

Adapt or Live

Adaptation, Convergent Evolution, and Plesiomorphy

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Introduction

Traditionally, the ecological theory of adaptive radiation relied on the fossil record to examine the relationship between increases in taxonomic diversity and the rates of evolution for, or emergence of, certain traits (Hunter and Jernvall 1995; Simpson 1953). A biological radiation was originally defined by observations of rapid diversification in taxonomic and trait diversity (Osborn 1902). Elaborating on this recurrent pattern, Simpson (1953) explained adaptive radiation as the result of high rates of diversification in species and traits after encountering ecological opportunity, followed by slowdowns in both rates as the opportunities were exhausted (Losos and Mahler 2010). The engine of diversity was therefore explicitly ecological, with new niches—whose sum Simpson called the “adaptive zone”—becoming available by reaching previously unexploited resources in new geographical areas, through the evolution of key innovations, or because of the extinction of competitors. As new species fill the niches in the new adaptive zone, their phenotypes diversify to match the diverse resources.

Based on these insights, and translating them to an explicitly phylogenetic framework, Schluter (2000) summarized the characteristics of adaptive radiation thus: (1) common ancestry, (2) correlated phenotype and ecology, (3) adaptive phenotype has higher fitness in new niche, and (4) rapid taxonomic and phenotypic evolution. Hence, ecological differentiation, competition, and opportunity are at the center of the theory, which remains the only general theory of how coupled taxonomic and ecological diversity evolve (Harmon et al. 2010; Rabosky et al. 2015). Schluter’s (2000) framework translates into a series of predictions to be tested using phylogenies and comparative data. In addition to the relatively easily tested monophyly

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of proposed adaptive radiations, these predictions include bursts of diversification in response to ecological opportunities (Wagner et al. 2012; Yoder et al. 2010), diversity-dependent decreases in rates of taxonomic and trait diversification (Harmon et al. 2010; Rabosky and Glor 2010), and deterministic evolution of convergent phenotypes in response to ecological opportunities (Mahler et al. 2013; Reynolds et al. 2016).

Given that phyllostomids make up the only mammalian family to include species that feed on diets ranging from blood to figs, and the cranial anatomy of different species closely matches their ecological specialization (Dumont et al. 2014), the entire family has traditionally been assumed to be an adaptive radiation (Freeman 2000). Nevertheless, this is more often asserted than tested (Dumont et al. 2012) and has not been definitely established using current phylogenetic methods. Here we apply the phylogenetic framework of the theory of adaptive radiation to review comparative analyses of phyllostomid taxonomic and trait diversity. We also introduce new analyses of an index of trophic level, aiming to test for both distinct trophic adaptive zones and convergence in the evolution toward those zones across the family. Finally, we review a series of proposed adaptations, all associated with dietary specialization, discussing their implications and the questions emerging from them. This synthesis reveals both the wealth of evidence for increased fitness associated with derived features characterizing most plant-visiting lineages and the long-term persistence of ancestral dietary habits and traits across the family.

Methods

We applied new comparative methods to evaluate adaptation, ecological convergence, and plesiomorphy (or conservation of traits/phenotypes) as mechanisms underlying the diversity of trophic levels characteristic of phyllostomids. The response in the model was obtained from a continuous trophic-level metric developed based on the natural history literature (Rojas et al., in review). Briefly, the trophic-level index is given by $\log_{10}\left\{\frac{(1 + \sum a_i + n_a)/n_a}{(1 + \sum h_i + n_h)/n_h}\right\}$, in which $\sum a_i$ sums the relative importance of animal diets, while $\sum h_i$ is the counterpart for herbivory, with n_a quantifying the number of animal items in this category, and n_h the number of plant items. The index ranges from negative

values or low trophic level, to positive for high-trophic-level diets. Although this variable will not capture changes from, for example, 100% frugivory to 100% nectarivory, it will illuminate the transition from the ancestral chiropteran diet of primarily insects to both the carnivorous and frugivorous extremes that characterize the family (Freeman 2000).

The evolutionary pattern expected under adaptive radiation is open to debate. While all models emphasize that rapid taxonomic diversification is expected in every case, trait evolution can be modeled in different ways. An early burst of evolution followed by subsequent slowdown, for instance, is a poor fit to most adaptive radiations, including several classical examples such as Caribbean *Anolis*, Galapagos finches, or cichlids of the Great Lakes of Africa (Harmon et al. 2010). In all these cases, models specifying either adaptive optima or random walk fit the data better than early burst models, probably because both adaptive and partitioned Brownian motion models can capture aspects of directional evolution (Collar et al. 2009; Harmon et al. 2010). In practice, an adaptive model specifying multiple peaks can better fit adaptive radiations and simultaneously test for the convergence expected when adaptation to the ecology occurs (e.g., Mahler et al. 2013). Here, we focus on the adaptation expected under adaptive radiation and therefore use directional models with more than one peak in phylogenetic tests. To test for adaptive evolution we fitted the data to the comprehensive phylogeny of Phyllostomidae by Rojas et al. (2016) using an Ornstein-Uhlenbeck (OU) process of evolution (Butler and King 2004). The OU model enriches the Brownian motion model of evolution for continuous traits by two parameters, a rate of directional evolution often interpreted as adaptation α , and one or several adaptive optima or regimes with their values given by one or several θ (Hansen 1997).

To speed the analyses of the data, we applied a recently published lasso implementation of the phylogenetic OU model (Khabbazian et al. 2016). The lasso method penalizes the absolute value of the parameters leading to multiple coefficients estimated at zero (Tibshirani 1996) and thus overcomes the nonidentifiable nature of phylogenetic OU models without predetermined regimes (Bastide et al. 2016). As with previous methods to test nonpredetermined OU models (e.g., Ingram and Mahler 2013; Uyeda and Harmon 2014),

the lasso method implemented in the R package *l1ou* (Khabbazian et al. 2016), examines shifts along all available branches. A newly developed phylogenetic Bayesian information criterion was applied to compare models (Khabbazian et al. 2016). Unlike commonly used criteria, such as the Akaike information criterion (AIC), the phylogenetic Bayesian information criterion accounts for phylogenetic correlation, and therefore its value is not strictly dependent on the number of shifts to different adaptive optima. Finally, to quantify the support for changes in adaptive zones along particular branches, we ran 100 bootstrap replicates. The method implemented in *l1ou* resamples the phylogenetic residuals of the regression of trait values against the inferred regimes (Khabbazian et al. 2016). To speed convergence and prevent parameter overfitting (Ho and Ané 2014), the ceiling on the number of regimes was set at the square root of the tips, or 13.

Additionally, we reviewed the literature on phyllostomid evolution since 2000, focusing on comparative analyses of either genes or continuous traits. In particular, we examined analyses in which specific adaptive or convergent hypotheses were tested, whether or not models such as the one applied here were used. Only analyses including at least three species were included; adaptations corresponding to single species are excluded from discussion as these are not comparative (e.g., ultraviolet-sensing cones in *Glossophaga soricina* Winter et al. [2003], or alternative splicing of genes linked to infrared sensation Gracheva et al. [2011]).

Results and Discussion

Parameters of the OU models fitted to the trophic-level data are summarized in table 7.1, and the inferred shifts

Table 7.1. Parameters inferred for Ornstein-Uhlenbeck (OU) models fitted to the trophic-level data on the phyllostomid phylogeny

Model	Number of regimes	pBIC
Ornstein-Uhlenbeck no convergence	6	-244.8
OU convergent evolution	5	-245.2

Note: Phylogenetic Bayesian information criterion, pBIC, lower values correspond to better-fitting models. A regime is a group of branches sharing a common adaptive optimum.

in adaptive optima are shown in figure 7.1. Five adaptive zones, two of them showing ecological convergence in trophic level, were identified. Comparative analyses of potential adaptations in quantitative traits are summarized in table 7.2. Comparative analyses finding potential genetic adaptation are summarized in table 7.3.

Evidence for Adaptive Radiation in Phyllostomids: Taxonomic and Trait Diversification

Analyses of traits for phyllostomid bats are relatively new compared to other classical examples of adaptive radiations, from fishes to anole lizards (e.g., Collar et al. 2014; Losos 2010; Near et al. 2013; Pinto et al. 2008), and consequently the literature to date has primarily focused on a few traits and even fewer genes. Nevertheless, several patterns emerge from both the new analyses of trophic-level evolution and the literature. In short: (1) a series of morphological, physiological and genetic adaptations to plant-based diets and frugivory in particular are well documented; (2) part of the evidence of adaptation arises from the convergent evolution of traits associated with lower trophic levels, from olfactory receptor repertoires to the decrease in lower trophic level itself; (3) although convergent, the evolution of higher trophic level from plesiomorphic ancestors is associated with distinct, nonconvergent morphologies; and (4) the only trophic adaptation associated with higher rates of diversification is that of the fig-eating stenodermatines. We begin by summarizing and examining the results of the new analyses, before reviewing the evidence for emerging patterns.

Although greatly advanced since 2000, quantitative analyses among phyllostomids have focused almost exclusively on taxonomic diversification and traits associated with feeding ecology. A single shift toward higher taxonomic diversification in stenodermatines was first reported by Dumont et al. (2012), then confirmed by Rojas et al. (2016) and Shi and Rabosky (2015). None of these studies found diversity-dependent declines in taxonomic diversification rates, and only Dumont et al. (2012) correlated the shift to ecologically relevant traits. In particular, and in support of stenodermatines as an adaptive radiation, Dumont et al. (2012) found a correlation between phenotype (cranial morphology) and ecology (trophic level) with the phenotype evolu-

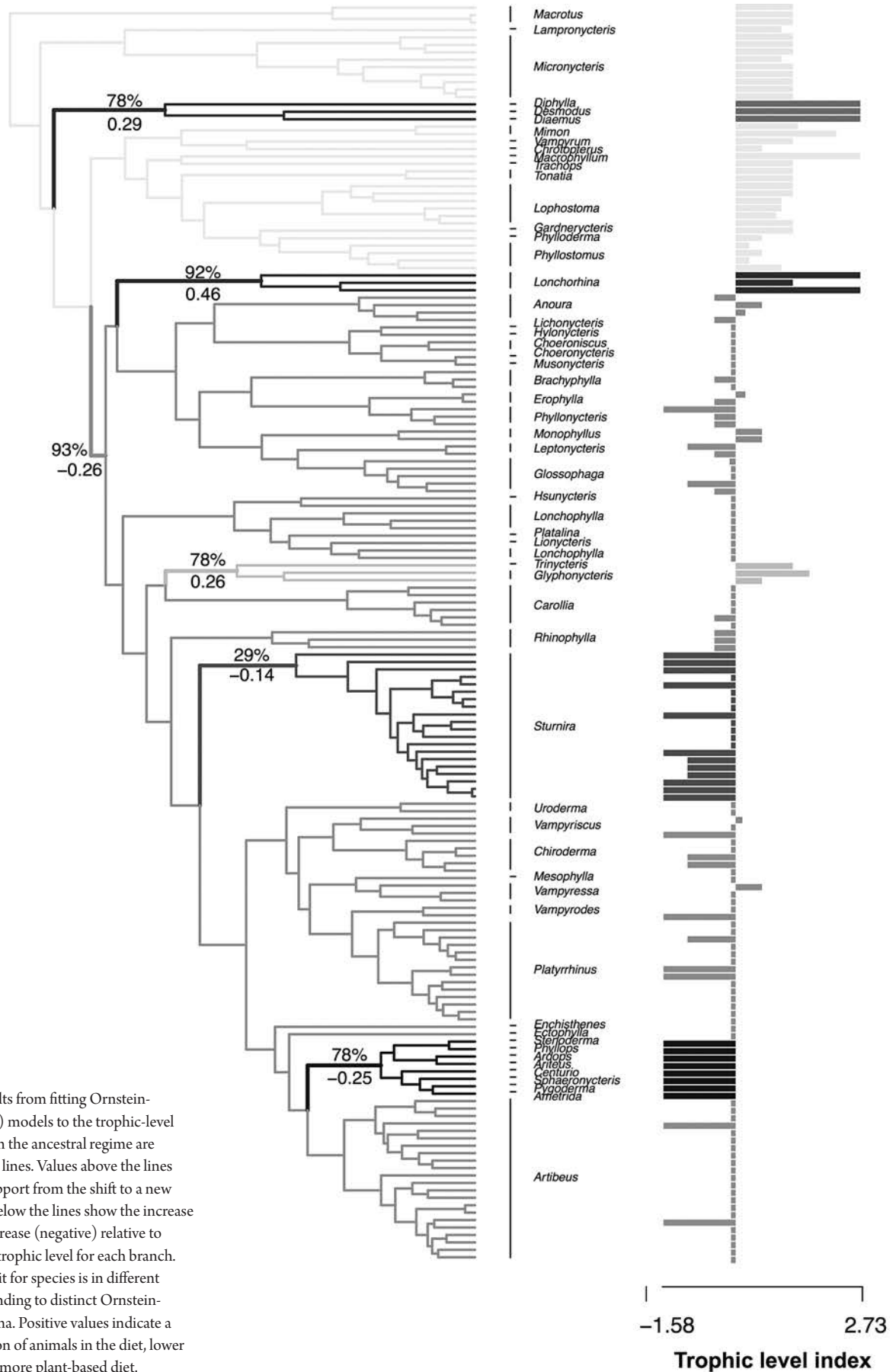


Figure 71. Results from fitting Ornstein-Uhlenbeck (OU) models to the trophic-level index. Shifts from the ancestral regime are shown in thicker lines. Values above the lines are bootstrap support from the shift to a new regime. Values below the lines show the increase (positive) or decrease (negative) relative to the background trophic level for each branch. Trophic-level trait for species is in different shades corresponding to distinct Ornstein-Uhlenbeck optima. Positive values indicate a greater proportion of animals in the diet, lower values point to a more plant-based diet.

Table 7.2. Annotated list of studies on (potential) trait adaptation, ecological convergence, or plesiomorphy in phyllostomids since 2000

Traits	Function	Taxonomic scope	Source
Skull proportions from linear measurements	Food acquisition	"Microchiroptera"	Freeman 2000
Intestinal sucrase, maltase and trehalase activity, relative medullary thickness of kidneys	Digesting nectar and fruit	Phyllostomidae	Schondube et al. 2001
Nephron and medullary morphology	Keep ions/concentrate urine	New World Chiroptera	Casotti et al. 2006
Intake response behavior for different sugars	Optimal nutrition	<i>Leptonycteris</i> , <i>Glossophaga</i> , <i>Artibeus</i>	Ayala-Berdon et al. 2008
Feeding habits	Food acquisition	Phyllostomidae	Rojas et al. 2011
Feeding habits and qualitative skull shape	Food acquisition	Phyllostomidae	Baker et al. 2012
Principal components of linear skull measurements, trophic level	Pierce through figs	Phyllostomidae	Dumont et al. 2012
Skull shape and loading behavior	Food acquisition	Phyllostomidae	Santana et al. 2012
Craniodental characters	Food acquisition	Phyllostomidae	Dávalos et al. 2012
Mechanical advantage and von Mises stress derived from engineering model of the skull	Food acquisition	Phyllostomidae	Dumont et al. 2014
Digestive capacity	Digestion	<i>Carollia</i> , <i>Sturnira</i> , <i>Artibeus</i>	Saldaña-Vázquez et al. 2015
Roost preference	Roosting	Amazonian Chiroptera	Voss et al. 2016

Table 7.3. Annotated list of studies on (potential) genetic adaptation and ecological convergence in phyllostomids since 2000

Traits	Function	Taxonomic scope	Source
Intestinal microbiome	Possibly digestion	New World Chiroptera	Phillips et al. 2012
Mitochondrial <i>cytochrome b</i> gene	Possibly optimize electron transport	Phyllostomidae	Dávalos et al. 2012
Olfactory receptor gene diversity analyzed as principal component of frequencies	Detect fruit	Chiroptera	Hayden et al. 2014
Intestinal microbiome	Possibly digestion	Phyllostomidae	Carrillo-Araujo et al. 2015
<i>SLC2A2</i> promoter deletion	Increase glucose transport to liver	Chiroptera	Meng et al. 2016

ing more slowly within the group than outside it. As expected when new ecological opportunities become available (Yoder et al. 2010), the rates of trophic-level evolution in stenodermatines were higher than among other phyllostomids (Dumont et al. 2012). Finally, while the fitness of the new cranial architecture of stenodermatines was not directly assessed, the correlation between cranial morphology and bite force was a measure of fitness for an adaptive zone including hard fruits (figs). Applying the formal definition of adaptive radiation, especially the expectation of higher specia-

tion rates, the results of diversification analyses shift the focus from all phyllostomids (e.g., Baker et al. 2012; Freeman 2000) to the subfamily Stenodermatinae.

Olfactory Receptor Genes

Olfactory receptor (*OR*) genes are the largest family of protein-coding genes in mammals (Buck and Axel 1991), and extensive gains and losses of gene subfamilies underlie ecological shifts across mammals (Hayden et al. 2010; Niimura and Nei 2007). Each gene codes for one olfactory receptor protein expressed in olfac-

tory epithelial cells, where odor perception occurs when volatile compounds bind to *ORs* in a combinatorial fashion (i.e., combinations of *ORs* recognize different odorants (Malnic et al. 1999). The composition and diversity of *OR* genes is therefore expected to reflect ecological adaptation across lineages, but, besides a handful of primate species, this had not been tested within a mammalian order (e.g., Matsui et al. 2010). To explore *OR* dynamics in an ecologically diverse clade, Hayden et al. (2014) sequenced paralogs within the gene family across bats. Focusing on hypotheses of exchange between sensory modalities, bat species were classified according to their use of laryngeal echolocation, as well as the presence of a functional vomeronasal organ. Additionally, the different species were also characterized by feeding ecology to investigate the interplay of sensory modalities and diet with *OR* genes.

Birth-death dynamics, in which genes easily duplicate and are lost even within populations (Nei and Rooney 2005), make it difficult to use standard measures of genetic adaptation in *OR* genes. With sequences from thousands of *OR* genes across 27 species of bats, Hayden et al. (2014) summarized the composition and diversity of genes by collapsing the data into phylogenetic principal components based on the relative frequencies across gene subfamilies and between putatively functional genes and pseudogenes—those with premature stop codons or shifts in the reading frame—as well as the proportion of pseudogenes found. After accounting for phylogeny, there were no significant differences between echolocating and nonecholocating bats for all *OR* genes, functional genes or pseudogenes, or the proportion of pseudogenes. Likewise, no differences were found between species with a functional vomeronasal organ versus those in which the vomeronasal organ is nonfunctional, for taxonomic groups such as Yinpterochiroptera versus Yangochiroptera, or between frugivorous and nonfrugivorous species across Chiroptera.

In contrast, examining *OR* gene diversity in frugivorous (Stenodermatinae) versus nonfrugivorous phyllostomids yielded significant results for all *OR* genes, functional genes, and pseudogenes. Relative to other phyllostomids, stenodermatines show increases in the relative diversity of *OR* gene subfamilies 1/3/7 and, to a lesser extent subfamilies 2/13. In non-stenodermatines, *OR* gene subfamilies 5/8/9 is better represented and

appears to be more important. Within Yinpterochiroptera, the *OR* gene subfamilies 1/3/7 and 2/13 lie on the main axis of differentiation between pteropodids and insectivorous Yinpterochiropteran bats for all genes and functional genes. In contrast to phyllostomids, significant differentiation in pseudogenes of Yinpterochiroptera lies on axes defined by *OR* gene subfamilies 11 and 52, separating pteropodids from other clades. In short, the greater representation of gene subfamilies 1/3/7 and 2/13 across clades of frugivorous bats evolving independently in the New World and Old World shows ecological convergence, while differences in patterns of pseudogenization lead to nonsignificant comparisons for all bats. In phyllostomids, similar patterns for all *OR* genes, functional genes, and pseudogenes may indicate adaptive changes linked to ecological specialization, while a similar pattern emerges in the frugivorous Pteropodidae but through greater pseudogenization in other gene subfamilies (Hayden et al. 2014). The results illuminate a clear genetic shift linked to the evolution of stenodermatines, and convergence with other frugivorous bats suggests adaptation to a specialized and previously unavailable diet.

Roosting Ecology

While most attention on phyllostomids in general and stenodermatines in particular has focused on diet and dietary diversity, Voss et al. (2016) analyzed the surprising diversity of roost types in the family. By assigning phyllostomid taxa to seven roosting guilds defined for Amazonia based on observations and data collected from the literature, Voss et al. (2016) found that roosts might be a limiting resource across lowland rainforest habitats, structuring communities to an extent hitherto unknown. Importantly, stenodermatines comprise almost all phyllostomid species known to roost in foliage and all species in the family known to make tents by modifying leaves. As the same skull architecture that enables stenodermatines to bite through figs despite their small size (Dumont et al. 2012) enables making leaf tents, the invasion into a new roost adaptive zone may be another factor contributing to the diversity of the subfamily. While the importance of these traits to adaptation or adaptive radiation remains unknown, a new roosting adaptive zone may contribute to increases in diversification rates at the base of the stenodermatine radiation.

Evidence for Adaptive Radiation in Phyllostomids: Multiple Adaptive Zones

Multiple adaptive zones corresponding to a variety of ecological resources are expected in adaptive radiations (Price and Hopkins 2015). As the Dumont et al. (2012) analyses focused on localizing shifts in taxonomic diversification and correlates of that shift, there was no exploration of the diversity of phenotypes known across phyllostomids. The first study to quantify diverse phyllostomid adaptive zones is the analysis of the evolution of functional cranial traits by Dumont et al. (2014). By fitting increasingly complex Ornstein-Uhlenbeck models to traits derived from engineering models of the skull, Dumont et al. (2014) tested whether these phenotypes corresponded to different adaptive zones for three traits: mechanical advantage and two types of biting stress. Using a set of predefined groups corresponding to (from longest to shorter skulls) nectarivores, other phyllostomids, stenodermatines except short-faced bats, and short-faced bats, Dumont et al. (2014) tested the fit of the data to increasingly complex models. A model with four adaptive zones was supported for mechanical advantage, although the adaptive optima of both other phyllostomids and most stenodermatines overlapped, supporting convergent evolution for this trait. In contrast, unilateral biting stress had optima corresponding to all stenodermatines, nectarivores, and other phyllostomids, and bilateral biting stress had two optima comprising nectarivores and all other phyllostomids (Dumont et al. 2014). There is, therefore, evidence of adaptation into divergent adaptive zones for mechanical advantage—a strong correlate of bite force—as expected if these traits contributed to the adaptive radiation of all phyllostomids.

The evolution of trophic level in phyllostomids has been intimately linked to changes in cranial morphology (Dumont et al. 2012; Freeman 2000) and traits associated with it, such as bite force (Dumont et al. 2014). But associated skeletal traits and their correlates are not the only ones relevant to shifts in trophic level. Santana et al. (2012) used Ornstein-Uhlenbeck models to relate dietary hardness to the evolution of the temporalis and masseter muscles and to relate phylogenetic regressions of loading behavior to muscles as well as skull shape. They found multiple distinct adaptive optima for both muscles and for observed mechanical advantage. Fur-

ther, skull and muscle measurements best explained loading behaviors (a quantitative summary of the type of bite bats take for each mouthful) across phyllostomid species. These analyses highlight the tight association among form, function, and behavior, which results in adaptive phenotypes given diets of very different hardness. Additionally, support for multiple adaptive optima for muscle configuration across phyllostomids corroborates the adaptive nature of cranial morphology in the family (Santana et al. 2012).

Evidence for Adaptive Radiation in Phyllostomids: Within-Family Ecological Convergence

Deterministic convergent evolution is one of the predictions from the phylogenetic formulation of the ecological theory of adaptive radiation that remains untested. Through analyses that fit free—not predetermined—Ornstein-Uhlenbeck models to trophic-level data, our results provide the first quantitative evidence for convergence across phyllostomids (table 7.1, fig. 7.1). Despite the obstacles posed by previous phylogenetic hypotheses, several previous studies had either asserted or at least hinted at trait convergence (Freeman 2000; Rojas et al. 2011). Freeman (2000), for example, attributed the morphological diversity of phyllostomids to the ancestral inclusion of some plant food, resulting in the subsequent evolution of specialized cranial types associated with different plant-based diets. The data show a few species with reduced trophic level, reflecting occasional plant consumption, even among phyllostomines such as *Phyllostomus* (fig. 7.1). Seven trophic-level regimes or optima can be differentiated corresponding to the ancestral niche (1), the higher trophic level of vampire bats (2), *Lonchorhina* (3), a shared low trophic level for all nectar-feeding and most frugivorous lineages (4), with a secondary increase in level of glyphonycterines (5), and the reduction in level among *Sturnira* (6) and short-faced bats (7). Of these, the increases in animalivory of vampire bats and *Lonchorhina* are convergent. The secondary increase in trophic level in glyphonycterines is particularly relevant, as it upends the widely held assumption that no lineage reverts to insectivorous habits (Baker et al. 2012).

Another way to quantify these results is to compare the rate of directional evolution α to the Brownian

motion rate of change σ , as the latter represents the background rate of random-walk change throughout the phylogeny. With a directional rate estimated at 0.32 units/million years, the adaptive rate of change is ~64 times higher than the background rate of change, indicating very strong directional tendencies to particular trophic-level optima. Hence, these first analyses of trophic-level evolution have shown there are shifts in trophic level across phyllostomids that have been shaped by natural selection, with two lineages converging on similarly high trophic levels. Thus, even in the absence of high taxonomic diversification rates during the early history of phyllostomids, the evolution of trophic level is consistent with adaptive trait diversification in diet for the family.

The analyses introduced here are the first to directly model convergence in an ecologically relevant trait, trophic level. These models, however, are not the first to propose ecological convergence to explain the evolution of morphological or even genetic traits. To address the persistent conflict between phylogenies based on morphology and those based on DNA sequences (see chap. 6, this vol.), Dávalos et al. (2012) examined methodological (e.g., taxonomic sampling, character sampling, methods of analysis) and biological (saturation, homoplasy, incongruence between gene trees, adaptive convergence) drivers of incongruence in the phyllostomid phylogeny. As the morphological data of Wetterer et al. (2000) supported clades composed of taxa that shared feeding behaviors strongly rejected by DNA sequences (Baker et al. 2003), there was already evidence for incongruence and conflict. Analyzing an updated set of 220 morphological characters for 80 species combined with roughly matching sequences, Dávalos et al. (2012) sought to minimize the effects of taxon and character sampling on incongruence by adding morphological characters and to reduce methodological conflict by using Bayesian algorithms for both the morphological and DNA sequence data. After removing these method-based sources of error, they found taxon choice produced some, but not all, of the significant phylogenetic conflict, and adding morphological characters and using the same algorithms had no effect on incongruence. In short, previously used methods and sets of taxa or characters were not responsible for the vast differences in phylogenetic results.

After ruling out methods as an explanation for phy-

logenetic conflict, Dávalos et al. (2012) searched for biological causes of incongruence. Here, it is important to consider two mechanisms underlying such conflicts: structural constraints imposed by development and natural selection causing ecological convergence (Wake 1991). Although reflecting very different processes, both mechanisms generate similar patterns of exhaustion in character states (Wagner 2000). Dávalos et al. (2012) found patterns of saturation in DNA substitutions and, more importantly, in morphological characters. But the pattern of morphological character-state evolution was not consistent with a hard ceiling on character states. Instead, the best fit to the character state to steps in tree curve was an ordered model, in which new character states only emerge from derived states (and not from other states). Subsequent analyses of exclusively dental characters revealed similar patterns, with the ordered model fitting the evolution of states for those characters (Dávalos et al. 2014). In short, the pattern of exhaustion of morphological character states is common to more than one data set collected by different teams. This suggests saturation in states may characterize phyllostomids more generally. Additionally, ordered states are also consistent with Ornstein-Uhlenbeck optima for cranial traits (Dumont et al. 2014): traits evolving toward an optimum are equivalent to derived states evolving from other derived states. Character-state saturation in phyllostomid characters, then, is consistent with the adaptive evolution toward a small number of optima. Additional analyses supported this interpretation, as morphological character changes supporting the monophyly of nectar-feeding phyllostomids or *Carollia* as sister to *Rhinophylla* were concentrated in suites of characters associated with feeding (Dávalos et al. 2012). To conclude, detailed analyses suggest that ecological convergence in morphological traits linked to feeding ecology influences the evolution of morphological character states and that adaptation helps explain conflict with phylogenies based on DNA sequences.

Natural selection is not the only process leading to exhaustion of character states in phyllostomids: developmental constraints also contribute to character-state exhaustion and phylogenetic conflict (Dávalos et al. 2014). Using distances between dental characters to quantify the similarity in state changes across characters, Dávalos et al. (2014) found an excess of similarities in change compared to similar analyses for DNA

sequences. That is, there are more correlated changes in states than expected if the different dental characters were evolving independently from one another. Anatomical systems are tightly integrated, and correlated evolution in anatomical traits is to be expected (Vojte et al. 2014, 2013). The result is convergence in form, even when natural selection is not involved. Phylogenetic analyses of morphological characters, however, assume the independent evolution of characters (O’Keefe and Wagner 2001). Although Dávalos et al. (2014) measured the expected excess of similarity, they did not exclude characters to assess their effects on phylogeny (e.g., Herrera and Dávalos 2016) or model the evolution of associated traits to determine the contribution of developmental constraint relative to natural selection in phyllostomid evolution (e.g., Bartoszek et al. 2012). The two latter approaches are promising avenues for disentangling the contributions of adaptation and constraint to evolution and uncovering the role of natural selection in trait diversity.

The pattern of ecological convergence among plant-visiting phyllostomids, and especially among nectar-feeding lineages, is not limited to morphological traits. Instead, there is evidence for convergent DNA substitutions in the mitochondrial *cytochrome b* gene (Dávalos et al. 2012). The first indication of ecological convergence emerged in the phylogenetic analyses of Dumont et al. (2012), which differed from the Baker et al. (2003) phylogeny in making nectar-feeding phyllostomids monophyletic (subfamilies Glossophaginae and Lonchophyllinae as sister taxa). To determine whether the relevant DNA substitutions were adaptive, Dávalos et al. (2012) fit models to estimate the ratio of nonsynonymous to synonymous (silent) substitutions at different branches and codons and ran simulations to determine if codons supporting the spurious nectar-feeding node underwent a shift toward more nonsynonymous changes. If codons with higher ratios tended to support the nectar-feeding node, it would be an indication of positive selection leading to convergent adaptation in both subfamilies. The results showed codons experiencing a ratio shift were massively overrepresented among codons supporting the nectar-feeding node. Further, the codons with shifting ratio were also concentrated in the carboxy-terminus of the protein, which is essential for correct assembly of the respiratory complex (di Rago et al. 1993). This study was the first to relate phylogenetic support for an

ecologically convergent node to shifts in selection and particular protein regions in phyllostomids.

Evidence of Adaptation in Phyllostomids

The previous sections have focused on predictions arising from Simpson’s (1953) ecological theory of adaptive radiation and, in particular, from the phylogenetic framework suggested by Schluter (2000) and developed subsequently (Mahler et al. 2013; Moen and Morlon 2014; Rabosky and Glor 2010; Yoder et al. 2010). However, many more studies have focused on adaptation in general, compared to explicit phylogenetic predictions of adaptive radiation (tables 7.1 and 7.2). Here, we summarize key analyses on adaptation across phyllostomids, seeking to place them within the framework of the ecological theory.

Comparative Physiology

In one of the first quantitative analyses in phyllostomids, Schondube et al. (2001) aimed to test if physiological changes in diet, digestion, and renal function were adaptations linked to changes from the ancestral diet. They collected detailed quantitative data from 16 phyllostomid species using the Wetterer et al. (2000) phylogeny, in which shifts to different diets from insectivory occurred once each (e.g., once to nectarivory, once to sanguivory). If the new diets resulted in physiological adaptations, then intestinal enzyme activity should match the corresponding diets after controlling for evolutionary relatedness. For example, there should be increasing sucrase and maltase activity for plant-based diets, increasing trehalase activity for insect-based diets—as trehalose is the primary insect storage sugar—and constant aminopeptidase N as a stand-in for protein digesting ability. Similarly, the ability to assimilate nutrients and meet metabolic demands would be reflected in the amount of nitrogenous waste and water that the kidney processes and in differences in kidney gross anatomy. Additionally, Schondube et al. (2001) examined the nitrogen isotopic composition of tissues, expecting nitrogen composition to reflect predominantly plant-based or animal-based diets. The data to test all predictions paired five shifts in diet against changes in the relative medullary thickness of the kidney, maltase, sucrose, trehalase, and aminopeptide-N activity.

In general, the adaptive hypothesis was supported

for most digestive traits, and the results for renal function were more nuanced (Schondube et al. 2001). First, shifts from insectivory to carnivory or sanguivory resulted in a large reduction in trehalase activity. While there was no detectable sucrase and maltase activity in the sanguivore, shifts from insectivory to nectarivory or frugivory resulted in increases in sucrase and maltase activity, as well as a decline in trehalase activity. Second, ^{15}N varied predictably with diet: species that ate a largely plant-based diet had lower values, while those that were omnivorous or had a largely animal-based diet had higher concentrations, reflecting the higher trophic level of their primary diets. Third, shifts from insectivory to nectarivory or frugivory were coupled with changes from the ancestral state of having well-developed papillae, and clearly differentiated outer and inner zones in the medulla (insectivory), to reduced medullary papillae and undifferentiated medullae (nectarivory, frugivory). And although no change was predicted to match the shift from insectivory to omnivory, a reduction in renal medullary thickness was also detected. Fourth, the lower water content of meat-based diets was not reflected in kidney anatomy, as there were no anatomical differences corresponding to shifts in diet from insectivory to sanguivory or carnivory. The changes in renal anatomy to reduced papillae and medullar thickness potentially reduce the ability to concentrate urine, and increase the ability to absorb electrolytes, matching the greater availability of water in diets containing nectar and fruit (Schondube et al. 2001). Finally, and despite not being originally highlighted, the ancestral traits linked to insectivory are themselves reflective of trophic specialization into this adaptive zone, to the point that including some plants results in increases in activity of maltase and sucrase and in reductions in medullar thickness. Although maintenance of ancestral phenotypes does not represent adaptation in phyllostomids, it does show how tightly optimized functional traits are to specific diets and how stable these traits have been through time across independent insectivore lineages such as *Macrotus* and *Micronycteris*. In short, there was systematic support for adaptive changes in digestive and renal function linked to the exploitation of new food sources, with the exception of certain kidney traits for lineages expanding their diet into higher trophic levels. This last result is not as surprising as first thought, given that the diets of carnivorous phyllostomids are just one end of

the gradient of animalivory and do not constitute a distinct ecological guild (Giannini and Kalko 2005).

To follow up on the groundbreaking work of Schondube et al. (2001), Casotti et al. (2006) delved into the renal morphology of 21 phyllostomid species, this time using stereology to assess the relative proportion of renal cortex, medulla, and vasculature and renal medullary thickness, as well as the proportions of nephron components and vasculature. Despite earlier findings of no changes in gross morphology in the transition from insectivory to omnivory, Casotti et al. (2006) expected changes in fine anatomy linked to these shifts and to those to frugivory or nectarivory. As the relative water content of the diet increases in each of these shifts, changes to smaller renal medullary surface and thickness and larger renal cortex were expected in each case. In contrast, and based on elements of nephron morphology conserved across vertebrates (Dantzler and Braun 1980), no changes to any nephron components or vasculature were expected with dietary shifts. Using a statistical approach based on Schondube et al. (2001), these predictions were tested using matrices of expected directional changes, one for cortex and medullary components and another for nephron components. The analyses test against a null probability of change in morphological elements at all transitions if all changes were independent and random.

As expected, shifts in diet away from ancestral insectivory to new diets were linked to reductions in the renal medullary surface and thickness and to enlargement of the renal cortex. Unexpectedly, and in line with previous results for gross anatomy by Schondube et al. (2001), no changes in these anatomical traits were found in the transition to omnivory. Also as expected, nephron elements were highly conserved and unrelated to shifts in diet. These results confirm at much finer scale the selective pressures of changes toward a primarily plant-based diet that result in adaptation, and the contrast with omnivory, which results in conserved morphology (Casotti et al. 2006).

The physiological effects of changing diets to include plenty of fruit and nectar are not limited to renal or digestive function. Instead, behavior of individuals should be integrated with their physiological adaptations to preserve metabolic homeostasis depending on relevant sources of energy. To test for this coupling of behavior and energy source, Ayala-Berdon et al. (2008) examined feeding responses in three species—the

nectar-feeding *Leptonycteris curasoae* and *Glossophaga soricina* and the fruit-feeding *Artibeus jamaicensis*. Their experiments varied the composition—sucrose, or a mix of glucose and fructose, both hexoses, at 1:1 ratio—expecting preference for hexoses matching the composition of New World flower nectars. Experiments varying the concentration of artificial nectars aimed to show an “intake response” (Castle and Wunder 1995), in which intake decreases as a function of increases in energy. While all species lacked a preference for sucrose or the hexose mix, like nectar-feeding birds, bats showed the expected intake response: as the concentration of sugar in nectar increased, the amount of nectar consumed, or intake, decreased.

Despite the predominance of hexose-dominated nectars in New World bat-pollinated flowers, the three bat species that Ayala-Berdon et al. (2008) studied exhibited indistinguishable intake responses to the sucrose and the hexose solutions. The intake response of these species to both sucrose and hexose solutions demonstrates that neither sucrose hydrolysis nor uptake of hexoses is the limiting factor in food intake for these three species. Instead, as Ayala-Berdon et al. (2008) suggest, numerous factors—including digestion, osmoregulation, and metabolic processes—may limit the ability of individuals in these species to vary ingestion to maintain a constant sugar intake (compensatory feeding), and, when confronted with a low sugar concentration diet or high energetic demands, they may suffer potential impacts to fitness.

The prevalence of intake response is intriguing by itself because at low sugar concentrations and corresponding high intake, nectar-feeding bats would need to rapidly eliminate large amounts—from one to five times their body mass—of water. But, as outlined earlier, the kidneys of both nectar- and fruit-feeding phyllostomid bats are morphologically well-suited for eliminating water but not for recovering electrolytes. Among other adaptations to a diet of high water content are an undifferentiated medulla, lack of medullary papilla reducing the concentration of urine and increasing electrolyte recovery, and the enlarged renal cortex furthering the absorption of electrolytes and other solutes (Casotti et al. 2006; Schondube et al. 2001). These adaptations lead to an osmoregulatory dilemma, as the rate at which their kidneys can eliminate water may limit the amount of dilute nectar these species can ingest (Ayala-Berdon et al. 2008). How these bats re-

solve the conflict between osmoregulation and intake of dilute nectar remains to be discovered.

Previous physiological studies have lumped plant-visiting bats into a single category, but there are important differences between frugivorous and nectarivorous species. Saldaña-Vázquez et al. (2015) focused on testing the link between dietary diversity and factors such as body mass, gut nominal area, and digestive capacity—defined as the ability to obtain nutrients from foods of different nutrient quality. Digestive capacity is the negative of the slope of the relationship between volumetric intake and nutrient concentration in log-log scale, such that high digestive capacity indicates a strong intake response and low digestive capacity a weaker intake response. Using a sample of five representative species in three frugivorous genera (*Sturnira*—*S. ludovici*, and *S. lilium*, *Artibeus*—*A. jamaicensis* and *A. toltecus*, and *Carollia sowellii*), Saldaña-Vázquez et al. (2015) first tested for phylogenetic signal in digestive capacity, body mass, gut nominal area, and dietary diversity. These variables are not strongly constrained by phylogeny, demonstrating variability (and the potential for adaptation) within genera. Instead, digestive capacity was the sole factor predicting dietary diversity. Species with greater digestive capacity (e.g., *Sturnira lilium* and *Artibeus jamaicensis*) feed on a wider variety of plant food, including plants of lower quality, than congeners with smaller digestive capacity. In species with lower digestive capacity, diet was more restricted, with higher-quality fruits comprising much more of the total intake. In effect, 77% of the variation in dietary diversity in the species they considered could be explained by digestive capacity alone (Saldaña-Vázquez et al. 2015). Digestive capacity, then, has a direct bearing on the width of the dietary niche of frugivorous phyllostomids. As bats must limit gut size due to constraints imposed by flight, congeners may differ in gut physiology by changing reaction speed or the affinity of enzymes for their substrates, thus modifying digestive capacity without changing gut size or body mass (even though changes in body size occur relatively rapidly in some genera such as *Artibeus*).

Genetic Basis of Physiological Adaptation

The analyses summarized so far have focused on the evolution of adaptive traits in lineages with derived diets, and among these there is only one potential convergent genetic adaptation (Dávalos et al. 2012),

without any functional tests. Only very recently have analyses integrating metabolic function, ecological characteristics, and functional genetics begun to include phyllostomid species. As the contemporary high-sugar diet of humans and resulting high-glucose levels in blood lead to type-2 diabetes, insulin resistance, and metabolic disease, among other diseases (Leturque et al. 2009), the search for biochemical adaptations to the derived plant-based diets of phyllostomids and other frugivorous bats has garnered interest beyond bat biology. Blood sugar regulation in bats with high-sugar diets is therefore a new focus of research in the quest for potential adaptations of value for future use in human treatments.

Meng et al. (2016) aimed to relate genotypes of the gene encoding glucose transporter 2 (*SLC2A2*), a transmembrane carrier protein responsible for bidirectional transport of glucose between the blood and liver (Shen et al. 2012), to the capacity to absorb glucose and maintain glucose homeostasis across bats. To this end, they began by exploring the relationship between blood glucose level, body size (measured by body mass or forearm length), and diet. Classifying 149 species of bats into frugivores, insectivores, and “others,” they found the frugivore group has the highest body mass index, insectivores the lowest, with the “other” category intermediate between the two. For the 16 species for which blood glucose–level data were available, including the phyllostomid *Artibeus intermedius*, the relationship between this trait and body size was negative both across all bats and within diet categories. Hence, larger bats, corresponding largely to frugivorous species, tend to have lower blood glucose levels, but the slope of the relationship for the group is steeper, indicating a greater drop in blood glucose per size unit compared to other bats. Meng et al. (2016) interpreted these results to hypothesize that bats have adapted to eating fruit—high in sugar and poor in protein—by maintaining high glucose in blood, so energy is readily available for flight. If this were the case, however, frugivores should have higher glucose levels than expected for their body size, and there is no evidence to this effect. Indeed, a phylogenetic regression of blood glucose level against body mass index finds the best-fit model determined using the AIC fails to include diet as a predictor (table 7.4). With only a few independent observations and despite the apparent relationship, the data set is underpowered. Covariation between blood glucose level and body

Table 7.4. Comparison of phylogenetic regression models of blood glucose levels as a function of predictors

Predictor	Degrees of freedom	AIC
Body Mass Index (BMI)	2	60.59599
Two intercepts by diet and BMI	3	62.58643
Two BMI slopes by diet	3	61.94425
Two intercepts and BMI slopes by diet	4	62.46562

Note: The phylogeny of Shi and Rabosky (2015) was used to account for the phylogenetic structure of residuals. AIC = Akaike Information Criterion.

mass index is not significant ($R^2 = 0.16$, $F_{(1,14)} = 3.902$, $p = 0.068$). The relationship between diet and blood glucose levels across bats, therefore, remains to be assessed with more data and using appropriate statistical approaches.

Assuming that adaptation to the high-sugar diet further requires demonstrating enhanced function in transporting sugar, if frugivorous bats have evolved to cope with the sudden influx of sugar in their diet, then they should have better sugar transport. Meng et al. (2016) tested the intra-peritoneal glucose tolerance of the yinpterochiropterans *Rousettus leschenaulti* (a frugivore) and *Hipposideros armiger* (an insectivore). Testing blood glucose levels at 0, 10, 60, 90, and 120 minutes after the injection across multiple individuals in each species, they found both enhanced transfer from intestine to blood, and then from blood. Glucose blood concentration in the frugivorous bat peaked earlier and at a significantly lower value than in the insectivorous species and decreased more rapidly. This functional demonstration strengthens the case for adaptation in sugar transport in frugivorous bats, even if only for yinpterochiropterans.

The final piece of evidence regarding adaptation for glucose homeostasis in frugivorous bats lies in the proximal promoter sequence (275 bp) of *SLC2A2*, coupled with measures of expression of the gene. Although highly conserved across the 16 species they sequenced, and therefore subject to negative selection (and not adaptation), an important potentially functional difference emerged between plant-visiting and other bats (Meng et al. 2016, table 2). An 11 base pair deletion overlaps a portion of sequence thought to bind a transcriptional repressor (*ZNF354C*; a putative binding site found only in nonfrugivorous species), suggesting

loss of the binding site causes increased transcription of *SLC2A2* in frugivores. The deletion, shared by phyllostomids (four plant-visiting bats and *Desmodus*) and Old World frugivorous bats, has evolved at least twice. The implications of the deletion in *Desmodus*, along with the evolution of the promoter across the diet shifts within Phyllostomidae, remain to be explored.

To close the chain of inference from the deletion to better sugar homeostasis for frugivorous bats, Meng et al. (2016) used quantitative PCR of *SLC2A2* mRNA to measure levels of the transporter in the liver. As expected if the deletion had functional effects, two insectivorous species lacking the deletion (*Hipposideros armiger*, *Myotis ricketti*) had much lower expression levels than the two frugivores (*Cynopterus sphinx*, *Rousettus leschenaulti*) with the 11-bp deletion (base level of 1 in the lowest values, 1.3 times higher in the other insectivore and 7.2 and 31 times higher in the frugivores). The convergent deletion, then, has substantial effects on transporter expression at least in yinpterochiropterans and is linked to better regulation in frugivorous species. Considering phyllostomids have evolved both nectarivory and frugivory more than once, as well as the intriguing presence of the high-expression deletion, there is great potential to explore this mechanism of adaptive glucose homeostasis in the family.

The Bacterial Microbiome

Gut bacterial communities are increasingly viewed as essential to host function because they provide nutrients to, regulate tissue development of, and interact with the immune system of their mammalian hosts (Eckburg et al. 2005). Although microbiome studies in humans have received the most attention (Kuczynski et al. 2012), comparative studies have grown in parallel to determine the ecological and evolutionary forces shaping these bacterial communities (Ley et al. 2008; Ochman et al. 2010). Sharing a similar method of sequencing the prokaryotic ribosomal RNA gene 16S, and based on samples from the New World, the two studies analyzing bat microflora have an excellent representation of phyllostomids (Carrillo-Araujo et al. 2015; Phillips et al. 2012). The pioneering analyses of Phillips et al. (2012) sampled seven families and focused on ecological and evolutionary covariates of bacterial diversity. Using phylogenetic distance (PD), or the total branch lengths contained in a sample for the overall 16S phylogeny, emballonurids have the most diverse

microbiomes, followed by vespertilionoids, and then noctilionoids, relating microbiome diversity to clade age. A non-phylogenetic regression of family PD against the time to the most recent common ancestor was significant, as were Kolmogorov-Smirnov tests comparing the PD subsample of each bat family to others. The sole exception to the latter pattern were noctilionoids, which shared similar values of PD. As measured by PD, the microfloral diversity of phyllostomids does not differ from that of mormoopids or noctilionoids, despite the much greater dietary diversity of the former.

Multiple factors seem to influence the bacterial diversity of the gut communities within phyllostomids. These factors, however, are not all related to diet; geographic locality, sex, sexual maturity, and reproductive condition also influenced the PD of various phyllostomids. Phylogenetic distance increased from sanguivores to insectivores, nectarivores, and frugivores, which had the highest PD values. This is consistent with the general mammalian pattern of increasing diversity from carnivory to omnivory to herbivory (Ley et al. 2008). Although not much can be said about the functional significance of particular bacterial lineages, the diversity of Lactobaccillales was higher in herbivorous phyllostomids, and bacterial species in this group could improve nutrient acquisition in herbivorous species (e.g., Famularo et al. 2005).

With hundreds of thousands of sequences clustered into hundreds of operational taxonomic units, the bacterial microbiome analyses of Phillips et al. (2012) defined the broad contours of diversity across diet specialists. New sequencing technology, however, quickly upended these first insights. Carrillo-Araujo et al. (2015) sequenced the same region of the prokaryotic 16S gene (hypervariable V4) and, in contrast with the longer-read method used by Phillips et al. (2012), sequenced using short-read technology (Illumina) for multiple individuals within species and at different regions of the intestine. With millions of sequences, grouped into thousands of operational taxonomic units, rarefaction analyses revealed lower bacterial diversity among the frugivorous *Carollia perspicillata* and *Artibeus jamaicensis* compared to the insectivorous *Macrotus waterhousii* and the sanguivorous *Desmodus rotundus*. This ordering of diversity was also reflected in analyses of phylogenetic diversity and cannot be explained by different measures of bacterial community richness. While intestinal region had no effect on

bacterial diversity for most species, there were important differences for the frugivorous species. Gamma-proteobacteria and firmicutes dominated the anterior intestine of *Carollia*, and cyanobacteria were prevalent in the posterior intestine. For *Artibeus*, tenericutes and firmicutes dominate the posterior intestine, with gammaproteobacteria prevalent in the anterior section. As in the previous microbiome study, phylogeny was a strong influence on the microbiome, with the effects of feeding strategy nested within the larger phylogenetic pattern. This is in line with analyses of the microbiomes of great apes, in which the gut microbiome recapitulates the host phylogeny (Ochman et al. 2010; Sanders et al. 2014). As the larger diversity of phyllostomids has not been sampled (e.g., Micronycterinae, Lonchorhinae, Lonchophyllinae, Rhinophyllinae), it is not yet possible to test if ecological convergence has reshaped the gut microbiome of ecologically similar mammals as it has in ant-eating lineages (Delsuc et al. 2014). As with the genomic basis of adaptation, much remains to be explored in the biology of phyllostomid microbiomes.

Conclusions

Since 2000, an important body of comparative work has focused on the adaptive radiation of phyllostomids and, in particular, on the diversity of adaptations linked to dietary specialization. Predictions from the ecological theory of adaptive radiation, however, have only been tested recently, and analyses of traits have focused mainly on feeding ecology and the morphology of the cranium. Using this framework, we find shifts in taxonomic diversification with the emergence of subfamily Stenodermatinae (Dumont et al. 2012; Shi and Rabosky 2015) and no diversity-dependent decrease in either this subfamily or phyllostomids as a whole (Rojas et al. 2016). Shifts in phenotype associated with the evolution in this subfamily include a new skull architecture leading to increased bite force (Dumont et al. 2012), a distinctive profile of olfactory receptor genes (Hayden et al. 2014), and a likely change in roosting behavior (Voss et al. 2016). Although there is no evidence of an increase in taxonomic diversification when phyllostomids first arose, several analyses reveal a diversity of adaptive zones across the family, including among cranial muscles and mechanical advantage (Santana et al. 2012) and in mechanical advantage inferred from engineering models (Dumont et al. 2014). Another line

of support for adaptive radiation in phyllostomids is the ecologically convergent evolution of phenotypes linked to particular adaptive zones. Quantitative analyses have documented convergence in trophic level (table 7.1, fig. 7.1), characters associated with feeding ecology for *Carollia* and *Rhinophylla* as well as nectar-feeding phyllostomids, and the possible adaptive evolution of the cytochrome *b* protein also in nectar-feeding lineages (Dávalos et al. 2012). The diversity of phyllostomid phenotypes corresponds to an adaptive radiation in traits and hence to the filling of various adaptive zones with distinctive niches, with stenodermatines comprising the fastest rate of taxonomic diversification in response to the completely new adaptive zone they occupy.

Evidence for adaptation to dietary specialization in phyllostomid subfamilies is more common but no less interesting: adaptations in feeding response to nectar concentration (Ayala-Berdon et al. 2008), digestive enzymatic activity and renal morphology—the latter especially in plant-visiting bats (Casotti et al. 2006; Schondube et al. 2001)—and digestive capacity defined by being able to extract nutrients from varying foods (Saldaña-Vázquez et al. 2015). All phyllostomids studied to date, including *Desmodus*, share a deletion in an 11-bp region of the promoter sequence for the *SLC2A2* gene that results in higher gene expression and faster sugar transport in Old World plant-visiting bats (Meng et al. 2016). The implications of this finding for phyllostomids remain to be fully explored. Similarly, variation in diversity of the microbiome suggests specialization whose functional significance remains unknown (Carrillo-Araujo et al. 2015; Phillips et al. 2012). Thus, the range of adaptations documented so far points to future lines of research, including exploring the limits some adaptations place on dietary intake, the regulation of sugar metabolism across lineages with different degrees of specialization, and determining the functional role of microbiome components in digestion, absorption, and metabolic regulation.

Acknowledgments

We thank N. Giannini and one anonymous reviewer for comments on the manuscript. LMD and SJR were supported in part by the National Science Foundation (DEB-1442142). DR was supported by Foundation for Science and Technology, Portugal (www.fct.pt),

fellowship SFRH/BPD/97707/2013. The Portuguese Foundation for Science and Technology supported CESAM RU (UID/AMB/50017) through national funds and FEDER funds, within the PT2020 Partnership Agreement.

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