Approach to the introduction of GM silkworms to sericulture farms for recombinant silk production in Japan

Keiko Kadono-Okuda

Institute of Agrobiological Sciences,

National Agriculture and Food Research Organization (NARO),

Japan

Advantage of GM silkworms

Fast growth

It takes less than one month from hatching to cocoon-spinning with 10.000x growth from egg to larvae.

Easy control

Silkworms can't survive or reproduce in nature.
Larvae don't escape even if they are starved.
Moths are unable to fly although they have wings.
High density rearing is available with artificial diet (1,000 larvae/m^2).

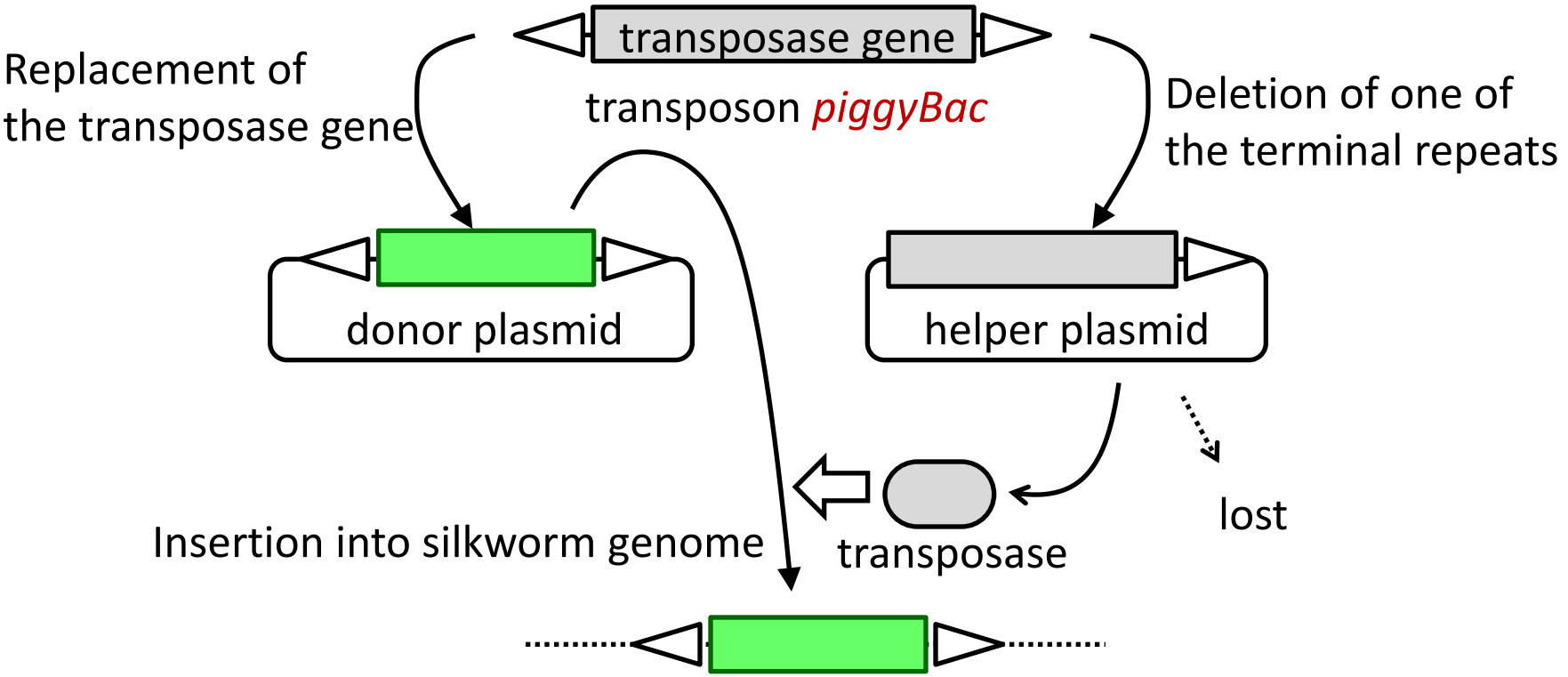
Highly active protein production in silk glands

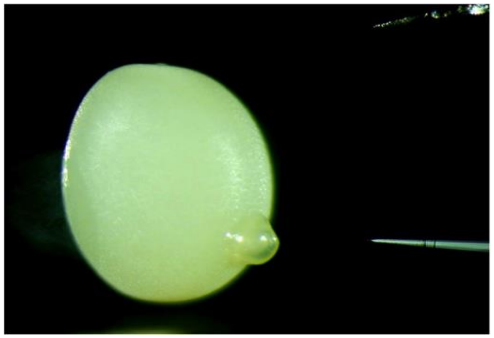
Silk glands occupy up to 30% of larval body weight and produce 100μg-10mg recombinant protein.

Safe

There has been no virus infectious to human.

Production of GM silkworms

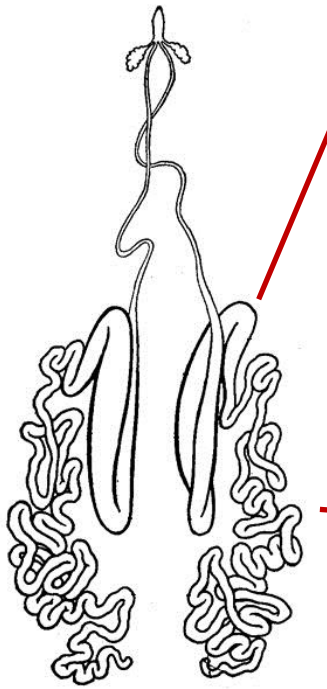




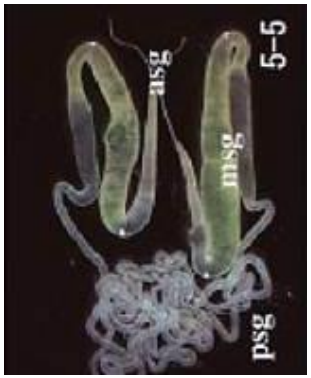
Injection of the two plasmids into silkworm eggs

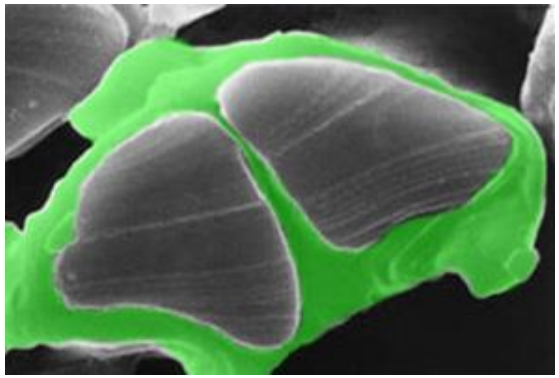
Two-layer structure of silk fiber

Fibroin and sericin are produced in different part of silk glands.

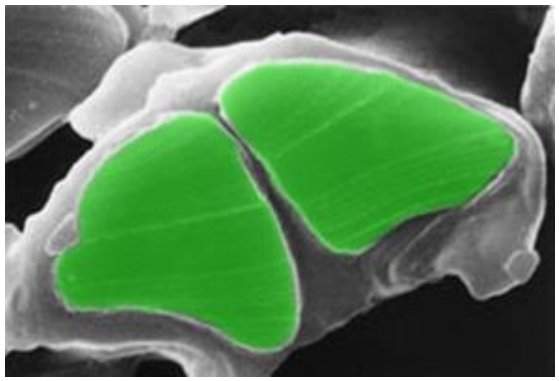


middle silk gland: sericin layer, water-soluble





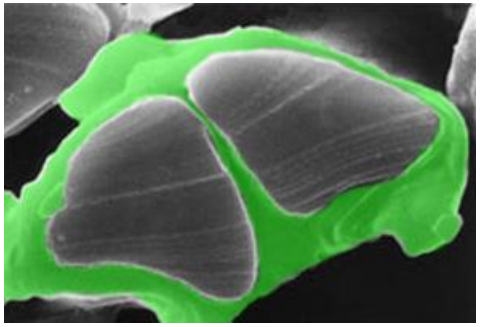
posterior silk gland: fibroin layer, water-insoluble



Application of GM silkworms

Application 1:

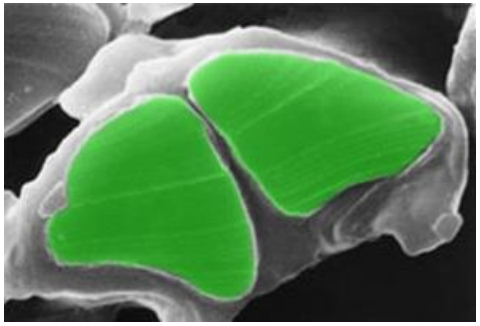
Recombinant protein production



- Expression in the **sericin** layer (water-soluble).
- Easy recovery and purification.
- Proteins for pharmaceutical, diagnostic or cosmetic use.
- Some have been already commercialized.
- Rearing in closed facilities to keep clean conditions.

Application 2:

New recombinant silk production



- Expression in the **fibroin** layer.
- Fluorescent silk, ultra-thin silk, spider silk, etc.
- Under development.
- Rearing in sericulture farms.

Recombinant protein production by GM silkworms

Example of application 1:

Some proteins are commercially produced by silkworms.

Components of kits for diagnosis and research
(enzymes, antigens, antibodies, etc.)

Osteoporosis (TRACP-5b)

Canine inflammation reaction (CRP)

Alzheimer's dementia (amyloid-ß)

Human collagen I for cosmetics

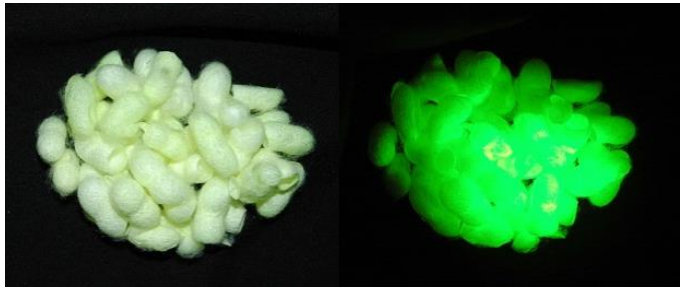
Ongoing researches of drug production

- Antibody drugs
- Vaccines
- Hematological drugs (fibrinogen, albumin, etc.)

Fluorescent silks

Example of application 2:

Expression of modified fibroin heavy chain gene



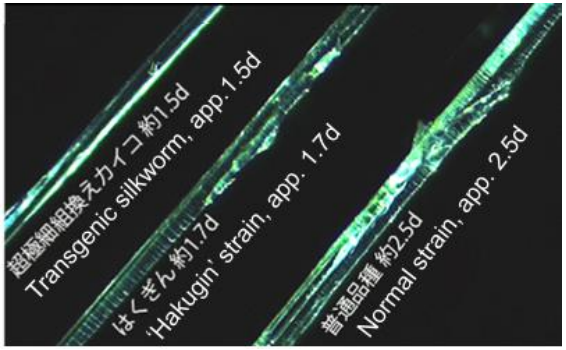
Sputniko!

Various recombinant silks

Example of application 2:

Ultra-thin silk

- Modification of amino acid composition of fibroin.
- Thread size is 1.5 denier.



Spider silk

- Fusion of spider dragline silk protein and silkworm fibroin.
- Toughness increases by 50%.

Regulation of GMOs in Japan

Conservation of biological diversity

- Cartagena Act*
- Ministry of the Environment and related ministries

Food safety

- Food Sanitation Act
- Food Safety Commission
- Ministry of Health, Labour and Welfare

Feed safety

- Feed Sanitation Law
- Food Safety Commission
- Ministry of Agriculture, Forestry and Fisheries

* Cartagena Act is the law that regulates the use of LMOs, to ensure the precise and smooth implementation of the “Cartagena Protocol” on the Biosafety to Convention on Biological Diversity.

GMO Regulation in the Cartagena Act

Type 2 Use

- Use of GMOs under closed system.
- Rearing of GM animals or culture of GM plants, cells or bacteria inlaboratories or factories, etc.
- Allowed if the containment measures are judged appropriate to prevent dispersal of the GMOs.

Type 1 Use

- Use of GMOs under open system.
- Cultivation of GM crops in the field, releasing of GM insects into the field, etc.
- Allowed if the GMOs are judged not to cause harmful effects on biological diversity.

Regulation and application of GM silkworms

Type 2 Use: Recombinant protein production in factories

- Clean condition.
- Rearing through the year.
- High value of the products.
- High cost of artificial diet and waste disposal.
  (autoclave or freeze waste to kill silkworms in it)

Type 1 Use: New recombinant silk production in sericulture farms

- Low-cost mass rearing.
- Feeding on mulberry leaves.
- Simple disposal of waste.

The necessity of Type 1 Use in sericulture farms

Type 1 Use: rearing under open system, without containment measures

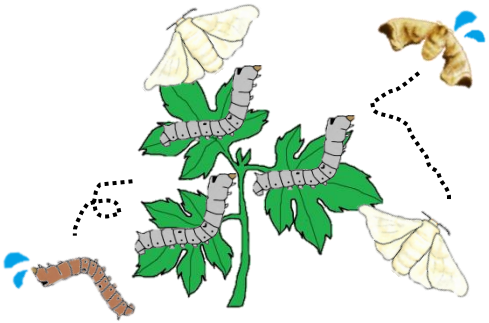
Although silkworms are reared indoor...

- Large amount of waste, mainly mulberry branches.
- In sericulture farms, waste is usually took out to compost.
- Impossible to remove all remaining silkworms from the waste.
- Type 2 Use (rearing under closed system) does not allow any possibilities to expose GMOs in the environment.

Risk assessment of GM silkworms for Biological Diversity

Competitive?

Silkworms can't survive or reproduce by themselves in nature, even in transgenic silkworms.



Predatory or parasitic?

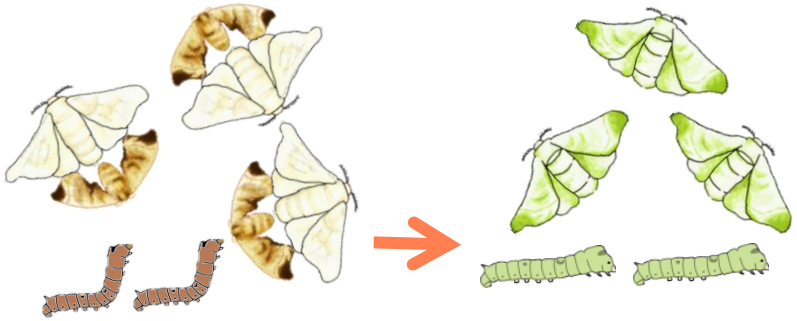
Silkworms can't feed by themselves in nature even in transgenic silkworms.

Toxic-substances producer?

It is necessary to assess each transgenic strain.

Cross-ability to wild species

There is a wild species in Japan but no hybrids have not been found in the nature.



Wild relative of the silkworm

Wild mulberry silkworm, *Bombyx mandarina*



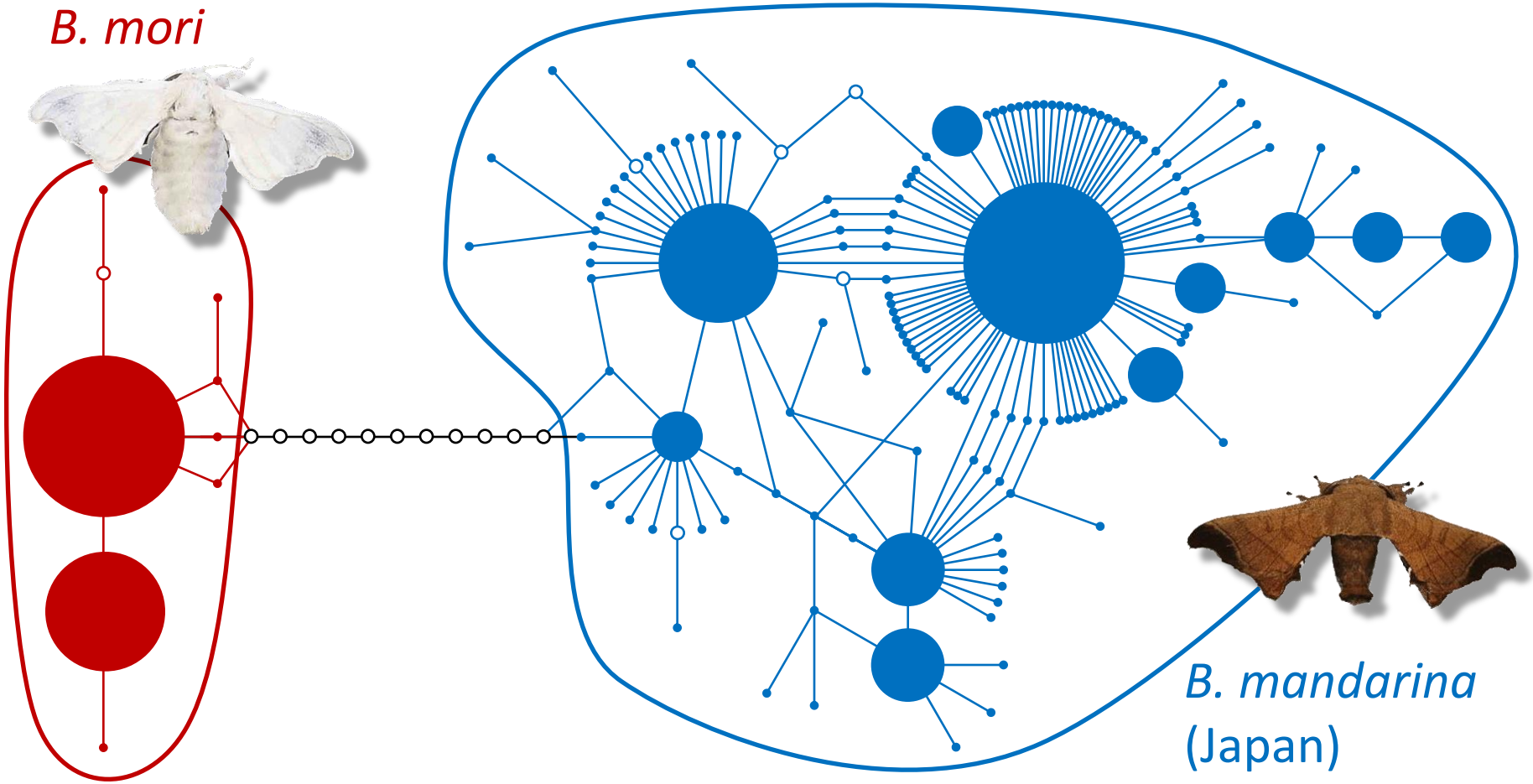
Larvae walk around actively, and moths can fly.

Domesticated silkworm, *Bombyx mori*



No gene flow from *B. mori* to *B. mandarina*

Mitochondrial *COI* haplotypes



Hybrids are non-existent or extremely rare.

No gene flows anywhere in Japan.

- No gene of *B. mori* has been found in the *B. mandarina* collected all around Japan.
- No F_1 moths have been found around active sericulture farms.

No moths in sericulture farms

- Sericultural farms sell cocoons (pupae in them).
- Adult-eclosion damages cocoons.
- Cocoons are dried by heating to kill pupae in silk mills.

Domesticated silkworms can't survive in nature.

- Larvae and moths of the domesticated silkworm are inactive and preyed easily by birds and other insects.

Test rearing of GM silkworms as Type 1 Use

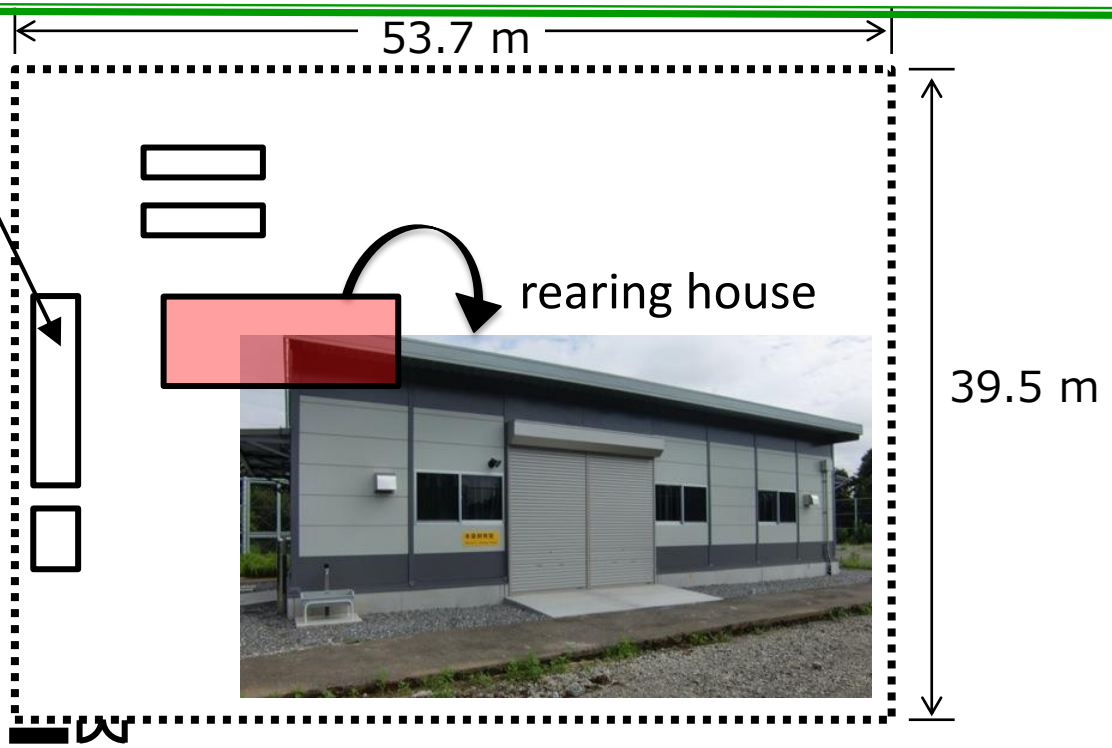
Test rearing in a restricted area

- Restricted areas in our institutes.
- From 4th-instar larvae to cocoon.
- Rearing in a condition similar to that of sericulture farms.
- The first GM silkworm strain to produce green fluorescent silk was approved in May 2014.

Data for further application and commercialization

- Test the expected performance (growth rate, healthiness, quality of the fluorescence silk, etc.)
- Test the possibility of influence on biological diversity (number of remaining silkworms in waste, emergence of moths, etc.)

A restricted area for test rearing by type 1 use



Test rearing of GM silkworms by type 1 use

feeding on 5th-instar larvae

collecting larvae for cocooning

waste left in a container

cocooning

Prospects of GM silkworm industry

Recombinant protein production in factories

- Regulation of GM silkworm rearing is simple in closed facilities, such as factories.
- Farmers can be involved in providing mulberry leaves for the production of artificial diet.

Recombinant silk production in sericulture farms

- Regulation of GM silkworm is an important issue.
- Farmers can rear approved GM silkworms in their own facilities throughout Japan.
- It will be a new hope for sericulture farmers in Japan.

Thank you for your attention