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Diet and the evolution of digestion and renal function in phyllostomid bats

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Abstract

Bat species in the monophyletic family Phyllostomidae feed on blood, insects, small vertebrates, nectar, fruit and complex omnivorous mixtures. We used nitrogen stable isotope ratios to characterize bat diets and adopted a phylogenetically informed approach to investigate the physiological changes that accompany evolutionary diet changes in phyllostomids. We found that nitrogen stable isotopes separated plant-eating from animal-eating species. The blood of the latter was enriched in ^{15}N. A recent phylogenetic hypothesis suggests that with the possible exception of carnivory, which may have evolved twice, all diets evolved only once from insectivory. The shift from insectivory to nectarivory and frugivory was accompanied by increased intestinal sucrase and maltase activity, decreased trehalase activity, and reduced relative medullary thickness of kidneys. The shift from insectivory to sanguinivory and carnivory resulted in reduced trehalase activity. Vampire bats are the only known vertebrates that do not exhibit intestinal maltase activity. We argue that these physiological changes are adaptive responses to evolutionary diet shifts.

Key words: Bats, comparative method, diet, digestive and renal function, stable isotopes.

Introduction

The family Phyllostomidae is a speciose (49 genera and more than 140 species) monophyletic group of New World bats (Simmons, 1998; Wetterer et al., 2000). Phyllostomids exhibit a diversity of feeding habits that is unparalleled by any other mammalian family (Gillette, 1975). Phyllostomid species can feed on blood, insects, small vertebrates, nectar, fruit and complex omnivorous mixtures (Gardner, 1977). This broad dietary spectrum provides a unique opportunity to investigate the influence of diet on physiological traits. Animals ingesting different diets must process contrasting arrays of nutrients, electrolytes, toxins and water. Thus, diet has the potential to act as a selective force that shapes digestive and renal function (Studier et al., 1983a; Karasov and Diamond, 1988; Diamond, 1991). The main purpose of this study is to describe the effect of evolutionary shifts in diet on the digestive and renal traits of phyllostomid bats.

Characterizing animal diets can be difficult. We suggest that the stable isotopic composition of animal tissues can be used as a quantitative covariate in comparative studies, and we use phyllostomid bats to examine this conjecture. Our study uses nitrogen stable isotope ratios as indirect indices of an animal's protein intake. Using the nitrogen isotopic ratio of a consumer's tissues to assess its protein intake relies on an ecological observation: animal tissues are enriched in ^{15}N relative to their food sources (by about 3.4‰; Ehleringer et al., 1986). This enrichment probably results from differential retention of ^{15}N during amino acid deamination and transamination (Gaebler et al., 1966; Hobson et al., 1993). We predicted that $\delta^{15}N$ would differentiate between animal- and plant-eating phyllostomid species (Herrera et al., 1998).

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Comparative physiological analyses often seek to test the hypothesis of evolutionary correlation between an independent variable, such as diet, and a variety of physiological traits (Harvey and Pagel, 1991; Losos and Miles, 1994). These analyses must be guided by phylogenetic data (Felsenstein, 1985). The phylogenetic history of the group under study constrains the comparative analysis and the inferences that can be derived from it (Losos and Miles, 1994). In following paragraphs we outline a current hypothesis about the phylogeny of phyllostomid bats (Wetterer et al., 2000). We use this hypothesis to trace the evolution of diet diversity in this group and to devise an approach to explore the role of diet in the evolution of digestive and renal traits.

Wetterer et al. (2000) found that the eight traditionally recognized phyllostomid subfamilies are monophyletic (Fig. 1). Mapping diets onto Wetterer et al's (2000) phylogeny indicates that insect-eating is the ancestral phyllostomid diet and that most diets evolved only once from insectivory. The only possible exception to this pattern seems to be carnivory, which may have evolved twice within the Phyllostominae (Fig. 1). Assuming that most phyllostomid diets evolved from insectivory, what are the physiological changes that resulted from the shift to blood, nectar, meat, and fruit diets?

Diet composition has a significant impact on both digestive and renal function. The amounts of water, carbohydrates, proteins and electrolytes assimilated from diet depend on the morphological and biochemical characteristics of the digestive tract (Karasov and Diamond, 1988; Karasov and Hume, 1997). The interaction of the nutrient content of diet, digestive abilities to assimilate these nutrients and metabolic demands of the animal determine the amount of nitrogenous waste and water that the kidney must process (Studier and Wilson, 1983; Martínez del Rio et al., 2000). Even though several authors have mentioned the existence of this relationship (Studier and Wilson, 1983; Martínez del Rio et al., 2000; McWhorter and López-Calleja, 2000), the interplay between diet, digestion and renal function is a relatively poorly developed field. In this paper we explore the effect that evolutionary dietary shifts have on both digestion and renal function and the interaction between these two physiological systems.

To examine the digestive and renal changes resulting from dietary shifts, we examined four digestive enzymes and the gross morphology of the kidney. We used intestinal sucrase and maltase activity as indicators of ability to assimilate plant sugars (Martínez del Rio and Karasov, 1990; Martínez del Rio et al., 1992; Martínez del Rio; 1994). Trehalase activity was used as an indicator of capacity for the digestion of trehalose (the primary insect storage sugar, Chippendale, 1978), and aminopeptidase-N was used as a proxy variable for protein digestion (see Kania et al., 1977 and Martínez del Rio et al., 1995). We used relative medullary thickness to estimate the ability to produce concentrated urine (Sperber, 1944; Geluso, 1978; Beuchat, 1990; Herrera et al., 2001a).

Hernandez and Martínez del Rio (1992) hypothesized that insectivorous diets are correlated with reduced ability to assimilate carbohydrates (excepting trehalose), relatively high ability to assimilate protein, and the capacity to produce concentrated urine (Studier and Wilson, 1983; Herrera et al., 2001a). We predicted that a change from eating insects to eating meat or blood would result only in the reduction of trehalase activity (Table 1). Because, with the exception of small amounts of glucose, blood lacks significant amounts of carbohydrates we also predicted that a dietary shift from insects to blood would be accompanied by decreased sucrase and maltase activities (Table 1). A change in diet from insects to nectar or fruit is accompanied by increased sugar intake, by a reduction in protein and trehalose ingestion, and by increased ingestion of preformed dietary water (Martínez del Rio et al., 2000). We predicted that these changes in dietary composition would lead to increased intestinal maltase and sucrase activity, a reduction in trehalase activity, and a reduction of the kidney's concentrating capacity (Table 1). Because protein is an essential nutrient, we predicted that the ability to digest proteins would remain constant among diets (Karasov and Diamond, 1988). Our predictions are summarized in Table 1.

Table 1. Predicted changes in physiological traits in response to shifts in diet for bats in the family Phyllostomidae. A + sign signifies an increase in the value of a trait, a – sign represents a decrease, and 0 represents no change.

	Maltase	Sucrase	Trehalase	Amino-peptidase-N	RMT
Change in Diet:					
Insects to Blood	–	–	–	0	0
Insects to Nectar	+	+	–	0	–
Insects to Omnivore	+	+	0	0	–
Insects to Meat	0	0	–	0	0
Insects to Fruit	+	+	–	0	–

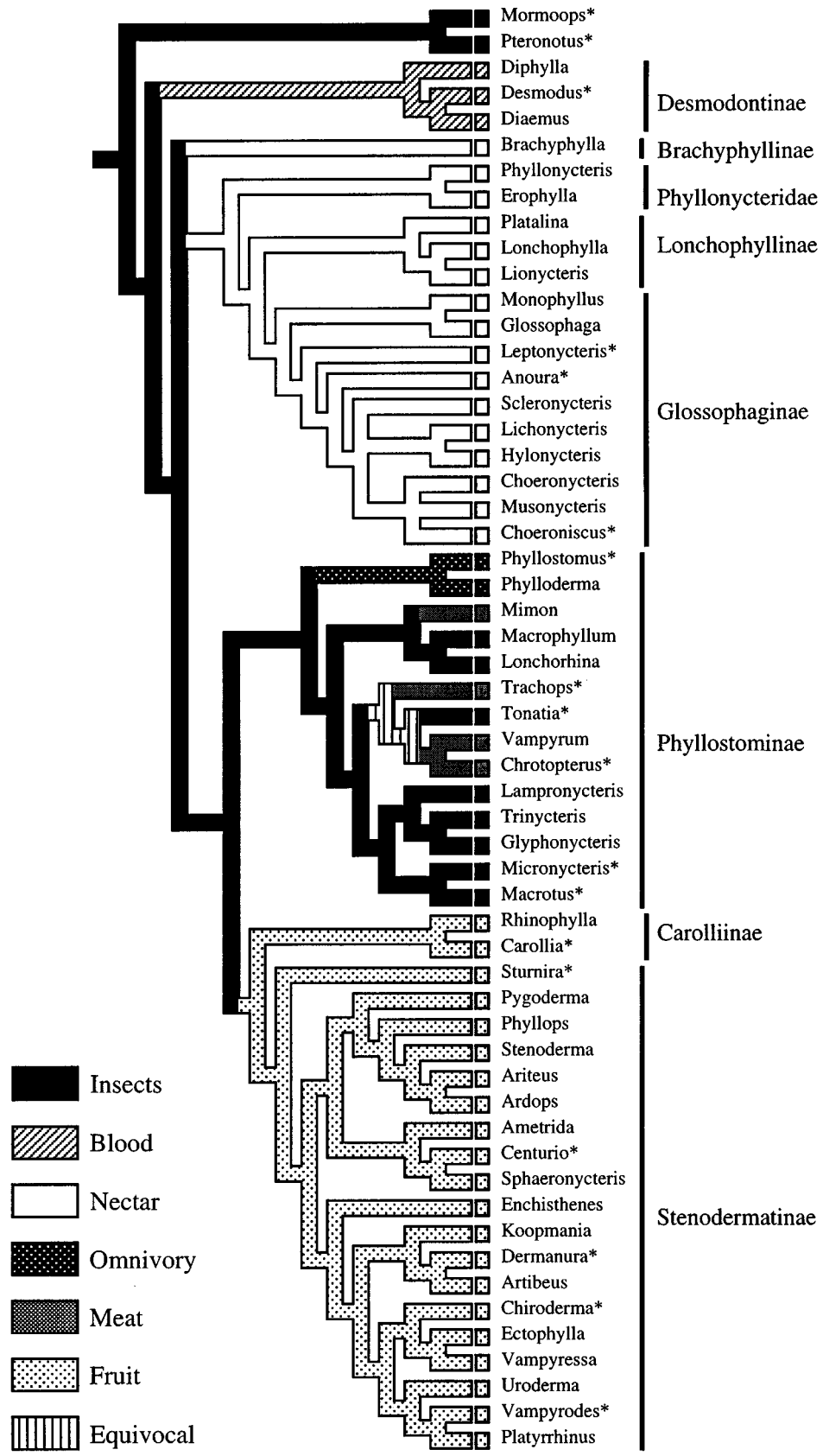

Fig. 1. A phylogenetic hypothesis for the relationships of the Phyllostomidae including its sister taxa (family Mormoopidae) according to Wetterer et al. (2000). We mapped the evolution of different diets into the phylogeny assuming parsimony and using MacClade (Maddison and Maddison, 1992). The tree includes all traditional genera in the family and several new generic groupings proposed by Wetterer et al. (2000). Subfamilies correspond with those proposed by Koopmann (1993). Only genera marked with * are included in our data set.

Material and methods

Collection of samples

Sixteen species of phyllostomid and 3 species of mormoopid bats were captured at five different localities in Mexico: Los Tuxtlas (18°35′N, 95°07′W) and Orizaba (18°51′N, 98°42′W), Veracruz; Jalalpan (18°19′N, 98°50′W) and Tlancualpican (18°27′N, 98°42′W), Puebla; and Chajul, Chiapas (16°06′N, 90°57′W). We collected ca. 80 microliters of blood from the antebrachial vein of the bats using a needle and glass capillary tubes. Blood was placed in a vial with 70% ethanol, refrigerated, and later dried to constant mass at 60 °C. Bats were euthanized with ether and their intestines and kidneys immediately removed. Intestines were chilled in ice-cold 0.9% saline and divided into three sections of approximately equal length. Each tissue section was slit longitudinally, laid flat, and length and width were measured to obtain an estimate of nominal area. The tissue was then blotted, weighed and stored in liquid N_2. The kidneys were cut longitudinally and preserved in a 4 : 1 mixture of formaldehyde and gluteraldehyde. Bats were collected with permission from the Secretaría del Medio Ambiente, Recursos Naturales y Pesca (SEMARNAP), México. All specimens were deposited in the zoological collection of the Instituto de Biología, Universidad Nacional Autónoma de México.

Stable isotope analysis

Dry blood was ground into a fine homogeneous powder. Samples (ca. 1 mg) were loaded in tin cups and combusted in a Robo-Prep elemental analyzer at 1800 °C. The resultant gases were separated and analyzed on a Europa 20 : 20 continuous-flow isotope ratio mass spectrometer (CFIRMS) for stable-nitrogen isotope ratio. CFIRMS involves the automated sequential measurement of samples (unknowns) together with reference material. We used two laboratory standards (egg albumen) for every 5 unknowns in sequence. Stable-isotope ratios were expressed in conventional notation: $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$. Where R_{sample} is the ratio $^{15}N : {}^{14}N$ of samples, and $R_{standard}$ is the ratio of atmospheric air. Based on several hundred replicates of laboratory standards, we estimated laboratory measurement error to be ±0.3‰. Herrera et al. (2001a and 2001b) provide more details of our isotopic analyses.

Kidney morphology

Kidneys were measured for length (L), width (W), and breadth (B) to the nearest millimeter with calipers. Longitudinal sections of each kidney were then projected onto a Sony monitor using a CCD video camera (Sony model CUE) mounted on a dissecting microscope. Sigma Scan Pro (Jandel) was used to captured the image. This measurement program was calibrated to measure in microns the medulla (M) and cortex (C) in each longitudinal section of the kidney. We use the cortico-medullary junction as the boundary between the cortex and the medulla. Relative medullary thickness (RMT) was calculated as $10 \times (M)/(L \times W \times B)^{0.33}$ following Sperber (1944).

Intestinal enzymatic activity

Intestinal tissues were thawed at 5 °C and homogenized (30 s, OMNI 5000 homogenizer at setting 6) in nine volumes of 350 mmol/l mannitol in 1 mmol/l Hepes/KOH, pH 7.5. Disaccharidase activities were measured following Martínez del Rio et al. (1995). In brief, tissue homogenates (100 µl) diluted with 350 mmol/l mannitol in 1 mmol/l Hepes/KOH were incubated at 37 °C with 100 µl of 56 mmol/l sugar (sucrose, maltose or trehalose) solutions in 0.1 mol/l maleate/ NaOH buffer, pH 6.5. After a 10–20 min incubation, reactions were arrested by adding 3 ml of a stop/developing Glucose-Trinder (one bottle of Glucose-Trinder 500 reagent [Sigma, St. Louis, Mo.] in 250 ml 1.0 mol/l TRIS/HCL, pH 7, plus 250 ml of 0.5 mol/l NaH_2PO_4/Na_2HPO_4, pH 7). After 15 min at 20 °C, absorbance of the resulting solution was measured at 505 nm with a Beckman DU-64 spectrophotometer. Aminopeptidase-N assays were done using L-alanine-p-nitroanilide as a substrate. Briefly, 100 µl of homogenate diluted with mannitol/KOH buffer were mixed with 1 ml of a pre-warmed (40 °C) assay mix (2.04 mmol/l L-alanine-p-nitroanilide in 0.2 mol/l NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 37 °C and arrested after 10 min with 3 ml of ice-cold 2 N acetic acid, and absorbance was measured at 384 nm.

To determine pH optima, we used a 0.1 mol/l maleate/ NaOH buffer system (for sucrose, maltose and trehalose), and a 0.2 mol/l NaH_2PO_4/Na_2HPO_4 buffer system (for aminopeptidase-N) with pH ranging from 5 to 8.5. Disaccharide (56 mmol/l) and L-alanine-p-nitroanilide (2.04 mmol/l) concentrations were held constant. Measurements reported in results were conducted at optimal pH (to the nearest 0.5, Appendix 1). Kinetics parameters were measured at concentrations ranging from 0.78 to 200 mM for sucrose, maltose and trehalose, and concentrations ranging from 1.5 to 40 mM for L-alanine-p-nitroanilide. We used a non-linear Gauss-Newton routine to obtain the maximal hydrolysis rates for each of the different substrates (V_{max}) and their apparent binding constants (K_m, the

concentration at which the rate of hydrolysis equals $V_{max}/2$, Appendix 1). On the basis of absorbance standards constructed for glucose and p-nitroanilide, we calculated intestinal activities standardized per unit of nominal area (μmol/min cm^2). Martínez del Rio et al. (1995) provide justification for our choice of standardization.

Comparative analysis

Many comparative phylogenetic methods rely on comparing the changes in a dependent variable (i.e. physiology) that result from evolutionarily independent shifts in a control variable (i.e. diet; Felsenstein, 1985; Garland et al., 1992; Pagel, 1994). Although phyllostomids are speciose and show a large diversity of diets, the structure of their phylogeny limits the use of standard comparative analyses. Each diet evolved once, or at most twice (Fig. 1). Single dietary evolutionary events reduce the sample size available for comparisons and greatly reduce the power of most available comparative methods (Garland et al., 1993). How can we use a phylogenetically informed approach to test the hypothesis that shifts in diet lead to physiological changes? In a critique of two species comparisons, Garland and Adolph (1994) suggested that at minimum, a two species comparison should involve several traits and a set of directional a priori predictions. Here we elaborate on this suggestion by combining it with Coddington's (1988) cladistic definition of adaptation.

Greene (1986) and Coddington (1988) argued that adaptation is an apomorphic ("derived") functional state of a trait promoted by natural selection. Thus, testing for adaptation requires identifying the ancestral, plesiomorphic condition and showing that a change in a selective regime leads to a trait that is functionally superior than the ancestral one under the new, derived conditions. Greene's (1986) and Coddington's (1988) criterion to determined adaptation was devised for a single trait. We extended their guidelines by testing concurrently the effect of five evolutionary shifts in diet on five physiological traits (Table 1).

We compared a 5×5 matrix of predictions (Table 1) with a similar matrix of physiological measurements (Table 2). Each of the 25 cells in these matrices is the change in a trait that accompanies a diet shift. Thus, each entry can register either a decrease (–), no change (0), or an increase (+) in a trait's value. The null hypothesis of no significant association between diet and physiological traits allows change to vary randomly among these three change states with equal probability ($p = 0.33$). A comparison of the predicted and observed matrices provides a test of the notion that physiological changes are adaptively correlated with evolutionary diet shifts.

To determine if a diet shift was associated with a change in physiological traits, we used observed values for insect-eating species as the "ancestral" state from which other diets evolved. To characterize the renal and digestive function of the ancestral state we used data from three insectivorous phyllostomid species (*Macrotus waterhousii, Micronycteris schmidtorum,* and *Tonatia brasiliense*) and data from three insect-eating species in the family Mormoopidae (*Mormoops megalophylla, Pteronotus parnellii,* and *Pteronotus davyi*), the sister group of the Phyllostomidae (Fig. 1). We compared the values of the ancestral state with those of the derived diets and scored evolutionary changes as positive, negative, or zero depending on the overlap in values among species. If there was no overlap between the 95% confidence intervals (CIs) of the values for the insectivores and the species with the derived diet, the change was scored as positive (e.g. maltase activity in the shift from insectivory to frugivory, Fig. 3b) or negative (e.g. trehalase in the shift from insectivory to carnivory, Fig. 3d). If there was overlap in the 95% CIs of any of the insectivores with any of the species with a derived diet, the change in the trait was scored as zero. Our test is conservative in that even small amounts of overlap between the traits of insectivores and the derived diet were scored as zeros.

After scoring the change in each trait, we compared the results matrix with the prediction matrix. Our null model was a binomial experiment with 25 trials (i.e.

Table 2. Predicted and observed changes (in parenthesis) in physiological traits in response to shifts in diet for bats in the family Phyllostomidae. Only one of our predictions (labeled with *) was falsified. Assuming that change can adopt a +, –, and 0 state with equal probability, the probability of making 24 correct predictions in 25 trials at random equals 5.9×10^{-11}.

	Maltase	Sucrase	Trehalase	Amino-peptidase-N	RMT
Change in Diet:					
Insects to Blood	– (–)	– (–)	– (–)	0 (0)	0 (0)
Insects to Nectar	+ (+)	+ (+)	– (–)	0 (0)	– (–)
Insects to Omnivore	+ (+)	+ (+)	0 (0)	0 (0)	0* (–)
Insects to Meat	0 (0)	0 (0)	– (–)	0 (0)	0 (0)
Insects to Fruit	+ (+)	+ (+)	– (–)	0 (0)	– (–)

cells in our 5 × 5 matrix) and a probability (p) of success (i.e. making the correct prediction in each trial) equal to 1/3. The probability of making i correct predictions was estimated as:

$$p(i) = \left(\frac{n!}{i!}\right) p^i q^{n-i}.$$

Using the mean value of each trait for each species, we also conducted a principal component analysis (PCA). The purpose of this analysis was descriptive rather than inferential. Our aim was to identify whether covarying suites of traits accompany evolutionary diet shifts.

Results

$\delta^{15}N$ values and their relation with diet

$\delta^{15}N$ varied in a predictable fashion with diet (Fig. 2). To avoid phylogenetic pseudoreplication, we used $\delta^{15}N$ for descriptive purposes only (Figs. 2 and 3). Bats feeding on animal tissues (insects, small vertebrates, and blood) had higher $\delta^{15}N$ values than those of bats feeding on plant products (fruit, nectar; Fig. 2). There were no apparent differences in the $\delta^{15}N$ values among species feeding on different animal diets. The nitrogen isotopic composition of *Phyllostomus discolor*, the only omnivorous species in our sample, was indistinguishable from that of animals feeding on insects, meat and blood (Fig. 2).

Kidney morphology and Relative Medullary Thickness (RMT)

Our predictions were largely confirmed (Table 2 and Fig. 3a). A shift from insectivory to blood and meat did not lead to a change in RMT (Fig. 3a). However, a shift to either nectarivory or frugivory was accompanied by a decrease in RMT (Fig. 3a). Contrary to our predictions, a shift from insectivory to omnivory did not cause a reduction in RMT (Fig. 3a).

Fruit- and nectar-eating species had kidneys with a large renal cortex, undifferentiated medullae possessing no distinct inner and outer zones, and a reduced medullary papillae that was replaced by a crest in some species (Fig. 2). In contrast, the kidneys of insectivorous, sanguinivorous, and carnivorous species had well-developed papillae and exhibited a medulla with two clearly differentiated zones (Fig. 2). Kidneys of insectivorous mormoopid bats were similar to those of animal-eating phyllostomids. They showed well-differentiated medullae, but presented a larger inner medulla and a smaller outer medulla than the kidneys of animal-eating phyllostomid bats (Fig. 2).

Intestinal enzymes

Our data set on intestinal enzymes also upheld our predictions (Table 2 and Figs. 3b–e). Enzyme activities standardized by intestinal nominal area (cm^2) and wet mass of tissue (g) for the four enzymes were linearly and tightly correlated both within ($0.94 < r < 0.99$) and among species ($r > 0.92$, $p < 0.001$, $n = 15$). Subsequently we standardize enzymatic activity by nominal area only. Intestinal nominal areas, body weights, and intestinal lengths are presented in Appendix 1.

A shift from insectivory to sanguinivory and carnivory was accompanied by a 10 to 15 fold decrease in trehalase activity, respectively (Fig. 3d). A shift from insectivory to carnivory did not result in a change in the intestinal activity of maltase, sucrase, and aminopeptidase-N (Fig. 3b–f). As predicted, the evolution of sanguinivory from insectivory involved a reduction in the activity of maltase and sucrase but not in aminopeptidase-N (Fig. 3b–c). *Desmodus rotundus*, the common vampire bat, did not show any detectable maltase or sucrase activity.

A shift from insectivory to nectarivory or frugivory was accompanied by an increase in maltase and sucrase activity, a decrease in trehalase activity, and no change in aminopeptidase-N activity (Fig. 3b–e). The shift from insectivory to omnivory involved only increases in maltase and sucrase activity (1.2 and 44 fold increases respectively; Fig. 3b–e) but no changes in aminopeptidase-N or trehalase.

Altogether, 24 of our 25 predictions were upheld (Table 2). The probablity of 1 miss in a binomial experiment with 25 trials and with a success probability of 1/3 equals:

$$P(24) = \left(\frac{25!}{24!}\right)\left(\frac{1}{3}\right)^{24}\left(\frac{2}{3}\right) = 5.9 \times 10^{-11}.$$

Thus, following Coddington's (1988) cladistic definition of adaptation, we conclude that evolutionary changes in diet in phyllostomid bats were accompanied by adaptive shifts in digestion and renal function.

Principal component analysis

Three principal component axes explained 94.6 % of the variance (Table 3). PCA axis 1 explained 70.3% of the variance. This axis was positively correlated with intestinal trehalase activity, aminopeptidase-N activity, and RMT, but negatively correlated with maltase and sucrase activity. Thus, this axis seems to define a gradient that spans from the ability to use food containing high carbohydrate and water levels to the ability to use food with high protein and low sugar and water content.

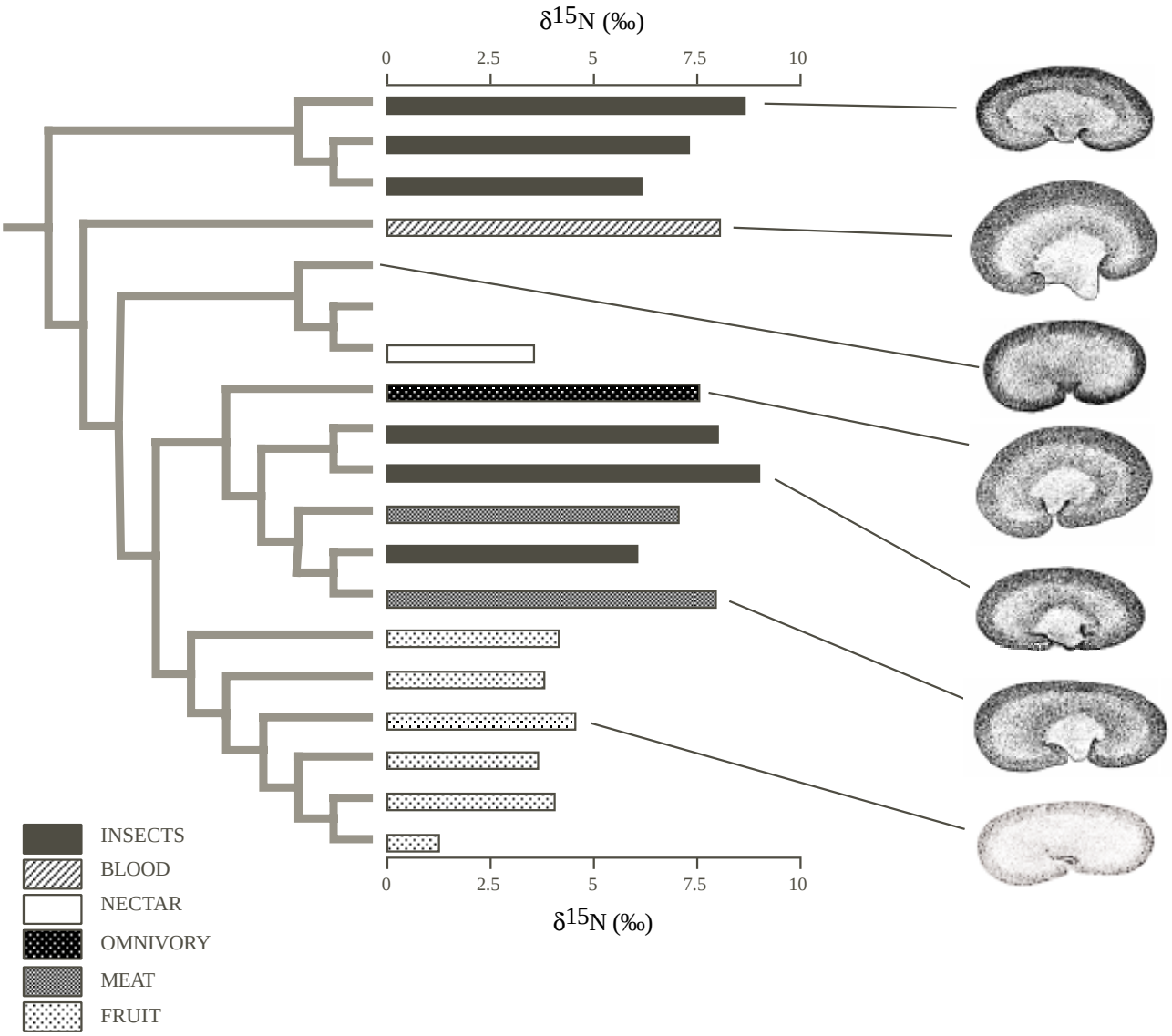

Fig. 2. Reduced phylogeny of the phyllostomids based on Wetterer et al. (2000). It includes only the species included in our data set. $\delta^{15}N$ values are shown as bars. Examples of typical kidney gross morphology were added to the right of the taxa. Two species of nectarivorous bats lack data for $\delta^{15}N$ values. Scientific names of taxa in the tree, from bottom to top, are: *Mormoops megalophylla, Pteronotus parnellii, P. davyi, Desmodus rotundus, Leptonycteris curasoae, Anoura geoffroyi, Choeroniscus godmani, Phyllostomus discolor, Micronycteris schmidtorum, Macrotus waterhousii, Trachops cirrhosus, Tonatia brasiliense, Chrotopterus auritus, Carollia brevicauda, Sturnira lilium, Centurio senex, Dermanura tolteca, Chiroderma salvini,* and *Vampyrodes caraccioli.*

Figure 3f suggests a positive correlation between PCA1 and $\delta^{15}N$ ($r_s = 0.77$, note that we use this correlation for descriptive purposes only). Bat species with low $\delta^{15}N$ values, and hence presumably low protein intakes, seem to be characterized by elevated ability to assimilate sugars and low renal concentrating capacity. Bats with high $\delta^{15}N$ values, in contrast, seem to be characterized by reduced ability to assimilate sugars and significant renal concentrating capacity.

Discussion

Our results demonstrate that evolutionary changes in diet were accompanied by adaptive shifts in digestion and renal function in phyllostomid bats. We begin our discussion by establishing the similarities and differences between the approach adopted in our study and other current comparative methodologies. A second section assesses the potential usefulness of stable isotopes in comparative studies. Two sections consider in some detail the

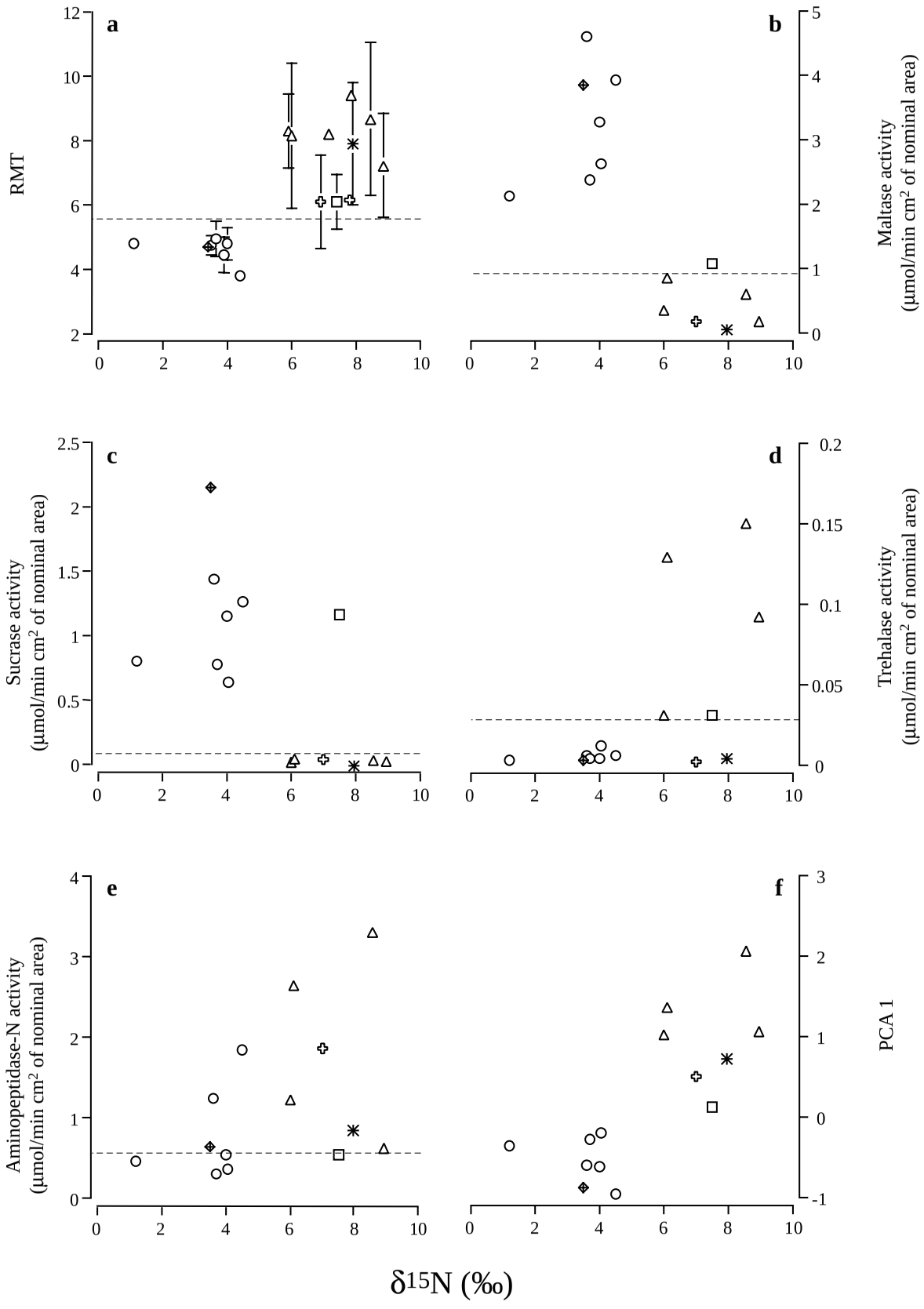
a
b
c
d
e
f
RMT
Maltase activity (µmol/min cm² of nominal area)
Sucrase activity (µmol/min cm² of nominal area)
Trehalase activity (µmol/min cm² of nominal area)
Aminopeptidase-N activity (µmol/min cm² of nominal area)
PCA 1
δ15N (‰)

Table 3. A principal components analysis of 5 physiological traits in 16 species of phyllostomid and 3 species of mormoopid bats yields 3 components that account for 94.6% of the variance. The first PCA seems to define a gradient that spans from the ability to use food containing high carbohydrate and water levels to the ability to use food with high protein and low sugar and water content.

	PCA Axis 1	PCA Axis 2	PCA Axis 3
Factor loadings:			
Maltase	-.44	.5	.11
Sucrase	-.47	.35	.30
Trehalase	.43	.43	.63
Aminopeptidase-N	.37	.65	-.63
RMT	.49	-.08	.3
Eigenvalue	3.51	.92	.28
Variance explained (%)	70.3	18.5	5.8
Cumulative variance explained (%)	70.3	88.8	94.6

physiological consequences of the renal and digestive changes unveiled by our study. In a final section, we explore the interplay between diet and physiology and examine how it may constrain the ecology of bats.

Do evolutionary diet shifts result in adaptive physiological changes?

In this study, we adopted a historical/phylogenetic approach to study physiological adaptation (Baum and Larson, 1991). We attempted to test the hypothesis that changes in physiological traits accompanying dietary shifts are adaptive (Martins, 2000). With some differences, we followed a more or less standard procedure: we focused on a set of traits for which we had a priori predictions about how they might function to increase the efficiency with which different diets were processed (Winkler, 2000). Using linear parsimony, we reconstructed an ancestral state for each diet (Swofford and Maddison, 1987) and found that insectivory was ancestral to all other diets. This is a more or less uncontroversial finding (Ferrarezi and Gimenez, 1996; Wetterer et al., 2000). We assumed that the physiological phenotype of the ancestral insectivore was similar to that of extant insectivorous species, and then compared this phenotype with those found in the derived diets. Our null model was constructed by assuming that evolutionary change in each trait could occur in three ways (+, –, and 0) with equal probability.

Thus, our method relies on two crucial assumptions: the use of parsimony to reconstruct ancestral diets and the hypothesis that the ancestral insectivore had traits similar to those of extant insect-eaters. We chose parsimony to reconstruct ancestral diets because the infrequency with which different diets have evolved in phyllostomid bats appears to indicate significant stabilizing selection with occasional "peak" shifts. This mode of evolutionary change seems to underlie linear parsimony (Swofford and Maddison, 1987). Reconstructing ancestral physiological states poses a significant challenge (reviewed by Losos, 1999 and Martins, 2000). Ancestral states are often reconstructed inaccurately and with uncertain confidence limits (Losos, 1999). We chose to accept these limitations and did not attempt to estimate ancestral physiological phenotypes from our comparative data. Our analysis provided support for the hypothesis that shifts in diet are accompanied by adaptive changes in digestive and renal traits. However, our conclusions are based on a phylogenetic hypothesis (Wetterer et al., 2000) and on an assumption-laden analysis. A different phylogenetic hypothesis and other comparative methods may reach different conclusions. To facilitate the task of others who may wish to re-examine our conclusions, we have provided all our data in Appendix 1.

δ^{15}N as a physiological covariate

Our results show that measurement of nitrogen stable isotopes are a useful tool to complement feeding habit studies. In our study δ^{15}N proved to be a convenient supporting variable that separated species with different feeding habits and allowed us to describe their diets quantitatively (Fig. 2). Specifically, and in support of our prediction, δ^{15}N was higher in animal-eating than in phytophagous species (Fig. 2). Because evolutionary diet changes in phyllostomid bats are relatively rare, this

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Fig. 3. Relationship between nitrogen isotopic composition (δ^{15}N) and several physiological traits. Points are species means and error bars are 95% confidence intervals. The dashed line in each panel represents the upper or lower 95% confidence interval for the value of insectivorous species. Panel a shows that renal relative medulary thickness decreased in the shift from insect- (triangles) to nectar- (diamond) and fruit-eating (circles). Panels b and c show that the intestinal disaccharidases maltase and sucrase increased in the shift from insectivory to nectarivory and frugivory. Note that maltase and sucrase were undetectable in vampire bats (star). Trehalase activity decreased in the shift from insectivory to all other diets, except omnivory (square; panel d). Values for the carnivorous species are shown as crosses. There was no consistent change in aminopeptidase-N with diet (panel e). The first axis of a principal components analysis clearly divided species into two groups: plant-eating species characterized by low PCA1 scores and low δ^{15}N values, and animal-eating species, characterized by higher PCA1 and δ^{15}N values (panel f). Note that the only omnivorous species in our sample exhibited intermediate PCA1 values, but δ^{15}N values that were indistinguishable from those of animal-eating species.

group does not allow a proper statistical test of a correlation between diet and nitrogen isotopic composition. Our data are consistent with this hypothesis, but our small phylogenetic sample makes our conclusion tentative. We envision two lines of evidence that can help to test our conjecture: 1) many species show changes in diet. Do these changes result in changes in $\delta^{15}N$? For example, some nectar-feeding bat species increase insect consumption seasonally and show a concomitant increase in $\delta^{15}N$ (Herrera et al., 1998; Herrera et al., 2001c). Also, an experimental increase in insects/meat should lead to increased $\delta^{15}N$ in the laboratory. 2) Our conjecture must be examined in other animal groups that exhibit diverse feeding habits (i.e. passerine birds and marsupials). Using $\delta^{15}N$ as a covariate in comparative studies has significant advantages and merits further investigation.

RMT and kidney morphology

We found that the kidneys of animal-eating bats, both in the Phyllostomidae and Moormopidae families, have thicker medullas and thinner cortices than those of phytophagous phyllostomids. Our results are consistent with Studier et al.'s (1983a) previous analyses of bat kidneys. We also found that protein ingestion seems to be positively correlated with the ability to concentrate urine as has been proposed by Carpenter (1969), Geluso (1978), and Studier and Wilson (1983; Fig. 3a). In contrast with animal-eating species, nectar- and fruit-eating bats show low RMT values and exhibit low urine concentrating capacity (Carpenter, 1969; Studier and Wilson, 1983; Studier et al., 1983b, Herrera et al., 2001a). Our results suggest that Studier and Wilson's (1983) hypothesis that renal morphology in bats is determined primarily by the protein and electrolyte content of the diet and secondarily by environmental water is correct.

Nectar- and fruit-eating bats must conserve the scanty electrolytes present in their diets in the face of copious urinary output (Carpenter, 1969; Studier et. al, 1983a, 1983b, Martínez del Rio et al., 2000). Differentiation of the medulla into inner and outer zones corresponds with the thin and thick ascending limbs of the loop of Henle respectively (Beuchat, 1996). Thus, the presence of an undivided medulla and the lack of a medullary papilla in phytophagous bats suggests a reduction in the number of loops of Henle of the long type (containing both thin and thick ascending limbs), and an increase in the number of the short type (with only thick ascending limbs; Sperber, 1944; Carpenter, 1969). Because thick ascending limbs are responsible for active sodium reabsorption (Beuchat, 1996), we hypothesize that this morphological modification reduces urine concentrating capacity and increases electrolyte recovery. Carpenter (1969) found that the large cortex found in *Leptonycteris curasoae* was associated with an increase in the length of the convoluted tubules, and suggested that this was a mechanism to increase reabsorption of electrolytes and other solutes. We hypothesize that the reduced RMT, the large cortex and the undivided medulla that characterizes phytophagous bats are features that allow filtering large amounts of dietary water while conserving electrolytes (Carpenter, 1969; Studier and Wilson, 1983; Beuchat, 1996). Similar renal traits have been reported in two species of frugivorous megachiropterans in the genus *Pteropus* (Sperber, 1944), suggesting convergent renal trait evolution in phytophagous mega- and microchiroptera.

Digestive traits

Digestive enzymes tracked dietary changes predictably, increasing and decreasing according to the prevalence of their substrate in the diet. Our results suggest the existence of an evolutionary match between the activity of specific enzymes and transporters and the ingested loads of their corresponding substrates as has been proposed by Karasov et al. (1985), Diamond (1991), and Diamond and Hammond (1992). The costs of synthesizing and maintaining the molecular machinery needed to assimilate nutrients should generate a selection pressure against unused physiological mechanisms (Karasov and Diamond, 1988; Diamond, 1991; Diamond and Hammond, 1992; Hammond et al., 1996). Our study provides several examples that are consistent with this hypothesis. Trehalase activity was reduced when bats shifted from insectivory to all other diets (Table 2 and Fig. 3d). Intestinal maltase and sucrase activity were absent in *Desmodus rotundus* whose diet is almost completely free of disaccharides and complex carbohydrates (Table 2). To our knowledge, *D. rotundus* is the only vertebrate known to lack maltase activity. In contrast, activity of the disaccharidases sucrase and maltase exhibited large increases in the shifts from insectivory to nectarivory and frugivory. If extreme changes in diet are accompanied by extreme changes in physiology, then perhaps we should expect intermediate physiological traits in omnivorous organisms. *Phyllosotomus discolor,* the only omnivorous species in our data set, provides provisional support for this idea. The diet of *P. discolor* includes insects, fruits, pollen, nectar and vegetative parts of flowers (Garder, 1977 and references therein) and most of its physiological traits seem to be intermediate between those of insect- and plant-eating species (Fig. 3).

Many, albeit not all, intestinal enzymes are modulated by levels of dietary substrates (reviewed by Caviedes-Vidal et al., 2000). Because our study used field-captured specimens, we cannot discard the hypotheses that the observed differences among diets are the result of physiological acclimatization to each diet rather than the result of evolutionary change. However, the magnitude of the difference in the expression of intestinal hydrolases indi-

cates that this hypothesis is unlikely. Karasov and Hume (1997) report that intestinal hydrolases vary up to 3-fold among diets as a result of dietary modulation. Our study revealed much larger differences in enzyme expression among species with different diets. Maltase activity was from 2.6 to 7.6 times higher in nectar- and fruit-eating species than in insectivores (Fig. 3). Sucrase activity was from 25 to 82 times higher in nectar- and fruit-eating species than in insectivores (Fig. 3). In contrast, trehalase was from 3 to 30 times higher in insectivores than in phytophagous, carnivorous and sanguinivorous bats. These large differences lend support to the hypothesis of evolutionary differences in enzyme expression, although do not demonstrate it conclusively.

Ecological consequences

Karasov and Diamond (1988) have proposed that physiology and diet interact with each other in a complex fashion, creating constraints and opportunities. Our results demonstrate that evolutionary diet shifts are accompanied by physiological changes that facilitate the use of the derived diet and that may constrain using the ancestral diet again. A shift from insectivory to frugivory, for example, led to increased sucrase and maltase activity, reduced trehalase activity, and a greatly reduced ability to concentrate urine. In the absence of large amounts of free water, the latter renal changes may constrain the ability of nectar- and fruit-eating bats to get rid of the urea produced by the catabolism of the large amounts of protein that characterize an insect diet (Studier et al., 1983a). Although several species of nectar- and fruit-feeding bats in mesic tropical habitats can become seasonally insectivorous when nectar and/or fruit are scarce (Gardner, 1977; Herrera et al., 2001c), to our knowledge no nectar- and fruit-eating bats survive in arid environments by feeding only on insects. Huey (1991) has emphasized thermal physiology as a determinant of habitat use. Our results indicate that renal and digestive physiology may play a role as well.

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Appendix 1. Data used in a comparative analysis of the effect of diet on the evolution of digestive and renal function in phyllostomid bats. Values are means and errors are standard deviations. Species were assigned to the following diets: blood (B), fruit (F), insects (I), meat (M), nectar (N), and omnivory (O).

Family/Species	n	BM (g)	Diet	$\delta^{15}N$	RMT	Gut Mass (g)	Gut Area (cm^2)
Phyllostomidae							
Desmodus rotundus	2	28.5 ± 2.12	B	7.05 ± 0.79	7.92 ± 0.85	1.69 ± 0.31	14.02 ± 0.64
Leptonycteris curasoae	4	24.8 ± 1.75	N		4.98 ± 0.82	0.92 ± 0.12	8.48 ± 0.40
Anoura geoffroyi	1	15.5	N		4.45	0.49	7.37
Choeroniscus godmani	1	10	N	3.551	4.73	0.46	3.89
Phyllostomus discolor	2	41.5 ± 0.70	O	7.6 ± 0.14	6.13 ± 0.85	1.94 ± 0.46	14.9 ± 1.62
Micronycteris schmidtorum	1	8	I	7.92	9.39		
Macrotus waterhousii	4	18.87 ± 1.03	I	8.56 ± 0.73	7.22 ± 1.66	0.97 ± 0.11	7.59 ± 0.69
Trachops cirrhosus	2	33.5 ± 2.12	M	6.8 ± 0.07	6.13 ± 1.45	1.80 ± 0.07	11.29 ± 1.45
Chrotopterus auritus	1	70	M	7.9	6.15		
Tonatia brasiliense	3	11.83 ± 0.76	I	6.27 ± 0.80	8.39 ± 1.16	0.65 ± 0.14	5.62 ± 0.45
Carollia brevicauda	3	17.23 ± 1.16	F	4.61 ± 0.70	4.81 ± 0.52	1.08 ± 0.21	7.50 ± 1.91
Sturnira lilium	3	16.83 ± 1.04	F	3.81 ± 0.62	4.95 ± 0.65	1.19 ± 0.17	9.58 ± 0.12
Centurio senex	1	26	F	4.0	3.79	1.09	15.01
Dermanura tolteca	3	15.16 ± 1.25	F	4.10 ± 0.65	4.77 ± 0.31	0.96 ± 0.06	7.76 ± 1.92
Chiroderma salvini	1	25	F	1.2	4.81	0.46	11.77
Vampyrodes caraccioli	2	31.0 ± 1.41	F	4.02 ± 0.10	4.45 ± 0.55	1.70 ± 0.32	15.64 ± 3.35
Mormoopidae							
Mormoops megalophylla	3	15.33 ± 1.52	I	8.51 ± 0.47	8.68 ± 2.36	0.77 + 0.09	7.15 ± 0.39
Pteronotus parnellii	2	20.10 ± 0.63	I	7.00 ± 0.28	8.21	1.18 ± 0.05	7.04 ± 0.08
Pteronotus davyi	1	10	I	7.22	8.18 ± 2.25		

Appendix 2.

Family/Species	Maltase total activity (µmol/min)	Maltase pH optima	Maltase K_m	Maltase total V_{max}	Sucrase total activity (µmol/min)
Phyllostomidae					
Desmodus rotundus	0	–	0	0	0
Leptonycteris corasoae	51.77 ± 4.96	6.5	12.07	74.11	12.88 ± 1.69
Anoura geoffroyi	34.32	6	6.65	42.48	12.06
Choeroniscus godmani	14.84	6	7.49	18.82	8.36
Phyllostomus discolor	15.46 ± 0.98	5.5	6.73	19.18	18.36 ± 1.91
Micronycteris schmidtorum					
Macrotus waterhousii	Trace	6.5	–	1.11	Trace
Trachops cirrhosus	1.42 ± 0.41	6.5	2.42	1.55	0.5 ± 0.13
Chrotopterus auritus					
Tonatia brasiliense	1.81 ± 1.14	6.5	2.70	1.99	Trace
Carollia brevicauda	22.90 ± 11.32	5.5	11.05	27.02	6.49 ± 2.41
Sturnira lilium	22.36 ± 11.92	6	12.10	32.04	7.51 ± 4.87
Centurio senex	58.40	5.5	16.24	92.28	18.96
Dermanura tolteca	45.94 ± 11.28	5	9.27	47.05	11.20 ± 1.85
Chiroderma salvini	24.42	5.5	14.60	37.17	9.49
Vampyrodes caraccioli	50.47 ± 8.29	6.5	12.71	73.38	17.99 ± 2.38
Mormoopidae					
Mormoops megalophylla	4.00 ± 0.74	6	1.16	4.17	Trace
Pteronotus parnellii	5.66 ± 1.03	6.5	0.77	5.82	Trace
Pteronotus davyi					

Appendix 3.

Family/Species	Sucrase pH optima	Sucrase K_m	Sucrase total V_{max}	Trehalase total activity (µmol/min)	Trehalase pH optima	Trehalase K_m
Phyllostomidae						
Desmodus rotundus	–	0	0	Trace	6.5	–
Leptonycteris corasoae	6.5	52.77	37.16	Trace	6.5	–
Anoura geoffroyi	6	11.03	16.82	Trace	6.5	–
Choeroniscus godmani	6.5	46.84	22.35	Trace	6.5	–
Phyllostomus discolor	6.5	31.3	36.76	0.447 ± 0.20	6.5	2.18
Micronycteris schmidtorum						
Macrotus waterhousii	6.5	–	Trace	0.838 ± 0.021	6.5	1.65
Trachops cirrhosus	6.5	20.07	0.86	Trace	6.5	–
Chrotopterus auritus						
Tonatia brasiliense	6.5	–	Trace	0.172 ± 0.005	6.5	1.56
Carollia brevicauda	6	57.33	14.85	0.085 ± 0.031	6.5	1.58
Sturnira lilium	5.5	48.48	20.51	Trace	6.5	–
Centurio senex	5.5	84.15	75.96	Trace	6.5	–
Dermanura tolteca	5.5	112.01	56.01	Trace	6.5	–
Chiroderma salvini	6	34.95	21.34	Trace	6.5	–
Vampyrodes caraccioli	6	51.05	50.79	Trace	6.5	–
Mormoopidae						
Mormoops megalophylla	6.5	–	Trace	1.072 ± 0.35	6.5	2.37
Pteronotus parnellii	6.5	–	Trace	0.900 ± 0.13	6.5	2.47
Pteronotus davyi						

Appendix 4.

Family/Species	Trehalase total V_{max}	Aminopeptidase-N total activity (µmol/min)	Aminopeptidase-N pH optima	Aminopeptidase-N K_m
Phyllostomidae				
Desmodus rotundus	Trace	12.21 ± 3.47	7	1.31
Leptonycteris corasoae	Trace	7.20 ± 1.12	7	2.01
Anoura geoffroyi	Trace	7.77	7	1.08
Choeroniscus godmani	Trace	2.58	7	0.22
Phyllostomus discolor	0.482	8.57 ± 1.12	7	0.57
Micronycteris schmidtorum				
Macrotus waterhousii	0.734	4.91 ± 0.77	7	1.91
Trachops cirrhosus	Trace	21.27 ± 9.16	7	1.05
Chrotopterus auritus				
Tonatia brasiliense	0.182	7.02 ± 2.42	7	0.56
Carollia brevicauda	Trace	2.98 ± 0.64	7	0.96
Sturnira lilium	Trace	3.12 ± 0.319	7	2.37
Centurio senex	Trace	28.11	7.5	0.63
Dermanura tolteca	Trace	9.88 ± 1.54	7.5	0.03
Chiroderma salvini	Trace	5.72	7.5	0.22
Vampyrodes caraccioli	Trace	8.82 ± 2.38	7	0.05
Mormoopidae				
Mormoops megalophylla	1.072	23.79 ± 3.56	7.5	1.32
Pteronotus parnellii	0.981	18.81 ± 3.50	7.5	0.51
Pteronotus davyi				

Appendix 5.

Family/Species	Aminopeptidase-N total V_{max}	Family/Species	Aminopeptidase-N total V_{max}
Phyllostomidae		*Carollia brevicauda*	3.09
Desmodus rotundus	12.78	*Sturnira lilium*	3.39
Leptonycteris corasoae	8.33	*Centurio senex*	28.74
Anoura geoffroyi	3.47	*Dermanura tolteca*	9.89
Choeroniscus godmani	2.61	*Chiroderma salvini*	5.77
Phyllostomus discolor	8.75	*Vampyrodes caraccioli*	8.83
Micronycteris schmidtorum			
Macrotus waterhousii	5.25	Mormoopidae	
Trachops cirrhosus	22.07	*Mormoops megalophylla*	24.91
Chrotopterus auritus		*Pteronotus parnellii*	19.15
Tonatia brasiliense	7.16	*Pteronotus davyi*	

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