

1 **No evidence that maximum fundamental frequency reflects selection for**
 2 **signal diminution in bonobos**

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4 Maxime Garcia^{1,+} & Jacob C. Dunn^{2,3,*,}

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6 ¹Animal Behaviour, Department of Evolutionary Biology and Environmental Science,
 7 University of Zurich, Winterthurerstrasse 190, 8051, Zurich, Switzerland

8 ²Behavioural Ecology Research Group, Anglia Ruskin University, East Road,
 9 Cambridge, CB1 1PT, United Kingdom.

10 ³Biological Anthropology, Department of Archaeology, University of Cambridge,
 11 Fitzwilliam St, Cambridge, CB2 1QH, United Kingdom.

12 ⁺These authors contributed equally

13 *Correspondence to: jacob.dunn@anglia.ac.uk

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16 Grawunder *et al.* claimed that short vocal folds and high fundamental frequency
 17 reflect selection for signal diminution in bonobos. Garcia & Dunn challenge their
 18 conclusions, highlighting issues in subspecies sampling, acoustic analyses, tissue
 19 fixation methods and allometric scaling principles, thus calling for reconsideration
 20 of the original data.

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Acoustic allometry consists of looking at how an organism's body size scales with the characteristics of its vocalizations. A typical finding based on this framework is that body size is reflected in the fundamental frequency (f_o) of vocalizations, whereby lower f_o indicates larger body size [1]. This relationship holds owing to the fact that vocal fold length generally scales with body size [2]. Cross species comparisons allow for the identification of interesting outliers from the body size – f_o regression [3]. Such cases are of particular relevance as they can provide insight into the selective forces potentially driving deviations from standard allometric principles [2]. In a recent study, Grawunder et al. [4] argue that selective pressure for higher f_o has led to the evolution of shorter vocal folds in bonobos than in chimpanzees. Thus, they claim, vocal fold length has evolved independently of body size in bonobos for the purposes of signal diminution. However, considering both the existing literature and their own data, this conclusion does not appear to be supported for several reasons.

First, the authors do not discuss the implications of known variation in body size among chimpanzee subspecies in their study. The authors argue for similarity in body size between chimpanzees and bonobos, but cite data specific to the smallest population of the smallest chimpanzee subspecies (*Pan troglodytes schweinfurthii* from Gombe) [5,6], thus minimizing species differences. While overlap in body size exists between the species, in general chimpanzees are bigger [7] and significant differences have been documented among chimpanzee subspecies and between chimpanzee subspecies and bonobos [6]. Indeed, the authors' own data indicate that their chimpanzee subjects (mean = 48.9 Kg, range 34.9-60.7 Kg) were substantially

bigger than their bonobos (mean = 39.3 Kg, range 26.7-55.3 Kg). Furthermore, the acoustic data they report come from a combination of *P. t. schweinfurthii* and *P. t. verus*, while anatomical data come from unknown subspecies, but are extremely unlikely to come from *P. t. schweinfurthii*, as this subspecies is thought to represent just 5% of chimpanzees in European zoos [8]. Given the large amount of variation, a thorough control for body size is required. Such control has not been provided by the authors, and should the bonobos be smaller than the chimpanzees, shorter vocal folds and higher fundamental frequencies would not deviate from standard acoustic allometry and, thus, tell us little about selection. Indeed, including the new data on chimpanzee and bonobo vocal fold length from Grawunder et al. [4] in a phylogenetic comparative analysis of body mass [7] and vocal fold length [3] across mammals, we find that bonobo vocal fold length is almost exactly what would be predicted by allometry (Figure 1; see supplementary information for methods and detailed results).

Secondly, even if the difference between maximum fundamental frequency in the two species were not to be fully explained by differences in body size, the methodology applied by Grawunder et al. [4] introduced a further potential bias that may have contributed to the conclusions of their study – their anatomical specimens were fixed before scanning, using two different solutions. This may have had a critical impact on their data, since significant differences in the effects on tissue volume have been shown between Bouin’s solution (decrease in volume of 14 % compared with fresh tissue) and formaldehyde-based reagents (increase in volume of 29-34 % compared

with fresh tissue), respectively [9]. Although the exact effects of the procedures are unknown here as the authors do not report which samples were placed into which solutions, such potential bias may affect the conclusions drawn regarding relative vocal fold length in these two species.

Finally, the authors focused exclusively on maximum fundamental frequency ($\max f_0$) in high and low frequency calls. We recognize the effort made by the authors to apply a conservative method aimed at minimizing species differences in f_0 . However, the physics underlying sound production mechanisms in mammals implies the use of $\min f_0$, rather than $\max f_0$, when investigating acoustic allometric principles. The reason for this is two-fold: (1) $\max f_0$ is not a reliable indicator of vocal fold length, as it strongly depends on vocal fold tension and subglottal pressure [10]. While collecting acoustic recordings in the field, there is no control over these two parameters and we cannot be certain that the upper range of f_0 capabilities has been reached. Looking at $\min f_0$ assumes minimal tension and subglottal pressure and thus corresponds to a 'resting' vocal fold condition, which alleviates measurement biases; (2) In line with (1), the vocal fold measurements obtained from the CT-scans are made on vocal folds in a resting position (i.e., no external tension is applied, which provides a reliable reference). As such, acoustic measurement should rely on the same rationale (i.e., the use of $\min f_0$ from vocal folds that are not under tension) as anatomical measurements.

Overall, the data presently available indicate that the higher f_0 in bonobos may simply relate to their smaller body size (reflected in smaller vocal folds), as pointed out by our body size-vocal fold length regression analysis. They do not provide any grounds to suggest a specific selection pressure acting upon laryngeal anatomy in bonobos. This does not mean that such selection pressure is non-existent, but only that the data collected to date do not provide evidence for it. We found the author's theoretical concept of higher f_0 for reduced aggression in bonobos very interesting, as it falls within the framework of self-domestication processes and would support the production of high frequency calls in negative contexts. In addition, the male/female difference they observe in $\max f_0$ in chimpanzees is intriguing. Given the level of sexual dimorphism in this species [6], males showing higher $\max f_0$ is counter-intuitive and worthy of further investigation.

Should further research be carried out comparing these species, we suggest that it needs to be done without pre-scanning tissue fixation, reporting $\min f_0$, and, crucially, controlling for body size. Such research should in addition include a broader phylogenetically controlled comparative approach for contextualizing species-specific data.

Author contributions

J.C.D and M.G contributed equally to all aspects of this manuscript.

Acknowledgments

JD is supported by a grant from the Royal Society (EP09_C189_RE815).

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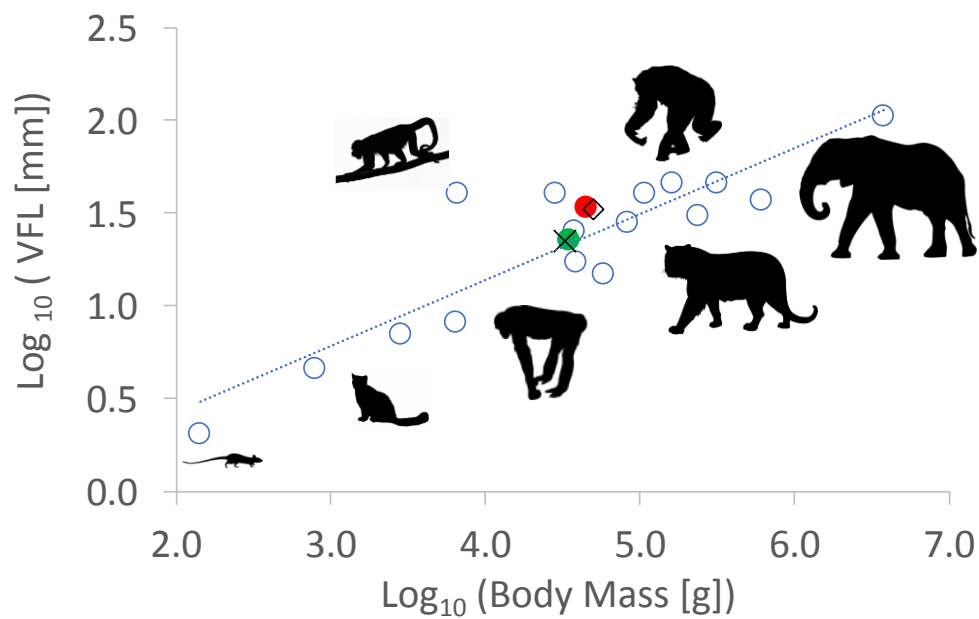
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Figure Legends

Figure 1. A log-log phylogenetic regression plot of body mass versus vocal fold length for a range of mammals (see supplementary information for methods and detailed results), showing that vocal fold length in bonobos (green marker = body mass data from [7]; black cross = body mass data from Grawunder et al. [4]) is what would be expected from body size. Chimpanzees (red marker = body mass data from [7]; black diamond = body mass data from Grawunder et al. [4]), are also indicated, for comparison.

Figure 1.



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Supplementary Materials

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Supplementary Statistical Methods

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 between body size (dependent variable) and vocal fold length (independent variable) with a Brownian motion model of evolution. The PGLS was based on a molecular phylogeny of mammals [1], and published data on body size [2] and vocal fold length [3]. These models use maximum-likelihood methods to estimate Pagel's lambda (λ) [4], 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 log transformed body size and vocal fold length owing to the large range of sizes found among species, which spanned an order of magnitude. We performed the analyses using the packages 'ape' [5] and 'caper' [6] in R version 1.0.153 [7].

Supplementary Results

Body size was strongly and positively correlated with vocal fold length across species (Figure 1: $R^2 = 0.79$, $F_{(1,16)} = 60.23$, $P < 0.001$). As $\lambda = 0.00$, then there was no evidence for a phylogenetic signal and PGLS is equivalent to ordinary least squares regression. Thus, the

regression lines of the linear models and the regression lines of the full PGLS models had the same intercept (-0.29) and slope (0.36).

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