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Progress in Genetic Engineering Research of Silkworms

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Abstract: The genetic breeding of silkworms has established a sound mechanism for silkworm breeding and preservation in previous research, which has steadily promoted the industrial development of silkworms. However, with the continuous advancement of technology, traditional genetic resource development has gradually encountered development bottlenecks and has reached saturation. Therefore, turning to new technological means has become an urgent task. In recent years, the rapid development of molecular biology, genomics, genetic modification, and genome editing technologies has opened up new opportunities for the genetic breeding of silkworms. The application potential of these modern technologies not only promotes the transformation and upgrading of silkworm genetic breeding, but also points out the direction for its future development. In summary, future research and applications in silkworm genetic breeding will focus more on transgenic and genome editing technologies, which will provide new opportunities and ideas for the improvement of varieties and the cultivation of new silkworm breeds.

Keywords: Silkworm; Genome Editing; Silk; Disease-resistant; Genomics.

1. Introduction

The contribution of the domestic silkworm, as an insect of great value to the human economy, has long transcended the single economic category. Since ancient times, the breeding of silkworms and the production of silk have not only contributed to China's economic prosperity, but also played an indispensable role in traditional politics and culture. During the development of Chinese civilization, silk, a luxury product, has not only served as one of the main commodities for trade, but has also become an important vehicle for cultural exchange, symbolizing wealth and prosperity. In the 21st century, with the rapid development of life sciences and biotechnology, the research value and importance of the silkworm, as a typical model species of Lepidoptera, has become more and more prominent. Scientists have gradually realized the potential of the silkworm in the fields of molecular biology, genetic research and bioengineering, providing an important experimental basis and theoretical support for the study of modern biology.

Over the past decade, the Autographa Genome Project has achieved a number of notable results, providing a wealth of genome-wide data for research (Xia et al., 2004). The landmark achievement of this series of efforts was the release of the improved houseworm genome assembly in 2008, with nine-fold sequence coverage, which greatly enhanced the completeness and precision of genomic information (Xia et al., 2008). And with the development of transgenic technology, the research value of the houseworm has been further enhanced, making it an important tool for gene function analysis and genetic improvement research. This series of achievements has not only promoted the in-depth development of the research related to the domestic silkworm, but also provided abundant data support and theoretical basis for related fields. Since the introduction of the first transgenic silkworm in 2000, transgenic technology has been widely used in gene function research and breed improvement of silkworm. The introduction of this technology has not only promoted the genetic research of the domestic silkworm, but also provided new ideas for improving silk quality and production efficiency. Because of its excellent mechanical properties, biocompatibility and biodegradability, silk has attracted widespread attention in several scientific fields in recent years, and has become an important object of research in materials science, biomedicine and environmental protection. However, domestic silkworm farming faces the threat of several diseases in actual production, among which the domestic silkworm nucleopolyhedrosis virus, as a major disease-causing factor, has caused significant economic losses to the farming industry (Ling et al., 2015). Therefore, breeding virus-resistant varieties is regarded as an economic measure to effectively deal with this series of sericultural diseases, and enhancing the disease resistance of the domestic silkworm through genetic improvement can help to guarantee the sustainable development of the aquaculture industry. In addition, the developmental process of insects is precisely regulated by ecdysteroids and sesquiterpene juvenile-preserving hormones, two key endocrine hormones that not only relate to the growth and development of insects, but also play an important role in the application of transgenic technology (Yang et al., 2021). Transgenic technology has important application prospects in silkworm research, and the strategy of breeding virus-resistant varieties against disease threats will provide a guarantee for the healthy development of the silkworm industry, while in-depth studies on the mechanisms of endocrine regulation in insects are expected to promote the further development of this field.

As one of the earliest species to realize genome editing in China, the domestic silkworm has important research value and application potential in the field of agriculture and biotechnology. In recent years, the continuous improvement of genome editing technology in the study of the domestic silkworm has enabled it to play an increasingly important role in genetic improvement and trait optimization (Li et al., 2023). This paper systematically summarizes the development of genetic breeding in the domestic silkworm, with special attention to the progress and effectiveness of transgenic technology and genome editing technology in practical applications. By looking forward to the future research direction and practical application of genetic breeding in the silkworm, this paper aims to promote the in-depth understanding and wide application of genetic improvement

technologies in the silkworm, thereby promoting the progress and development of related fields.

2. Organization of the Text

2.1. Progress in Insect Genetic Engineering Research

The Insecta are the largest group in the animal kingdom, with more than 850,000 known species, an astonishing number that accounts for about 80% of the total number of animals. This diversity allows insects to play an indispensable role in ecosystems, especially in areas closely related to human productive activities. Insects closely related to agriculture and ecosystems mainly include nine orders, namely Orthoptera, Coma, Homoptera, Hemiptera, Psittacidae, Lepidoptera, Coleoptera, Hymenoptera, and Diptera (Zhang Ran et al., 2010). Some of the common insects, such as locusts, crickets, katydids and mole crickets, belong to these orders. These insects not only play an important role in biodiversity, but also play an important role in agricultural production and ecological balance.

2.1.1. Progress in Insect Genomics

The analysis of genome sequences has provided rich data resources for the study of insect molecular biology, which not only covers the genetic information of insects, but also lays the foundation for an in-depth understanding of their biological characteristics, ecological adaptations and the diversity they exhibit in different environments. The progress of systematic biology in entomology is closely related to genomics research, and the accumulation of genomic data has enabled systematic biology research to more comprehensively reveal the phylogenetic relationships of insects and their complex biological processes. As a result, insect genomics has gradually emerged as a popular research field, attracting the attention and input of a large number of scientists. At present, the number of insect genome sequencing projects registered with NCBI has reached 494, of which 225 species have submitted raw sequencing data, 215 species have completed genome splicing, 65 species have been genetically annotated, and 43 related genome research articles have been published. These data and information show that insect genomics research is developing at a rapid pace, providing a solid foundation and abundant information for future biological research.

2.1.2. Progress in Insect Gene Editing Research

The CRISPR/Cas9 system has been widely used in the field of biological research for its simplicity, rapidity and efficiency, especially in a variety of insect studies, such as in a wide range of insects, such as fruit flies (Bombyx mori), silkworms (silkworm), Aedes aegypti and butterflies (Sudmant et al. 2015). The advent of this technology has provided a powerful tool for studying the genomes of insects, allowing scientists to perform genome editing accurately and rapidly. Currently, insects occupy an integral position in ecosystems, where they play an important pollination role in agriculture, as well as posing a threat to the spread of disease in public health. Along with the continuous advancement of insect genome sequencing, researchers are gradually deepening their understanding of various aspects of the growth, development, and evolution of these organisms. In addition, advances in genome editing technology have not only made it possible to precisely modify insect DNA sequences, but have also led to more diverse ways of studying entomology. Thus, genome editing technologies, especially CRISPR/Cas9, have become important tools for understanding and exploring entomology, providing new perspectives and methods for in-depth analysis of the biology of insects and their roles in the ecosystem.

2.1.3. Bombyx mori: A Promising Model Organism for the Life Sciences

The houseworm (Bombyx mori), an economically important insect, has gained increasing attention in the biological sciences in recent years (Golicz et al., 2020). This insect exhibits inexpensive advantages in terms of rearing costs, while its high fecundity and short generation time make the houseworm particularly prominent in biological research. In addition, the houseworm has a clear genetic background, which facilitates researchers in genome resolution and functional studies. With the abundance of genetic resources of the houseworm and the completion of genomic studies, the potential of the introduction of the houseworm as a model organism has been further enhanced. It is noteworthy that some of the houseworm genes are highly homologous to genes related to human genetic diseases, providing an ideal experimental model for studying human disease mechanisms. Therefore, it can be concluded that the silkworm has become an important model organism in the field of modern life sciences, which is particularly suitable for the study of various biological problems related to human diseases.

The domestic silkworm is a lepidopteran insect of great agricultural and economic importance and is widely used in silk production and related industries. Its position in the agricultural economy cannot be underestimated, and it provides lucrative economic benefits to the silk industry in many countries. In addition, the short generation time, clear genetic background and abundant genetic resources of the domestic silkworm make it an ideal target for life science research. In particular, the silkworm genome project initiated by China and Japan since 2003 has greatly promoted the development of sericulture and facilitated breakthroughs in areas such as genomics and transgenic technology (Kluge et al., 2008). With the progress of science and technology, the application of the silkworm in various fields such as antipathogenic drug screening, therapeutic evaluation and environmental safety monitoring has gradually increased. These applications not only enhance the research value of the domestic silkworm, but also provide new ideas and means for disease control and environmental protection. In summary, as an ideal model organism, the houseworm demonstrates broad application prospects, especially in life science research and its related fields, foreshadowing the potential future development direction.

2.2. Progress in the Genomics of the Silkworm, Bombyx Mori

On September 30, 2002, the International Lepidoptera Genome Project (ILGP) held a meeting in Tsukuba, Japan, during which the participating experts reached a unanimous decision to make the genome project of the houseworm a primary goal. This decision not only highlighted the status of the silkworm as an economically important insect, but also laid a solid foundation for subsequent genomic research. Subsequently, on November 15, 2003, the Chongqing Municipal People's Government and the Chinese Academy of Sciences (CAS) jointly announced that, after years of effort, the framework map of the silkworm genome had been successfully completed. This milestone achievement provided a clear guideline and structure for the in-depth study

and follow-up of the silkworm genome. Further, by December 10, 2004, a Chinese research team successfully and independently completed the 6X genome sequence of the houseworm and the corresponding biological analyses (Parent et al., 2018), driven by both international cooperation and independent research and development. This achievement not only marked the successful completion of the first phase of the silkworm genome project, but also won a reputation for the Chinese research team in the global genome research field.

2.2.1. Genomic Framework of the Chinese Silkworm (Bombyx Mori)

In 2004, Southwest Agricultural University successfully completed the genome framework map of the silkworm, which is an important progress in this field (Xia et al., 2004). Based on this framework map, the university also established the world's largest database of expressed sequence tags of the silkworm, which provided abundant data support for subsequent genomic research. In the course of the study, the research team has deeply excavated key functional gene clusters related to sex determination, developmental process, hormone regulation and resistance of the silkworm, which provides a new perspective to understand the complexity of the biology of the silkworm. In addition, the research work also achieved important theoretical results in genome structural characterization, gene organization, evolution and comparative genomics.

The genome size of the houseworm is approximately 428.7 Mb, a size that is remarkable among several biological species. Specifically, the genome size of the silkworm is 3.6 times larger than that of Drosophila and 1.54 times larger than that of the mosquito, showing the significant advantage of the silkworm in genome expansion (Wang et al., 2021). In addition, the number of genes possessed by the silkworm is also more abundant, reaching 18,510 genes, a figure that not only far exceeds the 13,379 genes of Drosophila, but also reflects the potential of the silkworm in terms of genetic diversity and functional complexity.

2.2.2. Large-scale Genome Resequencing and Genetic Variation Mapping of Silkworms

In 2009, Southwest University completed the genome sequencing of 40 different domestic silkworm strains, including the wild silkworm, marking an in-depth study of the genetic resources of the domestic silkworm. In this study, about 16 million single nucleotide polymorphisms (SNPs), insertion deletion variants (Indels) and structural variants were identified, comprehensively revealing the complexity and diversity of the silkworm genome. In addition, the research team constructed a (SNP) map of single-base genetic variation in the silkworm, which provides a powerful tool and reference for subsequent genetic studies (Gong et al., 2021). By analyzing these genomic data, the researchers proposed a "one-time event" for the origin of the silkworm, implying that the domestication of the silkworm may have been a relatively simple and rapid process. At the same time, this study also reveals the influence of domestication selection on the biological traits of Bombyx mori, providing a genomic imprint that demonstrates the important role of natural selection in the evolution of Bombyx mori.

As an economically important insect, the domestic silkworm was domesticated from the wild mulberry silkworm in China as early as 5000 years ago. Through a long domestication process, the domestic silkworm has gradually formed a variety of different geographic strains and mutation systems (Huang et al., 2018). These geographic strains showed significant differences in ecological adaptations and phenotypic characteristics, reflecting the changes in biological traits brought about by domestication selection. For example, domesticated Bombyx mori showed significant enhancement in cocoon size, growth rate, and digestive efficiency, but at the same time, their ability to survive in the wild, including flight ability and disease resistance, was significantly weakened. Such phenotypic differences are not only the result of genetic variation, but also provide rich material for further genetic studies. The analysis of genetic changes in different geographic strains or mutant systems of the silkworm, Bombyx mori, has far-reaching theoretical significance and important application value, which not only deepens the understanding of the domestication process of Bombyx mori, but also provides potential opportunities for future applications in the fields of agriculture and biology.

A single-base genetic variation map was successfully constructed through whole-genome resequencing studies, which not only revealed the genetic variation among different lines, but also provided valuable data support for subsequent genetic improvement and germplasm resource conservation (Huang et al., 2018). As an economically important insect, the houseworm has successfully become one of the few species that have completed the three stages of framework mapping, fine mapping, and resequencing of important genetic resources.

2.2.3. Construction of the Whole Genome Editing and Screening Platform of the Silkworm, Bombyx Mori

The establishment of a genome-wide editing and screening platform for the silkworm, Bombyx mori, marks an important advance in the field of functional genome research in Bombyx mori (Adrianos et al., 2013). Using this platform, we screened and characterized a series of key essential genes in Bombyx mori, including responsive genes that play important roles under biotic and abiotic stress conditions. This work lays the foundation for our understanding of the biological mechanisms of the silkworm under various environmental stresses. In addition, a CRISPR vector library and a cell library covering almost all the genes of the houseworm were constructed, which provides a powerful tool to support the subsequent validation of gene functions. On this basis, we have conducted several mutation screening experiments to further expand our understanding of the genome function of the silkworm, Bombyx mori.

2.2.4. Dynamic Mapping of Whole-Cell Heterogeneity and Gene Transcription in Silk Glands of the Silkworm, Bombyx Mori, At Single-Cell Resolution

As a natural and renewable protein resource, silk protein has received increasing attention in recent years. This protein has excellent mechanical properties, biocompatibility and biodegradability, which makes it show a wide range of application prospects in many fields such as medicine, material science and environmental protection. In fact, there are about 100,000 species of organisms capable of producing silk, including silkworms, spiders and ants. However, among these silk-producing organisms, the domestic silkworm is undoubtedly the only silk-producing animal that has been fully domesticated and has formed an important industry. The domestic silkworm's ability to synthesize silk proteins is extremely strong, making it a major source of silk protein research, development and application. The development and utilization of silk protein has great potential, especially under the drive of the domestic silkworm, the silk protein industry

has formed a relatively mature system. With the progress of science and technology and the increasing market demand, the application scope of silk protein is expected to be further expanded, promoting the sustainable development of related industries (Tan et al., 2013).

The whole-cell heterogeneity of the cells within the silk gland of the domestic silkworm, Bombyx mori, and their gene transcription profiles were revealed, emphasizing the roles of different cell types in the function of the silk gland. By the technical means of single-cell analysis, the silk-producing organ of the silkworm and its gene expression were analyzed at ultra-high resolution for the first time. This technological breakthrough has enabled the study of the silk gland of the silkworm to go deeper from the previous tissue level to the single-cell level, greatly enriching the understanding of its biological properties. This discovery not only promotes in-depth research in related fields, but also lays a solid foundation for future applied research, which is especially important in the related fields of silk protein production and genetic improvement in the silkworm (Xia et al., 2009).

2.2.5. Super-pan-genome Mapping of the Houseworm, Bombyx mori

A large-scale genome analysis of silkworm germplasm resources has been carried out, and a "super pan-genome of the silkworm" has been successfully mapped (Sadat et al., 2022). This work not only provides a more comprehensive understanding of the genome of the silkworm, but also digitizes the gene pool of the silkworm, creating a "digital silkworm". This digitization will allow for more in-depth functional genomic research and promote the development of the silkworm in modeling and design breeding. In the past, research has relied only on a single reference genome, a limitation that has prevented in-depth exploration of genomic variation and superior genes, especially in molecular breeding. Super pan-genomes, on the other hand, cover information from all genomes of a species, which not only provides an important basis for germplasm innovation, but also lays a solid foundation for the development of functional genome research. In this study, we provided the world's largest long read-length pan-genome data, containing detailed genomic information of the domestic silkworm and wild mulberry silkworm, and conducted in-depth analysis of genetic variation, population structure, etc., and obtained a number of innovative results (Sadat et al., 2022). The construction and digitization process of the super pan-genome of the domestic silkworm will greatly promote the functional genomic research of the silkworm, facilitate the innovation of breeding models, and enhance the economic traits of the silkworm.

2.2.6. SilkMeta, A Platform for Sharing and Analyzing Pan-Genomic and Multi-omics Data of the Silkworm, Bombyx Mori

The completion of the pan-genome of the silkworm, Bombyx mori, marks a major breakthrough in the field of genomics research, and the ensuing volume of genomics data has shown explosive growth, which provides a solid theoretical foundation for the analysis of the genetic basis of Bombyx mori and molecular breeding research. However, faced with the integration and analysis of multi-omics data, often facing high costs and complex technical challenges, which to some extent limits the progress of related research. In order to solve this series of problems, a pan-genomic and multi-omics database of the houseworm, SilkMeta (http://silkmeta.org.cn/), was created (Asadpour et al., 2020). The database successfully integrates multiple genomics data and provides rich analytical tools, which in turn support in-depth studies of comparative and functional genomics. SilkMeta is a leader in terms of data type and data volume, and has now become the largest and most comprehensive database of houseworm genomics. The establishment of SilkMeta database has effectively promoted the in-depth study of silkworm genomics, successfully overcame the technical bottleneck caused by the integration of multi-omics data, and promoted the genetic study of important traits of the silkworm and its application in the field of molecular breeding.

2.2.7. Complete Genome Sequence of the W Chromosome of the Silkworm, Bombyx Mori, and a New Mechanism for the Origin of the W Chromosome in Lepidoptera

The composition of sex chromosomes and their role in determining sex in the animal kingdom shows great diversity. In mammals (including humans), the sex chromosome composition is usually XY in males and XX in females, with sex determined by key genes on the Y chromosome. Birds, on the other hand, have a ZW chromosome system, with ZZ in males and ZW in females. sex chromosome combinations are also diverse in reptiles and amphibians, with the ZZ/ZW system predominating. In insects, the sex chromosome composition XX/XY system is predominant, whereas the sex chromosome composition of Lepidoptera is different from most of the other insects in that their sex staining is dominated by the ZZ/ZW system, with ZZ in males and ZW in females (Gholipourmalekabadi et al., 2019). Therefore, the origin and evolution of the W chromosome in Lepidoptera have long been of interest to evolutionary biologists.The W chromosome was first discovered in the silkworm in 1916, and since then, the classical genetic study of sex determination in the silkworm has been developed, and based on the female-determining role of the W chromosome, the sex morphology markers of the silkworm have been established by mutagenesis, which have been widely used in the cultivation of "sex-restricted varieties" of silkworm (Zhhong et al., 2011). varieties" breeding (Zhao et al., 2019). In the last 20 years, the research on the molecular mechanism of sex determination in Bombyx mori has been a hot issue, including the discovery of Fem (a piRNA) on the W chromosome, the downstream factor Masc on the Z chromosome, and several genes affecting the sex of Bombyx mori on the autosomal chromosome (Han et al., 2024). However, the overall sex determination mechanism of the silkworm is still not clearly resolved, which is not unrelated to the lack of information on the sequence of the W chromosome of the silkworm.

Sex chromosomes in the animal kingdom exhibit a rich diversity of characteristics. For example, mammals usually use the XY/XX system, while birds use the ZW system. For reptiles and amphibians, the corresponding sex chromosome pattern is predominantly ZZ/ZW, whereas insects are dominated by the XX/XY system, but Lepidoptera have a clear preference for the ZZ/ZW system in this group (Gholipourmalekabadi et al., 2019). This diversity not only reflects the complexity of biological evolution, but also provides a broad perspective for the study of sex determination mechanisms. Among many studies, the discovery of the W chromosome in the houseworm is closely related to the study of its sex determination mechanism. The role of the W chromosome in the silkworm for female sex determination has become an important research field, which not only reveals the basic mechanism of sex determination,

but also provides a reference for the study of sex determination in other species. In recent years, the molecular mechanism of sex determination in the silkworm, Bombyx mori, has received extensive attention, with the discovery of the strong female determinant Fem and its downstream factor Masc (He et al., 2017). The discovery of these factors has provided new perspectives for the understanding of the sex determination mechanism in the houseworm, however, the overall mechanism is still not fully resolved, leaving many unanswered scientific questions. In addition, the lack of sequence information on the W chromosome of the houseworm constitutes a major obstacle to the in-depth resolution of its sex determination mechanism (Zhao et al., 2019). Due to the lack of detailed sequence data, it is impossible to fully understand the specific function of the W chromosome in the sex determination process and its interaction with other factors (Han et al., 2024). Although the sex determination mechanism of the silkworm has gained some understanding, there are still many unanswered questions constrained by the problem of insufficient sequence information of the W chromosome. Therefore, further studies in the future should focus on obtaining sequence information of the W chromosome of Bombyx mori to promote in-depth exploration of its sex determination mechanism.

2.3. Progress of Research on Transgenic Technology in the Silkworm, Bombyx Mori

As an economically important insect, the resolution of the genome of the houseworm has provided strong support for further scientific exploration (Imamura et al., 2006). On this basis, the development of genomics technologies, especially the continuous advancement of genome precision editing and transgenic silkworm technology, has facilitated the widespread application of these cutting-edge technologies in silkworm research. These technologies have provided scientists with new tools for precise genetic manipulation of the silkworm, exploring the expression of functional genes as well as the improvement of economic traits of the silkworm, such as increased silk yield and enhanced stress tolerance. These studies are not only of great theoretical importance, but also have great economic potential. The sequencing of the silkworm genome and the development of genomics technology, as well as the application of transgenic technology, have made the study of functional genes of the silkworm an indispensable tool in this field.

2.3.1. Establishment of a Safe Transgenic System for Silkworm Based on Conditional Gene Targeting

Transgenic technology has been widely used in gene function characterization and breed improvement of the silkworm, which has greatly promoted research progress in related fields. This technology enables researchers to understand the genetic characteristics of the silkworm more precisely, thus providing new ideas and methods for breed improvement. However, with the deepening of research on transgenic silkworms, the scientific community has gradually come to realize the biosafety problems that may be brought about by genetically modified organisms (GMOs). These problems involve not only ecological impacts, but also potential threats to human health. Therefore, biosafety issues have become a key challenge in the popularization and application of new varieties of transgenic silkworms. In order to ensure the safe promotion of transgenic silkworms in basic and applied research, there is an urgent need to strengthen the attention to and assessment of their biosafety, and to ensure that this technology will not cause negative impacts on the ecological environment and human health while bringing economic benefits.

In recent years, gene editing technology of the silkworm genome has been significantly developed, resulting in several innovative systems for targeted gene deletion, integration and replacement.In 2011, researchers first proposed the FLP/FRT system, which provides an effective tool for targeted deletion of marker genes in the silkworm genome (Park et al., 2011).In 2013, scientists successfully established the phiC31/att system, which enabled the integration and replacement of exogenous genes in the houseworm genome in a targeted manner (Guha et al., 2020). Further advancing the field was in 2015, when researchers combined the phiC31/att system with the modified piggyBac system to successfully achieve stable integration and precise replacement of exogenous genes, providing greater flexibility and reliability in the editing of the houseworm genome (Eckermann et al., 2020). in 2016, the validation of the effectiveness of embryonic microinjection of FLP recombinase mRNA and sustained expression, significantly improving the efficiency of targeted deletion (Long et al., 2016). The innovation and continuous optimization of technologies based on conditional gene targeting have significantly improved the efficiency of screening and editing of genes in the silkworm, and promoted the in-depth development of genome research.

2.3.2. Progress in Editing the Genome of the Silkworm, Bombyx Mori, Using the CRISPR-Cas System

Wang et al. (2013) reported the first successful manipulation of the silkworm genome using CRISPR-Cas9 tools. Each complex formed by an sgRNA and Cas9 nuclease was injected at the preembryonic stage of the embryonic disc. Normally, the larval ectoderm is opaque, but when there is a loss of function of the BmBlos2 gene, the ectoderm becomes translucent. This effect can be seen as a phenotypic marker for mutant detection. In 94% and 95.6% of all individuals, respectively, successful editing was achieved through the use of these two sgRNAs (Dong et al., 2019). Li et al. (2015) investigated a specific 20E inactivating enzyme using the houseworm, and the CRISPR-Cas9 system was used to deplete the BmEo gene, which resulted in the observation of a prolongation of the fifth instar larval stage duration by 24 hours (Li et al., 2015). ling et al. (2015) investigated the function of miR-2 in the houseworm using CRISPR-Cas9 technology, overexpressing miR-2 using the Gal4/UAS system (Ling et al., 2015), which resulted in wing deformation in adult worms. In the houseworm, insulin-like growth factor-like peptides are closely related to the reproductive discs and are particularly involved in their growth. By knocking down BmFibH, severe changes such as naked pupae or thin cocoons were observed. All individuals exhibiting naked pupae died (Zeng et al., 2016). The absence of insulin-like growth factor-like peptide results in smaller ovaries and fewer eggs being laid compared to the wild type (Fujinaga et al., 2017). Zhang et al. (2017) used CRISPR-Cas9 to eliminate the genes encoding for JHE, specifically BmJhe, in the silkworm, Bombyx mori, in order to study its function. The data showed that by knocking out the gene, the fourth and fifth instar stages were prolonged due to delayed JH metabolism (Zhang et al., 2017). Considering the feasibility of expressing the spider silk gene in the domestic silkworm, the spider silk gene was successfully knocked into

the silkworm genome using CRISPR-Cas9 in order to obtain enhanced silk (Xu et al., 2018). CRISPR-Cas9 was used to target the BmCactus gene for targeted mutagenesis in a specific B. mori cell line. Expression of several antimicrobial proteins, such as lysozyme and lebocin, was stimulated by disrupting the BmCactus gene (Dong et al., 2018). A potential endocrine protein, Sgsf, that is specifically expressed by salivary glands but of unknown function, was identified by comparing the changes in the hemolymph proteome before and after salivary gland deletion in the absence of hemocytes.The Sgsf gene in Drosophila was knockout mutated using CRISPR/Cas9 technology. This mutation resulted in significant systemic growth inhibition during growth in Drosophila with phenotypic features highly similar to the deleted phenotype of salivary glands. This finding suggests that the Sgsf gene plays an important role in Drosophila growth and development (Li et al., 2022). On the other hand, it was shown that overexpression of the Sgsf gene was effective in rescuing the defective phenotype exhibited by Drosophila, further supporting its potential function in Drosophila growth regulation. Studies have shown that OSER1 plays a role in several species, including the houseworm, nematode and Drosophila, and that it contributes to the slowing down of the aging process and the extension of lifespan by boosting the organism's defenses against oxidative stress. More notably, studies on the effect of OSER1 on lifespan have also been validated in human experiments, demonstrating the importance of this gene in promoting healthy aging and prolonging lifespan, highlighting its promising role as a potential regulatory target.Knockdown of the Sgsf gene significantly affects the growth of Drosophila, whereas the newly discovered OSER1 gene demonstrates its great potential to slow down the aging process by enhancing the ability to combat oxidative stress (Song et al. potential (Song et al., 2024). These results provide important clues for further investigation of the aging mechanism and point to a bright future for OSER1 as a key regulatory target in healthy aging and life extension.

2.4. Progress in Silk Research on the Domestic Silkworm, Bombyx Mori

Commercial silk is considered to be much weaker and less malleable than spider tow, which is known as a "superfiber". When spun under controlled conditions, the mechanical properties of silk can approach those of spider silk. Silkworms may be able to produce silk comparable to spider silk by altering their spinning habits, rather than by changing their silk genes (Shao et al., 2002).

2.4.1. Regenerative Medicine Applications of Silk from the Domestic Bombyx mori silkworm

The domestic silkworm, as an insect that originated in China, is renowned for its rich silk protein resources and excellent silk properties. Its history of domestication and rearing has lasted for 5,000 years, which has not only promoted the prosperous development of silk culture, but also provided a steady stream of raw materials for the human textile industry. Over the course of its long history, the application of domestic silk has undergone significant expansion, extending from the traditional textile industry to a number of cutting-edge fields such as tissue engineering, biomedicine, food, and advanced materials. This transformation indicates that the value of domestic silk in modern technology and industry has been widely recognized and applied. The silk gland is a specialized organ for silk protein production in the domestic silkworm, which has a decisive impact on silk yield and quality. In-depth exploration of the efficient silk production mechanism of the silk gland has become an important scientific issue in the field of sericulture.

For decades, silkworm proteins have been recognized as potential materials for various advanced engineering and biomedical applications. Recently, silk has received a lot of attention in research due to its excellent mechanical properties. Studies have shown that silk has unique interactions with cells in tissues through biorecognition units. Natural silk contains both sericin and silk glue, which have been used in various tissues of the human body (skin, bones, nerves, etc.). In addition, silk has anticancer, anti-tyrosinase, anticoagulant, antioxidant, antimicrobial and antidiabetic properties (Khosropanah et al., 2002).

Silk is a very important material that has been used since ancient times not only in the textile industry but also in medicine. In Persian medicine, silk also plays an important role in the treatment of diseases. Due to its many properties, the potential of silk proteins for medical applications, especially in the field of regenerative medicine and tissue engineering, is still very promising. With such a wide range of applications in tissue engineering and regenerative medicine, it is highly expected that many incurable diseases and conditions will be cured in the near future, such as skin and wound diseases, skeletal tissue, nervous tissue, cardiovascular tissue, eye and ear tissue diseases, prevention of premature births, urinary tissue diseases, breast tissue diseases, drug delivery for chronic diseases, IBD (i.e., Ulcerative Colitis and Crohn's disease), organ failure, cancer, and many others. Further research in this area is still needed to gain a more accurate, better, and more in-depth understanding of the uses of filaments and their gradients.

2.4.2. Silk Spinning Mechanism of the Silkworm

Spider traction silk, a protein-like silk fiber synthesized by the large jugular gland, is known for its excellent mechanical properties and has shown potential for a wide range of biomimetic applications (Song et al., 2024). This filamentous material is known as "biosteel" in nature, and its excellent strength and toughness make it highly valuable for applications in biomedicine, defense and military industries, and high-strength composites. However, the specific biological function of the large jugular gland in the synthesis of traction filaments is still unclear. To address this challenge, recent studies have revealed the molecular mechanism of spider macropotent gland in the third-order synthesis of traction silk fibers through a multidimensional histological approach (Hu et al., 2023). This result constructed a chromosome-level, high-quality genome of the rodentia spider, with a size of 2.63 GB, and provided a detailed annotation of 37,607 protein-coding genes.

The efficient expression of spider and pouch moth silk proteins has been achieved through the establishment of a variety of silk gland expression systems in the domestic silkworm, marking an important breakthrough in the field of natural fibrous protein production. Fibrous proteins synthesized by a variety of animals in nature, including spiders and pouched moths, have demonstrated a wide range of potential applications in various fields such as national defense, aerospace, and medical care, but the difficulty of obtaining them has always been an urgent problem. The silk gland of the domestic silkworm, Bombyx mori, has become an ideal expression system due to its good protein expression

ability. Meanwhile, the introduction of gene editing technology provides unprecedented opportunities for the production of customized silk proteins. The contents of the spider traction silk protein MaSp2 and the bagworm silk protein EvFibH in individual cocoon layers reached 51.02% and 63.77%, respectively, which laid the foundation for large-scale production of related silk proteins (Yu et al., 2024). In addition, the incorporation of MaSp2 and EvFibH significantly improved the toughness of the recombinant silk fibers and endowed the materials with superior physical properties, which were enhanced by about 86% and 80%, respectively.

A high-resolution spatio-temporal transcriptional map of the silk gland of the silkworm Bombyx mori at different developmental stages, including the embryo, the young silkworm and the large silkworm, has been developed. This mapping not only reveals the dynamic changes of cell types and cell states in the silk gland, but also provides insights into the temporal and spatial characteristics of gene expression (Ma et al., 2024). The systematic analysis of the spatial location of cell populations and their heterogeneity enables a clearer understanding of the function and developmental process of the silk gland. In addition, the spatio-temporal biological research difficulties of the silk gland of the domestic silkworm due to its special morphology and structure were effectively overcome, providing important technical and data support.

2.5. Progress in Molecular Breeding for Disease Resistance in the Silkworm, Bombyx Mori

The domesticated silkworm is the first insect domesticated for silk production in human history, and its unique biological characteristics and economic value make it an important part of sericulture (Xiang et al., 2018). However, sericulture is facing serious challenges, among which the infestation of pathogens has become one of the major nuisances. These pathogens not only affect the health and growth of domestic silkworms, but also directly lead to economic losses for farmers. Therefore, it is particularly important to improve the resistance of the silkworm against pathogens. Gene editing technology can effectively enhance the ability of silkworms to resist pathogens by precisely modifying their genetic information, thus providing a new way for the sustainable development of sericulture.

2.5.1. Common Strategies Used in Breeding Research for Disease Resistance in Silkworms

The silkworm is not only an important experimental tool, but also a good research organism for genetics, molecular biology and other life sciences, and has become an excellent model organism for studying human physiology, diseases and genetics, as well as for the development of new drugs. However, the silkworm is susceptible to various bacterial, fungal and viral infections, which cause serious harm to sericulture and textile industry. There is a wide variety of diseases of the domestic silkworm, which can be categorized into two main groups based on their infectiousness (Wang et al., 2020). The first category is infectious sericulture diseases, which are subdivided into three subclasses: (1) viruses, (2) bacteria and (3) fungi. Infectious sericulture is a pathology in which infected silkworms are able to cause normal sericulture; that is, some diseased silkworms produce pathogenic microorganisms that are able to infect other silkworms through various vectors. There are three main types of strategies for breeding for disease resistance in the domestic silkworm: (1) RNAi, (2) overexpression of endogenous antiviral genes, and (3) gene editing techniques.

For different infectious or non-infectious diseases, taking different measures to improve feeding conditions and strictly eliminate the breeding environment of pathogens plays an important role in the breeding of silkworms. In addition to controlling external factors, more attention should be paid to the domestic silkworm itself. Transgenic technologies, including RNAi, have been widely used in improving the resistance of the domestic silkworm to a variety of pathogens, and their applications have become widespread (Li et al., 2023). However, the regulation of biotechnology is an issue that requires attention. In addition, after obtaining transgenic domestic silkworm lines, it is important to retain the good traits and disease resistance as well as to be environmentally friendly. So far, the application of gene editing technology in the study of disease resistance in the domestic silkworm has progressed well. Simultaneous editing of multiple pathogen genes to knock out host genes required for pathogen proliferation. Multiple knockout sites are used for a single target gene to enhance editing efficiency, and inducible promoters are currently used to improve editing efficiency and reduce damage to the host. A number of resistant strains have been realized (Wang et al., 2024). Therefore, in combination with the existing CRISPR gene editing technology, the use of inducible promoters to combine multiple genes and multiple targets can realize efficient resistance breeding.

2.5.2. Processes and Mechanisms of Vertical Transmission of Microparticle Disease in Bombyx mori via Eggs

Bombyx mori is a disease that poses a serious threat to sericulture and is caused by Nosema bombycis (Wang et al., 2023). This pathogen is capable of infecting the oocytes of the host, leading to severe consequences, which in turn cause the larvae to carry the virus, affecting the quality of the subsequent silkworm seed. Bombyx mori has been recognized as a legal quarantine in silkworm seed production. The infestation process of this pathogen has been revealed in academic studies, which show that the pathogen begins to infest the oviducts on day 3 of the pupal stage and enters the oviducts and follicular cells through the hemolymph. This complex infection mechanism makes the control of microparticle disease in silkworms very difficult and becomes a major quarantine risk that cannot be ignored in silkworm production.

2.6. Progress in the Regulation of Etamorphosis in the Silkworm, Bombyx Mori

Insect metamorphosis is a complex and delicate process that is mainly regulated by two endocrine hormones: juvenile hormone (JH) and ecdysone (20-hydroxyecdysone, 20E). Juvenile hormone is synthesized by the pharyngeal lateral body in insects, and its main function is to maintain the growth state of larvae and ensure their healthy development during the larval stage. Meanwhile, ecdysone is synthesized by the prothoracic gland and plays a crucial role in the molting process and the transition to pupa. These two hormones are not only distinctive in their respective functions, but also collaborate with each other to form a fine regulatory network

during insect growth and development (Liu et al., 2018).

2.6.1. Mechanism of Insect Ecdysteroid Biosynthesis

This study reveals a novel mechanism indicating that juvenile hormone (JH) inhibits ecdysone synthesis through the signaling molecule Krüppel homolog 1 (Kr-h1).JH and ecdysone are two endocrine hormones indispensable to insect growth and metamorphosis, with the former being secreted mainly by the lateral pharyngeal body and the latter being synthesized by the anterior thymus. The former is mainly secreted by the lateral pharynx while the latter is synthesized by the anterior thymus. The synthesized levels of JH and ecdysone alternate during insect development, and JH can indirectly regulate the synthesis of ecdysone. The role of juvenile preserving hormones in insect development is complex, especially in the regulation of ecdysone synthesis. Although the mechanism of action of JH has been explored, little is known about how JH acts directly on the prothoracic gland and regulates ecdysone synthesis.

Combining two model systems, the domestic silkworm and Drosophila, it was confirmed by tissue-specific genetic manipulation and biochemical experiments at the individual and cellular levels that JH can directly act on the anterior thymus and induce the expression of Kr-h1, which in turn directly binds to a key cis-acting element in the promoter region of the ecdysone synthase gene and induces the promoter to undergo DNA methylation, thereby inhibiting ecdysone synthase gene transcription and ecdysone synthesis, which ultimately hinders molt and metamorphosis development and maintains the larval growth state (Zhang et al., 2018).

JH is able to act directly on the anterior thymus to induce the expression of the key gene Kr-h1. This finding provides important clues to our understanding of the role of JH during development. The binding relationship of Kr-h1 to key elements in the promoter region of the ecdysone synthase gene, in turn, leads to the phenomenon of DNA methylation of the gene. This methylation alteration significantly represses the transcription process of the ecdysone synthase gene, which in turn affects ecdysone synthesis and ultimately acts on the molting and metamorphosis developmental processes in insects.

2.6.2. New Mechanisms of Epigenetic Regulation of Insect Metamorphosis and Development

The important role of H3K27me3 modification in insect hormone interactions and its regulation of metamorphosis and development and the corresponding molecular mechanisms. Studies have shown that juvenile hormone (JH) and ecdysteroid (20E) work together to synergistically regulate the metamorphosis of insects (Yang et al., 2021). Among them, the function of JH is to maintain the growth state of larvae, and its synthesis and secretion are mainly responsible by the pharyngeal lateral body. In contrast, 20E is responsible for promoting larval molting and metamorphosis to pupa, a process that is synthesized and secreted by the anterior thymus. In addition, the transcription factor Schlank plays a key role in this process, which effectively influences insect metamorphosis by regulating the shift from acetylation to methylation modification of H3K27 in the anterior thymus.

Focusing on the important role of the transcription factor Schlank in insect development. Specifically, Schlank influences endocrine hormone signaling pathways in insects by mediating modification changes of histone H3K27 from acetylation to trimethylation in prothoracic cells of larval final instar stages (Yuan et al., 2024). In this process, Schlank activity inhibits the transcription of the juvenile hormone signaling molecule Hairy. This inhibition allows the removal of the inhibitory effect of juvenile protective hormone on ecdysone synthesis, which ultimately promotes ecdysone synthesis and thus promotes metamorphosis in insects.

3. Summary and Discussion

The houseworm, one of the most important domesticated insects, has great potential as a biotechnology platform for the production of recombinant proteins and has also achieved great success as an experimental model organism (Li et al., 2022). The use of CRISPR-Cas technology in silkworms holds great future promise. Nowadays, significant progress has been made in better understanding the synthesis of silk proteins and sericin through genetic manipulation of the silkworm genome, but also in increasing the knowledge of the most important processes in the silkworm (Pacesa et al., 2024).

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