Evolutionary trade-off between vocal tract and testes dimensions in howler monkeys

Article (Published Version)

Dunn, Jacob C, Halenar, Lauren B, Davies, Thomas G, Cristobal-Azarate, Jurgi, Reby, David, Sykes, Dan, Dengg, Sabine, Fitch, W Tecumseh and Knapp, Leslie A (2015) Evolutionary trade-off between vocal tract and testes dimensions in howler monkeys. Current Biology, 25 (21). pp. 2839-2844. ISSN 0960-9822

This version is available from Sussex Research Online: http://sro.sussex.ac.uk/id/eprint/61147/

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher’s version. Please see the URL above for details on accessing the published version.

Copyright and reuse:
Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.
Evolutionary Trade-Off between Vocal Tract and Testes Dimensions in Howler Monkeys

Highlights
- Howler monkey hyoid volume varies significantly between sexes and among species
- Hyoid volume negatively correlates with number of males per group and testes volume
- Larger hyoids lower formant spacing, increasing the acoustic impression of body size
- Results provide the first evidence of a trade-off between vocal investment and testes

In Brief
Males often face a trade-off between pre- and postcopulatory investments for reproduction. Dunn et al. report the first evidence for a trade-off between vocal investment and sperm production—howler monkey species with harem groups have large vocal tracts and small testes, whereas those in multimale groups have small vocal tracts and large testes.

Authors
Jacob C. Dunn, Lauren B. Halenar, Thomas G. Davies, ..., Sabine Dengg, W. Tecumseh Fitch, Leslie A. Knapp

Correspondence
jacobcdunn@gmail.com (J.C.D.), tecumseh.fitch@univie.ac.at (W.T.F.)

Dunn et al., 2015, Current Biology 25, 2839–2844
November 2, 2015 ©2015 The Authors
http://dx.doi.org/10.1016/j.cub.2015.09.029
Evolutionary Trade-Off between Vocal Tract and Testes Dimensions in Howler Monkeys

Jacob C. Dunn,1,* Lauren B. Halenar,2,3 Thomas G. Davies,1 Jurgi Cristobal-Azkarate,1 David Reby,4 Dan Sykes,5 Sabine Dengg,6 W. Tecumseh Fitch,1,7,8,10,* and Leslie A. Knapp9,10
1Division of Biological Anthropology, University of Cambridge, Pembroke Street, Cambridge CB2 3QG, UK
2Department of Biology, Farmingdale State College, State University of New York, 2350 Broadhollow Road, Farmingdale, NY 11735-1021, USA
3New York Consortium in Evolutionary Primatology, American Museum of Natural History, Central Park West and 79th Street, New York, NY 10024, USA
4Mammal Vocal Communication and Cognition Research Group, School of Psychology, University of Sussex, Falmer, Brighton BN1 9QH, UK
5Imaging and Analysis Centre, Natural History Museum, Cromwell Road, London SW7 5BD, UK
6Clinic of Diagnostic Imaging, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210 Vienna, Austria
7Department of Cognitive Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria
8Haidlhof Research Station, University of Vienna/University of Veterinary Medicine Vienna/Messerli Research Institute, Haidhofer Strasse, 2540 Bad Vöslau, Austria
9Department of Anthropology, University of Utah, 270 1400 E, Salt Lake City, UT 84112, USA
10Co-senior author
*Correspondence: jacobcdunn@gmail.com (J.C.D.), tecumseh.fitch@univie.ac.at (W.T.F.)
http://dx.doi.org/10.1016/j.cub.2015.09.029
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

SUMMARY

Males often face a trade-off between investments in precopulatory and postcopulatory traits [1], particularly when male-male contest competition determines access to mates [2]. To date, studies of precopulatory strategies have largely focused on visual ornaments (e.g., coloration) or weapon morphology (e.g., antlers, horns, and canines). However, vocalizations can also play an important role in both male (e.g., antlers, horns, and canines). However, vocalizations can also play an important role in both male-male contest competition and female choice [3–5]. We investigated variation in vocal tract dimensions among male howler monkeys (Alouatta spp.), which produce loud roars using a highly specialized and greatly enlarged hyoid bone and larynx [6]. We examined the relative male investment in hyoids and testes among howler monkey species in relation to the level of male-male competition and analyzed the acoustic consequences of variation in hyoid morphology. Species characterized by single-male groups have large hyoids and small testes, suggesting high levels of vocally mediated competition. Larger hyoids lower formant frequencies, probably increasing the acoustic impression of male body size and playing a role analogous to investment in large body size or weaponry. Across species, as the number of males per group increases, testes volume also increases, indicating higher levels of postcopulatory sperm competition, while hyoid volume decreases. These results provide the first evidence of an evolutionary trade-off between investment in precopulatory vocal characteristics and postcopulatory sperm production.

RESULTS AND DISCUSSION

Large body size, weaponry, and/or ornaments can confer an advantage to males during reproductive competition, allowing them to better dominate precopulatory contests and increase the number of offspring they sire [3]. However, when multiple males copulate with the same female, postcopulatory sperm competition occurs. This favors adaptations in male reproductive physiology, such as the production of more numerous and larger ejaculates (facilitated by larger testes) or faster and more enduring spermatozoa, which increase the likelihood of fertilization by a given male over competitors [7]. Vocalizations are also an important component of sexual selection in many animal species, often playing a crucial role in determining the outcome of agonistic contests and/or female choice [3–5]. However, despite considerable interest in the idea of vocal trade-offs [8], little is known about the evolutionary dynamics favoring investment in vocal characteristics versus sperm production.

The powerful and characteristic roars of howler monkeys (genus Alouatta) are among the loudest vocalizations produced by any terrestrial animal (Figures 1A and 1B and Movie S1). All howler monkey species have a highly modified larynx with a greatly enlarged cup-shaped hyoid bone containing an air sac, which is thought to function as a resonating chamber for their calls [6, 9] (Figures 1C and 1D and Movie S2). The highly specialized anatomy of the vocal apparatus, coupled with the time and energy invested in vocalizing [10, 11], suggests an important role for roaring in howler monkey fitness—particularly given their energy-minimizing lifestyle [12–14]. Multiple studies suggest that howler monkey roars function in male-male competition as territorial displays, regulating the use of space by groups [10, 15–17], although their precise functional significance and evolution is debated [18].

The howler monkey hyoid bone differs considerably in size between the sexes and among species [19, 20], but the full extent of...
this variation and the selection pressures underlying variability have not been investigated quantitatively. For our core analyses, we collected comparative data on nine of the ten classically recognized *Alouatta* species [21], using laser surface scanning to produce virtual 3D models of 255 hyoids. We then used phylogenetic methods and average species level data on body weight, skull length, canine length, testes volume, and number of males per group (data from five to nine species, depending on the dataset) to examine whether differences in male hyoid volume were related to variation in male competition among species—the “vocal competition” hypothesis. We also tested an alternative “environmental adaptation” hypothesis, that howler monkey hyoids are adapted to produce different frequency vocalizations in different habitats [22], by analyzing data on net primary productivity. Finally, we used bi-acoustic methods to analyze recordings of male roars and examined the acoustic consequences of variation in male hyoid morphology among species, hypothesizing that a more voluminous hyoid bone reduces formant spacing (ΔF) and increases the acoustic impression of body size conveyed by roars [23–25] (i.e., the “size exaggeration” hypothesis [26, 27]). In order to provide broader comparative context to the core analyses described above, we performed CT and MRI on the cadavers of two adult male howler monkeys (*Alouatta sara* and *A. caraya*) and one adult male spider monkey (*Ateles fusciceps*). This allowed us to visualize the howler monkey vocal tract and measure vocal fold length and vocal tract length (VTL) for comparison with other mammals.

We found that hyoid volume is highly sexually dimorphic ($F_{(1,259)} = 497.6, p < 0.001$) and varies significantly among species ($F_{(7,259)} = 52.4, p < 0.001$). We also found a significant interaction between sex and species ($F_{(7,259)} = 30.1, p < 0.001$), with greater sexual dimorphism in species with larger hyoids (Figure 2 and Table S1). $\log_{10}$ male hyoid volume was significantly correlated with $\log_{10}$ female hyoid volume (phylogenetic $p < 0.005$; Figure 3A), consistent with precopulatory sexual selection of this trait. Testes volume also varied significantly among species ($F_{(4,86)} = 19.1, p < 0.001$) and correlated significantly and positively with the number of males per group (PGLS: $R^2 = 0.78, \lambda = 0.00, F_{(1,6)} = 10.45, p < 0.05$; Figure 3B), consistent with the hypothesized role for testes volume in postcopulatory sperm competition. Crucially, there was a significant negative correlation between male hyoid volume and testes volume (PGLS: $R^2 = 0.94, \lambda = 0.00, F_{(1,3)} = 43.84, p < 0.01$; Figure 3C). Canine length was sexually dimorphic ($F_{(1,107)} = 148.89, p < 0.001$) but did not vary across species ($F_{(8,107)} = 1.16, p = 0.33$), and there was no interaction between sex and species ($F_{(8,107)} = 1.38, p = 0.22$). At the species level, canine length was not correlated with body weight, number of males per group, hyoid volume, or testes volume (see the Supplemental Experimental Procedures), suggesting that sexual selection on canine weaponry does not vary across species in this taxon.

We found no support for the “environmental adaptation” hypothesis: hyoid volume was not predicted by net primary productivity (PGLS: $R^2 = 0.19, \lambda = 1.00, F_{(1,6)} = 1.44, p = 0.27$; general linear mixed model [GLMM] males: Akaike information criterion [AIC] model = 613.9, AIC null = 611.9, $\chi^2_{(1)} = 0.01, p = 0.90, n = 144$; GLMM females: AIC model = 351.7, AIC null = 349.9, $\chi^2_{(1)} = 0.19, p = 0.67, n = 111$).

As a result of their anatomical modifications, howler monkeys produce exceptionally low-frequency vocalizations for their body size compared with other mammals (Figure 4A). MRI-based measurements indicated that howler monkey vocal folds are extremely long for an animal of their size (4.08 cm in *A. caraya* and 3.55 cm in *A. sara*, Figure S1; human male vocal fold length is ~1.5 cm [30]). Based on a theoretical string model [30] of the vocal folds, we found that the vocal fold lengths obtained from the MRI-based measurements accurately predict the remarkably low fundamental frequency (F0) of howler monkey vocalizations (see the Supplemental Experimental...
Procedures). This explains how a howler monkey could produce an F0 similar to that of tigers or reindeer, despite major differences in body size (7 kg versus >100 kg; Figure 4 B). However, F0 is not typically measurable in howler monkey roars, which are noisy, broadband sounds presumably generated via deterministic chaos (Figure S2), and in terrestrial mammals, empirical evidence suggests that F0 is not typically a reliable index of body size within age and sex classes [31, 32]. In contrast, numerous studies suggest that formant frequencies can provide reliable information about body size within species [26] and that individuals attend to this information in both inter- and intra-sexual contexts [23–25]. Male log10 hyoid volume was significantly negatively correlated with ∆F in male roars (R² = 0.88, λ = 0.00, F₁,₅ = 35.14, p < 0.005; Figure 3 D). For example, in A. caraya, mean ∆F was 535 Hz, whereas in A. sara mean ∆F was 388 Hz (Table S1). These values predict VTLs of 33 cm and 45 cm, respectively (Table S2), even though total sitting height is only about 40–50 cm in this genus [33]. Although VTL is greater in howler monkeys than other similarly sized primates [26] as a result of their unusual vocal anatomy, these values are inconsistent with our MRI-based VTL measurements of 20.6 cm in A. caraya and 26.3 cm in A. sara (Figure S1). These findings are consistent with the hypothesis that large hyoids may have evolved to enable lower ∆F than expected for body size, thereby increasing the acoustic impression of body size conveyed by howler monkey roars.

Across species, hyoid volume did not correlate with body weight in either males (PGLS: R² = 0.06, λ = 1.00, F₁,₄ = 0.25, Figure 3. Relationship between Key Variables in Pre- and Postcopulatory Male Strategies across Howler Monkey Species

Regression plots showing (A) log₁₀ mean male hyoid volume versus mean number of males, (B) mean testes volume versus mean number of males per species, (C) log₁₀ mean male hyoid volume versus mean testes volume, and (D) log₁₀ mean male hyoid volume versus ∆F. Each point represents the mean value for a distinct howler monkey species: Alouatta macconnelli (orange), A. belzebul (black), A. sara (pink), A. guariba (red), A. seniculus (purple), A. caraya (dark blue), A. palliata (yellow), and A. pigra (light blue). The slopes and intercepts of the regression lines of the linear model and PGLS model were identical in all cases, so only one line is visible in each figure. Mean values ±SE are shown. Sample sizes are given in Table S1. See also Figure S2 and Tables S1 and S2.
Data sources are provided in the Supplemental Experimental Procedures. See vocalizations of howler monkeys are to be expected, given their remarkable from [29] with permission from Elsevier), showing that the low-frequency (B) Log-log plot of vocal fold length versus F0 for a range of mammals (adapted from [28] with permission from AAAS).

Figure 4. The Exceptionally Low Call Frequency of Howler Monkey Vocalizations

(A) Log-log plot of body weight versus F0 for a range of mammals, highlighting the low-frequency vocalizations of howler monkeys (adapted from [28] with permission from AAAS). (B) Log-log plot of vocal fold length versus F0 for a range of mammals (adapted from [29] with permission from Elsevier), showing that the low-frequency vocalizations of howler monkeys are to be expected, given their remarkable vocal fold length.

Data sources are provided in the Supplemental Experimental Procedures. See also Figure S1.

Our results provide strong evidence for the vocal competition hypothesis, consistent with Darwin’s suggestion that the vocal organs of male Alouatta have been sexually selected [17]. Females are likely to require large hyoids for some of the same reasons as males, e.g., inter-group resource defense (infants, food, and territory) and predator deterrence [18]. However, it is unclear why female hyoid volume should correlate with male hyoid volume. One reason could be that female hyoid volume is a “correlated response” of selection for large hyoids in males [36]. Another reason could be that there is independent selection for larger hyoids in the females of species in groups with fewer males (in which males also have large hyoids), e.g., as a strategy against male infanticide [37], or owing to variation in female contest competition among species [38]. These phenomena are not mutually exclusive, and further research would be necessary to disentangle this interesting question.

These data provide the first evidence in any species of an evolutionary trade-off between a precopulatory vocal-investment strategy and postcopulatory sperm competition. The phylogenetic correlations we observe are consistent with at least two non-mutually exclusive functional mechanisms, which may work at different phylogenetic levels. The first model, known as the “Y model,” or the acquisition-allocation model, holds that for a given amount of a resource, it is not possible to increase allocation to two traits at once [39]. Traits used in pre- and postcopulatory male-male competition may both be energetically expensive [1, 40], leading to a trade-off in resource allocation. The second mechanism results from trade-offs that occur when evolutionary change in one trait directly decreases the relevance or performance of another [39, 41]. Under this model, the coevolution of intense female monopolization and large hyoids in unimale species limits the opportunity for sperm competition, leading to relaxed selection pressure on testes. In contrast, a failure of precopulatory male-male competition to repel rivals results in increased postcopulatory competition. Matching data on testes and hyoids from the same males across multiple species would be required to fully explore the precise functional nature of trade-offs within and between species, providing an exciting avenue for future research.

EXPERIMENTAL PROCEDURES

Morphological Traits

We analyzed 255 (111 females and 144 males) apparently non-pathological, adult hyoids at a number of museums. Species were identified on the basis of geographic location of the site of provenance of the specimens. Following a standardized protocol, we scanned the bullate basihyoid bone using a 3D laser surface scanner and calculated hyoid volume from the resulting models (see the Supplemental Experimental Procedures). We used both new and published data on testes volume, canine length, and body weight, though the datasets were not matching, i.e., were not from the same individuals (see the Supplemental Experimental Procedures). However, where possible, we collected matching data on skull length for the hyoids analyzed in the dataset (see the Supplemental Experimental Procedures). Data on morphological traits are given in Table S1. In order to analyze VTL and vocal fold length, we also performed CT and MRI on the cadavers of two adult male howler monkeys of different species (Alouatta sara and A. caraya) and one adult male spider monkey (Ateles fusciceps) (see the Supplemental Experimental Procedures).

Group Size and Composition

We compiled data on group size and composition from the literature for each of the howler monkey species studied (see the Supplemental Experimental Procedures and Table S3). Given that local environmental factors, such as variations in climate and vegetation, may affect group size and composition within species, we calculated mean values per study site and then took the average...
across study sites. We also ran analyses using the mean values for all groups (rather than sites), and the results did not change (Table S4).

**Net Primary Productivity**

We used 2013 data from the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra satellite [42] to calculate the annual NPP for the location of provenance of each hyoid specimen (see the Supplemental Experimental Procedures).

**Acoustic Analyses**

We searched the Macaulay Library (http://macaulaylibrary.org/) and the British Library Sounds archive (http://sounds.bl.uk/) for high-quality recordings of lone adult male *Alouatta* roars. We selected the highest-quality recording available of an adult male for each species (see the Supplemental Experimental Procedures). Given the very small level of within species variation in hyoid volume (Table S1), we considered these single recordings to be representative. We extracted three roars per recording for analysis. From these, we calculated AF and apparent VTL using published methods (see the Supplemental Experimental Procedures). We could not routinely measure F0 in the roars of the males because of the deterministic chaos typically present. However, we were able to measure F0 in other call types in order to make a general comparison with other mammals (Figure 4). We performed all acoustic analyses in Praat version 5.3.51 [43].

**Statistical Methods**

We first used a general linear model to examine differences in hyoid volume and canine length between sexes and among species and a one-way ANOVA to examine variation in testes volume among species. Then, to analyze the covariance between variables while accounting for the non-independence of data points due to shared ancestry of species, we used PGLS regressions (see the Supplemental Experimental Procedures). In the analyses that included the mean number of males per species, we used this variable as the independent variable and the morphological traits (i.e., hyoid volume, canine length, and testes volume) as dependent variables. When analyzing the relationship between testes volume and hyoid volume, AF and hyoid volume, and skull length and hyoid volume, we assigned hyoid volume as the dependent variable. In order to account for potential error in the branch lengths used, we recalculated all of the PGLS analyses with branch lengths of 1, and the results did not change (Table S4). We present absolute hyoid volume and testes volume in the main text, as there was no correlation between either hyoid volume or testes volume and body weight accounted for very little variance and did not change our results (see the Supplemental Experimental Procedures). We log 10 transformed variables in those cases where this improved the linearity of the relationships and performed all statistical analyses in R version 2.15.2 [44].

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures, two figures, four tables, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.09.029.

**ACKNOWLEDGMENTS**

We are grateful to Alexander Sliwa, Catalina Gomez, Robert Wallace, Michael Pivak, Zelinda Braga Hirano, and Julio Cesar de Souza, Jr. for sharing data, Andrew Kitchener (National Museums Scotland) for loaning whole animal specimens, Michaella Gumpergen and Jaap Saers for support with CT and MRI, Carolyn M. Crockett, Mariana Raño, and La Senda Verde Animal Refuge Bolivia for providing photographs and videos, Nadja Kavcik for help with the figures, and Dieter Lukas for help with statistical analyses. J.C.D. was funded by a Cambridge Humanities Research Grant. W.T.F. acknowledges support of ERC Advanced Grant SOMACCA (#230604) and Austrian Science Fund (FWF) grant W1234-G17.

Received: July 2, 2015
Revised: August 17, 2015
Accepted: September 11, 2015
Published: October 22, 2015

**REFERENCES**

1. Parker, G.A., Lessells, C.M., and Simmons, L.W. (2013). Sperm competition games: a general model for precopulatory male–male competition. Evolution 67, 95–109.
2. Lupold, S., Tomkins, J.L., Simmons, L.W., and Fitzpatrick, J.L. (2014). Female monopolization mediates the relationship between pre- and post-copulatory sexual traits. Nat. Commun. 5, 3184.
3. Anderson, M. (1994). Sexual Selection (Princeton University Press).
4. Clutton-Brock, T.H., and Albon, S.D. (1979). The roaring of red deer and the evolution of honest advertisement. Behaviour 69, 145–170.
5. Davies, N., and Halliday, T. (1978). Deep croaks and fighting assessment in toads *Bufo bufo*. Nature 274, 683–685.
6. Schön, M.A. (1971). The anatomy of the resonating mechanism in howling monkeys: Folia Primatol. (Basel) 15, 117–132.
7. Birkhead, T.R., and Møller, A.P. (1998). Sperm Competition and Sexual Selection (Academic Press).
8. Simmons, L.W., Peters, M., and Rhodes, G. (2011). Low pitched voices are perceived as masculine and attractive but do they predict semen quality in men? PLoS ONE 6, e29271.
9. Kelemen, G., and Sade, J. (1960). The vocal organ of the howling monkey (*Alouatta palliata*). J. Morphol. 107, 123–140.
10. Da Cunha, R.G.T., and Byrne, R. (2006). Roars of black howler monkeys (*Alouatta caraya*): evidence for a function in inter-group spacing. Behaviour 143, 1169–1199.
11. Van Bele, S., Estrada, A., and Garber, P.A. (2014). The function of loud calls in black howler monkeys (*Alouatta pigra*): food, mate, or infant defense? Am. J. Primatol. 76, 1196–1206.
12. Dunn, J.C., Cristóbal-Azkarate, J., Schulte-Herbrüggen, B., Chavira, R., Vea, J.J., and Vea, J.J. (2013). Travel time predicts fecal glucocorticoid levels in free-ranging howlers (*Alouatta palliata*). Int. J. Primatol. 34, 246–259.
13. Cristóbal-Azkarate, J., and Arroyo-Rodríguez, V. (2007). Diet and activity pattern of howler monkeys (*Alouatta palliata*) in Los Tuxtlas, Mexico: effects of habitat fragmentation and implications for conservation. Am. J. Primatol. 69, 1013–1029.
14. Milton, K. (1980). The Foraging Strategy of Howler Monkeys: A Study of Primate Economics (Columbia University Press), p. 165.
15. Sekulic, R. (1982). The function of howling in red howler monkeys (*Alouatta seniculus*). Behaviour 81, 38–54.
16. Carpenter, C.R. (1934). Adaptive Radiation, Systematics, and Morphology, M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, and D. Youlatos, eds. (Springer), pp. 369–399.
17. Hershkovitz, P. (1949). Mammals of northern Colombia. Preliminary report no. 4: monkeys (primates), with taxonomic revisions of some forms. Proc. U. S. Natl. Mus. 98, 323–427.
18. Youlatos, D., Couette, S., and Halenar, L.B. (2015). Morphology of howler monkeys: a review and quantitative analysis. In Howler Monkeys: Adaptive Radiation, Systematics, and Morphology, M.M. Kowalewski, P.A. Garber, L. Cortés-Ortiz, B. Urbani, and D. Youlatos, eds. (Springer), pp. 133–176.
19. Cortés-Ortiz, L., Bermingham, E., Rico, C., Rodríguez-Luna, E., Sampaio, I., and Ruiz-García, M. (2003). Molecular systematics and biogeography of *Alouatta* in Los Tuxtlas, Mexico: ef-
22. Ey, E., and Fischer, J. (2009). The “acoustic adaptation hypothesis” - a review of the evidence from birds, anurans and mammals. Bioacoustics 19, 21–48.

23. Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W.T., and Clutton-Brock, T. (2005). Red deer stags use formants as assessment cues during intrasexual agonistic interactions. Proc. Biol. Sci. 272, 941–947.

24. Charlton, B.D., Zhihe, Z., and Snyder, R.J. (2010). Giant pandas perceive and attend to formant frequency variation in male bleats. Anim. Behav. 79, 1211–1227.

25. Charlton, B.D., Ellis, W.A.H., Brumm, J., Nilsson, K., and Fitch, W.T. (2012). Female koalas prefer bellows in which lower formants indicate larger males. Anim. Behav. 84, 1565–1571.

26. Fitch, W.T. (1997). Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. J. Acoust. Soc. Am. 102, 1213–1222.

27. Ohala, J.J. (1984). An ethological perspective on common cross-language utilization of F0 of voice. Phonetica 41, 1–16.

28. Herbst, C.T., Stoeger, A.S., Frey, R., Lohscheller, J., Titze, I.R., Gumpenberger, M., and Fitch, W.T. (2012). How low can you go? Physical production mechanism of elephant infrasonic vocalizations. Science 337, 595–599.

29. Charlton, B.D., Frey, R., McKinnon, A.J., Fritsch, G., Fitch, W.T., and Reby, D. (2013). Koalas use a novel vocal organ to produce unusually low-pitched mating calls. Curr. Biol. 23, R1035–R1036.

30. Titze, I. (1994). Principles of Voice Production (Prentice Hall).

31. Ey, E., Pfefferle, D., and Fischer, J. (2007). Do age- and sex-related variations reliably reflect body size in non-human primate vocalizations? A review. Primates 48, 253–267.

32. Taylor, A.M., and Reby, D. (2010). The contribution of source-filter theory to mammal vocal communication research. J. Zool. (Lond.) 280, 221–236.

33. Kelata, M., Dias, P.A.D., Aguilar-Cucurachi, Mdel.S., Canales-Espinoza, D., and Cortés-Ortiz, L. (2011). Impact of intrasexual selection on sexual dimorphism and testes size in the Mexican howler monkeys Alouatta palliata and A. pigra. Am. J. Phys. Anthropol. 146, 179–187.

34. Charlton, B.D., Ellis, W.A., McKinnon, A.J., Cowin, G.J., Brumm, J., Nilsson, K., and Fitch, W.T. (2011). Cues to body size in the formant spacing of male koala (Phascolarctos cinereus) bellows: honesty in an exaggerated trait. J. Exp. Biol. 214, 3414–3422.

35. Reby, D., and McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. Anim. Behav. 65, 519–530.

36. Lande, R. (1980). Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution (N. Y.) 34, 292–305.

37. Agoramoorthy, G., and Rudran, R. (1995). Infanticide by adult and sub-adult males in free-ranging red howler monkeys, Alouatta seniculus, in Venezuela. Ethology 88, 75–88.

38. Amundsen, T. (2000). Why are female birds ornamented? Trends Ecol. Evol. 15, 149–155.

39. Garland, T., Jr. (2014). Trade-offs. Curr. Biol. 24, R60–R61.

40. Fitzpatrick, J.L., Almbro, M., Gonzalez-Voyer, A., Kolm, N., and Simmons, L.W. (2012). Male contest competition and the coevolution of weaponry and testes in pinnipeds. Evolution 66, 3595–3604.

41. Roff, D.A., and Fairbairn, D.J. (2007). The evolution of trade-offs: where are we? J. Evol. Biol. 20, 433–447.

42. Zhao, M., Heinsch, F.A., Nemani, R.R., and Running, S.W. (2005). Improvements of the MODIS terrestrial gross and net primary production global data set. Remote Sens. Environ. 95, 164–176.

43. Boersma, P. (2001). Praat, a system for doing phonetics by computer. Glot. Int. 5, 341–345.

44. R Development Core Team (2008). R: a language and environment for statistical computing (R Foundation for Statistical Computing).
Current Biology
Supplemental Information

Evolutionary Trade-Off between Vocal Tract and Testes Dimensions in Howler Monkeys

Jacob C. Dunn, Lauren B. Halenar, Thomas G. Davies, Jurgi Cristobal-Azkarate, David Reby, Dan Sykes, Sabine Dengg, W. Tecumseh Fitch, and Leslie A. Knapp
Figure S1, related to Figure 4B. Magnetic resonance imaging of two adult male howler monkeys. Panel (A) and panel (B) show transverse plane images of vocal fold length (traced in red) in *Alouatta sara* and *Alouatta caraya*, respectively; panel (C) and panel (D) show saggital plane images of vocal tract length (traced in red) in *Alouatta sara* and *Alouatta caraya*, respectively. The white scale on the left hand side of the images is in centimetres.
Figure S2, related to Figure 3D. A) Waveform (top) and spectrogram (bottom) of the vocalisations of a male howler monkey (*Alouatta caraya*). In the left hand box (incipient roar), F0 is approximately 50Hz. However, in the right hand box (roar), F0 is no longer measurable; B) Spectrograms of loud calls by male howler monkeys. The formants are labelled F1 – 6.
Table S1, related to Figure 3. Comparative data on howler monkey social organisation, morphology and acoustics. Mean values are reported ± SD and sample sizes are given in parentheses. *For sources of data see Supplemental Experimental Procedures.
| Species      | Call | F1 (Hz) | F2 (Hz) | F3 (Hz) | F4 (Hz) | F5 (Hz) | F6 (Hz) | ∆F (Hz) | VTL (cm) |
|--------------|------|---------|---------|---------|---------|---------|---------|---------|----------|
| A. pigra     | A    | 340     | 717     | 1255    | 1635    | 1932    | 2426    | 449.6   | 38.9     |
|              | B    | 321     | 720     | 1250    | 1488    | 1900    | 2412    | 439.0   | 39.9     |
|              | C    | 367     | 737     | 1199    | 1594    | 1952    | 2418    | 446.6   | 39.2     |
| A. caraya    | A    | 401     | 998     | 1418    | 1918    | 2278    | 2542    | 506.1   | 34.6     |
|              | B    | 434     | 916     | 1488    | 1928    | 2540    | 2966    | 556.0   | 31.5     |
|              | C    | 398     | 907     | 1381    | 1898    | 2501    | 2906    | 543.0   | 32.2     |
| A. guariba   | A    | 426     | 746     | 1415    | 1625    | 2220    | 2425    | 474.0   | 36.9     |
|              | B    | 390     | 586     | 1218    | 1486    | 1750    | 2520    | 434.0   | 40.3     |
|              | C    | 410     | 802     | 1402    | 1694    | 2487    | 2824    | 525.0   | 33.3     |
| A. seniculus | A    | 314     | 530     | 1080    | 1571    | 1924    | 2122    | 412.0   | 42.5     |
|              | B    | 312     | 530     | 1080    | 1465    | 1920    | 2337    | 423.0   | 41.4     |
| A. macconnelli | A | 65     | 516     | 744     | 1010    | 1914    | 2326    | 386.0   | 45.3     |
|              | B    | 63      | 467     | 981     | 1250    | 1909    | 2346    | 406.0   | 43.1     |
|              | C    | 69      | 498     | 789     | 1006    | 1930    | 2337    | 389.0   | 45.0     |
| A. sara      | A    | 371     | 530     | 878     | 1518    | 1672    | 2186    | 392.0   | 44.6     |
|              | B    | 379     | 515     | 855     | 1500    | 1636    | 2155    | 386.0   | 45.3     |
|              | C    | 380     | 523     | 827     | 1510    | 1688    | 2108    | 385.0   | 45.5     |
| A. palliata  | A    | 358     | 712     | 1573    | 1916    | 2811    | 3340    | 600.0   | 29.2     |
|              | B    | 380     | 748     | 1244    | 1745    | 2528    | 3340    | 563.0   | 31.1     |
|              | C    | 380     | 754     | 1497    | 1871    | 2539    | 3340    | 579.0   | 30.2     |

**Table S2**, related to Figure 3D. Frequency of the first six formants, formants spacing (∆F) and apparent vocal tract length (VTL) in the three roars analysed per species.
| Species             | Study Site                                                                 | Gr. | M  | F  | N  | Ref. |
|---------------------|----------------------------------------------------------------------------|-----|----|----|----|------|
| Alouatta belzebul   | Cauaxi Ranch, Para, Brazil                                                 | 6.0 | 1.0| 2.0| 1  | [S1] |
| Alouatta belzebul   | Ferreira Penna Scientific Station, Para, Brazil                           | 9.0 | 1.0| 3.0| 1  | [S2] |
| Alouatta belzebul   | Ilha de Germoplasma, Para, Brasil                                         | 7.8 | 1.2| 2.6| 5  | [S3] |
| Alouatta belzebul   | Paranaixa, Mato Grosso, Brazil                                            | 7.0 | 1.0| 2.0| 1  | [S4] |
| Alouatta belzebul   | Sapé, Paraiba, Brazil                                                    | 7.4 | 1.2|    | 5  | [S5] |
| Alouatta caraya     | Brasiliera Island, Chaco, Argentina                                       | 10.9| 1.8| 3.4| 14 | [S6–9]|
| Alouatta caraya     | Carioca Island, Upper Paraná River, Brazil                                | 10.5| 2.5| 4.0| 2  | [S10]|
| Alouatta caraya     | Cerro dos Negros, Rio Grande do Sul, Brazil                               | 8.4 | 1.6| 2.4| 8  | [S11]|
| Alouatta caraya     | Corrientes Biological Field Station, Corrientes, Argentina               | 6.8 | 1.5| 2.5| 11 | [S7] |
| Alouatta caraya     | Guaycolec Ranch, Formosa, Argentina                                      | 7.5 | 1.3| 1.9| 15 | [S12]|
| Alouatta caraya     | Isla Guascara, Corrientes, Argentina                                     | 10.2| 2.7| 3.8| 11 | [S13]|
| Alouatta caraya     | Mutum Island, Upper Paraná River, Brazil                                  | 10.5| 2.5| 5.0| 2  | [S10]|
| Alouatta caraya     | Nhumirim Farm, Mato Grosso do Sul, Brazil                                 | 14.0| 2.0| 2.0| 1  | [S14]|
| Alouatta caraya     | Porto Rico Island, Upper Paraná River, Brazil                            | 11.0| 2.0| 3.0| 1  | [S10]|
| Alouatta caraya     | Rio Riachuelo, Corrientes, Argentina                                     | 7.2 | 1.6| 2.3| 46 | [S13, S15]|
| Alouatta caraya     | Upper Paraná River (left bank), Upper Paraná River, Brazil                | 9.7 | 2.0| 3.7| 3  | [S10]|
| Alouatta caraya     | Upper Paraná River (right bank), Upper Paraná River, Brazil               | 11.3| 2.0| 4.3| 3  | [S10]|
| Alouatta guariba    | Beco Xavier, Alegrete, Rio Grande do Sul, Brazil                         | 5.0 | 1.0| 1.0| 1  | [S16]|
| Alouatta guariba    | Campo de Instrução de Santa Maria, Rio Grande do Sul, Brazil             | 8.0 | 1.0| 2.7| 5  | [S17]|
| Alouatta guariba    | Cantareira Reserve, São Paulo, Brazil                                    | 5.9 | 1.8| 2.4| 26 | [S18, S19]|
| Alouatta guariba    | Chácara Payquerrê, Paraná, Brazil                                        | 4.6 | 1.0| 2.0| 7  | [S20]|
| Alouatta guariba    | El Piñaito Provincial Park, Misiones, Argentina                           | 7.5 | 1.0| 3.0| 4  | [S21]|
| Alouatta guariba    | Estação Biológica Caratinga, Minas Gerais, Brazil                       | 6.9 | 1.2| 2.2| 29 | [S22, S23]|
| Alouatta guariba    | Fazenda Barreiro Rico, São Paulo, Brazil                                 | 6.0 | 1.0| 2.0| 1  | [S24]|
| Alouatta guariba    | Intervales State Park, Sao Paulo, Brazil                                 | 5.0 | 2.0| 1.0| 1  | [S25]|
| Alouatta guariba    | Parque Estadual de Itapuí, Viamao, Rio Grande do Sol, Brazil             | 6.0 | 1.0| 2.0| 1  | [S26]|
| Alouatta guariba    | Porto Alegre, Viamao, Rio Grande do Sol, Brazil                         | 7.5 | 3.0| 3.0| 2  | [S27]|
| Alouatta guariba    | Santa Genebra Reserve, Campinas, Sao Paulo, Brazil                       | 6.0 | 1.0| 2.0| 1  | [S28]|
| Alouatta macconnelli| Anakoko Island, Venezuela                                                 | 5.0 | 1.0| 1.0| 1  | [S29]|
| Alouatta macconnelli| Nouragque Station, French Guiana                                         | 6.8 | 1.0| 1.8| 5  | [S30–32]|
| Alouatta palliata   | Barro Colorado Island, Panama                                            | 19.7| 3.2| 8.6| 73 | [S33, S34]|
| Alouatta palliata   | Cabo Blanco Absolute Natural Reserve, Puntarenas, Costa Rica             | 14.9| 2.5| 7.8| 8  | [S35, S36]|
| Alouatta palliata   | Finca Taboga, Guanacaste, Costa Rica                                     | 11.5| 2.4| 5.5| 22 | [S37]|
| Alouatta palliata   | Guanacaste, Costa Rica                                                  | 21.8| 3.1| 10.2| 11 | [S38]|
| Alouatta palliata   | Hacienda la Pacifica, Guanacaste, Costa Rica                             | 12.7| 2.1| 6.6| 92 | [S39–41]|
| Alouatta palliata   | Inland lowland forest, Chiriqui, Panama                                  | 18.9| 3.9| 8.0| 8  | [S42]|
| Alouatta palliata   | La Selva Biological Reserve, Heredia, Costa Rica                        | 11.0| 3.3| 4.0| 7  | [S43]|
| Alouatta palliata   | Los Tuxtlas, Veracruz, Mexico                                            | 9.1 | 3.0| 4.1| 17 | [S44, S45]|
| Alouatta palliata   | Santa Rosa National, Park, Guanacaste, Costa Rica                       | 13.8| 3.1| 5.7| 104| [S46–49]|
| Alouatta pigra      | Bermuda Landing, Gulf Coast, Belize                                     | 5.4 | 1.3| 1.6| 22 | [S50, S51]|
| Alouatta pigra      | Calakmul, Campeche Mexico                                               | 7.5 | 2.5| 2.2| 8  | [S52]|
| Alouatta pigra      | Community Baboon Sanctuary, Gulf Coast, Belize                          | 5.9 | 1.5| 2.0| 74 | [S53, S54]|

Gr. = Group size; M = Males; F = Females; N = Number of individuals; Ref. = Reference number.
| Species            | Location                                      | Group Size | Composition | Reference |
|--------------------|-----------------------------------------------|------------|-------------|-----------|
| *Alouatta pigra*   | Palenque, Chiapas, Mexico                     | 7.0        | 2.0 1.9 20  | [S55]     |
| *Alouatta pigra*   | Tikal National Park, Guatemala                | 8.7        | 2.2 2.9 10  | [S52]     |
| *Alouatta pigra*   | Yaxchilán, Chiapas, Mexico                    | 6.6        | 2.8 2.0 8   | [S52, S55]|
| *Alouatta sara*    | Madidi National Park, Bolivia                 | 5.0        | 1.4 2.5 162 | [S56]     |
| *Alouatta sara*    | Noel Kempff Mercado National Park, Bolivia    | 3.0        | 1.0 2.0 18  | [S57]     |
| *Alouatta seniculus* | Estación Biológica Caparú, Vaupés, Colombia | 7.0        | 1.0 2.0 1   | [S58]     |
| *Alouatta seniculus* | Finca Merenberg, Huila, Colombia              | 9.0        | 2.0 2.5 2   | [S59]     |
| *Alouatta seniculus* | Hato el Frío, Apure, Venezuela               | 7.6        | 1.8 3.0 5   | [S60]     |
| *Alouatta seniculus* | La Macarena National Park, Meta, Colombia    | 7.5        | 1.5 2.5 8   | [S61]     |
| *Alouatta seniculus* | Río Peneya, Meta, Colombia                    | 5.5        | 1.2 1.6 29  | [S62]     |
| *Alouatta seniculus* | Ríos Tuparro and Tomo, Vichada, Colombia     | 6.3        | 1.9 2.4 10  | [S63]     |

**Table S3**, related to Experimental Procedures. Review of group size and composition for study species.
| Dependent       | Independent       | Branch Lengths Normal | Branch Lengths = 1 | Average No. of males |
|-----------------|-------------------|-----------------------|--------------------|--------------------|
|                 |                   | $R^2$  | $\lambda$ | $F$  | $P$  | $R^2$  | $\lambda$ | $F$  | $P$  | $R^2$  | $\lambda$ | $F$  | $P$  |
| Male Hy. Vol.   | Female Hy. Vol.   | 0.94  | 0.00     | 97.17 | <0.001 | 0.94  | 0.00     | 97.17 | <0.001 | 0.94  | 0.00     | 97.17 | <0.001 |
| Male Can. L.    | Male Body Wt.     | 0.00  | 0.00     | 0.00  | 0.95   | 0.00  | 0.00     | 0.00  | 0.96   | 0.00  | 0.00     | 0.00  | 0.95   |
| Female Can. L.  | Female Body Wt.   | 0.06  | 1.00     | 0.33  | 0.59   | 0.06  | 1.00     | 0.33  | 0.59   | 0.06  | 1.00     | 0.33  | 0.59   |
| Male Can. L.    | No. of Males      | 0.00  | 0.00     | 0.00  | 0.99   | 0.00  | 0.00     | 0.00  | 0.99   | 0.00  | 1.00     | 0.00  | 0.98   |
| Female Can. L.  | No. of Males      | 0.08  | 1.00     | 0.51  | 0.50   | 0.08  | 0.00     | 0.51  | 0.50   | 0.06  | 1.00     | 0.40  | 0.55   |
| Male Can. L.    | Male Hy. Vol.     | 0.15  | 0.00     | 1.07  | 0.34   | 0.05  | 0.00     | 0.31  | 0.60   | 0.15  | 0.00     | 1.07  | 0.34   |
| Female Can. L.  | Female Hy. Vol.   | 0.00  | 1.00     | 0.01  | 0.95   | 0.01  | 1.00     | 0.08  | 0.78   | 0.00  | 1.00     | 0.01  | 0.95   |
| Male Can. L.    | Testes Vol.       | 0.52  | 0.00     | 3.22  | 0.17   | 0.52  | 0.00     | 3.22  | 0.17   | 0.52  | 0.00     | 3.22  | 0.17   |
| Male Hy. Vol.   | Male Body Wt.     | 0.06  | 1.00     | 0.25  | 0.65   | 0.06  | 1.00     | 0.25  | 0.65   | 0.06  | 1.00     | 0.25  | 0.65   |
| Female Hy. Vol. | Female Body Wt.   | 0.00  | 0.69     | 0.02  | 0.90   | 0.00  | 0.69     | 0.02  | 0.91   | 0.00  | 0.69     | 0.02  | 0.90   |
| Male Hy. Vol.   | No. of Males      | 0.91  | 1.00     | 54.18 | <0.001 | 0.92  | 0.00     | 54.18 | <0.001 | 0.91  | 0.00     | 49.00 | <0.001 |
| Female Hy. Vol. | No. of Males      | 0.89  | 0.00     | 49.13 | <0.001 | 0.89  | 0.00     | 49.13 | <0.001 | 0.90  | 0.00     | 53.28 | <0.001 |
| Testes Vol.     | No. of Males      | 0.78  | 0.00     | 10.45 | 0.05   | 0.78  | 0.00     | 10.45 | 0.05   | 0.76  | 0.00     | 9.45  | 0.05   |
| Male Hy. Vol.   | Testes Vol.       | 0.93  | 0.00     | 27.00 | 0.03   | 0.93  | 0.00     | 27.00 | 0.03   | 0.93  | 0.00     | 27.00 | 0.03   |
| Formant Spacing | Male Hy. Vol.     | 0.88  | 0.00     | 30.36 | 0.01   | 0.88  | 0.00     | 30.36 | 0.01   | 0.88  | 0.00     | 30.36 | 0.01   |

**Table S4**, related to Experimental Procedures and Figure 3. PGLS analyses using branch lengths equal to 1 in the phylogeny and using mean number of males from all groups (rather than mean per site). Hy. = Hyoid, Vol. = Volume, Can. = Canine, Wt. = Weight, No. = Number.
Supplemental Experimental Procedures

Hyoid species identification and volume calculation

We analysed hyoid volume for 255 (111 females and 144 males) apparently non-pathological, adult howler monkeys at a number of museums. Species were identified on the basis of geographic location of the site of provenance of the specimens. These coordinates were uploaded into the QGIS [S64] software package compared with the current distribution maps of *Alouatta* species from the IUCN Red List [S65].

Following a standardised protocol, we scanned the hyoids using either a NextEngine Desktop 3D laser surface scanner, with the software ScanStudio HD Pro Version 1.3.2, or a Minolta Vivid 910 laser surface scanner, with the software Geomagic Studio. We scanned the hyoids at the highest SD resolution, in macro mode. Scans were composed of 12 individual scan surfaces comprising a 360 degree rotation with ten viewpoints, and two single scans of the remaining uncaptured surfaces. We conducted initial scan trimming and alignment in ScanStudio HD. Subsequently we perfected the alignment of individual scan surfaces in Rapidform XOR with the Mesh Build-up Wizard, the Best-Fit Aligning function, and Accuracy Analyzer tool.

Owing to the difficulty in capturing the internal surface of the hyoid with the 3D scanner, we calculated volume from the external surface. Thus, any part of the internal surface of the hyoid that had been captured was trimmed away leaving just the external surface. Next, we fused the individual scan faces using the Merge function, and converted the model to a solid mesh in order to calculate the volume. We filed any holes in the mesh (due to the state of preservation of the hyoid) manually using the Fill Holes function and used the settings that best created continuity in the curvature of the surface. Finally, we applied a “Global Re-mesh” to provide a relatively flat
closing to the posterior opening of the basihyal. We then calculated the volume of the final closed hyoids automatically in the software. Finished (closed) hyoid models were composed of between approximately 7,000 and 250,000 individual poly vertices, or 15,000 to 500,000 poly-faces.

**Micro computed tomography (µCT) validation of volume calculations**

In order to test the accuracy of the surface scan estimates of hyoid volume from the external surface of the hyoid, relative to the actual internal volume of the hyoid, we obtained µCT scans of a subsample of 4 hyoids. We performed µCT scanning using a Nikon Metrology HMX ST 225 at the Natural History Museum, London. We scanned the samples using a tungsten reflection target, at an accelerating voltage of 210 kV and current of 190 µA using a 500 ms exposure time (giving a scan time of 25 minutes). We used copper filters between 2.5 and 4 mm, depending upon the density of the hyoids. Filtering reduces the number of artifacts in the data usually produced by higher density material such as scattering and beam hardening. Higher density objects require greater levels of filtering. Over the course of a scan we took 3,142 projections over a 360° rotation of the specimen. The voxel size of the resulting datasets ranged from 70 – 111 µm depending upon the size of the specimen, as the resolution is determined by geometric magnification. We reconstructed the 3D volumes using the Feldkamp back-projection algorithm [S66] through CT Pro (Nikon Metrology, Tring, UK) and exported TIFF stacks using VG Studio Max (Volume Graphics GmbH, Heidelberg, Germany).

In Avizo Fire 6.3, we constructed isosurfaces of the hyoids by thresholding the scans. We imported the isosurfaces into Rapidform XOR, where we cropped away the external surface of the hyoid, leaving only the interior surface. We carried out the same procedures of hole filling
and re-meshing, as described above, to create a counterpart solid model of the internal volume of the hyoid. In the case of one hyoid, a laser scan was not possible (as the hyoid was articulated with the skeleton) and thus we used the µCT scan to create both the internal and external volume models. The results of the comparison show that the volume based on the external surface had an error of $5.1 \pm 1.4\%$ compared with the true internal volume and the volumes calculated using the two different methods were highly correlated ($R^2 = 0.99, P < 0.001$). The volume based on the external surface consistently overestimated the internal volume. An overestimate was expected, and thus this falls very closely within the expected bounds, and also suggests the procedures for closing the hyoid are not creating substantial variation. It can be assumed therefore that the errors in volume arising from the lower resolution laser scan approach, and in the method applied to create a solid model, are subject to ca. 5% error from true properties. However, we would expect this to be consistent across species and sexes.

**Testes volume**

We used new and published data to calculate mean ± SD testes volume per species. Only adult males were considered in the analyses. Given the technical and logistical challenges of gaining these data, we were only able to report testes volume for 5 species (Table S1). The published data were used from *A. pigra* [S67], *A. palliata* [S67], and *A. caraya* [S68]. We collected new data for *A. guariba* at the Centro de Pesquisas Biológicas de Indaial, Brazil and for *A. seniculus* at Cologne Zoo, Germany and Parque Zoológico Santa Fe, Colombia. We followed the methods used by Kelaita et al [S67] to determine testicular volume. Briefly, we measured the width and length of each testicle, excluding scrotal skin folds, to the nearest millimeter using Mitutoyo Digital Calipers. We then used the following formula to calculate the volume of a prolate sphere:
\[ \pi LW^2/6 \]; where L is length and W is width. We used total testicular volume (sum of left and right testes) to account for any variability that exists between the left and right testes and to have data that are comparable to results presented in the literature.

**Canine length**

Raw data on canine length for *A. belzebul, A. caraya, A. guariba, A. palliata, A. pigra* and *A. seniculus* was kindly provided by J. Michael Plavcan from museum specimen. Methods used to collect these data have been reported elsewhere [S69]. Following these methods, we collected additional data on canine length for all remaining species from museum specimens and report the average length of left and right canines for males and females (Table S1).

**Body weight**

Data on body weight of wild males and females for each species were taken from a review of body size in primates [S70], with two exceptions. For *Alouatta pigra*, a more recent paper [S67] offered a much larger sample size, and for *Alouatta macconnelli* data were not reported in the original review, so they were taken from a more recent review paper [S71]. Data on body weight were not available for *Alouatta sara* or *Alouatta nigerrima* (Table S1).

**Skull length**

Where possible, we collected matching data on skull length for the hyoids analysed in the data set. We measured maximum skull length [S72] to the nearest mm using digital callipers. The sample consisted of 117 skulls with matching hyoids, representing 6 species (Table S1).
**Group size and composition**

We compiled data on group size and composition for each of the howler monkey species studied (Table S2). Much of the data came from a review paper [S71], but we complemented these data with as many additional studies as possible for each species (Table S4). We located additional records using Latin binomials as keywords in searches of Web of Science, Google Scholar and PrimateLit. Data on group size and composition were not available for *Alouatta nigerrima*. Given that local environmental factors, such as variations in climate and vegetation, may affect group size and composition within species, we calculated mean values per study site and then took the average across study sites (Table S2). We also ran the analyses using the mean values for all groups (rather than sites), and the results did not change (Table S5).

**Net Primary Productivity (NPP)**

We downloaded NPP data for 2013 from the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra satellite launched by NASA [S73]. These data are freely available from the Numerical Terradynamic Simulation Group (NTSG) [http://www.ntsg.umt.edu](http://www.ntsg.umt.edu). The data give an estimate of spatial variability in the amount of atmospheric carbon that is fixed by plants and, hence, a good estimate of forest productivity. Using the “Point Sampling Tool” in QGIS [S64], we calculated the annual NPP for the location of provenance of each hyoid specimen (Figure S2).

**Calculation of theoretical fundamental frequency**

The fundamental frequency (F0) of vocal fold vibration can be explained by using a simple piano-string model [S74]. In this model, vocal fold length is inversely and linearly related to F0
and can be approximated using the equation $F_0 = \left(\frac{1}{2L}\right) \times \left(\sqrt{\frac{\sigma}{\rho}}\right)$, where $L$ is vocal fold length in m, $\sigma$ is equal to the stress applied to the vocal folds in kPa, and $\rho$ is the tissue density of the vocal folds, which is approximately equal to 1.02 g/cm$^3$ [S75, S76]. If we assume that there is no stress on the vocal folds during sound production [S74, S77, S78] and apply the equation to the vocal fold length of howler monkeys (ca. 40 mm), then we could predict that howler monkeys may produce an $F_0$ as low as 15 Hz.

**Acoustic analyses and calculation of apparent vocal tract length**

We used bioacoustics methods to analyse the acoustic effect of variation in hyoid volume among male howler monkeys. We concentrated on males, because: (a) they howl more frequently than females, including vocalising in the context of male-male competition; (b) comparative acoustic data are much more widely available for males; and (c) the variation in hyoid volume is much greater among males.

We searched the Macaulay Library (www.macaulaylibrary.org) and the British Library Sounds archive (www.sounds.bl.uk) for high quality recordings of lone adult male roars. Many recordings were available and we screened over 300 for quality, but only a very small number were of high enough quality for reliable formant analyses. Therefore, we selected the highest quality recording of an adult male for each species. Given the very small level of within species variation in hyoid volume (Table S1), we considered these single recordings to be representative. From these recordings, we extracted three “roars” [S79] per recording for analysis.

The howler monkey roar is characterised by an introductory phase, which has classically been termed as an “incipient roar” [S79]. This section of the call contains some tonal segments and, as such, fundamental frequency can be measured in some high quality recordings. However,
incipient roars generally build in volume and grade into full roars, which are characterised by deterministic chaos and, therefore, lack periodicity and fundamental frequency may not be measured, even in high quality recordings (Figure S2). Therefore, though we could not routinely measure F0 in the roars of the males (because of the deterministic chaos typically present), we were able to measure F0 in other call types in order to make a general comparison with other mammals (Figure 4).

We extracted formant frequencies using the PRAAT 5.3.47 sound analysis package [S80]. The lowest frequency values of the first six formants were extracted using linear predictive coding (LPC) via the ‘LPC: To Formants (Burg)’ command in PRAAT. We did not attempt to measure formants higher than the sixth formant because these frequency components were often poorly defined. To measure the formants, we used the following analysis parameters: time-step, 0.01 s; maximum number of formants, 8-10; maximum formant frequencies, 3500–4000 Hz; window of analysis, 0.1 s. To check that the program was accurately tracking the frequency of formants, we compared the outputs with visual inspections of spectrograms and power spectra using cepstral smoothing at 200Hz. In order to ensure the reliability of our results, a bioacoustics expert with experience in formant analysis (D.R.) repeated the analyses blind to the species names. The results obtained by the two independent analysts were highly consistent.

The output of this analysis was transferred into a spreadsheet, and formant values were plotted against time and frequency and superimposed onto a narrow band spectrogram of each call. Spurious values were deleted, missing values were linearly interpolated and octave jumps were corrected for. For the first six formants, we then plotted the observed minimum frequency value of each formant against \((2i – 1)/2\) increments of the formants spacing, as predicted by the model of a vocal tract, approximated as a straight uniform tube closed at one end (the glottis) and
open at the other (the mouth) [S81]. Then, we fitted a linear regression line through the set of observed values, applying an intercept equal to 0 [S82]. Since $F_i = ((2i - 1)/2)\Delta F$, the slope of the regression gives the best estimate of $\Delta F$ for our vocal tract model. The frequency of each of the first six formants, for each of the 3 loud calls analysed per species, are given in Table S2. There was very little variation in $\Delta F$ across the three roars within species, so we took the average $\Delta F$ across the three roars for each species. In the final step, we deduced the estimated apparent VTL directly from the average $\Delta F$ by using the equation $VTL = C/2(\Delta F)$, where $c$ (350 m/s) is the approximate speed of sound in the warm humid air of a mammalian vocal tract [S74].

**Computed tomography (CT) and magnetic resonance imaging (MRI) of whole animals**

We performed computed tomography (CT) and magnetic resonance imaging (MRI) on the cadavers of two adult male howler monkeys of different species (*Alouatta sara* and *A. caraya*) and one adult male spider monkey (*Ateles fusciceps*). We chose to examine the *Ateles* specimen in order to demonstrate the simple larynx and hyoid, typical of most primates, in this closely related taxon, and to highlight the fact that the *Alouatta* hyoid and larynx are highly modified, derived traits. The two howler monkey species were chosen to represent the greatest extremes of hyoid volume possible, given the available material. We carried out both CT and MRI at the University of Veterinary Medicine, Vienna.

We performed CT examination using a Somatom Emotion multislice scanner (Siemens AG, Munich, Germany). The specimens were placed in ventral recumbency, and scanned, depending on body size, with the following parameters: 110 – 130 kV, 94 – 174 effective mA and 0.75 mm thick axial slices. We used Avizo Fire 6.3 and Somaris/5 Syngo CT2009E (Siemens AG, Berlin) to generate multiplanar reconstructions and 3D surface models.
We performed MRI examination using a Magnetom Espree 1.5 Tesla Open Bore Design MR-System (Siemens AG, Erlangen, Germany) using a 4-canal Neck-Matrix-coil in combination with a 24-canal Spine-Matrix-coil (Siemens AG, Medical Solutions, Erlangen, Germany).

Specimens were placed in ventral recumbency and scanned using the following sequences: T2-weighted 3D Turbo Spin Echo (TSE) [Repetition Time (TR): 1.500, Echo Time (TE): 225, Echo Train Length (ETL): 79, Band Width (BW): 476, Flip Angle: 130°, Slice Thickness (SL): 1 mm, Matrix: 256 x 250, Field of View (FoV): 250*250 mm], T1-weighted 3D Gradient Echo fast low angle shot [TR: 9.67, TE: 4.78, BW: 199, Flip Angle: 18.6, SL: 1 mm, Matrix: 512 x 512, FoV: 250*250 mm] and a Proton-weighted (PD) TSE [TR: 2.510, TE: 22, SL: 2 mm, ETL: 9, BW: 181, Flip Angle: 150, Matrix: 384 x 384, FoV: 250 x 250 mm]. In all cases the scanning-direction was sagittal. The phase encoding direction for the T2- and PD-weighted sequence was head-feet and for the T1-weighted anterior-posterior. We used Osirix v 5.8 [S83] to generate reconstructions and carry out anatomical measurements.

**Data sources for body weight, vocal fold length and fundamental frequency**

Data on body size and fundamental frequency (Figure 4a) were taken from Herbst et al. [S78].

Data on vocal fold length and fundamental frequency (Figure 4b) were from: rat [S84, S85], squirrel monkey [S86, S87], domestic cat [S74, S88], rhesus macaque [S75, S87], human[S74], sheep [S89], saiga antelope [S90], pig [S89, S91], red deer [S81, S92], cow [S89, S93], reindeer [S94], howler monkey – present study, Mongolian gazelle [S95–97], muskox [S98], tiger [S99, S100], elephant [S78] and the figure was adapted from [S77].

**Statistical methods**
We first used a general linear model to examine differences in hyoid volume and canine length between sexes, among species and the interaction between sex and species, and a one-way analysis of variance (ANOVA) to examine variation in testes volume among species. Then, to analyse the covariance between variables, while accounting for the non-independence of data points due to shared ancestry of species, we conducted phylogenetic generalised least squares (PGLS) regressions with a Brownian motion model of evolution, based on a published molecular phylogeny of howler monkeys [S101] (Figure 2). These models use maximum-likelihood methods to estimate Pagel`s lambda (\( \lambda \)) [S102], which can be used to assess the degree of phylogenetic signal in the PGLS and varies between 0 (phylogenetic independence) and 1 (species` traits covary in proportion to their shared ancestry). We used branch lengths and splitting dates from the published molecular phylogeny [S101]. Because \( A. \) nigerrima was not included in the published phylogeny, we used an additional molecular and karyotypic analysis of the genus, which shows that this species is more closely related to \( A. \) macconnellii than any other [S103]. We therefore positioned this species accordingly in the phylogeny for visual purposes (Figure 2a), but \( A. \) nigerrima was not used in any PGLS analyses. In order to account for potential error in the branch lengths used, we recalculated all of the PGLS analyses with branch lengths of 1 and the results did not change (Table S4). We present absolute hyoid volume and testes volume in the main text, as there was no correlation between either hyoid volume or testes volume and male body weight in our species level data, and, therefore, no effect of isometric scaling. When added to the models as a covariate, body weight accounted for very little variance and did not change our results (see below). The regression lines of the linear models and the regression lines of the full PGLS models had the same intercept and slope in all cases. Therefore,
only one line is presented in the figures. We log$_{10}$-transformed variables in those cases where this improved the linearity of the relationships.

In the analyses that included the mean number of males per species, we used this variable as the independent variable and the morphological traits (i.e., hyoid volume, canine length and testes volume) as dependent variables. When analysing the relationship between testes volume and hyoid volume, ΔF and hyoid volume, and skull length and hyoid volume, we assigned hyoid volume as the dependent variable.

Where possible, we also used a more complete data set, rather than relying on average species level data. Firstly, in order to test the “environmental adaptation” hypothesis, we used general linear mixed models (GLMM) to evaluate the effect of NPP on hyoid volume using matching data for NPP and hyoid volume from all 255 hyoids. We specified species as a random factor in the model to account for the non-independence of species and tested the model including NPP against a null model (not including NPP) using ANOVA. In order to account for phylogenetic effects, we also carried out a PGLS regression using mean NPP values per species as the independent variable. Secondly, we used matching data on skull length and hyoid volume for 117 individuals, and performed a GLMM using species and sex as random factors in the model and hyoid volume as the dependent variable. We then tested this model against a null model (not including the variable skull length) using ANOVA. We performed all analyses in the statistical package R version 2.15.2 [S104].

**Supplemental Results**

*The relationship between canine length and other traits*
At the species level, canine length was not correlated with body weight (male PGLS: $R^2 = 0.00, \lambda = 0.00, F_{(1,5)} = 0.00, P = 0.95$; female PGLS: $R^2 = 0.06, \lambda = 1.00, F_{(1,6)} = 0.33, P = 0.59$), the number of males per group (male PGLS: $R^2 = 0.00, \lambda = 0.00, F_{(1,6)} = 0.00, P = 0.99$; female PGLS: $R^2 = 0.08, \lambda = 1.00, F_{(1,6)} = 0.51, P = 0.50$), hyoid volume (male PGLS: $R^2 = 0.15, \lambda = 0.00, F_{(1,6)} = 1.07, P = 0.34$; female PGLS: $R^2 = 0.00, \lambda = 1.00, F_{(1,7)} = 0.01, P = 0.95$) or testes volume (PGLS: $R^2 = 0.52, \lambda = 0.00, F_{(1,3)} = 3.22, P = 0.17$).

Adding body weight as a covariate

After adding body weight as a covariate, the number of males per group was still a significant predictor of log$_{10}$ hyoid volume in both males (full PGLS model: $R^2 = 0.85, \lambda = 0.00, F_{(2,4)} = 11.25, P < 0.05$; number of males: estimate $\pm$ SE = -0.47 $\pm$ 0.10, $t$ = -4.71, $P < 0.01$; male body weight: estimate $\pm$ SE = 0.12 $\pm$ 0.15, $t$ = 0.81, $P = 0.46$) and females (full model: $R^2 = 0.92, \lambda = 0.00, F_{(2,4)} = 24.23, P < 0.01$; number of males: estimate $\pm$ SE = -0.44 $\pm$ 0.06, $t$ = -6.85, $P < 0.005$; female body weight: estimate $\pm$ SE = -0.002 $\pm$ 0.09, $t$ = -0.03, $P = 0.98$). Similarly, testes volume was still a significant predictor of hyoid volume (full PGLS model: $R^2 = 0.97, \lambda = 0.00, F_{(2,3)} = 34.55, P < 0.05$; testes volume: estimate $\pm$ SE = -3.02 $\pm$ 0.36, $t$ = -8.31, $P = 0.01$; male body weight: estimate $\pm$ SE = -8.64 $\pm$ 5.40, $t$ = 1.59, $P = 0.25$) and log$_{10}$ male hyoid volume was still a significant predictor of formant spacing (full PGLS model: $R^2 = 0.97, \lambda = 0.00, F_{(2,3)} = 43.41, P < 0.01$; male hyoid volume: estimate $\pm$ SE = -172.44 $\pm$ 19.45, $t$ = -8.86, $P < 0.05$; male body weight: estimate $\pm$ SE = -32.15 $\pm$ 15.03, $t$ = -2.13, $P = 0.12$) after adding body weight as a covariate. Although the fixed effect of the number of males was still a significant predictor of testes volume after adding body weight as a covariate (estimate $\pm$ SE = 13.21 $\pm$ 3.06, $t$ = 4.3, $P <
the overall model was only borderline significant (full PGLS model: $R^2 = 0.91$, $\lambda = 0.00$, $F_{(1,3)} = 9.58$, $P = 0.09$) because of the small sample size and the noise introduced by body weight, which did not explain any of the variation (estimate $\pm$ SE $= -6.25 \pm 3.79$, $t = -1.65$, $P = 0.24$).

Supplemental References

S1. Pinto, A. C. B., Ramos, C. A., and de Carvalho Jr., O. (2003). Activity patterns and diet of the howler monkey *Alouatta belzebul* in areas of logged and unlogged forest in Eastern Amazonia. Anim. Biodivers. Conserv. 2, 39–49.

S2. De Souza, L. L., Ferrari, S. F., Da Costa, M. L., and Kern, D. C. (2002). Geophagy as a correlate of folivory in red-handed howler monkeys (*Alouatta belzebul*) from eastern Brazilian Amazonia. J. Chem. Ecol. 28, 1613–21.

S3. Camargo, C. C., and Ferrari, S. F. (2007). Interactions between tayras (*Eira barbara*) and red-handed howlers (*Alouatta belzebul*) in eastern Amazonia. Primates. 48, 147–50.

S4. Pinto, L. P., and Setz, E. Z. F. (2004). Diet of *Alouatta belzebul discolor* in an Amazonian rain forest of Northern Mato Grosso State, Brazil. Int. J. Primatol. 25, 1197–1211.

S5. Bonvicino, C. (1989). Ecologia e comportamento de *Alouatta belzebul* (Primates, Cebidae) na Mata Atlântica. Rev. Nord. Biol. 6, 149–179.

S6. Bravo, S. P. S., and Sallenave, A. (2003). Foraging behavior and activity patterns of *Alouatta caraya* in the northeastern Argentinian flooded forest. Int. J. Primatol. 24, 825–846.

S7. Oklander, L. I., Kowalewski, M. M., and Corach, D. (2010). Genetic consequences of habitat fragmentation in black-and-gold howler (*Alouatta caraya*) populations from northern Argentina. Int. J. Primatol. 31, 813–832.

S8. Pavé, R., Kowalewski, M. M., Peker, S. M., and Zunino, G. E. (2010). Preliminary study of mother-offspring conflict in black and gold howler monkeys (*Alouatta caraya*). Primates. 51, 221–6.

S9. Pavé, R., Kowalewski, M. M., Garber, P. A., Zunino, G. E., Fernandez, V. A., and Peker, S. M. (2012). Infant mortality in black-and-gold howlers (*Alouatta caraya*) living in a flooded forest in northeastern Argentina. Int. J. Primatol. 33, 937–957.
S10. Aguiar, L. M., Ludwig, G., and Passos, F. C. (2009). Group size and composition of black-and-gold howler monkeys (Alouatta caraya) on the Upper Paraná River, Southern Brazil. Primates. 50, 74–7.

S11. Bicca-Marques, J. C., Prates, H. M., de Aguiar, F. R. C., and Jones, C. B. (2008). Survey of Alouatta caraya, the black-and-gold howler monkey, and Alouatta guariba clamitans, the brown howler monkey, in a contact zone, State of Rio Grande do Sul, Brazil: evidence for hybridization. Primates. 49, 246–52.

S12. Juárez, C. P., Dvoskin, R., and Fernández-Duque, E. (2005). Structure and composition of wild black howler troops (Alouatta caraya) in gallery forests of the Argentinean Chaco. Neotrop. Primates 13, 19.

S13. Rumiz, D. I. (1990). Alouatta caraya: Population density and demography in northern Argentina. Am. J. Primatol. 21, 279–294.

S14. Da Cunha, R. G. T., and Byrne, R. (2006). Roars of black howler monkeys (Alouatta caraya): evidence for a function in inter-group spacing. Behaviour 143, 1169–1199.

S15. Agoramoorthy, G., and Lohmann, R. (1999). Population and conservation status of the black-and-gold howler monkeys, Alouatta caraya, along the Rio Riachuelo, Argentina. Neotrop. Primates 7, 43–44.

S16. Bicca-Marques, J. C., Muhle, C. B., Prates, H. M., Oliveira, S. G., and Calegaro-Marques, C. (2009). Habitat impoverishment and egg predation by Alouatta caraya. Int. J. Primatol. 30, 743–748.

S17. Fortes, V. B., and Bicca-Marques, J. C. (2008). Abnormal pelage color in an isolated population of Alouatta guariba clamitans Cabrera, 1940 in South Brazil. Int. J. Primatol. 29, 717–722.

S18. Da Silva Jr, E. . (1981). A preliminary survey of brown howler monkeys (Alouatta fusca) at the Catareira Reserve, Sao Paulo, Brazil. Rev. Bras. Biol. 41, 897–909.

S19. Teixeira da Cunha, R. G., and Jalles-Filho, E. (2007). The roaring of southern brown howler monkeys (Alouatta guariba clamitans) as a mechanism of active defence of borders. Folia Primatol. 970, 259–271.

S20. Miranda, J. M. D., and Passos, F. C. (2005). Composição e dinâmica de grupos de Alouatta guariba clamitans Cabrera (Primates, Atelidae) em Floresta Ombrófila Mista no Estado do Paraná, Brasil. Rev. Bras. Zool. 22, 99–106.

S21. Holzmann, I., Agostini, I., and Bitetti, M. (2012). Roaring behavior of two syntopic howler species (Alouatta caraya and A. guariba clamitans): Evidence supports the mate defense hypothesis. Int. J. Primatol. 33, 338–355.
S22. Mendes, S. L. (1989). Estudo ecologico de *Alouatta fusca* (Primates: Cebidae) na Estacao Biologica de Caratinga, M.G. Rev. Nord. Biol. 6, 71–104.

S23. Strier, K. B., Mendes, S. L., and Santos, R. R. (2001). Timing of births in sympatric brown howler monkeys (*Alouatta fusca clamitans*) and northern muriquis (*Brachyteles arachnoides hypoxanthus*). Am. J. Primatol. 55, 87–100.

S24. Martins, M. M. (2008). Fruit diet of *Alouatta guariba* and *Brachyteles arachnoides* in Southeastern Brazil: comparison of fruit type, color, and seed size. Primates. 49, 1–8.

S25. Steinmetz, S. (2001). Drinking by howler monkeys (*Alouatta fusca*) and its seasonality at the Intervales State Park, Sao Paulo, Brazil. Neotrop. Primates 9, 111–112.

S26. Lopes, K. G. D., and Bicca-Marques, J. C. (2011). Extragroup copulations in *Alouatta guariba clamitans*. Neotrop. Primates 18, 52–53.

S27. De Souza Fialho, M., and Setz, E. Z. F. (2007). Extragroup copulations among brown howler monkeys in southern Brazil. Neotrop. Primates 14, 50–52.

S28. Chiarello, A. (1994). Diet of the brown howler monkey *Alouatta fusca* in a semi-deciduous forest fragment of southeastern Brazil. Primates 35, 25–34.

S29. Urbani, B. (2006). A survey of primate populations in northeastern Venezuelan Guayana. Primate Conserv. 20, 47–52.

S30. Julliot, C. (1996). Fruit choice by red howler monkeys (*Alouatta seniculus*) in a tropical rain forest. Am. J. Primatol. 282, 261–282.

S31. Julliot, C., and Sabatier, D. (1993). Diet of the red howler monkey (*Alouatta seniculus*) in French Guiana. Int. J. Primatol. 14, 527–550.

S32. Julliot, C. (1997). Impact of seed dispersal by red howler monkeys *Alouatta seniculus* on the seedling population in the understory of tropical rain forest. J. Ecol. 85, 431–440.

S33. Milton, K. (1982). Dietary quality and demographic regulation in a howler monkey population. In The ecology of a tropical forest: Seasonal rhythms and long-term changes, E. Leigh, S. A. Rands, and D. Windsor, eds. (Washington, DC), pp. 273–289.

S34. Milton, K. (1996). Effects of bot fly (*Alouattamyia baeri*) parasitism on a free-ranging howler monkey (*Alouatta palliata*) population in Panama. J. Zool. 239, 39–63.

S35. Lippold, L. (1988). A census of primates in Cabo Blanco Absolute Nature Reserve, Costa Rica. Brenesia 29, 101–105.

S36. Lippold, L. (1989). Primates in Cabo Blanco Absolute Nature Reserve, Costa Rica. Primate Conserv. 10, 23–25.
S37. Heltne, P., Turner, D., and Scott, N. J. J. (1976). Comparison of census data on *Alouatta palliata* from Costa Rica and Panama. In Neotropical primates: Field studies and conservation, R. Thorington and P. Heltne, eds. (Washington D.C.: National Academy of Sciences), pp. 10–19.

S38. Jones, C. B. (1985). Reproductive patterns in mantled howler monkeys: Estrus, mate choice and copulation. *Primates* 26, 130–142.

S39. Clarke, M. R., Zucker, E. L., and Scott, N. J. J. (1986). Population trends of the mantled howler groups of La Pacifia, Guanacaste, Costa Rica. *Am. J. Primatol.* 11, 79–88.

S40. Clarke, M. R., and Zucker, E. L. (1994). Survey of the howling monkey population at La Pacifica: A seven-year follow-up. *Int. J. Primatol.* 15, 61–73.

S41. Clarke, M. R., Collins, D. A., and Zucker, E. L. (2002). Responses to deforestation in a group of mantled howlers (*Alouatta palliata*) in Costa Rica. *Int. J. Primatol.* 23, 365–381.

S42. Baldwin, J. D., and Baldwin, J. I. (1976). Primate populations in Chiriqui, Panama. In Neotropical primates: Field studies and conservation, R. Thorington and P. Heltne, eds. (Washington D.C.: National Academy of Sciences), pp. 20–31.

S43. Elizabeth, K., and Stoner, E. (1994). Population density of the mantled howler monkey (*Alouatta palliata*) at La Selva Biological Reserve, Costa Rica: A new technique to analyze census data. *Biotropica* 26, 332–340.

S44. Estrada, A. (1982). Survey and census of howler monkeys (*Alouatta palliata*) in the rain forest of “Los Tuxtlas”, Veracruz, Mexico. *Am. J. Primatol.* 2, 363–372.

S45. Estrada, A. (1984). Resource use by howler monkeys (*Alouatta palliata*) in the rain forest of Los Tuxtlas, Veracruz, Mexico. *Int. J. Primatol.* 5, 105–131.

S46. Fedigan, L. M., Fedigan, L., and Chapman, C. A. (1985). A census of *Alouatta palliata* and *Cebus capuchinus* in Santa Rosa National Park, Costa Rica. *Brenesia* 23, 309–322.

S47. Fedigan, L. M. (1986). Demographic trends in the *Alouatta palliata* and *Cebus capuchinus* populations of Santa Rosa National Park, Costa Rica. In Primate Ecology and Conservation, J. Else and P. C. Lee, eds. (Cambridge University Press), pp. 285–293.

S48. Fedigan, L. M., Rose, L. M., and Avila, R. M. (1998). Growth of mantled howler groups in a regenerating Costa Rican dry forest. *Int. J. Primatol.* 19, 405–432.

S49. Fedigan, L. M., and Jack, K. (2001). Neotropical primates in a regenerating Costa Rican dry forest: A comparison of howler and capuchin population patterns. *Int. J.* 22, 689–713.

S50. Horwich, R. H., and Gebhard, K. (1983). Roaring rhythms in black howler monkeys (*Alouatta pigra*) of Belize. *Primates* 24, 290–296.
S51. Bolin, I. (1981). (*Alouatta palliata pigra*) in Belize and Guatemala. Primates 22, 349–360.

S52. Estrada, A., Luecke, L., Van Belle, S., Barrueta, E., and Meda, M. R. (2004). Survey of black howler (*Alouatta pigra*) and spider (*Ateles geoffroyi*) monkeys in the Mayan sites of Calakmul and Yaxchilán, Mexico and Tikal, Guatemala. Primates. 45, 33–9.

S53. Horwich, R. H., Brockett, R. C., James, R. A., and Jones, C. B. (2001). Population growth in the Belizean black howling monkey (*Alouatta pigra*). Neotrop. Primates 9, 1–7.

S54. Ostro, L. E. T., Silver, S. C., Koontz, F. W., Horwich, R. H., and Brockett, R. C. (2001). Shifts in social structure of black howler (*Alouatta pigra*) groups associated with natural and experimental variation in population density. Int. J. Primatol. 22, 733–748.

S55. Estrada, A., Castellanos, L., Garcia, Y., Franco, B., Munoz, D., Ibarra, A., Rivera, A., Fuentes, E., and Jimenez, C. (2002). Survey of the black howler monkey, *Alouatta pigra*, population at the Mayan site of Palenque, Chiapas, Mexico. Primates. 43, 51–8.

S56. Wallace, R. B. unpublished data.

S57. Wallace, R. B., Painter, R. L., and Taber, A. B. (1998). Primate diversity, habitat preferences, and population density estimates in Noel Kempff Mercado National Park, Santa Cruz Department, Bolivia. Am. J. Primatol. 46, 197–211.

S58. Palacios, E., and Rodriguez, A. (2001). Ranging pattern and use of space in a group of red howler monkeys (*Alouatta seniculus*) in a southeastern Colombian rainforest. Am. J. Primatol. 251, 233–251.

S59. Gaulin, S. J. C., and Gaulin, C. K. (1982). Behavioral ecology of *Alouatta seniculus* in Andean cloud forest. Int. J. 3.

S60. Braza, F., Alvarez, F., and Azcarate, T. (1981). Behaviour of the red howler monkey (*Alouatta seniculus*) in the Llanos of Venezuela. Primates 22, 459–473.

S61. Izawa, K., and Nishimura, A. (1988). Primate fauna at the study site La Macarena, Colombia. F. Stud. New World monkeys, La Macarena, Colomb. 1, 5–11.

S62. Izawa, K. (1976). Group sizes and compositions of monkeys in the upper Amazon basin. Primates 17, 367–399.

S63. Defler, T. R. (1981). The density of *Alouatta seniculus* in the eastern llanos of Colombia. Primates 22, 564–569.

S64. QGIS Development Team, T. (2014). QGIS Geographic Information System. Open Source Geospatial Found. Proj. Available at: http://qgis.osgeo.org.
IUCN (2013). The IUCN Red List of Threatened Species. Version 2013.1. Available at: http://www.iucnredlist.org/.

Feldkamp, L. A. (1984). Practical cone-beam algorithm. J. Opt. Soc. Am. A 1, 612–619.

Kelaita, M., Dias, P. A. D., Aguilar-Cucurachi, M. D. S., Canales-Espinosa, D., and Cortés-Ortiz, L. (2011). Impact of intrasexual selection on sexual dimorphism and testes size in the Mexican howler monkeys Alouatta palliata and A. pigra. Am. J. Phys. Anthropol. 146, 179–87.

Moreland, R. B., Richardson, M. E., Lamberski, N., and Long, J. A. (2001). Characterizing the reproductive physiology of the male southern black howler monkey, Alouatta caraya. J. Androl. 22, 395–403.

Plavcan, J. M., and van Schaik, C. P. (1992). Intrasexual competition and canine dimorphism in anthropoid primates. Am. J. Phys. Anthropol. 87, 461–77.

Ford, S. M., and Davis, L. C. (1992). Systematics and body size: implications for feeding adaptations in New World monkeys. Am. J. Phys. Anthropol. 88, 415–68.

Di Fiore, A., and Campbell, C. J. (2007). The atelines: Variation in ecology, behaviour, and social organization. In Primates in perspective, C. J. Campbell., A. Fuentes., K. C. MacKinnon., M. Panger., and S. K. Bearder, eds. (New York: Oxford University Press), pp. 155–185.

Fitch, W. T. (1997). Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. J. Acoust. Soc. Am. 102, 1213–22.

Zhao, M., Heinsch, F. A., Nemani, R. R., and Running, S. W. (2005). Improvements of the MODIS terrestrial gross and net primary production global data set. Remote Sens. Environ. 95, 164–176.

Titze, I. (1994). Principles of voice production (Prentice Hall).

Riede, T. (2010). Elasticity and stress relaxation of rhesus monkey (Macaca mulatta) vocal folds. J. Exp. Biol. 213, 2924–32.

Riede, T., and Titze, I. R. (2008). Vocal fold elasticity of the Rocky Mountain elk (Cervus elaphus nelsoni) - producing high fundamental frequency vocalization with a very long vocal fold. J. Exp. Biol. 211, 2144–54.

Charlton, B. D., Frey, R., McKinnon, A. J., Fritsch, G., Fitch, W. T., and Reby, D. (2013). Koalas use a novel vocal organ to produce unusually low-pitched mating calls. Curr. Biol. 23, R1035–6.
S78. Herbst, C. T., Stoeger, A. S., Frey, R., Lohscheller, J., Titze, I. R., Gumpenberger, M., and Fitch, W. T. (2012). How low can you go? Physical production mechanism of elephant infrasonic vocalizations. Science 337, 595–9.

S79. Baldwin, J. D., and Baldwin, J. I. (1976). Vocalizations of howler monkeys (Alouatta palliata) in southwestern Panama. Folia Primatol. (Basel). 26, 81–108.

S80. Boersma, P. (2001). Praat, a system for doing phonetics by computer. Glot Int. 5, 341–345.

S81. Reby, D., and McComb, K. (2003). Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. Anim. Behav. 65, 519–530.

S82. Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T., and Clutton-Brock, T. (2005). Red deer stags use formants as assessment cues during intrasexual agonistic interactions. Proc. Biol. Sci. 272, 941–7.

S83. Rosset, A., Spadola, L., and Ratib, O. (2004). OsiriX: an open-source software for navigating in multidimensional DICOM images. J. Digit. Imaging 17, 205–16.

S84. Riede, T., York, A., Furst, S., Muller, R., and Seelecke, S. (2012). Elasticity and stress relaxation of a very small vocal fold. J. Biomech. 44, 1936–1940.

S85. Jourdan, D., Ardid, D., Chapuy, E., Le Bars, D., and Eschalier, A. (1997). Audible and ultrasonic vocalization elicited by a nociceptive stimulus in rat: relationship with respiration. J. Pharmacol. Toxicol. Methods 38, 109–16.

S86. Brown, C. H., Alipour, F., Berry, D. A., and Montequin, D. (2003). Laryngeal biomechanics and vocal communication in the squirrel monkey (Saimiri boliviensis). J. Acoust. Soc. Am. 113, 2114.

S87. Fitch, W. T., and Hauser, M. D. (1995). Vocal production in nonhuman primates. Acoustics, physiology and functional constraints on honest advertisement. Am. J. Primatol. 37, 191–219.

S88. Turner, D., and Bateson, P. (2013). The domestic cat: the biology of its behaviour 3rd ed. D. Turner and P. Bateson, eds. (Cambridge: Cambridge University Press).

S89. Alipour, F., and Jaiswal, S. (2008). Phonatory characteristics of excised pig, sheep, and cow larynges. J. Acoust. Soc. Am. 123, 4572–81.

S90. Frey, R., Volodin, I., and Volodina, E. (2007). A nose that roars: anatomical specializations and behavioural features of rutting male saiga. J. Anat. 211, 717–36.

S91. Schrader, L., and Todt, D. (1998). Vocal quality is correlated with levels of stress hormones in domestic pigs. Ethology 876, 859–876.
S92. Frey, R., Volodin, I., Volodina, E., Carranza, J., and Torres-Porras, J. (2012). Vocal anatomy, tongue protrusion behaviour and the acoustics of rutting roars in free-ranging Iberian red deer stags (*Cervus elaphus hispanicus*). J. Anat. 220, 271–92.

S93. Kiley, M. (1972). The vocalizations of ungulates, their causation and function. Z. Tierpsychol. 31, 171–222.

S94. Frey, R., Gebler, A., Fritsch, G., Nygrén, K., and Weissengruber, G. E. (2007). Nordic rattle: the hoarse vocalization and the inflatable laryngeal air sac of reindeer (*Rangifer tarandus*). J. Anat. 210, 131–159.

S95. Frey, R., Gebler, A., Olson, K. a, Odonkhuu, D., Fritsch, G., Batsaikhan, N., and Stuermer, I. W. (2008). Mobile larynx in Mongolian gazelle: Retraction of the larynx during rutting barks in male Mongolian gazelle (*Procapra gutturosa*, Pallas, 1777). J. Morphol. 269, 1223–37.

S96. Frey, R. F., and Riede, T. R. (2003). Sexual dimorphism of the larynx of the Mongolian gazelle (*Procapra gutturosa*, Pallas, 1777). Zool. Anz. 242, 33–62.

S97. Frey, R., and Gebler, A. (2003). The highly specialized vocal tract of the male Mongolian gazelle (*Procapra gutturosa*, Pallas, 1777 - Mammalia, Bovidae). J. Anat. 203, 451–71.

S98. Frey, R., Gebler, A., and Fritsch, G. (2006). Arctic roars - laryngeal anatomy and vocalization of the muskox (*Ovibos moschatus*, Zimmermann, 1780, Bovidae). J. Zool. 268, 433–448.

S99. Klemuk, S. A., Riede, T., Walsh, E. J., and Titze, I. R. (2011). Adapted to roar: functional morphology of tiger and lion vocal folds. PLoS One 6, e27029.

S100. Titze, I. R., Fitch, W. T., Hunter, E. J., Alipour, F., Montequin, D., Armstrong, D. L., McGee, J., and Walsh, E. J. (2010). Vocal power and pressure-flow relationships in excised tiger larynges. J. Exp. Biol. 213, 3866–73.

S101. Cortés-Ortiz, L., Rodríguez-Luna, E., Sampaio, I., and Ruiz-García, M. (2003). Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Mol. Phylogenet. Evol. 26, 64–81.

S102. Pagel, M. (1999). Inferring the historical patterns of biological evolution. Nature 401, 877–84.

S103. Bonvicino, C. R., Lemos, B., and Seuánez, H. N. (2001). Molecular phylogenetics of howler monkeys (*Alouatta*, Platyrhini): A comparison with karyotypic data. Chromosoma 110, 241–246.

S104. Team Development Core, R. (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.