The method of DNA-seq analysis published in Okaiko-sama No.54.

Genomic DNA was extracted from CT05 and contaminated-p50 male larvae using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). DNA library preparation and sequencing were performed by Azenta (Beijing, China) using DNBSEQ (MGI, Shenzhen, China) and Novaseq(Illumina, San Diego, USA). The DNA sequence data for the Sakado strain (B. mandarina) was downloaded from the DDBJ DRA database (DRA004652). Raw DNA sequencing reads were subjected to quality checking and trimming to remove adaptor sequences, contamination, and low-quality reads using fastp version 0.22.0 (Chen et al., 2018) (-q 30). The trimmed reads were aligned to the publicly available reference genome of B. mori (Genome assembly (November 2016), http://silkbase.ab.a.u-tokyo.ac.jp) (Kawamoto et al., 2019) using minimap2 version 2.1.0 with default parameter settings (Li, 2018). SAMtools version 1.9 was used to convert SAM files and to sort and index BAM files (Li et al., 2009). Genomic variants including single nucleotide polymorphisms (SNPs) were called using SAMtools version 1.9, using the command line "mpileup -uf" and BCFtools version 1.9 (call -c) (Narasimhan et al., 2016). SNPs fewer than 20 mapped reads were filtered out using SnpSift version 4.3t using the following command: filter "(DP>=20)" (Cingolani et al., 2012a). Histograms were drawn using R packages (R Development Core Team, 2013)

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2024

Newsletter “おかいこさま” No.54

National Bio-Resource Project “Silkworm”

National Bio-Resource Project “Silkworm” Newsletter
April 8, 2024 Volume 54
http://www.nbrp.jp/index.jsp

DNA-seq analysis of the *Bombyx mori* consomic strain CT05

We employed DNA sequencing to analyze the CT05 strain, a *Bombyx mori* individual homozygous for the p50 type across all chromosomes except chromosome 5. Chromosome 5 exhibits homozygosity for the Sakado type（*Bombyx mandarina*）. The obtained DNA-seq data was mapped to the p50 reference sequence, followed by visualization of single nucleotide polymorphism (SNP) distribution across chromosomes using histograms with a bin width of 1 million base pairs. This analysis revealed a dense cluster of SNPs specifically on chromosome 5.

Report of Genetic Contamination in the p50 Strain: Future Actions

We report the identification of genetic contamination in the p50 strain maintained by the Silkworm Resource Division, Institute of Genetic Resources, Kyushu University. In January 2024, investigations confirmed the presence of W chromosome from *Bombyx mandarina* (Sakado strain) within the p50 strain.

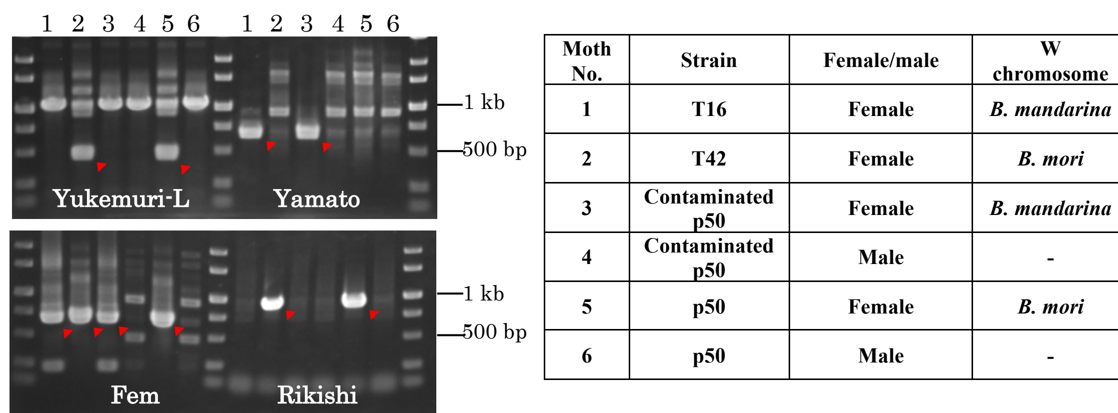
B. mandarina is a close relative of *B. mori*, allowing for interspecific crosses. Through controlled breeding, we have generated semiconsomic strains in which the p50 strain of *B. mori* serves as the recurrent parent, while the W chromosome originates from the Sakado strain of *B. mandarina* collected in Sakado City, Saitama Prefecture, Japan. The semiconsomic strains harbor predominantly p50 chromosomes, except for the W chromosome, derived from *B. mandarina*, rendering them phenotypically indistinguishable from the p50 strain, except in four lines. These four exceptions retain dominant visible trait *loci* on chromosomes 2, 17, 23, and 24 inherited from *B. mandarina*, allowing for differentiation. These observations suggest a potential inadvertent introduction of genetic material from the semiconsomic strains into the p50 strain.

Distribution of the p50 strain has been temporarily suspended following the identification of genetic contamination. To re-establish a verified p50 lineage, we obtained the p50T strain from the University of Tokyo's Insect Genetics Laboratory and initiated its rearing in February 2024. The p50T strain originates from the p50 strain previously maintained at the Silkworm Resource Division, Institute of Genetic Resources, Kyushu University. This strain (p50T) was derived from the original p50 strain maintained at Kyushu University through repeated inbreeding via single moth rearing and subsequent genome analysis for differentiation. Distributed to the Silkworm Resource Division, Institute of Genetic Resources, Kyushu University in 2002, this strain was managed and distributed under the name p50 since then. Consequently, researchers utilizing the p50 strain distributed after March 2024 should be aware that it is genetically identical to the p50 strain distributed prior to contamination (from 2002 onward).

PCR-based genotyping allows for the differentiation of W chromosomes between the Sakado and p50 strains, as detailed in Figure 1 and Table 1. We are currently investigating potential autosomal contamination. To facilitate identification, whole-genome re-sequencing and mapping to the p50 reference genome were performed on a consomic strain, the Sakado strain, and the genetically contaminated p50 strain (Figure 2, Cover page).

Our investigation revealed contamination within the p50 strain as early as 2019. However, the precise origin of the contamination remains undetermined. To address this issue, we are establishing a distribution system for verified p50 eggs, free of charge, to researchers who are currently utilizing p50 obtained from NBRP Silkworm.

Moving forward, we will implement stricter strain management and distribution protocols to prevent similar incidents.



Moth No.	Strain	Female/male	W chromosome
1	T16	Female	*B. mandarina*
2	T42	Female	*B. mori*
3	Contaminated p50	Female	*B. mandarina*
4	Contaminated p50	Male	-
5	p50	Female	*B. mori*
6	p50	Male	-

Figure 1. PCR-based genotyping of the W chromosome for p50 and Sakado strains.

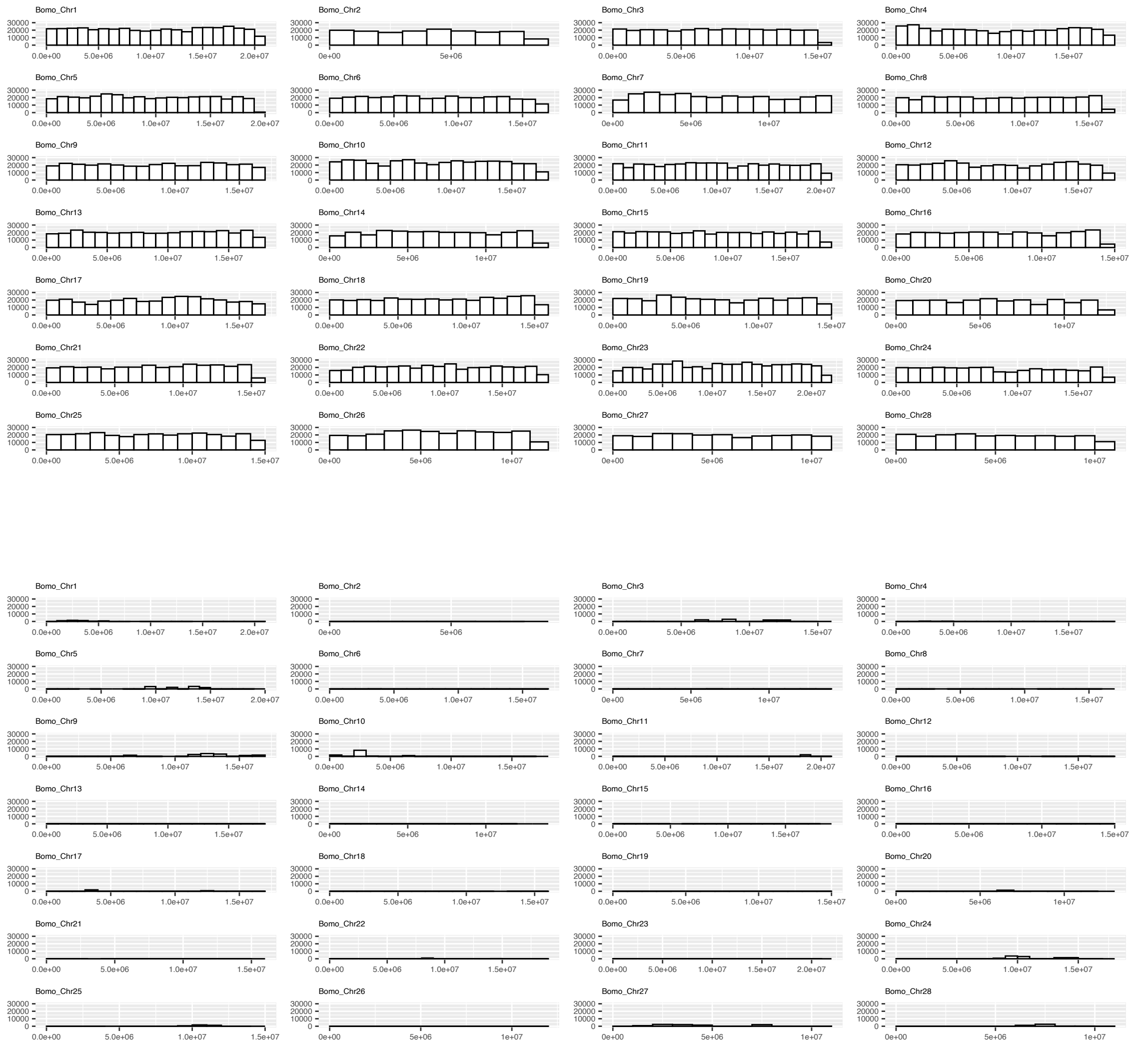


Figure 2. DNA-seq analysis of the Sakado and contaminated p50 strains.

DNA sequence data for the Sakado strain (top) was retrieved from the DDBJ DRA database (DRA004652), while data for the genetically contaminated p50 lineage reared in January 2024 (as shown on the cover page) and the CT05 strain (bottom) were sequenced from a single male of each strain using DNBSEQ and Illumina platforms. Following short-read mapping to the p50 reference genome and single nucleotide polymorphism (SNP) selection based on quality filters (minimum depth of 20 reads), the number of SNPs per chromosome was visualized in histograms with 1 million base pair bins. Detailed data analysis methods will be made available on the NBRP Silkworm website (https://shigen.nig.ac.jp/).

Table 1. PCR primers for genotyping the W chromosome

Marker name	Primer name	Sequence (5′-3′)	Product size (bp)	*B. mori* Wchromosome	*B. mandarina* Wchromosome	Reference
Yukemuri-L	Yukemuri-L-F1	AGTGAAGGTCCCAGAGTAACAACT	461	Amplification	No amplification	1
	Yukemuri-L-	AGGTTGCCAGAGCAACTCTATACT				
Yamato	yamato2	TGCCAGCCATTTCTAACTAC	700	No amplification	Amplification	2
	yamato1A	AGTCAGCGCTCACGGCCAAT				
Fem	Fem-1F	TCAAAAACGTATGTATCAGG	668	Amplification	Amplification	3
	Fem-2R	CTCGGATCGCACGAAATCAG				
Rikishi	Rikishi-A1	GGCGATGCTGTGTACCCAGAATGT	927	Amplification	No amplification	1
	Rikishi-B2	GTTCCTCTGCGATGGGTGGCACATA				

Reference 1. Insect Mol. Biol. 14, 339–352 (2005). https://doi.org/10.1111/j.1365-2583.2005.00565.x
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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.

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～next Jun	～100 eggs
	larva	Jun～Sep	～50 individuals
	pupa	Jul～Oct	～50 individuals
	Adult	Aug～Oct	～10 individuals
Antheraea pernyi	egg(non-diapause)	Apr～Aug	～100 eggs
	Larva	Jun～Sep	～50 individuals
	pupa(diapause)	Sep～next May	～50 individuals
	Adult	May～Oct	～10 individuals
Samia cynthia ricini	egg(non-diapause)	year round	～1000 eggs
	larva	year round	～100 individuals
	pupa(diapause)	year round	～100 individuals
	Adult	year round	～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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