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Advantages and Limitations of Silkworm as an Invertebrate Model in Aging and Lifespan Research

Jiangbo Song[1,2], Jianfei Zhang[1,2] and Fangyin Dai[1,2]*

[1]*State Key Laboratory of Silkworm Genome Biology, Southwest University, China*

[2]*Key Laboratory of Sericultural Biology and Genetic Breeding, Ministry of Agriculture, College of Biotechnology, Southwest University, China*

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***Corresponding author**: Fangyin Dai, State Key Laboratory of Silkworm Genome Biology, Southwest University, Chongqing 400716, China, Tel: +86 23 68250793; Fax: +86 23 68251128; email: fydai@swu.edu.cn

Abstract

Aging and lifespan study had been the intense subjects of biological science. By now, nematode (*Caenorhabditis elegans*) and fruit fly (*Drosophila melanogaster*) are the most widely used experimental invertebrates in aging and lifespan researches. Considering the complicated mechanism, utilization of more model organisms is indispensable for its evolutionary conservation and species specificity. Moreover, it is easier to make a breakthrough in the experimental animal with low cost, relatively integrated and differentiated physiological systems, and simple body composition which is more susceptible to exogenous regulation than mammals. The silkworm, *Bombyx mori*, has been regarding as a new choice for some aging and lifespan research, especially in longevity genes' function, signaling transduction and medicine efficacy evaluation study as its many features. Here, we discuss the objective conditions of this species as an invertebrate model for aging and lifespan research. Meanwhile, the major limitation reasons were also discussed for future work in research on aging and lifespan.

Keywords: Silkworm; Aging; Lifespan; Invertebrate Model

Abbreviations: CR: Calorie Restriction

Introduction

Animal model has become an indispensable tool in biology, medicine, pharmacy and other fields. However, the existing invertebrate experimental animals could not fully satisfy the needs for studying the complex mechanism of aging and lifespan regulation. Therefore, more experimental animal models need to be identified and developed [1]. Meanwhile, the conservative lifespan regulation pathway in animal kingdom provides a natural basis for the study of the complex lifespan decision mechanism utilizing the lower animals [2]. Silkworm (Bombyx mori) is one of the most important economic insects in agriculture and excellent experimental animals in classical genetics research [3]. Simultaneously, silkworm possesses many characteristics which make it suitable for study the mechanism of aging and lifespan decision. Silkworm has been artificially bred for more than five thousand years, so some unique biological characteristics and cultural connotation have been formed [4].

With the overwhelming need for expanding new model organisms in the whole biological and medical research, the silkworm has been emerging as an organism of choice for research into the molecular regulation of aging and longevity [5]. There are several laboratories and investigators exploring aging and lifespan using silkworm and our group is the first batch of using this organism to address our questions of interest [6,5]. Although the studies in aging reporting advances in silkworm is significantly less than that in *Caenorhabditis elegans, Drosophila melanogaster* and *Mus musculus*, the silkworm is emerging as a novel invertebrate model in the aging and lifespan research field, which will further expand the choice scope of the invertebrate model alongside its more classical brethren: *Caenorhabditis elegans* and *Drosophila melanogaster* [7-9].

Discussions

Some Advantages of Silkworm as a New Model for Aging and Lifespan Study

Silkworm has numerous advantages as a model organism for aging research, such as abundant mutant strains, clear genetic background, less ethical issues and low rearing cost, clear boundaries between different development stages, fasting model, and open blood circulation system, etc. There are numerous inherent beneficial traits in the use of silkworm to study aging, such as: the convenience of operation, the short growth and development cycle, the high propagation coefficient, and so on (Table 1). Major features and advantages are stated as following.

Table 1: Advantages of silkworm in the study of aging and lifespan.

Item	Advantages
Genetic background	Clear genetic background
Body size	Moderate body size
Food and environment	Oligotrophic (mulberry leaf)
	Adaption to highly density feeding in laboratory
Ethical issues and cost	Less ethical issues
	Low cost
Easy growth and development	Can grow on a clean diet
	Rapid development (larva stage: approximately 3 weeks)
Blood circulation system	Open hemolymph circulation system
Appropriate lifespan expectancy	Life cycle (5-7weeks)
	Maximum lifespan (approximately 8 weeks, 25°C)
	Abundant mutant strains with lifespan differences
Molecular genetics operation	Easy gene knockdown and knockout by RNAi and gene editing
	Easy gene overexpression: transgenics
	No change of phenotype between generations
Complete genome sequence	Full analysis platform
Fertile and reproduction speed	High fecundity (approximately 500)
	Rapid reproduction (in several hours after mating)
	No inbreeding depression
	Facilitates mutant isolation
Locomotion	Larval slow, Adult cannot fly

Some Trophic Facilitate Silkworm as an Invertebrate Model for Aging and Lifespan Study: The silkworm possesses a high reproductive rate, and the female moth can lay approximately 500 eggs in several hours after mating, finally these eggs will become the larva with the same developmental status and genetic background, combine with the low cost feature compared to mammalian model. This allows researchers to obtain easily a large number of uniform individuals for aging and lifespan investigations. Silkworms belong to oligotrophic animals, which only feed on mulberry leaves. In recent decades, researchers have developed a variety of silkworm artificial diets which lead to silkworm feed and reproduce easily standardized in the laboratory [10-13]. So that we can test the effect of drugs on the lifespan of silkworms by daubing drugs on mulberry leaves or adding drugs into artificial diets [6]. The above characteristics and standardized breeding of silkworm can ensure the reliability and repeatability of experimental results which are not disturbed by diet and culture conditions. The suitable individual size of silkworm contributes to observation of morphology and behavior, exogenous injection. In addition, due to the low mobility of larva and the moth cannot fly, which is convenient for breeding, management and lifespan measurement. Silkworms are entirely dependent on human feeding and unable to survive and reproduce in the wild environment. So it will not harm other species and the local ecological environment even if it escapes into the outside. Furthermore, silkworm is a non-emotional animal so that there has less ethical issues in the study of aging and lifespan.

Strains and Physiology, Gene Regulation, and Functional Genome Research Platform: The genetic resources available in silkworm are rich, which have a clear genetic background. The Silkworm Gene Bank at Southwest University in China maintains most of these genetic systems, in particular, there are a large number of genetic strains with tremendous differences in lifespan. The genome sequence was completed previously [14] and has been continually annotated providing an excellent bioinformatics resource including genome sequence information (Silk DB: http://www.silkdb.org) [15]. The whole-genome microarray was also constructed to analyze gene expression (http://www.silkdb.org/microarray/). There is numerous on-line resources information for silkworm strains (http://silkbase.ab.a.u-tokyo.ac.jp/cgi-bin/index.cgi). Transgenic silkworms can be easily generated by exogenous injection of DNA from micropyle of newly laid eggs. RNAi and gene editing methodology has advantage to study gene function in the genome for a variety of different phenotypes. These platforms have aroused the concern of silkworm researchers and become a forceful advantage in the study of the silkworm aging and lifespan.

The Short Life Cycle and Relatively Obvious Boundaries Between Different Development Stages: Silkworm possesses a short life cycle with approximately 5-7 weeks in a generation in standard feeding environment (25°C, approximately 75% relative humidity with a 12L: 12D photoperiod). Maximum lifespan is approximately 8 weeks. The whole life cycle of silkworm undergoes larval, pupa, and adult stage. The adult stage is equivalent to the aging stage of mammals. As the rapid generation, it is easy to carry out research on intergenerational aging and lifespan study. Compared with other more complicated organisms, silkworm lifespan is easier to be influenced by artificial manipulation, therefore, silkworm have a natural material advantage as an invertebrate model to study how external factor affect lifespan between intergeneration within a short period. Meanwhile, the number of larval instars appears to be firmly fixed at three in Drosophila melanogaster [16], while the number of larval in star varies from four to six in silkworm. This provides a better plasticity potential in lifespan study, which could expand the display strength of altered lifespan.

Silkworm Adult Stage is a Natural Model for Studying the Relationship of Fasting and Lifespan: Because silkworm begins and completes mating and reproduction quickly at the very early stage of the adult stage, so the adult stage is generally considered to be the initial origination stage of

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silkworm organism aging. The eating and alimentary related organs of adult silkworm are degraded and no feeding behavior in silkworm adult stage. However, their energy is sufficient to support mating, reproduction, and survival 1-2 weeks. So, it is a natural invertebrate animal model for studying the relationship between fasting and lifespan. Few species in nature have such feature. This primary advantage of the model is it could exclude factors such as food intake quantity and type from interfering with the lifespan statistical results in aging and lifespan studies. Meanwhile, this model provides great convenience for studying energy metabolism and lifespan.

Silkworm Possess an Open Hemolymph Circulation System: Silkworm has an open blood circulation system without blood vessels, such a system can quickly transport and transform all kinds of components and energy. At the same time, it can initiate the immune prevention and secrete a variety of proteins to effectively eliminate foreign pathogenic factors when stimulated by foreign pathogenic microorganisms. Although the open hemolymph system of silkworms is simpler in structure than that of mammals, they have a similar pharmacokinetic mechanisms of chemicals. This makes it possible for the silkworm to be used as a rapid screening model for bioactive natural components screening and drug efficacy evaluation. Microinjection can directly delivery the drug into the hemolymph of silkworm. Because of silkworm open hemolymph system and simpler structure compare to multi-layer wall structure of mammals, this structure reduces the loss of the drug as it passes through the walls of blood vessel, which can make the drug more quickly and efficiently spread to the whole body. Finally, the drug can directly reach various organs and is absorbed to function.

Limitations of Silkworm as a Model for Aging and Lifespan Researches

Despite the numerous advantages, there are several limitations in the use of silkworm as a model system for the study of aging and lifespan (Table 2).

Table 2: Liminations of *Bombyx mori* in the study of aging.

Item	Limitations
Evolutional relationship with mammals	More remote evolutional relationship with mammals
Orthologous genes	Some genes have no homologs in mammals
Physiological system	lower integrity of physiological system differentiation than mammals
	Many physiological system absence: e.g. acquired immunity

Long Divergence Time With Mammals in Evolution: Silkworm is a poikilotherm animal and own a far evolutionarily relationship with humans, being separated by hundreds of millions of years. More dominating factor is the fact that many important physiologically systems was absent in the silkworm, especially the acquired immune system. Moreover, the most intensely studied set of aging mutations in silkworm are those also specifying voltinism and moultinism development and there have little relevance between "voltinism and moultinism" state with mammalian aging and lifespan.

Some Human Orthologous Genes is Absent on Aging and Lifespan in Silkworm: The results of genome sequence showed that there were 14623 genes in silkworm. Through the identification of orthologous genes between silkworm and human, it was found that 8469 genes in silkworm had direct homologous genes in human [17]. However, there are still 6154 genes in silkworms without corresponding human homologous genes, accounting for 42% of the total genes of silkworms, which may contain some genes that strongly associate with aging and longevity. For example, sirtuin 1 is a gene involved in processes such as calorie restriction (CR), chromatin remodeling, stress response, and DNA repair, and there is no corresponding orthologous gene in silkworm [18,19]. The effects of these genes on aging and lifespan regulation cannot be carried out in silkworm. Therefore, this has brought some limitations to the exploration of silkworm as an invertebrate animal model for aging and lifespan research.

Summary and Prospect

With these advantages, silkworms is emerging as an invertebrate model for the aging and lifespan mechanism study, and could also serve as a novel replaceable laboratory animal to provide early reference for mammalian and clinical screening for lifespan extending drugs and pharmacodynamics evaluation. The more expandable application using silkworm would also rise in aging and lifespan field [20,21]. We hope that the advantages of the silkworm as an invertebrate model for aging and lifespan will be further exploited in the near future, and some limitations could be overcome gradually with the continuously efforts of the colleagues in the field. Studies using silkworm to unravel molecular mechanisms behind aging and lifespan will provide new insights into this field.

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