

# Chapter 9

## The Howler Monkey as a Model for Exploring Host-Gut Microbiota Interactions in Primates

Katherine R. Amato and Nicoletta Righini

**Abstract** The mammalian gut microbiota is essential to many aspects of host physiology, including nutrition, metabolic activity, and immune homeostasis. Despite the existence of numerous studies of the impact of the gut microbiota on human health and disease, much work remains to be done to improve our understanding of the host-microbe relationship in nonhuman primates. Howler monkeys (*Alouatta* spp.) are highly dependent on the gut microbiota for the breakdown of plant structural carbohydrates, and in this chapter we use new data describing the gut microbiome of captive and wild black howler monkeys (*A. pigra*) to develop and test two models of host-microbe interactions and bioenergetics. Improving our understanding of how spatial and temporal fluctuations in diet affect the nonhuman primate gut microbiota, and how this in turn influences host nutrition and physiology, has important implications for the study of the role that the gut microbiota plays in primate ecology, health, and conservation.

**Resumen** El papel de la microbiota intestinal es fundamental para muchos aspectos de la fisiología de los mamíferos, incluyendo la nutrición, la actividad metabólica y la homeostasis del sistema inmune. A pesar de la existencia de muchos estudios acerca de la microbiota intestinal humana debido a sus implicaciones para la salud, aún queda mucho por hacer para poder entender la relación huésped-microorganismos en primates no humanos. Los monos aulladores (*Alouatta* spp.) dependen de manera importante de los microbios intestinales para la digestión de los carbohidratos estructurales de las plantas. En este capítulo utilizamos nuevos datos sobre la composición de la microbiota de monos aulladores negros cautivos y silvestres (*A. pigra*) para desarrollar y poner a prueba dos modelos sobre las interacciones

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huésped-microbios desde un punto de vista ecológico y bioenergético. El análisis del efecto de las fluctuaciones espaciales y temporales de la dieta sobre la microbiota intestinal de los primates, y de cómo esto a su vez se refleja en la nutrición y fisiología del huésped, tiene implicaciones importantes para entender el papel de la microbiota en la ecología, salud y conservación de los primates.

**Keywords** Gut microbiome • Health • Nutrition • Growth • Reproduction

## 9.1 Introduction

Mutualistic microbial communities composed of bacteria, ciliate and flagellate protozoa, archaea, anaerobic fungi, and bacteriophages (Mackie 2002) are an essential part of the mammalian gut and play an important role in host physiology by influencing nutrition, metabolic activity, and immune homeostasis (Dethlefsen et al. 2007; Sekirov et al. 2010; Flint et al. 2011). These communities are dominated by bacteria, particularly in the colon, which in the case of humans, is estimated to contain more than 70 % of all of the microbes present in the body, with  $10^{11}$ – $10^{12}$  bacteria per gram of content (Sekirov et al. 2010). These bacteria contribute to host health by regulating xenobiotic metabolism (Bjorkholm et al. 2009), producing vitamins (Hill 1997), excluding pathogenic microbes, attenuating inflammation (Kelly et al. 2003), and affecting immune system development through the formation and modification of the intestinal epithelia and gut-associated lymphoid tissue (GALT) (Bauer et al. 2006; Neish 2009; Hooper et al. 2012). They also are thought to play a role in modulating brain development and function and affect behavior by altering gene expression and neuronal circuits involved in motor control and anxiety (Forsythe et al. 2010; Foster and McVey Neufeld 2013).

While each of these functions is important to the host, the role of the gut bacterial community, or the gut microbiota, in host energy and nutrient acquisition is the most well studied. Because all vertebrates lack the enzyme cellulase, which is required to break down cellulose, their ability to digest fiber is dependent on enzymes either present in their food or produced by intestinal microbes (Stevens and Hume 1995; Barboza et al. 2009). As a result, foods containing high proportions of plant cell wall material and resistant starches can only be digested if hosts maintain rich microbial communities. These microbial communities convert indigestible compounds such as cellulose into short-chain fatty acids (SCFA) such as acetate, butyrate, and propionate, which can be absorbed directly by the host and used as an energy source or stored as glucose in the liver (Mackie 2002). Short-chain fatty acids produced by the gut microbiota can supply hosts with up to 70 % of their daily energy needs (Flint and Bayer 2008) and have been reported to reduce the pH of the intestinal lumen to facilitate nutrient absorption and to prevent the accumulation of potentially toxic metabolic by-products (Neish 2009; Sekirov et al. 2010).

Like all mammals, primates rely on their gut microbiota to process low-quality resources such as woody plants, mature leaves, fungi, and plant exudates that are

difficult to digest and require greater handling and processing due to mechanical defenses, limited nutrients, and high concentrations of indigestible material or toxins (Lambert 2011). As a result, in addition to adaptations in dental morphology, specialized features of either the foregut or the hindgut have evolved independently in species of prosimians (indriids, *Lepilemur*), New World monkeys (*Alouatta*, *Callithrix*), Old World monkeys (colobines), and apes (gorillas) that regularly exploit low-quality resources. Specifically, fermentative processes are either pregastric (occurring before hydrolytic/enzymatic digestion), as seen in colobine monkeys, or post-gastric/cecocolic (occurring after hydrolytic/enzymatic digestion), as seen in some prosimians, New World monkeys, cercopithecines, apes, and humans (Chivers and Hladik 1980; Chivers and Langer 1994; Lambert 1998).

Howler monkeys (*Alouatta* spp.) are known for their ability to consume low-quality diets consisting of mostly leaves during some periods of the year (more than 80 % of feeding time in a given month) (Pavelka and Knopff 2004) and are post-gastric, or hindgut, fermenters (Milton 1980; Edwards and Ullrey 1999). They do not possess a particularly specialized gut morphology compared to foregut fermenters such as colobines (Kay and Davies 1994; Edwards and Ullrey 1999), but gut measurements for *A. palliata* (Chivers and Hladik 1980) reveal larger-than-expected cecum and colon volumes given their body mass (positive residuals from the least squares regression of cecum and colon volumes on body weight). Moreover, howlers are characterized by relatively long food transit times compared to other atelines (20.4 h for *A. palliata*) (Milton 1984). A large gut volume is usually associated with a greater production of microbial SCFA, and SCFA absorption and assimilation depend principally on the surface area available and on the length of time food is retained in fermenting chambers (Bourton and Perrin 1991; Kay and Davies 1994). Indeed, howlers are estimated to gain as much as 31 % of required daily energy from SCFA produced by the gut microbiota (Milton and McBee 1983).

Although it is widely accepted that the gut microbiota plays a critical role in howler nutrition, very little is understood about the dynamics of the howler-microbe relationship. For example, as energy minimizers, howler monkeys are able to persist in a wide range of habitats, including highly fragmented or anthropogenically impacted areas (Strier 1992; Phillips and Abercrombie 2003; Bicca-Marques 2003; Behie and Pavelka 2005; Zunino et al. 2007; Pozo-Montuy et al. 2011; Bonilla-Sanchez et al. 2012), and can endure marked seasonal changes in availability of food items such as mature fruit by exploiting hard-to-digest foods such as mature leaves and unripe fruits (Arroyo-Rodriguez et al. 2008), instead of dramatically increasing day range or time spent traveling [as observed in other atelines (Di Fiore et al. 2011)]. However, the role of the gut microbiota in allowing howlers to extract sufficient energy and nutrients from a wide range of resources across seasons and habitats is not well studied, and many questions remain to be answered. Does the composition of the howler gut microbiota shift in response to changes in diet? Do these shifts allow howlers to obtain the energy and nutrients they need under conditions of marked fluctuations in food availability? Do changes in the gut microbiota affect other aspects of howler health and behavior? Similarly, the influence of the gut microbiota on howler life history via nutrition has not been explored. Because

howler monkeys have an earlier age at first reproduction (42 vs. 84 months for *Ateles*), shorter gestation length (186 vs. 225 days for *Ateles* and *Lagothrix*), and shorter interbirth intervals (19.9 vs. 34.7 months for *Ateles*) than other atelines (Fedigan and Rose 1995), their daily nutritional demands for growth and reproduction are expected to be greater. Can differences in the composition of juvenile and female gut microbiota help compensate for some of these demands? If so, what triggers the gut microbiota to change? Are these changes important regardless of season and diet?

In this chapter, we begin by reviewing the factors that influence mammalian gastrointestinal microbial community structure and function and the impacts of the gut microbiota on host nutrition, physiology, and health. Using this information we develop two models—a general model of host-microbiota interactions and a revised bioenergetics model that includes gut microbiota effects—and use data from black howler monkeys (*A. pigra*) to test the predictions of these models. Finally, we discuss patterns that correspond to our models within and among other primate species and detail important avenues for future research that integrate gut microbiome analyses with ecological, nutritional, and physiological data to describe interactions between diet, behavior, nutrition, and health in wild primate populations.

## 9.2 The Mammalian Gut Microbiome

In recent years, the study of microbial communities has benefited from molecular approaches that use the extraction and amplification of microbial DNA to identify patterns in community composition across samples. Given these techniques, it is now possible to overcome the limitations associated with bacterial culturing methods, such as the small number of samples that can be processed at a time and the bias against strict anaerobes, many of which play an important role in the gut microbial community (Sekirov et al. 2010). Instead, analyses such as termination restriction fragment length polymorphism (T-RFLP) (Osborn et al. 2000), denaturing gel gradient electrophoresis (DGGE) (Fischer and Lerman 1979), automated ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett 1999), and high-throughput pyrosequencing (Ronaghi et al. 1998) allow researchers to describe in detail the taxonomic composition, function, and diversity of the fecal (i.e., mainly colonic) microbiota in a variety of animal species (see Sekirov et al. 2010 for a comparison among these techniques and their respective benefits and limitations).

### 9.2.1 Evolution of the Mammalian Gut Microbiota

Due to their involvement in host nutrient metabolism, gut microbes are thought to have played a primary role in host evolution by facilitating the adoption of a particular diet and providing specific metabolic pathways for the digestion of that diet

(Neish 2009; Yildirim et al. 2010). However, it is also possible that gut microbial communities co-diversified and coevolved with their hosts, leading to specializations and increased dependence between the host and its microbial colonists (Kau et al. 2011; Yeoman et al. 2011). Recent studies analyzing bacterial 16S ribosomal RNA gene sequences from 60 mammalian species (Ley et al. 2008a, b) indicate that gut bacterial diversity may be affected by host phylogeny since the fecal microbial communities of conspecific hosts are more similar to each other than to the communities of more distantly related hosts. Additionally, data from this study demonstrate an effect of diet on gut bacterial diversity. Herbivorous mammals exhibit a higher diversity of microbial phyla than omnivores, which in turn exhibit a higher diversity than carnivores. Separating these genetic and environmental influences from each other is crucial not only for understanding the role the gut microbiota has played in the evolution of mammalian dietary diversification but also for determining the impact of the microbiota on host diet and nutrition at different time scales (e.g., days, weeks, years).

### ***9.2.2 Factors Affecting Gut Microbiota Composition***

In all mammals the fetal gut is generally sterile, and microbial colonization occurs during and after birth via horizontal transfer of microbes from the surrounding environment (Mackie et al. 1999; Donnet-Hughes et al. 2010). In humans, the establishment of the gut microbial community takes approximately 1 year (Mackie et al. 1999). Initially, the gut microbiota exhibits a relatively simple structure (mainly composed of lactic acid bacteria (LAB), enterobacteria, and streptococci) and varies greatly among individuals and across time according to diet (Mackie et al. 1999; Sekirov et al. 2010; Spor et al. 2011). However, as weeks pass, gut microbiota composition stabilizes and begins to include higher numbers of obligate anaerobes (Mackie et al. 1999). Studies have shown that during this process there is a strong maternal influence on the structure of the gut microbial community. For example, among individuals fed the same diet, mouse gut microbiota composition is more similar between mother and weaning offspring than between unrelated individuals, even when the unrelated individuals share the same genotype for obesity traits while the mother and offspring do not (Ley et al. 2005). Increasing evidence suggests that this maternal influence is a result of microbial transfer via colostrum and breast milk. Enteric bacterial translocation and colonization of the mammary tissue have been documented in pregnant and lactating mice, and an analysis of human milk confirmed the presence of autochthonous ileal and colonic microbes (Donnet-Hughes et al. 2010). Therefore, nursing is crucial to gut microbial community development.

Although human microbial community composition stabilizes after about a year, it remains highly dynamic throughout an individual's lifetime. Rapid responses by the microbiota to changes in the selective pressures in the gut result in intra- and interindividual variation according to factors such as host diet, age, nutrition, health status, and genetics (Spor et al. 2011). Of these, diet has been shown to play a

particularly strong role in determining gut microbial community composition. For example, within the span of a day, mice that were switched from a low-fat diet rich in plant polysaccharides to a high-fat, high-sugar diet experienced a dramatic increase in the abundance of several classes of bacteria belonging to the Firmicutes phylum (Turnbaugh et al. 2009). Additionally, studies in humans show that there exist at least two stable, broad “enterotypes” which are linked to long-term dietary habits: the *Bacteroides* enterotype, associated with animal protein and saturated fat intake, and the *Prevotella* enterotype, associated with plant-based nutrition (Wu et al. 2011). However, shifting from a primarily plant-based diet to a primarily animal-based diet also affects the gut microbial community by increasing the abundance of bile-tolerant bacteria and reducing the abundance of bacteria from the Firmicutes phylum over a span of one week (David et al. 2014). Together these patterns suggest that host diet exerts strong selective pressure on the mammalian gut microbiota on time scales from hours to years.

In addition to environmental influences such as diet, host genetics appear to impact gut microbiota composition (Benson et al. 2010). This is especially evident when focusing on specific groups of microorganisms within the gut community. For example, variations in fecal abundance of LAB, a group of gram-positive microbes belonging to the phylum Firmicutes, are reported to be associated with particular mouse genetic lines, regardless of the maternal microbiota the mice are exposed to (Buhnik-Rosenblau et al. 2011). Additionally, mice that are genetically predisposed to obesity possess a higher proportion of Firmicutes and a lower proportion of Bacteroidetes compared to mice with a “lean” genotype (Ley et al. 2005; Turnbaugh et al. 2006). Obesity is thought to be a result of the ability of Firmicutes to harvest energy with higher efficiency from a given diet, thereby providing the host with surplus energy (Turnbaugh et al. 2006). As a result, hosts that are genetically predisposed to higher Firmicutes abundances are more likely to become obese. Of course, environmental effects can interact with these genetic effects. In humans, changes in diet leading to weight loss result in decreased proportions of Firmicutes (Ley et al. 2006). Similarly, Zoetendal et al. (2001) argue that genetics strongly influence gut microbiota composition since monozygotic twins living separately show more microbiome similarity than domestic partners, and profiles of domestic partners do not differ in similarity from those of unrelated individuals. However, many environmental effects (e.g., maternal effect, diet, lifestyle, illness) were not controlled for in twin pairs. Therefore, while host genotype appears to have some effect on mammalian gut microbiota composition, in many cases nongenetic effects are equally, if not more, important.

Aside from maternal influences and diet, other environmental factors can interact with the gut microbiota via shifts in host physiology. For example, a study of captive rhesus macaques (*Macaca mulatta*) indicated that physical and psychological stress alter gut microbial community composition (Bailey and Coe 1999). Six- to nine-month-old infants separated from their mothers showed stress-indicative behaviors (e.g., distress calls), increases in plasma cortisol, and a significant reduction in fecal lactobacilli starting the third day after separation (Bailey and Coe 1999). Similarly, rats and chicks exposed to stress from heat and crowding possess distinct gut microbiota

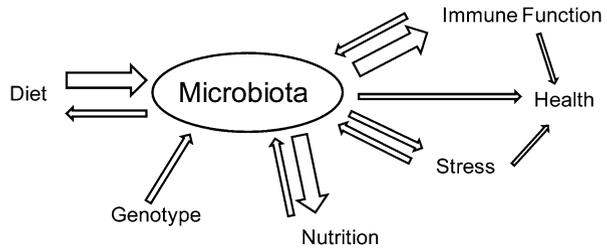
compared to individuals not exposed to these stressors (Suzuki et al. 1983) and mouse models of depression also exhibit changes in the gut microbiota (Park et al. 2013). However, the relationship between host stress and gut microbiota composition is not unidirectional. Studies of rodents and humans provide evidence that gut microbiota composition can influence host stress responses. Based on measures of plasma adrenocorticotrophic hormone (ACTH) and corticosterone responses, germ-free mice are more susceptible to stress when physically restrained than specific pathogen-free mice (Sudo 2006). As a result, it appears that the gut microbiota has a role in the development of hypothalamic-pituitary-adrenal (HPA) stress responsiveness. Similarly, the administration of certain bacteria strains (such as *Lactobacillus* and *Bifidobacterium*) has been shown to have beneficial effects on stress, anxiety, and depression in rats and humans, as indicated by reductions in anxiety-like behaviors and cortisol levels (Messaoudi et al. 2010). Thus, there may exist a positive feedback loop relating stress to depauperate gut microbiota.

Interactions between host physiology and gut microbiota composition also appear to occur via immune system function. The adaptive immune system constantly monitors the gut microbiota and stimulates the secretion of local strain-specific immunoglobulin A (IgA) across mucous membranes (Macpherson et al. 2008; Neish 2009). Secretion of IgA influences gut microbiota composition and protects beneficial microbiota from host immune attacks since IgA is used by the mammalian humoral immune system (i.e., mediated by antibodies produced by B cells) to recognize cells and tag only pathogenic invaders for destruction. However, as with the stress-microbiota relationship, this relationship is not unidirectional. The gut microbiota appears to play an active role in host immune function. IgA-secreting cells are significantly reduced (1–2 orders of magnitude lower) in germ-free animals and absent in neonates suggesting that intestinal IgA levels are regulated by the presence of gut microflora (Benveniste et al. 1971a, b; Macpherson et al. 2008). Furthermore, the gut microbiota is thought to contribute to the development of the host intestinal mucosal and systemic (i.e., peripheral) immune systems (Neish 2009; Forsythe et al. 2010; Sekirov et al. 2010; Hooper et al. 2012). For example, germ-free mice lack immune activity, and only colonization of their guts with specifically selected bacteria provokes the complete restoration of immune activity (Talham et al. 1999). Similarly, in humans, the Hygiene Hypothesis suggests that reduced exposure to microorganisms suppresses the normal development of the immune system, resulting in the increased rates of allergies or immune/inflammatory conditions associated with sanitation, antibiotic use, and other “Western” habitats (Strachan 1989; Sekirov et al. 2010).

### 9.3 General Model of Host-Microbiota Interactions

The mammalian studies described above indicate a wide variety of interactions between mammals and their gut microbiota. In general, host physiology and diet impart strong selective pressures on the gut microbiota. Therefore as host

**Fig. 9.1** General model of host-gut microbiota interactions. Size of arrows indicates relative size of effect



physiology or diet changes, selective pressures also change, influencing the competitive abilities of microbial taxa and inciting shifts in gut microbial community composition. In turn, these shifts can affect host nutrition and health. Based on these dynamics, we have developed a general model to predict host-gut microbiota interactions in mammals (Fig. 9.1). Because diet appears to play a stronger role in determining microbiota composition than host genotype or physiology (Friswell et al. 2010), in this model, host diet is presented as the main influence on gut microbiota composition. Likewise, because host nutrition and immune development and function depend so heavily on the gut microbiota, these factors in our model are the most strongly affected by changes in gut microbiota composition.

Using this framework as a guide, a series of predictions regarding host-gut microbiota interactions within mammalian species can be made. First, because diet varies spatially across habitats and temporally according to seasonal food availability (Ostfeld and Keesing 2000; Meserve et al. 2003), gut microbiota composition should vary among individuals of a species occupying distinct habitats or within individuals across seasons. These differences should be associated with differences in overall diet diversity or composition. For example, bacteria such as *Clostridium* and *Ruminococcus* have high cellulolytic capability and can outcompete other microbes in the presence of cellulose (Cavedon et al. 1990; Ohara et al. 2000; Louis et al. 2007). Therefore, we would expect individuals consuming a leaf-heavy diet to have higher abundances of these two genera compared to individuals consuming a fruit-heavy or lower-fiber plant-based diet. However, as diet shifts and leaf eating decreases, *Clostridium* and *Ruminococcus* may no longer be able to outcompete other microbes and survive in the gut, and microbiota composition should change as other microbes increase in abundance or invade the gut community. Similarly, a diverse host diet delivering a large array of nutrients and different types of carbohydrate substrates to the gut provides a variety of feeding niches to support microbial taxa or functional groups (Louis et al. 2007). Therefore, for an herbivore/frugivore, we would expect that the more plant species an individual is able to utilize in a particular habitat, the richer and more diverse its gut microbiota. In other cases, a specific food item, plant species, or set of plant species may determine the amount of one or two key macro- or micronutrients in the gut and strongly influence the composition of the gut microbiota.

Once changes in gut microbiota composition occur, our model predicts that host nutrition should be affected. Because previous studies suggest that certain gut

microbial communities are specialized for the digestion of certain host diets (Ley et al. 2008a; Wu et al. 2011), food digestion by the microbiota adapted to that diet should be highly efficient. Moreover, adaptation of the gut microbiota to changes in host diet can occur within days (Turnbaugh et al. 2009), meaning that increased digestive efficiency can be achieved rapidly and should aid hosts in acquiring sufficient energy and nutrients to meet metabolic demands despite variability in diet across habitats and seasons. If this is the case, we would expect host energy balances, body masses, and/or body conditions not to vary dramatically spatially or temporally, while the products of microbial fermentation such as SCFA should.

Finally, our model predicts that shifts in gut microbiota composition should have strong effects on host immune function. The literature suggests that depletion of the gut microbiota leads to decreased immune function, specifically decreased IgA secretion (Benveniste et al. 1971a, b; Moreau et al. 1978). Therefore, regardless of their nutritional status, we would expect individuals with low gut microbiota diversity and/or richness to exhibit low IgA levels compared to the rest of the population. Additionally, if depleting the microbiota reduces overall immune function, we would also expect individuals with reduced gut microbiota diversity and/or richness to contract more illnesses and to serve as hosts to more parasites. This has critical implications for primate conservation and survivorship. Similarly, reducing gut microbiota diversity is believed to facilitate colonization of the gut by gastrointestinal pathogens and parasites by reducing the number of feeding niches occupied by mutualistic microbes, increasing the number of niches available for colonization, and/or eliminating those bacterial taxa that actively exclude pathogens (Fons et al. 2000; Servin 2004; Costello et al. 2012). As a result, we would expect individuals with reduced gut microbial diversity to have higher occurrences and abundances of gastrointestinal pathogens and parasites. Although it is not directly linked to immune function, we would also expect gut microbial diversity to interact with host glucocorticoid levels as suggested in the literature (Bailey and Coe 1999; Sudo 2006). Whether microbiota composition influences glucocorticoid levels or vice versa is difficult to distinguish. However, individuals with reduced microbial diversity should exhibit higher glucocorticoid levels.

To explore the validity of our model, we used behavioral and gut microbiome data from an 8-week study of wild, black howler monkeys (*A. pigra*) in southeastern Mexico (Amato et al. 2013) to test the hypotheses that (1) differences in diet across habitats result in differences in gut microbiome composition and (2) differences in gut microbiome composition affect host health. Specifically, we expected that howlers consuming diets with a relatively higher proportion of leaves during the study period would exhibit relatively higher abundances of cellulose-degrading bacteria such as *Ruminococcus* compared to other howlers. We also expected howlers consuming a relatively more diverse diet in terms of plant species to exhibit a relatively more diverse microbiome. Finally, for those howlers with the lowest microbial diversity, we expected to find higher abundances of pathogenic bacteria.

Fecal samples analyzed in this study were collected from five groups of black howler monkeys occupying four habitats—a continuous evergreen rainforest; an evergreen rainforest fragment; a continuous, semi-deciduous forest; and a rehabilita-

**Table 9.1** Percent of total feeding time spent consuming plant parts by howlers in the continuous evergreen rainforest and semi-deciduous forest (adapted from Amato et al. 2013)

Food type	Feeding time (%)	
	Continuous evergreen	Continuous semi-deciduous
Mature fruit	53.03	30.68
Immature fruit		0.79
Mature leaf	21.25	11.70
Young leaf	13.22	56.83
Stem	9.17	
Other	3.34	

Howlers in the evergreen rainforest fragment consumed less mature fruits than those in the continuous forest, and the captive howlers were fed a mixture of mature fruits, cereal, and monkey chow

**Table 9.2** Percent of total feeding time spent consuming plant species by howlers in the continuous rainforest and semi-deciduous forest (adapted from Amato et al. 2013)

Plant species	Feeding time (%)	
	Continuous evergreen	Continuous semi-deciduous
<i>Acacia usumacintensis</i> (Fabaceae)		35.32
<i>Alseis yucatanensis</i> (Rubiaceae)		1.19
<i>Brosimum alicastrum</i> (Moraceae)		35.32
<i>Bursera simaruba</i> (Burseraceae)		9.92
<i>Cecropia peltata</i> (Urticaceae)	2.47	
<i>Ficus americana</i> (Moraceae)	23.99	
<i>Ficus aurea</i> (Moraceae)	11.37	
<i>Ficus</i> sp. (Moraceae)	8.34	
<i>Ficus yoponensis</i> (Moraceae)	14.61	
<i>Lonchocarpus castilloi</i> (Fabaceae)		2.28
<i>Manilkara zapota</i> (Sapotaceae)		1.19
<i>Metopium brownei</i> (Anacardiaceae)		26.58
<i>Monstera</i> sp. (Araceae)		0.26
<i>Poulsenia armata</i> (Moraceae)	15.58	
<i>Schizolobium parahyba</i> (Fabaceae)	1.82	
<i>Simarouba glauca</i> (Simaroubaceae)		1.78
<i>Vitex gaumeri</i> (Verbenaceae)		7.27
Unknown sp. 1		1.24
Unknown sp. 2	0.13	
Unknown sp. 3	1.04	
Unknown sp. 4	0.17	
Unknown sp. 5	3.38	
Unknown sp. 6 (Fabaceae)	3.6	
Unknown sp. 7 (Araceae)	2.70	
Unknown sp. 8	4.94	
Unknown sp. 9	3.04	
Unknown sp. 10		0.59
Vines	3.24	2.17

Species consumed by the howlers in the evergreen rainforest fragment were a subset of those consumed by the howlers in the continuous rainforest and also included several distinct species. Captive howlers were fed melon, mango, papaya, and banana in addition to cereal and monkey chow

tion center—in southeastern Mexico. Quantitative diet data collected using focal sampling were available for two of these habitats, and qualitative diet data were available for the other two (Tables 9.1 and 9.2). Bacterial community fingerprinting (ARISA) was used to detect broad patterns in overall microbiome composition while pyrosequencing provided information regarding which bacterial taxa were driving the patterns. Functional genes associated with the production of VFA's and other microbial fermentation products were measured using quantitative real-time PCR.

Analyses revealed that howler gut microbiome richness, diversity, and composition differed by habitat. Captive howlers ( $N=8$ ) exhibited the lowest microbial richness and diversity (Chao1=9,821, Shannon=6.82), and howlers in the continuous rainforest ( $N=14$ ) exhibited the highest microbial richness and diversity (Chao1=1,549, Shannon=4.78). Because the captive howlers came from distinct geographic regions of Mexico and were not genetically related, these patterns must be an effect of their captive environment. Indeed, gut microbiome variation was strongly correlated with howler diet both in terms of plant parts (Spearman's  $\rho=0.54$ ,  $p<0.001$ ; Table 9.1) and plant species (Spearman's  $\rho=0.34$ ,  $p<0.005$ ; Table 9.2), which differed according to habitat. Howlers consuming a more diverse diet also exhibited more diverse gut microbiome.

Diet composition also influenced gut microbiome composition. Cellulolytic *Ruminococcus* increased with the proportion of fiber-rich, mature leaves in the howler diet (Table 9.3). *Prevotella*, which degrades the monosaccharide xylose (Yildirim et al. 2010), was found in higher abundances in the captive howlers (Table 9.3). Simple sugars like xylose are typical of fruits, and since the captive howlers consumed a fruit-heavy diet, it is likely that these sugars favored the presence of *Prevotella*. Similarly, *Lachnospira pectinoschiza*, a Clostridia that utilizes pectin, was found in captive howlers in higher abundances (Nakamura et al. 2011). Pectin is a complex polysaccharide contained in many fruits such as apple and guava. *Lactobacilli* are benefited by dietary calcium (Bovee-Oudenhoven et al. 1999), and the captive howlers with high calcium content in their manufactured diet (Mazuri Leaf-Eater Primate Chow: 1.12 % Ca vs. 0.40 % and 0.30 % for young leaves and fruits, respectively (Righini, 2014) had the highest levels of *Lactobacilli* (Table 9.3). *Ficus* is also known to have high calcium content compared to other fruit species (O'Brien et al. 1998), and *Ficus* trees were present in every wild habitat except the semi-deciduous forest. Likewise, all howlers outside of the semi-deciduous forest possessed *Lactobacilli*.

Measures of howler health showed less clear patterns. In general, there was a low occurrence of pathogenic bacteria in all of our sampled howlers, and the presence of a pathogen was not related to gut microbiome diversity. However, the eight individuals (all captives and one continuous forest howler) with the lowest microbiome richness and diversity died within 6 months following the sampling period. Although

**Table 9.3** Percent of total bacterial sequences sampled belonging to bacterial genera for each howler group sampled

Bacterial genus	Cont. evergreen	Cont. semi-deciduous	Frag. evergreen	Captive
<i>Ruminococcus</i>	0.42	0.19	0.70	0.10
<i>Prevotella</i>	0.0060	0.0070	0.0030	12.98
<i>Lactobacillus</i>	0.0100	0.0040	0.029	0.28

this relationship cannot be assumed to be causative, it suggests a potential connection between the gut microbiome and howler health.

Shifts in metabolic functional genes across habitats reinforce this connection. The butyryl-CoA:acetate CoA-transferase gene involved in the microbial production of health-promoting butyrate was more prevalent in howlers in the continuous evergreen rainforest than in howlers at other sites. Similarly, the number of acetyl-CoA synthase genes used for microbial production of acetate, another important VFA, was significantly higher in the continuous evergreen rainforest than in other habitats. Increased hydrogen production is associated with increased microbial fermentation, and (Ni-Fe)-hydrogenase ((NF)*hyd*) genes for hydrogen production were most abundant in the continuous evergreen rainforest. Finally, hydrogen sulfide is a toxic gas produced by the consumption of hydrogen by sulfate-reducing bacteria that affects smooth muscle and has been linked to colonic disease (Medani et al. 2011; Carbonero et al. 2012). The dissimilatory (bi)sulfite reductase gene associated with hydrogen sulfide production was most abundant in the evergreen fragment howlers and the captive howlers.

The results from this initial study confirm that diet diversity and composition play an important role in determining howler gut microbiota composition and indirectly support the hypothesis that reductions in gut microbiota diversity negatively affect howler health. However, measurements of IgA levels, parasite abundance, or glucocorticoid levels would more accurately pinpoint the effect of microbiota depletion on howler health. Studies of wild, nonhuman primates have used fecal measurements of IgA to estimate immune function (Lantz et al. 2011), and this technique could be easily integrated into future microbiome studies. Similarly, measuring gastrointestinal parasite abundance in primates relies on fecal sample collection and could be easily incorporated into future protocols. Studies of several howler species, including black howlers in Palenque, have reported higher gastrointestinal parasite diversity and abundance in primates inhabiting degraded areas compared to those in relatively undisturbed habitats (Eckert et al. 2006; Stoner and Gonzalez Di Pierro 2006; Trejo-Macias et al. 2007; Vitazkova and Wade 2007), suggesting that there may be a connection between reduced gut microbial diversity and parasite abundance in these habitats. However, analyses of parasite diversity and abundance must be carried out simultaneously with analyses of gut microbiota composition to truly test this relationship. Similarly, Martinez-Mota et al. (2007) report that black howler monkeys living in small (<2 ha), highly disturbed forest fragments in Mexico have higher fecal glucocorticoid levels than monkeys inhabiting less-disturbed forest. Again, this relationship provides indirect support for the interaction of the gut microbiota and host stress responses, but fecal samples must be analyzed for glucocorticoids and gut microbiota composition concurrently to confirm the pattern.

Although the results from this study demonstrate that howler microbiota composition differs with diet across habitats, it is unclear whether these differences are associated with the expected shifts in microbial activity and digestive efficiency that would allow howlers to meet nutritional demands in all habitats. Measurements of gut microbiota activity as well as host nutritional status are necessary to clarify this relationship. Fecal volatile fatty acid (VFA, a subset of SCFA) and ammonia content

provide an estimate of carbohydrate and protein metabolism by the microbiota (Erwin et al. 1961; Chaney and Marbach 1962). Generally, the more VFA and ammonia detected in fecal samples, the more produced by the gut microbiota, and the more available to the forager. Similarly, the nutritional status of individuals from a variety of primates species has been described using C-peptide analyses from urine samples (Sherry and Ellison 2007; Deschner et al. 2008; Thompson and Knott 2008; Thompson et al. 2008; Harris et al. 2009; Girard-Buttoz et al. 2011). Urinary excretion levels of C-peptide are positively correlated with insulin production in humans (Kruszynska et al. 1987), and in nonhuman primates, high C-peptide levels are correlated with increased body mass and high food availability, among other factors (Sherry and Ellison 2007; Deschner et al. 2008; Thompson and Knott 2008; Girard-Buttoz et al. 2011). Therefore, if changes in the gut microbiome result in increased microbial activity and provide sufficient energy and nutrients to the host, we would expect spatial and temporal changes in gut microbiota composition to be associated with strong variations in fecal VFA and ammonia content and only weak variations in C-peptide.

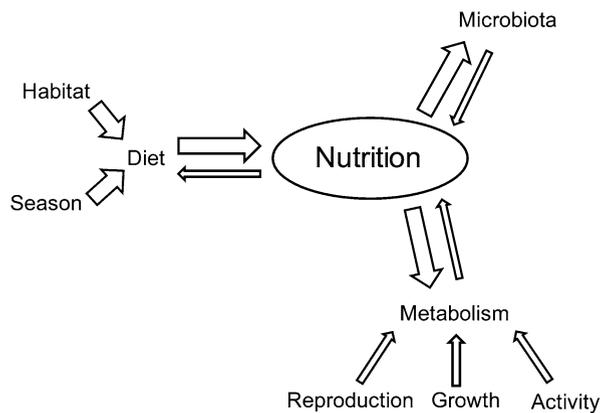
#### **9.4 Integrating the Gut Microbiota into Mammalian Bioenergetics Models**

In addition to knowing how the mammalian gut microbiota changes with habitat and diet, information regarding how the microbiota differs among individuals and within individuals over time is crucial to understanding the relationship between the gut microbiota and host nutrition, health, and ecology. Foragers face challenges in obtaining sufficient energy and nutrients as food availability varies across habitats in response to disturbance and fragmentation and within habitats across seasons. However, these challenges are compounded for individuals as their energy and nutrient requirements change due to processes like growth and reproduction. In primates, pregnancy and lactation are estimated to increase female daily energy requirements by 20–30 % and 37–39 %, respectively (Aiello and Wells 2002), and lactation is estimated to increase protein requirements by more than a third (Oftedal et al. 1991). Similarly, growth in weaned juveniles can require 50 % more energy and 100 % more protein than basal requirements (Altmann and Alberts 1987; Altmann and Samuels 1992). According to mammalian bioenergetics models, as these nutritional demands increase, individuals must (1) increase energy and nutrient intake, (2) decrease metabolic consumption of energy and nutrients, and/or (3) increase energy and nutrient assimilation efficiency to compensate (McNab 2002; Peles and Barrett 2008). Although a large number of mammalian studies have investigated changes in diet and activity in response to growth and reproduction (e.g., Mellado et al. 2005; Chilvers and Wilkinson 2009; Larimer et al. 2011), few explore differences in assimilation or digestive efficiency (Hammond and Kristan 2000; Jaroszewska and Wilczynska 2006). However, changing digestive efficiency in response to growth and reproduction is likely to be an important mechanism for meeting increased

nutritional demands, especially in cases where diet and/or activity are constrained. Although physiological changes that increase intestinal volume, surface area, or permeability can improve primate digestive efficiency, changes in gut microbiota composition, which lead to changes in SCFA production, may represent a faster, less energetically expensive, and more labile mechanism because they do not require host growth or physiological changes. Therefore, we propose a revised bioenergetics model, which incorporates shifts in gut microbiota composition and function.

As in traditional bioenergetics models, this model predicts that as nutritional needs change due to processes such as reproduction and growth, individuals must to consume more energy and nutrients, become less active, or increase gut volume, surface area, and permeability to compensate (Fig. 9.2). However, it also predicts that individuals should exhibit shifts in gut microbiota composition that result in the production of more energy and nutrients. Laboratory studies of mice and humans have demonstrated that the ratio of Bacteroidetes to Firmicutes bacteria influences host digestive efficiency since Firmicutes produce energy more efficiently (Ley et al. 2005; Armougom et al. 2009). Additionally, in humans, increased *Bacteroides* numbers in pregnant women have been associated with increased HDL cholesterol and folic acid, increased *Bifidobacterium* with increased folic acid, and increased *Enterobacteriaceae* and *E. coli* with increased ferritin and reduced transferrin (Santacruz et al. 2010). Therefore, we predict that juvenile and reproductively active female primates should alter the proportion of functional groups of microbes such as Firmicutes or *Bifidobacterium* in the gut to increase digestive efficiency and nutrient production. Although we do not expect changes in the microbiota to replace changes in diet, activity, and/or gut morphology as mechanisms for meeting increased nutritional demands, we do expect them to be most pronounced and most critical when these other mechanisms are constrained.

To test some of the predictions of our revised bioenergetics model, we again use data from wild, black howler monkeys in Mexico. Specifically, we investigate the relationship between diet, activity budget, and gut microbial composition and activity



**Fig. 9.2** Revised mammalian bioenergetics model. Size of arrows indicates relative size of effect

across howler age and sex classes during an 8-week period. As energy minimizers, howlers exhibit clear behavioral constraints with respect to activity levels. Additionally, howler studies indicate few changes in activity patterns and/or diet for juveniles or reproductively active females (pregnant or lactating) that would suggest compensation for increased nutritional demands (Nagy and Milton 1979; Schoeninger et al. 1997; Serio-Silva et al. 1999; Raguet-Schofield 2009; Dias et al. 2011). Therefore, it is likely that juvenile and adult female howlers rely to some extent on changes in digestive efficiency to meet nutritional demands and provide an excellent system for testing our model.

To compare behavioral and physiological mechanisms for meeting nutritional demands, K. Amato collected data describing diet, activity budget, and gut microbiome composition and activity from black howlers from different age, sex, and reproductive classes (pregnant, lactating) in Palenque National Park, Chiapas, Mexico. Approximately 159 h of behavioral data were collected during May–July 2009 from two neighboring groups of howlers: the Motiepa group ( $N=8$  individuals) and the Balam group ( $N=6$  individuals). Twenty-minute focal samples with activity recorded instantaneously every 2 min were used to describe feeding (active consumption of food resources), foraging (movement within a feeding tree), resting (periods of inactivity), traveling (movement between tree crowns), and social behavior (aggression, howling, play, sexual activity, etc.) between 6 am and 4 pm each day. During a feeding bout, the food type (young leaves, mature fruit, flowers, etc.) and plant species were recorded. Average daylight hours during the study period were used to calculate the average amount of time spent daily by the howlers in each activity based on the percent of time spent in each activity during focal observations. For feeding data, average ingestion rates collected across seasons (Amato 2013) were used to estimate the number of food items consumed per minute by each individual for each food type and plant species when possible. Average wet masses of food items were used to estimate the average daily amount of grams of food ingested by each individual for each food type and plant species (Amato 2013), and the average kcal and grams of protein ingested by each individual was calculated using general estimates for Neotropical food types (Norconk et al. 2009). Feeding data were standardized by metabolic body weight for each age/sex class before analysis (Kleiber 1975; Kelaita et al. 2011).

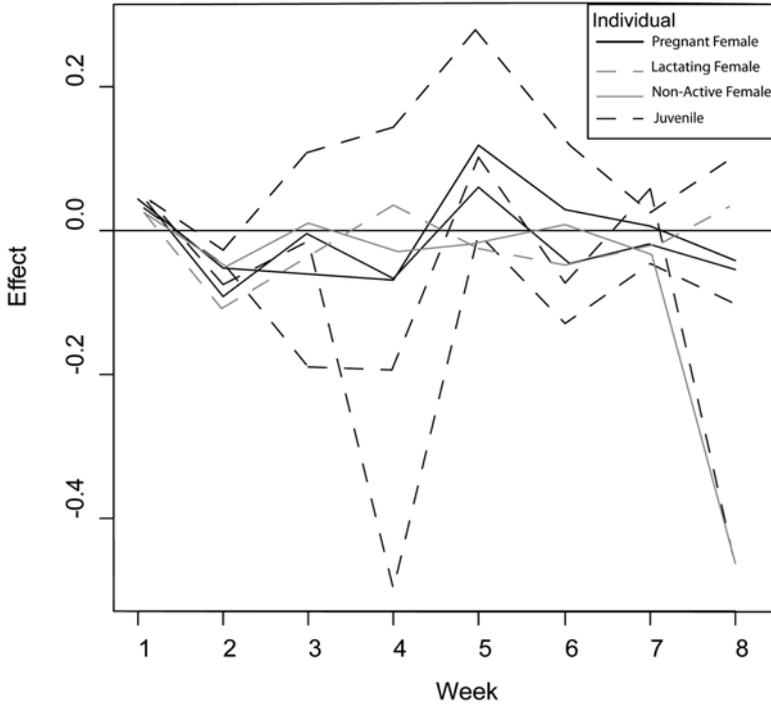
To determine whether gut microbial community composition and activity differed across age and sex classes, fecal samples were collected from each individual weekly over the course of 8 weeks (114 samples total,  $\approx 8$  samples per individual). Each fecal sample was preserved for the measurement of ammonia concentration and VFA content, as well as for microbial community fingerprinting (automated ribosomal intergenic spacer analysis, ARISA) and sequencing (Chaney and Marbach 1962; Erwin et al. 1961; Mackie et al. 1978; Ronaghi et al. 1998; Fisher and Triplett 1999; Yannarell and Triplett 2005). Fecal ammonia concentration estimates microbial protein metabolism, while VFA content can be used to estimate microbial carbohydrate fermentation. Because these values vary according to body size, all data were standardized by body weight for each age/sex class before analysis (Kelaita et al. 2011).

For both focal data and microbial data, dissimilarity between samples was visualized using nonmetric multidimensional scaling (NMDS) on PRIMER 6 for Windows v 6.1.10 (PRIMER-E, Plymouth, UK). NMDS plots for focal data were created using Euclidean distances, while those for microbial data were created using Bray-Curtis similarity indices. Overall activity budget, diet, and gut microbiome composition were tested for significant differences using analysis of similarity (ANOSIM), and nonparametric similarity percentage analysis (SIMPER) was used to determine which variables accounted most for observed differences in activity budget and diet (Clark and Gorley, 2006). Permutational (nonparametric) multivariate analysis of variance (PerMANOVA) was also used to detect significant patterns in microbial community composition across samples as well as to describe the amount of variation in microbial community composition explained by howler group, age, sex, and reproductive status as well as by sampling week (R software). Differences in kcal and grams of protein ingested, as well as differences in fecal ammonia concentration and VFA content, were tested for significance using ANOVA (R software) with Bonferroni corrections applied to  $p$ -values.

The behavioral data revealed few differences in activity budget among individuals. Male and female activity budgets did not differ (ANOSIM  $R=0.038$ ,  $p=0.35$ ), and the activity budgets of reproductively active females (pregnant or lactating) were the same as those of all other adults (ANOSIM  $R=-0.068$ ,  $p=0.58$ ). Activity budgets for juveniles were significantly different from adult activity budgets (ANOSIM  $R=0.791$ ,  $p=0.001$ ). Nonparametric SIMPER analyses revealed that 67.2 % of the variation between juvenile and adult activity budgets is a result of juveniles resting less than adults, while 25.1 % is a result of juveniles spending more time in social behavior (e.g., play) than adults.

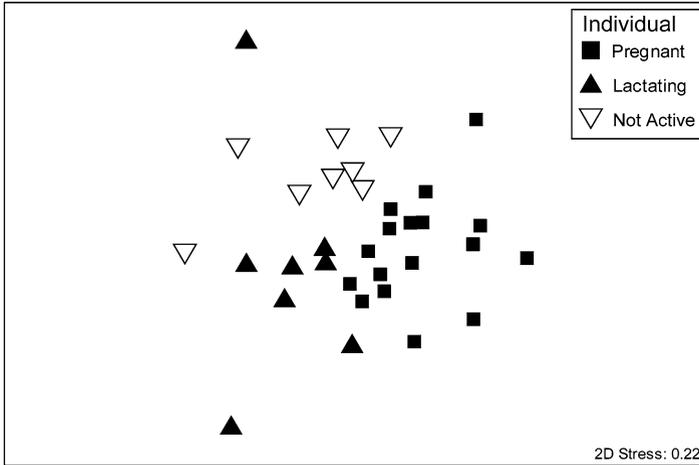
Few differences in diet existed among age and sex classes. Overall, there were no differences in the diets of males, females, or juveniles when analyzed by food type or by plant species (ANOSIM  $R=-0.069$ ,  $p=0.72$ ; ANOSIM  $R=-0.02$ ,  $p=0.45$ ). Reproductively active females also showed no overall diet differences when compared to other adults (ANOSIM  $R=0.198$ ,  $p=0.16$ , ANOSIM  $R=0.296$ ,  $p=0.083$ ). There were no age or sex differences in the number of kcal ingested per day ( $F_{2,11}=1.32$ ,  $p=0.3057$ ). Adult females tended to consume more grams of protein per day than juveniles and adult males ( $F_{2,11}=3.65$ ,  $p=0.060$ ), but this trend was not significant. Reproductively active females showed the same trend as nonreproductively active females.

Microbial analyses of fecal samples identified Firmicutes (68.4 %), Bacteroidetes (13.3 %), and Proteobacteria (0.92 %) in all individuals. Among Firmicutes, Clostridia were the most abundant (64.5 % of the entire microbiota). Microbial community fingerprinting revealed that gut microbiome composition clustered by individual over time, suggesting stability in the microbiota over the 2-month sampling period (ANOSIM  $R=0.384$ ;  $p=0.001$ ). Variation in gut microbiome composition within individuals from week to week was also detected, but there were no significant trends in gut microbiome composition across the study period ( $F_{1,72}=3.01$ ,  $p=0.42$ ). Similarly, while females and juveniles exhibited similar temporal shifts in gut microbiome composition that differed from males, these differences were small (Fig. 9.3).

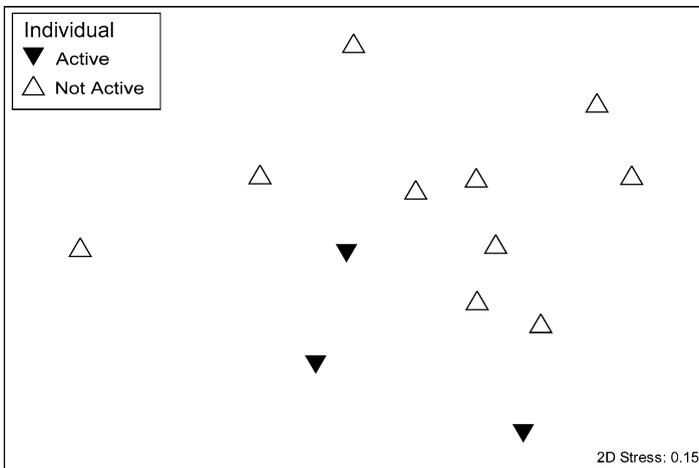


**Fig. 9.3** Principal response curves depict weekly variation in gut microbiota composition in adult females and juveniles when compared to adult males (baseline)

PerMANOVA revealed that howler group, age/sex, and individual identity as well as sampling week accounted for approximately 41.2 % of the variation in gut microbiome composition. Of this, approximately 16.0 % of the variation was explained by individual howler identity ( $F_{10, 105} = 2.09, p = 0.001$ ), 4.3 % by howler group ( $F_{1, 105} = 5.62, p = 0.001$ ) and age/sex class ( $F_{2, 105} = 2.79, p = 0.001$ ), and 2.1 % by sampling week ( $F_{1, 105} = 2.68, p = 0.001$ ). When female gestation was incorporated into the age/sex class data, it explained more variation than age/sex class alone ( $r^2 = 0.062, F_{3, 105} = 2.68, p = 0.001$ ). Similarly, when female gut microbiome patterns were depicted across the study period using NMDS, composition varied with reproductive status (ANOSIM  $R = 0.473, p = 0.001$ ; Fig. 9.4). Average gut microbiome composition across the study period was distinct for reproductively active females and other individuals (ANOSIM  $R = 0.262, p = 0.023$ ; Fig. 9.5). However, Firmicutes:Bacteroidetes proportions did not differ across age, sex, or reproductive classes. There were also no strong patterns in *Bifidobacterium*, *Bacteroides*, or *Enterobacteriaceae* (although the lactating female had higher *Enterobacteriaceae* abundances than any other individual sampled). *Oxalobacter*, a bacterium which increases calcium availability (Stuart et al. 2004; Nakata and McConn 2007), was present in both pregnant females and detected in only one other individual in lower abundance (0.007 % vs. 0.033 % of total sequences).



**Fig. 9.4** NMDS demonstrates clustering of female gut microbiota composition by reproductive status. Symbols represent all samples collected for each female by week. Pregnant females ( $N = 2$ ) exhibit different gut microbiota composition than the lactating female or the nonreproductively active female



**Fig. 9.5** NMDS demonstrates clustering of gut microbiota composition by individual reproductive status. Each symbol represents an average gut microbiota composition for each individual during the study period. Adult males, females, and juveniles that are not reproductively active exhibit distinct microbiota composition from reproductively active females

Gut microbiota activity assays revealed few differences. Fecal ammonia concentration did not vary significantly by age or sex ( $F_{2,10} = 2.04$ ,  $p = 0.18$ ) or by reproductive status ( $F_{1,11} = 0.016$ ,  $p = 0.90$ ). The proportions of fecal volatile fatty acids were similar for all individuals (average molar ratio of acetate to propionate

to butyrate=84.8:8.4:4.4) and generally matched those reported for *A. palliata* (Milton et al. 1980). However, adult females and juveniles had significantly higher total fecal VFA content than adult males (adult female  $1,218.96 \pm 126.67$   $\mu\text{g/g}$  fecal material/kg body weight, adult male  $907.70 \pm 26.08$  mM/kg body weight, juvenile  $1,427.34 \pm 368.51$   $\mu\text{g/g}$  fecal material/kg body weight;  $F_{2,7}=7.64$ ,  $p=0.017$ ). Pregnant females also had somewhat lower total fecal VFA content than nonpregnant females (pregnant  $1,120.89 \pm 97.13$   $\mu\text{g/g}$  fecal material/kg body weight, nonpregnant  $1,317.03 \pm 15.10$   $\mu\text{g/g}$  fecal material/kg body weight;  $F_{3,6}=6.02$ ,  $p=0.031$ ).

Together these data indicate that the gut microbiota may play an important role in satisfying nutritional demands in juvenile and reproductively active female black howlers. Howlers did not show strong differences in activity patterns or diet according to age, sex, or reproductive status that would imply they are reducing activity or increasing energy and nutrient intake to compensate for differences in nutritional demands. However, variation in gut microbiome composition was in part explained by howler age, sex, and reproductive status, and differences in gut microbial activity were detected across age and sex classes. Low variation in total microbiome composition associated with howler age, sex, and reproductive status is likely a consequence of shifts in only a subset of microbial taxa or functional groups and the somewhat limited resolution of community fingerprinting. However, as indicated by the sequencing results, some microbial taxa may be important for juvenile and reproductively active female nutrition. More research describing microbial function is necessary to understand these patterns as are studies with larger sample sizes and longevity.

Additionally, microbial activity data suggest that juvenile and female howler monkeys are processing the same diet differently (producing different amounts of VFA) even if shifts in microbiota composition are limited. Increases in fecal VFA content may indicate higher energy production by females and juveniles. However, variation in energy absorption among individuals is unknown. As a result, lower VFA levels for pregnant females may not indicate that they produce less energy but rather that they absorb more of it than other individuals. Additional measures of digestive efficiency are necessary to separate these processes. Nevertheless, the production of distinct amounts of VFA by individuals of different age, sex, and reproductive classes is likely to have important consequences for host digestive efficiency and ultimately nutrition.

Although this study provides preliminary evidence validating our revised bioenergetics model, additional data are necessary to confirm the roles of diet, activity, gut morphology, and the gut microbiota in allowing howlers to meet nutritional demands. Furthermore, because behavior and diet may change seasonally depending on climate and/or food availability (Overdorff et al. 1997; Altmann 2009; Grueter et al. 2009; Marshall et al. 2009), cross-seasonal data must be collected to examine how the relationships and importance of these mechanisms vary. Future studies should also integrate C-peptide measurements from juveniles and reproductively active females [or other estimates of host nutritional status since pregnancy is known to interfere with insulin production (Havel 1998)] to test whether changes in diet, activity, and/or digestive efficiency actually allow hosts to meet nutritional demands and maintain body condition.

## 9.5 Application of Microbiota-Centered Models to Other Primate Taxa

While howler monkeys are an ideal system for exploring host-gut microbe interactions, the models developed in this chapter should extend to other primate taxa as well and allow us to predict both intraspecific and interspecific relationships among gut microbiota composition and host diet, health, and nutrition. Although studies of rodent and human gut microbiomes are still the most numerous due to the implications of gut microbes in human health and disease, some initial investigations of nonhuman primate gut microbiota composition and function exist. These investigations have generally focused on catarrhines (Frey et al. 2006; Fujita and Kageyama 2007; Uenishi et al. 2007; Kisidayova et al. 2009; Nakamura et al. 2009; Szekely et al. 2010; Degnan et al. 2012; Moeller et al. 2012; McCord et al. 2013; Moeller et al. 2013), although other primate taxa, such as the pygmy loris (*Nycticebus pygmaeus*) (Bo et al. 2010; Xu et al. 2013) and the black and mantled howler monkeys (*A. pigra*, *A. palliata*) (Nakamura et al. 2011; Clayton et al. 2012; Amato et al. 2013) also have been sampled.

In most of these studies, the main goal is to provide data regarding the composition of the gut microbiota. As a result, we know that the three dominant bacterial phyla in human and nonhuman primate gut microbial communities are Firmicutes, Proteobacteria, and Bacteroidetes (Frey et al. 2006; Yildirim et al. 2010). However, because these studies present little to no information regarding host diet, health, age, sex, or reproductive status, patterns and functions of intraspecific gut microbiota variation are difficult to discern. For example, a study of 23 chimpanzees revealed differences in gut microbiome composition between wild and captive individuals (as indicated by different TGGE band profiles), with wild chimpanzees' feces containing more bacteria such as *Clostridium*, *Ruminococcus*, and *Eubacterium* (Firmicutes), which are known for their sugar-fermenting and cellulolytic activity (Uenishi et al. 2007). Similarly, studies of African apes suggest an influence of host geography on gut microbial community composition that results in convergence of the gut microbial community for individuals of the same host species inhabiting the same area as well as for individuals of distinct host species (Degnan et al. 2012; Moeller et al. 2013). Although these results indicate that diet may play a role in determining ape gut microbiota composition, no data describing differences in microbial richness and diversity or host diet are provided.

Literature-based investigations of the gut microbial influence on individual bioenergetics within primate species are equally limited. Only one study of 12 wild chimpanzees (*P. troglodytes schweinfurthii*), belonging to the same social group and including parent-offspring pairs, has to some extent provided detailed data at the individual level (Szekely et al. 2010). However, this study reported that while the most common bacterial phyla (Firmicutes and Bacteroidetes) were shared by all individuals, indices of microbial community similarity were only high among a few samples. Therefore, while the authors suggest that kinship might play a role in determining microbial community composition, the identification of patterns across age and sex classes in response to growth and reproduction is not possible.

Although the overall scarcity of data limits our ability to more formally test the predictions of our models *within* a given nonhuman primate species, there exists a growing data set which can be used to compare *across* species. For example, a recent survey compared fecal samples from 23 free-ranging great apes (*Pan troglodytes*, *P. paniscus*, *Gorilla gorilla*, and *G. beringei*) and two humans (Ochman et al. 2010). Since a phylogeny based on the microbiome composition in these samples matched the great ape species phylogeny (mtDNA) more closely than a phylogeny based on the chloroplast sequence diversity (an indicator of diet) in these samples, the authors argue that host phylogeny is the most likely determinant of primate gut microbiota composition. However, branch length in the mtDNA phylogeny only explained 25 % of the variance in the gut microbiome tree, leaving a large percentage of the variation unexplained. Additionally, because these plant DNA sequences cannot distinguish between leaves and fruits of the same species, and because diet data obtained from plant DNA in fecal material have been shown to differ somewhat from observational dietary data (Bradley et al. 2007), chloroplast diversity may not completely describe host diet. In fact, when the comparison was expanded to include populations of the same species of apes in different habitats, the data suggested that host geography affects the magnitude of differences in the gut microbiota across host species (Moeller et al. 2013). Further evidence is necessary to better understand the influence of diet on interspecific gut microbiota composition and revise our model.

In fact, other studies provide evidence in support of this aspect of our model. Although a molecular analyses of nine fecal samples collected from sympatric wild, foregut-fermenting *Colobus guereza* and *Ptilocolobus tephrosceles* and hindgut-fermenting *Cercopithecus ascanius* also revealed an influence of host phylogeny on gut microbiota composition (Yildirim et al. 2010), some effects of diet were clear. In particular, bacterial diversity and community composition analyses showed that the more-folivorous red colobus monkeys were characterized by the highest bacterial richness and highest diversity compared with the other two species. Similarly, a study by Lambert and Fellner (2012) reported significantly higher fecal acetate concentration in *Colobus guereza* (61 mol%) than in *Cercopithecus neglectus* (47 mol%). Although these data do not provide information on gut microbiota composition, they illustrate differential microbial activity possibly associated with dietary and digestive strategies.

Additionally, based on our model, we would predict that primate species specializing on a high-fiber diet such as gorillas or leafy diets such as colobines and indriids would harbor higher abundances of cellulolytic and proteolytic bacteria such as Clostridia and Eubacteria. Studies describing the gut microbiota composition of a variety of nonhuman primates verify these predictions. The fecal bacterial analysis of a wild male gorilla (*G. beringei*) (Frey et al. 2006) indicated a high abundance of Clostridia (51.5 %), as well as the presence *Ruminococcus flavefaciens* (a cellulolytic bacterium) and *Eubacterium oxidoreducens*, which decarboxylates gallate, a phenolic compound found in plant flavonoids, tannins, and lignin. These bacteria could confer an advantage to gorillas when consuming pith containing high cellulose concentrations (17.5–19.8 % of dry matter intake) (Rothman et al. 2007) and

condensed tannins (Rothman et al. 2006). Likewise, the pygmy loris, an insectivorous primate which also includes fruits, gums, and small mammals in its diet, possesses more gut bacteria with proteolytic activity such as *Bacteroides* (Bacteroidetes) and carbohydrate-degrading Proteobacteria (34.5 %) than other primate species (0.6–2.2 % reported in the study of the two colobus species and the guenon) (Bo et al. 2010; Yildirim et al. 2010). Gut microbiota analyses also uncovered several species of the genus *Pseudomonas* (Proteobacteria) in the pygmy loris, some of which are known to degrade organic solvents such as toluene (Marques and Ramos 1993) and might play a role in the digestion of toxic insects and other plant material. This capacity of the pygmy loris gut microbiota for processing plant toxins was recently confirmed using metagenomic analyses (Xu et al. 2013).

While results from current primate gut microbiome studies provide some general support for the importance of diet and genotype on gut microbiota composition on an interspecific level, more data from both within and across primate species are necessary to fully test the validity of these relationships in our model. Likewise, intra- and interspecific studies investigating the effects of microbiota composition shifts on host health and nutrition are critical for understanding the role that the gut microbiota plays in host ecology and evolution. Much work remains to be done to improve our understanding of the host-microbe relationship in primates, but we hope that the models presented in this chapter will provide a guide for future project designs.

## 9.6 Conclusions

The studies reviewed in this chapter provide important baseline data with regard to the interactions between the mammalian gut microbiota and host diet, nutrition, and health. However, many questions remain unanswered regarding the role of the gut microbiota in wild host populations, especially in the case of primates. In even the most comprehensive primate microbiome studies, data reflecting the dynamics of the gut microbiota across time and space are largely absent (but see Amato 2013; Amato et al. 2013; McCord et al. 2013), and studies that integrate behavioral and ecological data with microbiome analyses of primates in their natural habitats simply do not exist. Data obtained from laboratory studies in a variety of taxa have allowed us to develop models to predict patterns in host-gut microbiota interactions in natural habitats, which can then be tested in field settings. Howler monkeys are an excellent system for testing these models due to their presence in a wide variety of habitats with diverse types of plant resources and their nutritional reliance on the gut microbiota. Additionally, improving the understanding of how howlers adjust to changing nutritional demands when diet is constrained has important implications for the study and conservation of these primates. It has recently been suggested that conservation biology could benefit greatly from the research on the human microbiome, by applying its methods and frameworks to improve conservation goals such as reintroductions, captive breeding, and dealing with invasions of nonnative species (Redford et al. 2012). If gut microbiota diversity, composition, and turnover are

related to dietary flexibility, conservation of endangered howler species in the face of habitat fragmentation may depend, in part, on a better understanding of host-microbe relationships since fragmentation alters the amounts and types of resources to which the howlers have access (Cristobal-Azkarate and Arroyo-Rodriguez 2007; Dunn et al. 2009; Arroyo-Rodriguez and Dias 2010).

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