

The evolution of acoustic size exaggeration in terrestrial mammals

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1 The evolution of acoustic size exaggeration in terrestrial
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13 **Abstract**

14

15 Recent studies have revealed that some mammals possess adaptations that enable
16 them to produce vocal signals with much lower fundamental frequency (F0) and
17 formant frequency spacing (ΔF) than expected for their size. Although these
18 adaptations are assumed to reflect selection pressures for males to lower frequency
19 components and exaggerate body size in reproductive contexts, this hypothesis has
20 not been tested across a broad range of species. Here we show that male terrestrial
21 mammals produce vocal signals with lower ΔF (but not F0) than expected for their
22 size in mating systems with greater sexual size dimorphism. We also reveal that males
23 produce calls with higher than expected F0 and ΔF in species with increased sperm
24 competition. This investigation confirms that sexual selection favours the use of ΔF
25 as an acoustic size exaggerator, and supports the notion of an evolutionary trade-off
26 between pre-copulatory signalling displays and sperm production.

27

28

29 **Introduction**

30

31 Identifying the proximate and ultimate factors that underlie the extraordinary diversity
32 of mammal vocal signals is a key objective of animal communication research, and an
33 essential prerequisite for understanding the origins and evolution of human vocal
34 communication¹. Body size is known to exert major constraints on the frequency
35 characteristic of animal vocalizations, and as a consequence, size differences between
36 species explain a large proportion of the acoustic diversity of animal vocalizations^{2,3}.
37 Large animals tend to produce lower call frequencies than smaller ones because they
38 have larger larynges with longer vocal folds that can oscillated periodically at lower
39 frequencies and longer vocal tracts that produce lower resonance frequencies (termed
40 formant frequencies in animal vocalisations)⁴⁻⁷. While this general rule of acoustic
41 allometry is broadly verified across mammal species, several exceptions, typically
42 affecting male vocal signals, have been documented.

43 For example, some species possess anatomical innovations that enable males
44 to produce abnormally low fundamental frequency (hereafter F0), such as the fleshy
45 vocal pads of roaring cats⁸, Mongolian and goitered gazelles^{9,10}, or saiga antelope¹¹;
46 hypertrophied larynges in howler and colobus monkeys¹², fallow deer¹³, and hammer-
47 headed bats¹⁴, and even an additional, non-laryngeal set of vocal folds in the koala¹⁵.
48 In other species, males produce abnormally low formant frequency spacing (ΔF) for
49 their size by extending their vocal tracts using descended and/or mobile larynges^{10,13,16-}
50 ¹⁹, additional resonators^{20,21}, or nasal proboscises^{11,22,23}. Because these anatomical
51 adaptations are often only present or disproportionately large in males and involved in
52 the production of mating calls, it is generally assumed that they have evolved via
53 selection pressures for individuals to lower frequency components in order to
54 broadcast an exaggerated impression of their body size in reproductive contexts^{1,17,24}.
55 However, while this assumption has been verified experimentally within a small
56 number of species²⁵⁻²⁹, whether sexual selection pressures on male body size drive the
57 evolution of putative acoustic size exaggeration across a wider range of mammalian
58 taxa remains to be investigated.

59 Although phylogenetically controlled comparative analyses of vocal behaviour
60 exist for birds^{30,31}, previous comparative investigations of mammal vocalizations are
61 either restricted to one mammalian order (e.g. Primates^{3,32,33}, Rodentia³⁴) or family (e.g.

62 Cervidae³⁵, Felidae³⁶). In this paper we provide the first phylogenetically controlled
63 comparative examination of the selection pressures that lead to acoustic size
64 exaggeration across nine orders and 72 species of terrestrial mammals. We show that
65 the principle of acoustic allometry is generally observed across taxa, and that males
66 from mating systems with strong selection pressures for large male body size produce
67 lower ΔF , but not F_0 , than expected for their size. Our findings also confirm that
68 selection pressures to exaggerate size are relaxed in male species with larger testes
69 relative to overall body size, indicating that a high level of post-copulatory sperm
70 competition reduces the importance of pre-copulatory acoustic size exaggeration.

71

72 **Results**

73

74 **Model selection criteria**

75 To test our hypotheses we used phylogenetic generalised least squares (PGLS)
76 regressions that simulated five different evolutionary scenarios. To select the best
77 supported PGLS regression models we started with a ‘global’ model including male
78 body mass, habitat (arboreal or terrestrial), call-type (sexual or nonsexual), mating
79 system (polygynous, monogamous, polyandrous, promiscuous or variable), and sexual
80 size dimorphism or relative testes size depending on the hypothesis that was being
81 tested, and iterated through all variable combinations to explain variation in male F_0
82 and ΔF for each of the five different evolutionary scenarios. All models considered
83 included \log_{10} male body mass to control for body size differences across species and
84 a model selection criteria based on the Akaike’s Information Criteria corrected for
85 sample size (AICc) was used, in which the model having the lowest AICc value is
86 chosen³⁷ (Supplementary Tables 1-6).

87

88 **Male body size versus F_0 and ΔF across species**

89 Our model selection approach indicated that the best supported PGLS regression
90 models to test for the effect of male body size on male F_0 and ΔF were a Brownian
91 motion model of evolution using Pagel’s λ (λ) to model the covariance structure
92 (BM + λ) and a pure Brownian motion model (BM) with habitat included as a
93 covariate, respectively (Supplementary Tables 1 and 2). The PGLS regressions
94 showed that greater male body mass predicted lower $\log_{10} F_0$ (estimate \pm s.e. = $-0.50 \pm$

95 0.09, $\lambda = 0.87$, $t_{4,65} = -5.92$, $P < 0.001$) and $\log_{10} \Delta F$ (estimate \pm s.e. = -0.34 ± 0.05 , $t_{4,32} = -6.19$, $P < 0.001$), confirming that the expected acoustic allometry exists across
96 species (Fig 1). We also found that arboreal species produced significantly lower
97 $\log_{10} \Delta F$ than other terrestrial mammals (estimate \pm s.e. = 0.30 ± 0.11 , $t_{4,32} = 3.10$, $P =$
98 0.008).
99

100

101 **Male size dimorphism versus F0 and ΔF across species**

102 A BM + λ model of evolution including \log_{10} male body mass as a covariate best
103 explained the relationship between size dimorphism and F0 (Supplementary Table 3).
104 This model showed that size dimorphism was not significantly related to \log_{10} F0
105 (estimate \pm s.e. = -4.93 ± 3.11 , $\lambda = 0.87$, $t_{5,64} = -1.58$, $P = 0.119$) (Fig. 2A). The
106 relationship between size dimorphism and ΔF was best explained by a pure Brownian
107 motion model of evolution with \log_{10} male body mass and habitat included as
108 covariates (Supplementary Table 4). This model revealed that species with greater
109 male sexual size dimorphism produced sexual calls with lower $\log_{10} \Delta F$ (estimate \pm s.e.
110 = -3.58 ± 1.21 , $t_{5,31} = -2.97$, $P = 0.006$) (Fig. 2B, Supplementary Table 4), indicating
111 that males produce vocal signals with lower than expected ΔF for their size in mating
112 systems with sexual selection pressures for large male body size.

113

114 **Sperm competition versus F0 and ΔF across species**

115 The best supported models to examine the effect of postcopulatory sperm competition
116 on F0 and ΔF were an Ornstein-Uhlenbeck (OU) model and a BM + λ model of
117 evolution, respectively (lowest AICc values, see Supplementary Tables 5 and 6).
118 Both models included \log_{10} male body mass as a covariate. The relationship between
119 relative testes size and male acoustic values in the 42 mammal species for which
120 acoustic and testes data were available, revealed that \log_{10} relative testes size was
121 positively correlated with \log_{10} F0 (estimate \pm s.e. = 0.39 ± 0.16 , $\alpha = 0.02$, $t_{5,39} = 2.50$,
122 $P = 0.017$) (Fig. 3A). We also found that \log_{10} relative testes size was positively
123 correlated with $\log_{10} \Delta F$ (estimate \pm s.e. = 0.09 ± 0.02 , $\lambda = 1.02$, $t_{5,21} = 4.04$, $P <$
124 0.001) (Fig. 3B) for the 24 species with available acoustic and testes data. These
125 findings indicate that species with larger testes relative to body size produce calls with
126 higher F0 and ΔF .

127

128 **Discussion**

129

130 Several interesting results emerge from this phylogenetically-controlled examination
131 of the link between acoustic variation in mammal calls and putative pre- and post-
132 copulatory sexual selection pressures in a wide range of mammalian species. First,
133 the key predictions of the acoustic allometry are confirmed: males from larger species
134 produce calls with lower F0 and lower formants (Fig. 1). The analysis also revealed
135 that males of arboreal species give sexual calls with lower ΔF than other terrestrial
136 mammals. This finding is consistent with the notion that low frequency calls given
137 from relatively higher positions are less affected by ground interference³⁸ and/or that
138 lower frequencies propagate best in forest environments³⁹. Interestingly, the
139 relationship between body mass and F0 across nine orders of terrestrial mammals (Fig
140 1A) indicates that laryngeal enlargement and the concomitant lengthening of the vocal
141 folds is a more effective way of lowering F0 than thickening the vocal folds to
142 increase their mass⁴⁰, and suggests that vocal pads may primarily support the
143 production of high amplitude low F0 sexual calls, rather than lowering F0 *per se*.

144 A very close relationship between male ΔF and body mass was also revealed
145 (Fig. 1B), illustrating how strong anatomical constraints affect the correlations
146 between vocal tract length, skull size, and overall body size⁴¹. When species without
147 anatomical adaptations to lower formant frequencies are considered alone, the
148 relationship is even stronger (R^2 of 0.79) (Fig. 1B). Interestingly, species with
149 specific anatomical and/or behavioural adaptations that allow them to escape these
150 constraints follow a separate downward shifted trend that still, nevertheless,
151 represents a close relationship between ΔF and body mass (R^2 of 0.58) (Fig. 1B).
152 This is suggestive of secondary constraints acting on size exaggerators, such as the
153 sternum preventing any further laryngeal descent¹⁶ and/or other skeletal structures that
154 limit further enlargement of acoustic resonators, which in turn limits the extent of size
155 exaggeration and maintains an allometric relationship between ΔF and body mass. Of
156 particular interest are species that are not known to possess adaptations to lower F0 or
157 formants yet still produce call frequencies which fall way below the expected acoustic
158 allometry (e.g. European badgers and mole rats). Future studies should further
159 investigate these species' vocal anatomy in conjunction with the selection pressures
160 acting on their vocal communication systems.

161 When investigating the effect of sexual selection for large male body size we
162 found that sexual size dimorphism did not predict F0 across taxa. The lack of a
163 relationship between sexual size dimorphism and F0 is not surprising as F0 is
164 generally a poor predictor of adult male body mass within species⁵. Our results,
165 therefore, support the hypothesis that sexual selection does not systematically favour
166 the use of F0 as an acoustic size exaggerator. In contrast, sexual size dimorphism was
167 negatively correlated to formant frequency spacing, with greater male sexual size
168 dimorphism resulting in male sexual calls with lower than expected ΔF . This
169 relationship indicates that sexual selection for increased male body size is likely to be
170 a key force leading to the evolution of anatomical and/or behavioural adaptations that
171 enable male callers to acoustically exaggerate their apparent body size via formant
172 lowering. Although ΔF is known to function as a size exaggerator in some
173 mammalian species²⁵⁻²⁹, the findings of the current study constitute the first
174 demonstration that sexual selection is a key driver of acoustic variability across
175 mammals.

176 Our phylogenetic analysis also revealed that male mammals with relatively
177 large testes produced calls with higher ΔF , suggesting that pre-copulatory sexual
178 selection pressures to acoustically exaggerate body size are relaxed in species where
179 sperm competition predominates. This result confirms the evolutionary trade-off
180 between acoustic size exaggeration and testes size revealed by a recent study of
181 sexual calls in howler monkeys²⁰. The fact that F0 was also lower in species with
182 relatively smaller testes is consistent with previous observations that, while lower F0
183 may not function as a reliable cue to body size within mammal species, it can indicate
184 higher testosterone levels^{42,43}, threat potential⁴⁴ and dominance^{45,46}, and hence, remains
185 an important, sexually-selected component of pre-copulatory signalling in mammals.
186 Indeed, recent findings in anthropoid primates show how sexual dimorphism in F0
187 increases during evolutionary transitions towards polygyny and decreases during
188 transitions towards monogamy³³, further emphasizing that F0 is a sexually selected
189 component of mammal vocalisations.

190 Finally, our comparative investigation provides a useful background for
191 understanding the selection pressures contingent on our own species' vocal
192 communication. Although male humans do not appear to possess an exclusively
193 sexual call, it is now well established that F0 and formants are sexually-selected

194 components of the male voice that play a role in mate choice^{47,48} and intra-sexual
195 competition^{44,46}. However, unlike other primates, adult humans have a descended
196 larynx that results in a disproportionately long pharyngeal cavity¹. Moreover, a
197 secondary descent of the larynx that only affects adult men at puberty, and enables
198 them to produce even lower formant frequencies, has been attributed to sexual
199 selection for size exaggeration⁴⁹. Yet surprisingly, the observed ΔF of male humans is
200 quite far above, rather than below the value predicted from the acoustic allometry (Fig.
201 1B), indicating that the human male vocal tract is in fact shorter than expected for a
202 male terrestrial mammal that weighs around 75 kg (the average weight of a male
203 human⁵⁰). We suggest that selection pressures to decrease facial size may have
204 counter-balanced sexual selection pressures to exaggerate apparent body size, and
205 resulted in the relative overall shortening of the human vocal tract revealed by our
206 comparative investigation. Indeed, selection pressures linked to speech production,
207 thermoregulation or locomotion⁵¹ and facilitated by tool use and meat eating⁵², are
208 generally assumed to have led to the 1:1 ratio of the oral cavity relative to the
209 pharyngeal cavity that is considered to be a crucial prerequisite for the evolution of
210 complex speech articulation^{1,53}. Our phylogenetically-controlled investigation,
211 therefore, not only reveals how sexual selection for acoustic size exaggeration drives
212 the anatomical and acoustical diversification of terrestrial mammal vocal
213 communication systems, but also highlights the importance of the comparative
214 approach for providing the background necessary to fully understand the origins and
215 evolution of our own species' vocal apparatus¹.

216 **Methods**

217 **Data sources**

218 To test our hypotheses we collated acoustic data on mean F0 from 67 male species across 52 genera,
219 and mean formant frequency values from 35 male species across 25 genera from the literature
220 (Supplementary Table 7). We restricted the dataset to adult terrestrial mammals and noted whether
221 vocalisations function as sexual calls (i.e. those that are purported to have functional relevance during
222 intra-sexual or inter-sexual assessment). This allowed us to enter call-type (sexual or nonsexual) as a
223 covariate in the analysis, and control for any differences in the acoustic structure generated by males
224 using different modes of sound production and/or adopting different calling postures exclusively in
225 sexual calls (such as the use of non-laryngeal sources and/or vocal tract elongation by laryngeal

226 retraction or neck stretching). Humans were included on the basis that F0 and formants in the male
227 voice have also been shaped by sexual selection⁴⁶⁻⁴⁸.

228 In cases where mean F0 and formant frequency values were not directly reported in papers,
229 the acoustic values were obtained by contacting the lead authors of the respective studies. For five
230 species, mean F0 was estimated by taking the average of the minimum and maximum reported
231 values^{3,32}. To calculate formant frequency spacing (ΔF) we used the first two to nine formant
232 frequencies (mean = 5) and the regression method of Reby & McComb⁵⁴, in which the formant
233 frequency values are plotted against those that would be expected if the vocal tract was a straight
234 uniform tube closed at one end (the glottis) and open at the other (the mouth). This regression method
235 is an accurate way to estimate ΔF in species with unevenly spaced formants (as is commonly the case
236 in mammals^{17,22,54}).

237 Data on male acoustic features and body mass were obtained from the same published source
238 for 39 out of 67 taxa for the F0 data and 15 out of 35 taxa for the formant data. In addition, because the
239 physical environment also shapes the acoustic features of vocal signals^{32,38,39}, we collected data on the
240 typical habitat for each of the species in our comparative analyses from the Encyclopaedia of Life
241 website (<http://eol.org/>) to control for this factor in the analyses. We also collected data on the mating
242 system of each species in the analysis from the Animal Diversity Website (<http://animaldiversity.org/>).
243 If body weight data was not available from the acoustic studies we referred to the CRC handbook of
244 mammalian body masses⁵⁵ and the PANTHERIA v.1 database⁵⁶. We did not collect acoustic and body
245 weight data for farmed or domestic animals (e.g. cats, dogs, horses, sheep, goats) that are often
246 intensely bred and therefore subject to strong artificial selection.

247 The degree of sexual size dimorphism was used as an indicator of the intensity of sexual
248 selection pressures acting on male body size in a given species^{57,58} with greater values indicative of
249 selection pressures for larger male body size. Sexual size dimorphism was calculated for each species
250 by dividing \log_{10} male body weight by \log_{10} female body weight (to convert a cubic measure to a linear
251 measure of size^{57,58}). Body mass data were taken from several sources (Supplementary Table 7);
252 however, care was always taken to match male and female body mass data from the same population
253 when calculating sexual size dimorphism. Relative testis size was used as an index of post-copulatory
254 sexual selection pressures^{20,59,60}. Post-copulatory sexual selection is prevalent in promiscuous species
255 that live at high population densities and typically manifests itself as sperm competition, which in turn
256 leads to larger male testes relative to overall body size⁶¹. Relative testes size is thus assumed to be a
257 reliable index of the degree of sperm competition experienced by males within a species^{20,59,60}. We
258 calculated relative testes size for each species as the total mass of both testis in grams divided by the
259 overall body mass in grams, rather than generating residuals of male testes mass regressed on body
260 mass across taxa^{59,62}. Male body and testes mass data from the same population were collected for 42
261 species for the F0 analyses and 24 species for the ΔF analyses (Supplementary Table 7). In five cases
262 where data on testes mass were not directly available, the mass in grams was calculated by multiplying
263 the volume in mm^3 by 1.02^{60} .

264 **Statistical analyses**

265 Due to shared phylogenetic history, data from different species cannot be treated as statistically
266 independent^{20,57,58,63}. Accordingly, we conducted phylogenetic generalised least squares (PGLS)
267 regressions using the `gls` function (`nlme` package) in R⁶⁴ to test our hypotheses. To control for the
268 confounding effects of shared phylogenetic ancestry we used untransformed branch lengths and
269 splitting dates from a recent molecular phylogeny of mammals⁶⁵. Additional molecular phylogenies
270 were used to improve resolution within the *Cervinae*⁶⁶, *Alouatta*⁶⁷ and *Cercopithecinae*⁶⁸ species.
271 Figure 4 depicts the phylogenies used to control for shared ancestry among species in each of the
272 separate analyses.

273 In addition, for each formal hypothesis we computed five PGLS regression models that were
274 designed to test a different evolutionary scenario, and chose the most parsimonious model with the
275 lowest Akaike Information Criterion statistic corrected for sample size (AICc)^{37,69}. The different
276 models were an Ornstein-Uhlenbeck (OU) model of evolution, a non-phylogenetic Ordinary Least
277 Squares (OLS) model, a pure Brownian motion (BM) model, and two restricted maximum-likelihood
278 (REML) Brownian motion models that allow parameters to vary with the strength of the phylogenetic
279 signal, a Brownian motion + Pagel's lambda (BM + λ) and a Brownian motion + Grafen's rho (BM +
280 ρ) model. The OLS model assumes phylogenetic independence, the BM model assumes a Brownian
281 motion model of trait evolution (or pure Random Walk), the OU model uses alpha (α) to test the
282 strength of stabilizing selection: $\alpha = 0$ is equivalent to pure Brownian motion and larger values of α
283 indicate stronger stabilizing selection, the BM + λ model allows us to test if the best model falls
284 between pure Brownian motion ($\lambda = 1$) and phylogenetic independence ($\lambda = 0$), and the BM + ρ model
285 tests the rate of evolutionary change, with $\rho < 1$ indicating relatively more gradual recent evolution, $\rho >$
286 1 relatively faster recent evolution, and $\rho = 0$ indicates a star phylogeny, generated by a recent
287 population expansion event from a common ancestor.

288 For each PGLS regression the dependent variable was the acoustic measure ($\log_{10} F0$ or
289 $\log_{10} \Delta F$). \log_{10} transformed male body mass (in grams) was entered as a covariate to control for body
290 size differences across taxa, and the species-typical habitat (arboreal versus terrestrial), the call-type
291 from which the acoustic data was derived (sexual or nonsexual), and the mating system for each
292 species (monogamous, polygynous, polyandrous, promiscuous, or variable) were also entered into a
293 global PGLS model to control for these factors. For each formal hypothesis we then used the “dredge”
294 function in R (`MuMIn` package) to iterate through all variable combinations in the global model to
295 explain variation in $\log_{10} F0$ and $\log_{10} \Delta F$, and chose the model with the lowest AICc value⁶⁹. The
296 acoustic variables $F0$ and ΔF , and relative testes size were \log_{10} transformed to achieve a normal data
297 distribution. All other variables were normally distributed.

298

299 **Data availability**

300 The data that support the findings of this study are available in Supplementary Table 7 and also from
301 the corresponding author upon request.

302

303

304

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460 **Author contributions**

461 BDC and DR conceived and designed the study; BDC and DR wrote the manuscript; BDC collated the
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463

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465

466 **Figure legends:**

467

468 **Fig. 1.** Relationship between \log_{10} male body mass and \log_{10} F0 (A) and \log_{10} Δ F (B).
469 The dotted lines represent the slope and intercept of phylogenetic generalized least-
470 squares regressions of \log_{10} male body mass on \log_{10} F0 (A) and \log_{10} male body mass
471 and habitat on \log_{10} Δ F (B). In both plots, the blue dotted line shows the relationship
472 between male body mass and acoustic features for species without known adaptations
473 to lower frequency components of calls, the red dotted line shows the relationship
474 between male body mass and acoustic features for species that are known to possess
475 adaptations to lower frequency components of calls, and the black dotted line shows
476 the relationship between male body mass and acoustic features for all the species in
477 the dataset (Plot A: PGLS regression, $N = 67$, $P < 0.001$; Plot B: PGLS regression, N
478 $= 35$, $P < 0.001$). In both plots a black square indicates species with no known
479 adaptations to lower frequency components. A red triangle denotes species with
480 additional sound sources and resonators in plots A and B, respectively, a green circle
481 signifies species with an enlarged larynx in plot A and species with a descended
482 larynx in plot B, and a blue circle represents species with thickened vocal folds in plot
483 A and a nasal proboscis in plot B. A key for the icons representing each of the
484 mammal species is also provided in the lower panel (C).

485 **Fig. 2.** Relationship between sexual size dimorphism and residual F0 (A) and Δ F (B).
486 Residual F0 and Δ F refer to the residuals obtained from PGLS regressions of \log_{10}
487 male body mass on \log_{10} F0 and \log_{10} male body mass and habitat on \log_{10} Δ F,
488 respectively. For each plot, the dotted line represents the slope and intercept of the
489 PGLS model regressions (Plot A: $N = 67$, $P = 0.119$; Plot B: $N = 35$, $P = 0.006$). R^2
490 values are given in the bottom right-hand corner.

491

492 **Fig. 3.** Relationship between \log_{10} relative testes size and residual F0 (A) and ΔF (B).
493 Residual F0 and ΔF refer to the residuals obtained from PGLS regressions of \log_{10}
494 body mass on \log_{10} F0 and \log_{10} ΔF , respectively. The dotted line represents the slope
495 and intercept of the PGLS model regressions (Plot A: $N = 42$, $P = 0.017$; Plot B: $N =$
496 24 , $P < 0.001$). R^2 values are given in the bottom right-hand corner.

497

498 **Fig. 4.** The phylogenies used to control for shared ancestry between different species.
499 PGLS regressions testing the effect of size dimorphism and relative testes size on F0
500 used the phylogeny in the top panel (A); and those testing the effect of size
501 dimorphism and relative testes size on ΔF used the phylogeny in the lower panel (B).

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