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Relationships between renal morphology and diet in 26 species of new world bats (suborder microchiroptera)

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Abstract

The renal morphology of 24 species of mormoopid and phyllostomid bats feeding on six different diets was examined to test evolutionary changes in several structural traits presumably led by dietary shifts from ancestral insectivorous diets. The kidneys of a fish-eating vespertilionid and an insect-eating emballonurid were also examined but not included in the phylogenetic comparison. The length, width, and breadth of the kidneys were used to calculate relative medullary thickness (RMT). Tissues were processed for stereological analysis, and the volumes of the kidney, nephron components, and vasculature were determined. RMT did not correlate with body mass in either animal-eating or plant-eating phyllostomid and mormoopid bats. The shift from insectivory to frugivory and nectarivory was accompanied by a reduction in RMT, a reduction in the percent of renal medulla, and an increase in the percent of renal cortex. No changes in these traits were observed in bats that shifted to carnivorous, omnivorous or sanguinivorous habits. No changes were observed in renal vasculature, in the percentage of cortical and medullary nephron components or of capillaries surrounding the nephrons in any feeding group. Vespertilionid and emballonurid species had similar values in all traits examined as compared to insectivorous phyllostomids and mormoopids. Our data suggest that diet does not influence a single area of the nephron, but rather the entire nephron such that the relative amounts of renal cortex and medulla are affected.

Keywords: Kidney; Medulla; Stereology; Chiroptera

Introduction

The mammalian kidney has to dispose of salts and nitrogenous wastes, recover filtered metabolites, conserve water when it is scarce and discard it when it is ingested in excess (Yokota et al., 1985; Kanai et al., 1994). These conflicting demands can lead to tradeoffs in the morphology required to facilitate function. Consequently, there appear to be correlations between ecological characteristics such as diet and habitat, and body mass, renal morphology and physiology. For

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example, the renal medulla contains structures that are responsible for concentrating urine and this area of the kidney is more developed in carnivorous than in herbivorous mammals (Studier et al., 1983b).

The hypothesis that evolutionary dietary shifts led to changes in kidney morphology was recently tested in bats of the family Phyllostomidae (Schondube et al., 2001). The family Phyllostomidae presents the widest array of feeding habits of any mammalian family (Gardner, 1997) and their phylogeny is relatively well resolved (Wetterer et al., 2000). Members of this family evolved different feeding strategies (e.g. meat-, blood-, fruit- or nectar-eating) from their insectivorous ancestors (Ferrarezi and Gimenez, 1996; Wetterer et al., 2000). As a result of their dietary shifts, the medullary region (measured as relative medullary thickness or RMT) of frugivorous and nectarivorous species decreased whereas in carnivorous, omnivorous and sanguinivorous species it remained unchanged (Schondube et al., 2001).

To date, Schondube et al. (2001) have provided the only study that tested evolutionary changes in kidney morphology in bats, although several studies examined functional renal morphology. Moreover, several studies examined the renal anatomy and physiology within a single species (McFarland and Wimsatt, 1969; Studier et al., 1983a; Busch, 1988; Arad and Korine, 1993), while others examined multiple species (Carpenter, 1969; Geluso, 1978; Happold and Happold, 1988). These authors attempted to determine an association between various anatomical indices and urine concentrating ability. Most used RMT, medulla to cortex (M/C) ratio or percent medullary thickness as indices. Beuchat (1990), in an extensive review of medullary thickness and urine concentrating ability in mammals, found that the relative thickness of the renal medulla accounted for only 59% of the variability among species in concentrating ability. This finding indicates that there are other morphological or physiological factors that significantly influence urine-concentrating ability. Moreover, the ability to concentrate urine is correlated with diet, with bats taking in large quantities of fluid excreting urines with lower osmolalities (Geluso, 1978; Studier and Wilson, 1983). Studier and Wilson (1983) showed that frugivorous bats produced an average urine concentration of 563 mOsm compared to 1643 mOsm in non-frugivorous bats. Other studies on frugivorous bats showed mean osmolality values for fruit bats of 619 and 113 mOsm (Studier et al., 1983a; Arad and Korine, 1993). A study on insectivorous bats showed an average urine concentration of 3225 mOsm (Geluso, 1978). Although feeding on a liquid (i.e., blood), vampire bats (*Desmodus rotundus*) have a diet high in protein and as a result they produce a highly concentrated urine reaching a peak of 4656 mOsm (McFarland and Wimsatt, 1969).

Unlike previous studies with bats and other mammals, we used the unbiased tissue sampling techniques of stereology to examine the renal morphology of bats. Stereology enables the entire kidney to be sampled quickly and efficiently to arrive at an estimate of the percentage of morphological components present. We have used stereology previously and have shown a consistent correlation between renal morphology and diet in different species of birds (Casotti et al., 1993, 1998; Casotti and Braun, 2000). In this study we investigate whether similar correlations exist in mammals. As found in birds, we hypothesize that differences in renal morphology in bats are not the result of changes at the level of the nephron segment or component, but rather at a gross morphological level (i.e., changes in amounts of renal cortex and medulla). If this is the case in mammals, it is interesting because it suggests that birds and mammals (two different evolutionary lineages) have evolved similar anatomical mechanisms to cope with dietary shifts.

In our study, we had access to the same tissues as used by Schondube et al. (2001) and included species not previously considered. Previous studies on renal morphology in bats (including Schondube et al., 2001) examined morphological indices from mid-sagittal kidney sections. In our study, we examined kidney and nephron structures using stereology to probe evolutionary relationships between diet and kidney morphology. This is the first study to use stereology to quantify areas of the kidneys and nephron in mammals.

Most species ($n = 21$) were from the family Phyllostomidae, three species were from the family Mormoopidae, one species was from the family Emballonuridae, and one species was from the family Vespertilionidae. Diets ranged from those that are water-rich and carbohydrate-rich (frugivory and nectarivory), to those that are protein-rich and ion-rich (carnivory, insectivory, sanguivory, fish-eating) and those on a generalist diet (omnivory). This broad dietary spectrum provides a unique opportunity to investigate the influence of diet on morphological traits.

We used a phylogenetic approach to investigate renal morphological changes that may have occurred due to a change in diet. We restricted our analysis to the family Phyllostomidae as most species in our study belong to this family and most dietary changes in New World bats occurred in this taxa. The only exception was to include three species of the Mormoopidae as this is the sister family of Phyllostomidae. Recent phylogenetic evidence suggests that, with the possible exception of carnivory which may have evolved twice, all diets evolved only once from insectivory in phyllostomid bats (Ferrarezi and Gimenez, 1996; Wetterer et al., 2000). Therefore we tested whether shifts in diet from insectivory to frugivory, nectarivory, sanguivory, carnivory and omnivory were accompanied by changes in renal morphology. Omnivory

was represented by a species that regularly includes fruits, nectar, pollen and insects in its diet. We conducted the analysis at two separate anatomical levels: (a) the relative proportion of renal cortex, medulla and vasculature, and RMT, and (b) the relative proportions of the nephron components and vasculature (glomeruli, proximal tubule, distal tubule, cortical collecting tubule, cortical capillaries, medullary collecting duct, thick limb of Henle, thin limb of Henle, and medullary capillaries). We predicted a reduction in RMT and renal medulla surface, and an increase in cortex surface in frugivorous, nectarivorous and omnivorous bats compared to their ancestral insectivorous relatives. As carnivorous and sanguinivorous diets are close in ionic content to insectivory, we predicted no change from the ancestral condition in these species. Previous studies of birds (Casotti et al., 1993, 1998; Casotti and Braun, 2000) show no changes in the volume of renal vasculature with diet; therefore we predicted no change in this morphometric parameter in bats. No association between diet and nephron components was found in previous avian studies although no explicit phylogenetic hypothesis has been tested for these traits (Casotti et al., 1993, 1998; Casotti and Braun, 2000). Accordingly, we hypothesized that nephron components are relatively conservative traits in vertebrate kidneys and predicted no changes in these anatomical parameters associated with dietary shifts in bats. We also analyzed the effect of body mass on RMT separately for plant- and animal-eating species. We excluded from the analyses the fish-eating *Myotis vivesi* (Vespertilionidae) and the insectivorous *Rhynconycteris naso* (Emballonuridae) because we did not have material from their ancestral forms for this study and they are not closely related to mormoopids and phyllostomids. We did, however, present data of these species because no published information of their kidney structures exists.

Materials and methods

Kidneys

Kidneys from 26 species of bats in the families Phyllostomidae, Mormoopidae, Emballonuridae and Vespertilionidae were collected in Cuba and Mexico (see Appendix A and B). The Cuban bats (*Erophylla sezekorni*, *Brachyphylla nana*, *Phyllonycteris poeyi*, *Monophyllus redmani*, and *Pteronotus parnellii*) were collected in Cueva del Indio (23°02′N and 82°09′W) near La Havana, Cuba, *Rhynconycteris naso* was collected in Los Tuxtlas, Veracruz (18°51′N, 98°42′W), and *Myotis vivesi* was collected on Partida Norte Island in the Sea of Cortez (28°52′N and 113°02′W). All other bats were collected as listed in Schondube et al. (2001). They were captured in mist nets and killed by placing them in a sealed container with either halothane or ether. The kidneys were removed and preserved in 10% neutral-buffered formalin. Species collected fed on differing diets and included: insectivores (7 species), nectarivores (8), sanguivores (1), omnivores (1), carnivores (2), frugivores (6) and fish-eaters (1). The number of bats in each of these dietary groups varied,with 20 insectivores, 28 nectarivores, 2 sanguivores, 2 omnivores, 3 carnivores, 11 frugivores and 10 fish-eaters. Some of the bat tissue used in this study was the same as that used in two previous studies (Herrera et al., 2001; Schondube et al., 2001). For some species, only one complete kidney was available for examination. In these instances, that kidney was used for stereological measurement and not available to calculate RMT.

The length, width and breadth of each kidney were measured to ± 0.1 mm using Vernier calipers. The total volume of each kidney was calculated using water displacement (Scherle, 1970). Percentage counts obtained from stereology were multiplied by the total volume and used to obtain volume estimates for structures within the kidneys. Mid-sagittal sections were made of each kidney. Each half was photographed using a Nikon Coolpix 995 camera attached to an Olympus SZ40 dissecting microscope. Digital images were imported into Image J (National Institute of Health, Washington, DC), and the thickness of the renal cortex and medulla were calculated. These data were used to derive the RMT using the equation:

$$\mathrm{RMT} = \frac{10\ (\text{medullary thickness})}{(\text{length} \times \text{breadth} \times \text{width})^{1/3}}.$$

Tissue from the right kidney (and on occasion from the left kidney if the right one was unavailable) was sampled in an unbiased manner by placing the slices face-down and sectioning the tissue vertical to the horizontal plane and in random directions on that plane, thus making the tissue isotropic uniform random (IUR) (Henery and Mayhew, 1989). Tissue sections were processed for light microscopy and embedded in paraffin wax. The embedded tissue was sectioned in an unbiased manner at 10 equally spaced intervals through the wax block (Mayhew, 1991). The resulting sections were stained with hematoxylin and eosin and volumes of the kidney components, the cortical and medullary nephron components, and the vasculature estimated using the Cavalieri principle (Gundersen and Jensen, 1987; Gundersen et al., 1988).

Allometric relationships

We examined the relationship between body mass and RMT in mormoopid and phyllostomid species. Because

these bats differed in dietary habits, we conducted separate least-square regressions of RMT and body mass for animal-eating and plant-eating species. The omnivorous species was grouped with the animal-eating bats because its kidney morphology was similar to that found in insectivorous bats.

Phylogenetic analysis

We adopted the approach used by Schondube et al. (2001) to test for evolutionary changes in kidney morphology associated with changes in diet in phyllostomid bats (Fig. 1). Schondube et al. (2001) tested a series of predictions for evolutionary changes from ancestral to derived conditions in kidney and intestinal morphological and physiological traits using Greene's (1986) and Coddington's (1988) definition of adaptation (e.g., the derived functional state of a trait resulting from natural selection). Similarly, we developed two prediction matrices: a 4×5 matrix for RMT, cortex, medulla and vasculature surfaces (hereafter referred to as cortex-medulla matrix; Table 1), and a 10×5 matrix for the nephron components (hereafter referred to as nephron matrix; Table 2). The cortex-medulla matrix included only 19 values because quality tissues were not available for sanguinivorous bats for measurement of RMT. The number of bat species included in the cortex-medulla matrix was also lower than the maximum number because some tissues were not adequate for examination (see Appendix A and B for a full list of species and measurements). Each cell in the matrix represents an evolutionary change in the trait related to a dietary shift. Predictions were no change (0), a

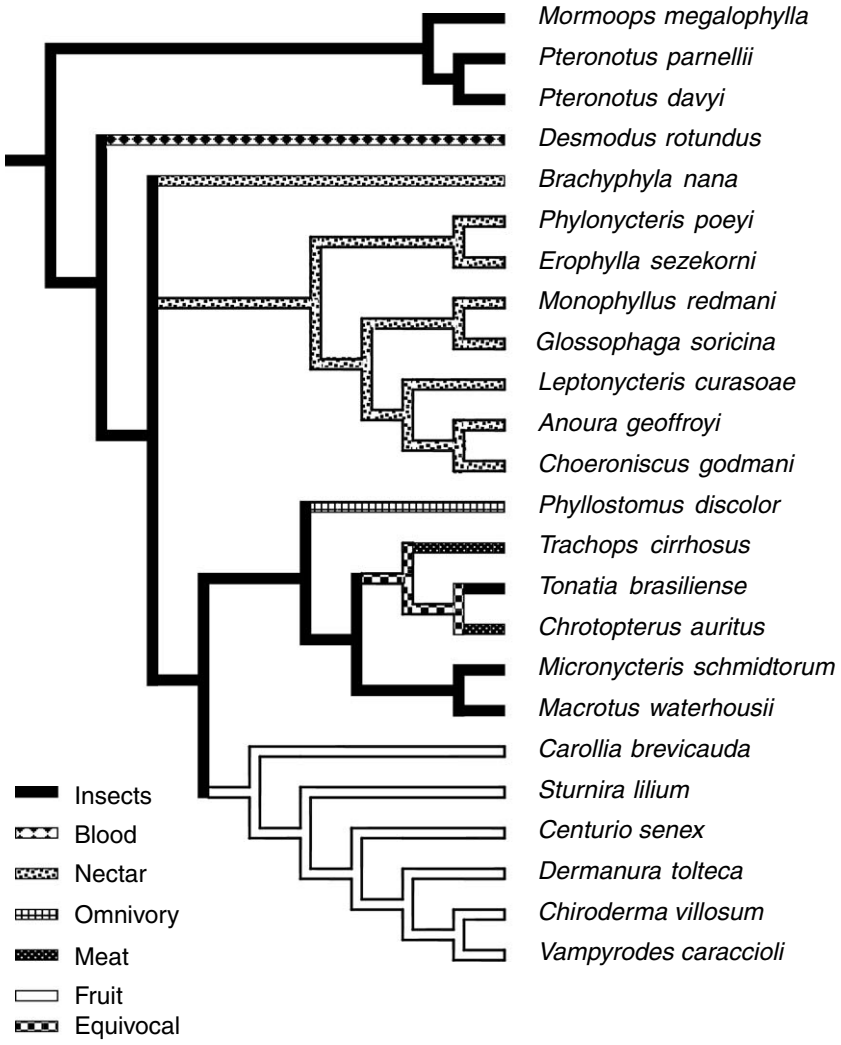

Fig. 1. Phylogenetic hypothesis for the relationships of the phyllostomid species considered in this test for evolutionary changes in kidney structure associated with changes in diet. Three species of the sister family Mormoopidae (*Pteronotus parnellii, P. davyi, and Mormoops megalophylla*) were also included. Evolution of different diets was mapped following Schondube et al. (2001).

Table 1. Cortex-medulla matrix: Predicted and observed changes (in parentheses) in relative medullary thickness (RMT), renal cortex (*C*), medulla (*M*) and vasculature (*V*) in phyllostomid bats as a response to dietary shifts from an ancestral insectivorous condition

Shift in diet	RMT	*C*	*M*	*V*
To fruits	− (−)	+ (+)	− (−)	0 (0)
To nectar	− (−)	+ (+)	− (−)	0 (0)
To meat	0 (0)	0 (0)	0 (0)	0 (0)
To blood		0 (0)	0 (0)	0 (0)
To omnivory	− (0)	+ (0)	− (0)	0 (0)

A + sign represents an increase in the trait, a − sign represents a decrease, and a 0 indicates no change. All measurements were made using stereology. The RMT shift to blood-eating was not included as adequate tissue was not available for study. Only three predictions were falsified. The probability of randomly making 16 correct predictions out of 19 equals 3.9×10^{-5}.

decrease (−) or an increase (+) in a given trait. Therefore, the probability of making the correct prediction (p) in each trial was 1/3. For each trait, we compared the value of the ancestral condition and each of the derived conditions using the phylogenetic hypothesis described by Schondube et al. (2001). To characterize the ancestral kidney morphology we used data from three insectivorous phyllostomid species (*Macrotus waterhousi*, *Tonatia brasiliense*, and *Micronycteris schmidtorum*) and three insectivorous species in the Mormoopidae (*Pteronotus parnellii*, *P. davyi*, and *Mormoops megalophylla*), the sister family of Phyllostomidae. If the 95% confidence intervals of the insectivorous species and the species of each derived condition overlapped, we scored the change as zero. If no overlap occurred, the change was scored as negative or positive depending on the direction of the change. After scoring the changes, we compared the prediction and the resulting matrices. The probability of making i correct predictions at random was estimated as: $p(i) = [n!/i!]\, p^i q^{n-i}$.

Results

Bat body mass

Bat body mass varied by a factor of 17.5 among the species (see Appendix A and B). Individual bats ranged in mass from 4 g in the insectivorous bat *Rhynconycteris naso* to 70 g in the carnivorous bat *Chrotopterus auritus*. Body mass also varied with diet. Carnivores had the highest body mass (47.8 g ± 20.3), followed by omnivores (41.3 g ± 0.6), sanguivores (28.5 g ± 2.1), piscivores (26.1 g ± 2.6), nectarivores (19.6 g ± 8.4), frugivores (18.8 g ± 5.9) and insectivores (13.4 g ± 4.9).

Allometric relationships

RMT was not correlated with body mass in either meat-eating or plant-eating bats (Fig. 2). The equation describing the regression line for meat-eaters is $\mathrm{RMT} = 8.0M^{-0.03}$ ($r = 0.223$). The equation describing the regression line for plant-eaters is $\mathrm{RMT} = 3.4M^{0.16}$ ($r = 0.426$). These data indicate little correlation between the relative thickness of the renal medulla and the body mass of the bats. However, meat-eating bats generally had a higher RMT than plant eaters (Fig. 2).

Renal morphology

Only 3 of the 19 predictions in the cortex-medulla matrix were falsified (Table 1). As predicted, RMT and percent medulla decreased and percent cortex increased in nectarivorous and frugivorous bats (Fig. 3). Medulla and cortex percentages did not change in carnivorous and sanguinivorous bats, RMT did not change in carnivorous bats, and the percent of major blood vessels did not change in any species (Fig. 3). Contrary to our predictions, no changes were found in RMT, and percent of cortex and medulla in omnivorous bats. The probability of making 16 correct predictions out of

Table 2. Nephron matrix: predicted and observed changes (in parentheses) in nephron components in phyllostomid bats as a response to dietary shifts from an ancestral insectivorous condition

Shift in diet	Cortical components					Medullary components				
	GL	PT	DT	CT	CC	MD	TK	TH	PT	MC
To fruits	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
To nectar	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
To meat	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (–)	0 (0)	0 (0)	0 (0)	0 (0)
To blood	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
To omnivory	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

A − sign represents a decrease in the trait, a + sign represents an increase, and a 0 indicates no change. All measurements were made using stereology. Only 1 prediction was falsified. The probability of randomly making 49 correct predictions in 50 trials equals 1.4×10^{-22}.
GL = glomeruli, PT = proximal tubule, DT = distal tubule, CT = cortical collecting tubule, CC = cortical capillaries, MD = medullary collecting duct, TK = thick limb of Henle, TH = thin limb of Henle, MC = medullary capillaries.

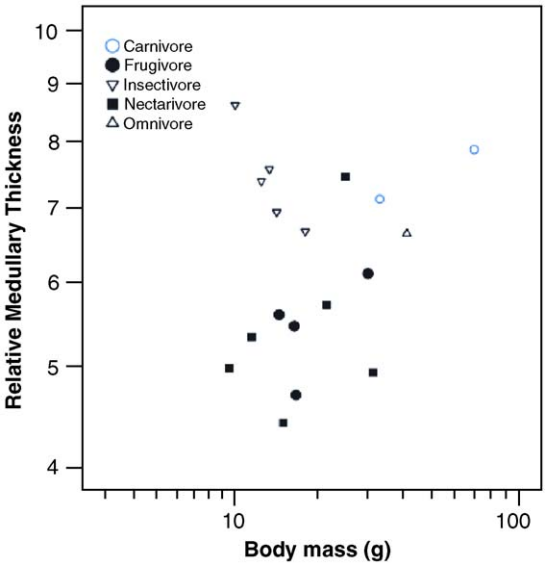

Fig. 2. Allometric relationship of relative medullary thickness (RMT) to body mass for 18 mormoopid and phyllostomid bat species of different dietary habits. Solid symbols represent plant-eating bats and open circles meat-eating bats. These data show that meat-eating bats generally had a higher RMT than plant eaters. The equation describing the regression line for meat-eaters is $RMT = 8.0M^{-0.03}$ ($r = 0.223$). The equation describing the regression line for plant-eaters is $RMT = 3.4M^{0.16}$ ($r = 0.426$). The omnivorous species was grouped with the animal-eating bats as its kidney morphology was similar to that found in insectivorous bats.

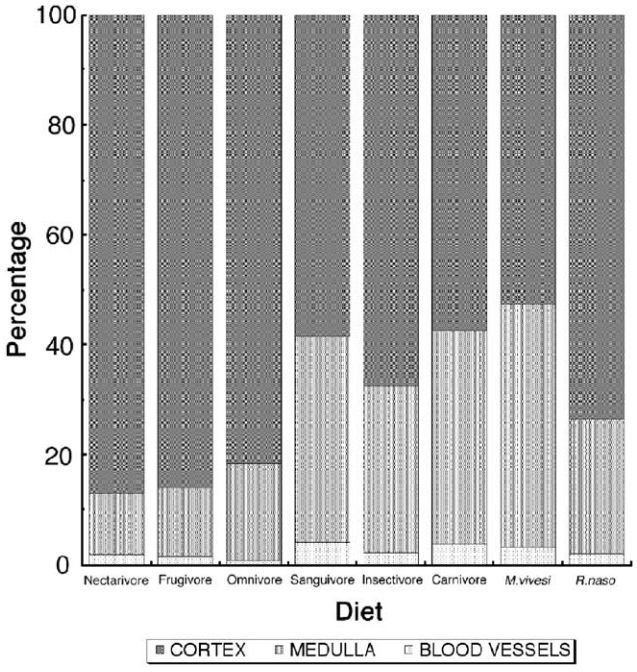

Fig. 3. Percent volume of cortex, medulla and major blood vessels within the kidneys of phyllostomid and mormoopid bats. The percentage of the medulla was lowest in bats feeding on a dilute diet, and highest in bats feeding on a concentrated diet. Data for *Myotis vivesi* (Vespertilionidae) and *Rhynconycteris naso* (Emballonuridae) are included for comparison.

19 is: $p(16) = [19!/16!]\ [1/3]^{16}[2/3]^3 = 3.9 \times 10^{-5}$. Therefore it can be assumed that at this anatomical scale renal morphology of phyllostomid bats changed with evolutionary dietary shifts.

The fish-eating and the insectivorous species not included in the matrix had values for all traits that were similar to insectivorous phyllostomids and mormoopids (Fig. 3; Appendix A).

In the nephron matrix all predictions but one were proven correct (Table 2). As predicted, no changes in nephron components were found after the transition from insectivory to any other dietary categories in virtually all cases (Figs. 4 and 5). On average, for all species, proximal tubules made up the majority of the cortical volume (69%), followed by renal corpuscles (10%), capillaries (9%), collecting ducts (7%) and distal tubules (5%). On average, the majority of the medulla consisted of thick limbs of Henle (55%), followed by capillaries (23%), collecting ducts (14%), proximal tubules (5%) and thin limbs of Henle (3%). Among the carnivorous bats, the percentage of the medullary-collecting duct was too low to detect using the sampling techniques of stereology we employed, thus falsifying the prediction for this trait. The probability of making 49 correct predictions out of 50 is: $p(49) = [50!/49!]\ [1/3]^{49}[2/3] = 1.4 \times 10^{-22}$. The traits examined at this

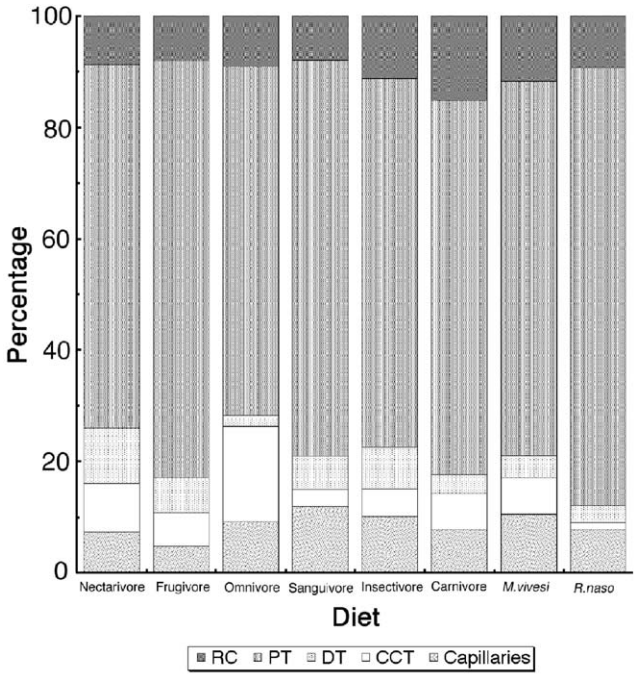

Fig. 4. Percentage of nephron components within the cortex of bat kidneys. As predicted, no changes in nephron components were found after the transition from insectivory to any other dietary categories in virtually all cases. CD, collecting ducts; Thick, thick limbs of Henle; Thin, thin limbs of Henle; PT, proximal tubules.

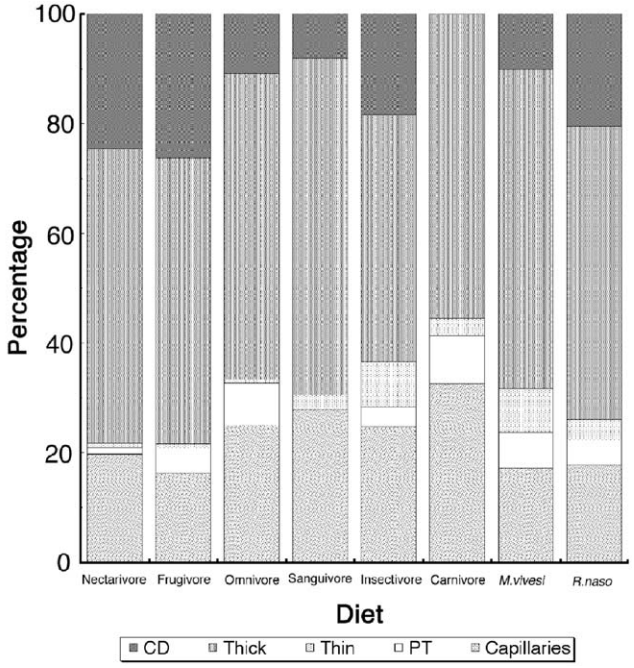

Fig. 5. Percentage of nephron components within the medulla of bat kidneys. As predicted, no changes in nephron components were found after the transition from insectivory to any other dietary categories in virtually all cases. CD, collecting ducts; Thick, thick limbs of Henle; Thin, thin limbs of Henle; PT, proximal tubules.

anatomical scale were of a conservative nature and not affected by evolutionary driven dietary shifts. The fish-eating and the insectivorous species not included in the matrix had values for all traits that were similar to phyllostomid and mormoopid bats (Appendix A, Table 2).

Discussion

The use of bats as a model to test the relationship between environmental factors and kidney morphology is not new. For example, renal morphology has been previously examined in 26 bat species from Central America and Central Africa (Studier et al., 1983b), in 28 species from Central Africa (Happold and Happold, 1988), and in 19 species from Mexico (Herrera et al., 2001; Schondube et al., 2001). However, these studies only examined morphological indices from mid-sagittal kidney sections. In addition to mid-sagittal sections, we examined kidney and nephron structures using stereology to probe evolutionary relationships between diet and kidney morphology. Our results showed that RMT and the proportion of medulla and cortex varied as a result of evolutionary dietary shifts in phylllostomid bats. In contrast, nephron structures did not change with dietary shifts. In the following paragraphs we will discuss these findings as well as allometric relationships determined for RMT.

Allometric relationships

Several comprehensive studies have documented the relationship between body mass and RMT. For example, Beuchat (1990) found a weak negative correlation between RMT and body mass. That study compared the kidney masses of different mammalian species over a wide range of body masses (2 g to 4000 kg). Similarly, Al-kahtani et al. (2004), studying rodents that ranged in body mass from 10 g to 50 kg found a negative correlation between RMT and body mass. In our study, body mass ranged from 4 to 70 g and RMT was not correlated with body mass in either meat-eating ($r = 0.223$) or plant-eating bats ($r = 0.426$). Over this body mass range (i.e., 4–70 g) in the other two studies, there was also no correlation between RMT and body mass. Similarly, a study by Herrera et al. (2001) using many of the same bat species as in the present study found little correlation between RMT and body mass ($r = 0.404$). The lack of a correlation between RMT and body mass in our study was not surprising, as correlations appear to be found only in multiple species over a wide range of body mass.

Cortex and medulla surfaces and dietary shifts

We found that RMT decreased in phyllostomid bats that shifted from their ancestral insectivory to nectar and fruit eating, but it remained unchanged when they adopted carnivorous or omnivorous habits. With the exception of omnivorous bats, these results were expected as carnivorous and insectivorous diets are high in protein and ions (Bell, 1990; Nicholson and Worswick, 1990), whereas nectarivorous and frugivorous diets are high in carbohydrates and fluid (Nicholson and Worswick, 1990; Martinez del Rio, 1994). This observation was consistent with studies showing that under dietary conditions of high salt or protein content, birds and mammals tend to excrete a more concentrated urine (Laverty and Wideman, 1989; Bankir and Kriz, 1995; Goldstein et al., 2001). In addition, bat species that concentrate urine well generally have a thicker medulla than species that produce a less concentrated urine (Geluso, 1978; Happold and Happold, 1988).

We also found that in species that shifted to nectar and fruit diets, the percent of renal medulla decreased and the percent of renal cortex increased. In contrast, in bats that shifted to carnivory, omnivory or sanguivory these traits did not change. Again, with the exception of the omnivorous species, these observations correlate with our initial predictions. As for sanguivores, although their diet is high in fluid, blood is not dilute, but iso-osmotic and high in protein content (Lumeij, 1985, 1987). Therefore a sanguinivorous diet would closely resemble the diet of insectivores. Previous studies

in mammals indicated that renal medullary hypertrophy occurred in species feeding on high protein diets (Bouby and Bankir, 1988; Bouby et al., 1988). That animals feeding on dilute and carbohydrate-rich diets have more renal cortex is a phenomenon well documented in avian species including honeyeaters and sparrows (Warui, 1989; Casotti et al., 1993, 1998; Casotti and Braun, 2000). Bats feeding on dilute diets with low ion concentrations must eliminate water from their diet, yet conserve ions. As the separation of ions from water occurs in the thick ascending limb of Henle's loop, we would expect species feeding on these diets to have expanded inner cortical and outer medullary regions of the kidney. Bats feeding on concentrated diets low in fluid and high in salt and protein would be expected to have thicker outer medullary regions for the same reason (area of ion and water separation), and to have well developed collecting ducts with receptors for antidiuretic hormone to facilitate the conservation of water.

Although previous studies on bats did not measure the percentage of renal medulla, they did measure such indices as RMT (Studier et al., 1983b; Bhati and Srivastava, 1994), the ratio of inner cortex to medulla (IC/M) (Geluso, 1978; Happold and Happold, 1988), and the thickness of the cortex and medulla (Carpenter, 1969). These studies demonstrate that species feeding on relatively concentrated diets have more renal medulla than species feeding on dilute diets.

The kidneys of fish-eating vespertilionid and insect-eating emballonurid species had similar RMT and cortex and medulla percentages as phyllostomid and mormoopid insectivorous bats, thus confirming the importance of diet in the shaping of these traits. Similar to insectivory, a fish-eating diet would be rich in ions and proteins.

Nephron components and dietary shifts

There was no evidence of evolutionary changes in the relative proportions of nephron components as a result of shifts from insectivory to nectar-eating, fruit-eating, blood-eating or meat-eating and omnivorous habits in phyllostomid bats. Similar patterns of nephron morphology as in phyllostomid bats were found in vespertilionid fish-eating and emballonurid insectivorous bats. We expected these results based on a previous study with 10 species of honeyeaters from wet and arid zones with presumably different feeding habits (Casotti et al., 1993). In contrast to this previous avian study, our study tested a set of evolutionary predictions in bats with distinct feeding habits.

Conclusion

This is the first study that tests the relationship between dietary changes and nephron morphology within a phylogenetic framework in vertebrates. Our data suggest that diet does not influence a single area of the nephron, but rather influences the amount of cortex and medulla within the kidney. As morphological changes are noted in the amounts of renal cortex and medulla, these evolutionary changes may be sufficient to allow bats to cope with dietary shifts without modification of nephron structure.

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Appendix 1. Summary of RMT data and volume data (Mean ± SD) for kidney components within bat kidneys

See Table A1.

Table A1

Species	Family	Sample size	Body mass (g)	RMT	Volume (mm^3)		
					Cortex	Medulla	Blood vessels
Nectarivores							
E. sezekorni	Phyllostomidae	2	15 (0)	4.4 (0.1)	4.0 (0.6)	0.6 (0.06)	0.2 (0.08)
B. nana	Phyllostomidae	6	31.2 (2.4)	4.9 (1.0)	10.1 (1.8)	3.5 (0.5)	0.2 (0.07)
C. godmani	Phyllostomidae	1	10	—	3.7	0.1	0.04
L. curasoae	Phyllostomidae	4	25 (1.7)	7.4 (0.2)	9.4 (3.4)	0.6 (0.2)	0.1 (0.01)
M. redmanii	Phyllostomidae	5	9.5 (1)	5.0 (0.7)	3.2 (0.6)	0.03 (0.01)	0.02 (0.01)

Table A1. (*continued*)

Species	Family	Sample size	Body mass (g)	RMT	Volume (mm^3)		
					Cortex	Medulla	Blood vessels
P. poeyi	Phyllostomidae	5	21.5 (1.9)	5.7 (0.6)	6.1 (1.0)	0.7 (0.3)	0.3 (0.4)
A. geoffroyi	Phyllostomidae	1	15	—	7.5	0.6	0.1
G. soricina	Phyllostomidae	4	11.5 (0.6)	5.3 (0.9)	3.5 (1.1)	0.1 (0.05)	0.05 (0.06)
Frugivores							
C. senex	Phyllostomidae	1	26	—	11.2	1.9	0.3
C. villosum	Phyllostomidae	1	25	—	7.7	1.2	0.06
V. caracciolli	Phyllostomidae	1	30	6.1	11.3	2	0.3
D. tolteca	Phyllostomidae	3	14.5 (1.2)	5.6 (0.8)	6.2 (0.6)	1.0 (0.2)	0.1 (0.07)
S. lilium	Phyllostomidae	3	16.7 (1)	4.7 (1)	6.8 (1.8)	1.7 (1.4)	0.1 (0.1)
C. brevicauda	Phyllostomidae	2	16.4 (0.5)	5.4 (1.6)	6.2 (1.5)	0.7 (0.2)	0.2 (0.2)
Carnivores							
T. cirrhosus	Phyllostomidae	2	33 (2.1)	7.1 (0.4)	7.9 (2.1)	4.3 (0.5)	0.4 (0.12)
C. auritus	Phyllostomidae	1	70	7.9	18.6	18	1.6
Insectivores							
M. waterhousii	Phyllostomidae	4	18 (1)	6.6 (0.5)	6.2 (1.7)	3.0 (1.0)	0.2 (0.05)
T. brasiliense	Phyllostomidae	3	12.5 (0.8)	7.3 (1.9)	5.1 (0.3)	1.5 (0.2)	0.14 (0.03)
M. schmidtorum	Phyllostomidae	1	8	—	2.4	1.6	0.1
P. parnellii	Mormoopidae	4	19.5 (3.7)	7.0 (1.9)	3.4 (1.5)	1.4 (0.3)	0.08 (0.03)
M. megalophylla	Mormoopidae	3	13.4 (1.5)	7.6 (1.0)	5.7 (0.8)	3.2 (1.0)	0.17 (0.05)
P. davyi	Mormoopidae	1	10	—	2.5	1	0.1
R. naso	Emballonuridae	4	4.3 (0.1)	5.3 (0)	1.9 (0.3)	0.6 (0.2)	0.04 (0.02)
Omnivore							
P. discolor	Phyllostomidae	2	41 (0.7)	6.6 (0.7)	12.3 (0.2)	2.6 (0.2)	0.01 (0.02)
Sanguivore							
D. rotundus	Phyllostomidae	2	30 (2.1)	—	1.7 (0.9)	0.7 (0.2)	0.1 (0.1)
Piscivore							
M. vivesi	Vespertilionidae	10	26.1 (2.1)	7.1 (0.7)	10.3 (2.0)	8.7 (2.0)	0.6 (0.3)

Appendix 2. Summary of volume data (Mean ± SD in parentheses) for renal nephron components within bat kidneys

See Table A2.

Table A2

Species	Body mass (g)	Sample size	Nephron components (mm^3)									
			GM	CPT	DT	CCT	CCAP	MCD	TLH	tLH	MPT	MCAP
Nectarivores												
E. sezekorni	15	2	0.2 (0.1)	2.8 (0.6)	0.2 (0.2)	0.6 (0.2)	0.2 (0.1)	0.1 (0.1)	0.4 (0.1)	0 0.0	0 0.0	0.1 0.0
B. nana	31.2	6	1.1 (0.3)	6.2 (1.5)	0.8 (0.5)	1.3 (0.6)	0.7 (0.2)	0.6 (0.4)	2.1 (0.5)	0 0.0	0 0.0	0.7 (0.1)
C. godmanii	10	1	0.1	2.9	0.4	0	0.3	0	0.05	0	0	0.03
L. curasoae	25	4	0.3 (0.4)	6.1 (2.0)	1.2 (0.9)	0.7 (0.4)	0.7 (0.4)	0.2 (0.1)	0.3 (0.1)	0 0.0	0 0.0	0.1 0.0

Table A2. (*continued*)

Species	Body mass (g)	Sample size	Nephron components (mm^3)									
			GM	CPT	DT	CCT	CCAP	MCD	TLH	tLH	MPT	MCAP
M. redmani	9.5	5	0.2 (0.1)	2.3 (0.4)	0.3 (0.1)	0.3 (0.1)	0.2 (0.1)	0.1 (0.1)	0.1 (0.1)	0 0.0	0 0.0	0.02 0.0
P. poeyi	21.5	5	0.6 (0.2)	3.5 (1.1)	0.9 (0.3)	0.6 (0.7)	0.5 (0.3)	0.2 (0.1)	0.3 (0.2)	0 0.0	0 0.0	0.1 (0.1)
A. geoffroyi	15	1	0.9	5.2	0.8	0.2	0.3	0.2	0.2	0	0.04	0.1
G. soricina	11.5	4	0.3 (0.1)	2.5 (0.8)	0.4 (0.4)	0.2 (0.2)	0.3 (0.1)	0.01 0.0	0.01 0.0	0 0.0	0 0.0	0.01 0.0
Frugivores											0	
C. senex	26	1	0.9	8.7	0.3	1	0.3	0.7	1	0	0	0.2
C. villosum	25	1	1.2	5.7	0.5	0	0.3	0	0.8	0	0	0.3
V. caraccioli	30	1	1.2	8	1	0	1.1	0.7	0.8	0	0	0.5
D. tolteca	14.5	3	0.4 (0.1)	4.7 (0.6)	0.5 (0.2)	0.6 (0.5)	0.1 0.0	0.2 (0.1)	0.3 (0.1)	0.01 0.0	0.1 (0.1)	0.2 (0.1)
S. lilium	16.7	3	0.5 (0.3)	5 (1.2)	0.2 (0.2)	0.5 (0.3)	0.5 (0.3)	0.4 (0.4)	0.7 (0.4)	0.02 0.02	0.3 (0.3)	0.3 (0.4)
C. brevicauda	16.4	2	0.4 (0.1)	4.4 (0.6)	0.6 (0.4)	0.3 (0.1)	0.4 (0.3)	0.3 (0.1)	0.3 (0.2)	0 0.0	0 0.0	0.1 (0.1)
Carnivores												
T. cirrhosus	33	2	1.2 (1.1)	5.6 (1.1)	0.05 0.0	0.4 (0.2)	0.6 (0.1)	0 0.0	2.4 (0.7)	0.1 (0.1)	0.3 (0.4)	1.5 0.0
C. auritus	70	1	3.3	10.9	1.5	1.4	1.5	0	10.2	1	2	4.8
Insectivores												
M. waterhousi	18	4	0.6 (0.3)	4.5 (1.3)	0.5 (0.1)	0.2 (0.2)	0.4 (0.2)	0.5 (0.5)	1.8 (0.8)	0.1 (0.1)	0.04 (0.1)	0.6 (0.1)
R. naso	4.35	4	0.2 (0.1)	1.5 (0.2)	0.2 (0.2)	0.02 (0.0)	0.1 (0.1)	0.2 (0.3)	0.3 (0.1)	0 0.0	0 0.0	0.1 (0.1)
M. schmidtorum	8	1	0.2	1.7	0.1	0.2	0.1	0.3	0.4	0.4	0.3	0.2
P. parnellii	19.5	4	0.3 (0.1)	2 (0.5)	0.3 (0.3)	0.2 (0.2)	0.5 (0.5)	0.3 (0.1)	0.6 (0.2)	0.2 (0.1)	0 0.0	0.2 (0.1)
M. megalophylla	13.4	3	0.6 (0.0)	3.9 (0.6)	0.5 (0.2)	0.1 (0.1)	0.7 (0.2)	1 (0.4)	0.7 (0.2)	0.4 (0.2)	0.1 (0.2)	1.1 (0.6)
P. davyi	10	1	0.4	1.6	0.2	0	0.2	0.02	0.8	0	0	0.2
T. brasiliense	12.5	3	0.7 (0.4)	3.1 (1.0)	0.3 (0.1)	0.4 (0.5)	0.5 (0.2)	0.1 (0.2)	0.6 (0.1)	0.1 (0.1)	0.1 (0.1)	0.5 (0.3)
Omnivore												
P. discolor	41	2	1.1 (0.3)	7.8 (1.5)	3.4 (4.6)	2.2 (2.0)	1 (0.7)	0.3 (0.3)	1.4 (0.5)	0.01 (0.0)	0.2 (0.3)	0.7 (0.2)
Sanguivore												
D. rotundus	30	2	0.9 (0.2)	8.3 (0.6)	0.7 (0.3)	0.4 (0.5)	1.4 (0.1)	0.7 (1.0)	4.6 (0.5)	0.2 (0.2)	0 0.0	2.1 (0.4)
Piscivore												
M. vivesi	26.1	10	1.2 (0.5)	6.8 (0.9)	0.4 (0.3)	0.8 (1.2)	1.1 (0.5)	0.9 (0.6)	5 (1.0)	0.7 (0.6)	0.6 (0.8)	1.5 (0.6)

GM, glomeruli; CPT, cortical proximal tubules; DT, distal tubules; CCT, cortical collecting tubules; CCAP, cortical capillaries; MCD, medullary collecting ducts; TLH, thick limbs of Henle; tLH, thin limbs of Henle; MPT, medullary proximal tubules; MCAP, medullary capillaries.

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