J Comp Physiol B (2012) 182:591–602
DOI 10.1007/s00360-012-0645-z

REVIEW

Diversity and function of the avian gut microbiota

Kevin D. Kohl

Received: 16 July 2011 / Revised: 30 December 2011 / Accepted: 4 January 2012 / Published online: 14 January 2012
© Springer-Verlag 2012

Abstract The intestinal microbiota have now been shown to largely affect host health through various functional roles in terms of nutrition, immunity, and other physiological systems. However, the majority of these studies have been carried out in mammalian hosts, which differ in their physiological traits from other taxa. For example, birds possess several unique life history traits, such as hatching from eggs, which may alter the interactions with and transmission of intestinal microbes compared to most mammals. This review covers the diversity of microbial taxa hosted by birds. It also discusses how avian microbial communities strongly influence nutrition, immune function, and processing of toxins in avian hosts, in manners similar to and different from mammalian systems. Finally, areas demanding further research are identified, along with descriptions of existing techniques that could be employed to answer these questions.

Keywords Avian hosts · Intestinal microbes · Symbiosis

Introduction

Vertebrate animals maintain complex and intimate associations with a diverse community of microbes residing in their intestinal tracts (Ley et al. 2008b). Previously, it was believed that the main benefit of hosting these microbes was to be able to utilize novel food sources, such as cellulose. However, recent research has revealed that these microbes play a large role in many aspects of an animal's physiology, including proper development of intestinal morphology and digestive function, as well as immune function (Leser and Mølbak 2009). Though the diversity of microbes, as well as their roles and importance in mammalian physiology have been elucidated, the biological significance of intestinal microbes in birds remains largely unknown.

Birds represent interesting study systems in which to investigate the roles of intestinal microbes, because they have extremely complex and unique diets, physiological traits, and developmental strategies. Additionally, the capacity for flight has been a strong selective pressure on many aspects of their physiology, perhaps changing the nature of their intestinal fauna. Many studies on microbial community function have been conducted on domestic bird species, which allow us to infer the biological role of intestinal microbes in wild birds. Taken together, results suggest that intestinal microbes have large effects on the nutrition, immune function, and development of their avian hosts. This review examines and compares microbial relationships between birds and mammals in order to highlight gaps in knowledge and identify experimental questions for the future.

Diversity of avian intestinal microbes

There have been several efforts to characterize the microbial diversity of the avian gut; however, the majority of these studies use selective, culture-based techniques to investigate microbial species of interest, and largely focus on identifying potentially pathogenic microbes (Craven et al. 2000; Gaukler et al. 2009). These techniques are not ideal, especially for the study of mutualistic microbial diversity, as it is estimated that 99% of microbial species

Communicated by H.V. Carey.

K. D. Kohl (✉)
Department of Biology, University of Utah, 257 S. 1400 East, Salt Lake City, UT 84112, USA
e-mail: kevin.kohl@utah.edu

cannot be cultured under laboratory conditions (Rappe and Giovannoni 2003). Fortunately, advances in culture-independent methods have allowed for more complete inventories of the intestinal microbial community.

The ideal method to conduct a microbial inventory is through 16S rDNA sequence analysis, which has only been carried out on the gut contents of eight avian taxa (Table 1). In contrast, a single study conducted inventories on 60 mammalian species (Ley et al. 2008a). For the most part, microbial communities at higher taxonomic levels are very similar between birds and mammals; most studies show 2 phyla, Firmicutes and Bacteroidetes, as dominant out of 75 known microbial phyla. This dominance is not surprising: it is believed that the common ancestor of amniotes (reptiles, birds, and mammals) maintained a microbial community mostly comprising Firmicutes and Bacteroidetes (Costello et al. 2010). At this point, comparing the abundance of microbes across studies is not possible due to the limited number of species, varied sources (feces vs. crop), and different techniques. It would be useful to adopt standardized approaches for future inventories of avian species (see Ley et al. 2008a for an example) to investigate the effects of host diet, taxonomy, and gut anatomy on intestinal microbial communities.

All phyla detected from birds using 16S sequencing have also been identified in mammalian microbial communities, suggesting that birds may not harbor unique microbial phyla. However, a recent study on the hoatzin crop, using DNA microarray techniques, documented for the first time the presence of phyla such as Aquificae, Coprothermobacteria, Thermodesulfobacteria and Caldithrix in a vertebrate gut system (Godoy-Vitorino et al. 2010). These techniques will need to be replicated on mammalian gut contents to determine if these phyla are unique to the hoatzin crop. At lower taxonomic levels, there are genera and species unique to birds. For example, 16S rDNA inventory of the hoatzin revealed that 94% of the phylotypes present represented completely novel microbial species and genera (Godoy-Vitorino et al. 2008). Additionally, researchers have found host-specific species in both the gull (*Larus* spp.) and Canada goose (*Branta canadensis*) (Lu et al. 2008, 2009).

In addition to bacteria, members of the domain Archaea are present in the intestinal communities of many birds. However, not all studies listed in Table 1 investigated the presence of Archaea. Targeted investigations have since shown the presence of Archaea in the hoatzin crop (Wright et al. 2009) and chicken cecum (Saengkerdsub et al. 2007). These isolates are often methanogens, which are important for removing the excess hydrogen ions produced by fermentation. The presence and functional roles of Archaea are often overlooked in basic microbial inventories (Baker et al. 2006), but should be investigated in avian systems.

Interactions between symbiotic microbes and avian physiology

In mammals, gut microbial communities are determined by host taxonomy, diet, and gut anatomy (Ley et al. 2008a), and the functions of these communities can be predicted based on 16S rDNA sequence inventories (Muegge et al. 2011). However, due to variability in gene content between even closely related strains of microbes (Nelson et al. 2010), as well as potential novelty in understudied avian systems, it may be difficult at this stage to assign putative functions to microbial populations based on avian 16S inventories alone. There have been several studies investigating the physiological functions and host interactions of microbes in wild birds and many well-developed experiments using germ-free chickens to investigate impacts on the hosts themselves. Results have elucidated that intestinal microbes play large roles in the nutrition, immune function, and processing of toxins of avian hosts; these results are described in greater detail below.

Nutrition

In many mammals, microbes aid in the host's ability to utilize plant polysaccharides, such as cellulose, as energy sources (Dehority 1997). However, in birds, the presence of fibrolytic microbes depends greatly on gut location and host phylogeny. Like mammals, some birds such as the hoatzin (Grajal et al. 1989) and ostrich (Matsui et al. 2010) maintain large, fibrolytic fermentation chambers. In these species, fermentation end products can supply large proportions of their total energy budgets (75% in the ostrich and 80% in ruminant mammals, compared to only 10–20% in other domestic fowl; Jozefiak et al. 2004). Cellulolytic microbes have also been isolated from the pigeon crop (Shetty et al. 1990), but due to low residence time in this chamber extensive fiber metabolism is not likely.

Microbes residing in the intestines of other bird species, however, are simply saccharolytic rather than cellulolytic, and thus only aid the avian host in utilization of substrates which it could otherwise digest itself (Vispo and Karasov 1997). Amylase activity, which is presumably microbial due to limited activity of salivary amylase in birds (Stevens and Hume 2004), has been detected in the crops of the chicken and turkey (Bolton 1965; Pinchasov and Noy 1994). Additionally, amylolytic, but not cellulolytic, microbes have been isolated from the crop of the green-rumped parrotlet (*Forpus passerinus*) (Pacheco et al. 2004). The ceca of most Galliformes are not thought to be extensive fibrolytic chambers, but do contain saccharolytic bacteria (Vispo and Karasov 1997). These microbes may conduct microbial fermentation of starches and simple sugars, which provides relatively less energy to the host

Table 1 Abundances of microbial taxa from the gut contents of previously studied birds and mammals

Species	Adelie Penguin	Gull	Canada Goose	Ostrich	Turkey	Chicken	Hoatzin	Parrots	Mouse	Cow	Horse
	Pygoscelis adeliae	*Larus spp.*	*Branta canadensis*	*Struthio camelus*	*Meleagris gallopavo*	*Gallus gallus*	*Opisthocomus hoazin*	Various species	*Mus musculus*	*Bos taurus*	*Equus ferus*
Source	Feces	Feces	Feces	Cecum	Cecum	Cecum	Crop	Cloaca	Cecum	Rumen	Hindgut
Microbial phyla	% of Community										
Firmicutes	39.2	54.6	71.6	50.9	32.3	70	66.3	72.9	58.9	22.3	36.8
Bacteroidetes	14.7	1.1	10.1	39.4	54.2	1.9	29.9	0.2	24.1	45.2	47.4
Actinobacteria	32.3	6.4	7.0	–	<0.1	4.9	0.8	12.0	–	–	–
Proteobacteria	9.8	23	10.4	–	3.4	21.5	1.6	14.9	2.6	26.9	–
Tenericutes	3.9	8.9	0.2	–	–	<0.1	0.2	–	13.9	–	–
Fibrobacteres	–	–	–	6.5	–	–	–	–	–	3.6	–
Deferribacteres	–	–	–	–	2.6	–	–	–	–	–	–
Spirochaetes	–	1.1	–	1	–	–	0.2	–	–	0.5	3.5
Fusobacteria	–	0.7	–	–	–	–	–	–	–	–	–
Planctomycetes	–	0.4	–	–	–	–	–	–	–	–	–
Cyanobacteria	–	0.4	–	–	–	–	–	–	–	–	–
Verrucomicrobia	–	–	–	0.3	–	–	<0.1	–	–	–	8.8
Synergistetes	–	–	–	–	–	–	0.2	–	–	–	–
TM7	–	–	0.1	–	–	–	<0.1	–	–	–	–
Lentisphaerae	–	–	–	–	–	–	<0.1	–	–	–	–
Archaea	–	–	–	1.9	–	–	–	–	–	1.5	3.5
Unknown	–	3.4	0.5	–	7.3	1.7	0.6	–	–	–	–
Source	(Banks et al. 2009)	(Lu et al. 2008)	(Lu et al. 2009)	(Matsui et al. 2010)	(Scupham et al. 2008)	(Zhu et al. 2002)	(Godoy-Vitorino et al. 2008)	(Xenoulis et al. 2010)	(Kibe et al. 2004)	(An et al. 2005)	(Yamano et al. 2008)

than hydrolysis by endogenous enzymes (Stevens and Hume 2004), but may still increase absolute energy extraction for the host.

Though present, these cellulolytic and saccharolytic microbes make up just a small proportion of the avian microbial community. Rather, microbes capable of degrading uric acid are much more abundant (Mead 1989). Uric acid is the main product of nitrogen metabolism in birds and can be moved into the lower intestinal tract and ceca through retrograde peristalsis. Here, it can be converted to microbially synthesized amino acids that can be reabsorbed by the host (Vispo and Karasov 1997). The process of uric acid metabolism by microbes is thought to be especially important for conserving nitrogen, especially in species with low protein diets. Indeed, uric acid metabolizing microbes have been isolated from the intestinal tract of the chicken, turkey, guinea fowl, duck, pheasant, and hummingbird (Barnes 1972; Preest et al. 2003). It is especially remarkable that these microbes have been isolated from the hummingbird because hummingbirds lack ceca and have extremely fast digesta throughput (Stevens and Hume 2004), which could make colonization by microbes challenging. However, whether this metabolic capability represents a significant contribution to the nitrogen economy of avian hosts remains to be explored.

Microbes are also known to increase nutrient absorption in mammals (Tennant et al. 1971). For example, gnotobiotic mice colonized with a single species of microbe (*Bacteroides thetaiotaomicron*) exhibited 2.6 times higher intestinal expression of the sodium-glucose transporter protein (SLGT-1) compared to germ-free mice (Hooper et al. 2001). However, colonization of the gut also greatly increases the integrity of the epithelial wall through upregulation of many cross-bridging proteins, presumably to decrease invasion by pathogenic microbes or absorption of endotoxins (Hooper et al. 2001). This epithelial fortification may actually inhibit nutrient absorption in avian species. Due to the selective pressure of flight, birds have decreased intestinal surface area compared to non-flying mammals, and rely more heavily on paracellular absorption, the process by which water-soluble nutrients are transferred between epithelial cells (Caviedes-Vidal et al. 2007). Studies with germ-free chickens have indeed shown that colonization by microbes decreases total absorption of glucose and vitamins (Ford and Coates 1971). The mechanisms of trade-offs between microbial colonization and nutrient absorption in birds remain to be explored.

Immune function

Intestinal microbes are known to greatly influence the cost, development, and effectiveness of mucosal and systemic immune responses in mammalian systems (Macpherson and Harris 2004). This trade-off is well evidenced by the fact that germ-free mammals overall have depressed immune functions. They have decreased cytokine production, systemic immunoglobulin levels, intraepithelial lymphocyte counts, and relative amounts of gut-associated lymphoid tissue (GALT). As a result, these animals are more susceptible to infection (O'Hara and Shanahan 2006). Interactions between intestinal microbes and the immune systems of avian hosts are often assumed to be similar to mammals, yet they have been largely undescribed (Brisbin et al. 2008). The relative lack of information on microbe–host immunity interactions is especially remarkable given that birds have many unique and interesting characteristics of their immune systems.

One unique aspect of the avian immune system is the bursa of Fabricius, the primary site of B cell development. Mammalian B cells develop in the bone marrow, far removed from the intestinal tract and commensal microbes. While sampling and transport of microbial antigens from the intestinal lumen have been shown in mammalian systems (Owen et al. 1986), the bursa of Fabricius is a diverticulum of the intestinal tract itself and is known to be colonized by microbes shortly after hatching (Kimura et al. 1986). These microbes may act as antigens themselves or induce production of cytokines, increasing the proliferation and maturation of bursal B cells (Ratcliffe 2006). When the bursal duct is experimentally ligated prior to hatching, chickens exhibit lower natural antibody production, suggesting that gut microbes can have systemic effects on immunity through this structure (Ekino et al. 1985). Likewise, infusion of killed bacterial antigens into the ligated bursal lumen recovered natural antibody production to greater than that of control chickens (Ekino et al. 1985). Further research must be done to determine if gut microbes play an increased role in B cell development in birds compared to mammals due to their intimate association with the bursa of Fabricius.

Birds also differ from mammals in several aspects of cell-mediated immunity. For example, birds have fewer gene families of the T-cell receptor gene (Lahti et al. 1991), which may influence the diversity of peptides recognized by T cells (Mwangi et al. 2010). Through normal development, the repertoire of T-cell receptors (TCR) shifts from polyclonal (recognizing many antigens) to oligoclonal (recognizing only a few antigens). This shift is thought to occur through the deletion of T cells that are reactive to food or commensal microbes so as to avoid costly or detrimental immune responses (Probert et al. 2007). However, this process depends on the presence of intestinal microbes, since germ-free rats maintain a polyclonal TCR repertoire, while those inoculated with microbes shift to an oligoclonal repertoire (Helgeland et al. 2004). In mammals, the shift from polyclonal to

oligoclonal could presumably take place during any life stage, as evidenced by the dominance of oral tolerance (uninducibility of the intestinal adaptive immune system by oral antigens) (Friedman 2008). Birds, on the other hand, have a very confined period of developmental oral tolerance, usually only about a week post-hatch (Friedman 2008). Germ-free chickens develop and maintain a polyclonal repertoire early in life (Mwangi et al. 2010). Thus, delayed colonization of microbes may permanently alter the TCR repertoire, causing costly and detrimental immune responses to harmless microbes later on (Probert et al. 2007). Interestingly, the complexity of the microbial community also greatly influences TCR repertoires, suggesting that variation in microbial species colonizing the chicken gut may greatly influence epithelial and systemic immune responses (Mwangi et al. 2010). In mammals, weaning affects the TCR repertoire, presumably through alterations of the microbial community (Probert et al. 2007). Chickens have a constant diet through development, and so it might be informative to investigate TCR patterns in avian species that undergo natural diet shifts during development.

Other components of the gut environment, sometimes considered innate immune defenses, also differ between mammals and birds and can have profound effects on the interactions between commensal microbes and the host. For example, mucins are glycosylated proteins produced by the intestinal tissues that serve as lubricants and protectants of the intestinal epithelium (Johansson et al. 2011). Mucins also provide nutrition and locations for adherence for commensal microbes (Deplancke and Gaskins 2001). These molecules vary in structure between birds and mammals (Verma et al. 1994), resulting in different host–microbe interactions. For instance, chicken mucins, but not human mucins, are able to mitigate the virulent properties of *Campylobacter jejuni*, causing it to assume a commensal role in avian tissues (Byrne et al. 2007). Additionally, it is believed that glycans, oligosaccharides produced by epithelial tissue, regulate microbial communities depending on their diversity and structure (Hooper and Gordon 2001). The presence of certain glycan structures vary between bird species (Ellström et al. 2009), and the avian fucosyltransferase gene important in determining glycan structure has only 50% sequence homology to mammals (Lee et al. 1996). Moreover, avian hosts produce novel defensins, a type of antimicrobial peptide, compared to mammals (Lynn et al. 2004). Allelic variants of avian defensin genes that differ in only several amino acids show functional differences in antimicrobial activities (Hellgren et al. 2010), and so larger differences in sequences between mammals and birds may correspond to functional differences. Together, these differences may regulate gut microbial diversity depending on specificity of their microbial targets and may result in colonization by novel microbes compared to mammals.

Detoxification

Birds consuming plants or invertebrates often ingest secondary metabolites that may act as toxins when absorbed (Karasov and Martinez del Rio 2007). Metabolizing these compounds is energetically expensive, and so it has been proposed that hosts may house detoxifying microbes to save energy (Dearing et al. 2005). Bacteria that degrade saponins have been found in the crop of the hoatzin (Garcia-Amado et al. 2007), and the microbial community of the chicken intestine has been found to metabolize several mycotoxins (Young et al. 2007). However, some microbes also express enzymes that make plant toxins more toxic to the host. For example, many microbes are able to cleave glycosides and glucosinolates, releasing a toxic compound (Hur et al. 2000). These toxic compounds then become more easily absorbed by the host, as shown by an experiment where control chickens absorbed significantly more glucosinolates compared to cecectomized chickens (Slominski et al. 1988). However, there has not been enough research to determine the role of avian commensal microbes in detoxification, or whether microbes play a role in diet diversification over evolutionary time (Dearing et al. 2005).

Additive effects

Microbes in the avian intestinal tract affect nutrition, immunology, and detoxification. However, bacteria seem to have both positive and negative effects in each of these areas, such as liberating nutrients yet decreasing absorption, inducing helpful yet energetically costly immune responses, and either reducing or increasing the toxicity of dietary toxins. Hence, studies with increased host taxonomic diversity must be conducted to elucidate the trade-offs involved in microbial colonization of birds, and specifically which functional roles are most important in terms of individual fitness.

Body temperature

Temperature can influence microbial communities due to differential growth rates and tolerances between microbial species (Mohr and Krawiec 1980). Birds maintain a higher body temperature compared to mammals (Clarke and Rothery 2008). Body temperature also varies between higher groups of birds; ratites show low body temperatures and passerines exhibit some of the highest (Clarke and Rothery 2008). It is likely that the higher body temperature

of birds selects for or inhibits the growth of certain microbial species. This notion is supported by the fact that *Borrelia garinii*, a Lyme's disease-causing agent hosted primarily by birds, is able to grow at higher temperatures compared to mammalian-hosted *Borrelia* species (Hubálek et al. 1998). This is thought to be an adaptation of the microbe to its avian host (Comstedt et al. 2011). However, studies have not yet investigated the role of body temperature in determining gastrointestinal microbial communities.

Variability in intestinal microbes in birds: a potential mechanism for developmental and phenotypic plasticity

Although they are illustrative experimental systems, germ-free animals do not occur in the natural world. Rather, there might be variation in the types and abundances of microbes that colonize individuals of a given host species. In mammals, the gut microbial community is 'inherited' from the mother through contact with fecal and vaginal microbes during the birthing process (Palmer et al. 2007). The importance of this one-time exposure is highlighted by differences in the microbial community structure of conventionally and cesarean-delivered humans from infancy up to at least 7 years of age (Dominguez-Bello et al. 2010; Salminen et al. 2004). Birds however, hatch from eggs, which are presumed to be internally sterile (van der Wielen et al. 2002), and so they may have many different potential sources of microbes. Microbial communities that inhabit eggshells may serve as sources of inoculum and can be modified by parental nesting behavior (Cook et al. 2005; Peralta-Sánchez et al. 2010; Ruiz-De-Castañeda et al. 2011). Additionally, vertical transmission may occur in birds that are fed via regurgitation, in which receiving transferred microbes from their parents is necessary for survival (Godoy-Vitorino et al. 2010; Kyle and Kyle 1993). Juvenile ostriches have been known to engage in consumption of adult feces, which may also aid microbial colonization (Cooper 2004). Yet chickens and turkeys are able to develop normal adult microbiota when hatched completely separately from adults and so they must obtain microorganisms from their surrounding environment (Lu et al. 2003; Scupham 2007). Thus, avian hosts may experience increased variation in the diversity and abundances of microbes that colonize their intestinal tract. This variation may have life-long effects on the phenotype of the host, mediated through altered microbial roles in host physiology discussed above.

With contrasting routes of colonization, the developmental succession of the intestinal microbiota of birds and mammals is also expected to differ. In most mammals and birds, the intestinal microbiota slowly transition to a stable adult-like community. In mammals, large changes in the microbial community structure are observed at points of weaning and transition to solid food (Palmer et al. 2007). However, in developing chickens and turkeys fed a constant diet, large shifts in microbial diversity still occur, presumably due to development of the intestinal environment (Lu et al. 2003; Scupham 2007). Interestingly, the crops of juvenile hoatzins fed by regurgitation have a microbial community with higher diversity compared to chicks and adults (Godoy-Vitorino et al. 2010), suggesting that successional profiles may also differ based on developmental or feeding strategies.

Development of a normal microbial community may be widely influenced by both genetics and environmental variation. For example, in Adelie penguins (*Pygoscelis adelie*), microbial community similarity is negatively correlated with both host genetic distance and geographic distance (Banks et al. 2009). Furthermore, a cross-foster experiment between great tits (*Parus major*) and blue tits (*P. caeruleus*) revealed that the environmental factors associated with a shared nest were more important than host species in determining microbial community structure (Lucas and Heeb 2005). However, at some point avian host genetic differences can have larger effects on the microbiota. For example, nestlings of a brood parasite, great spotted cuckoos (*Clamator glandarius*), and their host, magpies (*Pica pica*), sharing the same nest and parents have drastically different microbial communities (Ruiz-Rodríguez et al. 2009a). In mammals, host phylogeny seems to determine the intestinal microbial community more than environmental factors, as shown by similar microbial communities within mammalian host species housed in different zoos or even different continents (Ley et al. 2008a).

Environmental variation in the intestinal microbial community may have long-term effects on the developing avian and mammalian hosts. For example, artificial environmental variation in mammalian microbes was conducted by colonizing germ-free rabbits with mouse cecal microbes. These mouse-colonized rabbits have decreased body mass, lower digestibility, and are more susceptible to disease compared to those colonized with rabbit cecal microbes (Boot et al. 1985). However, in mammals, the strong vertical transmission of microbes reduces natural variation in community composition. Thus, in birds, natural selection may act on the parental behaviors which inoculate nestlings with optimal intestinal microbes (Soler et al. 2010). Abundances of certain intestinal microbes in nestlings have been correlated with several host phenotypic conditions such as wing asymmetry (Mills et al. 1999), nestling size (Moreno et al. 2003), body condition, and immune responses (Ruiz-Rodríguez et al. 2009b),

suggesting a significant role of specific intestinal microbes in avian development. In mammals, exposure to microbes early on can have developmentally plastic (i.e., irreversible) effects on phenotypic parameters such as immune and stress responses (Boissé et al. 2004; Shanks et al. 2000). These parameters have also been shown to be developmentally plastic in birds (Love and Williams 2008; Pitala et al. 2007), but the role of microbes in their development remains unclear.

Additionally, birds may experience variation in intestinal microbes as adults. Adult passerine birds show differences in their microbial communities due to geographic location, diet, and season (Klomp et al. 2008; Maul et al. 2005). These differences may simply be due to shifts in relative abundances of microbial species, and not necessarily inoculation of new microbes. However, as the cloaca serves as both an excretory, as well as copulatory organ, microbes can be transmitted between mates during sexual contact (White et al. 2010). Inoculation of new microbial species may cause host phenotypic variation through altered microbial roles in nutrition or immunity, and thus females may select for males with certain microbial assemblages (White et al. 2010). Though, in order for sexually transmitted microbes to colonize the adult avian intestinal tract, an immune response must be avoided. Developing oral tolerance is the most common way to avoid an immune response to new microbes, but this period, measured in chickens, is only a week in duration (Friedman 2008). Introduction of new microbes and their avoidance of an immune response in passerine birds clearly demands future study.

Future directions

16S rDNA sequence inventories

Many studies on the intestinal microbial communities of avian hosts use methods that underrepresent diversity. The majority of studies presented in this review used culture-based or molecular fingerprint techniques to correlate microbial diversity with various phenotypic traits. However, both of these techniques are known to underestimate microbial abundance and diversity. Culture-based experiments usually focus on microbial taxa of interest and cannot detect the 99% of microbes estimated to be unculturable. Molecular fingerprinting methods such as denaturing gradient gel electrophoresis (DGGE) and automated ribosomal intergenic spacer analysis (ARISA) make use of differences in sequence integrity or length between microbial taxa to create molecular banding patterns, which can be used to characterize a community. However, similarities in sequence integrity or length between members of disparate microbial taxa often result in large underestimates of diversity (Fisher and Triplett 1999; Smalia et al. 2007).

In order to more finely detect differences in microbial communities, investigators should rely on molecular cloning and sequencing of the 16S rDNA gene. The 16S rDNA sequence has a slow rate of change, is rarely transferred between microbial species, and is of sufficient size for bioinformatic and phylogenetic analysis, making sequence analysis a robust method for the characterization of microbial diversity (Head et al. 1998). To date, 16S rDNA inventories have only been conducted on 10 avian species (Table 1). There have been many microbial inventories of mammalian intestinal microbes, including one study alone that inventoried 60 mammalian species (Ley et al. 2008a). Additionally, advances in pyrosequencing now allow for large-scale inventories at relatively low costs (Dowd et al. 2008), but with increased error rates and shorter sequence lengths. Due to the potential novelty of avian microbes, researchers should also continue to create full-length, robust sequence libraries via Sanger sequencing.

Meta-"omics"

Due to the wide variation in genome content between even closely related strains of microbes (Nelson et al. 2010), it is difficult to assign putative functions to microbial populations based on 16S sequence inventories alone. To better deduce microbial functions, researchers now use metagenomic sequencing. This technique employs high-throughput, non-specific microbial DNA sequencing to inventory the many genes present in an environmental sample, and allows researchers to compare microbial functional diversity rather than solely taxonomic diversity. Recovered gene sequences can be assigned to functional categories (carbohydrate metabolism, membrane transport, xenobiotic metabolism, etc.), and the representation of different functional categories can be directly compared between samples. To date, metagenomic sequencing has only been conducted on the domesticated chicken (Qu et al. 2008).

Metagenomic sequences represent only the 'potential' of a microbial community and not necessarily the actual function. To monitor what genes are expressed, researchers now utilize metatranscriptomics and metaproteomics. Metatranscriptomics utilizes similar technology as metagenomics, but instead sequences microbial mRNA transcripts (via cDNA). However, differences in transcript abundances could be due to changes in either gene expression or different levels of microbial representation. To circumvent this problem, researchers must conduct a parallel metagenome to normalize abundances with transcript:gene ratios. Metaproteomics compares protein

abundances between environmental samples by separating proteins and sequencing those that have differential representation. However, due to limitations in technology, metaproteomics often focuses only on these differentially represented proteins (Wilmes and Bond 2006), making it useful for comparative studies, but not for gaining insight into the function of a whole microbial community.

There has also been interest in developing meta-metabolomics, the inventory of small molecule networks existing in an environmental sample (Turnbaugh and Gordon 2008). This technique involves identification of metabolites using nuclear magnetic resonance (NMR) or mass spectrometry (MS). In terms of microbe–host interactions, it is perhaps most relevant to investigate the host metabolome, because these profiles affect host physiology. For example, it has been found that colonization by microbes strongly influences the metabolomic profile of mammalian blood (Wikoff et al. 2009), but this has not yet been studied in avian species.

Physiological performance

Meta-"omics" approaches are often conducted to understand the staggering complexity of intestinal microbial communities. However, these metrics alone only allow insight into the potential metabolic capabilities of the microbiota. Therefore, investigators should continue to pursue other experimental methods to determine the functions of avian microbial communities and their roles in physiological performance.

Removal of microbial communities by antibiotic treatment, followed by nutrient supplementation has been used extensively to understand the function of microbial symbionts in insects. For example, microbe-free aphids show reduced growth and survival on diets lacking certain essential amino acids, while aphids with symbionts are less affected (Dadd and Krieger 1968; Mittler 1971). Likewise, bedbugs treated with antibiotics show decreased growth and survival, but these effects are reduced when diets are supplemented with B vitamins (Hosokawa et al. 2010). Microbial colonization is thought to be necessary for the growth and survival of passerine birds (Kyle and Kyle 1993), but nutrient supplementation of young birds hatched in sterile environments might reveal specific nutritional roles of the avian microbiota. In addition, there are bird species (e.g., hummingbirds, sunbirds) that feed on nutritionally incomplete diets. Insects feeding on such diets possess vertically transmitted microbes that are critical to their survival (Mittler 1971). It is possible that birds too have such obligate symbionts.

The use of germ-free organisms has also furthered the understanding of how microbial communities influence host physiology. Comparisons of germ-free and conventionally raised birds have been used to investigate numerous physiological processes (discussed above), but not all potential processes have been studied (stress response, behavior, etc.). Additionally, gene expression patterns in the intestines (Hooper et al. 2001) and livers (Claus et al. 2011) vary between conventionally raised and germ-free mice. However, similar gene expression studies using germ-free and conventional birds have not been conducted.

Stable and radioactive isotopes are additional tools for tracking compounds or nutrients of interest in microbial communities. One method for this technique is to expose complex microbial communities to labeled substrates. Microbes that are able to utilize the substrates of interest incorporate the labeled atoms into their DNA, and this 'heavy', labeled microbial DNA can easily be separated from other DNA by density gradient centrifugation. Isolated DNA can then be functionally or taxonomically characterized by sequence analysis (Radajewski et al. 2000). Labeled compounds have also been used to investigate the rates of oxidation of various nutritional compounds in birds (McCue et al. 2010). Comparing the fates and oxidation rates of labeled nutrients, toxins, and other compounds between conventional and germ-free birds would help to elucidate what role microbes play in the routing of nutrients and toxins.

Integrative approaches and utilizing host taxonomic diversity

Simultaneously conducting 16S inventories, meta-"omics" and physiological performance assays synergistically advances the knowledge gained in a single experiment. For example, correlating the abundance of a microbial species (based on 16S inventories) with host gene expressions or physiological assays can lead to hypotheses of how abundances of certain microbes influence host physiology (Claus et al. 2011).

Additionally, future research should embrace the diverse physiological strategies of avian hosts. Much of what we know about interactions between microbes and avian hosts are derived from studies on domesticated chickens. However, dietary strategies and gut anatomies vary widely between avian taxa. Pigeons (Columbiformes), parrots (Psittaciformes) and many fowl (Galliformes) are all granivorous, yet only Galliformes maintain cecal chambers that house microbes (DeGolier et al. 1999). Similarly, eagles, hawks, and falcons (Falconiformes) and owls (Strigiformes) share similar carnivorous diets, yet only owls maintain ceca (DeGolier et al. 1999). Comparative approaches between taxa will illustrate how dietary strategy, gut anatomy, as well as how variation in microbial communities might influence host–microbe relationships in avian taxa.

Summary

Our knowledge of the role of intestinal microbes in avian hosts lags far behind our understanding of mammalian systems. Studies that have been done, mostly in chickens, show that intestinal microbes play large roles in terms of host nutrition, immunity, and development. Researchers should now embrace host phylogenetic diversity, as well as quickly advancing methods to study avian intestinal microbial ecology. With a broader knowledge of avian hosts, we may be able to compare and contrast solutions of terrestrial vertebrate hosts to various physiological challenges, perhaps gaining insight into the evolution of the intestinal microbiota in a broader sense.

Acknowledgments I would like to thank M. Denise Dearing, Hannah Carey, and the two anonymous reviewers for comments that helped to improve this manuscript. This work was supported by a National Science Foundation Graduate Research Fellowship.

References

An D, Dong X, Dong Z (2005) Prokaryote diversity in the rumen of yak (*Bos grunniens*) and Jinnan cattle (*Bos taurus*) estimated by 16S rDNA homology analyses. Anaerobe 11:207–215

Baker BJ, Tyson GW, Webb RI, Flanagan J, Hugenholtz P, Allen EE, Banfield JF (2006) Lineages of acidophilic archaea revealed by community genomic analysis. Science 22:1933–1935

Banks JC, Cary SC, Hogg ID (2009) The phylogeography of Adelie penguin faecal flora. Environ Microbiol 11:577–588

Barnes EM (1972) The avian intestinal flora with particular reference to the possible ecological significance of the cecal anaerobic bacteria. Am J Clin Nutr 25:1475–1479

Boissé L, Mouihate A, Ellis S, Pittman QJ (2004) Long-term alterations in neuroimmune responses after neonatal exposure to lipopolysaccharide. J Neurosci 24:4928–4934

Bolton W (1965) Digestion in the crop of the fowl. Br Poult Sci 6:97–102

Boot R, Koopman JP, Kruijt BC, Lammers RM, Kennis HM, Lankhorst A, Mullink JWMA, Stadhouders AM, de Boer D, Welling GW, Hectors MPC (1985) The 'normalization' of germ-free rabbits with host-specific caecal flora. Lab Anim 19:344–352

Brisbin JT, Gong J, Sharif S (2008) Interactions between commensal bacteria and the gut-associated immune system of the chicken. Anim Health Res Rev 9:101–110

Byrne CM, Clyne M, Bourke B (2007) *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. Microbiol 153:561–569

Caviedes-Vidal E, McWhorter TJ, Lavin SR, Chediack JG, Tracy CR, Karasov WH (2007) The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. Proc Natl Acad Sci 104:19132–19137

Clarke A, Rothery P (2008) Scaling of body temperature in mammals and birds. Funct Ecol 22:58–67

Claus SP, Ellero SL, Berger B, Krause L, Bruttin A, Molina J, Paris A, Want EJ, de Waziers I, Cloarec O, Richards SE, Wang Y, Dumas M-E, Ross A, Rezzi S, Kochhar S, Van Bladeren P, Lindon JC, Holmes E, Nicholson JK (2011) Colonization-induced host-gut microbial metabolic interaction. MBio 2:e00271–e00310

Comstedt P, Jakobsson T, Bergström S (2011) Global ecology and epidemiology of *Borrelia garinii* spirochetes. Infect Ecol Epidemiol 1:9545

Cook MI, Beissinger SR, Toranzos GA, Arendt WJ (2005) Incubation reduces microbial growth on eggshells and the opportunity for trans-shell infection. Ecol Lett 8:532–537

Cooper RG (2004) Ostrich (*Struthio camelus*) chick and grower nutrition. Anim Sci J 75:487–490

Costello EK, Gordon JI, Secor SM, Knight R (2010) Postprandial remodeling of the gut microbiota in Burmese pythons. ISME J 11:1375–1385

Craven SE, Stern NJ, Line E, Bailey JS, Cox NA, Fedorka-Cray P (2000) Determination of the incidence of *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* in wild birds near broiler chicken houses by sampling intestinal droppings. Avian Dis 44:715–720

Dadd RH, Krieger DL (1968) Dietary requirements of the aphid *Myzus persicae*. J Insect Phsiol 14:741–764

Dearing MD, Foley WJ, McLean S (2005) The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Ann Rev Ecol Evol Syst 36:169–185

DeGolier TF, Mahoney SA, Duke GE (1999) Relationships of avian cecal lengths to food habits, taxonomic position, and intestinal lengths. Condor 101:622–634

Dehority BA (1997) Foregut fermentation. In: Mackie RI, White BA (eds) Gastrointestinal microbiology. Chapman and Hall, New York

Deplancke B, Gaskins HR (2001) Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. Am J Clin Nutr 73:1131S–1141S

Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci 107:11971–11975

Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeehan T, Hagevoort RG, Edrington TS (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol 8:125

Ekino S, Suginohara K, Urano T, Fujii H, Matsuno K, Kotani M (1985) The bursa of Fabricius: a trapping site for environmental antigens. Immunology 55:405–410

Ellström P, Jourdain E, Gunnarsson O, Waldenström J, Olsen B (2009) The "human influenza receptor" Neu5Acα2,6Gal is expressed among different taxa of wild birds. Arch Virol 154:1533–1537

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microbiol 65:4630–4636

Ford DJ, Coates ME (1971) Absorption of glucose and vitamins of the B complex by germ-free and conventional chicks. Proc Nutr Soc 30:10A–11A

Friedman A (2008) Oral tolerance in birds and mammals: digestive tract development determines the strategy. J Appl Poult Res 17:168–173

Garcia-Amado MA, Michelangeli F, Gueneau P, Perez ME, Dominguez-Bello MG (2007) Bacterial detoxification of saponins in the crop of the avian foregut fermenter *Opisthocomus hoazin*. J Anim Feed Sci 16:82–85

Gaukler SM, Linz GM, Sherwood JS, Dyer NW, Bleier WJ, Wannemuehler YM, Nolan LK, Logue CM (2009) *Escherichia coli*, *Salmonella*, and *Mycobacterium avium* subsp. *paratuberculosis* in wild European Starlings at a Kansas cattle feedlot. Avian Dis 53:544–551

Godoy-Vitorino F, Ley RE, Gao Z, Pei Z, Ortiz-Zuazaga H, Pericchi LR, Garcia-Amado MA, Michelangeli F, Blaser MJ, Gordon JI, Dominguez-Bello MG (2008) Bacterial community in the crop of the hoatzin, a neotropical folivorous flying bird. Appl Environ Microbiol 74:5905–5912

Godoy-Vitorino F, Goldfarb KC, Brodie EL, Garcia-Amado MA, Michelangeli F, Dominguez-Bello MG (2010) Developmental microbial ecology of the crop of the folivorous hoatzin. ISME J 4:611–620

Grajal A, Strahl SD, Parra R, Dominguez MG, Neher A (1989) Foregut fermentation in the hoatzin, a neotropical leaf-eating bird. Science 245:1236–1238

Head IM, Saunders JR, Pickup RW (1998) Microbial evolution, diversity, and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. Microb Ecol 35:1–21

Helgeland L, Dissen E, Dai KZ, Midtvedt T, Brandtzaeg P, Vaage JT (2004) Microbial colonization induces oligoclonal expansions of intraepithelial CD8 T cells in the gut. Eur J Immunol 34:3389–3400

Hellgren O, Sheldon BC, Buckling A (2010) In vitro tests of natural allelic variation of innate immune genes (avian β-defensins) reveal functional differences in microbial inhibition. J Evol Biol 23:2726–2730

Hooper LV, Gordon JI (2001) Glycans as legislators of host–microbial interactions: spanning the spectrum from symbiosis to pathogenicity. Glycobiology 11:1R–10R

Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI (2001) Molecular analysis of commensal host–microbial relationships in the intestine. Science 291:881–884

Hosokawa T, Koga R, Kikuchi Y, Meng X-Y, Fukatsu T (2010) *Wolbachia* as a bacteriocyte-associated nutritional mutualist. Proc Natl Acad Sci 107:769–774

Hubálek Z, Halouzka J, Heroldová M (1998) Growth temperature ranges of *Borrelia burgdorferi* sensu *lato* strains. J Med Microbiol 47:929–932

Hur HG, Lay JO Jr, Beger RD, Freeman JP, Rafii F (2000) Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genistin. Arch Microbiol 174:422–428

Johansson MEV, Ambort D, Pelaseyed T, Schütte A, Gustafsson JK, Ermund A, Subramani DB, Holmén-Larsson JM, Thomsson KA, Bergström JH, van der Post S, Rodriguez-Piñeiro AM, Sjövall H, Bäckström M, Hansson GC (2011) Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci 68:3635–3641

Jozefiak D, Rutkowski A, Martin SA (2004) Carbohydrate fermentation in the avian ceca: a review. Anim Feed Sci Technol 113:1–15

Karasov WH, Martinez del Rio C (2007) Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, Princeton

Kibe R, Sakamoto M, Hayashi H, Yokota H, Benno Y (2004) Maturation of the murine cecal microbiota as revealed by terminal restriction fragment length polymorphism and 16S rRNA gene clone libraries. FEMS Microbiol Lett 235:139–146

Kimura N, Yoshikane M, Kobayashi A (1986) Microflora of the bursa of Fabricius of chickens. Poult Sci 65:1801–1807

Klomp JE, Murphy MT, Bartos Smith S, McKay JE, Ferrera I, Reysenbach A-L (2008) Cloacal microbial communities of female spotted towhees *Pipilo maculatus*: microgeographic variation and individual sources of variability. J Avian Biol 39:530–538

Kyle PD, Kyle GZ (1993) An evaluation of the role of microbial flora in the salivary transfer technique for hand-rearing Chimney Swifts. Wildlife Rehabil 8:65–71

Lahti JM, Chen CL, Tjoelker LW, Pickel JM, Schat KA, Calnek BW, Thompson CB, Cooper MD (1991) Two distinct alpha beta T-cell lineages can be distinguished by the differential usage of T-cell receptor V beta gene segments. Proc Natl Acad Sci 88:10956–10960

Lee KP, Carlson LM, Woodcock JB, Ramachandra N, Schultz TL, Davis TA, Lowe JB, Thompson CB, Larsen RD (1996) Molecular cloning and characterization of *CFT1*, a developmentally regulated avian α(1,3)-fucosyltransferase gene. J Biol Chem 271:32960–32967

Leser TD, Mølbak L (2009) Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host. Environ Microbiol 11:2194–2206

Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Bircher JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JI (2008a) Evolution of mammals and their gut microbes. Science 320:1647–1651

Ley RE, Lozupone CA, Hamady M, Knight R, Gordon JI (2008b) Worlds within worlds: evolution of the vertebrate gut microbiota. Nature Rev Microbiol 6:776–788

Love OP, Williams TD (2008) Plasticity in the adrenalcortical response of a free-living vertebrate: the role of pre- and postnatal developmental stress. Horm Behav 54:496–505

Lu J, Idris U, Harmon B, Hofacre C, Maurer JJ, Lee MD (2003) Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl Environ Microbiol 69:6816–6824

Lu J, Santo Domingo JW, Lamendella R, Edge TA, Hill S (2008) Phylogenetic diversity and molecular detection of bacteria in gull feces. Appl Environ Microbiol 74:3969–3976

Lu J, Santo Domingo JW, Hill S, Edge TA (2009) Microbial diversity and host-specific sequences of Canada goose feces. Appl Environ Microbiol 75:5919–5926

Lucas FS, Heeb P (2005) Environmental factors shape cloacal bacterial assemblages in great tit *Parus major* and blue tit *P. caerulus* nestlings. J Avian Biol 36:510–516

Lynn DJ, Higgs R, Gaines S, Tierney J, James T, Lloyd AT, Fares MA, Mulcahy G, O'Farrelly C (2004) Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 56:170–177

Macpherson AJ, Harris NL (2004) Interactions between commensal intestinal bacteria and the immune system. Nature Rev Immunol 4:478–485

Matsui H, Kato Y, Chikaraishi T, Moritani M, Ban-Tokuda T, Wakita M (2010) Microbial diversity in ostrich ceca as revealed by 16S ribosomal RNA gene clone library and detection of novel *Fibrobacter* species. Anaerobe 16:83–93

Maul JD, Gandhi JP, Farris JL (2005) Community-level physiological profiles of cloacal microbes in songbirds (Order: Passeriformes): variation due to host species, host diet, and habitat. Microb Ecol 50:19–28

McCue MD, Sivan O, McWilliams SR, Pinshow B (2010) Tracking the oxidative kinetics of carbohydrates, amino acids, and fatty acids in the house sparrow using exhaled $^{13}CO_2$. J Exp Biol 213:782–789

Mead GC (1989) Microbes of the avian cecum: types present and substrates utilized. J Exp Biol 3(Suppl):48–54

Mills TK, Lombardo MP, Thorpe PA (1999) Microbial colonization of the cloacae of nestling tree swallows. Auk 116:947–956

Mittler TE (1971) Dietary amino acid requirement of the aphid *Myzus persicae* affected by antibiotic uptake. J Nutr 101:1023–1028

Mohr PW, Krawiec S (1980) Temperature characteristics and Arrhenius plots for nominal psychrophiles, mesophiles and thermophiles. Microbiology 121:311–317

Moreno J, Briones V, Merino S, Ballesteros C, Sanz JJ, Tomás G (2003) Beneficial effects of cloacal bacteria on growth and fledging size in nestling pied flycatchers (*Ficedula hypoleuca*) in Spain. Auk 120:784–790

Muegge BD, Kuczynski J, Knights D, Clemente JC, González A, Fontana L, Henrissat B, Knight R, Gordon JI (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332:970–974

Mwangi WN, Beal RK, Powers C, Wu X, Humphrey T, Watson M, Bailey M, Friedman A, Smith AL (2010) Regional and global changes in TCR$\alpha\beta$ T cell repertoires in the gut are dependent upon the complexity of the enteric microflora. Dev Comp Immunol 34:406–417

Nelson KE, Weinstock GM, Highlander SK, Worley KC, Creasy HH, Wortman JR, Rusch DB, Mitreva MES, Chinwalla AT, Feldgarden M, Gevers D, Haas BJ, Madupu R, Ward DV, Birren BW (2010) A catalog of reference genomes from the human microbiome. Science 328:994–999

O'Hara AM, Shanahan F (2006) The gut flora as a forgotten organ. EMBO Rep 7:688–693

Owen RL, Pierce NF, Apple RT, Cray WC Jr (1986) M cell transport of *Vibrio cholerae* from the intestinal lumen into Peyer's Patches: a mechanism for antigen sampling and for microbial transepithelial migration. J Infect Dis 153:1108–1118

Pacheco MA, Garcia-Amado MA, Bosque C, Dominguez-Bello MG (2004) Bacteria in the crop of the seed-eating green-rumped parrotlet. Condor 106:139–143

Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO (2007) Development of the human infant intestinal microbiota. PLoS Biol 5:1556–1573

Peralta-Sánchez JM, Møller AP, Martin-Platero AM, Soler JJ (2010) Number and colour composition of nest lining feathers predict eggshell bacterial community in barn swallow nests: an experimental study. Funct Ecol 24:426–433

Pinchasov Y, Noy Y (1994) Early postnatal amylolysis in the gastrointestinal tract of turkey poults *Meleagris gallopavo*. Comp Biochem Physiol A 107:221–226

Pitala N, Gustafsson L, Sendecka J, Brommer JE (2007) Nestling immune response to phytohaemagglutinin is not heritable in collared flycatchers. Biol Lett 3:418–421

Preest MR, Folk DG, Beuchat CA (2003) Decomposition of nitrogenous compounds by intestinal bacteria in hummingbirds. Auk 4:1091–1101

Probert CSJ, Williams AM, Stepankova R, Tlaskalova-Hogenova H, Phillips A, Bland PW (2007) The effect of weaning on the clonality of $\alpha\beta$ T-cell receptor T cells in the intestine of GF and SPF mice. Dev Comp Immunol 31:606–617

Qu A, Brulc JM, Wilson MK, Law BF, Theoret JR, Joens LA, Konkel ME, Angly F, Dinsdale EA, Edwards RA, Nelson KE, White BA (2008) Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. PLoS One 3:e2945

Radajewski S, Ineson P, Parekh NR, Murrell JC (2000) Stable-isotope probing as a tool in microbial ecology. Nature 403:646–649

Rappe MS, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394

Ratcliffe MJH (2006) Antibodies, immunoglobulin genes and the bursa of Fabricius in chicken B cell development. Dev Comp Immunol 30:101–118

Ruiz-De-Castañeda R, Vela AI, Lobato E, Briones V, Moreno J (2011) Bacterial loads on eggshells of the pied flycatcher: environmental and maternal factors. Condor 113:200–208

Ruiz-Rodríguez M, Lucas FS, Heeb P, Soler JJ (2009a) Differences in intestinal microbiota between avian brood parasites and their hosts. Biol J Linn Soc 96:406–414

Ruiz-Rodríguez M, Soler JJ, Lucas FS, Heeb P, Palacios MJ, Martín-Gálvez D, de Neve L, Pérez-Contreras T, Martínez JG, Soler M (2009b) Bacterial diversity at the cloaca relates to an immune response in magpie *Pica pica* and to body condition of great spotted cuckoo *Clamator glandarius* nestlings. J Avian Biol 40:42–48

Saengkerdsub S, Anderson RC, Wilkinson HH, Kim W, Nisbet DJ, Ricke SC (2007) Identification and quantification of methanogenic archaea in adult chicken ceca. Appl Environ Microbiol 73:353–356

Salminen S, Gibson GR, McCartney AL, Isolauri E (2004) Influence of mode of delivery on gut microbiota composition in 7 year old children. Gut 53:1388–1389

Scupham AJ (2007) Succession in the intestinal microbiota of preadolescent turkeys. FEMS Microbiol Ecol 60:136–147

Scupham AJ, Patton TG, Bent E, Bayles DO (2008) Comparison of the cecal microbiota of domestic and wild turkeys. Microb Ecol 56:322–331

Shanks N, Windle RJ, Perks PA, Harbuz MS, Jessop DS, Ingram CD, Lightman SL (2000) Early-life exposure to endotoxin alters hypothalamic–pituitary–adrenal function and predisposition to inflammation. Proc Natl Acad Sci 97:5645–5650

Shetty S, Sridhar KR, Shenoy KB, Hegde SN (1990) Observations on bacteria associated with pigeon crop. Folia Microbiol 35:240–244

Slominski BA, Campbell LD, Stanger NE (1988) Extent of hydrolysis in the intestinal tract and potential absorption of intact glucosinolates in laying hens. J Sci Food Agric 42: 305–314

Smalia K, Oros-Sichler M, Milling A, Heuer H, Baumgarte S, Becker R, Neuber G, Kropf S, Ulrich A, Tebbe CC (2007) Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: do the different methods provide similar results? J Microbiol Methods 69:470–479

Soler JJ, Martín-Vivaldi M, Peralta-Sánchez JM, Ruiz-Rodríguez M (2010) Antibiotic-producing bacteria as a possible defence of birds against pathogenic microorganisms. Open Ornithol J 3:93–100

Stevens CE, Hume ID (2004) Comparative physiology of the vertebrate digestive system. Cambridge University Press, Cambridge

Tennant B, Reina-Guerra M, Harrold D (1971) Influence of microorganisms on intestinal absorption. Ann NY Acad Sci 176:262–272

Turnbaugh PJ, Gordon JI (2008) An invitation to the marriage of metagenomics and metabolomics. Cell 134:708–713

van der Wielen PWJJ, Keuzenkamp DA, Lipman JA, van Knapen F, Biesterveld S (2002) Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microb Ecol 44:286–293

Verma M, Madhu M, Marrota C, Lakshmi CV, Davidson EA (1994) Mucin coding sequences are remarkably conserved. Cancer Biochem Biophys 14:41–51

Vispo C, Karasov WH (1997) The interactions of avian gut microbes and their host: an elusive symbiosis. In: Mackie RI, White BA (eds) Gastrointestinal microbiology. Chapman and Hall, New York

White J, Mirleau P, Danchin E, Mulard H, Hatch SA, Heeb P, Wagner RH (2010) Sexually transmitted bacteria affect female cloacal assemblages in a wild bird. Ecol Lett 13:1515–1524

Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, Siuzdak G (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci 106:3698–3703

Wilmes P, Bond PL (2006) Metaproteomics: studying functional gene expression in microbial ecosystems. Trends Microbiol 14:92–97

Wright AG, Northwood KS, Obispo NE (2009) Rumen-like methanogens identified from the crop of the folivorous South American bird, the hoatzin (*Opisthocomus hoazin*). ISME J 3:1120–1126

Xenoulis PG, Gray PL, Brightsmith D, Palculict B, Hoppes S, Steiner JM, Tizard I, Suchodolski JS (2010) Molecular characterization of the cloacal microbiota of wild and captive parrots. Vet Microbiol 146:320–325

Yamano H, Koike S, Kobayashi Y, Hata H (2008) Phylogenetic analysis of hindgut microbiota in Hokkaido native horses compared to light horses. Anim Sci J 79:234–242

Young JC, Zhou T, Yu H, Zhu H, Gong J (2007) Degradation of trichothecene mycotoxins by chicken intestinal microbes. Food Chem Toxicol 45:136–143

Zhu XY, Zhong T, Pandya Y, Joerger RD (2002) 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. Appl Environ Microbiol 68:124–137