

Trends in Insect Studies in Life Science

SHIN-ICHI MOGI, JUNKO SHIMADA AND SATOSHI TAKEDA (*Affiliated Fellow*)
Life Science and Medical Research Unit

3.1 Introduction

Insect studies in biology and life science have been carried out mainly with silkworms (*Bombyx*) and fruit flies (*Drosophila*). These two insects contributed especially to classic genetics in the 1910s. For example, while Mendel's laws in plants were discovered in 1865, those in animals were first discovered in *Bombyx* silkworm by Dr. Kametaro Toyama of Tokyo Imperial University in 1910. Also, Dr. Thomas H. Morgan of Columbia University, U.S., used fruit flies to conduct basic research in genetics such as studies on mutation induction and salivary chromosome engineering. In 1972, Dr. Yoshiaki Suzuki of the Carnegie Institution of Washington succeeded in isolating the messenger RNA of fibroin, a silk protein, from the silkworm for the first time in eucaryote. Suzuki's research was a remarkable achievement in molecular biology.

In the meantime, studies with fruit flies have played crucial roles in the development of biology through improvement of the chromosome engineering technology using salivary chromosomes, the gene-transferring technology, and establishment of experimental methods with mutation and gene functions employed. The whole genome of the fruit fly was sequenced in 2000, the first time in insects, thereby ensuring its position as a model organism in the molecular biology of insects.

However, we cannot unveil functions and biological phenomena peculiar to each insect with studies only on the fruit fly, a model organism. Accordingly, we need to enhance researches on various insects with special functions. For example, fruit flies do not have dormancy although it is an essential characteristic of insects. Furthermore, most of the biomolecules involved in specific functions of insects have not

been identified yet and are biological resources to be developed. Analysis of properties specific to insects, which may lead to the discovery of new biological resources, will not only contribute to life science but also greatly advance industry through applied researches for exploiting biological resources.

In this report, we will introduce to you the trends in insect studies in life science.

3.2 Position of insects in the Animal Kingdom and importance of insect studies

An insect is defined as an organism comprising of a body divided into three parts (head, thorax and abdomen) and six legs. Insects belong to the Arthropoda, which includes crustaceans, such as prawns and crabs, and arachnids, both of which have legs with segments as insects do.

3.2.1 Position of the insect in the Animal Kingdom

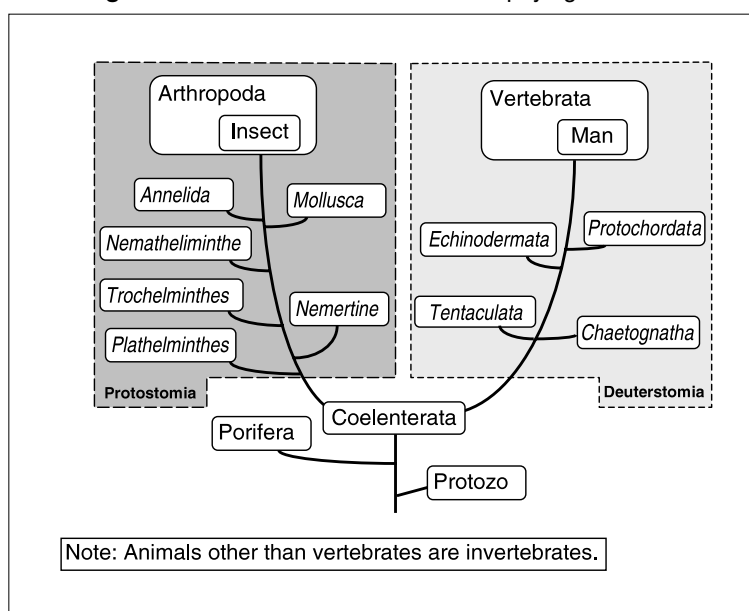
The earth was born 4.6 billion years ago and life began to exist about four billion years ago. The birth of the insect is estimated to be 350 million years ago, while the birth of man five million years ago.

While man is the most highly evolved animal among vertebrates in the phylogenetic tree, the insect is regarded as the most highly evolved invertebrate because it has excellent sensory functions and includes species having sociality (Figure 1).

Meanwhile, among all animals, which presumably contain one million species, the number of insect species accounts for about 70%, while the number of vertebrate species including man is about 4% (Figure 2).

There are many factors for such prosperity of insects on the earth. For instance, they have

Figure 1: Position of insects in animals' phylogenetic tree



Source: Prepared based on material made by Dr. Shunji Natori, an invited researcher of the Institute of Physical and Chemical Research, with some added changes

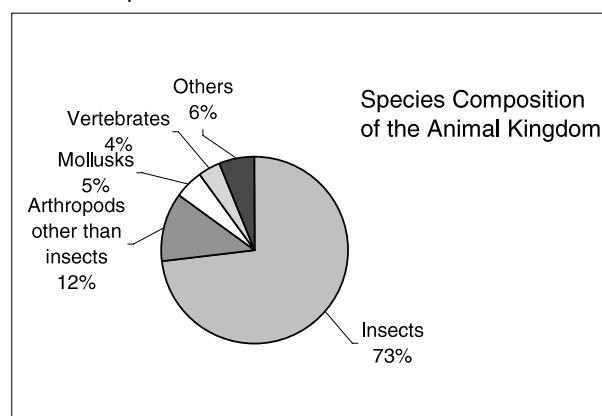
short life cycles (10-14 days in fruit flies and 50 days in *Bombyx* silkworm), high reproductivity, diapause and metamorphosis, which help them to survive severe environments, and defense mechanisms effective in protecting themselves from enemies. Insects have obtained these functions in their long evolutionary process to adapt to the environment. Insects, which have infinite diversity obtained through such variable adaptation, are very attractive research objects as biological resources, too.

3.2.2 Importance of insect studies in life science

Basic knowledge in developmental biology, genetics and molecular biology has been obtained through studies using fruit flies and silkworms. Such studies realized, for instance, gene recombination in fruit flies and *Bombyx* silkworms. Useful substances produced by the recombination of the silkworm genes are thought to have high safety because silkworms do not have pathogenic microbes in common with man and domestic animals and, thus, these substances do not contain microbes harmful to us. Actually, the technology for producing useful substances like interferon in the silkworm using transgenic viruses has been improved, and interferon for cats has already been commercialized.

In the meantime, the insect, the most highly evolved invertebrate, has many biological

Figure 2: Insects account for about 70% of all animal species



Source: Prepared based on E. O. Wilson (1992), "The Diversity of Life" (Harvard University Press, Cambridge, 1992) translated by Masako Oonuki and Shunichi Makino (Iwanami Shoten Publishers) with some added changes.

mechanisms different from us. Analysis of such mechanisms specific to insects will give us a fresh insight into life science. For example, a bacteria-resistant substance produced by an insect that does not have an immune system with the antigen-antibody reaction, which vertebrates have, shows resistance to methicillin-resistant *Staphylococcus aureus* (MRSA), and its application to insect-derived pharmaceuticals is greatly expected.

In addition, applied researches on insects in the fields of medicine and industry are necessary. It is also crucial to study insects that serve as vectors of diseases, like *Anopheles* mosquitos transmitting

malarial parasites and tsetse flies transmitting trypanosomes that cause sleeping sickness. The anopheles is the second insect whose whole genome was sequenced following the fruit fly. If artificial control of insect growth becomes possible through studies on insect hormones, extermination of these vectors will be enhanced.

Furthermore, insects are focused on as a reservoir of new compounds beneficial in industry as their various biologically active substances and useful biopolymers are discovered. For instance, it is hoped that useful substances produced by symbiotic microorganisms of insects will be applied as pharmaceuticals for man and livestock.

3.3 Major fruits and trends in recent insect studies in life science

In this chapter, we will introduce to you major fruits and trends in recent insect studies in life science. Specifically, we will deal with “Molecular Biological Studies on Insects,” which serve as the base of every research in life science

including fundamental and academic studies led by universities. Then, we will refer to “Research on Growth Control and Hormones of Insects,” “Research on the Defense Mechanism of Insects” and “Research on Symbiotic Microorganisms of Insects” as studies for analyzing functions peculiar to insects.

The Insect Technology Project of the Ministry of Agriculture, Forestry and Fisheries of Japan is an example of an applied research based on such basic studies as described above. This project aims at exploiting and industrializing insect properties. It includes such research objects as the promotion of the *Bombyx* silkworm genome sequencing, development of new agrichemicals for selectively exterminating targeted vermin by making good use of genomic information, mass production of useful proteins of insects by silkworms with gene recombination applied and improvement of new materials that can be employed in the field of medicine by engineering and processing substances specific to insects (Figure 3).

Figure 3: Trends in insect studies in life science

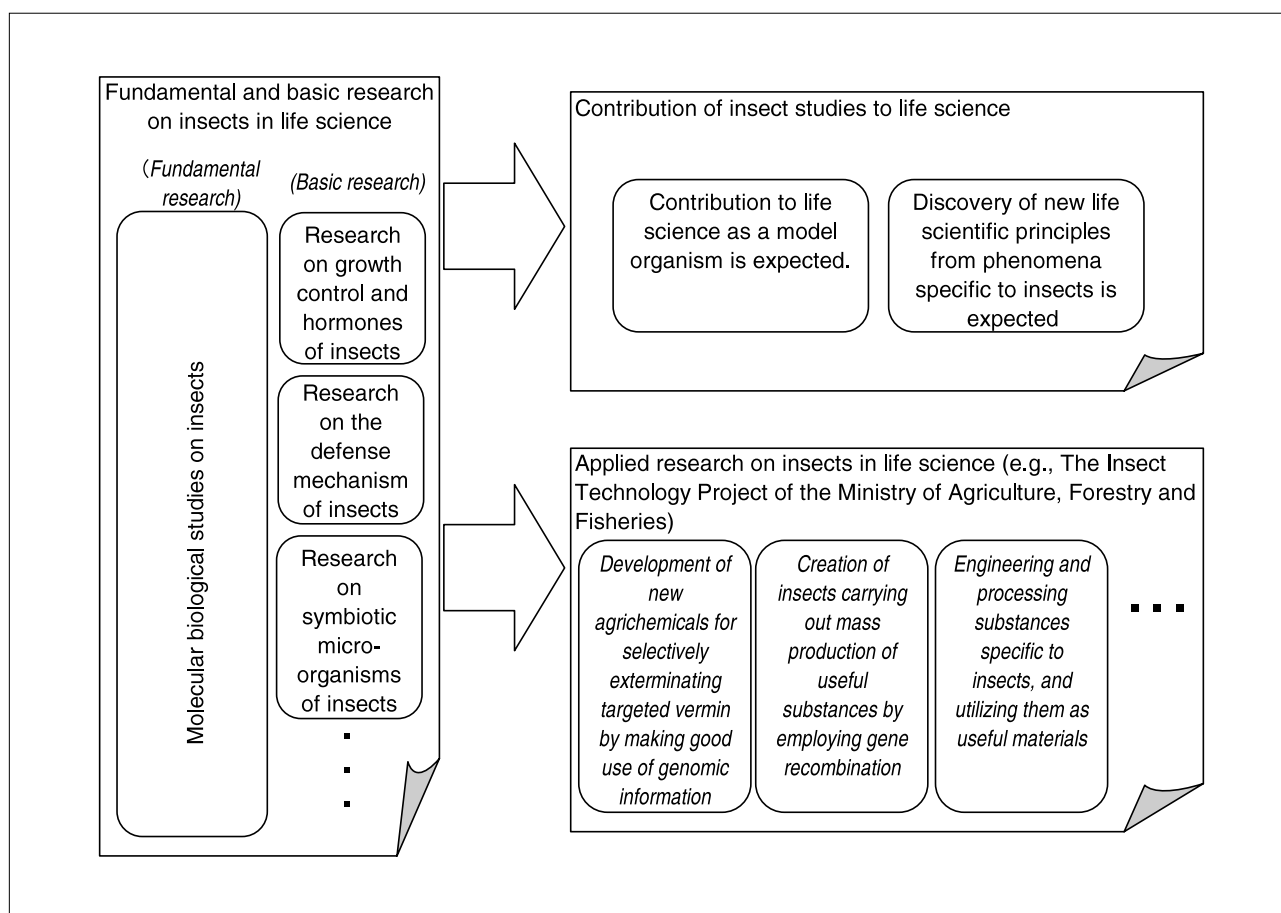


Table 1: Current status of insect genome sequencing projects

	Classification of the insect	Genome size	Genome sequencing project
Silkworm (<i>Bombyx mori</i>)	Lepidoptera	540Mbp	In progress (Japan)
Fruit fly (<i>Drosophila melanogaster</i>)	Diptera	180Mbp	Completed in 2000 (U.S.)
Anopheles (<i>Anopheles gambiae</i>)		280Mbp	Completed in 2002 (Europe)

Source: Reference^[3]

3.3.1 Molecular biological studies on insects^[1,2]

Research on fruit flies has been the most advanced among molecular biological studies on insects.

Fruit flies have been focused on as experimental materials of biology since Dr. Thomas H. Morgan of Columbia University employed them as materials for genetic studies in the 1910s. Since then, fruit flies have acquired their importance as experimental materials and have been used in the improvement of the mutation induction method, gene mapping of salivary chromosomes and application of balancer chromosomes for maintaining mutation and even lethal genes.

Furthermore, a transposon, or a moving gene, called P factor was discovered and transformation technology in fruit flies using it was established in the 1980s. In 2000, the fruit fly's whole genome was sequenced, a first for insects, and the position of the fruit fly as a model organism was secured. Now, gene functions of the fruit fly are investigated with genome informatics based on the genomic sequence, gene expression analysis using microarray, gene expression suppression with RNA interference and gene targeting (gene knockout).

Completion of the whole genome sequencing in a model organism radically changed the gene function analysis method. That is, it became possible to estimate the function of a particular gene from the genome database. This method is called reverse genetic analysis and enables the supposition of the functions of other organisms' homologous genes from the fruit fly's genomic information. In this way, whole genome sequencing of a species not only helps unveil every biological feature of the species but also gives various advantages to other fields of study.

In insects, whole genome sequencing of the fruit fly was completed in 2000 by a consortium

of Celera Genomics, U.S., and university researchers, and whole genome sequencing of the anopheles was completed in 2002 by an international consortium including Celera Genomics and European research institutions. Meanwhile, Japanese researchers are promoting *Bombyx* silkworm genome studies (Table 1).

The silkworm belongs to Lepidoptera, whereas the fruit fly and the anopheles, whose whole genome sequencings have been completed, belong to Diptera. Lepidoptera and Diptera are thought to have diverged at least 240 million years ago. This distance corresponds to that of mammals and birds, so even though whole genome sequencings of the fruit fly and the anopheles have been completed, whole genome sequencing of the silkworm is still highly necessary.

Researchers not only in Japan but also in other countries recognize silkworm genome studies to be crucial in life science of insects. The Ministry of Agriculture, Forestry and Fisheries of Japan included the silkworm's whole genome sequencing using the shotgun method into the extra budget for FY 2002. The ministry will vigorously promote the sequencing with the shotgun method even in and after FY 2003 in the Insect Technology Project to complete the whole genome sequencing as soon as possible (Table 2). However, silkworm genome sequencing has just been launched and we need to complete it immediately in order, for example, to apply the genomic information to gene function analysis and genomic-based drug discovery. Further cooperation of Japanese institutions that have experiences in human, mouse and microbe genome sequencing is essential in order to complete the silkworm's whole genome sequencing as early as possible.

The silkworm is thought to be more favorable than the fruit fly in industrializing the production

Table 2: Projects which have promoted the silkworm's genome research and their results

Year	Projects	Results
FY 1996 – 1998	CREST: "Mechanism of molecular responses between viruses and host insects"	Construction of the silkworm's EST ^{*1} database, construction of a BAC ^{*2} library, etc.
FY 1999 – 2003	Research for the Future Program: "Gene network upon sex decision of insects"	Analysis of the structure of the silkworm's sex chromosomes, construction of the silkworm's EST database, analysis of EST microarray, construction of BAC contig, etc.
FY 1999 – 2002	Ministry of Agriculture, Forestry and Fisheries: "Analysis of animal genomes--insect genomes"	
FY 2000 – 2004	Bio-oriented Technology Research Advancement Institution: "Establishment of the gene function analysis system of the silkworm"	
FY 2002	Ministry of Agriculture, Forestry and Fisheries: "Analysis of animal genomes — insect genomes" (subsidized from the extra budget)	Initiation of the silkworm's whole genome sequencing using with the whole genome shotgun method
FY 2003 –	Ministry of Agriculture, Forestry and Fisheries: "Insect Technology Project"	

***1 Expressed sequence tag (EST)**

Partial nucleotide sequence of complementary DNA (cDNA) of mRNA is called EST. Since cDNA reflects the nucleotide sequences of the genes expressed in the cell, EST helps estimate which genes are expressed in the cell.

***2 Bacterial artificial chromosome (BAC)**

Bacteria-derived artificial chromosomes into which genome fragments of more than 100kbp can be inserted. Since BAC can be easily handled, it is used to construct the genomic library covering the whole genome sequences.

Note: Some names in this table are abbreviated.

Table 3: Functions and structures of major insect hormones

Names of insect hormones	Molecular classification	Chief functions	Isolation of the hormone and clarification of its structure
Molting hormone	Steroid	Regulation of molt and metamorphosis	Butenandt et al. (Germany 1954)
Juvenile hormone	Terpenoid	Regulation of molt, metamorphosis and reproduction	Röler et al. (Germany 1967)
Peptide hormone			
Bombyxin	Peptide	Induction and enhancement of cell proliferation	Hiromichi Nagasawa, Akinori Suzuki et al. (Japan 1984)
Prothoracicotropic hormone	Peptide	Regulation of molting hormone secretion	Atsushi Kawakami, Akinori Suzuki et al. (Japan 1990)
Diapause hormone	Peptide	Induction of the silkworm's egg diapause	Kunio Imai, Okitsugu Yamashita et al. (Japan 1992)

of useful substances with insects by gene engineering, mainly because it produces and secretes a large amount of uniform silk proteins outside the body. Yet, the hard shell of the silkworm's egg is unfavorable for conducting gene engineering on the egg.

3.3.2 Research on growth control and hormones of insects

Among insect hormones, it has been known for a long time that the molting hormone and the juvenile hormone control molt and metamorphosis. Recently, researchers unveiled the structures of various peptide hormones, which serve as brain hormones for regulating

the molt and juvenile hormones. As shown in Table 3, Japanese researchers play a leading role in studies on the insect hormone, especially the peptide hormone. For example, they clarified the structures of the prothoracicotropic hormone and the diapause hormone.

Particularly, since the prothoracicotropic hormone holds the key to insect growth control, its research bears a profound meaning. We should note that such remarkable fruits could be obtained because large amounts of silkworms, which serve as materials for the purification of hormones, were available in Japan.

In advance of world researchers, Dr. Akinori Suzuki of the Faculty of Agriculture, The University

of Tokyo, and his colleagues successfully unveiled the structures of various peptide hormones in the brain, such as the prothoracicotrophic hormone, in the “Structures, functions and dynamics of insect brain peptides, especially the prothoracicotrophic hormone” project that was supported by the Grant-in-Aid for Scientific Research (Specially Promoted Research) of the then Ministry of Education, Science, Sports and Culture from FY 1989 to 1992. His group also advanced functional analysis of these hormones.

Moreover, analysis of hormone action in insects such as the operation mechanism of the silkworm’s diapause hormone, environmental response and neuroendocrine regulation of the hormone was accelerated in the “Molecular mechanism of metamorphosis and diapause of insects” project (representative: Dr. Okitsugu Yamashita; FY 1996-1999), which was supported by the Grant-in-Aid for Scientific Research on Priority Areas. In this study, Dr. Yamashita and his colleagues analyzed biosynthesis, secretion control, dynamics in the blood and action in the targeted organ of peptide hormones and expression of molting hormone receptor genes. These research objects have been taken over and further promoted by the “Expression mechanism of insect-specific functions and their development” project (representative: Dr. Okitsugu Yamashita; FY 1999-2003) of the Research for the Future Program.

3.3.3 Research on the defense mechanism of insects

A highly developed defense mechanism is a major factor of insects’ prosperity.

When the body surface of an insect is injured, antibiotic compounds are produced to combat invading microbes. Dr. Shunji Natori of the Institute of Physical and Chemical Research has pursued his research on antibiotic proteins of the flesh fly *Sarcophaga peregrina* in the “Defense mechanism of insects” project, which was supported by the Grant-in-Aid for Scientific Research on Priority Areas in FY 1996-1999, and the “Defense mechanism” project in FY 1999-2002 in the Core Research for Evaluational Science and Technology (CREST) of the Japan Science and Technology Corporation (JST). In

this study, he found that *Sarcophaga* produces various defensive chemical compounds from low-molecular-weight compounds to peptides and proteins in order to protect itself from enemies. Furthermore, he suggested that production of these defensive compounds is induced not only when the body surface of *Sarcophaga* is injured; they also serve as growth factors of the imago primordium at the molting period.

Defensive compounds have been discovered from many insects such as the silk moth and the beetle as well as *Sarcophaga*. More than 200 kinds of such compounds have been reported since a defensive compound of an insect was first found in the 1980s.

Table 4 shows some examples of antibiotic proteins among defensive compounds of insects. As this table indicates, antibiotic proteins are classified into several groups according to the homology of amino acid sequences.

Dr. Minoru Yamakawa of the National Institute of Agrobiological Sciences has isolated antibiotic proteins from the silkworm and the beetle, and investigated their operational mechanism in the “Research on utilization of insects’ functions” project from FY 1996, which was supported by the Special Coordination Funds for Promoting Science and Technology for encouraging Centers of Excellence (COE). This research has been taken over and put forward by the “Property analysis and engineering of antibiotic proteins of insects” project of the Bio-oriented Technology Research Advancement Institution from FY 2001. Among antibiotic proteins, cecropin- and defensin-type proteins show bactericidal effects by perforating the cell membrane of the bacteria and are even effective against methicillin-resistant *Staphylococcus aureus* (MRSA; a Gram-positive bacterium), which causes a nosocomial infection. Their application as insect-derived pharmaceuticals is highly expected.

3.3.4 Research on symbiotic microorganisms of insects

Insects account for about 70% of all animal species, and 60% of all insects presumably have symbiotic microorganisms. Symbiotic microorganisms

Table 4: Examples of insect-derived antibiotic proteins

Classification of the protein	Examples of insects in which the protein is found	The protein is active to:
Cecropin type	Silk moth, <i>Sarcophaga peregrina</i> , <i>Drosophila melanogaster</i>	Gram-positive bacteria and Gram-negative bacteria*
Defensin type	<i>S. peregrina</i> , <i>Apis mellifera</i> , <i>Aeschna mixta</i> , a kind of stinkbug, beetle	Mainly Gram-positive bacteria
Attacin type	Silk moth, <i>S. peregrina</i> , <i>D. melanogaster</i>	Gram-negative bacteria
High-glycine-containing antibiotic protein type	<i>S. peregrina</i> , <i>D. melanogaster</i> , <i>A. mellifera</i> , a kind of Tenebrionidae, a kind of Pyrrhocoridae	Gram-negative bacteria
High-proline-containing antibiotic protein type	Silk moth, Fruit fly , <i>A. mellifera</i> , a kind of stinkbug	Gram-negative bacteria

*** Gram-positive and Gram-negative bacteria**

Bacteria can be classified into Gram-positive and Gram-negative bacteria. Gram-positive bacteria are dyed purple with the Gram staining method. Lactic acid bacteria and MRSA are examples of Gram-positive bacteria. On the other hand, Gram-negative bacteria are not dyed with the Gram staining method. *Escherichia coli* is an example of Gram-negative bacteria.

Source: Prepared based on Reference^[4] with some added changes.

living in insect bodies are regarded as a reservoir of unknown useful substances. In addition, further advancement of studies on insect-microorganism symbiosis will help us to understand not only the basic mechanisms of biological phenomena but also the process of evolution.

Dr. Hirofumi Watanabe of the National Institute of Agrobiological Sciences revealed that termites as well as their symbiotic microorganisms have cellulase (cellulose-decomposing enzyme). Research for utilizing the cellulase of termites and their symbiotic microorganisms is accelerating in order to globally exploit cellulose as biomass.

Meanwhile, research on symbiotic microorganisms of insects in evolutionary biology has been conducted on *Wolbachia*, which is a symbiotic microorganism of about 17% of arthropods including insects and controls sex and reproduction of its host in several ways, and on *Buchnera*, a symbiotic microorganism of the aphid. These studies were promoted in the “Analysis and utilization of the molecular mechanism of the insect-microbe parasitic and symbiotic system” project (representative: Dr. Hajime Ishikawa; FY 1996-2000) of the “Promotion project of basic research for creation of new technology and realms” of the Bio-oriented Technology Research Advancement Institution. Recently, horizontal gene transfer (a phenomenon that the gene of a particular

organism is transferred to the genome of another species) from *Wolbachia*, which is a procaryote and intracellular symbiotic microorganism, to *Callosobruchus chinensis*, an eucaryote, was experimentally proved, and *Wolbachia* came to be focused on as a key to unveiling the mechanism of gene transfer. In the meantime, sequencing of the whole genome of *Buchnera*, a symbiont of the aphid, was completed in 2000 for the first time in the world as a symbiotic microorganism of the insect.

Research on symbiotic microorganisms of insects has fallen behind because their artificial cultivation has been impossible. Yet, as technology to amplify their DNAs and sequence them has been improved, researchers have discovered interesting phenomena without artificially cultivating symbiotic microorganisms. For example, it was realized that a general start codon of AUG is unnecessary to initiate translation in a parasitic RNA virus of an insect. This is a significant discovery that may change the principle of the translation mechanism. In this way, studies on symbionts can trigger the new development of life science. Symbiotic microorganisms of insects are regarded as a reservoir of future pharmaceuticals and agrichemicals, and construction of their gene libraries will be a base for research in pioneering new insect-related industries.

3.4 Conclusion

Life science of insects is about to enter the post-genome era in which researches are based on the whole genome sequence of each insect. If, following the fruit fly and the anopheles, we complete whole genome sequencing of the silkworm, which is far distant from the fruit fly and the anopheles on the phylogenetic tree, we can further enhance the clarification of every biological feature of model organisms like the fruit fly and the silkworm. Moreover, our understanding of insects in the realm of life science will be greatly deepened. Therefore, it is meaningful to promote the silkworm's whole genome sequencing project in Japan, where researchers have pursued various biological studies on this insect.

Furthermore, on such research bases, it is also crucial as a field of life science to enhance analysis of functions specific to insects, such as research on growth control and hormones, the defense mechanism, and symbiotic microorganisms of insects.

Acknowledgements

This report has been compiled based on

"Trends and Prospects in Studies Using Insects," a lecture by Dr. Shunji Natori, an invited researcher of the Institute of Physical and Chemical Research, held on April 16, 2003 at the National Institute of Science and Technology Policy and with our own investigations. We would like to extend our sincere gratitude to the researchers of the National Institute of Agrobiological Sciences for providing us with invaluable information.

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(Original Japanese version: published in June 2003)