A report of a high proportion of *naked pupae* in *Bombyx mori* without the *Fib-H* gene mutation

Yanzhi Xia[a,1], Qingyu Wang[b,1], Echo Xu[c,1], Zhenxia Wang[d], Bo Wang[a,e*]

[a] College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, PR China

[b] Wuhan Foreign Languages School, Wuhan, Hubei, PR China.

[c] James Ruse Agricultural High School, Sydney, New South Wales, Australia.

[d] Primary School Attached to HUST, Wuhan, Hubei, PR China.

[e] Medical Genetics Center, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei, PR China.

[1] These authors contributed equally to this work.

*Corresponding author.

Bo Wang

College of Life Science and Technology

Huazhong University of Science and Technology

ABSTRACT: We report a high proportion of *naked pupae* in *Bombyx mori* without the *Fib-H* gene mutation. Environmental factors (high temperature and high humidity) should be the main reason for the high proportion of *naked pupae*.

KEYWORDS: *Bombyx mori; naked pupa*; *Fib-H* gene

The silkworm (*Bombyx mori*) belongs to the order Lepidoptera, family Bombycidae, genus Bombycidae, is a monophagous insect that feeds only on mulberry leaf (Xia, 2023).

Cocoon is produced from *Bombyx mori* to protect itself from outer environment (Zhou 2020). Cocoon is a type of unique and important biopolymer composite in nature with excellent microstructure and ecological functions (Wang, 2021).

The first *Bombyx mori* fibroin-deficient mutant was identified in 1933 and known as a *Naked pupa* (*Nd*, a dominant mutation) (Nakano, 1951). Since then, the *Nd* locus (0.0 cM) has become a ubiquitous 25th chromosomal marker used by *Bombyx* geneticists to track mutations. Molecular genetic studies have revealed the molecular mechanism of *Nd* is the mutation of fibroin heavy chain gene (*Fib-H*) (Takasu, 2023).

Now we report a high proportion of *naked pupae* in *Bombyx mori* without the *Fib-H* gene mutation.

About 50 eggs of *Bombyx mori* came from the previous study (Xia, 2023). After feeding for one month, 29 five-instar silkworms are obtained. These silkworms have spun 26 cocoons and 3 *naked pupae* (Figure 1). About 20 days later, 26 silkworm moths emerged from 26 cocoons and 1 silkworm moths emerged from 1 *naked pupa*.

The silkworm moths from *naked pupa* is a male silkworm moth whose wings have been improperly unfolded (The silkworm moths that emerge from *naked pupae* often cannot fully developed their wings because they have not undergone the squeezing process of emerging from the cocoons, resulting in the inability of the body fluid to fully enter the

wings), and it can mate normally (Figure 2). In the next few days, all the silkworm moths died, and the remaining two *naked pupae* failed to become silkworm moths (Figure 3).

Apart from *Fib-H* gene mutation, poor environmental conditions and feeding management (such as high temperature, high humidity, poor ventilation, excessive carbon dioxide, insufficient oxygen supply, etc)often lead to the occurrence of *naked pupae* (Hu, 2019; Ruan, 2021).

The results of the genetic testing showed that no *Fib-H* gene mutation was found in these silkworm moths, and no drugs were involved in the breeding process.

During the cocoon spinning period, the city experienced high temperatures and heavy rainfall. Environmental factors should be the main reason for the high proportion of *naked pupae*.

Figure 1: Three *naked pupae*.

Figure 2: The male silkworm moths from *naked pupa* is located below the female silkworm moth.

Figure 3: All the silkworm moths died, and the remaining two *naked pupae* failed to become silkworm moths.

DECLARATIONS

Availability of data and materials: Please contact the corresponding author for data requests.

Conflict of interest: The authors have no conflicts of interest relevant to this article.

Authors' contributions:

Bo Wang and Zhenxia Wang are responsible for silkworms rearing.

Bo Wang and Yanzhi Xia are responsible for examination.

Qingyu Wang and Echo Xu are responsible for thesis writing.

LITERATURE CITED

Hu, W., Chen, Y., Lin, Y., and Xia, Q. 2019. Developmental and transcriptomic features characterize defects of silk gland growth and silk production in silkworm *naked pupa* mutant. *Insect biochemistry and molecular biology* 111: 103175.

Nakano, Y., 1951. Physiological, anatomical and genetical studies on the “Naked” silkworm pupa. *The Journal of Sericultural Science of Japan* 20(3): 169–179.

Ruan, J., Wu, M., Ye, X., Zhao, S., Liang, J., Ye, L., You, Z., and Zhong, B. 2021. Comparative mRNA and LncRNA Analysis of the Molecular Mechanisms Associated With Low Silk Production in *Bombyx mori*. *Frontiers in genetics* 11: 592128.

Takasu, Y., Yamada, N., Kojima, K., Iga, M., Yukuhiro, F., Iizuka, T., and Yoshioka, T. 2023. Fibroin heavy chain gene replacement with a highly ordered synthetic repeat sequence in *Bombyx mori*. *Insect biochemistry and molecular biology* 161: 104002.

Wang, W. H., Lin, W. S., Shih, C. H., Chen, C. Y., Kuo, S. H., Li, W. L. and Lin, Y. S. 2021. Functionality of Silk Cocoon (Bombyx mori L.) Sericin Extracts Obtained through High-Temperature Hydrothermal Method. *Materials (Basel, Switzerland)* 14(18): 5314.

Xia, Y. Z., Liang, X. T., Wang, Q. Y. and Wang, B. 2023. A Report of *Bombyx mori* Double Cocoon with Internal Eclosion, Mating and Oviposition. *JOURNAL OF THE KANSAS ENTOMOLOGICAL SOCIETY* 96(1): 1-5.

Zhou, B. and Wang, H. 2020. Structure and functions of cocoons constructed by eri silkworm. *Polymers* 12(11): 2701.