



SYMPOSIUM INTRODUCTION

With a Little Help from My Friends: Microbial Partners in Integrative and Comparative Biology—An Introduction to the Symposium

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Synopsis The role that host-associated microbes play in animal biology is gaining attention in comparative biology. Numerous research groups study the roles that microbes play in human health and nutrition, or in enhancing the production of agricultural animals. However, inclusion of host-associated microbes into research questions of integrative and comparative biology has lagged behind. We hosted a symposium to bring together top researchers in the field of host-associated microbes who also incorporate aspects of integrative and comparative biology. In this introduction, we highlight recent research demonstrating the profound roles that host-associated microbes play in many aspects of animal biology, such as immune function, endocrinology, and even behavior. It is our hope that integrative and comparative biologists will begin to include aspects of host-associated microbes into their research programs, enhancing both the fields of comparative biology and host–microbe interactions.

Introduction

Recent studies have revealed that animals are not individual organisms, but rather are collectives that host highly diverse and interactive communities of microbes. These microbial communities contain bacteria, archaea, protozoa, fungi, and viruses that provide a number of services and functions to their hosts (McFall-Ngai et al. 2013). It has been proposed that animals and their microbes may collectively form a “holobiont” upon which natural selection acts (Bordenstein and Theis 2015; Shapira 2016; Theis et al. 2016), though this idea has been debated (Moran and Sloan 2015; Douglas and Werren 2016). For the most part, investigations into host–microbial interactions have been focused on humans or model systems targeted at human health (Colston and Jackson 2016). However, research into the roles that microbes play in the ecology and evolution of their hosts is a rapidly growing area. We conducted a symposium that brought together and galvanized a number of

researchers that investigate host–microbe interactions in the context of ecology and evolution. The work presented at our symposium was highlighted in *Science* magazine (Pennisi 2017), and is reported in subsequent articles in this issue. The purpose of this article is to introduce the area of host–microbe interactions and to present the justification for the symposium.

Studies focused in model laboratory animals and humans have revised our understanding of how host-associated microbes can impact physiology, performance, and health. These studies provide the proof of concept and impetus for studying host-associated microbes in natural systems. Below, we highlight recent studies relating to numerous divisions of the Society for Integrative and Comparative Biology that supported our symposium. While this paper largely focuses on examples of gut microbial communities, similar studies have been conducted on other body sites (skin [Kueneman et al. 2014], oral cavity [Stothart et al. 2016], scent glands (Theis et al. 2013), etc.).

These studies are only a sampling of the recent investigations that are beginning to uncover the importance of host-associated microbes in the integrative biology of natural systems.

Ecology and evolution

Numerous studies have investigated how environmental characteristics and relatedness between host species dictate the composition of host-associated microbial communities. Controlled, experimental trials within single host species have demonstrated the role that diet (Kohl et al. 2016), temperature (Chevalier et al. 2015; Kohl and Yahn 2016), and other environmental variables can play in determining the structure of host-associated microbial communities. Additionally, large-scale, comparative studies have demonstrated that many factors contribute to structuring microbial communities across animal hosts. For example, the diversity and function of the mammalian gut microbiota are sculpted by host diet, phylogeny, and gut anatomy (Ley et al. 2008; Muegge et al. 2011). Across distantly related mammals, a diet of ants (myrmecophagy) has resulted in convergence of the gut microbial community structure (Delsuc et al. 2014). The avian gut microbiota is shaped by host taxonomy and diet (Kohl 2012; Waite and Taylor 2014; Hird et al. 2015). In fish, gut microbial community structure is affected by salinity, trophic level, and host phylogeny (Sullam et al. 2012). The repeated finding that host taxonomy/phylogeny influences microbial community composition gave rise to the hypothesis of “phylosymbiosis”, which states that similarities in community composition of host-associated microbes should be concordant with the phylogeny of host species. Phylosymbiosis has indeed been demonstrated in several clades of host species (Brooks et al. 2016), though the mechanistic basis for phylosymbiosis is an open area ripe for future investigation. Last, gut microbial communities can impact ecological interactions, such as plant–herbivore interactions (Kohl et al. 2014), or the capacity for insects to serve as vectors for diseases (Azambuja et al. 2005).

Ecoimmunology and disease ecology

Host-associated microbial communities can have large impacts on immune function and associated disease susceptibility. Microbial colonization of the gut drastically alters the gene expression and function of the gut, especially in regards to training of the immune system (Hooper et al. 2001). In turn, microbes may provide protection against pathogens and parasites. For example, locusts hosting microbial communities with higher diversity exhibit enhanced resistance to a pathogenic bacterium (Dillon et al. 2005). Additionally, bumble

bees transmit a gut microbial community that offers protection against a parasitic protozoan (Koch and Schmid-Hempel 2011). Last, the microbes that live on amphibian skin are thought to be important in susceptibility to the emerging pathogen of chytrid fungus (Harris et al. 2009). An area begging for further research is the integration of host-associated microbial communities into theories and methods associated with the field of ecoimmunology. One promising study system is the use of various stickleback populations that exhibit differential immune responses to gut microbial populations (Milligan-Myhre et al. 2016).

Invertebrate zoology

The associations between invertebrate hosts and microbial partners have been of great interest to ecologists and evolutionary biologists. Insect hosts harbor endosymbiotic bacteria that synthesize essential vitamins or amino acids for their hosts (Douglas 2009). Additionally, a number of marine invertebrates harbor chemosynthetic bacterial symbionts that provide nutrients to their hosts (Dubilier et al. 2008). More recently, studies have investigated the roles that complex microbial associations play in invertebrate zoology. For example, gut bacterial communities likely facilitated the repeated evolution of herbivory in ants (Russell et al. 2009), and the gut microbiota of locusts can improve resistance to pathogens (Dillon et al. 2005). Invertebrate model systems have greatly enhanced our understanding of the basic principles of host–microbe interactions (e.g., *Drosophila* fruit flies [Ridley et al. 2012], *Nasonia* wasps [Brucker and Bordenstein 2013], hydra [Bosch 2012], nematode worms [Berg et al. 2016], squid [McFall-Ngai 2014], and others). Given the large diversity of invertebrates and their associated ecology, they represent promising systems for expanding our knowledge of the role microbial partnerships play in ecology and evolution.

Comparative physiology and biochemistry

Host-associated microbes can have large impacts on hosts at the physiological and biochemical levels (Kohl and Carey 2016). Colonization by microbes significantly alters an animal’s metabolome, or the collection of small metabolites in the body (Wikoff et al. 2009). Many of the physiological interactions between gut microbes and hosts relate to nutrition and energy homeostasis. For example, after exposure to cold temperatures, mice exhibit longer intestines, altered intestinal gene expression, and increased insulin sensitivity (Chevalier et al. 2015). Remarkably, these physiological changes can be recapitulated in naïve, germ-free mice

by transplanting the gut microbiota from cold-acclimated mice (Chevalier et al. 2015). Similarly, germ-free mice that receive the gut microbiota from summer bears (preparing for hibernation) gain more fat mass than mice inoculated with the microbiota from hibernating bears, demonstrating a functional role of the microbiota in pre-hibernation fattening (Sommer et al. 2016). There have also been demonstrated links between host-associated microbes and respiratory (Moreno-Indias et al. 2015; O'Connor et al. 2016), cardiovascular (Crawford et al. 2009), and hepatic physiology (Claus et al. 2011) that could be further studied in an integrative and comparative context.

Comparative endocrinology

Host-associated microbes can interact with the endocrine system in a myriad of ways (Clarke et al. 2014). The field of “microbial endocrinology” was proposed over 20 years ago (Lyte 1993), and subsequently reviewed (Lyte 2013), to investigate the roles that microbially-produced neuroactive compounds play in host physiology. Conversely, host-produced hormones can influence microbial physiology and community structure (Freestone et al. 2008; Escallón et al. 2017). Catecholamines, such as norepinephrine, are produced by hosts in response to stressful stimuli, and enteric bacteria exposed to these compounds increase rates of horizontal gene transfer (Peterson et al. 2011). Gut microbes may also modify host-produced signaling molecules: the human gut microbe *Clostridium scindens* can convert glucocorticoids, such as cortisol, into androgens (Ridlon et al. 2013). Other studies have also demonstrated potential connections between endocrinology and host-associated microbes. For example, transplanting the microbiome from male mice into female mice resulted in higher testosterone levels in recipients (Markle et al. 2013). Additionally, squirrels with higher measurements of fecal glucocorticoid metabolites harbored oral microbial communities with lower diversity (Stothart et al. 2016).

Neurobiology, neuroethology, and sensory biology

As mentioned above, gut microbes can produce, modify, and respond to a number of neuroactive compounds, which could in turn influence animals' neurobiology. Germ-free mice lacking a gut microbiota exhibit altered concentrations of neurotransmitters and differential gene expression in the brain (Heijtz et al. 2011; Clarke et al. 2013). Microbial effects on neurobiology appear to scale up to influence animal behavior. For example, gut microbes have been shown to influence mating preferences in flies (Sharon et al.

2010) and anxiety behavior in mice (Bravo et al. 2011). The social interactions of baboons can have profound effects on the structure of gut microbial communities (Tung et al. 2015). Also, microbial communities associated with the scent glands of hyenas may mediate communication between individuals (Theis et al. 2013). Understanding how microbial communities might influence animal behavior in an ecological and evolutionary sense is a growing area of interest (Ezenwa et al. 2012; Shropshire and Bordenstein 2016).

Microscopy

Most microbiome studies utilize sequencing techniques to inventory microbial communities. However, such sequence-based approaches only provide simple snapshots into the community composition and lack information about spatial arrangement, changes over time, or potential interactions between microbial members. Advances in microscopy and imaging techniques have helped to expand our understanding of the organization of these communities. Fluorescent *in situ* hybridization allows researchers to visualize particular microbial members to investigate spatial organization (Moter and Göbel 2000). These images can also be quantified to assess the impacts of diet or other variables on the spatial arrangement of microbes (Earle et al. 2015). Additionally, a recently developed technique, light sheet fluorescence microscopy, allows researchers to visualize microbial communities colonizing the guts of live zebrafish, allowing both spatial and temporal resolution (Jemielita et al. 2014).

Conclusion

It is now being appreciated that host-associated microbes can influence many aspects of animal biology. The time is ripe for integrative and comparative biologists to incorporate aspects of microbial ecology into their study questions. The examples here and in the following papers offer the proof of concept, as well as the tools and techniques for including host-associated microbial communities into future research programs.

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