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EXAMINING THE MORPHOLOGICAL, ECOLOGICAL, AND ENVIRONMENTAL PREDICTORS OF BRAIN EVOLUTION IN THE ORDER CHIROPTERA

2023 | VALENTINA SANTAMARIA

B.Sc. HONOURS THESIS – BIOLOGY

THOMPSON RIVERS UNIVERSITY

EXAMINING THE MORPHOLOGICAL, ECOLOGICAL, AND ENVIRONMENTAL PREDICTORS OF BRAIN EVOLUTION IN THE ORDER CHIROPTERA

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

BACHELOR OF SCIENCE (HONS.)
in the
DEPARTMENT OF BIOLOGICAL SCIENCES
(Ecology and Environmental Biology)

THOMPSON RIVERS UNIVERSITY

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Dated this 15th day of June, 2023, in Kamloops, British Columbia, Canada

ABSTRACT

Brains evolve in conjunction with an array of morphological, ecological and environmental factors, ranging from diet to habitat. Although the details vary, most theories relating to brain evolution suggest that increasing variation, complexity or unpredictability in a species' environment favours a larger brain or an increase in the size of a specific brain region. In this phylogenetic comparative study, we investigated the relationship between ecology and brain size across 204 bat species (Order Chiroptera) using variables related to diet, habitat, climate, and range. Our findings suggest that different evolutionary patterns exist between the two major groups of bats, Microchiroptera and Pteropodidae. Diet itself did not have an influence on the whole brain size of bats from either clade. However, frugivorous species of Microchiroptera have larger olfactory bulbs and smaller medullas than insectivorous species. In addition to the constituents of the primary diet, we found that diet breadth contributes to brain evolution: frugivorous Microchiropterans with narrower diets (i.e., lower diet breadth) had larger olfactory bulbs. Among Pteropodid species, those with narrower diets had larger hippocampi than those with broader diets. The size of some brain regions was associated with variables other than diet; for example, within Pteropodidae, medulla size was impacted by whether species lived on islands. This study highlights how different brain regions are under different selective pressures, which is consistent with the mosaic theory of brain evolution. We also found evidence supporting the cognitive buffer hypothesis, as temperature variability and island-dwelling seemed to have influenced the evolution of certain brain regions. General patterns of brain evolution are difficult to elucidate even within a single order, but overall, the dominant result seems to be that diet has had the largest impact on the individual brain regions, particularly within the Order Chiroptera.

Thesis Supervisor: Professor Dr. Matthew Reudink
Thesis co-supervisor: Professor Dr. Nancy Flood

ACKNOWLEDGEMENTS

I would like to acknowledge that this research was conducted on the unceded territories of the Tk'emlúps te Secwépemc within Secwépemc'ulucw, the traditional and unceded territory of the Secwépemc. Thank you to Dr. Matt Reudink and Dr. Nancy Flood for supervising my project and to Dr. Mark Rakobowchuk for acting as my external supervisor. I would also like to thank Dr. Mateen Shaikh and Sean Mahoney for their continued support with my statistical analysis, and Dr. Andrew Iwaniuk for his help developing the methodology of this project.

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INTRODUCTION

Among animal species, brains vary substantially in both size and morphology, and their evolution is driven by an array of morphological, ecological or environmental factors. Multiple theories have been proposed to explain how these factors influence the evolution of brain size and morphology (Eisenberg and Wilson 1978; Iwaniuk and Arnold 2004; Jones and MacLarnon 2004; Pitnick et al. 2006; Sayol et al. 2016; Sayol et al. 2018). For example, the cognitive buffer hypothesis proposes that unpredictable environments may favour larger brains due to the instability or dynamic nature of resource availability within them (Sayol et al. 2018). Similarly, the habitat complexity hypothesis suggests that closed, dense, or cluttered habitats are more complex, thus these habitats favour an enlargement of the brain (Safi et al. 2005). The common thread among many hypotheses is that the more dynamic, unpredictable, or demanding the environment, the more resourceful the animal must be in order to find food, survive to sexual maturity and reproduce. Thus, selection may favour changes in brain size and morphology that improve inventiveness and resourcefulness in these unpredictable environments.

Rather than changing the brain as a whole, selection may alter individual parts of the brain, as described by the mosaic evolution theory (Barton and Harvey 2000). This theory states that varying selection pressures over evolutionary time can result in parts of the brain evolving as a patchwork (Barton and Harvey 2000; Rojas et al. 2013; Sayol et al.2018). In other words, different parts of the brain may become larger or smaller according to their use or disuse, respectively. For example, nut-caching birds tend to have a larger hippocampus—the brain region associated with spatial memory—likely because a larger hippocampus allows the birds to find the nuts they have hidden (Gould et al. 2013). As such, large-scale comparative approaches that examine the relative strength of ecological, biological, and environmental factors on the evolution of different brain regions may provide crucial insights into brain evolution.

Bats (Order Chiroptera) comprise a speciose mammalian, whose approximately 1,400 species live in a broad range of environments (Safi et al. 2005; Dechmann and Safi 2009). As a result, the order encompasses wide variation in body morphology, behaviour, life history, and brain size and structure. Due to bats' success in colonizing a range of habitats, they are a useful model for studying adaptive radiation and how different selective pressures can change morphology, including that of the brain (Hutcheon et al. 2002; Dechmann and Safi 2009).

As seems to be true for birds, bat brain evolution might be constrained by the energetic costs associated with flight (Dechmann and Safi 2009; McGuire and Guglielmo 2009). For example, migratory birds have smaller brains than non-migratory birds after controlling for body size; this is likely due to the trade-offs between the energetic demands of long-distance migration and growing expensive neural tissues (Vincze 2016). So much energy is required for flight that any changes in the size of the brain or its parts must have strong selective advantages. Thus, comparative study of bats may provide insight into the selective pressures that most influence the mammalian brain.

Absolute brain size is strongly correlated with body size (Dechmann and Safi 2009). Allometry accounts for most of the variation in the brain size; thus, the remaining percentage of brain volume and how brain mass is distributed across different brain regions must be explained by evolutionary pressures other than those affecting body size overall (Dechmann and Safi 2009). This correlation with body size can be controlled for by using residual brain mass (Jones and MacLarnon 2004; Pitnick et al. 2006; McGuire and Ratcliffe 2011; Smaers et al. 2012; Sayol et al. 2018) or less commonly, relative brain mass (Yao et al. 2012; Todorov et al. 2021). In this study we used whole brain volume as a response variable and control for body weight including it as a main effect within our analysis. Similarly, to control for brain size when analyzing different brain parts, we used volume of the rest of brain (ROB) as a predictor variable in the model (methods follow: Chambers et al. 2021; DeCasien and Higham 2019).

Diet is likely associated with brain evolution. For example, phytophagous bats (those that feed on nectar, leaves, and fruit) have been shown to have larger brains relative to their body size than do carnivorous bats (Eisenberg and Wilson 1978; Hutcheon et al. 2002; Jones and MacLarnon 2004). Eisenberg and Wilson (1978) hypothesized that demanding foraging strategies (e.g., searching for fruit or nectar) would select for larger brains. They suggest that large brains in frugivores may have evolved since the location of fruit is more unpredictable throughout the year than are insects; locating fruit requires more sensory input and spatiotemporal memory than does echolocation. Consistent with this, Eisenberg and Wilson (1978) found that aerial insectivores had the smallest brains. A more recent study by Rojas et al. (2013), using phylogenetic generalized least squares (PGLS) models to compare the dietary specialization and relative brain size of two bat families did not find any significant correlations between diet type, the degree of diet specialization and brain size; they also did not find that phytophagous bats had larger brains than insectivorous bats. The authors proposed that future studies should focus on the relative size of specific brain structures in bats, rather than on the overall size of the brain (Rojas et al. 2013).

When examining specific brain regions, Hutcheon et al. (2002) found that fruit bats had larger olfactory bulbs and hippocampi than non-fruit eaters. Furthermore, echolocating bats had larger auditory nuclei than fruit bats, which cannot echolocate (Hutcheon et al. 2002). This suggests bats' brains are morphologically adapted for the most efficient form of food seeking for their diet (Hutcheon et al. 2002). For example, phytophagous bats detect fruit by scent and have large hippocampi, which increases their spatial memory and ability to recall the location of ripe fruit.

Though selection may favour increases in overall brain size or the size of specific brain regions, due to the high metabolic demands of brain tissue, brain size may be constrained by the energetic needs of other organs and tissues—a trade-off referred to as the direct metabolic constraint hypothesis or the expensive tissue hypothesis (Jones and MacLarnon 2004). However, there is little evidence that energetic constraints have impacted brain size significantly in bats (Jones and MacLarnon 2004; Dechmann and Safi 2009). When tested against covariates such as basal metabolic rate (BMR), intestine length, and litter size, and brain size does not seem to be related to any of these variables (Jones and MacLarnon 2004). In addition, Jones and MacLarnon (2004) found no support for the maternal energy hypothesis which proposes that a higher BMR and longer gestation period will result in a larger brain. Another study found that among polyandrous species, males have large testes and small brains due to an energetic tradeoff between tissue types (Pitnick et al. 2006). However, in a reanalysis using different covariates than those selected by Pitnick et al. (2006), Dechmann and Safi (2009) found no correlation between bats' mating system and brain size. It has been suggested that the original correlation was due to a lack of covariates that considered the foraging ecology of the bat species examined (Healy and Rowe 2007; Dechmann and Safi 2009). These findings suggest that future comparative studies should include a multitude of covariates that may directly or indirectly influence brain size (Healy and Rowe 2007; Dechmann and Safi 2009).

Just as is true of expensive tissues, expensive behaviours such as migration can constrain brain size. For example, migratory bat species have smaller brains and neocortices than non-migratory species, perhaps due to a trade-off between the high energetic demands of sustaining brain tissue and traveling long distances (McGuire and Ratcliffe 2011); alternatively, non-migratory bats that face a harsh, cold environment could benefit from having a larger brain. Which might increase their chances of survival by allowing them to be more resourceful (McGuire and Ratcliffe 2011). Such a relationship between migratory status and brain size has also been found in many bird species (McGuire and Guglielmo 2009). However, for bats, migratory status is difficult to characterize due to high variability at both a population and individual level (McGuire and Ratcliffe 2011; Fleming 2019).

Regardless of the energetic costs associated with larger brains, the cognitive buffer hypothesis proposes that heightened intelligence allows an individual to adapt quickly to a changing habitat (Sayol et al. 2018). Though named differently in different papers (flexibility hypothesis, migratory precursor hypothesis, island hypothesis, foraging hypothesis), this idea has been tested using migration, habitat complexity, and island occupation as covariates influencing brain size (Safi et al. 2005; McGuire and Guglielmo 2009; Sayol et al. 2018). Safi and Dechmann (2005) found that a larger hippocampus was associated with larger wing area and a larger wing area was correlated with greater habitat complexity (Safi and Dechmann 2005). They suggest that those traits would enhance the ability to maneuver and forage (respectively) in closed habitats. However, they did not incorporate habitat complexity, wing area and brain size within the same analysis; therefore, the correlation between habitat complexity and brain size was never directly tested (Safi and Dechmann 2005).

Living on islands may also influence brain evolution, as island habitats restrict dispersal more than similar mainland habitats, limiting gene flow and enhancing local adaptation. In addition, large brained, and thus more innovative individuals, may be more resilient to the environmental changes on islands as they are unable to disperse when unfavorable conditions arise

(Sayol et al. 2018; Gavriilidi et al. 2022). When comparing island bird species with sister taxa found on the mainland, Sayol et al. (2018) found a strong relationship between bird brain size and island occupation: island birds had larger brains (Sayol et al. 2018). Their results suggest that this increase in brain size evolved after colonization, which supports the theory that islands are characterized by different selective pressures than the mainland (Sayol et al. 2018).

Previous studies on bat brain evolution have mostly used Phylogenetic Independent Contrasts, a method of analysis that assumes complete independence among taxa (ie., it constructs sets of taxa that are independent with respect to the evolution of the traits of interest). However, Rojas et al. (2012) suggested a realistic approach should account for the varying degrees of independence among taxa. This approach is best accomplished by a Phylogenetic Generalized Least Squares (PGLS) analysis; thus, it has gained popularity in comparative studies over the past decade (Rojas et al. 2013; Sayol et al. 2018). To our knowledge, there have been no large-scale phylogenetic studies on bat brain evolution that incorporate multiple aspects of bat ecology while using PGLS. Using PGLS we can examine patterns of evolution across whole families, orders, or clades. PGLS does not, however, allow us to discern whether specific predictor variables have a direct or indirect effect on brain size. To parse the nature of the relationships among covariates and changes in brain morphology, we can adopt a Phylogenetic Path Analysis (PPA). This allows us to determine whether the factors evolutionarily associated with brain size, as identified in the PGLS, have a direct or indirect effect, as well as determine the strength and directionality of the relationships (van der Bijl 2018). This type of analysis could aid in determining which hypothesis or hypotheses for brain evolution best explains the patterns seen in bats.

In this study we ask how ecological and biological traits influence brain size and morphology across the entire Order Chiroptera. Specifically, using PGLS we test whether variables, such as diet, climate, or island-dwelling are correlated with a change in overall brain size, as well as the size of specific brain regions. Based on previous studies, we predict that diet and habitat will be important predictors of brain size (Safi et al. 2005, Safi and Dechman 2005). In addition, we examine temperature and annual precipitation, factors that have been linked to body size, and potentially to energetic constraints (Jones and MacLarnon 2004). Because birds and bats are both volant species that may share similar energetic constraints and occupy similar niches, we predict that they may also experience similar changes in brain morphology associated with their ecology, such as an increase in brain size in island species (McGuire and Guglielmo 2009; Sayol 2018). After identifying factors associated with differences in brain morphology, we then employ a path analysis to determine the strength of these relationships and whether these relationships are direct or indirect.

MATERIALS AND METHODS

Data

Data on the brain volume of 204 species of bats were obtained from Comparative Neurobiology in Chiroptera **(**Baron et al. 1996). This source includes data on the volume of the following brain parts for each species: the hippocampus, olfactory bulbs (one data point for both bulbs), and medulla. The overall body size for each species is also provided.

Information on the ecology of bats was gathered from the Handbook of the Mammals of the World (2019) and from the International Union for Conservation of Nature (IUCN 2022). The data includes primary diet (fruit, seeds, insects, nectar/pollen, vertebrates, fish, or blood), habitat (from among forest, artificial/terrestrial, savannah, open or cave), and range size estimates (km^2) from IUCN range maps. See Table A1 in Appendix A for counts of the number of species in each category for diet and habitat variables. Bats with diets consisting of fish (n=1), blood (n=2) or vertebrates (n=5) were combined into a new diet category denoted as "Other" due to small sample sizes for each. Seed diets (n=3) were recategorized as fruit diets (now n=52) for the same reason. For a full list of diet and habitat categories with sample sizes please refer to Table A1 in Appendix A.

The data from the IUCN also included information on island occupancy. Islands were defined as land masses with areas less than or equal to 785 753 km^2, which is the size of Papua New Guinea (Benitez-Lopez et al. 2021). Island (n=49) and mainland (n=155) species were designated based on whether at least 70 % of their range fell into one of these 2 categories (Table A1). If the species' range was evenly divided between island and mainland environments, it was denoted as a mainland species.

Climate data was obtained from the World Clim2 website (www.worldclim.org). Climate variables included temperature seasonality (changes in temperature over the year), annual temperature range, isothermality (which relates day-to-night temperature oscillations to summer-to-winter oscillations), mean temperature of the coldest quarter, mean temperature of the coldest month, mean temperature of the driest quarter, precipitation seasonality, precipitation in the driest month, precipitation in the driest quarter, precipitation in the coldest quarter, and annual precipitation. A Principal Component Analysis (PCA) performed on the total set of temperature and precipitation variables. These variables were then used as covariates in our Phylogenetic Generalized Least Squares (PGLS) analysis and Phylogenetic Path Analysis (PPA).

Because diet could have a significant influence on brain morphology, the diet breadth of the bats was considered along with their primary food source. We obtained data on bat diet breadth data from Wilman et al. (2014) and Sayol et al. (2018). The dietary usage data estimated the frequency with which each species consumed different foods (invertebrates, vertebrates, fish, fruit, nectar, and seeds) and scaled them from 0 (not used) to 100 % (primarily used [Wilman et al. 2014]). Next, we created a matrix for each type of food the bats consumed and used the matrix to calculate overall diet breadth. Rao's quadratic entropy, a formula from the R package *indicspecies* was used to characterize the diversity (i.e., breadth) of a species' diet was used (De Caceres and Legendre 2009).

Only one phytophagous species (*Brachyphylla cavernum*) included non-fruit plant matter in their diet, thus plant matter was reclassified to fruit to simplify the analysis. One species, *Phylloderma stenops*, had a diet breadth 4 times larger than the next highest species (0.1 vs 0.025); it was thus considered an outlier and removed from subsequent analyses.

Linear Regression

A simple linear regression was performed on brain volume and body weight in the statistical analysis software JASP (JASP Team 2023).

Phylogenetic Generalized Least Squares (PGLS)

To examine the effects of phylogeny we began with a full mammalian phylogeny from Upham et al. (2019) and trimmed it to only include bat species. We then performed a series of analyses using R studio. A phylogenetic generalized least squares (PGLS) model from the *nlme* package in R was used to control for relationships among taxa (Revell 2012). When testing whole brain size as a response variable, allometric correction was performed by placing body weight directly in the model as a predictor variable. To control for brain volume when using a portion of the brain (e.g., amygdala), the rest of brain (ROB) method was used (DeCasien and Higham 2019). Specifically, the weight of the brain part was subtracted from the whole brain volume and used as a predictor variable in the model. Next, we performed a stepwise elimination of non-significant terms to arrive at a final best fit model.

Phylogenetic Path analysis

Using the R package *phylopath,* we performed a phylogenetic path analysis (1) to identify the strength and directionality of the factors identified as important in the PGLS and (2) to determine whether the effects were direct or indirect (van der Bijl 2018). The C statistic ((ΔCIC2) was used to rank the path models. The best model was selected for analysis if ΔCIC2 was > 2; however, we performed model averaging of the top models when ΔCIC2 was < 2.

Since categorical data cannot be included in path analysis, diet, habitat, and island/mainland classification data were changed to binary numerical variables. Specifically, diet was recategorized as either frugivorous or other and habitat was recategorized as either open or closed. Open habitats included those described as artificial/terrestrial, savannah, or open, and closed habitats included caves and forests. The Pteropodidae species included in the analysis all lived in open environments; thus, the habitat data was recategorized to be other and forest. open and artificial/terrestrial habitats were included in the "other" category.

RESULTS

Linear regression analysis showed that 90.5 % of the variation in brain size could be explained by variation in body mass. This finding suggests that 9.5 % of the variation in brain mass is due to other factors or potentially random noise. The mean brain volume was 837.08 ± 1444.56 cm^3 SD.

Principal Component Analysis

We performed a Principal Component Analysis (PCA) to collapse our climate variables. PC1 explained 49.7 % of the variation in our climate variable and related primarily to variation in temperature. The variables which loaded heavily on PC1 wer temperature seasonality, temperature annual range, isothermality, mean temperature of the coldest quarter-year, minimum temperature of the coldest month, and mean temperature of the driest quarter-year (Table 1). PC2 explained

21.6% of the variation in our climate variables and related mainly to variation in precipitation. As the loadings on PC2 show, it describes mainly precipitation seasonality (the amount of variation in precipitation within a year), total annual precipitation, and the amount of precipitation during driest month of the year, and driest quarter-year (Table 1. Together, PC1 and PC2 account for 71.3 % of the variation in our climate data

Table 1. The largest loadings for each variable on PC1 and PC2 (n=204).

Component Loadings/Variables	PC1	PC2
Temperature Seasonality	-1.11	
Annual Range in Temperature	-0.97	
Isothermality	0.97	
Mean Temperature of the Coldest Quarter	0.82	
Minimum Temperature of the Coldest Month	0.80	
Mean Temperature of the Driest Quarter	0.72	
Precipitation Seasonality		-1.05
Precipitation in the Driest Month		0.93
Precipitation in the Driest Quarter		0.93
Precipitation in the Coldest Quarter		0.77
Annual Precipitation		0.46

Predictors of brain size and morphology: Microchiroptera

Within the Microchiroptera, diet type ($X^2 = 9.58$, $p < 0.05$, $n = 168$) and PC1 ($X^2 = 7.4$, $p < 0.01$, $n = 168$) showed an association with whole brain size (Table 2). Diet type ($X^2 = 26.45$, $p < 0.0001$, $n = 168$) was the only variable other than the rest of brain (ROB) volume that showed a significant correlation with hippocampal volume (Table 2). As seen in Table 2, the size of the medulla was also significantly correlated with diet type ($X^2 = 11.58$, $p < 0.01$, $n = 168$) and also with range area ($X^2 = 4.22$, $p < 0.05$, $n = 168$). Finally, diet breadth ($X^2 = 13.02$, $p < 0.001$, $n = 168$) and diet type ($X^2 = 18.77$, $p < 0.001$, $n = 168$) were significantly correlated with olfactory bulb volume (Table 2). We did not find a difference in the size of any brain parts among species inhabiting different habitats or those living on islands rather than on the mainland.

Table 2. Top Reduced PGLS models of the brain including Microchiroptera (n=168).

Response Variable	Predictor Variables	X^2	p	Degrees of Freedom
Brain	Weight	358.20	<0.0001	1
	Diet Type	9.58	0.022	3
	PC1	7.40	0.0065	1
				1
Hippocampus	ROB Volume	708.55	<0.0001	1
	Diet Type	26.45	<0.0001	3
Medulla	ROB Volume	667.19	<0.0001	1
	Diet Type	11.58	0.0090	1
	Range Area	4.22	0.04	1
				1
Olfactory bulbs	ROB Volume	503.27	<0.0001	1
	Diet Breadth	13.02	0.00031	1
	Diet Type	18.77	0.00031	3

Predictors of brain size and morphology: Pteropodidae

In the fruit bat family Pteropodidae, none of the ecological predictors tested were associated with whole brain size (Table 3). Pteropodid species with broader diets had a larger hippocampal volume ($X^2 = 5.92$, $p < 0.05$, $n = 36$). Medulla volume was marginally associated with island-dwelling ($X^2 = 3.95$, $p < 0.05$, $n = 36$). Olfactory bulb volume had the most complicated associations with the covariables, as diet breadth ($X^2 = 21.03$, $p < 0.0001$, $n = 36$), diet type ($X^2 = 7.64$, $p < 0.01$, $n = 36$), habitat ($X^2 = 5.84$, $p < 0.05$, $n = 36$)., and island-dwelling ($X^2 = 16.76$, $p < 0.0001$, $n = 36$) all emerged as significant correlates in the reduced model (Table 3).

Table 3. Top Reduced PGLS models of the brain including Pteropodidae (n=36).

Response Variable	**Predictor Variables**	**X^2**	**p**	**Degrees of Freedom**
Hippocampus	ROB Volume	565.29	<0.0001	1
	Diet Breadth	5.92	0.015	1
Medulla	ROB Volume	317.37	<0.0001	1
	Island-Dwelling	3.95	0.047	1
Olfactory bulbs	ROB Volume	487.39	<0.0001	1
	Diet Breadth	21.03	<0.0001	1
	Habitat Type	5.8435	0.016	2
	Diet Type	7.638	0.0057	1
	Island-Dwelling	16.7555	<0.0001	1

Path analysis: Microchiroptera

From the path analysis, we were able to determine the strength and directionality of the relationships identified in the PGLS. Within the Microchiropteran clade, the best model showed a negative and direct effect of PC1 (the climate variable which represents temperature variation) on whole brain volume and weight; however, the effect for both was small (Figure 1a, Table 4). Our diet variable, which was changed to a binary variable representing a frugivorous or non-frugivorous diet, did not have an effect on any variable, but PC1 had a negative affect on diet (Figure 1a, Table 4). These findings suggests that diet does not have an impact on brain size, and that temperature and weight are much stronger determinants of whole brain size than diet.

Diet had a direct effect on ROB volume, but an indirect effect on hippocampus volume (Figure 1b, Table 4). Diet does not seem to directly influence hippocampus volume in Microchiroptera. Hippocampus size is largely determined by the size of the brain, and only indirectly by diet.

Diet had a direct and negative effect on medulla size (Figure 1b, Table 4): the path analysis showed that frugivorous Microchiropterans have smaller medulla than insectivorous bats. Range area also had a direct negative effect on medulla size, but it also had a small positive effect on diet (Figure 1c, Table 4).

Olfactory bulb size was strongly correlated with diet type and breadth in Microchiroptera. First, frugivory had a negative effect on diet breadth; thus, fruit eating species are more specialized than species with other diets (Figure 1d, Table 4). Second, frugivory also has a direct and positive effect on brain size. Third, diet breadth had a negative and direct influence on olfactory bulb size (Figure 1d, Table 4). Thus, species with large olfactory bulbs had smaller diet breadth. Our analysis suggests that frugivorous species with specialized diets, or small diet breadths had the largest olfactory bulbs among the Microchiroptera.

Fig 1.

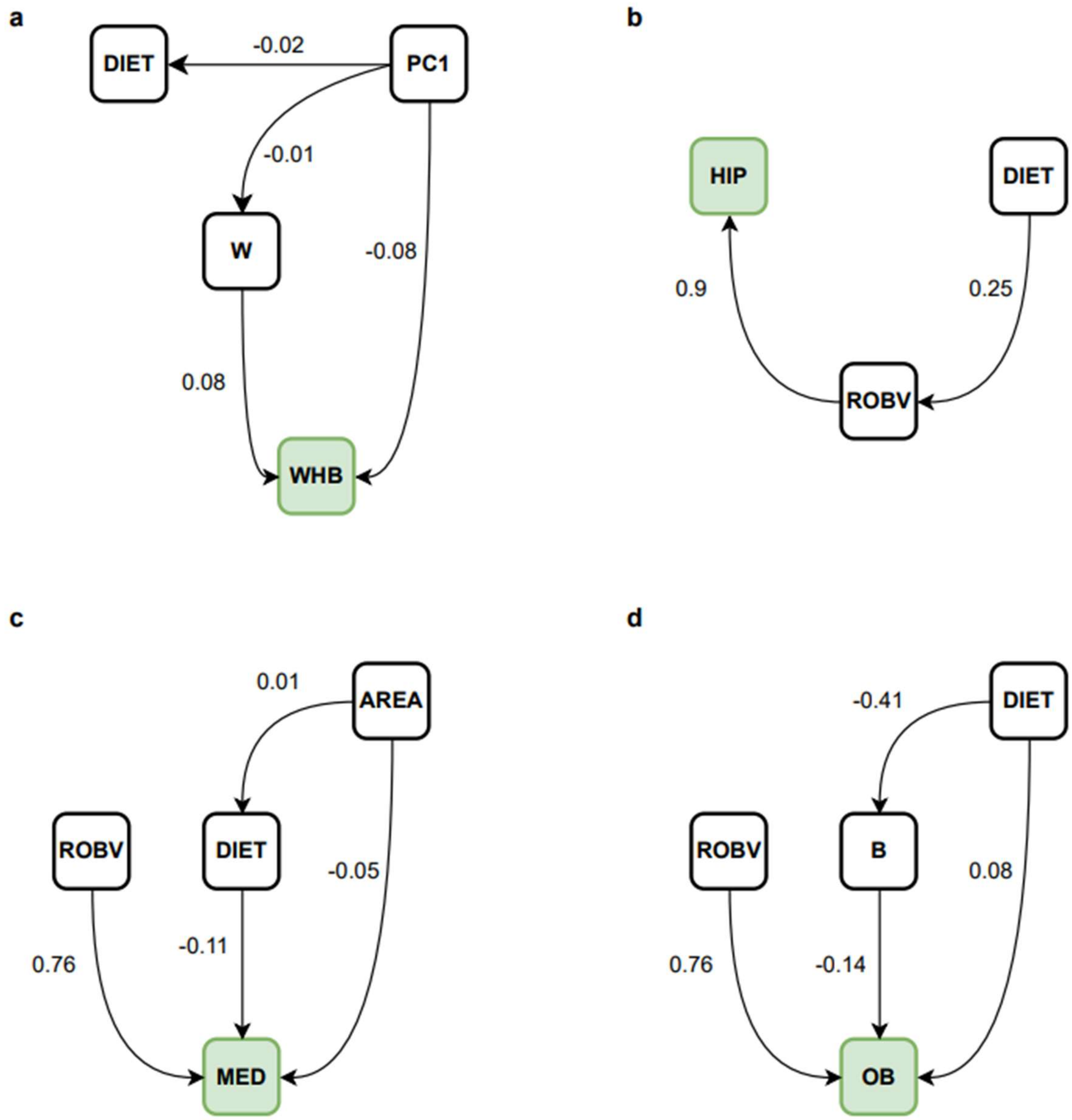

Figure 1. Path analysis results for Michrochiroptera for **a** Whole brain **b** Hippocampus **c** Medulla and **d** Olfactory bulbs (n=168). The response variable is outlined in green. Abbreviations: W weight, WHB whole brain, HIP hippocampus, AREA range area (km^2), ROBV rest of brain volume, MED medulla, B breadth, OB olfactory bulbs. Arrows indicate direction of the relationship, while numbers indicate the strength of the relationship and whether the effect is positive or negative.

Table 4. Path analysis coefficients for Microchiroptera (n=168).

Response Variable	**Factor 1**		**Factor 2**	**Path Coefficient**	**Standard Error**
Brain	Weight	→	Brain Volume	-0.75	0.03
	PC1	→	Diet	-0.02	0.03
	PC1	→	Weight	-0.10	0.06
	PC1	→	Brain Volume	-0.08	0.03
Hippocampus	ROB Volume	→	Hippocampus	0.90	0.03
	Diet Type	→	ROB Volume	0.25	0.16
Medulla	ROB Volume	→	Medulla	1.00	0.03
	Diet Type	→	Medulla	-0.11	0.07
	Range Area	→	Medulla	-0.05	0.02
	Range Area	→	Diet Type	0.01	0.03
Olfactory bulbs	ROB Volume	→	Olfactory Bulbs	0.76	0.03
	Diet Breadth	→	Olfactory Bulbs	-0.14	0.05
	Diet Type	→	Olfactory Bulbs	0.08	0.07
	Diet Type	→	Diet Breadth	-0.41	0.10

Path Analysis: Pteropodidae

Within the Family Pteropodidae, we found patterns opposite to those found for the Microchiroptera. Unlike the other bat families, the hippocampus size of Pteropodidae is directly influenced by diet breadth (Figure 2a, Table 5). Unexpectedly, fruit bats with a large breadth seem to have a smaller hippocampus than those with a small breadth (Figure 2a, Table 5). We also found a negative relationship between island-dwelling and medulla size (Figure 2b, Table 5). Fruit bats that live on islands have smaller medulla than those that live on islands.

Thus, overall, the path analysis, showed that the olfactory bulbs of Pteropodids are indirectly and directly influenced by diet type, diet breadth, habitat type, and island-dwelling (Figure 2c, Table 5). Olfactory bulb evolution is more complicated for Pteropodidae than for

Microchiroptera. Our path analysis showed that frugivorous species with a larger diet breadth had small olfactory bulbs (Figure 2c, Table 5). Thus, among the Pteropodidae, fruit eating generalists with small olfactory bulbs seem to be the norm. From this analysis it is not possible to know if specialist fruit bats have larger olfactory bulbs as in Microchiroptera. However, our results suggest that pollen/nectar eaters that are specialists have larger olfactory bulbs than those that are generalists. Our findings suggest that phytophagous (plant-eating; i.e., nectivores and frugivores) species from both bat clades could face similar selective pressures affecting olfactory bulb size, but these patterns are complex and difficult to understand.

Fig 2.

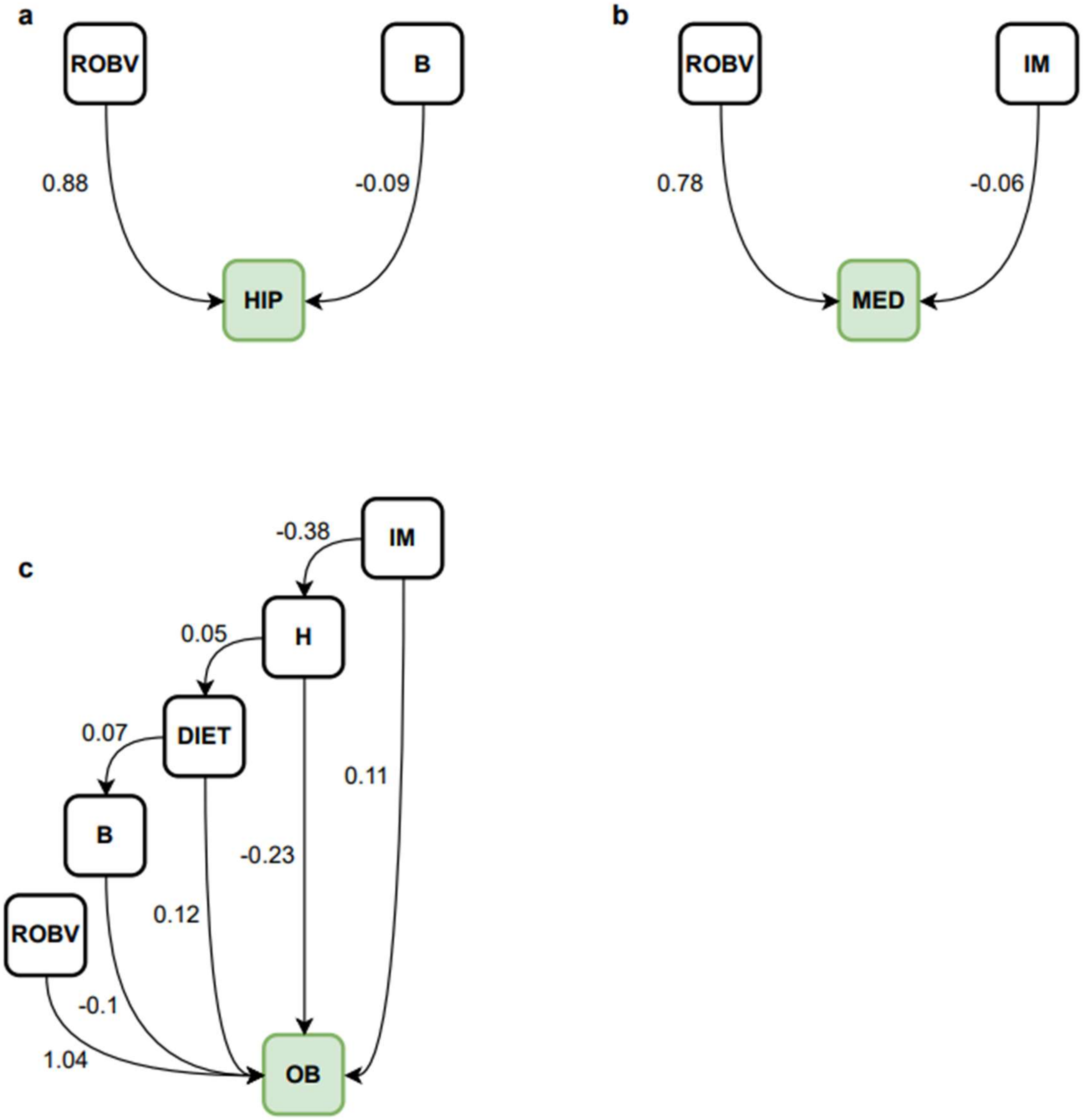

Figure 2. Path analysis results for Pteropodidae for **a** Hippocampus **b** Medulla and **c** Olfactory bulbs (n=36). The response variable is outlined in green. Abbreviations: ROBV rest of brain volume, HIP hippocampus, AREA range area (km^2), B breadth, IM Island mainland classification, MED medulla, H Habitat, OB olfactory bulbs. Arrows indicate direction of the relationship, while numbers indicate the strength of the relationship and whether the effect is positive or negative.

Table 5. Path analysis coefficients for Pteropodidae (n=36).

Response Variable	Factor 1		Factor 2	Path Coefficient	Standard Error
Hippocampus	ROB Volume	→	Hippocampus	0.88	0.04
	Diet Breadth	→	Hippocampus	-0.09	0.38
Medulla	ROB Volume	→	Medulla	0.78	0.04
	Island Dwelling	→	Medulla	-0.06	0.03
Olfactory bulbs	ROB Volume	→	Olfactory Bulbs	1.04	0.05
	Island Dwelling	→	Olfactory Bulbs	0.11	0.0
	Island-Dwelling	→	Habitat Type	-0.38	0.34
	Habitat Type	→	Olfactory Bulbs	-0.23	0.05
	Habitat Type	→	Diet Type	0.05	0.18
	Diet Type	→	Olfactory Bulbs	0.12	0.04
	Diet Type	→	Diet Breadth	0.07	0.18
	Diet Breadth	→	Olfactory Bulbs	-0.10	0.04

DISCUSSION

Whether the size of the brain, or the size of specific areas of the brain, in bats is influenced by diet, habitat, or energetic constraints has been widely debated (Healy and Rowe 2007). While diet, habitat, and environment were all associated with brain evolution, our findings demonstrate that the nature of the selective pressures that have influenced whole brain, hippocampus, medulla and olfactory bulbs size are complex and differ between the clades Microchiroptera and Pteropodidae. Taken together, our results support the mosaic evolution theory, suggesting that the evolution of individual brain parts has been influenced by different selective pressures in a variety of ways.

Whole brain

We found that whole brain size in Microchiroptera was negatively associated with temperature (PC1). The climate variable PC1, which is comprised primarily of temperature variables, had a direct effect on brain size in the Microchiroptera. Many of the PC1 component loadings pertained to temperature variability, such as isothermality and temperature seasonality. Thus, bat species with a higher PC1 score likely come from regions where temperature is variable over the year. A larger brain size could allow bats living in regions with larger fluctuations in temperatures to have more flexible behaviours or a wider range of behaviours, which would help to buffer them against environmental changes, consistent with the predictions of the cognitive buffer hypothesis (Sayol et al. 2018).

Few studies have investigated the effects of climate change on the mammalian brain, and the few that exist have mainly focused on human evolution. One recent macroevolutionary study on the Genus *Homo* found that, even after controlling for the effects of body size, sex, phylogeny, and geography (Stibel 2022), larger brains evolved during periods of global cooling. It is possible that both bat and human evolution are affected by environmental stochasticity in that daily and seasonal temperature fluctuations may influence the size of the brain. The cognitive buffer hypothesis suggests that stochasticity and fluctuations in climate could favor a capacity for individuals to produce novel behaviours that would improve the chances of survival in a changing environment. However, Stibel's (2002) results did not reveal if temperature variation influenced the evolution of brain size directly, or only indirectly through an effect on body size.

Rubalcaba et al. (2022) found that there was a trade off between flight ability in bats and thermoregulation in colder environments; larger wing surface area increases heat loss but reduces energy expenditure since it makes flight easier. Bats that live in colder environments tended to have a lower wing surface area due to the energetic constraints associated with living in cold environments (Rubalcaba et al. 2022). The authors suggested that selection related to thermoregulation was stronger in colder regions where bats expended more energy on heat production; however, they did not test if the effect changed in regions with highly variable temperature as they primarily used yearly temperature averages in their analysis (Rubalcaba et al. 2022). Our study suggests that the temperature itself may not constrain the size of the brain in Microchiroptera, but rather the fluctuations in temperature may have an important influence on brain evolution. Our finding could be the result of thermoregulation and energy tradeoffs, or because a larger brain is required for bats to survive in a wide range of environmental conditions.

Previous studies have found a correlation between diet type and whole brain size; however, our analysis suggests that diet has no influence on the brain. Among Pteropodids, whole brain size was not associated with any ecological factors at all. Thus, our results suggest that in general, diet type has not directly influenced the evolution of brain size in bats.

Hippocampus

The size of the hippocampus within Microchiroptera was associated with diet type; however, the path analysis showed that the effect of diet on the hippocampus is indirect, acting through its direct effect on the rest of brain (ROB) volume. This result provides a more comprehensive understanding of the evolution of the hippocampus in frugivorous Microchiropterans, as the only other study to examine hippocampus size across multiple bat species did not include PGLS or path analysis (Hutcheon et al. 2002). Like Hutcheon et al. (2002), we did find an association between diet and hippocampus size; however, the relationship is indirect, and driven by the effect of diet on the ROB volume.

In contrast to the findings for Microchiroptera, diet breadth had a direct and negative effect on hippocampus size in the Pteropodids. Specialist fruit bats have larger hippocampi than nectivores with broader diets breadths. The hippocampus is responsible for spatial awareness and memory (Liberti III et al. 2022). Thus, we presumed that bats with larger diet breadths would require a larger hippocampus to forage for food from various locations and to remember these locations. The relationship between brain size and diet breadth in other animals is unclear; however, research suggests a tendency for generalist species to have larger brain size residuals or score higher on tests of cognitive ability (Chambers et al. 2021; Henke-von der Malsburg 2020; Mac Lean et al). We found the opposite to be true among the Pteropodidae.

There are two possible explanations for our results: (1) fruit contains more calories than nectar and pollen, which could loosen the energetic constraints on the brain (DeCasien et al. 2017), and (2) food is more unpredictably and patchily distributed for longer periods of the year for fruit or flower eating species (Heithaus 1982). First, similar to our result, DeCasien et al. (2017) found that among primates, frugivorous species had larger brains than folivores. They also found that diet type was a better predictor of brain size than other behavioural factors such as sociality (DeCasien et al. 2017). These authors suggested that frugivorous diets are less energy constrained than other diet types because fruits are rich in calories and do not require large energy input for digestion. Lower energy constraints could allow for other tissues, such as those of the brain, to grow. Although DeCasien et al. (2017) investigated whole brain size, energy-dense foods such as fruit may also be influencing the evolution of the hippocampus in Pteropodidae. Second, although obligate fruit eaters only eat one type of food source, there is a wide variation in the size, shape, color, smell, seasonality, distribution, and nutrient content of fruit. The variation among plants, and the fact that fruit distribution can change overnight could result in greater selection pressures related to finding food and remembering its location, driving the evolution of a larger hippocampus (Heithaus 1982).

Medulla

After completing the path analysis for Microchiroptera, we found that the effect of diet on the medulla was negative and direct: non-frugivorous bats have larger medullae (or medullas) than frugivorous bats. In mammals, the medulla oblongata is part of the brainstem and relays information from the brain to the spinal cord (Sricharoenvej et al 2008). It controls involuntary physiological processes such as heart rate, blood pressure and respiration (Sricharoenvej et al. 2008). It is unclear why insectivores have a larger medulla than frugivores, but it could be due to trade-offs with other brain parts that seem to be associated with frugivory, such as the olfactory bulbs.

Range size also had a direct and negative effect on the medulla size in Microchiroptera; bats species with more restricted ranges have larger medullae. In Pteropodids, medulla size was correlated with island-dwelling. The relationship was direct and negative, meaning that bats living on islands have smaller medullae than bats living on the mainland. Because islands inherently have a limited area it is possible that range size is still responsible for this relationship, however it is unclear why. Islands restrict species ranges and gene flow and can have more erratic climates than the mainland (Sayol et al. 2018). These characteristics make living on islands a strong selective force; thus, living on islands may be impacting certain species of bats within the Pteropodidae.

Olfactory bulbs

Like other studies, we found that fruit eating bats in both Microchiroptera and Pteropodidae have larger olfactory bulbs than bats that eat other types of food (Bhatnagar & Kallen 1974; Barton et al. 1995; Eiting et al. 2014; Hall et al. 2020). However, we did not find that frugivorous

Pteropodids had larger olfactory bulbs than nectivores. Interestingly, we also found that Microchiropteran fruit eating bats with smaller diet breadths had larger olfactory bulbs than those with larger diet breadths. Bats specializing on nectar and pollen also had larger olfactory bulbs than generalist frugivores in the Pteropodidae. We could expect the opposite outcome if we consider that larger olfactory bulbs may contain a wider diversity of olfactory receptors for finding a variety of food types. However, it may be that olfactory bulb size provides bats with higher sensitivity to smell, rather than the ability to detect a broader spectrum of smells. Frugivorous bats rely heavily on olfaction to gather their food, especially the species that are specialists. As mentioned, specialist fruit or flower eaters encounter a wide variety of fruit or flower types and display flexible behaviours when foraging for fruit (Heithaus 1982). In this case, the absolute size of the olfactory bulbs could represent increased sensitivity for, and focus on, a particular food source.

The similarities between Microchiropteran and Pteropodid olfactory bulb evolution end with diet; although habitat complexity also plays a role in the evolution of the Pteropodidae olfactory bulbs, this is not true for Microchiropterans. Island dwelling has a positive and direct effect on the size of the olfactory bulbs in the Pteropodidae. In accordance with the cognitive buffer hypothesis, bats may require stronger olfaction when there is environmental stochasticity in order to adjust to changes in food sources. Islands may provide increased habitat complexity and environmental stochasticity, which would drive an increase in the size of the olfactory bulbs over evolutionary time. Perhaps the fruit sources on islands are less predictable than those on the mainland, which results in bats requiring an enhanced sense of smell.

We also found that living in a forested environment seems to result in a decrease in the size of the olfactory bulbs, compared to living in shrublands or artificial/terrestrial habitats such as pastureland or urban areas. Thus, our results reject the cognitive buffer hypothesis, as forested areas are the most complex (Safi et al. 2005). However, many bats classified as living on "artificial/terrestrial" lands also inhabit other habitat types such as forests. It might be useful to investigate this relationship further with a higher resolution index of habitat variability and complexity such as an Enhanced Vegetation Index which was used by Sayol et at. (2018).

Limitations

In contrast to a previous study (Safi et al. 2005), we did not find a strong relationship between habitat complexity and the size of various brain parts in bats. It is possible we simply did not examine the brain structures that are influenced by habitat complexity. Alternatively, the lack of support could be due to limitations in our habitat data. In nature, habitat type or preference is not neatly divided into categories for each species, as it was in our analysis. A more accurate estimation of the complexity of the habitats in which each species lives should be made, as we did for diet, in which “breadth” was considered.

To make sense of the results, we had to separate the Microchiroptera from the Pteropodidae (Yao et al. 2012). The large differences between Pteropodidae and the rest of the bats makes it very difficult to find broad patterns for all bats. If we chose to partition the bats in a different way—using only monophyletic groups, for example, it may have changed our conclusions.

As with any correlational study using PGLS and path analysis, these analytical tools themselves have limitations. PGLS results depend on the variables included in the model and the observations chosen, such as habitat or diet type of the species, for the models (Wartel 2019). Thus, if we had classified habitat type differently, or included more variables such as hibernation, migration, or basal metabolic rate, our results may have been different.

We had much more data available on the brain sizes of frugivorous Pteropodids (n=28), but far less for the pollen and nectar eaters (n = 8). It is possible that nectar eaters and fruit eaters share similar patterns of evolution as both depend on patchily distributed food sources from plants that may not be available year-round. A smaller sample size of nectivores than frugivores may not be ecologically meaningful, as differences may be found where none really exist as the statistical power of an analysis decreases.

Many phylogenetic comparative studies on the evolution of the brain assume that brain size is a direct index of cognitive ability. However, the relationship between brain size and cognition is unclear (Healy & Rowe 2021). For future studies, other brain measurements, such as neuron density, could be used to compare the cognitive abilities of animals (Dicke & Roth 2016).

Our results indicate that climate, foraging methods, diet breadth, range size, and habitat can influence the size and morphology of the brain in bats. Different selective pressures have impacted the evolution of the hippocampus, medulla and olfactory bulbs, within the groups Microchiroptera and Pteropodidae. These findings are consistent with the mosaic evolution hypothesis, which proposes that each part of the brain evolves independently to a certain degree. The medulla and olfactory bulbs were both directly influenced by living in stochastic environments such as islands or regions with high temperature variability—thereby supporting the cognitive buffer hypothesis. We also found that diet type and diet breadth were associated with the size of the olfactory bulbs in both bat clades. Although body size is the primary determinant for brain size in bats, many other ecological factors can limit or enhance the evolution of a section of the brain. This study represents the largest, most comprehensive study of bat brain evolution to date. Our study demonstrates that brain evolution in Chiroptera is complex, varying between subgroups of the order, and among different brain regions.

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APPENDIX A

Table A1. Counts for each discrete ecological variable used (n=204).

Variable	**Count**
Primary Diet	
Blood, Fish, and Vertebrates	8
Fruit	52
Insects	126
Nectar/Pollen	18
Habitats	
Open	22
Artificial/Terrestrial	47
Savana	10
Forest	101
Caves	24
Island/Mainland Classification	
Island	49
Mainland	155
N	**204**