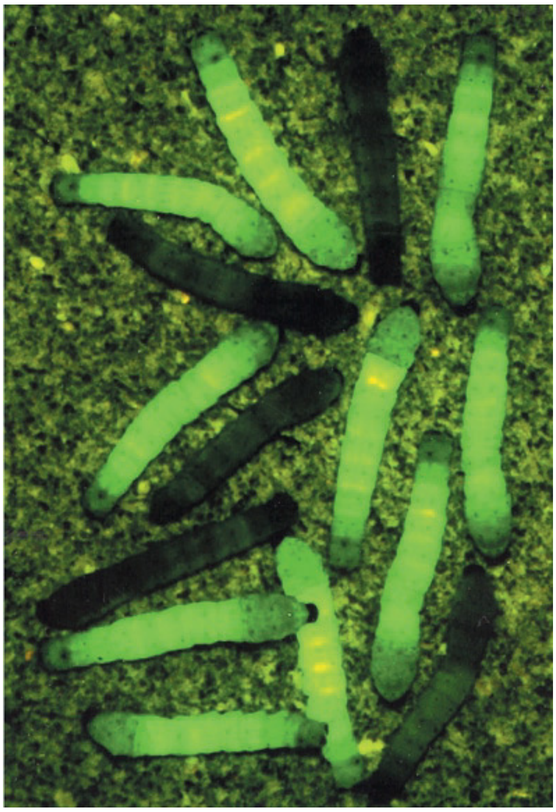
The Silkworm Turns: A New Era for Japanese Sericulture

Japan gave considerable attention to its sericulture industry during the Meiji period (1868–1912), and in the 1930s the country was the world's second largest producer of silkworm cocoons. With the sericulture technologies it had been developing since the Meiji period as its foundation, Japan is today pursuing biotechnology-based sericulture, which could be labeled a new sericulture industry. **Toshiki Tamura**, director of the Transgenic Silkworm Research Center at the National Institute of Agrobiological Sciences, who successfully produced the world's first transgenic silkworms, describes the new potential of silkworms.



Germline transformation of the silkworm *Bombyx mori* L. using a piggyback transposon-derived vector. Toshiki Tamura, Thibert Chantal, Royer Corinne, et al., (2000), *Nature Biotechnology*, Vol.18, January 2000, pp. 81–84.

The history of sericulture dates back as far as around 5000 BCE, and sericulture technologies were brought to Japan from mainland China around the first century (mid-Yayoi period). Subsequently, sericulture developed throughout Japan. During the Meiji period, silk thread became one of Japan's most important exports. The national government promoted silk thread production and sericulture technology expanded significantly. The establishment of sericulture training centers where human resources were developed and the development of technology at production laboratories built for original silkworm species and sericulture laboratories served as major driving forces. By the early twentieth century, around 1,000 species of silkworm existed in Japan. In 1906, a Japanese researcher achieve a world first by verifying that Mendel's principles of genetics also apply to silkworms, and created a hybrid species called the "first filial generation." This is also referred to as a crossbreed or hybrid. The principle of heterosis, or hybrid vigor, was at work, which led to stronger, larger and higher-quality cocoons, and this resulted in dramatic gains in sericulture productivity.

Sericulture technologies originating in Japan are diverse, such as the identification of the genes that determine silkworms' eye and egg colors, experiments that verified the fact that genes are associated with many properties of living organisms, and the development of technologies to adjust the time of pupation and the size of cocoons based on the structure and functions of prothoracicotropic hormone, which works in the process of metamorphosis.

Creating Transgenic Silkworms

Propelled by the accumulation of sericulture technologies and the development of genetics, research into creating transgenic silkworms has progressed in Japan in recent years.

Transgenic silkworms cannot be made only by injecting DNA as is done with other animals and plants, so the gene transposon that moves over the chromosomes is used. Of several types of transposon, the DNA type can be used for making a recombinant. Two types of DNA, vector and helper, are injected. The vector has been recombined with the green fluorescent protein (GFP) gene, a marker gene that identifies transgenic silkworms, and the helper has the transposase gene, which works to shift the gene recombined with the vector onto the chromosomes of silkworms. Transgenic silkworms made by injecting the vector and helper are genetically stable, so next-generation silkworms have the same properties. As a result, a path toward the development and production of new silk fibers with advanced functions has opened up. For example, while silk has been used as a surgical suture material because it is highly biocompatible, research is now being conducted to develop artificial veins, tendons and ligaments by advancing silk fibers functionally. Progress has also been made with the development of technology that uses silk as materials for artificial cornea, teeth, bones, cartilage and skin.

Practical Application of Transgenic Silkworms

Diverse studies are underway at research institutes such as the National Institute of Agrobiological Sciences, the University of Tokyo and Kyushu University, as well as at companies, for multifaceted application of transgenic silkworms.

One example is the use of transgenic silkworms as test animals. To date, mammals such as mice and rats have been used to evaluate the effects of new drugs. The advantages of silkworms over mammals have been noted, in terms of costs and animal protection considerations. Silkworms also have similar reactions to drugs as mammals. Silkworms offer the advantage of being the only domesticated insects, and technologies for large-volume cultivation have been established. They are also relatively large and can be treated and injected by human hands. Of particular note is that silkworms have blood, eyes, mouth, intestines, liver, brain, muscle and other tissues and organs that correspond to those of humans, and sickness models can be configured with silkworms. Knowledge found on sickness models for viral infection, liver ailments, diabetes and others have been publicized, and silkworms are expected to be used as test animals in drug development.

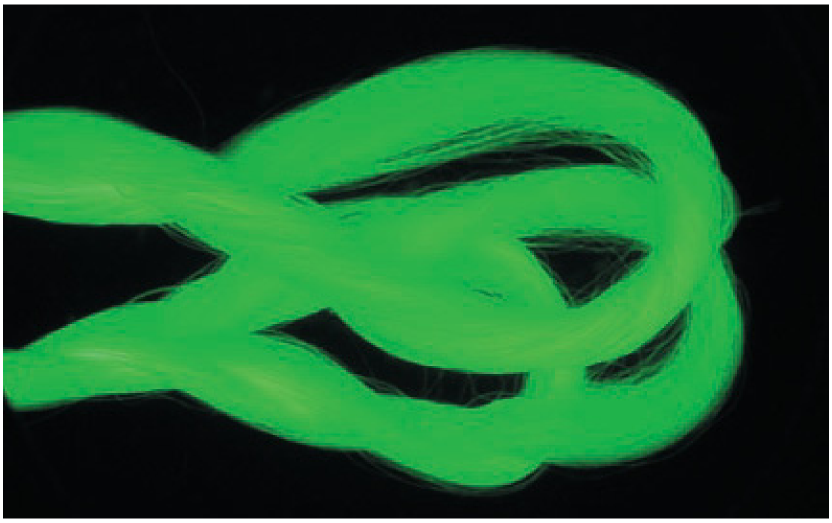
Research has also progressed at insect factories, where useful proteins are produced. About 97% of cocoons spun by silkworms are useful proteins, and studies are underway to find application for them as useful resources. Many proteins that are useful for humans, such as interferon, are difficult to produce and obtain in large volume and entail enormous cost. Methods are therefore being studied and developed for recombining genes of useful proteins into silkworms and reproducing useful proteins in large volumes instead of silk proteins.

One example is an approach that uses insect pathogens and viruses (particularly baculovirus). The expression system for baculovirus has been confirmed to express exogenous genes efficiently. When silkworms are used as a host, however, it is difficult to remove the virus because it is difficult to extract useful proteins, expressive substances that are secreted into the blood (bodily fluid). The method whereby useful proteins are produced in the silk gland using transgenic silkworms has an advantage in that it is easy to refine proteins and it is suited for volume-geared production. The proteins for which expression has been reported so far include collagen, interferon, human fibroblast growth factor and human albumin. Also, a method to produce active recombinant proteins in the posterior silk gland, using fibroin H-chain genes that produce large amounts of protein, has been developed. This is evidence that useful proteins produced from transgenic silkworms can be used not only as new silk fibers but also in diverse areas including medical treatment, biotechnology-based foods and cosmetics.

Silkworms are the only domesticated insects, and their larvae have atrophied legs, so they cannot climb mulberry trees to eat the leaves. Adult insects have atrophied wings and cannot fly to make contact with partners for reproduction. As such, the likeliness of transgenic silkworms turning wild and thriving is almost zero. There is no risk of environmental biohazard. Another feature of silkworms is that they do not incubate contagious diseases for humans. For all these reasons, production using transgenic silkworms is safe in every way.

Other examples include development of environment-friendly agrichemicals that use transgenic silkworms. The demands placed on safe foods are expected to heighten in the future, leading to increased demand for safe and efficient control methods against pests that eat and damage crops. It is important to develop agrichemicals that are better than existing agrichemicals from a safety standpoint. Development of new agrichemicals and research of pest control are being pursued using transgenic silkworms, through clarification of the insects' juvenile hormone actions and viral infection mechanism.

About 80% of the silkworm genome has been deciphered, as of the end of 2008. Based on the results, more sophisticated silkworms with diverse exogenous genes injected into the genome will likely be produced in the future. Specific examples include the creation of new species injected with genes that can withstand illness, agrichemicals and other stress, as well as genes of other insects, and production of growth hormones, vaccines and other useful substances. The potential of new biotechnology-based sericulture is indeed likely to emerge.



Silk made from cocoons expressed with the green fluorescent protein (GFP) gene

Toshiki Tamura is director of the Transgenic Silkworm Research Center of the National Institute of Agrobiological Sciences.