

Review Article

Progress in Insect-Microbe Relationships: A Novel Approach in Entomology

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A B S T R A C T

Entomology, the scientific study of insects, has been a cornerstone of biological research for centuries. Recent advancements in molecular biology, genomics, and microbiology have revolutionized our understanding of insects, particularly in the realm of insect-microbe interactions. These interactions are crucial for various ecological processes, insect physiology, and even human health. This review delves into the latest research on insect-microbe interactions, highlighting their significance and potential applications in agriculture, medicine, and biotechnology. By exploring mutualistic and parasitic relationships, the influence of microbiomes on insect health, and the technological advances driving this field, we provide a comprehensive overview of the current state and future directions of insect-microbe research. Understanding these interactions not only enhances our knowledge of insect biology but also offers innovative solutions for sustainable agriculture, novel medical therapies, and advancements in biotechnology. The integration of modern molecular techniques with traditional ecological approaches is uncovering the complexity and importance of these relationships, paving the way for transformative applications that address global challenges. As we continue to unravel the intricate dynamics between insects and their microbial partners, the potential for groundbreaking discoveries and practical innovations grows, marking a new frontier in entomological science.

Keywords: Insect-Microbe Interactions, Entomopathogenic Fungi, Microbiome, Biological Control, Genetic Engineering, Vector Control

Introduction

Insects, constituting over a million described species, are the most diverse group of organisms on Earth. They inhabit almost every conceivable environment and play essential roles in ecosystems, including pollination, decomposition, and serving as food sources for other animals. A critical aspect of their success is their intricate relationships with microorganisms, including bacteria, fungi, viruses, and protozoa. These interactions can be mutualistic, commensal, or parasitic, profoundly influencing insect biology and ecology.

The study of insect-microbe interactions has gained significant momentum in recent years, propelled by advancements in molecular biology, genomics, and microbiology. The advent of high-throughput sequencing technologies has allowed researchers to uncover the vast diversity of microbial communities associated with insects, shedding light on their functional roles and evolutionary significance. Understanding these interactions is crucial not only for basic biological research but also for developing practical applications in various fields.

In agriculture, for example, insect-associated microbes can be leveraged to enhance crop protection and productivity. Beneficial microbes can promote plant health by facilitating nutrient acquisition and protecting against pests and diseases. Conversely, understanding the microbial associates of pest insects can lead to the development of targeted biological control strategies, reducing the reliance on chemical pesticides and mitigating their environmental impact.^{1,2}

In the realm of public health, insect vectors such as mosquitoes transmit diseases like malaria, dengue, and Zika. The microbiome of these vectors can influence their capacity to harbor and transmit pathogens. By manipulating the microbial communities within vector populations, it may be possible to reduce disease transmission, offering a novel approach to vector control.

Moreover, insect-microbe interactions hold promise for biotechnological applications. Insects and their symbiotic microbes produce a wide array of bioactive compounds, including enzymes, antibiotics, and metabolites, which can be harnessed for industrial and pharmaceutical purposes. The enzymes involved in insect digestion, for instance, have potential applications in biomass conversion and biofuel production.

Despite the burgeoning interest and significant progress in this field, many aspects of insect-microbe interactions remain poorly understood. The complexity of these interactions, influenced by factors such as insect species, developmental stage, diet, and environment, presents a formidable challenge for researchers. Integrating interdisciplinary approaches and leveraging emerging technologies will be essential for unraveling the intricacies of these relationships and harnessing their potential benefits [3, 4].

Mutualistic Interactions

Mutualistic interactions between insects and microbes are widespread and diverse, playing critical roles in the survival, reproduction, and ecological success of many insect species. These symbiotic relationships can enhance nutritional intake, provide defense against pathogens, and even influence insect behavior and physiology. Here, we explore some of the most well-known and impactful mutualistic interactions between insects and their microbial partners.

Symbiotic Bacteria

One of the most studied mutualistic relationships is between insects and their symbiotic bacteria. These bacteria often reside in specialized organs or tissues and provide essential nutrients or other benefits to their insect hosts.

Aphids and *Buchnera aphidicola*

Aphids rely on *Buchnera aphidicola*, an obligate endosymbiont, for the synthesis of essential amino acids that are

scarce in their phloem sap diet. *Buchnera* resides within specialized cells called bacteriocytes and has co-evolved with aphids for millions of years. This relationship is so interdependent that neither the aphid nor the bacterium can survive without the other. The metabolic integration between the aphid and *Buchnera* is a prime example of how mutualistic interactions can drive evolutionary processes.

Termites and Gut Microbes

Termites harbor complex microbial communities in their guts that aid in cellulose digestion, a process crucial for their survival and ecological role in nutrient cycling. These microbes include bacteria, archaea, and protozoa that work synergistically to break down the tough cellulose fibers in wood, allowing termites to access the resulting sugars. This symbiosis not only supports the termite's nutritional needs but also contributes to the recycling of plant material in ecosystems.^{5,6}

Fungal Symbionts

Fungal symbionts also play a vital role in insect biology. Many insects engage in sophisticated mutualisms with fungi, which can provide nutrients, protection, and other benefits.

Leaf-Cutter Ants and Fungus Gardens

Leaf-cutter ants cultivate fungal gardens that degrade plant material, providing a nutritious food source for the colony. These ants cut leaves and bring them back to their nests, where the plant material is used to grow a specialized fungus. The ants feed on the fungal hyphae, which are rich in nutrients. This mutualism involves complex agricultural practices, including the use of antimicrobial compounds to protect the fungal crops from pathogens and ensure the health of the colony.

Ambrosia Beetles and Ambrosia Fungi

Ambrosia beetles, which bore into wood, cultivate fungal gardens within their galleries. These fungi, known as ambrosia fungi, break down wood and provide the beetles with a reliable food source. The beetles carry fungal spores in specialized structures called mycangia, ensuring that their fungal partners are introduced into new galleries. This mutualism allows the beetles to exploit a nutrient-rich but otherwise inaccessible resource, highlighting the intricate dependencies that can evolve between insects and fungi.

Yeast Symbionts

Yeast symbionts are also important for some insect species, particularly those that feed on nutrient-poor or specialized diets.

Drosophila and Yeast

Drosophila fruit flies have a mutualistic relationship with various yeast species found on their food substrates, such

as rotting fruit. These yeasts help break down complex carbohydrates and produce essential nutrients, including vitamins and amino acids, which are then available to the flies. In return, the flies disperse the yeast to new food sources, facilitating their reproduction and spread.

Microbial Influence on Insect Behavior

Microbes can influence insect behavior in ways that benefit both the host and the symbiont. These interactions often involve complex biochemical signaling and can have profound effects on insect ecology.

Wolbachia and Reproductive Manipulation

Wolbachia, a genus of bacteria that infects a wide range of arthropods, is known for its ability to manipulate host reproduction. Wolbachia can induce phenomena such as cytoplasmic incompatibility, parthenogenesis, and feminization, which enhance the spread of the bacteria through host populations. These manipulations can have significant impacts on insect population dynamics and are being explored as tools for pest control and disease vector management.

Parasitic and Pathogenic Interactions

Parasitic and pathogenic interactions between insects and microbes can have significant impacts on insect populations, health, and behavior. These interactions can be detrimental to the insect hosts, often leading to disease or death. However, they also play a crucial role in regulating insect populations and can be harnessed for biological control in agriculture and disease management. This section explores key examples of parasitic and pathogenic interactions between insects and various microbial pathogens, including fungi, bacteria, viruses, and protozoa.

Entomopathogenic Fungi

Entomopathogenic fungi are a diverse group of fungi that infect and kill insects. These fungi can penetrate the insect cuticle, proliferate within the host, and ultimately cause death, making them valuable biological control agents.

Beauveria Bassiana

Beauveria bassiana is a well-known entomopathogenic fungus that infects a wide range of insect hosts. The spores of *B. bassiana* adhere to the insect cuticle, germinate, and penetrate the host through enzymatic degradation of the cuticle. Once inside, the fungus proliferates and produces toxins that disrupt the host's immune system and physiology, leading to death. *B. bassiana* is widely used in biopesticides for controlling agricultural pests such as aphids, whiteflies, and beetles. Its specificity and effectiveness make it a valuable tool in integrated pest management (IPM) programs.

Metarhizium Anisopliae

Metarhizium anisopliae is another entomopathogenic fungus with a broad host range. It infects insects in a manner similar to *B. bassiana*, through spore adhesion, germination, and cuticle penetration. *M. anisopliae* is used for controlling various pests, including termites, grasshoppers, and mosquitoes. Research on the molecular mechanisms of pathogenicity and host resistance is ongoing, aiming to enhance the efficacy and sustainability of these fungal biocontrol agents.

Bacterial Pathogens

Bacterial pathogens can cause diseases in insects, leading to significant mortality in pest populations. These pathogens can be exploited for biological control, either through direct application or genetic engineering.

Bacillus Thuringiensis (Bt)

Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces crystalline endotoxins, known as Bt toxins, during sporulation. These toxins are ingested by insect larvae, particularly Lepidoptera, Diptera, and Coleoptera, and act by binding to receptors in the insect midgut, causing cell lysis and death. Bt toxins are used extensively in biopesticides and genetically modified crops (Bt crops) to control pest populations. However, the evolution of resistance in target insect populations poses a significant challenge, necessitating ongoing research into resistance management and the development of novel Bt toxins.

Wolbachia and Pathogen Interference

Wolbachia is an intracellular bacterium that infects many arthropods and nematodes. While Wolbachia is primarily known for its reproductive manipulations, it can also interfere with the transmission of insect-borne pathogens. For instance, Wolbachia-infected *Aedes* mosquitoes have reduced susceptibility to dengue and Zika viruses, making Wolbachia a promising tool for controlling vector-borne diseases. Field trials are underway to release Wolbachia-infected mosquitoes in areas affected by these diseases, with the aim of reducing disease transmission.

Viral Pathogens

Viruses can infect insects and cause diseases that can decimate populations. Understanding viral pathogenesis and host-virus interactions is crucial for developing viral biocontrol agents.

Baculoviruses

Baculoviruses are a family of large DNA viruses that specifically infect insects, particularly Lepidoptera. These viruses have been used as biological control agents due to their high specificity and efficacy. Baculoviruses infect insect larvae through ingestion, leading to systemic infection and

death. The occlusion bodies containing the viral particles are released into the environment upon the host's death, continuing the infection cycle. Baculoviruses are used in biopesticides for controlling pests such as the gypsy moth and cotton bollworm.

Protozoan Pathogens

Protozoan pathogens can also infect insects, causing diseases that impact insect health and population dynamics.

Nosema spp.

Nosema spp. are microsporidian protozoa that infect various insect hosts, including honeybees and silkworms. Nosema infections can lead to chronic diseases, reducing host vigor, longevity, and reproductive success. Nosema apis and Nosema ceranae are significant pathogens of honeybees, contributing to colony collapse disorder (CCD). Managing Nosema infections is crucial for maintaining healthy bee populations and ensuring pollination services in agriculture.⁷⁻¹⁰

Microbiome and Insect Health

The concept of the microbiome, which refers to the community of microorganisms residing in and on an organism, has gained significant attention in the study of insect health and physiology. Insects harbor diverse and complex microbial communities that can influence a wide array of biological processes. The insect microbiome plays crucial roles in digestion, immunity, reproduction, and even behavior, underscoring the integral relationship between insects and their microbial partners. This section explores the composition and functions of insect microbiomes and their impacts on insect health.

Composition of the Insect Microbiome

The composition of the insect microbiome varies widely among different insect species, life stages, and environments. It typically includes bacteria, fungi, viruses, and protozoa, with bacteria being the most extensively studied.

Gut Microbiome

The gut microbiome is one of the most critical microbial communities in insects. It aids in the digestion of food, detoxification of harmful substances, and synthesis of essential nutrients. For example, the gut microbiota of termites includes bacteria and protozoa that break down cellulose, allowing termites to derive energy from wood. Similarly, the gut microbiota of herbivorous beetles assists in degrading plant cell walls and detoxifying plant secondary metabolites.

Endosymbionts

Endosymbionts are microorganisms that live within the cells or tissues of insects. These symbionts can provide essential nutrients that are missing from the insect's diet.

For instance, aphids harbor *Buchnera aphidicola*, which supplies them with essential amino acids not found in their phloem sap diet. Other examples include *Wolbachia*, which can manipulate the reproductive systems of their hosts and impact population dynamics.

Functions of the Insect Microbiome

The functions of the insect microbiome are diverse and can significantly influence the health and fitness of the host. Key functions include:

Nutritional Support

Microbes can enhance the nutritional intake of insects by breaking down complex molecules and synthesizing essential nutrients. For instance, in the tsetse fly, the endosymbiotic bacterium *Wigglesworthia glossinidia* synthesizes B vitamins that are critical for the fly's reproduction and development. Similarly, the gut microbiota of honeybees aids in the digestion of pollen and nectar, contributing to the overall health and productivity of the colony.

Immune Modulation

The microbiome can modulate the insect immune system, enhancing the host's ability to resist pathogens. In *Drosophila*, gut bacteria stimulate the production of antimicrobial peptides, which help protect against infections. Additionally, microbial symbionts can compete with pathogenic microbes for resources and space, providing a form of colonization resistance.

Reproductive Manipulation

Some microbes can manipulate the reproductive systems of their hosts to enhance their own transmission. *Wolbachia*, for example, can induce cytoplasmic incompatibility, parthenogenesis, and feminization in various arthropod species. These manipulations can affect host population structure and dynamics, with potential applications in pest and vector control.¹¹⁻¹⁴

Impact on Insect Health

The impact of the microbiome on insect health is profound, influencing various aspects of physiology and behavior.

Disease Resistance

The microbiome can confer disease resistance by outcompeting pathogens or by priming the host's immune response. In mosquitoes, the presence of certain gut bacteria has been shown to reduce the susceptibility to malaria parasites, offering potential strategies for vector control. Similarly, in bees, a healthy gut microbiome is associated with resistance to pathogens such as *Nosema* and American foulbrood.

Development and Metabolism

Microbes can influence the development and metabolism of their insect hosts. In fruit flies, the gut microbiota af-

fects larval growth rates and adult body size. Insects with disrupted microbiomes often exhibit developmental delays and metabolic imbalances, highlighting the importance of a stable microbial community for normal physiological functions.

Behavior

Microbial communities can also influence insect behavior. For instance, the microbiome of honeybees affects foraging behavior and social interactions within the hive. Changes in the microbiome can alter pheromone production and perception, impacting communication and colony cohesion. In fruit flies, gut bacteria have been shown to influence mating preferences, suggesting a role for microbes in sexual selection.

Technological Advances and Future Directions

Recent technological advances, particularly in high-throughput sequencing and metagenomics, have greatly enhanced our understanding of insect microbiomes. These tools allow for comprehensive characterization of microbial communities and their functions, providing insights into the complex interactions between insects and their microbes.

Metagenomics and Microbiome Analysis

Metagenomic approaches enable researchers to identify and functionally analyze the genes present in microbial communities, offering a deeper understanding of their metabolic capabilities and ecological roles. By comparing the microbiomes of healthy and diseased insects, researchers can identify microbial signatures associated with health and disease states.

Manipulating the Microbiome

Advances in genetic engineering and synthetic biology provide opportunities to manipulate insect microbiomes for beneficial outcomes. For example, introducing or enhancing beneficial microbes in pest insects can reduce their fitness or reproductive capacity, offering novel pest control strategies. In vector control, altering the microbiome of disease vectors to reduce their ability to transmit pathogens is an area of active research.¹⁶⁻¹⁸

Technological Advances

The study of insect-microbe interactions has been significantly propelled forward by technological advancements in molecular biology, genomics, and microbiology. These technologies have provided new tools and methodologies that allow researchers to explore the complexity of these interactions at unprecedented levels of detail. This section discusses key technological advances that have revolutionized the field of entomology, particularly in the study of insect-microbe interactions.

Genomics and Metagenomics

High-Throughput Sequencing

High-throughput sequencing technologies, such as Illumina, PacBio, and Oxford Nanopore, have revolutionized the study of insect-microbe interactions. These technologies allow for the rapid and cost-effective sequencing of entire genomes, including those of insects and their associated microbes. This has enabled researchers to catalog the diversity of microbial communities (microbiomes) associated with different insect species, developmental stages, and environmental conditions.

Metagenomics

Metagenomics involves the sequencing of genetic material recovered directly from environmental samples, bypassing the need for culturing individual microorganisms. This approach has been particularly useful in studying complex microbial communities within insect guts, where many of the microbes cannot be easily cultured in the lab. Metagenomic analyses provide insights into the functional potential of these communities, identifying genes involved in processes such as digestion, nutrient synthesis, and immune modulation.

Transcriptomics and Proteomics

RNA Sequencing (RNA-seq)

RNA sequencing (RNA-seq) allows researchers to analyze the transcriptome—the complete set of RNA transcripts produced by an organism at a given time. This technology provides insights into the gene expression profiles of both insects and their microbial symbionts, revealing how these interactions affect host physiology and microbial activity. For example, RNA-seq can identify changes in gene expression in response to microbial infections or symbiotic relationships, shedding light on the molecular mechanisms underlying these interactions.

Proteomics

Proteomics involves the large-scale study of proteins, including their structures, functions, and interactions. Advances in mass spectrometry and bioinformatics have made it possible to identify and quantify thousands of proteins in complex biological samples. In the context of insect-microbe interactions, proteomics can reveal the protein-based communication and metabolic exchanges between insects and their microbes, providing a deeper understanding of these relationships at the molecular level.

CRISPR and Genetic Engineering

CRISPR/Cas9

The CRISPR/Cas9 system has revolutionized genetic engineering by allowing precise and targeted modifications of the genome. In entomology, CRISPR/Cas9 has been used to

manipulate genes in both insects and their microbial symbionts, facilitating functional studies of specific genes and their roles in insect-microbe interactions. This technology has the potential to create genetically modified insects with enhanced resistance to pathogens or altered microbiomes that reduce their capacity to transmit diseases.

Gene Drives

Gene drives are genetic systems that increase the likelihood of a particular gene being passed on to the next generation. This technology can be used to spread beneficial traits through insect populations, such as resistance to pathogens or reduced fertility in pest species. Gene drives, combined with CRISPR/Cas9, offer powerful tools for controlling vector-borne diseases and managing agricultural pests.

Imaging and Microscopy

Advanced Microscopy Techniques

Advances in microscopy, including confocal microscopy, electron microscopy, and super-resolution microscopy, have enabled detailed visualization of insect tissues and their associated microbes. These techniques allow researchers to observe the spatial organization and interactions of microbial communities within insect hosts, providing insights into the physical and structural aspects of these relationships.

In Vivo Imaging

In vivo imaging techniques, such as fluorescence and bioluminescence imaging, allow for the real-time observation of microbial dynamics within living insects. These techniques can be used to track the colonization, proliferation, and movement of microbes within their hosts, providing a dynamic view of insect-microbe interactions over time.

Bioinformatics and Computational Biology

Data Analysis and Integration

The explosion of genomic, transcriptomic, and proteomic data has necessitated the development of advanced bioinformatics tools and computational approaches for data analysis and integration. Bioinformatics pipelines enable the processing, visualization, and interpretation of large-scale data sets, facilitating the identification of patterns and relationships in complex biological systems. Computational models and simulations can also predict the outcomes of insect-microbe interactions under different environmental conditions.

Systems Biology

Systems biology approaches integrate data from multiple levels of biological organization, including genes, proteins, metabolites, and ecological interactions. By constructing comprehensive models of insect-microbe interactions, systems biology can provide a holistic understanding of

these complex relationships and identify key regulatory networks and pathways.

Synthetic Biology

Synthetic biology involves the design and construction of new biological parts, devices, and systems, or the redesign of existing biological systems for useful purposes. In the context of insect-microbe interactions, synthetic biology can be used to engineer microbes with specific functions, such as producing antimicrobial compounds or degrading environmental toxins. These engineered microbes can be introduced into insect populations to enhance their health, reduce their capacity to transmit diseases, or manage pest populations.^{19,20}

Applications and Future Directions

The study of insect-microbe interactions has significant potential for practical applications in various fields, including agriculture, public health, and biotechnology. Leveraging these interactions can lead to innovative solutions for pest control, disease management, and environmental sustainability. This section explores current applications and future directions in the field of insect-microbe interactions.

Agricultural Applications

Biological Control

One of the most promising applications of insect-microbe interactions in agriculture is biological control. Beneficial microbes can be used to target and suppress pest populations, reducing the need for chemical pesticides and mitigating their environmental impact.

Entomopathogenic Microbes

Entomopathogenic fungi (e.g., *Beauveria bassiana*, *Metarhizium anisopliae*), bacteria (e.g., *Bacillus thuringiensis*), and viruses (e.g., baculoviruses) have been developed into commercial biopesticides. These microorganisms can infect and kill a wide range of insect pests, providing an environmentally friendly alternative to synthetic chemicals.

Paratransgenesis

Paratransgenesis involves modifying symbiotic microbes within insect pests to produce substances that can control or kill the pest. For example, researchers are exploring the use of genetically engineered symbionts in aphids to disrupt their reproduction or reduce their survival rates.

Plant Growth Promotion

Beneficial microbes associated with insects can promote plant growth and health. For instance, some endophytic bacteria and fungi found in insects can enhance nutrient uptake, stimulate plant growth hormones, and provide resistance to plant pathogens. Utilizing these microbes in agricultural practices can improve crop yields and reduce the dependency on chemical fertilizers and pesticides.^{15, 16}

Public Health Applications

Vector Control

Microbial symbionts of disease vectors, such as mosquitoes, can be manipulated to reduce the transmission of pathogens that cause diseases like malaria, dengue, and Zika.

Wolbachia-Based Strategies

Wolbachia, a bacterium that infects many insect species, has been shown to interfere with the transmission of viruses by mosquitoes. Releasing Wolbachia-infected mosquitoes into the environment can reduce the incidence of mosquito-borne diseases. Field trials have demonstrated the effectiveness of this strategy in reducing dengue transmission in several countries.

Microbiome Engineering

Engineering the gut microbiome of vectors to produce antipathogenic compounds can inhibit the development of pathogens within the vector, thereby reducing disease transmission. This approach is still in the experimental stage but holds great promise for future vector control strategies.

Biotechnological Applications

Production of Bioactive Compounds

Insects and their associated microbes produce a wide range of bioactive compounds, including antibiotics, enzymes, and secondary metabolites. These compounds have potential applications in medicine, industry, and environmental management.

Antimicrobial Agents

Microbes associated with insects have been a source of novel antimicrobial agents that can be used to combat antibiotic-resistant pathogens. For example, certain entomopathogenic fungi produce compounds with strong antimicrobial properties that are being investigated for pharmaceutical applications.

Industrial Enzymes

Enzymes derived from insect-associated microbes have potential applications in various industries. For example, enzymes involved in the digestion of plant material by insect gut microbes can be used in biofuel production, improving the efficiency of biomass conversion.

Synthetic Biology

Synthetic biology approaches can be used to engineer insects and their microbiomes for beneficial purposes. This includes the production of valuable compounds, bioremediation of pollutants, and the development of bioindicators for environmental monitoring.

Future Directions

Integrative Approaches

The future of insect-microbe interaction research lies in integrative approaches that combine traditional entomology with advanced molecular biology, genomics, and computational biology. Understanding the complex networks and interactions at multiple levels will provide a comprehensive picture of these relationships.

Personalized Microbiome Management

Advances in microbiome research may lead to personalized approaches in managing insect health and behavior. This could involve tailoring microbiome compositions to enhance beneficial traits or mitigate harmful ones, both in natural and agricultural settings.

Climate Change Adaptation

As climate change impacts insect populations and their associated microbiomes, research will need to focus on understanding and mitigating these effects. Developing resilient insect-microbe systems that can withstand environmental changes will be crucial for maintaining ecosystem stability and agricultural productivity.

Ethical and Regulatory Considerations

The application of genetic engineering and synthetic biology in insects and their microbes raises important ethical and regulatory questions. Ensuring the safe and responsible use of these technologies will require robust regulatory frameworks and public engagement to address potential risks and societal concerns.

Conclusion

Insect-microbe interactions represent a dynamic and rapidly evolving field of entomology. The integration of modern molecular techniques with traditional ecological approaches is uncovering the complexity and importance of these relationships. As our understanding deepens, the potential to exploit these interactions for the benefit of agriculture, medicine, and biotechnology becomes increasingly apparent. Future research should focus on elucidating the mechanisms underlying these interactions and translating this knowledge into practical applications.

By embracing the complexity of insect-microbe interactions, entomologists can contribute to solving some of the most pressing challenges facing humanity, from food security to disease control. The frontier of insect-microbe research promises to be as diverse and impactful as the insects themselves.

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