fruit bat should contain saccharides, but pupae can grow only depending on yeast. *Drosophila melanogaster* is a dipster, is short in life story and easy to breed, fast in multiplication and small, and has few chromosomes and more mutants. It is a useful genetic experiment material as well as a model organism. *Drosophila melanogaster* originally appear in tropical or subtropical areas and spreads wide. The female is 2.5 mm long. The male is smaller and has dark hind legs, which can differentiate it from female. The female fruit bat lays 400 eggs of 0.5 mm at one time, and the eggs are covered with a chorion and a layer of vitelline membrane, whose development speed is susceptible to the ambient temperature. At 25 °C, larva will break out the eggshell in 22 hours and look for food immediately. As the female fruit bat will lay eggs on the rotten fruit or other fermented organic matters, their primary food comes from microorganisms on rotten fruit such as saccharomycetes and bacteria and sugar-containing fruits. In 24 hours, larvae will cast off skin and grow continuously to the second development stage of the larva. Through the third stage and four days of pupa, the larva will develop into imago in 24 hours at 25 °C. In the field of modern developmental biology study, the developmental genetics of the fruit bat takes the lead. The fruit bat is an ideal model for digging the relation amongst genes, nerves (brain), and behaviors. Over a century, the fruit bat has provided abundant resources for study of different levels of genetics and researchers know it better than other organisms. As the classic model organism, the fruit bat is about to play a greater and irreplaceable role in genetics study of 21st century.

(2) *Colibacillus*. *Colibacillus* is also known as *Escherichia coli* and is a normal flora in intestinal tracts of humans and most of warm-blooded animals. It is a gram-negative bacillus and $0.5 \times (1-3) \mu\text{m}$ in size, is covered with flagella and active, has no spores, and is aerobic or facultative anaerobe. No spore or capsule develops, and a round, smooth and white bacterial colony is formed on agar medium. *Colibacillus* can be obtained from wide sources, and is high in separation and multiplication, short in development period and simple in structure, and is an important model organism. It plays a critical role in modern study of life science, in particular molecular genetics and bioengineering. Just as other bacterial cells, *Colibacillus* is composed of a cell wall, a cell membrane, cytoplasm, and nucleoid. At the center of a cell is a nucleus area having neither karyotheca nor nucleolus, so called nucleoid, where DNA is stored. In September 1997, a whole gene map of *Colibacillus* is drawn out, sequencing of a complete sequence of genomes is completed, and a genome is 4.7×10^6 , including 4,288 genes, whose genetic functions of 60% of which have been explained. In addition, except the nucleus

area, there is a double-chain DNA molecule, which is a genetic matter outside the nucleus and called plasmid. The plasmid contains 2–200 genes of genetic information and can self duplicate. Sometimes, the plasmid can be integrated into DNA. As for bacteria, metabolism without plasmid is not different. Therefore, in modern genetic engineering, the plasmid is always used as a carrier for recombination of DNA.

(3) Retrovirus. The retrovirus is basically characterized by a replication process from RNA to DNA during the life activity, that is, the process of reverse transcription. Under the action of RT (reverse transcriptase), the virus takes RNA as a template, a complementary minus-strand DNA polymerase is synthesized and RNA is formed in the virus. The DNA midbody, Rnase H is hydrolyzed and under the action of DNA polymerase, DNA is duplicated into double-chain DNA. The retrovirus is one of RNA viruses. Its genetic information is not stored on DNA, but on RNA. Most of viruses of this type have reverse transcription polymerase. The retrovirus is 100 nm and has a layer of lipoprotein envelop with surface protruding on the outside. Inside the envelope is an icosahedral nucleocapsid, in which there is ribonucleoprotein of helix structure. It was found under a negative-staining electronic microscope that most of retroviruses (especially those cause leukemia and tumor) are pleomorphic virions and are sensitive to change of osmotic pressure, so they are easily broken up during sample treatment. In recent years, scientists have designed some defective viruses, so that the retroviruses have been useful gene vectors, and can successfully transfer drug-resistance genes into hematopoietic precursor cells and express it in the cells.

(4) Zebrafish. Zebrafish is also known as blue-stripe fish, stripe fish, or zebra danio and originated from India and Bangladesh. Zebrafish is slender and 3–4 cm long and has no strict requirements for water. It will be sexually matured in 3 months after being hatched. Matured fish will lay eggs once every few days. Eggs are fertilized and develop in vitro. Embryos develop synchronously and fast and are transparent. The development temperature should be 25–31 °C. Zebrafish is small and low cost for breeding and can multiply in large scale. The technologies of cell marking, tissue transplantation, mutation, monadic breeding, genetic modification, and gene activity suppression have developed well and hundreds of zebrafish embryo mutants provide rich resources for studying the molecular mechanism of embryo development and some of them could be human disease models. Zebrafish has been one of the mostly emphasized developmental biology model of vertebrates and greatly potential for other disciplines, particularly the employment in saturation mutagenesis and screening of positive genes in large scale. These features make zebrafish be one of the important model vertebrates in the study of life science in the functional genome era. Internationally, zebrafish as the model organism has been used widely to researches of development, functions, and diseases (such as neurodegenerative diseases, hereditary cardiovascular diseases, and diabetes) of various life systems (for example, nervous system, immune system, cardiovascular system, and reproductive system) and has applied to massive screening of new drugs of molecular compounds. The

similarity between the genes of zebrafish and humans is as high as 87%, which means that the results of medical experiment on zebrafish are applicable to humans in most cases, and this is the reason why zebrafish is so valued by biologists. The embryo of zebrafish is transparent and biologists can easily observe the effects on internal organs from medicine. In addition, the female zebrafish can lay 200 eggs and embryos can develop into shape in 24 hours, so biologists can carry out different tests on the same generation of zebrafish to study the pathological evolution process and find out the disease cause.

(5) Rat. The rat belongs to the family of Muridae in the order of Rodentir of the class of Mammalia. A rat evolves from a house mouse and spreads widely all over the world. It has developed into 1,000 inbred lines and independent outbred stock through selection by artificial breeding. Early to 17th century, the rat had been used in experiments. To date, the rat has been the mammal experiment animal that is used in the largest number and gives the most elaborative research results. As a vertebrate for study, especially a model organism of mammals, the rat has its advantages. More importantly, by the transgenic technology and gene targeting, the genetic information of the rat can be modified and transformed in vivo in an expected way. The gene targeting technology can lead any mutation into the genome. In most cases, the phenotype of these mutant rats is similar to the clinical symptoms of patients of some type of diseases. This makes these mutant rats possibly be a good model for human diseases. The disease phenotype of the rat model obtained by gene targeting can maintain stable in the inbred line. Compared with the models prepared by changing nutrition, medicine or operation, these genetically modified rat models are consistent and stable and can reflect the pathological process and molecular change of some human diseases in reality.

(6) Saccharomycete. Saccharomycete is a type of unicellular fungi and not a unit of system evolution. It is the earliest used microorganism in human civilization and can survive in the anaerobic environment. There are 1,000 varieties of Saccharomycetes. Saccharomycete cells are spherical, ovate, linear, oval, lemon-shaped, or bamboo-shaped. Saccharomycete is much larger than a single cell of bacteria, generally at 1–5 μm and has no flagella and can swim. It is of typical eukaryotic structure, composed of a cell wall, a cell membrane, a nucleus, cytoplasm, vacuole, and mitochondrion and some have microbodies. The colony characteristics of most saccharomycetes are similar to bacteria, but the colony of saccharomycete is much larger and thicker than that of bacteria and has smooth, humid, and sticky surface. It is also easy to lift and uniform in the texture, and the front and back sides, the edges and the center are in the same color, most are milky white, some are red and very few are black. The most direction work of saccharomycete as the high-grade eukaryotic organism, particularly the model organism for human genome study reflects in the field of bioinformatics. If someone discovered a new functionally unknown human gene, he can retrieve a homologous functionally known saccharomycete gene in any saccharomycete genome database and acquire related

information about its function in short time, which speeds up the function study of this human gene. The study found that most genes related to hereditary diseases are homologous to saccharomycete genes. Researches on the physiological functions of protein of these gene codes and the interaction with other proteins will deepen the knowledge on the hereditary diseases of humans. In addition, most of serious diseases such as early diabetes, carcinoma of small intestine, and heart diseases are polygenetic diseases. Disclosing all genes related to these diseases is a difficulty and long process, and similarity between saccharomycete genes and related genes of polygenetic diseases will help improve the diagnosis and disease treatment level. The most appropriate case of saccharomycete as the model organism is reflected in researches of related genes of human genetic diseases that are sequenced, verified and acquired by linkage analysis and positional cloning. The nucleotide sequence of the latter and homology of saccharomycete genes provide clues for function study. For example, related genes of human hereditary nonpolyposis carcinoma of small intestine are homologous to MLH1 and MSH2 genes of saccharomycetes, related genes of ataxia-telangiectasia to TEL1 genes of saccharomycetes, and related genes of Bloom's syndrome to SGS1 of saccharomycetes. The genes of hereditary nonpolyposis carcinoma of small intestine express cell phenotype with ribotide short repetitive sequence in the tumor cells. Before this gene was cloned, researchers separate out gene mutations (MSH2 and MLH1 mutants) with same phenotype from the saccharomycetes. Enlightened by the result, researchers inferred that the carcinoma gene is a homologous gene of MSH2 and MLH1 and its homology on the ribotide sequence also proved this inference. Bloom's syndrome is a hereditary disease clinically manifested as precocious puberty. Cells of patients manifested a phenotype of short life cycle when cultured in vitro, and its related gene is homologous to SGS1 of coded Snailase in saccharomycetes. Similar to culture cells of Bloom's syndrome, the yeast cells of SGS1 gene mutation expresses significantly shorter life cycle. Francoise *etc.* studied 170 human genes obtained by functional cloning and discovered that 42% of them are homologous to the saccharomycete genes and the code products of these human genes are mostly related to signal transduction pathway, membrane traffic or synthesis and restoration of DNA and those human genes not homologous to the saccharomycete genes mainly encode some membrane receptors, blood or immune system components or some important enzyme and protein in human special metabolic pathways.

(7) *Arabidopsis thaliana*. Though not an eye-catching flowering plant, *Arabidopsis thaliana* plays an important role of model organism in study of plant genetics and plant development. The genome of *Arabidopsis thaliana* is small but useful to gene positioning and sequencing. Its genome has about 12,500 basic group pairs and 5 pairs of chromosomes, and is small in the plant world. *Arabidopsis thaliana* became a first plant with genome completely sequenced in 2000. To date, it contributes a lot to exploring functions of extant 25,500 genes. Small size and short life cycle are also the advantages

of *Arabidopsis thaliana*. The commonly used specie of *Arabidopsis thaliana* in labs takes 6 weeks from germination to seed maturation. The small strain is easy to culture in a limited space and a single strain has thousands of seeds. Moreover, self-pollination is favorable for genetic experiments. All of these make *Arabidopsis thaliana* one of the important model organisms in genetic study.

7.2 *New Model Organism, Domesticated Silkworm*

Lepidoptera has more than 100,000 species of insects, only second to Coleoptera and is the most bio-diversified cluster in the animal world as well as the most harmful insects in agricultural production. The silkworm is a representative being in Lepidoptera of insecta and is a complete metamorphosis insect of great economic value. It has 28 pairs of chromosomes (the fruit bat has 4 and human has 23) and abundant genetic characters. The silkworm genome is 485 million bp and is only 1/6 of human genome and 4 times of the genome of the fruit bat. Tentatively, the silkworm has 18,500–20,000 genes (the fruit bat has 13,600). In 2002, International Society of Invertebrates officially identified the domesticated silkworm as a Lepidoptera insect.

Chinese people invented silkworm breeding, which benefited the world. Over the past thousands of years, the sericulture industry was only for reeling silk and making into clothes and quilt. However, as the human genome is cracked up, the sericulture workers were enlightened and surprisingly found that the domesticated silkworm is a perfect model organism. Our ancestors left a treasure, which still shines today. Definitely, scientists thought that it was better to class the domesticated silkworm into the Lepidoptera, and cracking up the silkworm genome and explaining the gene functions of the silkworm can provide a mirror for treatment of Lepidoptera insects. As study digs deeper, scientists found it was more suitable for taking the domesticated silkworm as the model organism in life science study. The domesticated silkworm as the model organism has the following advantages.

(1) Abundant species. There are more than 1,000 species of silkworms all over the world. There are 500–800 gene mutation lines stored in labs, including variants of eggs, larva, pupa, cocoon, and moth in shape, physiological and biochemical aspects. These are all good materials for study of life science.

(2) Short life cycle. A generation lasts 40–50 days and can be bred at any time by the artificial hatching technology. 6 generations can be bred continuously in a year and subculture is easy. The silkworms can be bred indoors massively. Except mulberry leaves as feed, artificial feed can be prepared for the time in winter lack of mulberry leaves or if needed at any time and any places. A room about 20 m² large can accommodate rearing frames of multiple layers and breed 20,000–30,000 silkworms at a time. Each female moth lays 500–700 eggs and a male moth can mate with female moths one by one.

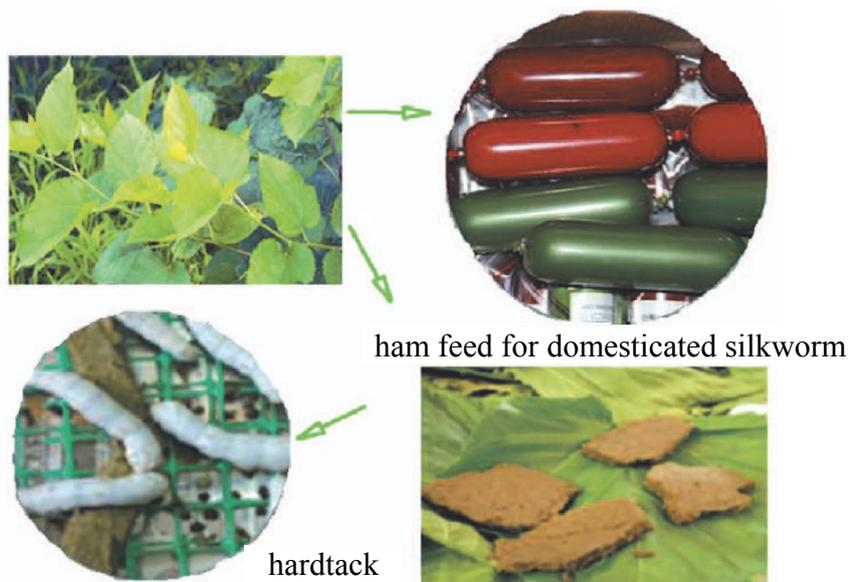


Figure 104. Domesticated silkworm.

(3) Reproduction can be easily controlled artificially. By experimental biological means, female moths can reproduce in an apomixes, that is, to lay eggs without mating with the male moths. Eggs laid by the female moths can be controlled to be all female or male by temperature. This is not only suitable for rerogenetic study but also can obtain a pure line and provides good materials for study of heterosis.

(4) Moderate size. A larva of the fifth instar is as big as a middle finger. Its organs and tissues can be conveniently taken out for culturing in vitro or as experimental materials. The female and male silkworms are easily differentiated and can be as gender study models. A biologist studying mosquitoes told me that he could use a small scalpel to dissect organs of a mosquito, which surprised me a lot. Compared with the mosquitoes, as the biological materials, the silkworms are more convenient to dissect.

In 1906, a Japanese scholar Sotoyama conducted hybrid experiment and chimera study on the silkworm in terms of stripes and color of larva and color of cocoons. He made the silkworm be a first animal to prove Mendelian inheritance. In addition, matrocliny was found on the silkworms, for example, egg color and voltinism. Therefore, we always emphasized the domesticated silkworm at the same level of the fruit bat in genetics. In agricultural production, heterosis was first known during silkworm breeding and massive popularization (1930) of crossbreed applied in the sericulture is earlier than that (1943) of corns. The law of linkage in genetics was first discovered by Bateson in 1906. The law of linkage of the fruit bat was found by Morgan in 1910. In 1916, Toyama used a translucent silkworm and a domesticated silkworm to conduct a crossbred experiment, discovered the sex linkage and proposed that allosome of a female silkworm is ZW and that of a male silkworm is ZZ. About allosome, living beings in the nature are different. The male allosome of mammals, most of dioecism plants, some fish and

amphibians is XY while the female allosome is XX. The allosome is ZW for some other females and ZZ for males. This way commonly exists in Lepidoptera insects, amphibians, reptiles, and birds. In 1930, scientists found a first linkage group of Z chromosome of the domesticated silkworm and at the same time it was found that the W chromosome has an only gene determining female gender. This was the earliest finding in the animals. In 1940, scientists induced translocation of the chromosome by X-ray, moving a gene on an autosome to a chromosome W of the domesticated silkworm. As long as this gene is moved to the chromosome, W it will link with the W. Chromosomes of most living beings exchange through mating. But the silkworm doesn't. Its chromosome W is a complete linkage and will not exchange. Likewise, the chromosome Y of the fruit bat determining the male gender is also in a complete linkage. The chromosome W of the domesticated silkworm is in a complete linkage, so a gene on the autosome easy to be identified according to its shape will be moved to the chromosome W and will not separate. Thus, genes if marked are female. In 1975, scientists of the Soviet Union carried out the study of "chromosome engineering" of the silkworms with "chromosome on atom", and this was the earliest "chromosome project" in the world biology development history. Later, the sericulture workers consecutively developed egg-color determined gender, body-color determined gender, and cocoon-color determined gender. Then what advantages of gender-limited species are there? On production, the domesticated silkworm is mostly first-crossbred. The first crossbred needs to separate female from male during egg production and this is an exhausting work for a silkworm yard. The main job is to split a cocoon with a knife and identify the gender with naked eyes according to the sex organs. It needs a very practiced skill and should be completed in a short time, or else the egg production will be affected. With the gender-determined species, egg production companies can separate the silkworms during the larva stage according to the gender characteristics during breeding. If gender is determined by cocoon color, male and female silkworms can be separated during cocoon selection, and this method is suitable for culturing first crossbred. From this, it can be seen how great sericulture scientists are!

Since the middle of 20th century, the study on silkworms plays an important role in the development of insect science. For example, the prothoracic gland and the corpora allata were discovered, the factors of environment and hormone of ecdysis and metamorphosis were studied for explaining the nutrition needs, researches on digestion and metabolism of silkworms realized artificial breeding of silkworms, 200 gene mutants including shape, physiology, and biochemical characters were positioned, a genetic linkage map of 20 linkage groups was drawn, the structure and biological functions of thyroid-stimulating hormone, diapause hormone, sbombycin, neuropeptide hormone, and physiological activators were separated and identified and the silk synthesis and silk fibrillation mechanism was expounded. On production, hormone regulation on growth and development of silkworms is realized. To be specifically, ecdysone is used to promote

Break the Cocoon

precocity and cocooning if lack of mulberry leaves in the process of silkworm breeding. But these cocoons will be smaller. If with sufficient mulberry leaves, juvenile hormone can be used to let silkworms eat more leaves and the cocoons they made will be bigger. In addition, the transgenic technology is built up and gene recombination is employed to study genetics, physiology, and molecular biology.

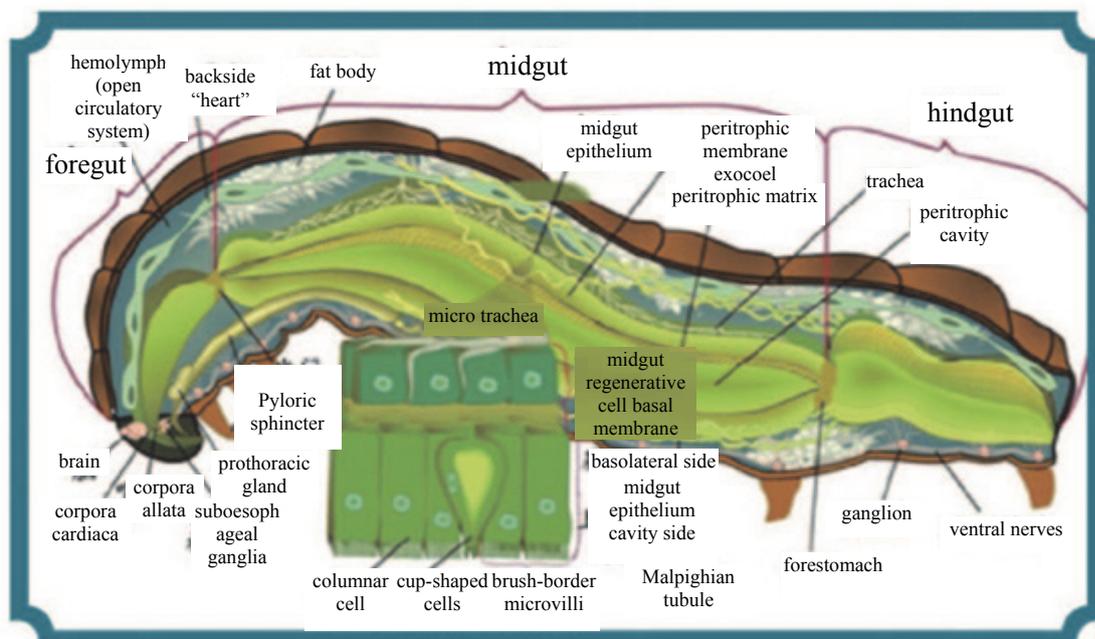


Figure 105. Internal organs of silkworms.

The silkworm has organs and tissues similar to mammals and humans, including brain, heart, liver, kidney, stomach, intestine, nerves, and muscle. These organs play similar functions as humans'. The physiological conditions of the silkworm are approximate to humans' and reaction to drug and receptivity to pathogens are the same as humans'. The practice indicated that the silkworm as the model organism for researches on human diseases and development of new drugs has good results in the first stage and testing is quick and convenient. In the second stage employs a rat for test but the necessary test is effectively carried out in a small scale with special purposes according to the test results of the first stage. The third stage is still a clinical test.

The silkworm as the model organism for researches on human diseases and development of new drugs ha the following advantages. First, compared with rats, the silkworms are low in cost and can be easily attained for the sake of abundant sources. A large number of silkworms can be used at a time and meets the requirements of experimental statistics and many test samples can be evaluated or judged at a time. Second, different from mammals, silkworms, as the model organism, will not cause problems about protection or escape. Third, the test is quick and convenient, and results of a small test can be achieved fast and judged. Fourth, the silkworms are in proper size

convenient for injection and have open blood circulation, and blood injection (equivalent to intravenous injection), intestinal injection, and oral feeding can be conducted on test matters. Fifth, the silkworms cannot escape and survive naturally, so that during test we don't need special breeding and observation equipment and have no worry about "biological disaster". Sixth, the silkworms have organs and tissues similar to humans', whose fat body (equal to the liver functionally) and intestines can be taken out for drug absorption test in vitro.

7.3 Pathogenic Microorganism Evaluation Model

British and Irish scientists found that major cells of mammals and insects will defend after infected with pathogens. 80% of the previous drug trials on rats can be replaced with insects. Compared with the fruit bat, the silkworm has a larger genome and is more complicated. It has abundant mutants in the early stage of embryo and larva and is similar to mammals in metabolism of fundamental substance and energy. These make the silkworm an effective model for testing efficacy and safety of the tested drugs. Japanese scholars found through the intestinal absorption test of the silkworm that metabolism inside the silkworm is similar to humans' and *Candida* and *aspergillus* pathogenic to humans can also infect silkworms. In light of this, the silkworm can be a fungus infection model for selecting antifungal drugs.

Scientists established a virus infection model with silkworm larvae for study of drug treatment and prevention. They injected virus into blood of the silkworm larvae, fed antivirus substance and found that nalidixic acid, and cinnzeylanine, an ingredient of Cassia bark, can significantly suppress virus reproduction and prevent virus from infecting silkworms and causing diseases. Furthermore, they compared the treatment effects of two compounds similar to nalidixic acid and proved that only nalidixic acid has antivirus activity. Nalidixic acid is one of Quinolone antibacterial agents of the first generation. Herpes virus and cytomegalovirus (CMV) of humans can be treated with antivirus drugs and patients can be treated. The antivirus drugs target DNA polymerase and can effectively prohibit Baculovirus of the similar DNA virus. The facts indicate that Taking Baculovirus as a model virus is feasible in developing human antivirus drugs.

During breeding of silkworms, silkworms are susceptible to fungal diseases, for example, white muscardine and yellow muscardine. Humans are also vulnerable to fungi as well. The fungal diseases are commonly seen in clinical skin diseases, caused by *Microsporum*, *Trichophyte*, *Candida*, and *malassezia*. Some *Candida* and *aspergillus* fungi infected humans through skin. Susceptibles with immunity compromised will be infected with deep-seated dermatomycosis. Scholars study the pathogenic mechanism of these fungal diseases with the silkworm as the model organism and select treatment medicine. They injected these fungi into silkworm blood and silkworms would die in a

few days. At the same time, antifungal drugs (such as Fluconazole) with clinical efficacy for humans are also effective to silkworms. Therefore, new antifungal drugs can be selected targeting the treatment effect in a mode of fungi infecting silkworms. The research found that trichodermin, mycophenolic acid, and myrothecium are antifungal.



Figure 106. Silkworm disease models.

Bacterial infection is one of causes threatening human health. There are a surprising number of bacteria in the nature. So to analyze these pathogenic factors and to research and develop related drugs only depending on rats costs tremendously and will kill a large number of rats, which may result in the ethic problems. Therefore, in the clinical experiment, rats are replaced with small invertebrates such as fruit bats, bees, and silkworms, small plants such as *Arabidopsis thaliana*, *Acrasiales* of protozoa, which are used as pathogenic bacteria infection model organisms. This will be the development direction of the bacteria infection study model organisms in the future.

The research team of the author used the silkworm as the model organism and collected abundant bacteria samples from local hospitals. We injected these bacteria into silkworm blood and found that different bacteria have different toxicity on the domesticated silkworms. Some silkworms would die in a few hours, and only a few of silkworms would die in several days. In addition, silkworms fed with bacteria would not die. Larvae just infected with bacteria would eat less and turn slow in action, and then their bodies would swell and turn black and soft. Some larvae would have rigid limbs after their bodies turn swollen and finally the bodies become all black even have black liquid flowing out. This was the result of septicemia and melanism due to bacterial infection.

7.4 Toxin Evaluation Model

If toxins enter human bodies, human bodies will excrete them outside through the detoxification mechanism. Likewise, the silkworm has the same detoxification mechanism. Some bacteria that can kill silkworms can also kill rats. The intestinal absorption of silkworms is similar to humans'. Vancomycin taken orally has no efficacy on humans, neither on silkworms. Chloramphenicol humans take orally has efficacy on humans as well as on silkworms by feeding through the mouths.

The direct method to identify toxins is conducted on organisms. In the early years, European miners would bring a birdcage with three canaries each time they went into a coalmine. The canaries are sensitive to carbon monoxide and will chirp if smell it and the chirp sounds like an alarm. They also make sound if they feel vibration caused by displacement of mine walls and mine bottom. In modern time, scientists use silkworms as vector and trace toxins in the agricultural products, food or environment, just as European workers bringing canaries into the coalmine. It is convenient to detect toxins with the domesticated silkworms as vectors, and harmful substances and dosage can be quickly detected. In production life, heavy metal and other toxic substances are mercury, cadmium, lead, chromium, and substances with significant biotoxicity like arsenic, sulfide, cyanide, and phenol, and wastewater of petrochemistry, electronic instruments, plastics, paintings, and metallurgy always contains these toxic substances. The toxic heavy metals can generate toxins at extreme low concentration (0.001–10 mg/L). Those highly toxic heavy metals like mercury, chromium, lead, and cadmium will be toxic at the concentration of 0.001–0.01 mg. The toxic heavy metals cannot be degraded by any organisms but can only be absorbed by microorganisms to become ingredients of organisms or be attached to the surface of activated sludge full of microorganisms. Under a certain condition, some heavy metals (e.g. mercury) can be transformed into organic matters of higher toxicity under the action of some microorganisms. Toxic heavy metals can stay and accumulate in the environment for a long time, enrich through the food chain, and finally enter human bodies. Some even hurts babies through heredity or nursing. The major manifestation is that it accumulates in some organs to cause chronic poisoning and the harms may show up in 10–30 years. Identification of heavy metals and other harmful substances with the domesticated silkworm as vector is low in cost, fast and effective.

7.5 Immunity Model

Defense of living things against pathogenic microorganisms can be divided into acquired immunity caused by antibody and innate immunity without antibody. The latter widely exists in invertebrates. The latest study proved that the silkworm has the same

molecular mechanism as humans. The same as common insects, silkworms have no acquired immunity system generated by antibody. In a long evolution process, inside silkworms there is an effective in-vivo defense system against attacks from microorganisms or eukaryotic parasites. The defense system includes natural barriers including skin and intestines, immune response of blood cells, and a blood immune response composed of blood cells, fat body, and immune protein synthesized and secreted by other cells. This is an innate immune system. The innate immunity is an in-vivo defense mechanism playing an important role in the early stage of pathogens like bacteria, fungi, and viruses before antibody is formed. Scientists gradually understand the relation between these diseases and the innate immunity mechanism during their study on various metamorphosis diseases such as allergy. The silkworm is an invertebrate and cannot generate antibody, but it can deal with the attacks from pathogens with the innate immunity mechanism. Therefore, the silkworm without the acquired immunity system is a useful model material for studying the innate immunity. It was reported that the active substance of innate immunity could lead to contraction of muscle of silkworms. By decapitating a silkworm and injecting the active substance of innate immunity from the decapitated end, the innate immunity system of the silkworm was activated, muscle contracted and as time passed, the silkworm body turned shorter and shorter. In addition, the contraction of muscle had no response to LPS of bacteria and testers had no worry about false positive caused by bacterial pollution. Hence the silkworm is suitable for studying the innate immunity.

7.6 Drug Metabolism Model

People usually believed that humans and silkworms have different responses to efficacy of drugs. Actually, it is not true. Plenty of researches indicated that the in-vivo dynamics of taking silkworms as vector for analyzing drugs are the same as the results of taking mammals as vector. The conclusion in drug treatment effect in the silkworm-as-vector study can predict the treatment effect of drugs on mammals. The silkworm can be inoculated in different ways such as blood injection, intestine injection, and mouth feeding. The latter two are equivalent to oral administration of humans. To evaluate efficacy of drugs from the perspective of drug injection, the silkworm is more convenient than the fruit bat and nematode. The fruit bat and nematode should be injected under a microscope and in a short time it is impossible to test a large number of samples. In addition, in light of anatomy, the intestinal canals compare to those of humans' and the fat body of the silkworm to a liver of mammals. Moreover, the intestinal canals and fat body of the silkworm can be taken out for in-vitro drug test. It is important to observe in-vivo dynamics of the treatment drugs by evaluating the treatment effect with the silkworm as vector. The experiment manifested that concentration change of

compounds in the silkworm blood and metabolism are similar to mammals' and compounds stable in silkworms are also stable in mammals. In light of this, we can test reversely. We carried out stability test in the silkworms and looked for stable compounds in mammals with the stability in the silkworm as an index, setting up a candidate drug reservoir. Then for the compounds in the reservoir, in an activity test of taking mammals as vector and killing pathogens one by one, scientists usually injected extract of a tested object into the midgut of the silkworm, and searched the effective compounds by detecting the conditions of compounds in the silkworm blood in a few hours. In this way, time and cost for developing drugs can be greatly reduced.

7.7 Diabetes Model

The SSPG of the silkworm hemolymph is exposed to food impacts. By adding glucose or canesugar into silkworm feed, the SSPG will go up immediately, and the developmental growth will be suppressed. If glucose addition is suspended, the blood sugar will be slowly down giving mulberry leaves to silkworms. The fact shows that the silkworm can regulate the blood sugar of its own. Inside the silkworm exists hypoglycemic hormone, called bombyxin. It is similar to insulin in humans. We commonly know that insulin is used for diabetes treatment. The research indicates that insulin can lower the high blood sugar of the silkworms, which can be seen that the molecular mechanisms of the silkworms and humans in regulating blood sugar are similar. Feeding melbine and 5-Aminoimidazole-4-carboxamide-1- β -D-ribofuranoside, diabetes treatment drugs, to the silkworms can also alleviate the symptoms of high blood sugar and resume the normal growth of the silkworms. Testers injected glucose solution at the concentration of 5%, 10%, and 15% in vitro to regulate blood sugar concentration of the silkworms and detected blood of different time points with a sulfuric acid-phenol method and glucose oxidase method. The results showed that high blood sugar could seriously impact the weight and growth of the domesticated silkworms. The phytochemical parameters comply with the standard of the high blood sugar model. Injecting pure water to the domesticated silkworms shows no abnormalities in growth. However, injected with glucose, the silkworms grow slow and turn smaller as the concentration is up.

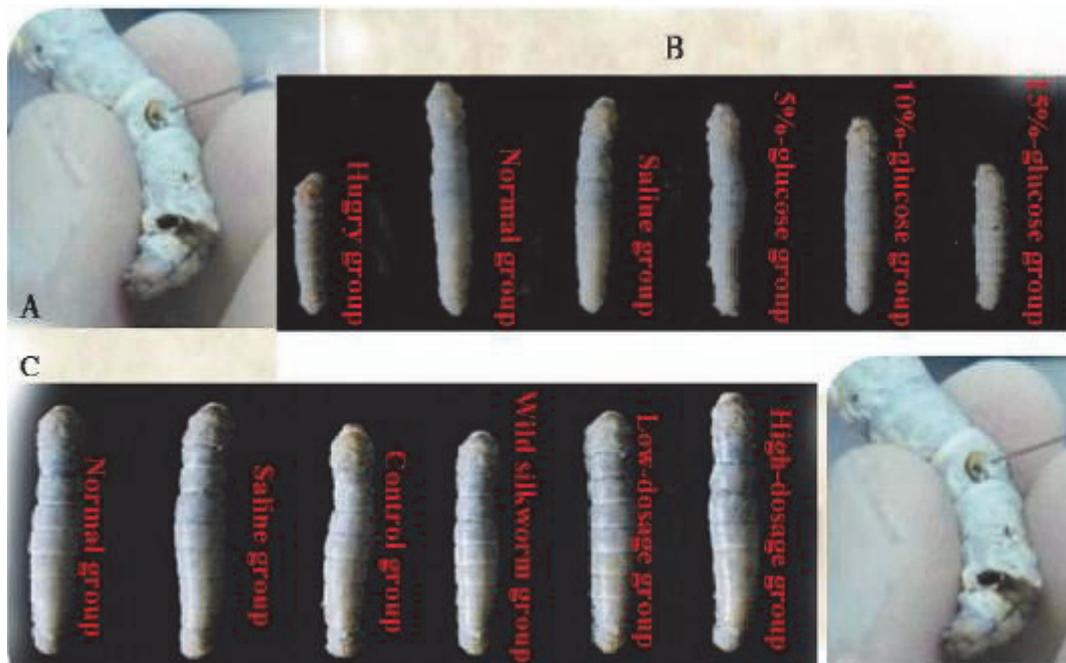


Figure 107. Diabetes model of domesticated silkworms.

The test shows that high-dosage glucose solution can evidently inhibit development and growth of the domesticated silkworms, especially in the 15%-glucose group, the weight and length of the domesticated silkworms are significantly drop drastically. In case the total sugar concentration in the silkworm blood is 9 mg/ml, it needs 3 days to establish a model and costs low. If rats are used instead, the cost may be several times higher. Some scholars added glucose into a fish pond to establish a zebrafish high blood sugar model and a tilapia high blood sugar model so as to evaluate the hypoglycemic effect of the oral administration of extract of *Ocimum Sanctum*, a tropical plant. However, compared with the glucose injection method of the silkworms, they take longer time.

Since the establishment of the high blood sugar model of the silkworms, the research team inserted wild brevibacterium into human proinsulin protein to obtain recombinant spores. Adding proinsulin protein into feed of silkworms, the silkworms of the high blood sugar model have the blood sugar down and weight resumed. The team verified with a rat diabetes model and got the similar results. This shows that the domesticated silkworm as the diabetes model for humans is feasible and also implies that probiotics can be used as a vector and recombining the insulin into probiotics of oral administration can lower the blood sugar and adding the recombinant insulin probiotics can resume normal of the silkworms of the diabetes model.

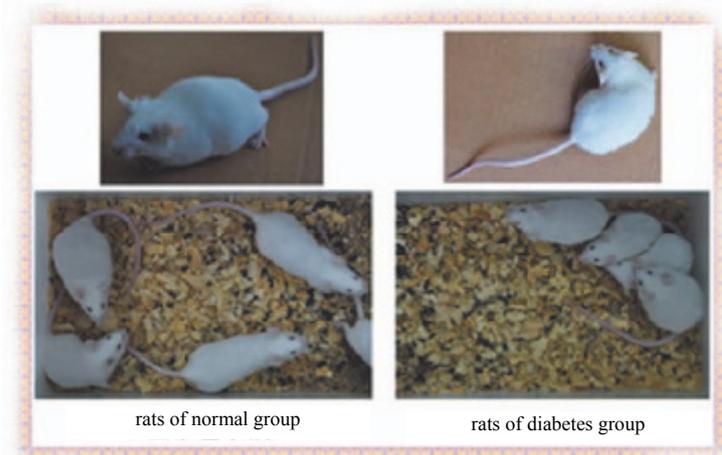


Figure 108. Adding the recombinant insulin probiotics can resume normal of the silkworms of the diabetes model.

7.8 Hepatopathy Model

The liver is a largest digestion gland in a human body and a central station of physical energy metabolism in the human body. As estimated, there are more than 500 chemical reactions in the liver. The liver is able to secrete bile to digest food, to absorb amino to synthesize protein and energize us in a whole day of work, and to store and burn up fat in vivo to control our figure. It is an organ storing liposoluble vitamins and able to oxidize, reduce, and decompose toxins in human body and to swollen bacteria taken in by accidents and is the largest detoxification organ of the human body. The experiment shows that animals with the liver removed can only survive 50 hours at most even if being given corresponding treatment. This proves that the liver is an indispensable organ for maintaining life activities. In modern times, drugs for treating the liver diseases are far from enough. The liver diseases are not easily found and patients have no symptoms appearing in the early time, so that the liver is called a “silent organ”. Medically, rapid and simple hepatotoxicity tests to develop drugs for treating the liver diseases or functional food are in urgent need. The Pharmaceutical Department of University of Tokyo worked with the Institute of Genome and Pharmaceuticals to select the pro-drugs for treating the liver diseases with the hepatopathy model. They inject extract of a certain plant to a “liver disease stricken silkworm” due to carbon tetrachloride and then found that alanine aminotransferase caused by carbon tetrachloride dropped immediately and manifested possible curative effect. Further, they fed the silkworms with A-contained feed and injected carbon tetrachloride. Later, it can be found that the concentration of alanine aminotransferase in the blood has no rise, which said that this matter has a preventive effect.

7.9 Nervous Model

Parkinson's disease is a common degenerative disease of the nervous system and mostly hits the elderly. The average onset age is around 60 years old and youths younger than 40 years old are seldom hit by Parkinson's disease. The morbidity of population over 65 years old is about 1.7%. Most of patients with Parkinson's disease are sporadic cases, and less than 10% of patients have family history. The major pathological change of Parkinson's disease is dopamine causing degeneration death of neurons and resulting in drastic reduction of the content of striatum dopamine. The accurate cause of this pathological change is still unknown and the heredity, environment, aging, and oxidative stress may involve in the degeneration death of dopaminergic neuron leading to the Parkinson's disease. In recent years, scholars also valued using "lemon lethal mutation" of the silkworm as a study model for tetrahydrobiopterin deficiency and Parkinson's disease. The tetrahydrobiopterin deficiency can cause growth disorder and dyskinesia of humans or mammals. Interestingly, normal larvae are bluish white, and the larvae of the second instar of the lemon lethal mutation is dark yellow all over the body and will die of failing to take in food. The uranidin here is xanthine. The cause of the mutation is a mutation in a tetrahydrobiopterin reductase gene and results in significant drop of its activity. The lemon lethal mutation silkworm is fed with tetrahydrobiopterin through the mouthpart, the larva of the second instar can grow normally and some of them can develop to be imagoes. Likewise, to feed the silkworm with dopamine, then testers observe its growth. The results indicated that the cause of death of the lemon lethal mutation silkworm is that deficiency of the tetrahydrobiopterin reductase lowers the activity of tyrosine oxidase, and further reduces the synthetic amount of dopamine and resultantly the silkworm cannot take in food. It is known that the reduction in secretion of the dopamine is also one of the causes of Parkinson's disease. As the lemon lethal mutation silkworm can be treated with tetrahydrobiopterin and dopamine, the silkworm must have value as a model organism for researching the treatment methods of tetrahydrobiopterin deficiency. So far Chinese scholars have started this research.

7.10 Arthrolithiasis Model

Arthrolithiasis is a disease of high morbidity and brings huge mental pain and economic burden on humans. Over time, Arthrolithiasis drugs are usually selected with rats as the model, however, the purine metabolism pathways and products of rats are slightly different from humans'. This brings a series of problems for drug screening for Arthrolithiasis treatment. Urate oxidase can metabolize urate into soluble Allantoin. Mammals mostly have urate oxidase genes. In case of deficiency of the urate oxidase genes, urate will accumulate and the metabolism level of urate is one of critical indexes

to judge whether people have Arthrolithiasis. Generally, scientific study simulates a rat Arthrolithiasis model with a method of adding urate oxidase inhibitor into food and then screens drugs for treating Arthrolithiasis. However, the metabolism conditions of urate cannot be simulated in reality and the rat model is expensive and causes the ethic and moral problems. Therefore, it is necessary to develop an animal model more suitable for screening drugs. The domesticated silkworm has no urate genes as humans and the final metabolism product urate of purine is the same as humans', so that it is believed that it can be used for screening the drugs for Arthrolithiasis drugs. Some scholars fed the silkworm orally with Allopurinol (a type of inhibitor for xanthine oxidase) for treating human Arthrolithiasis. The result showed that Allopurinol could significantly lower down the urate level in the silkworm. Also, others fed sodium bicarbonate orally out of the consideration of promoting urate excretion and the result showed that the sodium bicarbonate could speed up the urate metabolism of the silkworm and lower down the urate level as well. These experiments indicated that the silkworm model for screening and evaluating the Arthrolithiasis drugs is cost effective.



Figure 109. Skin (of mutant) with urate accumulation and skin (of normal silkworm) without urate accumulation.

As study proceeds, more and more research models for study of life science came into being. The silkworm model is also a new trend, but has shown great potential. Scholars have different views. They believe the domesticated silkworm is too sensitive to exogenous microorganisms and harmful substances and is not feasible as the research model. However, the author thinks that animals sensitive to the exogenous microorganisms should be better for being models. The development of natural science always goes from quality to quantity. Similarly, detection usually starts from insensitivity to sensitivity. Metaphorically, from a big ocean, you take water with a small bowl, and then throw a stone into the ocean and the bowl respectively, so what is the result? The stone thrown into the ocean looks nothing different, however, the stone into the bowl can splash water in all directions. Now think deeper, both with outside stones, but one makes

response, and the other doesn't. Just like the silkworms and some macroorganisms, the silkworms will respond to attack of very few of exogenous substances and can detect, however, macroorganisms have no response to intrusion of a small number of microorganisms. Therefore, it is feasible and effective to use the domesticated silkworm as the research model for detecting intrusion of exogenous substances. Though different from humans, the silkworm can quickly or tentatively identify the intrusion of exogenous substances and analyze the toxicological effect. Then the result can be verified with mammals. Thus, cost and time can be saved much and the silkworm as the research model must be promising.

From *Colibacillus*, yeast, *Arabidopsis thaliana* and nematode to zebrafish, fruit bat and rats, these model organisms play an important role in the study of life science. As the modern study of life science develops, new research models are forming in order to break through limitations of original model organisms in specific study, for example, hydroid may become the research model for formation of the nervous system network and action mechanism, and *euprymna scolopes* are becoming a model for researching symbiosis, and Australian pademelon is the research model of embryonic development.

There are many similarities between the domesticated silkworms and humans in metabolism of fundamental substances, energy metabolism, and inheritance patterns. The domesticated silkworms are higher in the degree of differentiation and bigger than the fruit bats and provides a research model of moderate complexity and feasibility for some basic disciplines and applied disciplines of life science. Based on extant abundant mutation gene resources of the domesticated silkworms and transgenosis, new mutation types are created, the individual silkworms with mutation genes related to the growth and development, diseases, and environmental receptivity can be selected, their genetic, pathological and molecular biological characteristics should be studied in depth to form the silkworm as the model organism. The model organism of the domesticated silkworm has the advantages of innovation from the origin and unique resources. In the posterior era of genome, even if with a single scientific purpose, there will be multiple model organisms for comprehensive analysis. New model organisms will show up increasingly. China's HGP of domesticated silkworms will develop the systematic study of the domesticated silkworm as the model organism. The establishment of the domesticated silkworm model organism system with Chinese characteristics and its application in prevention of Lepidoptera insects, human disease organism models, drug screening, and environment detection will add advantages to related industries of life science and biology in China.

8. Old Materials, New Functions

8.1 New Concept of Bulletproof Vest

The silk glands are “workshops” manufacturing silk. They are located on two sides of a digestive tract and are a pair of tubular glands that are slender, curved and as beautiful as butterfly wings. They are the source of colorful and elegant silk.

The silk gland of a newly hatched silkworm is only 0.01 mg and grows up over the instars. By the end of the fifth instar, the silk gland is up to 1.6 g, 160,000 times of that of the newly hatched silkworm. This speed is faster than the human liver synthesizing protein. The silk gland is an interesting silk making mill, composed of 900 cells. These 900 cells are busy in synthesizing fibroin. The silkworms are cute, and the cell number of a young silkworm is also 900. As the instar increases, these cells will grow up, in which DNA will continue to split and increase in times. The law is yet to know. The silk is comprised of fibroin and sericin, the silk gland in the middle generates sericin and the silk gland at the end generates fibroin. The fibroin is secreted into the gland cavity and moves forward to the silk gland in the middle and then is wrapped by the sericin. In other words, the outer layer of a silk is sericin and the inner is fibroin. The liquid fibroin just synthesized is secreted to the silk cavity and moves forward under pressure, and finally a solid silk is spit out under the action of mechanical traction of a spinneret below the mouth of a silkworm. A silk is 800–1,500 m long, up to 3,000 m at the most. The silk has amazing strength and it looks soft and thin but is tough inside. Its tensile force can be comparable to steel wires. The silk strength of the silkworm is not the highest. But compared with the physiochemical properties of silk of nine-color spider (*Latrodectus mactans*), the silkworm silk has more unique advantages. The spider silk has the strength approximated to that of carbon fibers and high-strength synthetic fibers, but is better in tenacity. As with good tenacity and rigidity, scientists want to make bulletproof vests with spider silk. The problem is, spider silk mainly comes from cobwebs and it is low in production. Moreover, the spiders eat one another and cannot be bred as many as silkworms. Scientists used to dream of transferring cobweb protein genes into domesticated silkworms so that the silkworms can spit silk like spiders. This dream will come true soon. Through experiments, scientists have obtained the cobweb gene expression in the silkworms.

If growing to the fifth instar, the silkworm will look a cocooning place. Generally speaking, an ideal cocooning space is a place where its head can contact with an object and its silk can wind around. Locating an ideal place, the silkworm will squeeze out a drop of pasty and directionless liquid silk onto an object beside as a fixing point and a starting point, and then it swings its head and thorax to continuously move a spinning tube from the

fixing point, and pull out silk with its own strength to gradually form a cocoon stent. Hence the silkworm will excrete feces and urine in its digestive tract and make itself ready for nesting and sleeping. The silkworm spinning silk on the stent curves its body towards its back and turns its urosome outwards to be a C-shape. At this time, the silkworm has 8 prolegs and 2 hind legs fix the urosome and tail still, and only its head and thorax move. The silkworm head swings reciprocatingly as “8”-shape or “S”-shape. The silk spinning tube pulls silk out continuously. The 8-shaped or S-shaped silk rings overlap one another, and 15–25 silk rings overlap to be one silk sheet. The fibroin is pulled out from the front silk gland and the sericin on the outer layer sticks and fixes cocoon fibers one by one. Meanwhile, the silkworm keeps using blood circulation and muscle contraction to lift the thorax and urosome to intrude the silk sheet, so that the loose cocoon layers will be compacted and form a cocoon finally. What a scene in front of us.



Figure 110. Adorable silk gland.



Figure 111. Nephila, spiderman.



Figure 112. Silkworm spinning silk, benefiting all people.

8.2 Colorful Cocoons

Cocoons look not the same and are spherical, oval, elliptical, pocket-shaped, peanut-shaped, spindle-shaped, olive-shaped *etc.* in general, cocoons of Chinese species are spherical and oval, those of Japan species are mostly pocket-shaped and peanut-shaped, those of European species are elliptical and slight peanut-shaped, and those of multivoltine species are mostly spindle-shaped and olive-shaped.

The cocoons are colorful, including white, yellow, golden yellow, orange, pink, green, and light green *etc.* genetically, colored cocoons show dominance to white cocoons, and blood color is related to the cocoon color. The cocoon colors are complicated. Yellow of the cocoon layers of colored cocoons mainly comes from carotenoid in the mulberry leaves. The carotenoid enters the silkworm and combines with the protein into compound, the compound gets into the blood and then the gland cavity of the silk gland in the middle, is dissolved in the sericin, and finally turns yellow cocoons. The pigments are instable and easy to be oxidized or fade away by exposure. Therefore, the colored cocoons will fade in color when stored in the air at the room temperature. During silk reeling, the color will fade away, and the silk will become colorless. Scientists are devoting to transforming these pigments into the fibroin so as to maintain the color on the sericin layer during silk reeling and produce natural colored silk.

The pigment ingredients of the colored cocoon mainly include fat-soluble carotenoid pigment (yellow and red cocoons) and water-soluble flavonoids (green cocoons), and these pigments mostly come from mulberry leaves.

After the silkworms take in mulberry leaves, carotenoid contained in the mulberry leaves will enter blood through the midgut and adsorbed by the sericin through the silk gland in the middle to show colors. Why will the same silkworms eating the mulberry leaves have cocoons in different colors? This is due to the difference in species, just like

different races in the world, Mongoloid (yellow race), Europoid (white race), and Negroid (black race). The penetration and absorption of these pigments are decided by multiple alleles, blood color gene (Y), inhibitor gene (I), and cocoon color gene (C). For example, the gene Y enables Lutein to pass the midgut and the silkworm is a yellow-blood silkworm. Then under the action of the gene C, the pigment passes through the central area of the middle silk gland and enters the gland cavity to be adsorbed by the sericin and make yellow cocoons. If the inhibitor gene I exists, cocoons are white as carotenoid cannot pass through the silk gland tissue and enter the gland cavity. Therefore, yellow cocoons must be yellow blooded, but yellow-blood silkworms are not always making yellow cocoons. Green or light green cocoons contain flavonoid. The flavonoid comes from mulberry leaves. It is combined with glucose to be flavonoid glucoside after being absorbed and transferred by UDP-glucosyl transferase. In the silkworm genome there are more than 40 types of UDP-glucosyl transferase or homologues. Light yellowish green cocoons are dominated by two different genes Ga and Gb, Ga is responsible for transporting the flavonoid to the silk gland from blood and Gb is for transporting it from the intestinal tubes to blood. If both Ga and Gb are dominant, the flavonoid moves to the silk gland to be light yellowish green cocoons. When only Ga or Gb is dominant or recessive, the cocoons are white.



Figure 113. Colorful cocoons.

Carotenoid is one of xanthin made by plants and bacteria and widely spreads in the nature. Animals need it but cannot make it. Carotenoid is a hydrophobic substance insoluble in water because inside organisms there are full of water. It can only dissolve in water when its hydrophobic part is totally overwhelmed and generally gaps hidden in

protein are covered. Human skin and eyes have carotenoid. The transport process of the carotenoid transport system in the human blood is similar to insects' and is undertaken by apolipoprotein. Nowadays, it is still unclear about how carotenoid is taken in and transported in cells. An optional transport system only taking in carotenoid, which is dominated by a certain tissue and similar to the silkworms', may exist. For example, macula lutea is caused by the condition that the apolipoprotein only accepts lutein and zeaxanthin that are accumulated. Recently, CBP protein similar to the silkworms' was discovered in the macula lutea. About 1% of the elderly in Europe and America is stricken with aged macular degeneration. The macular degeneration has been the primary cause of blindness of the elderly. For this, with the study results in the silkworm model, it is an important medical subject to find out the conveying mechanism of carotenoid.

8.3 Second Skin of Humans

The outer layer of the silk is sericin and the inner is fibroin. The sericin is synthesized and secreted respectively in different sections of the middle silk gland and at different development stages of larva. Most of sericin contains glycan. When the liquid fibroin synthesized in the rear silk gland flows through the middle silk gland, the sericin secreted in different sections of the middle silk gland will cover the fibroin sequentially layer by layer. From the physiological perspective, cocooning of the silkworm is actually spitting out excessive protein, or the silkworms will die of protein poisoning. From the perspective of silkworms themselves, cocooning is for protecting pupae from being eaten by pests. But why does silk contain sericin and fibroin? The sericin will be melted later in the silk reeling and cocoon cooking, but why insects keep the sericin in evolution? We still don't know the answers. The latest research shows that the sericin has many special purposes for humans.

Human skin is soft, moisten, and elastic because of 15–20% of water, natural moisturizing factor, and lipid contained inside. Upon the impacts of age, nutrition, health, and environmental factors, as the water content of skin cuticle is lower than 10%, the skin will turn rough and dry, even abnormal and easy to age if the problems linger on. The natural moisturizing factor is a kind of protein existing in the epidermis, and Filaggrin disintegrates and generates hydrophilic moisture-retentive substance in keratinocytes of the cuticle. It plays a role of making skin soft and elastic. The cortical membrane in the cuticle can prevent water from evaporating to some degrees. The natural moisturizing factor in the cuticle plays a key role in keeping moisture of the skin. The moisturizing factor contains many free amino acids, in particular much serine. This is similar to the amino acid composition of the sericin. The domesticated silkworms evolve from the wild silkworms, and scientists inferred that in ancient age, the wild silkworms lived in the field, and the sericin of cocoons can prevent water from

evaporating and was uvioresistant and antioxidant and served a natural barrier for protecting pupae. And this inference made sense.

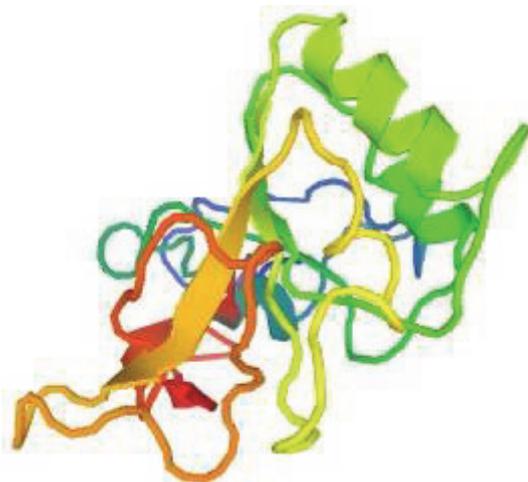


Figure 114. Sericin tertiary structure. (Source: <http://www.wikipedia.org>).

Human skin is usually exposed to the sunlight and air and easily generates ROS (reactive oxygen species). The stress response to oxidation of ROS is closely related to cancer, arteriosclerosis, and diabetes and also to skin care. This is also one of the reasons why most skincare products are mixed with antioxidant substances. In the tissue homogenate is added silkworm sericin, which can significantly inhibit peroxidation of fat, will not be inactivated by heating and is stable in antioxidation. As the sericin has no SOD, glutathione peroxidase, and catalase activity, the antioxidant activity of the sericin is different from the mechanism of common antioxidase. The antioxidation of the sericin at least partially chelated with copper ions generating ROS. The experiment of peroxidation inhibition of peroxidation substances for linoleic acid indicates that with the inhibition effect of 0.02 mg of BSA (bovine serum albumin) as 100% of reference, the inhibition effect of 1.5 mg of sericin is 80%, the number is 90% of 3.0 mg of sericin, is 20% of 1.5 mg of albumin, and is 30% of 3.0 mg of albumin. In view of this, the antioxidation of the sericin is significantly superior to the albumin.

As we have known, ascorbic acid, that is Vitamin C and kojic acid we usually say, can inhibit synthesis of melanin. The melanin itself has a function of preventing increase of ROS similar to antioxidant. Its accumulation is a defense mechanism of the body preventing increase of ROS due to the ultraviolet. The melanin synthesis system will not be active because of the antioxidant, its activity is inhibited and the accumulation of the melanin is reduced, so the skin can be whitened. Tyrosinase in the melanin cells is closely related to melanin synthesis and is a critical enzyme to oxidize Tyrosin into dopamine and then dopamine into Dopaquinone, and the ultraviolet in the sunlight can trigger this reaction, so we always turn dark under the sunlight. 1.0% of sericin can significantly suppress the activity of the Tyrosinase to prevent blackening. The

Tyrosinase contains copper, which inhibits the activity of the Tyrosinase, then further prohibits the activity of the melanin synthesis system, and reduces the accumulation of the melanin, so the skin will be whitened. The sericin is good for the hair and can form a layer of protective transparent film on the surface of the hair. It can protect the hair from damage by external factors and improve luster and elasticity of the hair, and has the advantages of moisture absorption, antistatic behavior, and shape-preserving effect. It is resistant to ultraviolet and good for maintaining health and beauty of the hair.

Women wear bras, but some bra materials on the market will cause atopic dermatitis. But for those with the sericin coating, the symptoms can be significantly alleviated. The onset of the atopic dermatitis is related to the increase of skin ROS and reduction in moisture retention. The alleviation may be resulted from the antioxidation and moisture retention effect of the sericin. An experiment was conducted on patients with contact dermatitis for 3 years and 5 years respectively, whose affected parts were applied with 0.5% of sericin water solution. The result showed that the symptoms were evidently alleviated in 20 days and disappeared in 60 days. To date, Japan has successfully developed skin drugs for external use with sericin and yeast autolysate.

With the development of modern biology, people get to know another function of sericin. The glass vessels with sericin coatings can help fiber-forming cells of rats attach and adding sericin extracted by hot water into culture solution of cell lines of humans and hybridoma of rats can speed up cell reproduction. In the hybridoma of rats and culture cells of insects, the sericin can serve as the additive for cell culture and take place of BSV. An experiment of applying 2.5 μg or 25 μg of sericin on each square centimeter of the surface of a glass vessel and then culturing fiber-forming cells of early test in the glass vessel for 72 hours came to a conclusion that the cell number is 2.5 times of that in a glass vessel without sericin coating. This shows that the sericin is a good culture material of fiber-forming cells. The sericin is hydrophilic and is still soluble even in case of degeneration without gathering after being treated at 120 $^{\circ}\text{C}$ for 20 min. It can be sterilized by high pressure, which is its special function.

Protein rich in hydrophilic amino acid is closely related to the water retention of the cells. It can be dehydrated under the conditions of freezing and drying for the sake of protecting cells. Peptide obtained by hydrolysed sericin is added into lactic dehydrogenase solution sensitive to freezing, and then treated by freezing and dissolving (30 $^{\circ}\text{C}$ for 5 min). The active acidohydrogenase added with sericin peptide is over 90%. Without antifreezing agent, the residual activity of the lactic dehydrogenase is lower than 10% through 5 times of freezing-dissolving treatment. The antifreezing capability of the sericin peptide is the same as BSV. The Colibacillus culture system added with the sericin peptide for 4 hours stands still for 3 days at -20°C . The DNA expression of the taken-out Colibacillus is 2 times higher than that without sericin. The hydrophilic amino acid areas in the sericin are combined with hydrone that can inhibit formation of ice crystal. The sericin peptide protects cells and protein and inhibits the freezing stress response of them.

It is good in storing antibody and vaccines with sericin. This application is promising in the antibody field. You must hear about the cold-chain logistics, that is, to transport food and medicine to be frozen. The food and medicine will always degenerate or lose efficacy during the freezing and cold-chain transport. However, if the sericin is added during freezing, they can be stored well.

The dermal carcinogenesis model system induced by DMBA and promoted with acetate of myristoyl crotyl alcohol is used to discuss the effect of inhibiting skin cancer with the application of sericin. The result showed that the occurrence and metastasis area of cancer of a rat with the affected part applied with sericin in advance are much lower than that of the control group. The sericin has the protection effect on acute skin injury and canceration induced by ultraviolet. In an experiment, hairless rats are radiated with ultraviolet of 180 mJ/cm² for consecutive 7 days, the skin of a rat in the control group has “sunburn focus”, and the red degree and area of the focus of a rat applied with 5mg of sericin on the skin are suppressed significantly. The immunohistochemistry method has proved that the sericin can evidently suppress rising of 4-hydroxynonenal induced by ultraviolet and the expression of epoxidase. This indicates that the sericin has the light resistance effect and can reduce the oxygen stress reaction and acute skin injury and canceration caused by ultraviolet irradiation.

8.4 Sericin and Medicine

Epoxidase can suppress excretion of waste in organisms and causes astriction. The experiment conducted on the astriction model of rats fed with epoxidase shows that the rats fed with sericin can completely have no astriction. The analysis indicated that water content in feces is increased due to the sericin and sericin is hard to digest and water retentive so that the astriction of the rats is eliminated. Increase of ROS in the intestinal mucosa will lead to rise of the occurrence rate of colorectal cancer, 1.5% of sericin plus 21.5% of casein and 3.0% of sericin plus 20.0% of casein were added into the feed of rats respectively. For rats of the control group, 23% of casein was added and carcinogen 1,2-Dimethylhydrazine was injected, the rats are fed for 5 weeks to investigate the number and area of canceration indexes, and the finding shows that compared with the control group, the folliculus focus of rats fed with food containing 1.5% and 3.0% of sericin respectively are 20% and 40% lower respectively. In light of this, the sericin can evidently suppress the occurrence of colorectal cancer and will be better as the sericin content in the feed is increased. The above two groups of rats were further injected with carcinogen for 115 days, and the result shows that the number of rats fed with 3.0% of sericin having tumor is much lower.

The sericin contains much serine, aspartate, hydroxyl, and carboxyl and can be chelated with multiple minerals. The sericin expresses resistance against protease and

undigested sericin may leave in the digestive tract. Thus, the sericin can improve solubility of minerals in the digestive tract and is good for the body to absorb minerals. To prove this point, researchers added 3.0% of sericin into feed of rats. Compared with the control group, the result showed that the absorptivity of the rats having sericin to ferrite, zinc, magnesium, and calcium is improved remarkably (by 20–40%). Some drugs can increase absorption of the body to a certain mineral, but can also suppress the absorption to the other one. The sericin is of great significance in promoting absorption of multiple minerals. The clinical test also found that Fructooligosaccharide, Lactulose, and Maltitol also could be promoters for mineral absorption. However, from this respect, the sericin shows no inferior.

8.5 Charming and Elegant Silk

The silk is different from cotton and wool. The cotton is not azelon and wool is keratin. The silk is synthesized by cells of the silk gland, fibroin is secreted first and then the silkworms spin it into fibers. This is a characteristic different from other natural fibers. The silk is composed of two silk cores, chemically, of macromolecular protein (-NH-RCH-CO-)n of complicated fine structure. The species of silkworms and positions of cocoon shells vary, the thickness and section shape of silk may be different. The silk has a special-shape section. With the special shape and structure, the silk has elegant luster, draping, softness, stylish, and dyeability. The silk fiber has high molecule aggregation power and has appropriate degree of crystalline. The silk fiber has excellent mechanical performance, and the breaking strength of the unit sectional area is comparable to the steel wire's and also to some synthetic fibers.

The silk has beautiful luster and is as elegant as pearls and ivory. The pearl and ivory are formed by accumulation of protein of many thin layers. The silk fibers are different in thickness and sectional shape and have fine complicated structure on the surface and abundant fine fibers to make the optical phenomena complicated. Under the sunlight, different levels of reflection rays on the surface and inside can be absorbed, curved, diffracted, reflected, scattered, and interfered. As the result of integration of multiple physic phenomena, the silk always shows elegant, reserved, and beautiful luster as moonlight. The dyeability of the silk is excellent among fibers. The silk can adsorb various kinds of natural or synthetic dyes sufficiently, and the fine part can be distinctly dyed. This is the result of that in the shapeless area of the silk exists active groups highly compatible with dyes and the fine structure with dye particles or molecules to be easily accepted. Many fibers are restricted to the types of dyes and dyeing methods in case of dyeing. In addition, compared with synthetic fibers, the silk has no striking contrast in dyeing and usually presents a style of profoundness, cold, and elegance. The drawback is that the dyed silk will fade away under sunlight after washed. People prefer using “ill

fated beauty” to describe the fading of the silk. This is a considerable drawback.

The luster, softness, draping, and feeling of the silk can be concluded into a word “elegance”. The elegance is an important value index of the fabric and the result of complicated interaction of composition, shape, property, fabric structure of the raw material. It is difficult to evaluate this point only from measured physical data. It also needs the examination of feeling organs. For example, the clothing fibers have requirements of flexibility and rigidity. When people wear it, they can feel soft and smooth. One of the most important characteristics of the silk fabric is the perfectness of softness and smoothness. Chinese drama costumes and Japanese kimono look beautiful and feel comfortable. They show the advantages of shape stability of the silk fabric. This aspect can be evaluated with reference to the draping coefficient. The determination method of draping coefficient is to cover a circular fabric on a smaller round plate in such a way that a projection of the fabric draping along the periphery of the round plate can be acquired, the area of the projection can be measured by the integration method and the draping coefficient can be draped according to the formula. The luster, softness, draping and comfortable feeling jointly present the elegance of the silk fabric. This is unrivaled by other fiber fabric and is also the reason why the silk is honored as “queen of fibers”. The serine also plays an important role in forming the elegance of the silk. According to different refining processes and degrees of sericin, various silk fabrics of different elegant styles can be produced.



Figure 115. Models in silk dresses at the MoMo Falana fashion show. (Source: <http://www.wikipedia.org>).

8.6 Silk Framework

In general, natural silk is defined as protein polymer. Some organisms can synthesize and spin fibers, such as silkworms, spiders, scorpions, mites, and mussels. Due to different characteristics, the natural silk can be used for making cocoons, webs, traps, nests, traction fibers, and egg-case silk. The natural silk has excellent tenacity and strength and in particular has the environmental stability, biocompatibility, controllable biodegradability, and easy molding of the structure. All of these make them a unique structural protein family. In addition to applications in the traditional textile industry, it provides a special position of widespread biomaterial applications. The repetitive primary structure of the fibroin leads to a high degree of homogeneity. Their secondary structure is applicable to the structural characteristic requirements of different materials. A few of well-aligned globulins can provide the functions of catalyzing and molecular identification. In the secondary structure of the natural silk the hydrogen bonds are connected between protein chains to generate β -lamella and form a hydrophobic area in a crystal protein domain. Given with strength and elasticity, the natural silk can effectively be crystallized and is insoluble in most of solvents. Beautiful fibers in fibroin contain three proteins, a heavy chain, a light chain, and a non-covalent combination of protein and glycoprotein P25. Compared with other proteins, the fibroin has the repetitive primary structure, for example, $(\text{GLY-Ala-Gly-Ala-Gly-Ser})_n$, $(\text{Gly-Ala-Gly-Ala-Gly-Tyr})_n$, and AGVGYGAG motif, the crystal domain is rigid and firm and is full of reversely parallel β -folded lamella.

The Nature made a comment on an article about silk entitled Old Materials, New Hope that the recent basic study results have broadened the application range of natural silk (silk and cobweb) and have promising future in the application of biomedicine. Leizu invented silkworm breeding and silk at the time was used as clothes and improved life quality. In modern times, the applications of silk have been rapidly broadened, and it is believed that Leizu will amaze at her offspring in developing silk.

The silk as a biomaterial in tissue regeneration has been applied in many labs. The silkworm is an invertebrate insect and can be taken orally. Though humans are generally not allergic to silk, the experiment indicates that the sericin on the surface of the silk can cause a slight allergic reaction. Therefore, to apply the silk, it should remove the sericin first so as to prevent inflammation (allergy) of humans. In fact, it is simple to remove sericin. The sericin can be removed by boiling the silk with alkaline substance. The sericin removal can remarkably affect the viability and biocompatibility of the cells on silk fibroin materials and three-dimensional fibroin porous frames.

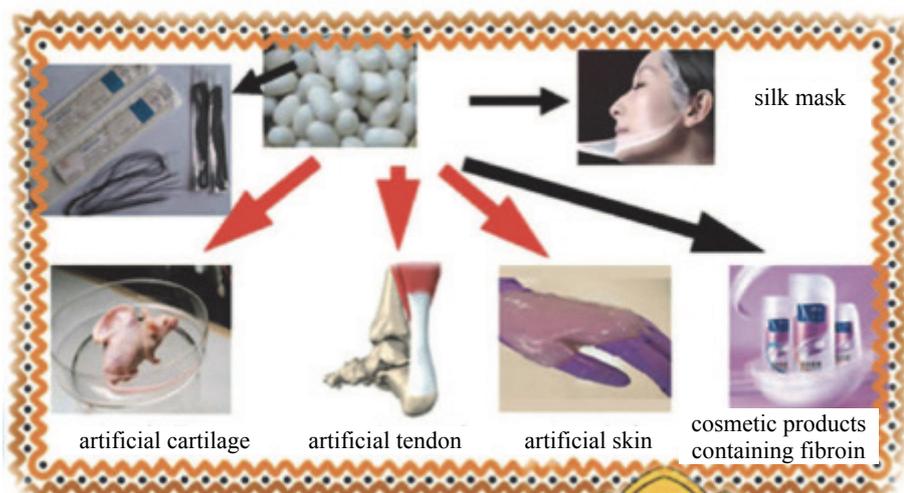


Figure 116. The application of natural silk.

So far, the sponge scaffolds made of silk have been used for the tissue engineering system. The silk sponge scaffolds are useful for regeneration of bones and organs. To achieve this goal, scientists concern about its biocompatibility, mechanical performance, chemical multifunctionality, bioactive molecules generated by water processing, and degradability of cell control. In general, the aperture and porosity of the scaffolds are critical factors. After the silk scaffold is manufactured, cells are put into it and grow on the silk scaffold. According to the size and mobility of the cells, the aperture larger than 100 μm is the minimum value of the tissue scaffold. Porosity is directly related to interaction between seed cells and ability of signal transmission. In view of applications of the scaffolds, the mechanical characteristics and degradation speed are important for the tissue functions, integrity and growth.

The fibroin tissue engineering scaffolds are applied to culture of high-density connective tissues of bones, cartilage, anadesma, and tendons. The experiment has proved the application effect. Researchers culture with a fibroin 3D scaffold and implant human “mesenchymal stem cells”, hoping to induce bone cell scaffolds for tissue engineering. The result shows that the stem cells can grow on the scaffold. The actin silk is dyed red with rhodamine-phalloidine dye and the nucleus is dyed into green with Hoechst33342. The cell can grow normally and develop.

8.7 Silk Nanoparticles

Fibroin can also be used for producing nanoparticles. Compared with synthetic polymer or other natural degradation materials, the fibroin has excellent mechanical performance, adjustable in-vivo degradation speed, and biocompatibility. For drug delivery, micromolecular medicine and protein medicine can effectively combine and

enter with the fibroin, and medicine release can be regulated by controlling the β -lamella content of the fibroin. 1 mg/ml of fibroin solution was treated with phosphate buffer and alcohol and can produce fibroin particles at nanometer level (200–600 nm), and the fibroin particles are uniform in diameter. The experiment on nano-fibroin gel's drug transport and release proves its role in delayed release of drugs and the time for drug release is prolonged. The treatment experiment on ulcerative colitis with the fibroin carrier drug has proved that the fibroin nanoparticles have the function of delayed release of drugs.

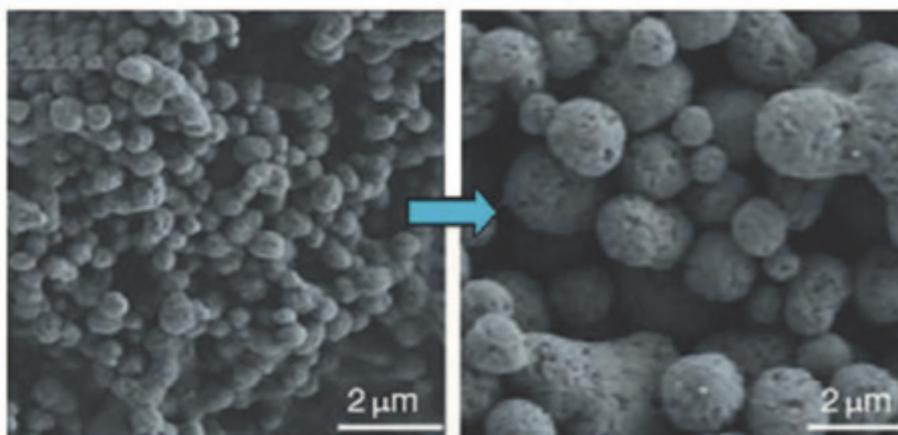


Figure 117. Preparation of silk nanoparticles.

Silk is not only a tenacious natural material but also has striking surface flatness and optical transparency. The fibroin mask is 40 cm² large and 40–100 μm thick and is transparent. The inorganic glasses or semiconductor materials are synthetic organic polymers. The organic polymer materials will have negative biological doping impacts under chemical treatment or high-temperature treatment. Nowadays, the silk photobiology materials have been applied to biomedicine and photobiology. As the optical members made of silk are of biodegradation and biocompatibility, a new equipment system for humans has been developed. For example, the silk optical components are applied to optical sensors for monitoring spectral response of embedded biochemical compounds and to cornea tissue engineering, the fibroin mask can be used for regenerating cornea mesenchyme, and so on. The reproduction, multiplication and growth of human and rabbit cornea fiber-forming cells on the fibroin mask have manifested the potential of the silk biomaterial system in the application of cornea tissue regeneration.

8.8 Silk Mask

The fibroin mask is a perfect material for artificial skin. The laboratory detection and clinical application show that the fibroin mask has high performance and has

protection and treatment effect for the skin wound such as superficial burn and plastic surgery. Generally speaking, the wound will be mostly infected by bacteria in 24 hours, even locally infected, common wound coverage materials cannot be directly applied on the infected wound but non-infective wound of the early stage and sterile operation wound. Scientists have developed a novel silk coverage material for the burn wound that has the anti-infection function and can speed up healing of the wound. This fibroin mask is tenacious, moist, and permeable and is adhesive and compatible with the wound of human. It has been clinically tested as the wound coverage materials. After the sterilization drug is embedded, the drug will slowly release from the fibroin mask and better effect can be achieved. The clinical test shows that the drug fibroin mask plays a role of protecting the wound and has the effects of eliminating bacteria and controlling infection.

8.9 Functional Food

The food function of the fibroin includes nourishment, taste, and regulation on the physiological functions and the immunity system. According to the composition and value of the fibroin, the fibroin can be a high-end food with the adding function. The fibroin as food can be dissolved in water and used in a manner of 200–300 small peptides composed of amino acids. The small peptides are easy to digest and use in human bodies and taste sweet or sour and smell good. They are moisture absorptive and have been added into dim sum, sugar, flour, porridge, bean curd, and cold drink and sold in the market. Fibroin itself is a pure protein, silk is dissolved in calcium chloride solution of specific concentration and treated into colorless, tasteless and odorless edible fibroin powder through purification and sterilization. Then added with proper ingredients or mixed with juice, sugar, pigment, essence and organic acid, it can be condensed into jelly at the room temperature. It will be melted in the mouth immediately and tastes smooth like jam and comparable to the jelly products. In Japan, waste silk is processed into fibroin powder and then added into maltose, pancake, noodles, buns, and medicated porridge. This is popular among Japanese customers. Companies are trying to produce fibroin drink and sell to the market. Factories producing edible fibroin powder and fibroin food have been set up and manufactured products including fibroin soy sauce, fibroin drink, cookie, and candies. In particular, the fibroin is added into food for the elderly and children so as to strengthen them of these groups of people. I believe that in near future, the fibroin must be developed into an ideal functional food and delicious healthcare food with the study going deeper in fibroin as food and its functions.

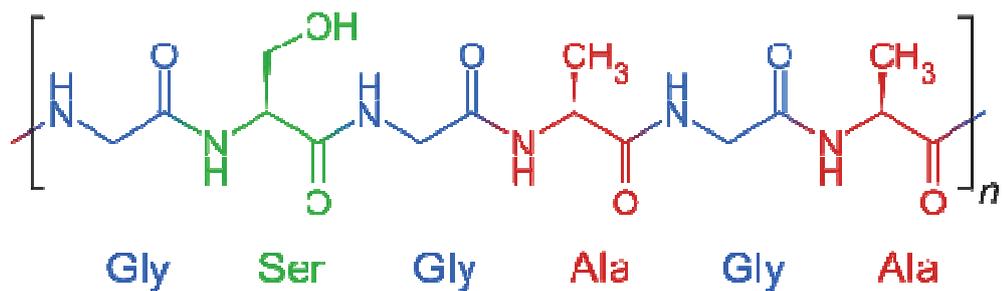


Figure 118. Primary structure of fibroin (Gly-Ser-Gly-Ala-Gly-Ala)_n. (Source: <http://www.wikipedia.org>).



Figure 119. Waste silk is processed into fibroin powder and then added into buns, pancake, noodles, maltose and medicated porridge.

9 More About Applications of Mulberry and Silkworms

“Birds chirp in mulberry trees, leaf shoots are sprouting”, this is a description about mulberry trees in a poem entitled Mulberry Leaf Pickup of Wang Jian, a poet of Tang Dynasty. The mulberry trees originated in Central and Northern China, now widespread from Northeast to Southwest and to Xinjiang in Northwest. In North Korea, countries in Central Asia and Europe are also planted mulberry trees. The mulberry trees blossom in May with catkin. China is the earliest country of planting mulberry trees and the mulberry trees have a long history in China. They are multipurpose, including breeding silkworms and preparing drugs.

9.1 Mulberry Leaves as “Longevity Panacea”

As recorded in Compendium of Materia Medica about mulberry leaves, “mulberry is the spirit of Ji star, cicada living on it can sing a song and old man eating it looks as young as a child”. Mulberry leaves are also called “longevity panacea”, and Japanese call mulberry leaf tea as “longevity tea”. Mulberry leaves were honored as “King of Plants”, and can “clear heat” on the contrary to tonifying of ginseng. It contains 17 amino acids, crude protein, and crude fat, has been identified as “homologous” plant by the Ministry of Health and listed as one of “Top Ten Health Care Food of 21st Century” by International Food and Health Organization, and has become a new green food source of humans.

Mulberry tea is a new-rising health care drink highly praised by people. To make mulberry tea, mulberry leaves should be processed to remove bitterness and sourness of organic acid, then brewed with boiling water and made into tea. The mulberry tea is limpid, sweet, tasty and refreshing in favor of the elderly and people who are not suitable for drinking tea. It is healthy and conducive to longevity and capable of reducing accumulation of lipofuscin (senile plaques) in skin or internal organs. The mulberry leaves contain flavones that can strengthen blood capillary and reduce blood viscosity, and this is able to lose weight and alleviate hyperlipidemia and capable of preventing myocardial infarction and cerebral hemorrhage.

The mulberry tree was also called as “longevity trees” in ancient times. It was reported there was a longevity village near the Black Sea, where villagers lived to 120 years old and the eldest was even 160 years old. Through investigation, mulberry trees were planted all over the village and villagers took mulberry as food and drank mulberry tea from generations to generations. Coincidentally, in a mountain area of Moscow were

planted mulberry trees, villagers there ate mulberries and drank mulberry tea and quite a few old men lived to 140 years old. There was a Xiyan Village in Xiajin County, the Yellow River Communist-held Forest Park, where mulberry trees were planted everywhere and 8 villagers lived over 90 years old and many people lived over 80 years old. In the fourth population census of 1990s, a county in Xinjiang had long-lived old men, the major reason is that mulberry trees were planted there and local people preferred mulberry drinks and mulberries. The mulberry leaves contain much folic acid. Each gram of mulberry leaves contains 105 μg of folic acid. The folic acid is a medical material and can be synthesized with nuclein. It has the antianemia effect, promotes growth, can prevent gastric cancer, bowel disorder, anorexia, malnutrition, and dermatitis herpetiformis and is of great significance for human health.

One of the Four Great Beauties in ancient China, Xi Shi, had a good looking that can “sink fish” and long hair. It was said that she played in the mulberry garden and washed her hair with water on the mulberry trees, so her beautiful hair might be related to the mulberry leaves. Another research indicates that mulberry leaves are good for skin, especially having curative effect on acnes and speckles. Mulberry leaves contain flavone compound, phenols, amino acid, organic acid, carotene, vitamins, and many trace elements indispensable for humans. They are conducive to regulating metabolism of skin tissues and particularly suppressing occurrence and development of chromatosis.



Figure 120. Mulberry tea and Xishi.

9.2 Celestial Fruit—Mulberry

As explained in *Shuo Wen Jie Zi* (Explaining Graphs and Analyzing Characters), Dan or Shen, is the black of mulberry fruits. Agricultural Archaeology also explained Dan/Shen is the color black. The mulberry is purplish black and so named “Sang Shen” (black mulberry) in Chinese. It is the fruit of mulberry trees, also called mulberry fruit. It is 1–2.5 mm long with small oval or obround fruit balls densely distributed, and is green at the beginning and turns fleshy and blackish purple or red after ripe. Their seeds are

small, the flowering period lasts 4–5 months, and the fruiting period lasts 5–6 months. Early in 2,000 years ago, the mulberry had been royal food for Chinese emperors. The special growth environment of the mulberry trees keeps the mulberries away from pollution. In the past, the mulberry was also called “celestial fruit” in the folk.

As recorded in Ben Cao Shi Dao, the mulberry has the following effects. It could “satisfy hunger, make peaceful and intelligent, whiten skin, and prolong life”. Ben Cao Jing Shu also had mentioned, “it tastes sweet and tonifies blood to clear heat, and must be a drug for cooling and tonifying blood and good for Yang”. Modern healthcare books always talk about mulberries. For example, Pharmaceutical Action and Application of Anticancer Traditional Chinese Medicine said, “the mulberry is rich in carotene, which can prevent cell mutation caused by cancerogenic substances and burst up lysosome in cells to release hydrolase, and the hydrolase can dissolve cancerogenic cells”. It can be seen that the health care effect of the mulberry is not a groundless rumor, but instead, there are many scientific supports from in all ages. The mulberry is rich in active protein, vitamins, amino acid, carotene, and minerals. Its nutrition is 5–6 times higher than apple and 4 times higher than grapes. It has multiple effects and was honored as “the Best Health Care Fruit of 21st Century” in the academic circle. The research also shows that taking mulberries usually can improve human immunity, delay ageing and beautify skin. There are orange red and purplish black mulberries. In general, black mulberries are better for brewing wine.

The mulberry wine and mulberry red wine made of mulberries contains abundant active ingredients and nutrition such as anthocyanidins, resveratrol, amino acid, and vitamins. According to the analysis of the authority, the mulberry wine contains 18 amino acids humans need and multiple microelements. The ingredients include most of in red wine made of grapes. Of them the anthocyanidins content in the mulberry wine is 5 times higher than grape wine, protein is 8 times, and lysine is 9.23 times. The content of selenium good for treatment and protection of the heart and immunity system is 12 times higher than grape wine's. Resveratrol, antioxidant, calcium, ferrite, and zinc are much higher than those in grape wine. From this, the mulberry wine is contamination-free high-end fruit wine rich in nutrient ingredients.

The mulberry can improve blood supply of skin (including scalp) and tonify to whiten the skin and blacken the hair and can also delay aging. It is a healthy beautify and antiaging fruit and good medicine for the middle-aged and aged people. Always eating mulberries can improve eyesight and alleviate fatigue and dry eyes. The mulberries have the immunological enhancement effect, gain weight for the spleen and enhance the hemolysis. With mulberries as daily food, arteriosclerosis and arthrosclerosis of bones can be avoided and metabolism can be improved. The mulberry can speed up growth of red blood cells and avoid reduction of leukocytes and have the assistive effect on anemia, hypertension, hyperlipidemia, coronary disease, and panasthenia. It also can help produce saliva and slake thirst, improve digestion and excretion and can promote

secretion of gastric juice and peristalsis and clear dry heat. The traditional Chinese medicine believes that the mulberry is sweet and cold and has the effects of tonifying the liver and kidney, producing saliva and moistening intestines, blackening hair and improving eyesight. As recorded in an ancient medical book Bao Shen Yao Lu, there was a youth-retaining receipt, “Mulberry Medicine Balls”. The book said, the Balls could beautify skin, blacken hair, tonify essence, prevent diseases and delay aging. In its receipt, there was mulberry leaves. Modern days, mulberry balls assist in treating stain and dry skin clinically.



Figure 121. Enjoy life and cherish time.

9.3 Magic Hypoglycemic Duo

The mulberry leaf contains al-kaloid 1-DNJ, which only exists in mulberry leaves. Every a hundred grams contain 100 mg. 1-DNJ was discovered on the mulberry trees and is an inhibitor with α -glycosidase of special function. Japanese academy circle called it DNJ. Its main actions include inhibiting decomposition of invertase, maltase, α -glucoside, and α -amylase, stimulating secretion of insulin and reducing the decomposing speed of insulin. Except mulberry trees, scientists also discovered a small amount of DNJ on hyacinth, commelina communis and bacillus. However, the DNJ content in the mulberry is much higher than that found in other plants and microorganism. The DNJ spreads in leaves, roots, and branches of mulberry trees. The leaves have the highest content of DJ, about one thousandth of the dry weight. At the same time, the mulberry leaves account for the largest percentage of the whole dry matter of the mulberry tree, about 65%. Therefore, the mulberry leaves have been the major source of natural DNJ and widely

recognized as a food material for regulating blood sugar. Since the ancient time, the mulberry leaves have been clinically used as traditional Chinese medicine for treating diabetes. Over 20 years of study, scientists unveiled the secret of mulberry leaves in lowering blood sugar. In 1976, Yoshiaki first detected DNJ molecules in twigs and branches of a mulberry tree. In 1994, Asano separated 6 kinds of al-kaloid the DNJ molecule. In 1995, Kimura confirmed that the DNJ molecule has the most striking hypoglycemic action. That is to say, the DNJ is the major active ingredient for lowering blood sugar. In 1997, Kato confirmed the molecular structure of DNJ and proposed that DNJ can alleviate symptoms of insulin resistance by maintaining healthy blood sugar, correcting lipid metabolism, and improving insulin sensitivity.

The mulberry leaf compatible with *potentilla discolor* has good curative effect on diabetes. It can be easily taken and made into tea as drinking. The mulberry leaves and *potentilla discolor* in compatibility can help produce insulin and lower blood sugar. The *potentilla discolor* belongs to the rosaceae family and is called “chicken-leg roots” in *Jiu Huang Ben Cao* and “wild lotus root” in *Ye Cai Pu* (Book of Edible Wild Herbs). It is called “chicken-leg seedlings” and “white chicken leg” in Jiangsu, spreads wildly all over China and can be eaten with or without cooking and for medical use.

9.4 “Living Gold” *Phellinus* (Sang Huang)

There is a bacterial parasite, called *Phellinus*. The *phellinus* is a fungus. It got its Chinese name Sang Huang as it is parasitic to the mulberry trees and is also known as Husunyan, Sang'er, Zhencengkongjun, Sangchen, Sang Huang Mushroom, Sang Jisheng, Shuji, Sang Huang Gu, and Meishujun. Its seed is free of stems and the cap is oblate or U-shaped. The *phellinus* is woody and light liver brown until dark grey or black and widely spreads in Northeast, North and Northwest China, Sichuan and Yunnan, as well as in Japan, South Korea, and DPRK. It is always parasitic to rotten parts in mulberry trees, pines, aspen, birches, and oaks and causes rot of heartwood. Ancient people also called it “tongue of trees”.

As an ancient Chinese tradition medicine herb, *phellinus* can be comparable to *cordyceps sinensis* in effect despite not as famous as it. The *phellinus* has complicated ingredients, also including β -glucan, chitin (chitosan), and heteropolysaccharide (pectin, hemicellulose, polyuronide and other dietary fibers) in addition to polysaccharides. It is a precious Chinese herbal medicine, capable of tonifying organs, softening hardness, detoxifying, stanching, invigorating the circulation of blood and harmonizing the stomach to check diarrhea and conducive to treatment of clap, metrorrhagia and metrostaxis, abdominal mass, phlegm-fluid retention, and spleen-deficiency diarrhea. Sheng Nong's Herbal Classic, the earliest herbalism work of China, has had a record of effects of “Sangjisheng (Sang Huang)”. Compendium of Materia Medica recorded that Sang Huang

(phellinus) can “tonify five organs and purge qi of the intestines and stomach and toxic qi”. Modern study has confirmed that phellinus polysaccharides can alleviate symptoms of cancer patients, such as pain, poor appetite, weight reduction, and fatigue, and improve life quality. The phellinus also has special effects on treatment of fatty liver and cirrhosis caused by virus hepatitis. In Japan and South Korea, phellinus micro-powder capsules have long been used for clinically treating the fatty liver or hepatic fibrosis, and the curative effect is impressively good. In addition, the phellinus seed contains abundant natural amino acids, vitamins, and minerals and can advance metabolism of the liver and regeneration of hepatic cells and weaken the reproduction of hepatitis virus.



Figure 122. Phellinus essence.

In addition to phellinus fermented mycelium, in Japan and South Korea phellinus seeds are wall-broken and processed into ultrafine powder capsule type oral products and mainly used for treating refractory hyperglycaemia as western drugs are ineffective. This is a new application area of phellinus preparations. So far, the annual sales of phellinus healthcare products are 500 million to 1 billion USD globally. The United State is a super country of consumption. It was reported that US has imported wall-broken phellinus ultrafine powder from Japan and South Korea since 2000 for filling capsules for sales. There are tens of products including phellinus polysaccharides, phellinus seed ultrafine powder, and phellinus mycelium powder.

9.5 *Twin of Cordyceps Sinensis*

The fungus is parasitic to the mulberry trees and grows into phellinus of great medical value. Silkworms also can be parasitic to fungus and turn to be cordyceps sinensis. As we all know, cordyceps sinensis is a valued wild Chinese herbal medicine in China, has the effects of tonifying deficiency, essence and qi, protecting the lungs and kidneys, stanching bleeding, reducing phlegm, and strengthening, and is listed as Three Major Restoratives together with ginseng and pilose antler. As recorded in Ben Cao Cong Xin, written by Wu Yiluo of Qing Dynasty, the cordyceps sinensis “tastes sweet and has the effects of protecting the lungs and kidneys, tonifying marrows, stanching bleeding, reducing phlegm, and treating consumptive cough and diaphragmatic obstruction. There are varieties of cordyceps sinensis, 350 of them have been known in the world and 62 in China. The most expensive are cordyceps and cordyceps militaris. There is an old saying, “a bunch of cordyceps is better than a cart of gold”. About growth of the cordyceps, people usually felt mysterious in the past. Pu Songling wrote a poem “Dong Chong Xia Cao (winter worms summer herb, cordyceps) is true to its name, turning into qi going one way. One thing could either be animal or plant, making truth hard to trace the end”. In fact, cordyceps is a product of an insect and fungus. The insect is a larva of *Hepialus armoricanus oberthur*, and the fungus is cordyceps fungus. Every summer, the *hepialus armoricanus oberthur* lays thousands of eggs on flowers and leaves as ice on the snow mountain of 3800m high melts. Then eggs grow into larvae warming into the loose and humid soil, absorbing nutrition of plant roots and gaining weight gradually. At this time, spherical ascospore drills into the larvae, absorbing nutrition and germinating hypha. Sometimes, larvae that eat leaves of cordyceps fungus will turn into cordyceps too. Nowadays, the sericulture researchers develop silkworm pupa cordyceps.



Figure 123. *Cordyceps sinensis*.

With living domesticated silkworm pupae as hosts, the silkworm pupa cordyceps is cultured by artificially inoculating pupa cordyceps militaris and simulating the ecological environment. The success rate of inoculation and the rigidity rate after inoculation are both over 95%, the germination rate of seeds is over 90%, and it needs 35–45 days from inoculation to matureness of the seeds. The medical analysis shows that the silkworm pupa cordyceps has no substantial difference with the cordyceps sinensis in chemical ingredients, and the contents of cordycepin, mannitol and SOD are comparable to or even higher than those in the cordyceps sinensis. The toxic test indicates that the silkworm

pupa cordyceps is basically non-toxic and has no side effects such as teratogenesis. The pharmaceutical test shows that in the silkworm pupa cordyceps is comparable to cordyceps sinensis the anti-hypoxia, sedation, and anti-inflammation effect, but superior to it in the anticancer aspect. The acute and subacute toxicity test shows that the silkworm pupa cordyceps is non-toxic. The chronic toxicity test indicates that the silkworm pupa cordyceps is low in toxicity and is safe. To sum up, the silkworm pupa cordyceps has the actions of anti-fatigue and anti-aging, and enhancing immunity and sexual functions and can kill cancer cells.



Figure 124. Silkworm pupa cordyceps.

9.6 Resurrection of Muscardine Silkworms

In general, silkworms are susceptible to white muscardine in the humid environment. The white muscardine is highly infectious. Once silkworms are infected with *beauveria bassiana*, they will be white muscardine silkworms. Modern medical study shows that the white muscardine silkworms have many purposes. As recorded in *Ben Cao Jing Shu*, “white muscardine silkworms are curative to stroke, lockjaw, fluttering, deficiency of blood, and fast-circulation meridians...” The study also found that 10% of white muscardine silkworms decocted with 2 g/kg of liquid given to rats orally has evident resisting action to convulsions caused by strychnine nitrate. The ethyl alcohol solution extract of white muscardine silkworm has hypnosis action to rats and rabbits. The hypnosis effect of 0.5 g/20 g taken orally and 0.25 g/20 g of hypodermic injection is equal to that of hypodermic injection of 50 mg/kg of phenobarbitone. As recorded in the ancient medicine book, the “white muscardine silkworms could remove black spots and beautify the skin”. It can be seen that the white muscardine silkworm is traditional Chinese medicine for ancient women in beautifying skin and removing speckles. The white muscardine silkworms have amino acids, vitamin E, and active fibroin, can

regulate hormone secretion and eliminate radicals and have good effect of dispelling chloasma caused by endocrine dyscrasia, senile plaques caused by anti-lipid peroxidation. At the same time, cell metabolism can be accelerated, skin micro-circulation can be improved, and dark skin and sunburn can be treated and skin elasticity can be improved.



Figure 125. White muscardine silkworms.

9.7 Silkworm Excrement and Health

Despite not pleasant, silkworm excrement is very useful. The excrement of young silkworms is boiled with water and disinfected and cools down. Mixed with traditional Chinese medicine, the excrement can be made into a medical pillow with the effects of removing turbidity, clearing heat, and refreshing. China and Japan have started extracting chlorophyll and carotene for industrial production from the silkworm excrement. The extracts are used as food additives, toothpaste, soap colorant, and deodorant and can protect skin and teeth and deodorize and are anti-bacteria. The extracted chlorophyll can be made into sodium copper chlorophyll, sodium ferrous chlorophyll, and sodium cobalt chlorophyll and used for treating hepatitides, gastric ulcer, duodenal ulcer, acute pancreatitis, chronic glomerulo nephritis, and reduction in leukocytes and can induce synthesis of hemoglobin. For example, Ganbao for Liver from Guangzhou Xingqun Pharmaceutical Co., Ltd, Weiganlv for Stomach from Guangzhou Foshan Pharmaceutical Plant, and Shengxuebao developed by Zhejiang Institute of Traditional Chinese Medicine are all sodium copper chlorophyll preparations. Sodium ferrous chlorophyll and sodium cobalt chlorophyll are of similar medical value similar to sodium copper chlorophyll. Sodium ferrous chlorophyll has good curative effect on Iron-deficiency anemia, and sodium cobalt chlorophyll is curative for leukopenia of cancer patients due to chemotherapy and radiotherapy. In addition, chlorophyll can be also prepared into ointment for treating burn, scalding, vasculitis, and haemorrhoids. There are more than 30 varieties of chlorophyll derivative drugs in the Drug List of the United States.

9.8 Delicacy

Pupa is a new nutrition source and is the only insect food listed in New Source List of Food Managed as Common Food approved by the Ministry of Health. Pupa is of great nutrition and contains abundant protein. Fresh pupa contains 51% of crude protein and 29% of crude fat and other indispensable vitamins for humans. The protein content in pupae is much higher than other food and there are 18 amino acids, which are comprehensive. 8 of them indispensable for humans are high in content, about 2 times of meat, 4 times of eggs, and 10 times of milk. These 8 amino acids are balanced and in proper ratio and accord with the requirements of FAO/WHO for food nutrition. Therefore, the protein is very suitable for humans and is a high-quality insect protein. In addition, the pupa also contains microelements, which are indispensable, including potassium, sodium, calcium, magnesium, ferrite, copper, manganese, zinc, phosphorus, and selenium. Japanese scholar Koyanagi Teruichi analyzed that each ton of pupa apolipoprotein is equivalent to 0.5 ton of beef. American people used pupa as “chocolate” filling, and Australians love to eat pupae, and French like pupae just as edible snails. People from Northeast China love fried pupae. The pupae have been cooked in various ways and make mouths watery.



Figure 126. Pupae as food delicacy.

The medical value of pupae has been mentioned in different parts in the ancient medical book of China Compendium of Materia Medica that the pupa can “dispel the wind and clearing damp and mainly treat rheumatism and cure patients with wandering arthritis”, “taken as food, malnutrition of children can be cured, muscle grows, heat can be cleared away, parasites can be got rid of; brewed it with water, it can quench the thirst”, “pupa tastes salty and spicy and is neutral and non-toxic, people eating it can maintain health and pupa made into drugs can cure diseases, tonify qi and blood, strengthening waist and kidneys, and moisten the lungs and intestines”. The pupa is the life essence of a silkworm and pupa oil can be the essence of the essence. The pupa oil is mixed oil refined from pupae and is yellowish red. It contains 60% of unsaturated fatty acid and ingredients of α -linolenic acid, oleic acid, and linoleic acid and has the characteristics of deep-sea fish oil. The food function and nutriology study shows that the α -linolenic acid has an important function of health care and is able to reduce blood fat, cholesterol, and blood pressure, prevent cardiovascular diseases, thrombosis, stroke and sight problems, inhibiting platelet aggregation, enhancing the reflection of retina and memory, and so on.



Figure 127. Pupa oil like deep-sea fish oil.

9.9 Sacred Moths

Li Shizhen called male moths “sacred insect and national treasure” in Compendium of Materia Medica and described its effects of nourishing the liver and kidney, tonifying Yang and anti-aging. There is male moth liquor made of sorghum and male moths sold in Chinese market. With the traditional brewing technique, ginseng, lycium barbarum, and eucommia ulmoides, together with hormone and protein in male moths, are immersed in the white spirit, and by reasonable storage, purification, and filtering, the male moth liquor is made. After drinking the male moth liquor, an unknown poet had written a poem Ode to Male Moths, to gain reputation for the male moth liquor.



Figure 128. Ode to male moths.



Figure 129. Delightful moth wine

9.10 Versatile Silk

The silk as additive to cosmetics has been long put into use at home and abroad. The silk products containing fibroin, such as foundation cream, facial oil, lipsticks, lotion, and vanishing cream, have been sold on the market for a long time. The structure of natural silk is similar to human skin. So, the mask made of the silk is known as “second

skin” and is the essence that the nature endows. The application of silk in facial beauty has a long history in China. As early as Ming Dynasty of China, the silk had been used for daily facial beauty and health care of imperial concubines due to its medical value. As recorded in Compendium of Materia Medica, fibroin powder can remove black spots and treat suppurative dermatitis. Modern medical test has confirmed that the fibroin content is much higher than that of pearls as well as the nitrogen content and the contents of the major amino acids are 10 times higher than that of the pearls. These amino acids can directly be absorbed by hair and skin, and can more easily penetrate the human skin and speed up skin metabolism. In addition, tyrosine in the silk can effectively inhibit the generation of melanin.

The natural silk peptide taking the silk as raw materials has been an ideal additive to popular nutrition skincare and facial beauty cosmetics and widely applied to production of high-end beauty cosmetics of natural nutrition. The silk peptide is easily absorbed by the skin and can advance metabolism of skin cells and provide necessary nutrients for skin. It can make skin smooth, soft and white and has the magic effect of delaying and preventing skin aging. The silk mask can be more attached to skin than other common nonwoven mask, can absorb more essence and will be more fitted to the skin. It can stick tight to the skin and prevent the essence from dripping off. As the silk mask is the most soft and smooth, it can closely fit to the skin texture and stick tight. It is thin, transparent, and permeable and can tightly fill into nose wings and corners of the mouth, retaining water long and moisturizing. The silk mask can speed up skin repairing. The skin is sensitive after exposed to the sun. Applied with a silk mask, it can quickly repair itself as the silk fibers are smoothly fitted. In addition, the skincare products made of silk peptide can facilitate secretion of collagen and have the effect of whitening, UV resistance, anti-dermatitis, and pock pustule resistance.

Since the ancient times, pure silk has been renowned as “Queen of Silk”. To date, people call the pure silk “fiber of health” or “health care fiber”. The health care function of the pure silk is incomparable and irreplaceable by other fibers. The pure silk contains 18 amino acids that humans need and nearly the same as those skin has. The silk clothing is luxurious and elegant and expensive. It contains protein as human skin has, so it is a good option for manufacturing women underwear. The silkworm silk is the best in natural silk and silk from silkworm cocoons is white mixed with a little yellow, feels smooth and fine and smells a slight fragrance of animals. Quilts made of silk are quite soft and fit.

With the development of the computer technology and the printing technology, silk is applied to a new area. In recent years, some cocoon silk manufacturers in Jiangsu of China have printed ancient poems and drawings on silk with the computer control and printing technology and the picturesque silk products are so breathtaking.



Figure 130. Silk picture.



Figure 131. Silk wadding quilts.

Afterword

Subtitle: Dream of Science of Sericulture

People have a lot of dreams of pursuing beautiful things and memorizing the past. I have devoted myself to the science of sericulture for long and always dreamed of wandering in the fantastic sea of science. I am close to sixty years old and have felt unable to do as well as I would wish, but I am still ambitious. Marshal Ye Jiaying made a poem in his the 80th birthday feast, “I was old, but still singing the praises of the era, looking over mountains afar, mountains are brighter under the setting sun”. He compared the old age to the setting sun, which still emits light and heat. How ambitious he was. Cao Cao, a statesman, strategist, and writer in the Period of Warring States, said in his poem *Though the Tortoise Lives Long*, “an old war-horse may be stabled, yet still it longs to gallop a thousand li; and a noble-hearted man though advanced in years, never abandons his proud aspirations”. The poem inspired so many old men. Over 30 years, I have engaged in the sericulture science and forged a bond with “Tian Chong (silkworms)”. It attracted me to investigate the life mystery and explore an abyss-deep subject. I cannot help roll in the deep. Everything in the world cannot be perfect. Just as two lines of a poem, “men have sorrow and joy; they part or meet again; the moon is bright or dim and she may wax or wane; there has been nothing perfect since the olden days”, so with the science. There is a proverb, “making another step at the top of a pole hundred feet high”, urging us in pursuit of broader boundary. “Science has no limits, neither our pursuit”. I didn’t mean to boast my devotion, but to popularize Chinese sericulture, promote traditional Chinese culture, and carry for the Belt and Road Initiative. However, words unfinished, dreams refreshed me again. The following I will talk about may dream A, B, and C.



Figure 132. The pursuit of broader boundary.

Dream A: Reasonable nutrition partitioning of silkworms make needs and wants meet

A silkworm leaf is thrown into silkworms and drools their mouth. They greedily enjoy food from the nature. Scientific study indicates that the domesticated silkworm is oligophagous and loves mulberry leaves. It eats leaves in three steps. The first step is to smell the leaves, that is, food calling. The second step is to start biting if the leaves smell good. The third step is to swallow. The silkworms complete selecting food after three steps. A silkworm can eat 24 g of mulberry leaves during its lifetime, 80% of which is taken in by the silkworm of the fifth instar. The silkworm eats mulberry leaves for its own tissue growth and development. The purpose of humans breeding silkworms is to reel silk and fabric and manufacture clothes. During breeding, most of them expect more silk from the silkworms. During sericulture, breeders prefer first-generation crossbred species, and people want silkworm species of high reproduction coefficient, so that the female moths can lay more eggs. I was involved in tackling key problems in sericulture during the Eighth Five-Year Plan and discovered a domesticated silkworm strain from the national silkworm resource. A single female moth of this strain can lay 900–1100 eggs. The mother species are bred in a ring, and 900–1100 eggs usually are full of the ring, so the strain is usually called a “full moon”. A single cocoon of the crossbred species is 2–2.5 g, while there are cocoons of 3 g in the species resource of domesticated silkworms. Since I first had contact with bombycology, I dreamt of inventing a regulator that can regulate nutrition partitioning of silkworms. So far, we are still yet to culture a species with eggs as many as the “full moon” and silk production up to 3g. “You cannot have your cake, and eat it”, as Mencius. The natural law has told us, all living things reinforce and neutralize each other. However, during agricultural production, scientists skillfully follow the natural laws to have and eat the cake at the same time, for example, broilers and layers in chickens, and boars and pork pigs. In spite of this, the silkworms are somewhat different. We hope the silkworms of the same strain can lay more eggs and produce larger cocoons in production after bred into the first-generation crossbred species. This dream is not easy and must be pursued by sericulture scientists. We believe in near future, there must be some substances in the nature capable of regulating nutrition partitioning in the domesticated silkworms as the scientific technology is advancing. One of substances fed to the silkworms can enable the silkworms to distribute nutrition to eggs, and this substance is used in the breeding farm. Another is used for production and can enable the silkworms to distribute nutrition to the silk, and the silk production can be increased.

Dream B: To treat diseases keep domesticated silkworms healthy

Nosema bombycis was first separated from *bombyx mori* by Naglli in 1857. It got its name, as it was parasitic on the *bombyx mori*. The *nosema bombycis* can infect through food and embryos and are lethal to sericulture production. Therefore, in the sericulture production the *nosema bombycis* was determined as the only statutory

inspection subject. The nosema bombycis is a critical and difficulty problem in the sericulture study. There are lots of legends on the nosema bombycis, one of which is about a great man Pasteur people will never forget in the sericulture history. In 1845, pebrine hit Vaucluse in France for the first time and later spread quickly to other silkworm breeding farms in Europe and caused destructive loss to the European sericulture in 17th century. At this critical moment, Pasteur was entrusted with a mission to deal with the pebrine. In fact, Pasteur had no idea about the bombyx mori and even didn't see it before. After taking the mission, he made every endeavor to seek for solution days and nights. At last, Pasteur observed a tiny oval green fluorescent particle with a microscope. It was the particle that infected the bombyx mori through the mouths and infected the next generation through eggs. Since then he invented a method of Pebrine Mother Moths Sampling Inspection to rule out sick moths and prevent embryo infection. This method is still used today in the sericulture. From then on, scientists started study in the morphology, life history, serology, pathology, and genetic evolution, but fruited no substantial breakthrough. I used to investigate in the breeding farms in China and saw the farm workers were exhausted to worry about the pebrine. The leaves they picked up should be rinsed with chlorinated lime and conducted Pebrine Mother Moths Sampling Inspection after the production of species. Nonetheless, pebrine is still hard to ward off. I used to dream that one day another "Pasteur" would show up and defeat the pebrine for good and all. As the genome of the nosema bombycis is decoded, scientists will definitely find out the biological target of chemical treatment or conquer the last defense line by modifying the genome of bombyx mori.

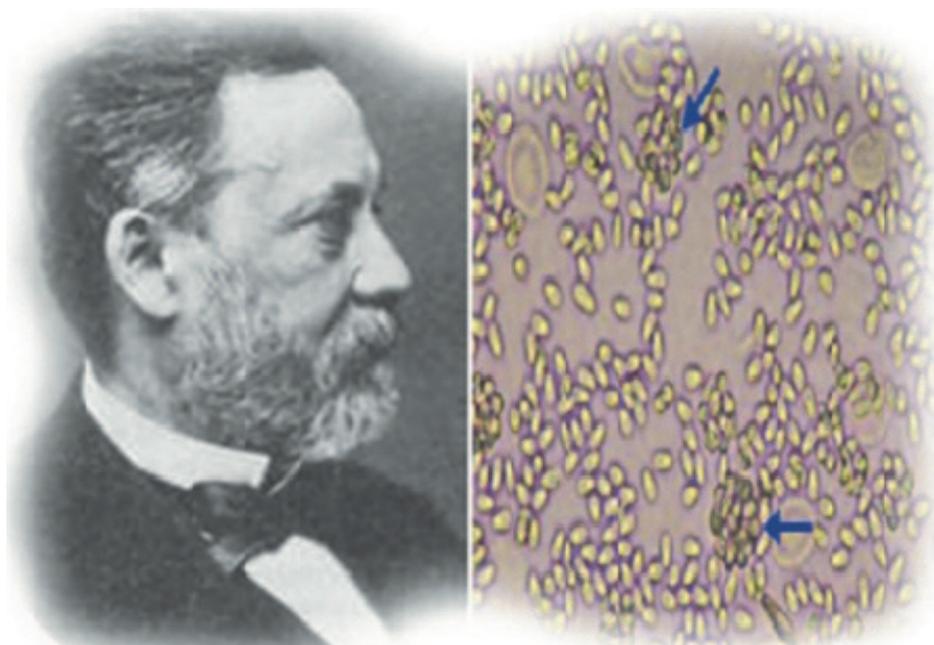


Figure 133. Pasteur and nosema bombycis.

Another disease affecting the silkworm production is nuclear polyhedrosis. It is a virus disease and usually called BmNPV. The major communication way is the mouth and the second is wounds. The disease is fast in morbidity and highly infectious. Once attacking, it cannot be cured just as “cancer” for humans. The loss caused by the BmNPV in the sericulture production accounts for 60% of the total loss. BmNPV usually hits India and China in summer and autumn. After graduated from the university, I took part in the “Yellow River-Huaihe River-Haihe River Sericulture Development Project” in the north of Jiangsu and knew how destructive this disease is and witnessed the hardship the silkworm farmers had been through when the disease hit there in large area. I was determined to solve BmNPV and comprehended the natural law of mutual engendering and restriction and the principle of individuality and characteristics. I inferred that since the silkworms can be infected with BmNPV, there must be silkworm strains against the infection and this is so-called biodiversity. In 1999, by evaluation of disease resistances of 344 strains in the silkworm species library of China, I found a local strain has resistance against the BmNPV. I worked on genetic analysis while conducting system purification as well. The early genetic experiment was unstable and reached a conclusion similar to the previous ones that the virus resistance of BmNPV is under control of host genes and synergic action of several modifiers. In fact, at the time a simple truth was ignored, that is, the disease-resistant strain is a blended group, because people didn’t select the disease resistance. In the group most are disease-resistant individuals, and a few of them are inductive. Therefore, the genetic experiment can confirm that the disease resistance against the BmNPV is dominant inheritance, but fail to determine which genes dominates, host genes or single gene. Afterwards, I found the problem of material sophistication and attained the pure lines of resistance through 8 generations of separation and purification. The result of another genetic test clearly stated that the disease resistance of the special species against BmNPV is dominated by a single dominance gene, corrected the theory of multi-gene domination on the BmNPV resistance and guided the breeding of the disease-resistant diseases of the silkworms. It sounds easy today. In fact, I was doubted when proposing there are disease-resistant resources against BmNPV existing in the silkworms. Of course, the proposal has been widely recognized. Many other scientists have successfully cultured new BmNPV-resistant species worldwide. However, what is a BmNPV resistant gene? How does the resistance mechanism work? Over the past 10 years, I have studied them and seen some breakthroughs, but I may need more time to unveil the “truth”.

Dream C: to construct a biology model turns silkworm production into industrial production

It is a complicated and hard process from planting mulberry trees, breeding to producing silk. Whether do plants simulate silkworm production and abandon the natural silk production process? This dream sounds impossible. We expect with the advancing of the bioengineering technology, the dream may come true. In retrospect to the

development history of scientific technology, those impossible but realized still amaze us. You must have heard about a big bang in biology, synthetic biology. The traditional biology studies the internal structure by dissecting living beings, but the synthetic biology goes in the opposite direction. It starts building parts from the most basic element step by step and reshapes life. This is the core idea of the synthetic biology. In 2002, virologist Eckard Wimmer from New York University announced that he and his team purchased short fragments of DNA from a bio-tech company and connected them with the aid of a DNA synthesis company and produced artificially synthesized poliovirus. In 2010, the research team led by Craig Venter of J. Craig Venter Institute created a new bacterial species, Synthia.

Nowadays, we can boldly envision the industrial silk production. The silkworms eat the mulberry leaves, which go into the digestive tract, decomposed by enzyme inside, and are partially absorbed and partially discharged. A part of the absorbed part left in the silkworms is used for growth and development, and another part of the absorbed enters the silk gland and synthesizes the fibroin. Definitely, this process must be complicated, dynamic, and variable. For this, we conceive that various enzymes are added into mulberry ingredients artificially made, then pass an unknown black hole and are synthesized into fibroin. I don't know how possible this dream will be, but I think, the genome of the mulberry trees has been formed, as well as that of the silkworm, and the structure and formation process of fibroin have been clear. As the synthetic biology technology advances, it is possible to artificially synthesize the silk, even silk better than that of silkworms. To realize this great dream and write an end, I have to quote Sun Yat-sen as saying that the twisted roads lead to a bright prospect.