2024

Newsletter “おかいこさま”

Overseas Distribution

National Bio-Resource Project “Silkworm”

Special version for overseas researchers

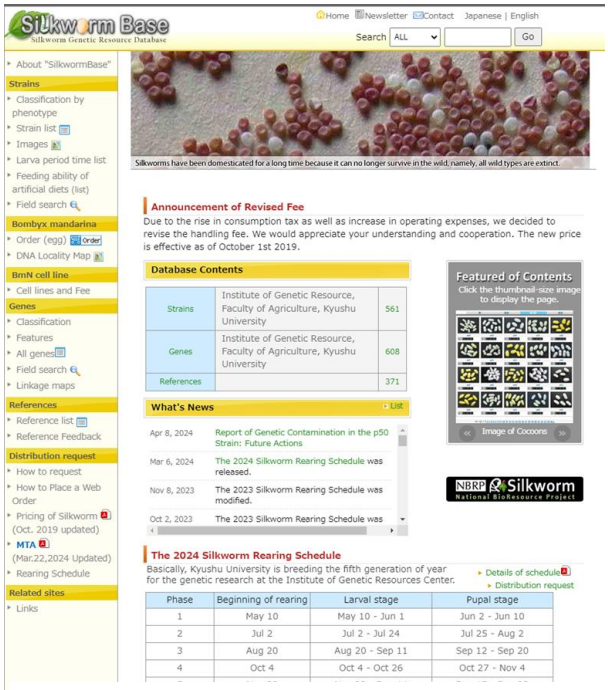
National Bio-Resource Project “Silkworm” Newsletter
August 1, 2024
http://www.nbrp.jp/index.jsp

***Bombyx mandarina* (Oki)**

The *Bombyx mandarina* Oki strain is large and is collected from Oki Island, Japan. They have been maintained at Kyushu University since 2022.

NBRP "Silkworm" resources

Kyushu University, the core institute of the NBRP "Silkworm," rears over 500 mutant strains of *B. mori* annually, and we are able to provide any mutant strain. Upon request, two Japanese strains (Oki and Sakado) of wild silkworms (*B. mandarina*) are also available (p. 3–5). In addition, we have consomic and semiconosomic strains, in which one pair of *B. mori* autosomes has been replaced with a *B. mandarina* chromosome (p. 3). Since last year, two types of BmN cell lines have been prepared and are available for distribution (p. 6). We look forward to receiving your orders.



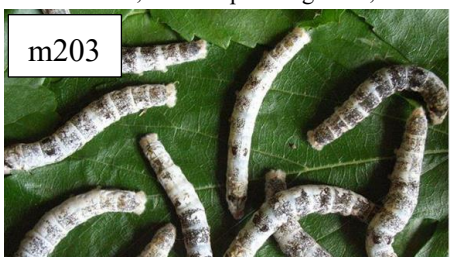
Silkworm strains: Classification by phenotype

Symbol	Phenotype	Number of related strains
NBRP	Rearing is easy	3
S	European strain	8
T	Consomic strain	26
a	lethal: embryo & larvae	20
b	cocoon: shape & quality	15
c	cocoon: color	28
d	egg: shape & chorion	38
e	egg: serosa color	29
f	larvae: appendage & marking	39
g	larvae: marking	15
i	larvae: eye-, head-, & tail spot	13
k	larvae: body color	22
l	larvae: body color	24
m	mosaic & malformation	18
n	larvae: body shape	30
o	larvae: translucent skin	41
p	local cultivar(landrace)	28
r	chromosomal aberration	20
t	growth, voltinism, moltinism	24
u	pupa & adult	25
w	multi marker line	27
x	new mutant in analysis	61
他	other	7
	Total	561

Classification by phenotype and the number of each strain.
You can view the strains available for distribution and their features on the website. More than 500 silkworm strains are available for distribution. https://shigen.nig.ac.jp/silkwormbase/top.jsp

Characteristic phenotypes

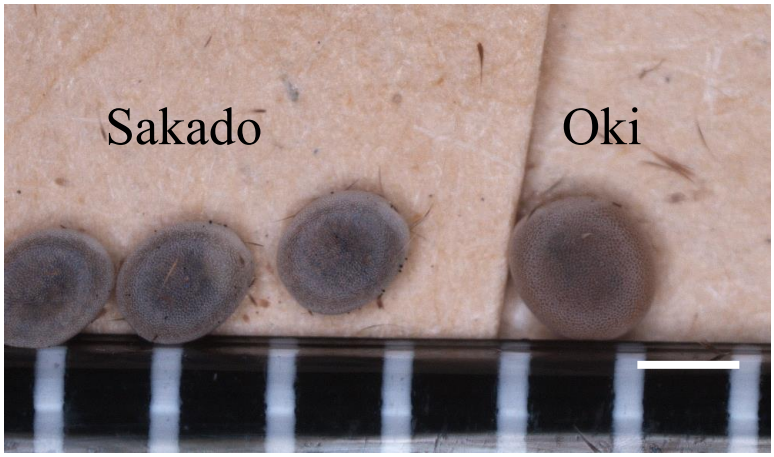
a60: Gene that affects the cuticle hardening at the first ecdysis.
Spontaneous; homozygotes hatch and develop normally during the first instar, and distinct albino features appear soon after the first ecdysis and die with shrinking. The cuticle layer has incomplete hardening and a porous structure. They are unable to eat leaves and maintain body water. The independent spontaneous mutation al^2 is like *al*. Symbol: *al*
c43: Spontaneous; with a pink cocoon, and coexistence with Y, $+^C$ (or CI), and F is needed for manifestation. Symbol: *F*, *Pk*
k31: Spontaneous; with the dorsal and lateral sides of the larva that are covered with dark brown pigments, resulting in a white longitudinal band above the dorsal vessel. When in combination with p, they are almost white except for small black spots on the side of the thorax, and in a p^2 background, there is a wavy pattern on the side of the abdomen. Symbol: *U*



m203: Includes many mosaic individuals, which is presumed to be caused by the presence of fragmented chromosomes.
t70: Adult silkworms usually live for approximately a week after emersence; however, the t70 strain is lethal within a few days. The exact cause is unknown, but the responsible region is located on chromosome 25.
u42: Introduced from *B. mandarina*. Spontaneous; with a black spot on the apex of the wings, and when heterozygous, it is less conspicuous, especially in females. Symbol: *Ws*

Bombyx mandarina and other silk moths

B. mori are insects that were domesticated from wild silkworms (*B. mandarina*) over 5,000 years ago. We provide two strains of *B. mandarina* that were collected from Sakado, Saitama Prefecture, and Oki, Shimane Prefecture. We present the characteristics of the two strains.

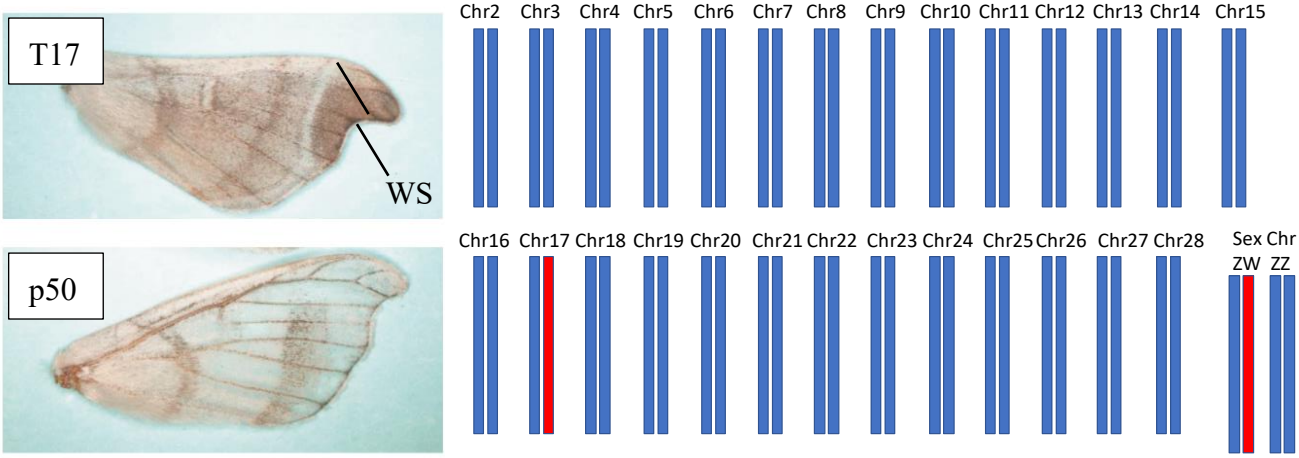


Introduction of the Sakado and Oki strains
NBRP "Silkworm" has two *B. mandarina* strains available for distribution. The Sakado strain was collected in Sakado City, Saitama Prefecture, and the Oki strain was collected in Oki Island, Shimane Prefecture, and they have been reared at Kyushu University. Sakado is the origin of the CT and T strains and is similar in size to the common Japanese *B. mandarina*, whereas Oki is larger than Sakado. Sakado is reared from summer to autumn, and Oki is reared in autumn.

Shinshu University is the curator of the wild silk moths. Information on Japanese wild silkworm moths are available at https://shigen.nig.ac.jp/wildmoth/.
More details are on pages 7–8.

Consomic and semi-consomic strains

We have consomic (CT) and semiconsomic (T) strains that have been constructed from the female Sakado strain of *B. mandarina*. These strains have a single or a pair of *B. mandarina* autosomes and a female-specific sex chromosome (W), and the remaining chromosomes are from the standard strain p50 of *B. mori*. *B. mandarina* in Japan has fused chromosomes that correspond to Chr14 and Chr27 of *B. mori*. Thus, there are no CT27 and T27. Details of these lines are summarized in the article *Journal of Insect Biotechnology and Sericology* 90, 33–40 (2021).
DOI: https://doi.org/10.11416/jibs.90.2_033

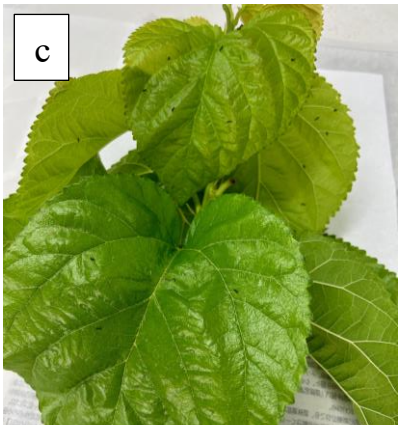


Characteristics of the T17 strain: wing spot (WS) and chromosome composition.
The red chromosomes are from the Sakado strain of *B. mandarina,* and the blue chromosomes are from the p50 strain of *B. mori*.

The dominant traits on the *B. mandarina* chromosome are easy to determine.
The CT website has not yet been published. Kindly contact us via email or other means to request a distribution. Pricing is the same as for regular eggs.

How to rear *B. mandarina*

B. mandarina is more sensitive to stress than *B. mori*. We present here how we rear and mate them.



a: Incubation of eggs

B. mandarina eggs are maintained at a temperature of 25°C and a humidity of approximately 75% for 14 days.

b: Move newly hatched larvae to mulberry leaves

Use bird feathers to gently move the larvae to the mulberry leaves. *B. mandarina* eggs do not hatch simultaneously; therefore, this should be done over several days. The hatching rate is approximately 50%.

c: Larvae rearing immediately after hatching

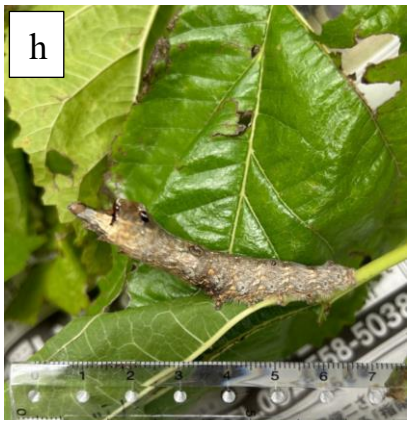
They are reared on fresh and soft mulberry leaves that are used by the branch. The rearing temperature is 25°C.

d: Humidity control

Larvae do not eat dry leaves; therefore, they should be kept under high humidity conditions. However, high humidity causes mold growth, and rearing cages should be kept clean. Mulberry feeding is done once a day.

e and f: Cleaning of rearing cages

Remove larval excrement and leftovers daily, as this can cause disease. When moving larvae, move them with the mulberry and do not touch them directly because this can cause ecdysis failure and disease.



g: Use of humidity retention bags

A bag that can maintain humidity (such as a vegetable bag) can be used as a substitute for rearing cages.

h: Third to last instar larvae

The final instar of *B. mandarina* is generally 4th instar. Some individuals of the Oki strain are nearly 7 cm in length.

i: Population density and mulberry feeding

If the number of individuals in the final instar is too large, the number of mulberry leaves may become insufficient, and the humidity may become too high. It is better to maintain a small number of individuals.

j: Wandering stage

Individuals who have eaten sufficient mulberry leaves necessary for the metamorphosis into pupae are active and very mobile. Care must be taken to prevent escape.

k and l: Spinning period

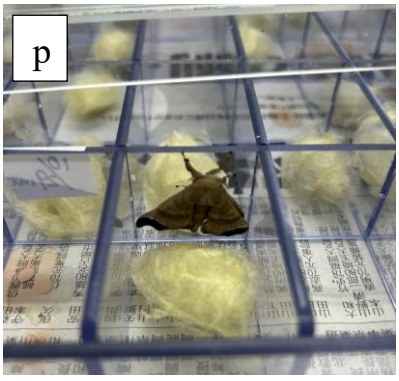
Larva produces feces and urine with a lot of water before it creates a cocoon. The cocoon-making process requires a three-dimensional frame. There are other frames that can be used, such as wavy paper or other materials, but generally, mulberry leaves are used to make the cocoons.

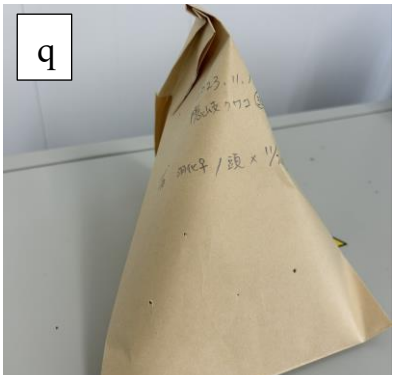
m and n: Cocoon of the Oki strain

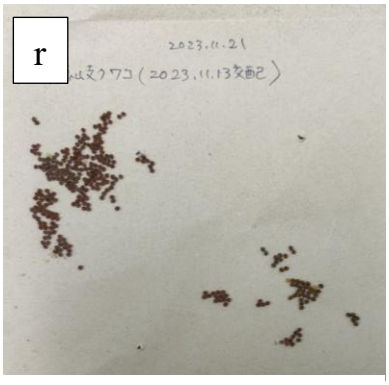
After the cocoon is completed, it takes approximately five days for the larvae to become pupa. During this period, the cocoon should not be moved. The cocoon size varies greatly among individuals, but in general, the cocoons of Oki tend to be larger.

o: Cocoon storage

Remove the paper, mulberry leaves, etc., and move only the cocoons to a separate room. However, if there is a possibility that the cocoons will be damaged, move them without removing them from the frame. The cocoons should be maintained at a temperature of 25°C and a humidity of approximately 60% until the emergence of the moths.







p and q: Mating

After identifying the male and female moths, 1–2 pairs are placed in a bag to allow for mating to occur. The bags are punctured with small air holes, and the egg-laying status is checked 3–5 days later. Note that some pupae are in summer diapause, and the moths may appear sluggishly.

r: Egg check

The first moths to emerge often produce non-diapause or unfertilized eggs; therefore, the eggs need to be checked.

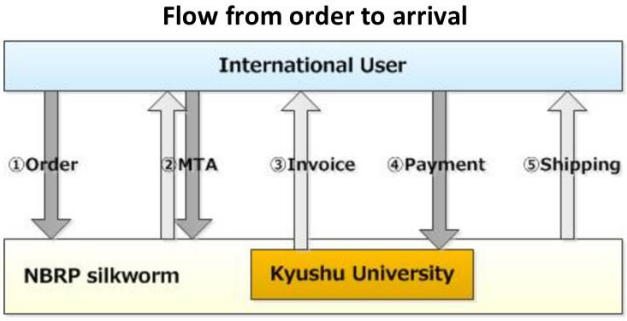
Diapause eggs are used for the following year's rearing, and they should be treated in the same manner as *B. mori*. Diapause breakage should be performed (cold treatment for about 3 months) according to the rearing time of the following year.

European strains

Kyushu University also has some non-Japanese silkworm strains, including many European silkworm strains. We have seven Spanish strains (S01, S02, S03, S04, S05, S06, and S13), two Italian strains (c42 and c44), and one French strain (u48). We introduce some of these strains here.

c44: This Kyushu University strain produces the reddest-looking cocoons.
S01: This strain produces the deepest yellow cocoons, and the larvae and cocoons are larger.
u48: This strain originates from the Papillion noire in France and is a slightly darker moth.

Overview of Distribution request



1: Order
Ordering from the Silkworm base is simple. Silkworm strains and characteristics can be selected by viewing the photographs.
2: Material transfer agreement (MTA)
A contract must be signed before our resources can be used (silkworms, BmN cells, etc.). We also accept some commercial use agreements.
3: Invoice
An invoice will be generated once the shipping details and method have been determined. This may take approximately a week to prepare.
4: Payment
The amount on the invoice in Japanese yen (JPY) should be paid. Kindly note that you are responsible for any other external fees. You can pay by bank transfer or WISE.
5: Shipping
After confirming payment, we will ship the order. This takes approximately one week, although the number of days varies depending on the shipping area.

It takes approximately 4 weeks from order to arrival.
Please contact us at least one month before the date you wish to rear the silkworm.

Q&A

The following is a list of frequently asked questions about distribution.

① Differences in shipping methods

We ship via EMS or FedEx. EMS has a shipping cost of about 3,150 yen (as of April 2024). If you are in an area with a hub airline, it will take approximately one week to arrive. However, considerable delays occur during peak season. FedEx has an indefinite shipping cost; however, it often costs more than 10,000 yen. It arrives faster than EMS, and the delivery days are more stable. Please indicate your shipping preference when placing an order.

	EMS	FedEx
Place (JPY)	3,150	About 10,000
Shipping days	1~2 weeks	3~10 days

※Place and days vary depending on location and time of season

② Two types of rates: academic and non-academic

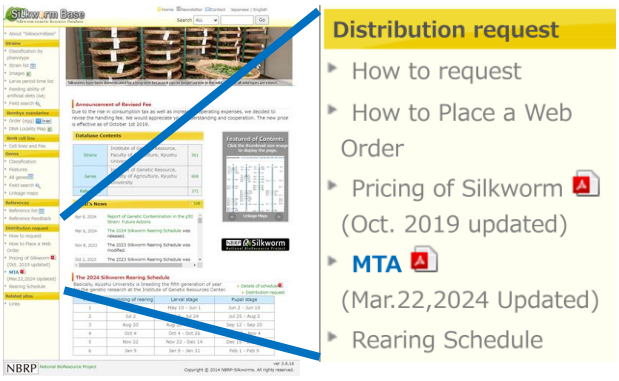
Academic rates apply to educational and research institutions when using the product for educational or academic research purposes.

Name	Number of lots offered	Fee (JPY)※3	
		Academic	Non-academic
BmN (With serum)※1	1 lot (2ml×2)	2,950	5,900
BmNp (Without serum)※2	1 lot (50ml×1)	3,950	7,900

		Unit	1	2	3	4	5	6
Egg	Price	Academic	1,520	1,670	1,830	1,990	2,150	2,310
		Non-academic	3,040	3,340	3,660	3,980	4,300	4,620
Larva	Price	Academic	2,230	3,040	3,850	4,770	5,570	6,380
		Non-academic	4,460	6,080	7,700	9,540	11,140	12,760
Pupa, Adult, Cocoon	Price	Academic	2,230	3,040	3,850	4,770	5,570	6,380
		Non-academic	4,460	6,080	7,700	9,540	11,140	12,760
DNA	Price	Academic	2,170	2,650	3,130	3,610	4,090	4,570
		Non-academic	4,340	5,300	6,260	7,220	8,180	9,140

③ More information on how to order

Ordering instructions and required documentation are summarized in the Distribution request section of our website.



When submitting a research article using resources distributed by NBRP "silkworms," please clearly mention the use of NBRP "silkworms" in your manuscript.

④ Invoice, payment, and shipping

Once the MTA has been signed and the shipping method is fixed, an invoice will be prepared. Please let us know if you require an address or VAT number on the invoice. We can also generate separate shipping and resource invoices.

Payment Notes

1. **Remittance fees are the client's responsibility.**
2. **Please make all payments in Japanese yen (JPY).**

Shipping Notes

1. **If you request delivery by FedEx, the shipment will be shipped pay-on-delivery.**
2. **Delivery is not possible during the summer (over 30°C) because the temperatures are too high.**
3. **In areas where delivery takes longer, we only deliver during the winter.**
4. **Refunds and reshipments are not available if eggs hatch because of shipping problems.**

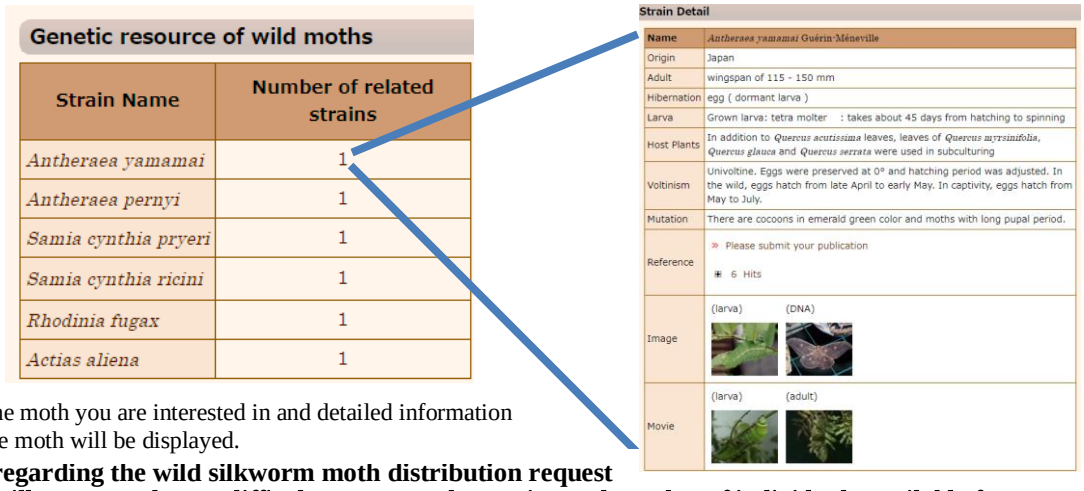
⑤ How to rear *B. mori*

It is easier to rear *B. mori* than *B. mandarina*. For details on rearing *B. mori*, kindly refer to previous publications (https://shigen.nig.ac.jp/silkwormbase/newsletter/No43_20190415.pdf). In addition, we do not distribute mulberry and artificial diet overseas. Please prepare the feed locally.

This QR code leads to information about how to rear *B. mori*. ⇒

Japanese wild silkworm moths

Shinshu University, the sub-core facility of the NBRP "Silkworm," maintains and distributes 6 wild silkworm species including *Samia cynthia pryeri* Butler, *Samia cynthia ricini* Donovan, *Antheraea yamamai* Guérin-Méneville, *Antheraea pernyi* Guérin-Méneville, *Actias aliena* Butler and *Rhodinia fugax* Butler.



Select the moth you are interested in and detailed information about the moth will be displayed.

Note regarding the wild silkworm moth distribution request

① **Wild silkworm moths are difficult to rear, so the strains and number of individuals available for distribution varies greatly.**
② **We are only shipping eggs due to the risk of insects escaping during shipping.**
③ **In some countries, we may not be able to ship to resource users.**
④ **Maximum care should be taken to prevent insects from escaping.**

Introduction of providing silkworms resources

●Kyushu University

Researchers can inquire by referencing this table as a guide. For time-sensitive requests, please contact Kyushu University directly.

Rearing schedule in 2024th

Phase	Larval stage	Pupal stage
1	May 10～Jun 1	Jun 2～Jun 10
2	Jul 2～Jul 24	Jul 25～Aug 2
3	Aug 20～Sep 11	Sep 12～Sep 20
4	Oct 4～Oct 26	Oct 27～Nov 4
5	Nov 22～Dec 14	Dec 15～Dec 23
6	Jan 9～Jan 31	Feb 1～Feb 9

We can provide DNA samples of *B. mori* and *B. mandarina*.

Our institute maintains a DNA repository of *B. mori* mutant strains (approximately 500) and *B. mandarina* collected over 40 locations across Japan (Hokkaido to Kagoshima). This resource offers researchers valuable DNA material for studies where rearing live silkworms is challenging or for investigations into genetic polymorphisms within strains due to the individual-level purification of DNA.

●Shinshu University (Sub-core facility)

The Shinshu University sub-core facility curates a collection of saturniid species native to Japan, including *Rhodinia fugax*, *Actias aliena*, *Actias gnoma*, *Saturnia janasii*, *Samia cynthia pryeri*, and *Aglia japonica*. For a complete species list, please visit our website.

http://www.shigen.nig.ac.jp/wildmoth/index.jsp

Contact: Zenta Kajiura (zkajiur@shinshu-u.ac.jp)

Strain name	Stage	Period	Offer
Antheraea yamamai	egg(diapause)	Sep～nextJun	～100eggs
	larva	Jun～Sep	～50 individuals
	pupa	Jul～Oct	～50 individuals
	Adult	Aug～Oct	～10 individuals
Antheraea pernyi	egg(non-diapause)	Apr～Aug	～100eggs
	Larva	Jun～Sep	～50 individuals
	pupa(diapause)	Sep～nextMay	～50 individuals
	Adult	May～Oct	～10 individuals
Samia cynthia ricini	egg(non-diapause)	yearround	～1000eggs
	larva	yearround	～100 individuals
	pupa(diapause)	yearround	～100 individuals
	Adult	yearround	～10 individuals

About the newsletter "おかいこさま"

Silkworms have held a significant role in Japanese agriculture. Traditionally, farmers meticulously raised silkworms, even keeping them in their home parlors, and bestowed upon them the respectful title “Okaiko-sama（おかいこさま）” (“sama” signifying respect). Silkworms hold a special place in Japanese culture. For generations, the Empress of Japan has personally overseen the care of “Okaiko-sama” at the Imperial Palace silkworm farm. This tradition underscores the significance of silkworms as a unique bioresource developed in Japan. In recognition of this heritage, we named our information magazine dedicated to sharing recent advancements in silkworm resources.

Newsletter “おかいこさま” Edited and published by

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