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Toward Practical Use of Silkworm Cryopreservation Technology

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Problems Associated with Maintenance of Silkworm Strains

Plant resources are generally maintained in long-term preservation as seeds, and the next generation is obtained by dissemination. Such plant resources can be preserved for approximately 10 years, although the preservation period differs according to the plant species. For silkworms (*Bombyx mori*), their eggs are preserved but the effective preservation period is only 1 year. Therefore, to preserve a silkworm strain, its eggs are hatched, the hatched silkworms are bred, and the bred adults are mated to obtain new eggs. This procedure must be performed every year. Furthermore, it is difficult to manage a mulberry plantation to obtain leaves as feed for silkworms. Therefore, the development of a long-term preservation method for silkworm strains had been strongly desired before establishment of the NBRP-Silkworm.

Utility of the Cryopreservation of Ovaries and Sperm

Long-term preservation of silkworm strains has been performed to some extent. Fig. 1 shows a method in which an undeveloped ovary is excised from a silkworm during its larval stage, preserved in liquid nitrogen, and then the next generation is obtained by its transplantation after thawing. Another useful method is collection of sperm from an adult. The collected sperm is cryopreserved, and the next generation is obtained from the cryopreserved sperm after thawing. However, the success rates (the number of eggs/ number of eclosed individuals) of these methods are very low and require advanced technical skills. Therefore, these methods are not practical. The NBRP-Silkworm has applied practical methods using sperm since 2004 and ovaries since 2006. This article introduces the history of the NBRP-Silkworm toward the practical use of long-term preservation technologies for silkworm strains and findings obtained during their practical use.

History of Practical Use

In 2006

- Mr. A (a post-doctoral researcher in his late twenties) and Ms. B (a part-time employee in her mid-forties), who were staff members of the NBRP-Silkworm, received training on ovary cryopreservation technology from its developer.
- These staff members performed ovary cryopreservation during the winter season, but the cryopreservations failed continuously.

In 2007

- Mr. A was transferred to another institute in a foreign country and Ms. C (a technical staff member in her early fifties) was employed as Mr. A's successor. Ms. C learned the technology from Ms. B. Thus, the technology was somewhat inherited.
- Ms. D (a post-doctoral researcher in her late twenties) joined the team and proposed a change of cryoprotective agent. Consequently, there was an increase in the average number of eggs. Ms. D eventually left the team at which time the success rate was slightly below 10% (Fig. 1).

In 2008

- Ms. E (a technical staff member in her late twenties) joined the team as Ms. D's successor. Owing to her experience at her previous workplace (preservation of cells), she proposed a method to gradually decrease the freezing temperature for cryopreservation of ovaries. Consequently, there was an increase in the number of eggs obtained from frozen/thawed ovaries.
- Adding a new step to retain a long string-shaped tissue, called the ovarian string duct, attached to an ovary in a donor and entwining it with another ovarian string duct in a host individual was expected to increase the success rate. This step greatly contributed to the advancement of the NBRP-Silkworm and success rate increased to 30% (Fig. 1).

In 2009

- Ms. E took maternity leave. Ms. F (a technical staff member in her late twenties) was temporarily employed during Ms. E's maternity leave. Ms. F and Ms. C performed further assessments of the two methods that had been considered useful in 2008. Consequently, these methods entered a stage where practical use was expected.

From 2010 onwards

- Only hybrid strains had been used for the development of ovary cryopreservation technologies until 2010. A hybrid strain is obtained by mating two different strains, and it possesses strong vitality because of heterosis effects. However, if the developed technology cannot be used to preserve strains other than hybrid strains, the developed technology is useless for strain preservation. Therefore, we investigated the number of eggs and the success rate after cryopreservation and thawing of 500 strains (core resources) with different histories and origins. At present, approximately 50% of the strains can be cryopreserved for a long period using the developed technology. Regarding the remaining strains, the number of eggs decreased after freeze/thawing of some strains, and Ms. B, Ms. C, and Ms. E have been tasked with solving this problem.

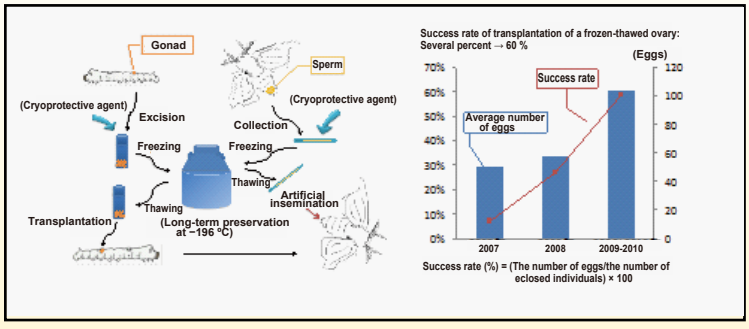

Fig. 1: Outline of the cryopreservation technology and improvement of the success rate

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Collaboration and Participation of Female Staff Members

The long-term preservation technology for silkworm resources using ovaries has been in development for five years. At present, this technology has reached the stage of practical use. The development process of this technology was briefly introduced in the previous chapter. Of the six staff members who were involved in the NBRP-Silkworm, many readers might have already realized that five members were female, except for Mr. A, Ms. B and Ms. C had already completed their childcare responsibilities to some extent and have been continuously engaged in the project. They have displayed their abilities to introduce, develop, and continue the long-term preservation technology. Ms. D, Ms. E, and Ms. F contributed to the project as female researchers who had completed studies at biology-related graduate schools. These three members could only participate in the project for a short period because of childcare responsibilities.

However, Ms. B and Ms. C, who were experienced members, improved the technology and advanced the project. The project may be an example of a strain preservation project that needs continuity and introduction of novel ideas. We are grateful to the female staff members for their collaboration.

In addition, the results of this technological development have been published in *Cryobiology* 66 (2013) 283−287.

Mobile Website Emulation Utilizing Desktop Browsers

Ongoing Column [No. 86] 10min

As the market for mobile devices such as smartphones and tablets rapidly expands, an increasing number of websites are being developed for these devices. However, mobile devices have varying screen resolutions, and the perceived resolution (CSS pixels) also varies widely. Furthermore, the height and width of the browser are inverted when a mobile device is rotated sideways (Table 1)
Thus, it is important to validate whether a mobile website is rendered correctly under various mobile device resolutions. While there would still be a need to validate how websites are ultimately rendered using physical mobile devices, current desktop browsers now enable developers to easily emulate the display of a mobile website. This article introduces examples of mobile device emulation, utilizing Internet Explorer (IE) 11, Firefox 26, and Google Chrome 32 on a Windows PC.

Table 1. Example visual resolutions of mobile devices

Device	Resolution (height ×width px)
Smartphones	
iPhone5s	568×320
iPhone4s	480×320
Xperia Z1 SO-01F	640×360
Tablets	
iPad Air	1024×768

IE 11

1. After starting IE11, press the [F12] key to display the Developer Tools console.
2. Select Emulation from the left-hand side menu, at the bottom.
3. From the Emulation screen, you can specify the display orientation, resolution, as well as the geolocation (GPS).

Fig. 1. Example of mobile website emulation utilizing IE 11

Firefox 26

1. After starting Firefox, press [Ctrl + Shift + M] on the keyboard to display the Responsive Design View.
2. The display orientation and resolution can be changed from the menu at the top of the display. Additionally, there is an option to convert mouse events to touch events.

Fig. 2. Example of mobile website emulation utilizing Firefox 26

Chrome 32

1. After starting Chrome, press [Ctrl + Shift + I] to display the Developer Tools console.
2. Select the cog icon displayed at the bottom of the window to display the Settings screen.
3. Select Overrides from the menu on the Settings screen, and select the “Show 'Emulation' view in console drawer” option, then close the Settings screen.
4. Select “Show console” located on the left of the cog icon to show the console.
5. By selecting Emulation from the control screen, you can select options for emulating various mobile devices. You can configure display orientation and resolution, as well as geolocation (GPS) and accelerator sensor settings.

Fig. 3. Example of mobile website emulation utilizing Chrome 32

[Figs are the Japanese version]

As demonstrated above, there are numerous attributes that you can configure, such as display orientation and resolution, depending on the browser. Please use the appropriate browser based on your needs.

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Editor's Note

This month, successful establishment of silkworm cryopreservation technology using ovaries was introduced in this newsletter. It was achieved by six staff members who transferred knowledge to one another. Although some team members were replaced by others, the technology has certainly been inherited. This achievement appears to be a good example of a strain preservation project. These achievements by staff members were probably made by trial and error during their daily routines, and Associate Professor Yutaka Banno has supervised carefully. In fact, the NBRP-Silkworm was briefly introduced along with some photographs in the August issue of this newsletter in 2012 and I would recommend you to read it. (Y. Y.).

BioResource Information

(NBRP) www.nbrp.jp/
(SHIGEN) www.shigen.nig.ac.jp/
(WGR) www.shigen.nig.ac.jp/wgr/
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