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Animal-microbial symbioses in changing environments

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ABSTRACT

The environments in which animals have evolved and live have profound effects on all aspects of their biology. Predictable rhythmic changes in the physical environment are arguably among the most important forces shaping the evolution of behavior and physiology of animals, and to anticipate and prepare for these predictable changes, animals have evolved biological clocks. Unpredictable changes in the physical environment have important impacts on animal biology as well. The ability of animals to cope with and survive unpredictable perturbations depends on phenotypic plasticity and/or microevolution. From the time metazoans first evolved from their protistan ancestors they have lived in close association with a diverse array of microbes that have influenced, in some way, all aspects of the evolution of animal structure, function and behavior. Yet, few studies have addressed whether daily or seasonal rhythms may affect, or be affected by, an animal's microbial symbionts. This survey highlights how biologists interested in the ecological and evolutionary physiology of animals whose lifestyles are influenced by environmental cycles may benefit from considering whether symbiotic microbes have shaped the features they study.

1. Introduction

The environments within which animals have evolved and live have profound effects on all aspects of their biology. Predictable rhythmic changes in the physical environment (daily and seasonal) are arguably among the most important forces shaping the evolution of behavior and physiology of animals. To anticipate and prepare for these predictable changes in the physical environment, life on Earth evolved biological clocks. Zeitgebers (e.g., temperature cycles, photoperiod) entrain the biological clock whose outputs manifest as biological rhythms. Unpredictable changes in the physical environment (e.g., catastrophic weather and geologic events, global climate change) also have profound impacts on animal biology. The ability of animals to cope with and survive these unpredictable perturbations depends on phenotypic plasticity and/or microevolution (Reed et al., 2010, 2011; Vander Wal et al., 2013; Wingfield et al., 2011).

From the time metazoans first evolved from their protistan ancestors they have lived in close association with a diverse array of microbes. Microbes are a vital component of the environments in which animals evolved; they have colonized animals inside and out, and thus have likely influenced in some way all aspects of the evolution of animal structure, function and behavior (Gilbert et al., 2012; McFall-Ngai et al., 2013). Research on biological rhythms has focused primarily on the animal and the environmental cues that entrain its rhythms. To date, only a few studies have addressed the possibility that an animal's biological rhythms may influence, or be influenced by, its microbial symbionts (Heath-Heckman et al., 2013; Mukherji et al., 2013; Wier et al., 2010).

Until recently, examinations of a host's microbiota have been limited in part by the techniques available to study the microbial community. Culture-dependent analyses (e.g., BIOLOG®, culture plating) result in significant underestimations of diversity and population size (Vaughan et al., 2000) because the vast majority of gut microbes are unculturable with current techniques. Culture-independent DNA fingerprinting techniques (e.g., T-RFLP, DGGE) eliminate culture bias, allow for rapid assessment of diversity, and when paired with additional analytical methods (e.g., cloning and sequencing), allow identification of community members; however, these techniques miss less abundant members of the community and are only semi-quantitative. The more recent development of culture-independent high-throughput next-generation (next-gen) sequencing techniques (e.g., Roche 454, Illumina) now allows for an in-depth analysis of microbial community structure (via 16 S rRNA genes), physiological potential (metagenomics), and function (community transcriptomics). Although the cost-per run of next-gen sequencing methods can be high (depending on sample number and desired sequencing depth), costs are rapidly declining, 1000s of sequences are generated per sample (compared to 1/sample via

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traditional Sanger sequencing), and price per base is significantly less than Sanger sequencing (by $\geq 200\times$) (Liu et al., 2012). In addition to next-gen sequencing techniques, methods to analyze the microbial metabolites that allow communication between animals and their symbionts are being developed (metabolomics). Finally, the development and use of specialized animal models (e.g., germ-free mice) facilitate design of experiments to uncover mechanistic relationships between specific microbes and changes in host biology (Faith et al., 2014).

The development of new tools to deeply interrogate diverse microbial communities has produced a wealth of knowledge on the ways microbial partners affect animal biology and how animal hosts shape the biology of their symbionts (McFall-Ngai et al., 2013). In this paper, we provide examples of how an animal's biological rhythms may impact the structure and/or function of its microbial community, and how activity of symbiotic microbes may influence seasonal or daily rhythms of the host. We focus primarily on non-laboratory, vertebrate animals and their gut symbionts, although other examples are included in which significant progress has been made toward understanding the mechanisms by which microbes influence their host's biological rhythms. This survey highlights how consideration of animal–microbe symbioses can enrich studies of the evolutionary and ecological physiology of animals whose lifestyles are influenced by environmental cycles.

2. Gut microbiota

The majority of vertebrate's microbial symbionts are found in the gut. Microbes that reside in the gastrointestinal tract of animals are members of complex ecosystems in which microbes can number in the trillions and vastly outnumber host cells (Ley et al., 2008; Muegge et al., 2011). The gut microbiota comprises two communities, the "luminal microbiota" that is associated with bulk contents in the lumen, and the "mucosal microbiota" that resides within the mucus layer that overlies the intestinal epithelium. To meet their nutritional needs, gut microbes metabolize dietary components ingested by their hosts that either cannot, or have not been digested in the small intestine, or endogenous substrates such as mucin glycans and nutrients in sloughed epithelial cells. Products of microbial metabolism are utilized by other members of the community, and some, such as short-chain fatty acids (SCFA) and vitamins, can be absorbed and utilized by the host to meet energetic and nutritional needs (Bugaut and Bentejac, 1993). It is becoming increasingly clear that beyond this nutritional input, the resident microbiota protects their hosts from invading pathogens and influence a diverse array of host characteristics including innate and adaptive immunity, cell signaling and proliferation, neural function, behavior and various aspects of organ physiology (Backhed et al., 2004; Crawford et al., 2009; McFall-Ngai et al., 2013; Velagapudi et al., 2010). Gut microbes can detoxify exogenous and endogenous compounds and alter expression of host metabolic genes that affect glucose and lipid metabolism (den Besten et al., 2013). Given its location, the mucosal microbiota is thought to be more strongly affected by host secretions than is the luminal community (Schluter and Foster, 2012), and to exert a greater influence on the host's immune system and other physiological traits (Van den Abbeele et al., 2011; Wang et al., 2010).

The abundance and composition of the microbiota are affected by several factors including host genetics and immune status, colonization history, physical aspects of the gut microenvironment and host diet (Campbell et al., 2012; Deloris Alexander et al., 2006; Friswell et al., 2010; Ley et al., 2006). Diet plays a dominant role among these factors (Scott et al., 2013; Sonnenburg et al., 2005) in large part because different microbial species are better equipped, through the complement of metabolic enzymes encoded in their genomes, to metabolize different substrates in support of growth (Flint et al., 2012). Dietary shifts driven by season or other environmental perturbations (such as drought or habitat fragmentation) can alter the gut microbiota through changes in taxonomic composition, and/or changes in expression of metabolic genes within the same species. For example, some members of the genus *Bacteroides* can alter their expression of carbohydrate-degrading enzymes depending on the availability of complex plant-derived substrates vs. endogenous host substrates such as mucins (McNulty et al., 2013; Salyers et al., 1977; Sonnenburg et al., 2005).

The microbial contribution to host nutritional physiology may be particularly important in fasted animals (Crawford et al., 2009), or in metabolic states that catabolize large amounts of body protein (Singer, 2003). Under these conditions, microbes can assist by "recycling" endogenous compounds lost in the feces (e.g., mucin and epithelial glycans), or what would normally be waste products (e.g., urea), and thus contribute to host energy balance and survival (Fuller and Reeds, 1998; Singer, 2003). This may benefit animals in environments where food availability varies significantly on a seasonal basis leading to substantial reductions in food intake or to complete cessation of feeding, such as species living in high latitudes, migratory species, and hibernators. Some examples of effects of seasonal environmental rhythms on gut microbiota are described below.

3. Circannual hibernation rhythms

Seasonal hibernators that rely on endogenous fuels during winter typically exhibit circannual cycles of hibernation, reproduction, growth and fattening (Lyman et al., 1982), as exemplified by many ground-dwelling sciurid rodents such as ground squirrels and marmots. Ground squirrels are homeothermic during most of the active season and become heterothermic during the hibernation season, which is characterized by weeks of torpor when animals profoundly decrease body temperature (T_b) and metabolic rate (MR). Torpor is periodically interrupted by interbout arousals of <24 h when animals resume normothermia and high MR. Hibernating squirrels fast for 5–9 months, depending on the species and sex, until arousal in spring. Thus, seasonal hibernators shift from reliance on a mixed diet during the active season to a primarily lipid-based metabolism with no dietary intake during hibernation. The physical environment within the gut lumen, and thus the ecosystem in which gut microbes exist, changes during hibernation. Many microbes (e.g., mesophiles) have limited or no growth at the T_b typical of deep torpor (<10 °C) whereas others are psychrophilic or psychrotolerant and are able to grow at temperatures regularly experienced by torpid animals (-2 to 10 °C) (Morita, 1975). Interbout arousals provide brief periods that return thermal, metabolic, and physiologic conditions to levels similar to the active season, and include conditions in which most gut microbes readily proliferate. Small intestine and, to a lesser extent, hindgut tissues undergo substantial atrophy during hibernation although overall architecture of the mucosa is well maintained (Carey, 1990). Transport of nutrients and electrolytes is depressed during torpor, but increases during interbout arousals to levels similar to or greater than those in active season squirrels (Carey and Sills, 1992). Thus, despite the absence of food intake during the hibernation season, solute transport can still occur in the hibernator gut, allowing absorption and utilization of molecules present in the lumen such as microbially derived SCFA, vitamins and ammonia. Compared with active season squirrels, gut microbes of hibernators have access only to endogenous, host-derived substrates – primarily glycans and proteins found in mucins and sloughed epithelial cells (Johansson et al., 2011). Although enzyme