

RESEARCH ARTICLE

Intraspecific variation in semicircular canal morphology—A missing element in adaptive scenarios?

Lauren A. Gonzales¹  | Michael D. Malinzak^{2,3} | Richard F. Kay² 

¹Department of Biomedical Sciences, University of South Carolina School of Medicine-Greenville, Greenville, South Carolina

²Department of Evolutionary Anthropology, Duke University, Durham, North Carolina

³Department of Radiology, Duke University School of Medicine, Durham, North Carolina

Correspondence

Lauren A. Gonzales, Department of Biomedical Sciences, University of South Carolina School of Medicine-Greenville, 701 Grove Road, Greenville, South Carolina 29605, USA.
Email: lgonzales@greenvillemed.sc.edu

Funding information

The Leakey Foundation; National Science Foundation, Grant/Award Number: BCS-1552848, BCS 1440742

Abstract

Objectives: Recent evidence suggests that the amount of intraspecific variation in semicircular canal morphology may, itself, be evidence for varying levels of selection related to locomotor demands. To determine the extent of this phenomenon across taxa, we expand upon previous work by examining intraspecific variation in canal radii and canal orthogonality in a broad sample of strepsirrhine and platyrrhine primates. Patterns of interspecific variation are re-examined in light of intraspecific variation to better understand the resolution at which locomotion can be reconstructed from single individuals.

Materials and Methods: Data was collected from high-resolution CT scans of 14 size-matched, related species. Six of these taxa have existing data on rotational head speeds.

Results: The level of intraspecific variation was found to differ in strepsirrhine and in platyrrhine species pairs, with larger ranges of variation generally observed for the slower moving taxon than the faster moving one. Taxa that are classified as relatively agile can to some extent be separated from those who are slower-moving, but only when comparing similarly sized, closely related species with more extreme forms of locomotion.

Discussion: Our findings agree with previous research showing that canal intraspecific variation can fluctuate according to species-specific locomotor behavior and extends this further by identifying behaviors that may be under unusual selective pressure. It also demonstrates the complexity of interpreting inner ear morphology in the context of broadly applicable locomotor “categories” of the kind commonly used in behavioral studies. We suspect that simplified models predicting vestibular sensitivity may be unable to differentiate behaviors when only a single specimen is available.

KEYWORDS

inner ear variation, primates, selective constraints, vestibular labyrinth

1 | INTRODUCTION

Characterizing intraspecific variation in morphological traits in extant species is an important component for understanding the “natural” limits of variation and is particularly critical when working with fossil species, which are often represented by only one or a few specimens. Without knowledge of intraspecific variability, confidence cannot be placed on the value of predictive models for inferring behavior from morphological proxies in extinct species. The semicircular canals (organs of balance in the inner ear) play a primary role in gaze-stabilization during locomotion, and it is proposed that patterns of

locomotor agility are reflected in canal morphology (Graf, 1988; Jones & Spells, 1963; Spoor et al., 2007; Spoor, Wood, & Zonneveld, 1994). The membranous canal ducts are encased inside a dense bony labyrinth, making it possible to reconstruct some aspects of membranous duct morphology in osteological specimens and fossils (Figure 1), facilitating broad comparative analyses. While adult interspecific differences in canal functional morphology have been widely documented in primates and other mammals (Berlin, Kirk, & Rowe, 2013; Malinzak, Kay, & Hullar, 2012; Spoor et al., 2007), the extent of canal variation within species is not well understood. A common assumption in comparative studies of canal shape is that intraspecific

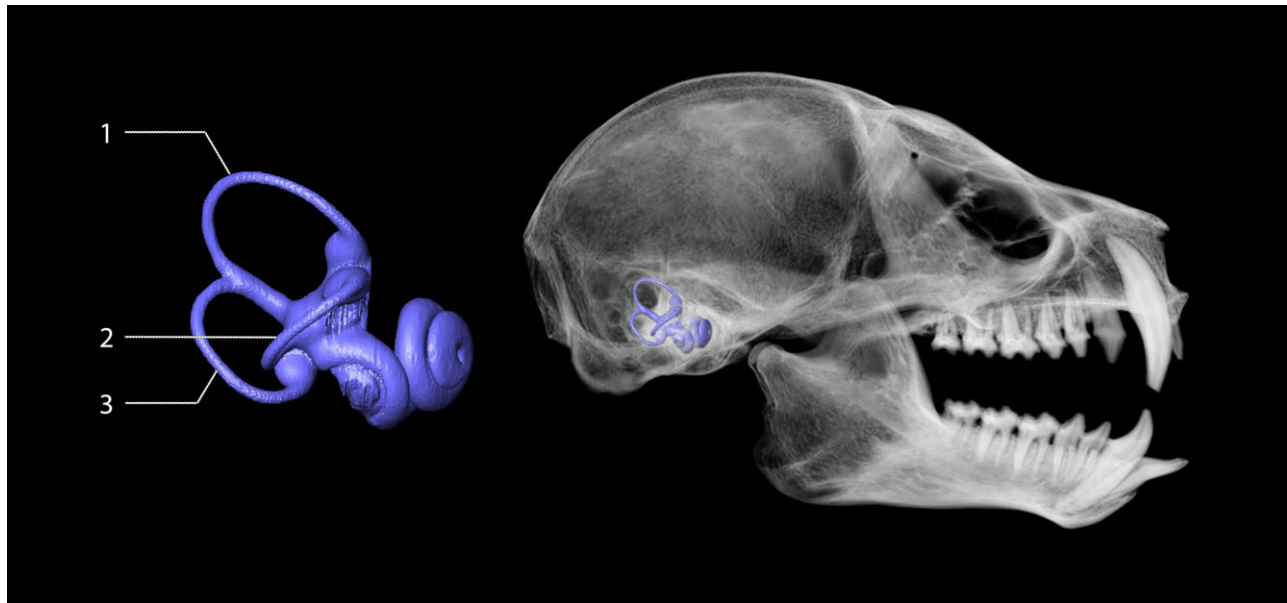


FIGURE 1 Bony anatomy of the semicircular canals in the skull of *Nycticebus coucang* (USNM 355347). (1) Anterior canal; (2) horizontal canal; (3) posterior canal

variation should be relatively low compared to interspecific variation. Conservative systems, such as the semicircular canals, are generally assumed to contain little intraspecific variation as a result of adaptive constraints, with outliers quickly selected against. Therefore, the proposition has been advanced that within-species variation in semicircular canal dimensions should be limited (Welker, Orkin, & Ryan, 2009). This, in turn, has justified the use of comparative models for inferring locomotor behavior in single specimens of extinct species based on either single or a small number of individuals per species in extant animals (Berlin et al., 2013; Malinzak et al., 2012; Spoor et al., 2007).

Currently, two models for reconstructing locomotor behaviors have achieved prominence: Spoor's model using canal radius of curvature (Spoor et al., 2007), and Malinzak's model of canal orthogonality (Malinzak et al., 2012). In Spoor's predictive model, canal radius of curvature is used to infer behavioral agility, building upon experimental literature focusing on semicircular canal radius of curvature as a determinant of vestibular sensitivity (Spoor et al., 2007). When body size is accounted for, larger mean canal radii are said to be indicative of more agile movements, while smaller mean canal radii reflect slower, more deliberate behaviors. Differences in radii among canals (within an individual) are also predicted to relate to the rapidity of habitual head rotations about different axes (Spoor et al., 2007). Since Spoor first published his seminal work on inner ear morphology, canal radii have been widely used as a proxy to reconstruct the locomotor behaviors of extinct vertebrates, with a particular focus on basal primates and early human ancestors (Ryan et al., 2012; Spoor et al., 1994; Spoor, Hublin, Braun, & Zonneveld, 2003; Spoor & Zonneveld, 1998; Walker, Ryan, Silcox, Simons, & Spoor, 2008).

More recently, Malinzak et al. (2012) proposed a model of canal orthogonality, wherein the collective orientation of the canal angles is indicative of the average angular velocities that are experienced by the head. This study showed that species with relatively fast average rotational head speeds have more orthogonal angles between the canals compared to species that experience slower average rotational

head speeds. More orthogonal canals equalize the sensitivity of the six-canal system to rotations about all axes (Ifediba, Rajguru, Hullah, & Rabbitt, 2007; Rabbitt, 1999), such that an animal with perfectly orthogonal canals of identical size would be just as sensitive to rotations about the roll axis as it would be to rotations about the pitch axis, or yaw axis, or any possible axis in between. To explain its findings, Malinzak et al. (2012) posits that slow-moving species are under less selective pressure than fast-moving species to have equalized sensitivity to rotations about all axes.

Both models are based on precise measurements of anatomical features that are believed to be functionally important, specifically, canal radius of curvature and canal orthogonality. However, the amount of intraspecific variation which exists in these measurements is not well characterized and is not addressed in either model.

With the recent increased feasibility of obtaining high-resolution CT scans, sample sizes large enough to evaluate the variation in these metrics within-species is finally attainable. Interestingly, the few studies to have taken advantage of this increase in data are beginning to report quite a bit more variability in canal functional traits than previously suspected (Billet et al., 2012; Ekdale, 2010; Osipov et al., 2013; Perier, Lebrun, & Marivaux, 2016; Welker et al., 2009). For example, previous assumptions inherent all physiological models include (a) low variation in canal dimensions between the right and left sides within an individual and (b) low variation between males and females belonging to species with low sexual dimorphism in size (Rabbitt, Damiano, & Grant, 2004; Yang & Hullah, 2007). These assumptions are supported by observations in short-tailed shrews where less than 5% variation was reported between left and right canal dimensions within individuals and no significant canal size differences were found between males and females, despite slight differences in body size (Welker et al., 2009). Yet, a study of humans (Osipov et al., 2013) has noted significant differences between males and females (particularly in the radii of the posterior canal) and significant bilateral asymmetry in several canal traits (e.g., radii of the anterior canal). These findings were found to extend across

mammals by Berlin et al. (2013), who described significant deviations in canal angle symmetry and coplanarity across 39 mammal species. And more recently Bernardi and Couette (2017) reported high intraspecific variation in canal orthogonality compared to canal radii when examining seven *Adapis parisiensis* specimens from the Late Eocene of Europe. This variation was high enough to lead the authors to disregard the orthogonality data in the conclusions of their study.

Interestingly, a few studies now suggest that the amount of intraspecific variation in functionally important canal measurements may also be correlated with interspecific difference in locomotor behavior. Billet et al. (2012) compared levels of intraspecific variation in canal measurements in xenarthrans (armadillos and sloths) and *Sciurus vulgaris* (red squirrels), documenting significantly larger ranges of intraspecific variation in canal angles in slower-moving species (e.g., sloths) than in faster-moving ones (Billet et al., 2012). The authors attribute their findings to a release of selective constraints and hypothesize that this may be representative of a more general pattern across Mammalia. Perier et al. (2016) have provided more recent support for this hypothesis by documenting a similar trend in two extremely slow moving primates (*Nycticebus coucang* and *Perodicticus potto*) when compared to the relatively more agile *Microcebus murinus* and *Callithrix jacchus*. Additionally, Schutz, Jamniczky, Hallgrímsson, and Garland (2014) found that mice artificially selected for increased running capabilities (thus increasing agility) show corresponding changes in aspects of canal shape but not radii of curvature after just a few generations. This added morphological variation, occurring within the same species and over a relatively short time scale, highlights the potential for selective pressures to affect sensitivity.

All of this begs the question, do different amounts of intraspecific variation actually reflect the strength of selection on canal shape depending on varying levels of head movement? As a test of this possibility, we quantified intraspecific variation in functionally relevant canal morphology in a sample of 14 primate species. Our first objective was to compare intraspecific variation in canal radius of curvature and canal angles in animals with interspecific variation in rotational head speeds or relative “agility”. We hypothesized that greater intraspecific variation would be observed for slow-moving species than fast-moving species in keeping with the proposal of Billet et al. (2012). Our second objective was to document patterns of interspecific variation in canal angles and radii of curvature while taking into account intraspecific variation. This allows us to determine whether canal morphology that has been deemed functionally relevant separates “fast” from “slow” animals across different taxonomic and body-size groups in a consistent “functional” manner. These analyses are undertaken with a broader goal of understanding how intraspecific variation may factor into the existing models of canal functional morphology and what implications it holds for behavioral reconstructions based on one or a few fossils.

2 | MATERIALS AND METHODS

2.1 | Sample selection

Study species were selected to represent a dichotomy of habitually fast- versus habitually slow-moving animals based on a general

consensus from the behavioral literature and/or recorded differences in rotational head speeds from experimental work (Table 1). Because the only existing comparative data on primate rotational head speeds is for strepsirrhines (Malinzak et al., 2012), we have chosen to focus on that taxonomic group for validation of the agility ranks assigned by Spoor et al. (2007). As a second step, closely related and similarly sized platyrrhines were included to extrapolate more confidently the findings associated with strepsirrhines. In both strepsirrhine and platyrrhine comparisons, we took a narrow allometric approach, where species pairings were selected to maximize differences in locomotor agility at a similar body size (small, medium, or large). This was carried out to minimize body size effects that are known to influence statistical analyses when using canal radius of curvature (Jones & Spells, 1963).

Semicircular canal dimensions were calculated from osteological specimens of seven extant strepsirrhine species and seven extant platyrrhine species. Computed Tomography (CT) scans of museum specimens were collected for a range of 10–16 individuals per species with roughly equal numbers of males and females (5–8 males and 5–8 females). Due to sexual dimorphism in body size for *Alouatta palliata*, five additional male and female specimens were included for this species to increase statistical robusticity for examination of sexual dimorphism. Specimens were scanned at Duke University. A full list of specimens (with museum attributions) can be found in Supporting Information Table S1.

2.2 | Data collection

2.2.1 | CT scanning and digital image preparation

Specimens used in this study were either downloaded from Morphosource.org or scanned at Duke University using a Nikon Xth 225ST high-resolution CT scanner housed in their Shared Material Instrumentation Facility (SMIF; Supporting Information Table S1). Joint Photographic Experts Group (JPEG) images were rotated, translated, and cropped to maximally distinguish all six semicircular canals in each specimen (Supporting Information Table S1). Post-scan processing was completed using the digital imaging software ImageJ (NIH) and Avizo 9.1 (Visualization Sciences Group). CT scans produced for this study can be accessed through the Gonzales Skull Project at Morphosource.org.

2.2.2 | Morphologic data collection and analyses

Radii of curvature and canal angles were calculated from landmark data collected in MATLAB (r2012a) following the protocol outlined by Yang and Hullar (2007) and Malinzak et al. (2012). In this procedure, landmarks were manually selected on each CT slice at the center of each canal lumen. Radius of curvature and orientation for each canal were calculated in MATLAB by fitting both a plane and a circle to scaled and rotated landmark data (Kandel & Hullar, 2010; Kay et al., 2012; Yang & Hullar, 2007). These methods were performed on all six canals (three right, three left) to allow for calculation of angles between ipsilateral canal pairs (Supporting Information Table S1). A description of canal measurements is given in Table 2.

To standardize measurements of radius of curvature so that comparisons can be made for different inner ear proportions, radii for each canal were divided by the geometric mean (GM) of the canal radii for the left and right sides respectively (Sokal & Rohlf, 2009) in a modification of Billet et al. (2012). In functional space, the locomotor signal in radius of

TABLE 1 Taxonomic comparative groups investigated in this study

Species	Taxa	N	Size Category	Agility	BM (m)	BM (f)	Source
<i>Galago senegalensis</i>	Strepsirrhine	9	Small	Fast	200	188	Gebo (1987); Malinzak et al. (2012); Isler et al. (2008) ^a
<i>Nycticebus coucang</i>	Strepsirrhine	11	Small	Slow	679	626	Gebo (1987); Glassman and Wells (1984); Malinzak et al. (2012); Smith and Jungers (1997) ^a
<i>Eulemur fulvus</i>	Strepsirrhine	15	Medium	Slow	2250	2333	Gebo (1987); Dagasto (1994); Dagosto and Yamashita (1998); Malinzak et al. (2012); Isler et al. (2008) ^a
<i>Hapalemur griseus</i>	Strepsirrhine	10	Medium	Fast	748	670	Gebo (1987); Malinzak et al. (2012); Smith and Jungers (1997) ^a
<i>Lepilemur mustelinus</i>	Strepsirrhine	13	Medium	Fast	931 (m-f mean)		Warren and Crompton (1997); Smith and Jungers (1997) ^a
<i>Propithecus verreauxi</i>	Strepsirrhine	11	Large	Fast	2930	2980	Gebo (1987); Lawler (2006); Malinzak et al. (2012); Gordon (2006) ^a
<i>Varecia variegata</i>	Strepsirrhine	15	Large	Slow	3630	3520	Gebo (1987); Dagosto (1994); Malinzak et al. (2012); Smith and Jungers (1997) ^a
<i>Aotus lemurinus</i>	Platyrrhine	14	Small	Medium fast	753	716	Wright (1989); Isler et al. (2008) ^a
<i>Saimiri sciureus</i>	Platyrrhine	10	Small	Fast	855	742	Fontaine (1990); Boinski (1989); Johnson and Shapiro (1998); Isler et al. (2008) ^a
<i>Pithecia pithecia</i>	Platyrrhine	15	Medium	Medium	1940	1580	Fleagle and Meldrum (1988); Fleagle and Mittermeier (1980); Smith and Jungers (1997) ^a
<i>Chiropotes chiropote</i>	Platyrrhine	15	Medium	Slow	2900	2580	Fleagle and Meldrum (1988); Fleagle and Mittermeier (1980); Smith and Jungers (1997) ^a
<i>Lagothrix lagotricha</i>	Platyrrhine	16	Large	slow	7280	7020	Cant, Youlatos, and Rose (2001); Cant, Youlatos, and Rose (2003); Smith and Jungers (1997) ^a
<i>Alouatta palliata</i>	Platyrrhine	18	Large	Slow	7150	5350	Gebo (1992); Johnson and Shapiro (1998); Bezanson (2009); Bezanson (2006); Smith and Jungers (1997) ^a
<i>Ateles geoffroyi</i>	Platyrrhine	16	Large	Fast	7780	7290	Cant (1989); Fontaine (1990); Johnson and Shapiro (1998); Mittermeier (1978); Smith and Jungers (1997) ^a

^aSources for body mass estimates are denoted by an asterisk. Sources for classifying agility are unmarked. For *Lepilemur mustelinus*, only the species mean for combined male and female body weight estimates was available. Behavioral data were not available for all species. Locomotor budgets from *Saimiri oerstedii* and *Saimiri boliviensis* were used to infer relative agility for *Saimiri sciureus*, and data from *Chiropotes satanas* were used to infer agility for *Chiropotes chiropotes*.

curvature comes from the disproportionately small or large size of the canals for a given body mass (BM; Silcox et al., 2009; Spoor et al., 2007), so we have also examined intraspecific variation for each canal radii divided by the cube root of the species sex-specific BM mean (Smith &

Jungers, 1997; Table 1). BM means were used instead of predicting individual body mass estimates from osteological proxies (i.e., molar length and/or skull length) because of evidence showing low correlations between individual body mass and predictor variables (Cuozzo, 2001; Yapuncich, 2017).

TABLE 2 Definitions and abbreviations of vestibular characters

Variable	Description
HSC/ASC	Angle between the Horizontal Canal and Anterior Canal
HSC/PSC	Angle between the Horizontal Canal and Posterior Canal
PSC/ASC	Angle between the Posterior Canal and Anterior Canal
ASC R	Radius of Anterior Canal/Geomean
HSC R	Radius of Horizontal Canal/Geomean
PSC R	Radius of Posterior Canal/Geomean
ASC R/BM	Radius of Anterior Canal/Cube Root of BM
HSC R/BM	Radius of Horizontal Canal/Cube Root of BM
PSC R/BM	Radius of Posterior Canal/Cube Root of BM

ASC = anterior canal; HSC = horizontal canal; PSC = posterior canal; BM = body mass.

For a subset of our study species (those species having mostly unobstructed views of both left and right vestibular labyrinths) the percent difference between the right and left sides of the head within individuals was calculated to assess bilateral symmetry in canal angles and radii of curvature and to determine whether information provided by a single side adequately captures vestibular morphology. The percent difference between males and females of the same species was also calculated to examine the effects of sexual dimorphism on vestibular morphology.

To visualize and assess differences in intraspecific variation for each metric across species, the variance of each unlogged measurement was calculated in JMP Pro (version 11.0), SAS Institute Inc. (Cary, NC; Figure 2). The significance of the differences in levels of variance between species was tested using a Brown-Forsythe Unequal

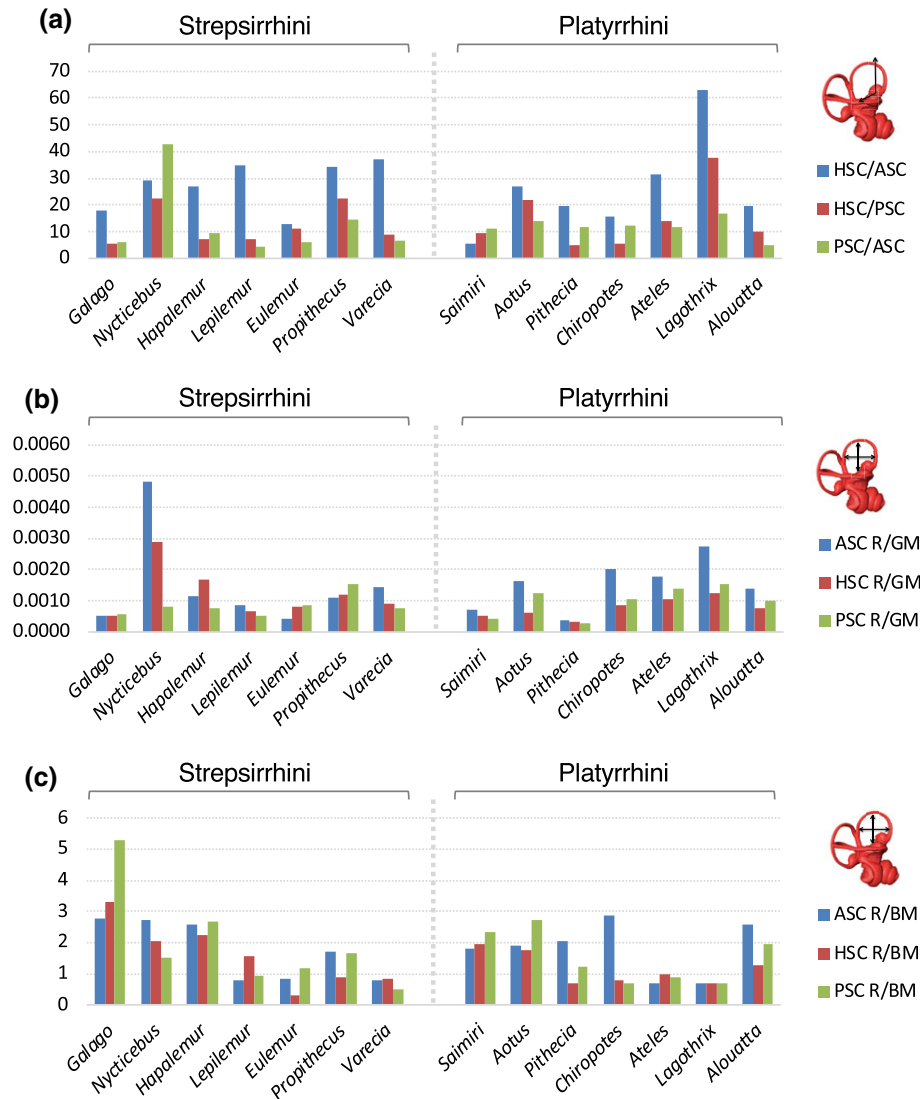


FIGURE 2 Calculated variance for (a) canal angles, (b) radii of curvature, and (c) radii of curvature with body mass accounted for

Variances test in JPM (SAS Institute Inc., Cary, NC). This statistic tests for the equality of deviation from the median of two or more samples. In the case of multiple pair-wise comparisons, a Bonferroni correction was applied to adjust the significance level.

Taxonomic- and locomotor-specific patterns in vestibular morphology were evaluated using Principal Components Analysis (PCA). Analyses were run with all angular and linear variables included in the PCA. A second set of analyses were run to include only those variables with low intraspecific variation and can be found in Supplemental Figure 1. To normalize the distribution data for each species sample, linear and angular variables included in the PCA were log transformed to create a log shape variable (Jungers, Falsetti, & Wall, 1995). The PCA was carried out on the correlation matrix using *JMP* (SAS Institute Inc., Cary, NC). PCA species means (eigenvalues) were calculated for each axis by averaging PCA scores computed for individuals.

2.2.3 | Behavioral categories

A locomotor agility rank was assigned to each species using the categorization originally allocated by Spoor et al. (2007), where a rank of

1 (very slow) through 6 (very fast) was assigned based on visual estimates of head rotational speeds corrected for locomotor budget (Table 3). Head rotations during locomotion are the stimulus detected by the semicircular canals and have been reported as angular velocity means (AVM) for six of the strepsirrhine species in our sample (Malinzak et al., 2012). For these six species, published estimates of agility ranks (Spoor et al., 2007) were compared with the available head rotation data to arrive at an adjusted agility rank. Behavioral studies of *Aotus lemurinus* are limited to anecdotal accounts of behavior (describing moderate amounts of leaping) and to inferences based on postcranial anatomy (Wright, 1989), so an agility score of four (medium to fast moving) was assigned following Spoor et al. (2007).

3 | RESULTS

3.1 | Intra-individual morphologic comparisons

The percentage difference between values obtained from the right and left sides of the head were calculated for all vestibular characters.

TABLE 3 Percentages of major locomotor modes observed for extant strepsirrhine and platyrrhine species

Species	Source	QWR	CL	L	S	Other	AVM Malinzak et al. (2012)	Spoor Agility Rank	Adjusted Agility Rank
<i>Galago senegalensis</i>	Gebo (1987)	5	26	63	4	2	235	6	6
<i>Nycticebus coucang</i>	Gebo (1987)	24	21	0	54	1	68	2	2
<i>Nycticebus coucang</i>	Glassman and Wells (1984)	44	53	0	1	3	68	2	2
<i>Eulemur fulvus</i>	Gebo (1987)	39	17	34	10	0	87	4	4
<i>Eulemur fulvus</i>	Dagasto (1994)	25	11	62	0	2	87	4	4
<i>Eulemur fulvus</i>	Dagosto and Yamashita (1998)	22	9	66	0	3	87	4	4
<i>Eulemur fulvus</i>	Dagosto and Yamashita (1998)	36	17	44	0	3	87	4	4
<i>Hapalemur griseus</i>	Gebo (1987)	24	15	56	5	1	147	4	5
<i>Lepilemur edwardsi</i>	Warren and Crompton (1997)	8	30	55	4	3		6	6
<i>Propithecus verreauxi</i>	Gebo (1987)	6	30	46	15	3	124	6	5
<i>Propithecus verreauxi</i>	Lawler (2006)	0	18	82	0	0	124	6	5
<i>Varecia variegata</i>	Gebo (1987)	35	31	21	13	1	56	4	2
<i>Varecia variegata</i>	Dagasto (1994)	34	11	54	0	2	56	4	2
<i>Aotus lemurinus</i>	Wright (1989); Spoor et al. (2007)							5	5
<i>Saimiri boliviensis</i>	Fontaine (1990)	74	3	21	3	1		6	6
<i>Saimiri oerstedii</i>	Boinski (1989)	85	4	7	0	3		6	6
<i>Saimiri oerstedii</i>	Johnson and Shapiro (1998)	56	20	23	1	0		6	6
<i>Pithecia pithecia</i>	Fleagle and Meldrum (1988)	25	5	70	0	0		4	4
<i>Pithecia pithecia</i>	Fleagle and Mittermeier (1980)	20	0	75	5	0		4	4
<i>Chiropotes satanas</i>	Fleagle and Meldrum (1988)	80	2	18	0	0		3	3
<i>Chiropotes satanas</i>	Fleagle and Mittermeier (1980)	80	2	18	0	0		3	3
<i>Lagothrix lagothricha</i>	Cant et al. (2001); Cant et al. (2003)	29	45	4	21	2		3	3
<i>Alouatta palliata</i>	Gebo (1992)	47	37	3	13	1		2	2
<i>Alouatta palliata</i>	Johnson and Shapiro (1998)	63	11	6	20	0		2	2
<i>Alouatta palliata</i>	Bezanson (2009)	82	10	3	5	0		2	2
<i>Alouatta palliata</i>	Bezanson (2006)	79	7	4	10	0		2	2
<i>Ateles geoffroyi</i>	Cant (1989)	52	8	1	29	10		4	4
<i>Ateles geoffroyi</i>	Fontaine (1990)	52	9	13	24	2		4	4
<i>Ateles geoffroyi</i>	Johnson and Shapiro (1998)	38	25	0	38	0		4	4
<i>Ateles geoffroyi</i>	Mittermeier (1978)	22	40	11	26	1		4	4

QWR = arboreal quadrupedal walk, bound, run; CL = clamber, vertical climb; L = leap, drop, hop; S = bridge and suspensory locomotion. Agility ranks for strepsirrhines have been adjusted to more reflect available measurements of angular velocity mean (AVM). Locomotor budgets for *Saimiri oerstedii* and *Saimiri boliviensis* were used to infer relative agility for *Saimiri sciureus* and *Chiropotes satanas* to infer agility for *Chiropotes chiropotes*.

TABLE 4 The percent difference between radius of curvature for each canal from the left and right sides for species means

Species	N	% Diff HSC/ASC	% Diff HSC/PSC	% Diff PSC/ASC	% Diff ASC R	% Diff HSC R	% Diff PSC R
<i>Galago senegalensis</i>	9	0.38	0.39	0.41	0.32	0.57	0.41
<i>Nycticebus coucang</i>	11	0.44	0.43	0.35	0.72	0.93	0.64
<i>Eulemur fulvus</i>	15	0.28	0.26	0.34	0.23	0.24	0.31
<i>Varecia variegata</i>	15	0.33	0.40	0.32	0.26	0.31	0.27
<i>Aotus lemurinus</i>	14	0.56	0.19	0.35	0.27	0.24	0.18
<i>Saimiri sciureus</i>	10	0.49	0.30	0.41	0.17	0.19	0.22
<i>Pithecia pithecia</i>	15	0.38	0.29	0.33	0.23	0.25	0.22
<i>Chiropotes chiropotes</i>	15	0.49	0.43	0.38	0.22	0.24	0.26
<i>Lagothrix lagothricha</i>	16	0.80	0.50	0.29	0.49	0.81	0.51
<i>Alouatta palliata</i>	18	0.55	0.47	0.47	0.27	0.33	0.32
<i>Ateles geoffroyi</i>	16	0.55	0.55	0.28	0.36	0.47	0.50

This difference was calculated as the absolute value of the difference between the left and right canals divided by the mean of both sides and multiplied by 100. Missing values indicate species with data available for only one side of the head.

TABLE 5 The percent difference between radius of curvature for each canal in male and female species means

Species	Sex	N	% Diff HSC/ASC	% Diff HSC/PSC	% Diff PSC/ASC	% Diff ASC R	% Diff HSC R	% Diff PSC R
<i>Lepilemur mustelinus</i>	F	7	0.76	0.38	0.40	0.73	0.35	0.45
	M	5						
<i>Aotus lemurinus</i>	F	7	0.60	0.12	0.34	0.35	1.05	0.54
	M	7						
<i>Chiropotes chiropotes</i>	F	6	0.14	0.31	0.33	0.73	0.57	1.17
	M	7						
<i>Lagothrix lagotricha</i>	F	9	0.30	0.79	0.08	1.39	0.48	1.12
	M	6						
<i>Alouatta palliata</i>	F	9	0.96	0.68	0.53	0.69	0.43	1.61
	M	8						
<i>Ateles geoffroyi</i>	F	7	1.31	0.22	0.01	2.16	0.57	0.74

The percent difference was calculated as the absolute value of the difference between male and female means divided by the combined species means and multiplied by 100. Species were only included if the sex of more than five individuals ($f = 5, m = 5$) could be identified.

An average difference of less than 1% was found for all variables (Table 4). Due to the low amount of variation within individuals, we chose to examine measurements from the left side of the head in all other analyses. Restriction of analyses to one side of the head reduces the likelihood of Type 1 errors by decreasing the number of pair-wise comparisons.

3.2 | Male versus female comparisons

The average percentage difference between values obtained for males and females (left side of the head only) were calculated for all vestibular characters. For all species, differences between sexes are less than 2.2% (Table 5). Despite marked body size sexual dimorphism in *Lagothrix lagotricha* and *Alouatta palliata*, variation was not found to be higher than in species where males and females are more similar in size (e.g., *Ateles geoffroyi*). Given the evident lack of sexual dimorphism, species means (including both males and females) are used in all subsequent analyses to allow for increased sample sizes.

3.3 | Fast versus slow comparisons

Comparisons were divided into three body size categories. For strepsirrhines, the groups are: small (*Nycticebus coucang* and *Galago senegalensis*), medium (*Lepilemur mustelinus*, *Eulemur fulvus*, and *Hapalemur griseus*), and large-bodied strepsirrhines (*Varecia variegata* and *Propithecus verreauxi*). Comparable size groupings of platyrrhines are: small (*Aotus lemurinus* and *Saimiri sciureus*), medium (*Chiropotes chiropotes* and *Pithecia pithecia*), and large (*Alouatta palliata*, *Ateles geoffroyi*, and *Lagothrix lagotricha*). Each size category was examined separately to limit potential variation introduced by body size differences. Data for the Brown–Forsythe pairwise comparisons are summarized in Table 6.

3.3.1 | Strepsirrhine variation

Results show that differences in intraspecific variation are greatest in smaller-bodied strepsirrhines (Figure 2). Undoubtedly the most dramatic behavioral comparison in this study, the slow-moving *Nycticebus coucang* has significantly higher levels of intraspecific variation in canal angles than the vertical clinger and leaper, *Galago senegalensis*

(PSC/ASC and HSC/PSC: $p < .05$). Though not statistically significant, *N. coucang* still exhibits nearly twice as much variance in radius of curvature for each canal than does *G. senegalensis*.

No significant differences are found in levels of variation for canal angles or canal radii in either medium- or large-bodied strepsirrhines, despite experimental evidence documenting substantial differences in “agility”. In medium-bodied strepsirrhines, the comparatively slower-moving, *Eulemur fulvus* does not exhibit higher levels of variation than either *Hapalemur griseus* or *Lepilemur mustelinus* in either canal angles or canal radii. The same is true for large-bodied more slow-moving *Varecia variegata* when compared to the faster-moving *Propithecus verreauxi*.

Controlling for body mass in radii of curvature (SCR/BM), statistical differences were also low, with the only significant difference found between *Lepilemur* and *Eulemur* (HSC R/BM: $p < .05$, Bonferroni significance criterion: $p < .017$). However, across all body size comparisons, there is an observable increase in levels of intraspecific variation for smaller animals, with the variance more than doubling between the large and small-body size categories. This is particularly evident for *G. senegalensis*, the smallest species in this sample, which has the highest variance for any included strepsirrhine in our sample, unlike previous patterns viewed for either canal angles or canal radii divided by GM.

While intraspecific variation was not compared directly between pairs of canal angles or radii, it is of note that the HSC/ASC angle exhibits far more variation in our sample than does any other canal metric. This trend is consistent across species. Furthermore, the disparity in levels of variation between canal angle pairs is much less for animals that are categorized as faster-moving than those that are categorized as slow-moving. Unlike this canal angle, no singular canal radius metric consistently shows more (or less) variation than any other.

3.3.2 | Platyrrhine variation statistics

Compared to strepsirrhines, the platyrrhine sample shows much greater differences in intraspecific variation for both canal angles and for canal radii (Figure 2). The small-bodied platyrrhine *Aotus lemurinus* has significantly larger variance than that of the more agile *Saimiri sciureus* for HSC/ASC. Among medium-bodied platyrrhines, the variance in canal angles is more similar, with differences instead found in canal radii (ASC R: $p < .05$).

TABLE 6 Brown-Forsythe test for equality of variances

Taxa	Body size category	Group 1	Group 2	HSC/ASC p value	HSC/PSC p value	PSC/ASC p value	ASC R p value	HSC R p value	PSC R p value	ASC R/BM p value	HSC R/BM p value	PSC R/BM p value
Strepsirrhini	small	Golago	Nycticebus	0.587	0.0343*	0.0054*	0.081	0.103	0.294	0.575	0.816	0.377
	medium	Lepilemur	Eulemur	0.101	0.192	0.421	0.323	0.875	0.880	0.684	0.0092*	0.542
		Lepilemur	Hapalemur	0.750	0.934	0.280	0.642	0.132	0.987	0.025	0.048	0.224
Platyrrhini	large	Eulemur	Hapalemur	0.171	0.255	0.650	0.144	0.174	0.893	0.021	0.040	0.151
		Varecia	Propithecus	0.807	0.175	0.138	0.661	0.914	0.335	0.229	0.544	0.051
		Aotus	Saimiri	0.0019*	0.072	0.982	0.323	0.878	0.098	0.992	0.948	0.539
	medium	Chiropotes	Pithecia	0.849	0.643	0.920	0.0043*	0.056	0.082	0.303	0.775	0.391
		Alouatta	Ateles	0.383	0.989	0.308	0.486	0.524	0.541	0.039	0.705	0.092
		Alouatta	Lagothrix	0.031	0.062	0.0196*	0.078	0.555	0.433	0.036	0.166	0.0108*
large	Ateles	Lagothrix	0.190	0.109	0.289	0.252	0.983	0.854	0.935	0.279	0.192	

* denotes significant p-values.

Large-bodied platyrrhines have a much more complicated pattern of variation. The relatively slow-moving *Lagothrix lagotricha* has the highest variance for all canal angles. Surprisingly *Alouatta palliata*, documented as having more deliberate locomotion than *L. lagotricha*, shows the least amount of variation in canal angles, with significantly less PSC/ASC variance than *L. lagotricha* ($p < .05$: Bonferroni significance criterion: $p < .017$). *Ateles geoffroyi*, which incorporates more dynamic suspensory behaviors than other atelids (Cant et al., 2003; Fontaine, 1990; Mittermeier, 1978), demonstrates levels of variation similar to, yet still higher than *Al. palliata*. For canal radii, the variance among these larger bodied platyrrhines is not statistically significant.

Controlling for body mass in canal radii (SCR/BM), the only statistical difference was found between *Alouatta* and *Lagothrix* (PSC R/BM; $p < .05$, Bonferroni significance criterion: $p < .017$). Again, included taxa show a trend toward increased levels of intraspecific variation in smaller-bodied animals compared to larger animals. However, unlike patterns viewed in either canal angles or canal radii divided by GM, *A. palliata* has higher variance in all canal measurements than any other Atelid.

Among canal angle pairs, HSC/ASC was again found to have notably high levels of intraspecific variation in all examined species (with the exception of *Saimiri sciureus*), similar to strepsirrhines. Unlike strepsirrhines, high levels of variation were also observed for ASC R compared to other canal radii.

3.4 | Principle components analyses

To explore taxonomic and locomotor specific patterns in semicircular canal variation, a PCA was performed to reveal overall patterns in the morphological shape space. All angular and linear variables were included in the PCA analyses except for canal radii controlled for body size, which we chose to omit due to the extreme inflation of variance in small body size categories. (PCA plots with this measurement can still be found in Supplementary Figure 2.) Taxon-specific trends were identified by analyzing strepsirrhines and platyrrhines separately. Results and species mean PCA values are listed in Tables 7 and 8 and Figure 3a–h.

TABLE 7 Principal component factor loadings for the first two axes of the PCA including both canal angles and radii of curvature

Taxa	Measurement	Factor 1 (34.6%)	Factor 2 (25.7%)
Strepsirrhines	Log ASC R	0.87095	-0.3804
	Log HSC R	-0.93942	-0.22453
	Log PSC R	0.13552	0.84819
	HSC/ASC	0.38724	-0.3764
	HSC/PSC	0.27929	0.69012
	PSC/ASC	-0.43632	0.0952
		Factor 1 (42.8%)	Factor 2 (17.2%)
Platyrrhines	Log ASC R	0.8138	0.00733
	Log HSC R	-0.97271	0.04729
	Log PSC R	0.74229	-0.09034
	HSC/ASC	0.45193	0.47481
	HSC/PSC	0.30959	0.47095
	PSC/ASC	0.33347	-0.75956

Significant loading variables are in bold.

TABLE 8 Principal component scores for species means

Taxa	Species	PC1 (species means)	PC2 (species means)
Strepsirrhines	<i>Galago senegalensis</i>	-2.89	0.83
	<i>Nycticebus coucang</i>	-0.28	-1.46
	<i>Eulemur fulvus</i>	0.21	-0.66
	<i>Haplemur griseus</i>	0.23	0.68
	<i>Lepilemur mustelinus</i>	0.37	-0.40
	<i>Propithecus verreauxi</i>	0.18	1.16
	<i>Varecia variegata</i>	1.12	0.27
Platyrrhines	<i>Aotus lemurinus</i>	-1.62	0.58
	<i>Saimiri sciureus</i>	-2.35	-0.32
	<i>Chiropotes chiropotes</i>	-0.26	0.46
	<i>Pithecia pithecia</i>	-0.97	-0.37
	<i>Alouatta palliata</i>	0.54	-0.09
	<i>Ateles geoffroyi</i>	2.39	-0.23
	<i>Lagothrix lagotricha</i>	1.04	-0.07

3.4.1 | Strepsirrhines

Taken together the first two principal components account for 60.3% of the variation in the data (Figure 3a–d, Table 7). The first principle component (PC1) accounts for approximately 34.6% of the variation, effectively separating the small-bodied *Galago senegalensis* from other strepsirrhines, whereas there is considerable overlap for medium and larger-bodied strepsirrhines. Factor loadings (eigenvalues) on the PC1 axis are most weighted by ASC R (positively) and HSC R and PSC/ASC (negatively; Table 7). The second principal component axis (PC2) accounts for approximately 25.7% of the variance, and while there is significant overlap among species ranges as a whole, mean PC2 scores within similarly sized taxa separate those incorporating some leaping behaviors (higher agility scores) from those exhibiting more antiprone behaviors (lower agility scores; Table 8). Factor loadings on this axis are primarily weighted (positively) by PSC R and HSC/PSC (Table 7).

In general, higher mean scores for PC2, and to a lesser extent, lower mean scores for PC1 separate similarly sized animals that incorporate greater amounts of leaping in their behaviors from those animals that would be categorized as generalized arboreal quadrupeds. Slower-moving species for each body-size group tend to have larger radii for ASC R and slightly more obtuse angles for HSC/ASC (*N. coucang* morphology). In turn, more agile species have larger canal radii for HSC R and PSC R and more obtuse angles for PSC/ASC and HSC/PSC (*G. senegalensis* morphology). Not surprisingly, the range of variation for the small-bodied, slow-moving *N. coucang* spans the entire range of all included species, particularly along PC1. Of note, the supposedly fast-moving *L. mustelinus* clusters with the slower-moving *E. fulvus* on both axes. Unlike our other included strepsirrhine species, head angular velocity was not available for *L. mustelinus* limiting our interpretation of “agility”.

3.4.2 | Platyrrhines

The first two principal components account for 60.3% of the variation within the sample (Figure 3e–h, Table 7). PC1 accounts for approximately 42.8% of the variation and separates small-sized (Cebidae: *Saimiri sciureus*, *Aotus lemurinus*) and medium-sized (Pitheciidae: *Pithecia*

pithecia, *Chiropotes chiropotes*) platyrrhines from larger platyrrhines (Atelidae: *Ateles geoffroyi*, *Lagothrix lagotricha*, and *Alouatta palliata*). Factor loadings on this axis are most heavily weighted by ASC R, PSC R, and the angles HSC/ASC (positively) and HSC R (negatively; Table 7). PC2 accounts for approximately 17.2% of the remaining variation and despite significant overlap in species ranges, mean values separate animals that engage in more leaping behaviors (higher agility scores) from other slow-moving, more generalized arboreal quadrupeds (lower agility scores; Table 8). Factor loadings on this axis weighted by HSC/PSC and HSC/ASC (positively) and PSC/ASC (negatively; Table 7).

Interestingly, the distribution of taxa along PC1 strongly resembles a body-size gradient, meaning shape differences are not isometric (a ratio may change with size, reflecting different allometric trends). The difference between body size categories is mostly dictated by a trade-off between the relative size of ASC R and PSC R (positive factor loadings in larger-bodied atelids) and HSC R (negative factor loadings in smaller-bodied cebids) on PC1. Along PC2, functional differences appear to correlate with a trade-off between PSC/ASC (negative factor loadings) and HSC/PSC and even HSC/ASC (positive factor loadings). Slower-moving species for each body-size category have more obtuse angles for HSC/PSC and HSC/ASC but a smaller angle for PSC/ASC. In turn, more agile species have smaller angles for HSC/PSC and HSC/ASC and a wider angle for PSC/ASC. The exception on both axes is larger-bodied atelids, who display a pattern of variation *opposite* of that seen for small and medium-bodied platyrrhines. In particular, the slow-moving *Al. palliata* (AG = 2) displays functional canal morphology more similar to other, more agile platyrrhines than either *At. geoffroyi* (AG = 4) or *L. lagotricha* (AG = 3).

The pattern of morphological variation seen in platyrrhines is different from that observed in strepsirrhines. In platyrrhines, morphological differences are primarily characterized by changes in canal radii (body size allometry) and secondarily by differences in canal angles (function) whereas, in strepsirrhines, changes to radii of curvature and canal angles occur in tandem.

4 | DISCUSSION

In this work, we examine intraspecific variation in semicircular canal shape as it relates to locomotor differences in strepsirrhine and platyrrhine primates. We also re-examine interspecific differences in canal radii and canal angles in light of new information on intraspecific variation. Study species were specifically selected to dichotomize species that engage in slow versus fast locomotor movements. Additionally, species pairs were size-matched to eliminate any possible variation introduced by differences in body size, given that canal shape and size is known to scale with negative allometry. Our results lend some support the hypothesis put forth by Billet et al. (2012), that selection on canal morphology is relaxed in animals with slow locomotor behaviors, as evidenced by their having higher levels of intraspecific variation in canal metrics. Differences in intraspecific variation are greatest when comparing species with the most extreme differences in locomotion. Our results also highlight the complexity of locomotor signals typically used in reconstructive models. While some patterns are consistently repeated across taxonomic groups (thus suggesting some kind of

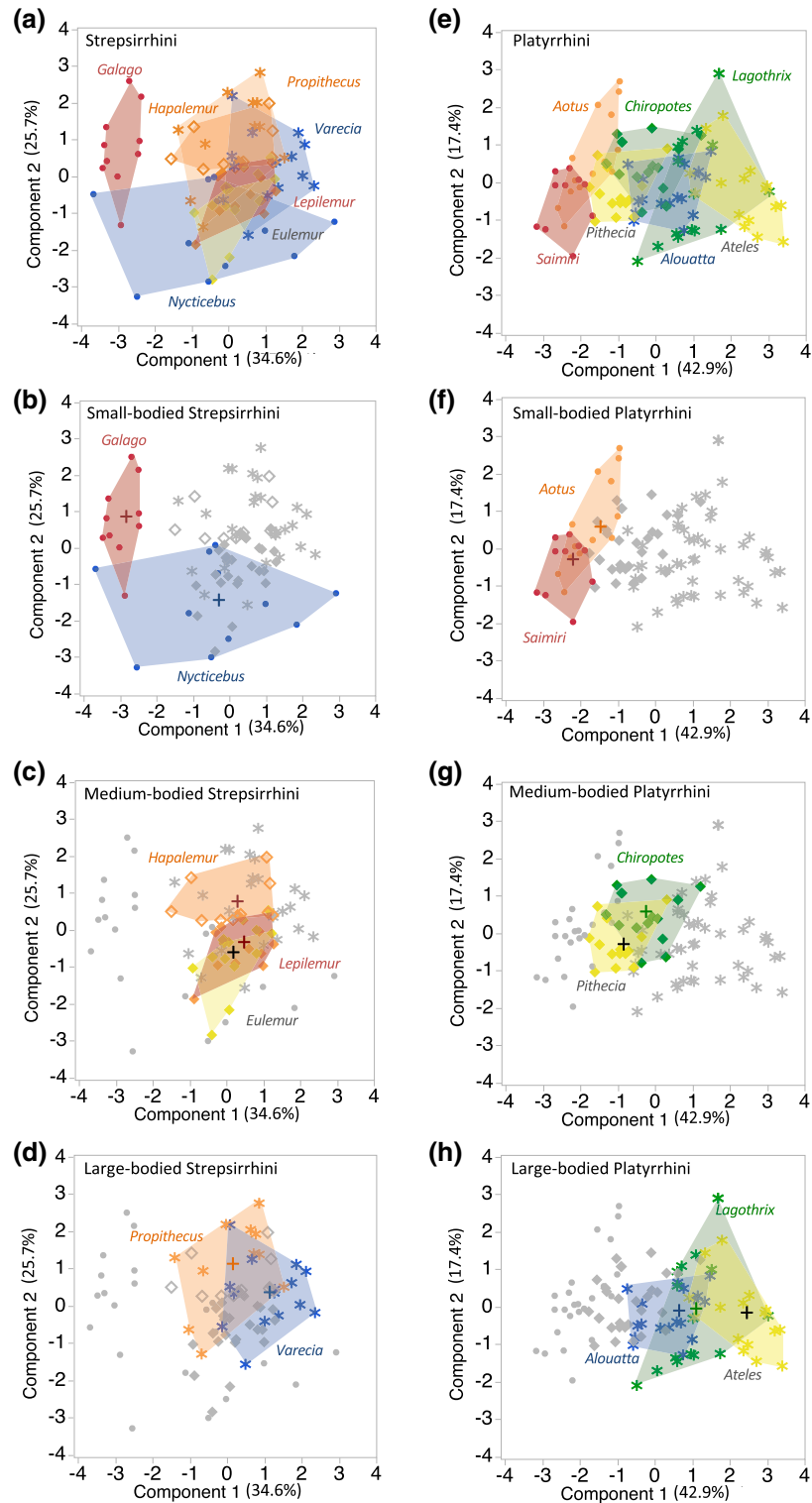


FIGURE 3 PCA including both canal angles and radii of curvature with polygons showing the distribution (in morphospace) of small, medium, and large (a–d) strepsirrhines and (e–h) platyrrhines. The mean for each species PC score is marked by a cross (+)

functional relevance), further variation between strepsirrhine and platyrrhine primates suggests either the adoption of different approaches to signaling rotational head movements or that interactions with other biological systems (body size, head size, or brain size, for example) are heavily influencing canal morphology. This work emphasizes the importance of critically assessing comparative groups used for inferring behaviors in both extinct and extant animals.

4.1 | Patterns of intraspecific variation

4.1.1 | Right versus left

Differences for measurements taken from the left versus right side of the head varied by less than 1% for all studied species despite differences in locomotor behaviors and assigned agility scores. This finding agrees with that reported by Welker et al. (2009) for *Blarina*

brevicauda (northern short-tailed shrew), who found no significant asymmetry in radii of curvature and also by Rodgers (2011) who reported the same findings for radii of curvature and canal angles in single individuals spanning 39 mammal species. Our results, combined with that of Welker et al. (2009) and Rodgers (2011) indicate that past studies using canal measurements from just one side of the head were still able to sufficiently capture canal variation representative of a target individual and validates this practice in general. This is particularly important regarding information obtained from fossil specimens, when material is limited.

4.1.2 | Males versus females

Variation between males and females was also found to be low (<3%) in all evaluated vestibular metrics, even among taxa like *Alouatta* that display large amounts of adult sexual dimorphism in body and skull size. These results are also consistent with the findings of Welker et al. (2009) and we speculate that this may be due to the early ossification of the semicircular canals during ontogeny when size differences between sexes are potentially more trivial. Our data provide support for the use of species means in analyses of canal morphology, however, caution should be employed before taking such an approach as more information is needed about variation related to sexual dimorphism among species with body sizes larger than our test sample, as evidenced by sexual dimorphism in the vestibular morphology of humans (Osipov et al., 2013).

4.1.3 | Fast versus slow

In general, slower moving species are expected to be under less selective pressure to maintain a strict morphological state (the optimum for a system of canals designed to detect head movement without error). The data reported here show some support for the hypothesis that levels of intraspecific variation in canal morphology covaries with locomotor behaviors. We found that “slow-moving” species tend to have higher levels of intraspecific variation than similarly sized animals who engage in more “active” leaping behaviors. However, this trend is only statistically significant in animals displaying the more extreme forms of each behavior. For example, in strepsirrhines, the slow-moving loris, *Nycticebus coucang*, has unusually high variation, displaying a 19° range of variation in the angle between the posterior and the anterior canals compared to the similarly sized but more agile *Galago senegalensis* (8° range of variation). A similar trend occurs for radii of curvature, particularly in the anterior and horizontal canals. Yet intraspecific variation for other strepsirrhines, in both canal radii and canal angles, is quite similar despite differences in relative “agility”. When extrapolated to similarly sized platyrrhines, the larger woolly spider monkey *Lagothrix lagotricha* has a much higher level of intraspecific variation than the similarly sized but faster-moving *Ateles geoffroyi* for both canal angles and radii of curvature. Small- and medium-sized platyrrhines share this trend toward greater variation in angles and radii among taxa perceived to move more deliberately, although not always rising to the level of statistical significance.

Strangely, these trends in levels of intraspecific variation do not always hold, suggesting that at least some taxa may be subject to some form of selection not previously recognized. *Alouatta palliata*, a

species noted for its slothful lack of activity displays surprisingly high orthogonality and low levels of variation that would be more typical of faster-moving animals. In turn, *Propithecus verreauxi*, known for extended bouts of leaping, displays unusually high amounts of intra-specific variation for canal angles, larger than that observed in its large-bodied slower moving relative *Varecia variegata*.

Though not specifically tested, one notable pattern appears consistently across all taxa is a marked increase in intraspecific variation for the angle between the horizontal and anterior canals compared with that seen for the other two canal angles. In platyrrhines, we found an additional increase in variation for the radius of curvature of the anterior canal (sensitive to rotations in the roll direction) compared to either the horizontal or posterior canals (sensitive to rotations in the yaw and pitch directions). Why some of these functional morphological characters have higher levels of intraspecific variation than others is not apparent. One possibility is that this variation reflects corresponding changes in the surrounding cranial base, and therefore may be a consequence of some other phenomenon, like differences in relative brain size or brain proportions, or to a general developmental constraint (Jeffery, Ryan, & Spoor, 2008; Jeffery & Spoor, 2006; Malinzak, 2010). Inside the skull, the canals are in direct contact with the developing basicranium as well as the petrosal lobule of the paraflocculus, an extension of the brain that runs through the arc of the anterior canal to sit within the inner ear (Jeffery et al., 2008; Jeffery & Spoor, 2006). Changes in one system could have profound effects on the morphology of the other systems and minute fluctuations in either system, particularly with the anterior canal and the paraflocculus, might be enhanced.

Finally, we briefly examined a third metric, canal radius of curvature divided by the cube root of body mass in an additional attempt to remove any confounding body size effects from our data. The results of the Levene test give a strong indication that this additional metric has not removed body size as intended, but instead significantly inflates the level of intraspecific variation in smaller animals. This is particularly obvious for *Galago senegalensis*, which has far more intraspecific variation (in just this one metric) than any other primate in our sample. The functional signal appears lost because intraspecific variation in the more extreme examples (i.e., *Galago* and *Nycticebus*) is driving the observed patterns. It is becoming increasingly obvious that body size is very difficult to control for when examining inner ear morphology, and we feel a narrow allometric approach seems the most appropriate solution for reducing body size effects in.

4.2 | Patterns of interspecific variation

A second objective in this study was to document patterns of interspecific variation incorporating both canal angles and radii of curvature to determine: (a) how effective canal-based predictions of vestibular sensitivity are at separating species with different locomotor behaviors; and (b) how variation in strepsirrhine canals compares with that seen in platyrrhines. Despite evidence of fluctuating intraspecific variation in both canal angles and radii of curvature, the PCA demonstrate that canal morphology covaries with relative agility to some degree. According to the predictive models of Spoor et al. (2007) and Malinzak et al. (2012), similar-sized species classified

as relatively agile should gain an advantage by having larger radii of curvature (Spoor et al., 2007) and more orthogonal canal angles (Malinzak et al., 2012), osteological features associated with increased sensitivity to rotational movements. In this study, Strepsirrhine species that were classified as relatively agile tend to have comparatively larger horizontal and posterior canal radii and more acute angles between the horizontal and the anterior canals—a pattern generally consistent with the expectations of the models of Spoor et al. (2007) and Malinzak et al. (2012). However, the range of species-specific morphospace overlaps considerably and it is only when similarly sized animals are compared that these differences are apparent.

Within platyrrhines, patterns of variation are similar but not necessarily the same as those observed in strepsirrhines, with functional changes in canal morphology mostly driven by changes in the canal angles. In general, within each body size category, relatively more “agile” platyrrhine species have smaller angles between the horizontal and the anterior canals and the horizontal and posterior canals. In contrast, slower-moving platyrrhine species have much wider angles for these same canal pairs. These patterns are consistent with the functional morphological pattern proposed by Malinzak et al. (2012) but not with that of Spoor et al. (2007). Yet the variation that occurs across all platyrrhine species is primarily driven by increases in canal radii of curvature with a secondary (and slight) increase in canal angles, which separates large-bodied atelids from medium-sized pitheciids and even further from smaller-bodied cebids. These changes appear more closely correlated with either difference in body size and/or even phylogeny than with agility. And again, it is only when similarly sized animals are compared that any apparent functional signal emerges.

4.3 | Functional implications of variation in semicircular canal morphology

Based on the physics that govern vestibular sensitivity, changes to semicircular canal morphology should dramatically affect an animal's ability to sense the speed of head rotational movements (Billet et al., 2012; Jones & Spells, 1963). The intraspecific variations documented here support Billet et al.'s hypothesis that animals displaying deliberate and slow locomotion (i.e., *Nycticebus coucang* in our sample) have reduced selection to maintain a strict functional shape. Evidently, “slower” movements are interpreted with sufficient accuracy that greater variation in canal morphology is tolerated, that is, the system is not “challenged” as much as it would be in a species with more extreme acrobatic movements.

Yet, despite instances of high intraspecific variation in some slower moving taxa, interspecific variation between “fast” and “slow” animals is highly complex. Current canal-based hypotheses for sensitivity to rotational head movements predict increases in overall arc size for similar-sized faster-moving animals and more orthogonal canals compared with their slower-moving close relatives (Malinzak et al., 2012; Spoor et al., 2007). The results presented here show that while some canals do increase in arc size as predicted for “fast” species, other canals do not; this is also the case for canal angles. We also note distinct taxon-specific functional patterns with few shared overlapping morphologies (i.e., strepsirrhines versus platyrrhines).

Following Spoor et al. (1994), this could suggest that a trade-off in individual canal arc size is related to directional differences in an animal's behavior, especially given that platyrrhines and strepsirrhines move very differently. However experimental evidence from Malinzak et al. (2012) has shown that changes in individual radii of curvature do not line up with axes of fast or slow rotational head speeds in animals, casting doubt onto the viability of this explanation.

Alternatively, it may be possible for animals to adopt more than one morphological strategy to gain information about rotational head speeds. Several recent studies have documented divergent vestibular adaptations in fossorial caecilians, snakes and amphibians (Maddin & Sherratt, 2014; Singh et al., pers. comm.; Yi & Norell, 2015) providing tentative support for this hypothesis. It may also be true that many of the differences documented in this study are not necessarily related to locomotor function, but rather other biological systems (components of brain or skull size). The size of the subarcuate fossa has long been known to have a strong correlation with the size of the anterior canal in primates (Jeffery et al., 2008; Jeffery & Spoor, 2006) and Muller (1999) has theorized that interspecific differences in mean canal size are a side-effect of interspecific differences in skull size. And, more recently, Lebrun, de León, Tafforeau, and Zollikofer (2010) and Billet, Hautier, and Lebrun (2015) have shown that phylogeny plays a distinctly larger role in shaping “functional” canal morphology in strepsirrhines and xenarthrans than previously suspected.

4.4 | Outliers and alternative selective pressures

Larger-bodied platyrrhines (atelids) are the exception to all locomotor patterns described here, displaying canal morphology in complete contrast to that seen for any other comparative group. While *Lagothrix lagotricha* and particularly *Ateles geoffroyi* engage in more climbing, leaping, and suspensory behaviors, *Alouatta palliata* engages in mostly in arboreal quadrupedal activities (Bezanson, 2006, 2009; Gebo, 1992; Johnson & Shapiro, 1998). Yet, *Al. palliata* exhibits extremely low levels of intraspecific variation in all canal metrics and has canal angles that most closely approximate an optimal 90°, a morphological pattern that is more similar to smaller-bodied, more agile species. If the interpretation of Malinzak's predictive model of vestibular sensitivity can be extrapolated to platyrrhines, this morphology would suggest that *Al. palliata* experiences angular velocities most comparable to *Galago senegalensis*, which seems highly unlikely.

An alternate explanation for this group may be that differences in canal angles are the result of differences in absolute skull size, in which case the smaller canal angles in *Al. palliata*, could be attributed to its relatively smaller cranial vault (resulting from a relatively small brain size) compared to *L. lagotricha* or *At. geoffroyi* (Isler et al., 2008). This idea was first introduced by Muller (1999) and has found some support in colobine monkeys (Rae, Johnson, Yano, & Hirasaki, 2016) and in birds (Benson, Starmer-Jones, Close, & Walsh, 2017). Another more functional interpretation may be that the larger canal variation and the wider canal angles seen in *L. lagotricha* and *At. geoffroyi* are the result of a deviation from more quadrupedal activities and the adoption of more suspensory (antipronograde) behaviors. All analyses of canal angles consistently group *Al. palliata* with smaller bodied arboreal quadrupeds. Compared to *Al. palliata*, *L. lagotricha*, and *At.*

geoffroyi engage much more frequently in suspensory activities, spending more of their time traveling and feeding below branch while suspended from a prehensile tail and thus have increased the dimensional space in which they navigate. Should this hypothesis be true, it might suggest that canal morphology is influenced by specific locomotor behaviors rather than simple generalizations about how fast or slow an animal moves. We would also predict that when canal angles are examined in suspensory hominoids (i.e., gibbons and siamangs), they will be found to be comparatively more obtuse.

Unfortunately, nearly all current assessments of “agility” are based on qualitative impressions of locomotion without sufficient knowledge of head movements during locomotion. This categorization of broad, complex locomotor behaviors into simplistic “agility” scores, is problematic for many reasons. While it may be relatively easy to identify when an animal is leaping, it may be nearly impossible to accurately gauge how fast or how far, or how rapidly its head is rotating. Additionally, changes in locomotor behaviors can occur very rapidly, and movements (specifically of the head) might be difficult to record or to even perceive, particularly in small arboreal animals in natural settings. These problems can easily result in observational bias and very likely a misinterpretation about how “agile” living species are in the metric that matters most: head rotational acceleration. Until more in vivo data on head movements can be collected to better understand the influence of specific behaviors rather than overall agility categories on canal morphology, our projections about semicircular canal variation, particularly in platyrrhines, must fall in the category of informed speculation.

5 | CONCLUSIONS

1. We characterize the levels of intraspecific variation in functional parameters of semicircular canal morphology in fast (agile) versus slow pairs of similarly-sized strepsirrhine and platyrrhine species. Two functionally relevant suites of canal measurements were examined, radius of curvature for individual canals and the angles between ipsilateral canal pairs.
2. When body size is controlled for, levels of variation distinguish between some broad locomotor or movement modes in both strepsirrhine and platyrrhine species. The selective forces hypothesized to be controlling for variation in the inner ear is a reduction in the relative agility (or rotational speed of head movement) of locomotor behaviors. As predicted, higher levels of intraspecific variation are typically seen in the slower-moving species within each body size pair, although this relationship is most apparent in platyrrhines (with the exception of small-bodied strepsirrhines).
3. When interspecific differences in canal morphology are considered, only minimal similarities are found between strepsirrhine and platyrrhine taxa. In strepsirrhines, both canal radii of curvature and canal angles are informative as to locomotion. In platyrrhines, canal radii of curvature seem to be highly influenced by body size while differences in canal angles correspond with differences in locomotor behaviors.
4. The results of this study show that canal functional morphology is highly complex and that species may adopt several strategies to

gain information about rotational head speeds. Furthermore, simplified models predicting vestibular sensitivity may be unable to differentiate slower-moving species from faster-moving ones when only a single specimen is available, as individual morphologies of various species overlap extensively. Finally, caution must be taken in choosing the best-fit comparative species, particularly accounting for body size, and perhaps (more speculatively) other factors like brain size before making any sort of behavioral interpretation.

ACKNOWLEDGMENTS

This study was funded by the LSB Leakey Foundation and in part by the National Science Foundation. We thank Christine Wall, Blythe Williams, Louis Roth and Michael Granatosky for statistical advice and constructive comments to the manuscript. We also thank Jimmy Thostenson, Doug Boyer, Christine Fantauzzi, and Crystal Terry for their generous help with computed tomography scanning of specimens and for technical help and advice. Darbi Griffith for her time spent uploading CT scans to the Gonzales Skull Project in Morphosource. We thank Tum Hullar M.D. for giving us access to the MATLAB code. Many thanks to Eileen Westwig, Darren Lunde, and Esther Langan for access to osteological specimens from the American Museum of Natural History and the Smithsonian Institution (Natural History). We also give a special thanks to Lynn Copes, Liz St. Clair, Eva Garrett, and Kari Allen who gave permission to use CT scans of their specimens for this study. Finally, we are very grateful to Mary Silcox and Eric Ekdale for their comments during the review process.

AUTHOR CONTRIBUTIONS

L.G. and R.K. initiated and designed the project, L.G. scanned specimens, collected data, and analyzed results, L.G., R.K., and M.M. discussed results and wrote the manuscript.

ORCID

Lauren A. Gonzales  <https://orcid.org/0000-0003-3304-0634>

Richard F. Kay  <https://orcid.org/0000-0002-4219-7580>

REFERENCES

- Benson, R. B., Stamer-Jones, E., Close, R. A., & Walsh, S. A. (2017). Comparative analysis of vestibular ecomorphology in birds. *Journal of Anatomy*, 231(6), 990–1018.
- Berlin, J. C., Kirk, E. C., & Rowe, T. B. (2013). Functional implications of ubiquitous semicircular canal non-orthogonality in mammals. *PLoS One*, 8(11), e79585. <http://doi.org/10.1371/journal.pone.0079585>
- Bernardi, M., & Couette, S. (2017). Eocene paleoecology of *Adapis parisiensis* (primate, Adapidae): From inner ear to lifestyle. *The Anatomical Record*, 300(9), 1576–1588.
- Bezanson, M. (2009). Life history and locomotion in *Cebus capucinus*. *American Journal of Physical Anthropology*, 140, 508–517.
- Bezanson, M. F. (2006). Leap, bridge, or ride? Ontogenetic influences on positional behavior in *Cebus* and *Alouatta*. In A. Estrada, P. A. Garber, M. M. Pavelka, & L. Luecke (Eds.), *New perspectives in the study of Mesoamerican Primates* (pp. 333–348). New York: Springer.
- Billet, G., Hautier, L., Asher, R. J., Schwarz, C., Crumpton, N., Martin, T., & Ruf, I. (2012). High morphological variation of vestibular system accompanies slow and infrequent locomotion in three-toed sloths.

- Proceedings. Biological Sciences/The Royal Society*, 279(1744), 3932–3939. <http://doi.org/10.1098/rspb.2012.1212>
- Billet, G., Hautier, L., & Lebrun, R. (2015). Morphological diversity of the bony labyrinth (inner ear) in extant xenarthrans and its relation to phylogeny. *Journal of Mammalogy*, 96(4), 658–672.
- Boinski, S. (1989). The positional behavior and substrate use of squirrel monkeys: Ecological implications. *Journal of Human Evolution*, 18(7), 659–677.
- Cant, J. G. H. (1989). Locomotion and feeding postures of spider and howling monkeys: Field study and evolutionary interpretation. *Folia Primatologica*, 46(1), 1–14.
- Cant, J. G. H., Youlatos, D., & Rose, M. D. (2001). Locomotor behavior of *Lagothrix lagotherica* and *Ateles belzebuth* in Yasuni National Park, Ecuador: General patterns and nonsuspensory modes. *Journal of Human Evolution*, 41(2), 141–166. <https://doi.org/10.1006/jhev.2001.0485>
- Cant, J. G. H., Youlatos, D., & Rose, M. D. (2003). Suspensory locomotion of *Lagothrix lagotherica* and *Ateles belzebuth* in Yasuni National Park, Ecuador. *Journal of Human Evolution*, 44, 685–699.
- Cuozzo, F. P. (2001). Craniodental body mass estimators in the dwarf bushbaby (*Galagoides*). *American Journal of Physical Anthropology*, 115(2), 187–190.
- Dagosto, M. (1994). Testing positional behavior of malagasy lemurs: A randomization approach. *American Journal of Physical Anthropology*, 94(2), 189–202. <https://doi.org/10.1002/ajpa.1330940204>
- Dagosto, M., & Yamashita, N. (1998). Effect of habitat structure on positional behavior and support use in three species of lemur. *Primates*, 39, 459–472. <https://doi.org/10.1007/BF02557569>
- Ekdale, E. G. (2010). Ontogenetic variation in the bony labyrinth of *Monoelphis domestica* (Mammalia: Marsupialia) following ossification of the inner ear cavities. *Anatomical Record*, 293(11), 1896–1912. <http://doi.org/10.1002/ar.21234>
- Fleagle, J. G., & Meldrum, J. (1988). Locomotor behavior and skeletal morphology of two sympatric pitheciine monkeys, *Pithecia pithecia* and *Chiropotes satanas*. *American Journal of Primatology*, 16, 277–249.
- Fleagle, J. G., & Mittermeier, R. A. (1980). Locomotor behavior and comparative ecology of seven Surinam monkeys. *American Journal of Physical Anthropology*, 52, 301–314.
- Fontaine, R. (1990). Positional behavior in *Saimiri boliviensis* and *Ateles geoffroyi*. *American Journal of Physical Anthropology*, 82, 485–508.
- Gebo, D. L. (1987). Locomotor diversity in prosimian primates. *American Journal of Primatology*, 13, 271–281.
- Gebo, D. L. (1992). Locomotor and postural behavior in *Alouatta palliata* and *Cebus capucinus*. *American Journal of Primatology*, 26, 277–290.
- Glassman, D., & Wells, J. (1984). Positional and activity behavior in a captive slow Loris: A quantitative assessment. *American Journal of Primatology*, 7, 121–132.
- Gordon, A. D. (2006). Scaling of size and dimorphism in primates II: Macroevolution. *International Journal of Primatology*, 27, 63–105.
- Graf, W. (1988). Motion detection in physical space and its peripheral and central representation. *Annals of the New York Academy of Sciences*, 545, 154–169.
- Ifediba, M. A., Rajguru, S. M., Hullar, T. E., & Rabbitt, R. D. (2007). The role of 3-canal biomechanics in angular motion transduction by the human vestibular labyrinth. *Annals of Biomedical Engineering*, 35(7), 1247–1263. <http://doi.org/10.1007/s10439-007-9277-y>
- Isler, K., Christopher Kirk, E., Miller, J. M. a., Albrecht, G. a., Gelvin, B. R., & Martin, R. D. (2008). Endocranial volumes of primate species: Scaling analyses using a comprehensive and reliable data set. *Journal of Human Evolution*, 55(6), 967–978. <http://doi.org/10.1016/j.jhevol.2008.08.004>
- Jeffery, N., Ryan, T. M., & Spoor, F. (2008). The primate subarcuate fossa and its relationship to the semicircular canals part II: Adult interspecific variation. *Journal of Human Evolution*, 55(2), 326–339. <http://doi.org/10.1016/j.jhevol.2008.02.010>
- Jeffery, N., & Spoor, F. (2006). The primate subarcuate fossa and its relationship to the semicircular canals part I: Prenatal growth. *Journal of Human Evolution*, 51, 537–549. <http://doi.org/10.1016/j.jhevol.2006.07.003>
- Johnson, S. E., & Shapiro, L. J. (1998). Positional behavior and vertebral morphology in atelines and cebines. *American Journal of Physical Anthropology*, 105(3), 333–354.
- Jones, G. M., & Spels, K. E. (1963). A theoretical and comparative study of the functional dependence of the semicircular canal upon its physical dimensions. *Proceedings of the Royal Society of London. Series B*, 157(968), 403–419.
- Jungers, W. L., Falsetti, A., & Wall, C. E. (1995). Shape, relative size, and size-adjustments in morphometrics. *Yearbook of Physical Anthropology*, 38, 137–161.
- Kandel, B. M., & Hullar, T. E. (2010). The relationship of head movements to semicircular canal size in cetaceans. *The Journal of Experimental Biology*, 213(Pt 7), 1175–1181. <http://doi.org/10.1242/jeb.040105>
- Kay, R. F., Perry, J. M. G., Malinzak, M., Allen, K. L., Kirk, E. C., Plavcan, J. M., & Fleagle, J. G. (2012). The paleobiology of Santacrucian primates. In S. Vizcaino, R. Kay, & S. Bargo (Eds.), *Early Miocene Paleobiology in Patagonia* (pp. 306–330). New York: Cambridge University Press.
- Lawler, R. R. (2006). Sifaka positional behavior: Ontogenetic and quantitative genetic approaches. *American Journal of Physical Anthropology*, 131(2), 261–271.
- Lebrun, R., de León, M. P., Tafforeau, P., & Zollikofer, C. (2010). Deep evolutionary roots of strepsirrhine primate labyrinthine morphology. *Journal of Anatomy*, 216(3), 368–380. <http://doi.org/10.1111/j.1469-7580.2009.01177.x>
- Maddin, H. C., & Sherratt, E. (2014). Influence of fossoriality on inner ear morphology: Insights from caecilian amphibians. *Journal of Anatomy*, 225(1), 83–93.
- Malinzak, M. D., Kay, R. F., & Hullar, T. E. (2012). Locomotor head movements and semicircular canal morphology in primates. *Proceedings of the National Academy of Sciences of the United States of America*, 109(44), 17914–17919. <http://doi.org/10.1073/pnas.1206139109>
- Malinzak, Michael, D. (2010). *Experimental analyses of the relationship between Semicircular Canal morphology and locomotor head rotations in primates*. PhD dissertation, Biological Anthropology and Anatomy, Duke University, 236 p.
- Mittermeier, R. A. (1978). Locomotion and posture in *Ateles geoffroyi* and *Ateles paniscus*. *Folia Primatologica*, 30, 161–193.
- Muller, M. (1999). Size limitations in semicircular duct systems. *Journal of Theoretical Biology*, 198, 405–437. <http://doi.org/10.1006/jtbi.1999.0922>
- Osipov, B., Harvati, K., Nathana, D., Spanakis, K., Karantanas, A., & Kranioti, E. F. (2013). Sexual dimorphism of the bony labyrinth: A new age-independent method. *American Journal of Physical Anthropology*, 151(2), 290–301. <http://doi.org/10.1002/ajpa.22279>
- Perier, A., Lebrun, R., & Marivaux, L. (2016). Different level of intraspecific variation of the bony labyrinth morphology in slow- versus fast-moving Primates. *Journal of Mammalian Evolution*, 23(4), 353–368. <http://doi.org/10.1007/s10914-016-9323-3>
- Rabbitt, R. D. (1999). Directional coding of three-dimensional movements by the vestibular semicircular canals. *Biological Cybernetics*, 80(6), 417–431.
- Rabbitt, R. D., Damiano, E. R., & Grant, J. W. (2004). Biomechanics of the semicircular canals and otolith organs. In S. M. Highstein, R. R. Fay, & A. N. Popper (Eds.), *The vestibular system* (pp. 153–201). New York, NY: Springer.
- Rae, T. C., Johnson, P. M., Yano, W., & Hirasaki, E. (2016). Semicircular Canal size and locomotion in Colobine monkeys: A cautionary tale. *Folia Primatologica*, 87, 213–223. <http://doi.org/10.1159/000449286>
- Rodgers, J. C. (2011). *Comparative morphology of the vestibular semicircular canals in therian mammals*. PhD dissertation, Geological Sciences, The University Texas at Austin.
- Ryan, T. M., Silcox, M. T., Walker, A., Mao, X., Begun, D. R., Benefit, B. R., ... Spoor, F. (2012). Evolution of locomotion in Anthropoidea: The semicircular canal evidence. *Proceedings. Biological Sciences/The Royal Society*, 279(1742), 3467–3475. <http://doi.org/10.1098/rspb.2012.0939>
- Schutz, H., Jamniczky, H. a., Hallgrímsson, B., & Garland, T. (2014). Shape-shift: Semicircular canal morphology responds to selective breeding for increased locomotor activity. *Evolution*, 68(11), 3184–3198.

- Silcox, M. T., Bloch, J. I., Boyer, D. M., Godinot, M., Ryan, T. M., Spoor, F., & Walker, A. (2009). Semicircular canal system in early primates. *Journal of Human Evolution*, 56(3), 315–327. <http://doi.org/10.1016/j.jhevol.2008.10.007>
- Smith, R. J., & Jungers, W. L. (1997). Body mass in comparative primatology. *Journal of Human Evolution*, 32(6), 523–559.
- Sokal, R. R., & Rohlf, F. J. (2009). *Introduction to biostatistics: Second edition*. New York: Dover Publications.
- Spoor, F., Garland, T., Krovitz, G., Ryan, T. M., Silcox, M. T., & Walker, A. (2007). The primate semicircular canal system and locomotion. *Proceedings of the National Academy of Sciences of the United States of America*, 104(26), 10808–10812. <http://doi.org/10.1073/pnas.0704250104>
- Spoor, F., Hublin, J.-J., Braun, M., & Zonneveld, F. (2003). The bony labyrinth of Neanderthals. *Journal of Human Evolution*, 44(2), 141–165.
- Spoor, F., Wood, B., & Zonneveld, F. (1994). Implications of early hominid labyrinthine morphology for evolution of human bipedal locomotion. *Nature*, 369, 645–369.
- Spoor, F., & Zonneveld, F. (1998). Comparative review of the human bony labyrinth. *American Journal of Physical Anthropology*, 41, 211–251.
- Walker, A., Ryan, T. M., Silcox, M. T., Simons, E. L., & Spoor, F. (2008). The semicircular canal system and locomotion: The case of extinct lemuroids and lorisooids. *Evolutionary Anthropology: Issues, News, and Reviews*, 17(3), 135–145. <http://doi.org/10.1002/evan.20165>
- Warren, R., & Crompton, R. (1997). Locomotor ecology of *Lepilemur edwardsi* and *Avahi occidentalis*. *American Journal of Physical Anthropology*, 104, 471–486.
- Welker, K. L., Orkin, J. D., & Ryan, T. M. (2009). Analysis of intraindividual and intraspecific variation in semicircular canal dimensions using high-resolution x-ray computed tomography. *Journal of Anatomy*, 215(4), 444–451. <http://doi.org/10.1111/j.1469-7580.2009.01124.x>
- Wright, P. C. (1989). The nocturnal primate niche in the New World. *Journal of Human Evolution*, 18(7), 635–658.
- Yang, A., & Hullar, T. E. (2007). Relationship of semicircular canal size to vestibular-nerve afferent sensitivity in mammals. *Journal of Neurophysiology*, 98(6), 3197–3205. <http://doi.org/10.1152/jn.00798.2007>
- Yapuncich, G. S. (2018). Alternative methods for calculating percentage prediction error and their implications for predicting body mass in fossil taxa. *Journal of Human Evolution*, 115, 140–145.
- Yi, H., & Norell, M. A. (2015). The burrowing origin of modern snakes. *Science Advances*, 1(10), e1500743.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Gonzales LA, Malinzak MD, Kay RF. Intraspecific variation in semicircular canal morphology—A missing element in adaptive scenarios? *Am J Phys Anthropol*. 2018;1–15. <https://doi.org/10.1002/ajpa.23692>