



universität
wien

DISSERTATION / DOCTORAL THESIS

Titel der Dissertation / Title of the Doctoral Thesis

„Mechanisms and functions of vocal communication in mammals“

verfasst von / submitted by

Maxime Garcia, Licence, Master

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Doctor of Philosophy (PhD)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on the student
record sheet:

A 794 685 437

Dissertationsgebiet lt. Studienblatt /
field of study as it appears on the student record sheet:

Biologie

Betreut von / Supervisor:

Univ.-Prof. W. Tecumseh Fitch, PhD

To my grandmother, who never fully understood why I wanted to work with wild animals but always seemed proud of it.

Acknowledgements

First I want to thank my supervisor, Prof. W. Tecumseh Fitch, for offering me this PhD position in the Department of Cognitive Biology. Thank you for believing in what I could achieve and for giving me the freedom of choosing my research questions and model species. Thank you for the shared insights and advice that helped me make the best of the various projects I conducted. Thank you for your support when it came to travel to conferences where I could disseminate my work and connect with other researchers who share my enthusiasm for animal behavior and bioacoustics. Thank you for encouraging me to challenge myself and to get experience in seeking additional funding sources. Finally, thank you for your patience and tolerance for the countless times I came knocking at your door with multiple questions in mind!

My sincere gratitude goes to Dr. Christian Herbst and Dr. David Reby. I consider both of them my additional, unofficial advisors and I have benefited incredibly from knowing them. To both: thank you for the many discussions that we've had, these were always insightful and helped me mature both as a scientist and a person. To Christian, thanks for teaching me how important it is to sometimes take a step back in order to keep going forward, and thanks for making me discover a new (to me) approach to animal vocal communication! To David, thanks for sharing your experience and philosophy, for involving me in your research and introducing me to the fantastic animal that is red deer. You told me once "let the animals tell you what is interesting" and I've made my best to keep this in mind while working in the field!

Thank you to Dan Bowling, post-doc at the Department of Cognitive Biology, for the stimulating discussions, his guidance, for being available no matter the time and the issue and of course for always having the words to boost me up!

Thanks to my colleagues and collaborators for their invaluable help. None of this work could have been done without you!

Thanks to all my friends and colleagues of the department of Cognitive Biology, those that are still around and those that left since I started this PhD. It is rare I think to find an environment as inspiring and thriving as the Cogbio family. Thanks for sharing a part or all of this 4-year period and for making it truly unforgettable!

To my family and friends in Bretagne, thank you! It is not easy to live away from the people you care about but you have made it possible and it gave me considerable strength all along the road. A special thank you goes to my wonderful parents for always seeing the best in me and for their faith in what I can achieve. Thank you for pushing me towards my dreams!

Finally, I want to thank Céline Jain, without whom none of this could have been possible. From the beginning, thank you for encouraging me to seize the opportunity of getting into this PhD program, for understanding my passion and for helping me achieve my goals. You have been the one facing all of my ups and downs and have provided me with your unconditional support and trust. For always standing by my side, knowing all of what doing a PhD abroad implies, thank you!

***" We are beginning to understand how
the structure of animal signals relates
to the function they serve "***

Peter Marler, 1967

Table of contents

General Introduction	7
References	11
 Chapter 1: <i>Acoustic recordings and vocal repertoires</i>: Providing a rigorous and sensible foundation for analysis of a species communication system	14
Introduction	17
Material and methods	20
Results	30
Discussion	33
References	40
Supplementary material	47
 Chapter 2: <i>Acoustic recordings and anatomical measurements</i>: Identifying the potential information content encoded within acoustic signals	55
Introduction	58
Material and methods	61
Results	69
Discussion	75
References	83
Supplementary material	89
 Chapter 3: <i>Playback experiments and signal re-synthesis</i>: Exploring the perceptual and behavioral role of specific acoustic features in red deer vocal communication	100
Introduction	101
Material and methods	102
Results	103
Discussion	103
References	106
Supplementary material	108

Chapter 4: <i>Playback experiments</i>: Exploring the roles of different call types in red deer vocal communication	109
Introduction	110
Material and methods	111
Results	112
Discussion	112
References	113
Supplementary material	114
 Chapter 5: <i>Excised larynx experiments</i>: Empirically examining the connection between vocal production mechanisms and acoustic structure	122
Introduction	125
Material and methods	127
Results	133
Discussion	135
References	140
 General Discussion	143
References	150
 Publication overview	155
 <i>Curriculum vitae</i>	156
 Zusammenfassung	159
 Abstract	161

General introduction

Background on communication systems and mammal vocal communication

How and why do animals communicate? This simple question has been a central element of the scientific enterprise that intends to understand both our living environment and the way we interact with it. Studying animal communication involves approaches ranging from behavioral observations to *in-vitro* physiology and is interconnected with various fields such as physics or environmental ecology. Irrespective of the modality employed (visual, olfactory, acoustic, tactile or electrical), the general process of communication implies an information exchange between a sender and a receiver: the signal, produced by the sender, carries information through a medium and is perceived by a receiver, which can retrieve (at least partly) the embedded information content of the signal (Bradbury & Vehrencamp 1998).

Acoustic signals can be seen as a mechanical wave that requires a medium (typically air or water) to propagate. The production of a disturbance in the medium leads to alternate events of compression and rarefaction of the molecules (Titze 1994), and it is this disturbance, defined as an acoustic wave, that can be perceived biomechanically and processed by the receiver, provided its sensory machinery is well adapted to perceive the signal (Bradbury & Vehrencamp 1998). Because acoustic signals have multiple advantages – they travel relatively fast, over a wide range of distances, through or around many objects, via different mediums and at any time of the day (Catchpole & Slater 2008) – acoustic communication has become widespread across vertebrates and arthropods (Bradbury & Vehrencamp 1998).

The understanding of mammalian vocal communication in particular has benefited greatly from the contribution of the source-filter theory, originally designed for human speech (Titze 1994; Fant 1960) and successfully applied to

mammals (Fitch & Hauser 1995; Taylor & Reby 2010). This theory states that the production of a voiced signal results from the combination of two processes, that are most often independent from one another. First, the sound wave is generated by the vibrating vocal folds in the larynx, whose rate of vibration defines the *fundamental frequency* (henceforth 'F0') of the signal (quite commonly called 'pitch', although pitch relates to the perception of sound and strictly speaking should only be used when dealing with psychoacoustics). The repetitive collisions of the vocal folds create pressure disturbances, i.e. acoustic energy within a certain frequency range, which will then travel through the air column within the vocal tract. During the second stage of voice production, the length and the shape of the air column within the vocal tract act as a filter on the acoustic frequencies created at the source, selectively attenuating some frequencies, and enhancing others (called resonances of the vocal tract or *formants*). The signal eventually produced and radiated is the resulting combination of the sound source properties overlaid by the filter characteristics of the caller's vocal apparatus.

This theoretical framework has allowed abundant studies which examine in different species how various attributes of the caller could be encoded by source-related features of mammal vocalizations (e.g. age (Erb et al. 2013; Charlton 2015), sex (Titze 1994; Charlton et al. 2009), dominance (Neumann et al. 2010), individual identity (Charrier et al. 2002; Torriani et al. 2006; Schibler & Manser 2007; Mathevon et al. 2010; Digweed et al. 2012), emotional content (Briefer 2012), hormonal level (Evans et al. 2008; Charlton et al. 2012)) as well as filter-related features (e.g. age (Fitch 1997; Briefer et al. 2010) and age-related body size (Ghazanfar et al. 2007), sex (Rendall et al. 2005; Charlton et al. 2009), body size (Fitch 1997; Reby & McComb 2003; Charlton et al. 2011b), individual identity (Vannoni & McElligott 2007; Charlton et al. 2011a; Townsend et al. 2014)). The source-filter theory has therefore provided clear insights into the production mechanisms underlying vocal communication and enabled bioacousticians to deepen our understanding of the origin and function of acoustic signals in mammals (Morton 1977; Fitch & Hauser 2002; Taylor & Reby 2010).

However, in-depth studies of the physical and physiological production mechanisms of mammal vocalizations have been relatively neglected, probably due to methodological limitations. Several studies have started to explore this question by focusing on the physical laws that govern vocal-fold oscillation (either according to the myoelastic-aerodynamic theory, so-called “flow-driven” mode (van den Berg 1958), or via active muscular contraction (Remmers & Gautier 1972)), or on the detailed biomechanics involved in vocal production (either through direct laryngeal observations (Fitch & Reby 2001; Weissengruber et al. 2002; Frey & Gebler 2003); or through modeling techniques (Ishizaka & Flanagan 1972; Titze & Riede 2010)). In this context, excised larynx experiments (ELE henceforth) have only rarely been carried out on nonhuman animals, although they provide an innovative and powerful tool to complement and expand the field of research aforementioned. This approach, which is technically challenging, consists in inducing an excised larynx to phonate while accurately controlling input parameters (such as subglottal pressure and adduction level) and measuring output (such as audio and electroglottographic signals) parameters (see for instance Herbst et al., 2012). Although mostly oriented towards a physical-clinical application and human voice research (Zhang et al. 2006; Alipour & Jaiswal 2008; Alipour & Jaiswal 2009; Döllinger et al. 2011), ELE have recently been conducted with the goal of getting a better understanding of acoustic signals produced *in vivo* (Riede et al. 2000; Brown et al. 2003; Titze et al. 2010; Herbst et al. 2012). It is important to note that the physiological traits and anatomical structures considered in ELE are typically bound to the internal conditions of the caller (for instance, subglottal pressure is influenced by contractions of laryngeal and respiratory muscles, which in turn depend on the emotional state of the caller (Scherer 1986; Briefer 2012)). By removing this uncontrolled element, ELE provide a unique methodology to relate animal physiology and anatomy to sound production mechanisms and acoustic signal structure.

Focus of the thesis and overview of chapters

Ultimately, a broad combination of the various approaches described above (including ELE, apprehension of the physio-anatomical processes involved, empirical investigation of the function of acoustic parameters and signals, together with behavioral observations) is required to allow us to reach a complete understanding of mammal vocal communication systems and the evolutionary pressures acting upon them. The goal of this thesis is to apply such a multi-faceted approach over a wide range of mammalian species, in order to provide a representative outline of the methods and questions involved in bioacoustics research. Accordingly, my work follows three main axes that I will explore throughout the chapters of this dissertation. I illustrate 1) how to quantify and qualify a species' vocal system, 2) how to investigate the information content of acoustic signals (either available within the structure or used functionally), 3) how to examine the ways in which physiology and anatomy can affect sound production and thus determine the structure of acoustic signals.

In the first chapter, we establish the vocal repertoire of wild boars (*Sus scrofa*), a species whose vocal communication has been relatively neglected. Call classification is a prerequisite to further understanding and examination of a species' vocal communication system. This requires a careful methodology, avoiding such pitfalls as anthropomorphism, in order to provide a solid foundation for further study of the functions associated with vocalization.

In the second chapter, we use a closely related model species (the domestic pig, *Sus scrofa domesticus*) to investigate the potential information that can be conveyed by pig grunts, one of the common call types identified in the wild boar repertoire. Using radiographs and acoustic recordings in a longitudinal acoustic allometry approach, we focus on identifying filter-related acoustic cues to body size and individuality.

Chapters 3 and 4 are two studies highlighting how to progressively improve our understanding of a species vocal communication. Using an already well-studied model species (red deer, *Cervus elaphus*) we implement playback

experiments and signal manipulation to investigate the role carried by a particular acoustic parameter (chapter 3) or by different classes of acoustic signals (chapter 4).

Finally, in the last chapter we extend our bioacoustic investigations to the anatomical constraints imposed on production mechanisms in mammal vocal communication. In this comparative study, using CT-scans and excised larynx experiments, we empirically investigate the relationship between vocal anatomy and the structure of acoustic signals in nonhuman primates.

References

- Alipour, F. & Jaiswal, S. 2008: Phonatory characteristics of excised pig, sheep, and cow larynges. *J. Acoust. Soc. Am.* **123**, 4572-4581.
- Alipour, F. & Jaiswal, S. 2009: Glottal airflow resistance in excised pig, sheep, and cow larynges. *J. Voice* **23**, 40-50.
- Bradbury, J. W. & Vehrencamp, S. L. 1998: *Principles of Animal Communication*. Sinauer Associates, Sunderland, MA.
- Briefer, E., Vannoni, E. & McElligott, A. G. 2010: Quality prevails over identity in the sexually selected vocalisations of an ageing mammal. *BMC Biol.* **8**, 1-15.
- Briefer, E. F. 2012: Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1-20.
- Brown, C. H., Alipour, F., Berry, D. A. & Montequin, D. 2003: Laryngeal biomechanics and vocal communication in the squirrel monkey (*Saimiri boliviensis*). *J. Acoust. Soc. Am.* **113**, 2114.
- Catchpole, C. & Slater, P. J. B. 2008: *Bird song : biological themes and variations*. Cambridge University Press, Cambridge [England]; New York.
- Charlton, B. D. 2015: The Acoustic Structure and Information Content of Female Koala Vocal Signals. *PLoS ONE* **10**, e0138670.
- Charlton, B. D., Ellis, W. A., McKinnon, A. J., Brumm, J., Nilsson, K. & Fitch, W. T. 2011a: Perception of male caller identity in Koalas (*Phascolarctos cinereus*): acoustic analysis and playback experiments. *PLoS ONE* **6**, e20329.
- Charlton, B. D., Ellis, W. A., McKinnon, A. J., Cowin, G. J., Brumm, J., Nilsson, K. & Fitch, W. T. 2011b: 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.
- Charlton, B. D., Swaisgood, R. R., Zhihe, Z. & Snyder, R. J. 2012: Giant pandas attend to androgen-related variation in male bleats. *Behav. Ecol. Sociobiol.* **66**, 969-974.

- Charlton, B. D., Zhihe, Z. & Snyder, R. J. 2009: The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim. Behav.* **78**, 893-898.
- Charrier, I., Mathevon, N. & Jouventin, P. 2002: How does a fur seal mother recognize the voice of her pup? An experimental study of *Arctocephalus tropicalis*. *J. Exp. Biol.* **205**, 603-612.
- Digweed, S. M., Rendall, D. & Imbeau, T. 2012: Who's your neighbor? Acoustic cues to individual identity in red squirrel *Tamiasciurus hudsonicus* rattle calls. *Current Zoology* **58**, 758-764.
- Döllinger, M., Kobler, J., Berry, D. A., Mehta, D. D., Luegmair, G. & Bohr, C. 2011: Experiments on Analysing Voice Production: Excised (Human, Animal) and In Vivo (Animal) Approaches. *Current bioinformatics* **6**, 286-304.
- Erb, W. M., Hodges, J. K. & Hammerschmidt, K. 2013: Individual, contextual, and age-related acoustic variation in Simakobu (*Simias concolor*) loud calls. *PLoS ONE* **8**, e83131.
- Evans, S., Neave, N., Wakelin, D. & Hamilton, C. 2008: The relationship between testosterone and vocal frequencies in human males. *Physiol. Behav.* **93**, 783-788.
- Fant, G. 1960: Acoustic theory of speech production. Mouton, The Hague.
- 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.
- Fitch, W. T. & Hauser, M. D. 1995: Vocal production in nonhuman primates: acoustics, physiology, and functional constraints on "honest" advertisement. *Am J Prim* **37**.
- Fitch, W. T. & Hauser, M. D. 2002: Unpacking "Honesty": Vertebrate Vocal Production and the Evolution of Acoustic Signals. In: *Acoustic Communication*. (Ed. Simmons, A. M., Fay, R. R. & Popper, A. N.), ed). Springer, New York. pp. 65-137.
- Fitch, W. T. & Reby, D. 2001: The descended larynx is not uniquely human. *Proc. Biol. Sci.* **268**, 1669-1675.
- Frey, R. & Gebler, A. 2003: The highly specialized vocal tract of the male Mongolian gazelle (*Procapra gutturosa* Pallas, 1777--Mammalia, Bovidae). *J. Anat.* **203**, 451-471.
- Ghazanfar, A. A., Turesson, H. K., Maier, J. X., van Dinther, R., Patterson, R. D. & Logothetis, N. K. 2007: Vocal-tract resonances as indexical cues in rhesus monkeys. *Curr. Biol.* **17**, 425-430.
- Herbst, C. T., Stoeger, A. S., Frey, R., Lohscheller, J., Titze, I. R., Gumpenberger, M. & Fitch, W. T. 2012: How Low Can You Go? Physical Production Mechanism of Elephant Infrasonic Vocalizations. *Science* **337**, 595-599.
- Ishizaka, K. & Flanagan, J. L. 1972: Synthesis of Voiced Sounds From a Two - Mass Model of the Vocal Cords. *Bell system technical journal* **51**, 1233-1268.
- Mathevon, N., Koralek, A., Weldele, M., Glickman, S. E. & Theunissen, F. E. 2010: What the hyena's laugh tells: Sex, age, dominance and individual signature in the giggling call of *Crocuta crocuta*. *BMC Ecol.* **10**, 1-16.
- Morton, E. S. 1977: On the Occurrence and Significance of Motivation-Structural Rules in Some Bird and Mammal Sounds. *Am. Nat.* **111**, 855-869.
- Neumann, C., Assahad, G., Hammerschmidt, K., Perwitasari-Farajallah, D. & Engelhardt, A. 2010: Loud calls in male crested macaques, *Macaca nigra*: a signal of dominance in a tolerant species. *Anim. Behav.* **79**, 187-193.

- Reby, D. & 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.
- Remmers, J. E. & Gautier, H. 1972: Neural and mechanical mechanisms of feline purring. *Respir. Physiol.* **16**, 351-361.
- Rendall, D., Kollias, S., Ney, C. & Lloyd, P. 2005: Pitch (F0) and formant profiles of human vowels and vowel-like baboon grunts: The role of vocalizer body size and voice-acoustic allometry. *J. Acoust. Soc. Am.* **117**, 944.
- Riede, T., Herzel, H., Mehwald, D., Seidner, W., Trumler, E., Böhme, G. & Tembrock, G. 2000: Nonlinear phenomena in the natural howling of a dog-wolf mix. *J. Acoust. Soc. Am.* **108**, 1435-1442.
- Scherer, K. R. 1986: Vocal affect expression: a review and a model for future research. *Psychol. Bull.* **99**, 143-165.
- Schibler, F. & Manser, M. B. 2007: The irrelevance of individual discrimination in meerkat alarm calls. *Anim. Behav.* **74**, 1259-1268.
- Taylor, A. M. & Reby, D. 2010: The contribution of source-filter theory to mammal vocal communication research. *J. Zool.* **280**, 221-236.
- Titze, I. R. 1994: *Principles of Voice Production*. Prentice Hall.
- Titze, I. R., Fitch, W. T., Hunter, E. J., Alipour, F., Montequin, D., Armstrong, D. L., McGee, J. & Walsh, E. J. 2010: Vocal power and pressure-flow relationships in excised tiger larynges. *J. Exp. Biol.* **213**, 3866-3873.
- Titze, I. R. & Riede, T. 2010: A cervid vocal fold model suggests greater glottal efficiency in calling at high frequencies. *PLoS Comput. Biol.* **6**.
- Torriani, M. V., Vannoni, E. & McElligott, A. G. 2006: Mother-young recognition in an ungulate hider species: a unidirectional process. *Am. Nat.* **168**, 412-420.
- Townsend, S. W., Charlton, B. D. & Manser, M. B. 2014: Acoustic cues to identity and predator context in meerkat barks. *Anim. Behav.* **94**, 143-149.
- van den Berg, J. 1958: Myoelastic-Aerodynamic Theory of Voice Production. *J. Speech Lang. Hear. Res.* **1**, 227-244.
- Vannoni, E. & McElligott, A. G. 2007: Individual Acoustic Variation in Fallow Deer (*Dama dama*) Common and Harsh Groans: A Source-Filter Theory Perspective. *Ethology* **113**, 223-234.
- Weissengruber, G., Forstenpointner, G., Peters, G., Kübber - Heiss, A. & Fitch, W. 2002: Hyoid apparatus and pharynx in the lion (*Panthera leo*), jaguar (*Panthera onca*), tiger (*Panthera tigris*), cheetah (*Acinonyx jubatus*) and domestic cat (*Felis silvestris f. catus*). *J. Anat.* **201**, 195-209.
- Zhang, Z., Neubauer, J. & Berry, D. A. 2006: The influence of subglottal acoustics on laboratory models of phonation. *J. Acoust. Soc. Am.* **120**, 1558.

Chapter 1 |

Acoustic recordings and vocal repertoires:

Providing a rigorous and sensible foundation for analysis of a
species communication system

This chapter is an article currently in press in a peer-reviewed journal.

Citation: **Garcia M**, Bowling DL, Gingras B, Herbst CT, Böckle M, Locatelli, Y & Fitch WT. *In Press*. Structural classification of Wild Boar (*Sus scrofa*) vocalizations. *Ethology*.

Title: Structural classification of Wild Boar (*Sus scrofa*) vocalizations

Maxime Garcia§, Bruno Gingras§, Daniel L. Bowling§, Christian T. Herbst#, Markus Boeckle§^a, Yann Locatelli*^b & W. Tecumseh Fitch§

§Department of Cognitive Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.

#Voice Research Lab, Department of Biophysics, Faculty of Science, Palacký University, Olomouc, Czech Republic

^aDepartment of Psychotherapy and Biopsychosocial Health, Danube University Krems, 3500 Krems, Austria

*Réserve de la Haute Touche, Muséum National d'Histoire Naturelle, Obterre, France

^bEquipe Interactions Cellulaires et Fertilité, UMR0085 Physiologie de la Reproduction et des Comportements, Institut National de la Recherche Agronomique, 37380 Nouzilly, France.

Corresponding author:

maxime.garcia@univie.ac.at

+431427776160

Running title: The vocal repertoire of wild boars (*Sus scrofa*)

Abstract

Determining whether a species' vocal communication system is graded or discrete requires definition of its vocal repertoire. In this context, research on domestic pig (*Sus scrofa domesticus*) vocalizations for example has led to significant advances in our understanding of communicative functions. Despite their close relation to domestic pigs, little is known about wild boar (*Sus scrofa*) vocalizations. The few existing studies, conducted in the 1970's, relied on visual inspections of spectrograms to quantify acoustic parameters and lacked statistical analysis. Here, we use objective signal processing techniques and advanced statistical approaches to classify 616 calls recorded from semi-free ranging animals. Based on four spectral and temporal acoustic parameters - quartile Q25, duration, spectral flux and spectral flatness - extracted from a multivariate analysis, we refine and extend the conclusions drawn from previous work and present a statistically validated classification of the wild boar vocal repertoire into four call types: grunts, grunt-squeals, squeals and trumpets. While the majority of calls could be sorted into these categories using objective criteria, we also found evidence supporting a graded interpretation of some wild boar vocalizations as acoustically continuous, with the extremes representing discrete call types. The use of objective criteria based on modern techniques and statistics in respect to acoustic continuity advances our understanding of vocal variation. Integrating our findings with recent studies on domestic pig vocal behavior and emotions, we emphasize the importance of grunt-squeals for acoustic approaches to animal welfare and underline the need of further research investigating the role of domestication on animal vocal communication.

Introduction

A central goal of animal communication research is to understand the function of vocalizations within a species (Bradbury & Vehrencamp 1998) and/or between species (e.g., Zuberbühler 2000). In this context, whether a communication system is better characterized as graded or discrete is a fundamental question (Marler 1975; Morton 1982; Cheney & Seyfarth 1990; Fitch 2010). While graded systems show continuous variation in acoustic structure, lacking strict boundaries between call types, discrete systems show acoustically distinct call types generally lacking structurally intermediate forms (Marler 1975; Marler 1977; Keenan et al. 2013). To investigate this issue, a key step is to define a representative acoustic repertoire of the study species (Hauser 1997). Many studies, especially on primates, have defined vocal repertoires and explored this topic, documenting both graded (Rowell & Hinde 1962; Marler 1976; Fischer & Hammerschmidt 2002) and discrete (Zuberbühler et al. 1997; Arnold & Zuberbühler 2008) repertoires. Some mixed cases have also been reported and showed varying levels of gradation depending on call types and/or sex (Bouchet et al. 2010; Lemasson & Hausberger 2011; Keenan et al. 2013).

Acoustic continuity has also been investigated in non-primate mammalian species vocalizations (Volodina 2000; Boisseau 2005; Stoeger-Horwath et al. 2007; Nair et al. 2009). For example, the first description of the domestic pig (*Sus scrofa domestica*) vocal repertoire by Kiley (1972), identified a degree of acoustic gradation in their definition of 'grunt-squeals': an intermediate vocalization between 'grunts' and 'squeals'. More recent work revisited the pig repertoire (Tallet et al. 2013), similarly concluding that gradation between the acoustic categories is prominent. However, most of the work carried out on domestic pig has focused on a single call type and specific conditions, thus neglecting much of the potential acoustic variability in this species. One reason is that the domestic pig is among the most intensively farmed species on the planet, and studies often focus on specific circumstances (i.e., those particularly relevant to welfare; Whittemore & Kyriazakis

2006). Examples of such focused studies include castration (Puppe et al. 2005), mother-offspring recognition (Illmann et al. 2002; Illmann et al. 2008), nursing (Algers 1993), experimentally-induced stress (Marchant et al. 2001), or discomfort (Hillmann et al. 2004). This approach has been productive in that it has improved our understanding of how vocalizations reflect the physiological and emotional status of pigs (Schrader & Todt 1998; Döpjan et al. 2008) as well as their inter-individual interactions (Kiley 1972; Schön et al. 1999; Melisova et al. 2014). It has also stimulated better assessment practices for housing conditions and overall treatment (Weary et al. 1998; Manteuffel et al. 2004; Puppe et al. 2005; Leidig et al. 2009).

Much less is known about the acoustic signals of the domestic pig's close relative and presumed wild forebear, the wild boar (*Sus scrofa*), from which *domesticus* traces its ancestry (Rothschild et al. 2011). Wild and domestic forms remain closely related: hybridization events occur under natural conditions (Scandura et al. 2011), and a given pair of domestic and wild animals will not necessarily be more genetically divergent than two wild animals drawn from geographically distinct populations (Scandura et al. 2008).

Given this close relationship, the investigation of vocal communication in wild boars is needed to establish a comparative foundation for understanding the evolutionary origins and the potential effect of domestication on domestic pig vocalizations. Nonetheless, the vocal repertoire of wild boars has received limited attention, with the only existing publications dating from the 1970s (Klingholz & Meynhardt 1979; Klingholz et al. 1979). These studies were based on data gathered over several years in the wild by ethologist Heinz Meynhardt and colleagues. They classified boar vocalizations into ten call types split between three main groups: voiced sounds or grunts (including contact), unvoiced sounds or squeals/screams (including fear, complaint, defense, fight, isolation and hunger calls) and intermediate sounds (including alert, alarm and advertisement calls) (Klingholz et al. 1979). Even though this classification implies discrete call types, in a follow-up paper these authors discussed acoustic gradation between call types, concluding that although many aspects of wild boar vocalization are continuous, there are also

acoustic invariants that may identify calls as discrete entities (Klingholz & Meynhardt 1979). Despite the impressive expertise and understanding these authors developed, their conclusions are potentially limited by a number of factors. First, they relied on visual inspection of spectrograms to define acoustic parameters, which may not provide an objective and consistent measurement method. Second, they performed no statistical analyses on their data. And third, because some of these call types were defined by a single utterance and/or were produced by a single individual, generalizations to the population level may be questionable.

The aim of the current study was to revisit the wild boar vocal repertoire based on the work of Klingholz et al. (1979), taking advantage of advances in digital sound recording and analysis, as well as modern statistical methodologies used in classification designs (Boisseau 2005; Bouchet et al. 2010; Gingras & Fitch 2013; Tallet et al. 2013; Baotic et al. 2014). Our goal was to provide an objective description of the wild boar vocal repertoire and call types, with consideration of acoustical intermediates, as suggested by research on domestic pig vocalization (Kiley 1972). We also applied a more cautious approach to the call type evaluation, which avoids assumptions about the potential meaning of calls. Thus, following the lead of some recent work with domestic pigs (Tallet et al. 2013), we did not assign call types based on behavioral contexts. Rather, we took an acoustic/analytical approach, using multinomial logistic regression modeling and hierarchical cluster analysis of objectively defined acoustic parameters to evaluate a perceptual classification based on the one developed by Klingholz et al. (1979). With this work, we thus establish an acoustically based classification of wild boar vocalizations and provide a basis for further research comparing wild boar and pig vocal behavior. We examine our results in the context of acoustic continuity in animal vocalizations and discuss potential contributions of this study to animal welfare and our understanding of the relationship between domestication processes and vocal communication.

Material and methods

Study site and animals

Recordings analyzed in this study were made during spring 2014 (from late February to mid May) on the property of a wild boar breeder located in Urciers (46.54°N, 2.13°E, 280m elevation), France (Société Eric Pradat, hereafter EP), associated with the La Haute-Touche animal reserve (which belongs to the French Museum National d'Histoire Naturelle). EP provided the first author with full access to wild boar keeping facilities. The largest enclosure at EP (hereafter EP1) measures 110,000 m² and is composed of mixed deciduous forest, a plateau of grassland and bush, and an area with a feeder where food was provided *ad libitum*. EP1 animals' only exposure to humans was a single capture/tagging event (at the age of about 3 months). The EP1 animals thus lived in semi-free ranging conditions with minimal human contact. This EP1 wild boar group was composed of 3 old adult males and 6 old adult females more than one and a half year old (all visually estimated to weigh between 80 kg and 150 kg by E. Pradat, based on years of experience capturing and selling wild boars to game parks), approximately 55 other young adults of about one year in age (all estimated to weigh between 45 kg and 65 kg by E. Pradat; gender unspecified) as well as between 30-50 newborn piglets (the number increased during the season). In early May 2014, 19 individuals (9 from EP1 and 10 from another park owned by the same breeder) were captured and placed together in a 30 m² indoor pen for 3 days before being sold to a game park. In both the EP1 and the indoor pen, the population sex ratio was approximately 50% males and 50% females (E. Pradat, personal communication). Finally, 8 one-year-old females were kept in another small enclosure of about 300 m² (hereafter EP2) for 6 days, before being transferred to La Haute-Touche. Recordings were made at all 3 locations (EP1, the indoor pen, and EP2).

Data collection

Recordings were made with a Sennheiser ME-67 directional microphone (frequency response 40-20000 Hz $\pm$ 2.5 dB; Sennheiser Electronic GmbH & Co. KG, Wedemark Germany) powered by a LR6 battery, connected to a Zoom H4N digital recorder (48 kHz sampling frequency and 16-bit quantization; Zoom Corporation, Tokyo, Japan); these recordings were stored as uncompressed WAV files. For shock and wind-noise reduction, the microphone was mounted on a Rycote Modular Windshield WS 7 Kit for Shotgun Microphones, which includes a windshield with suspension system and a synthetic fur windjammer (Rycote Microphone Windshields Ltd, Stroud, UK).

At EP2, recording sessions were made from locations around the edges of the enclosures, mostly at dawn and dusk. At the much larger EP1, recordings were made day and night, whenever individuals showed up to the feeder, mostly from inside the first author's car parked 15-25m away from the feeder, with the microphone protruding from the window and directed towards the group. In these two outdoor locations, the microphone was positioned 5-40 m from the source individual, whenever the weather conditions permitted a high recording quality. In the indoor pen, recordings were made at a distance of 1-5 m, mostly between 22:00 and 6:00 (in darkness), with the microphone protruding into the pen above the enclosure wall.

Both in the indoor pen and at the outdoor locations (EP1 and EP2), the main obstacle to recording the animals arose from the fact that they were most active at night. Accordingly, the majority of recordings were obtained at night, when lighting conditions made it impossible to clearly identify individual animals. Even under these conditions however, it was possible to roughly observe size and thus retrieve some information regarding the approximate age of animals (i.e., one year olds were typically smaller than older adults). While recording during the daytime it was sometimes (in 17% of the cases) possible to determine the sex of the animals

(through visualization of testes and/or penile brush), though the identification of specific individuals remained difficult due to the large group size and the rapid succession of interactions. Accordingly, individual identity of the signalers was not considered in this study.

Whenever possible, the type of interaction and/or context in which a vocalization was produced was noted. Unlike individual identification, interaction type and context were easier to assess, since observations of movement, call loudness and/or the sequence in which calls were emitted did not depend as much on lighting. Using these types of observations and considering the existing literature (McGlone 1985; Meynhardt 1990; Oczak et al. 2013), vocal behavior was sorted into seven classes: (1) alarm; (2) alert/nervous; (3) attacked; (4) chased; (5) contact; (6) scared/threatened; and (7) submissive. When the reaction of the whole group was to flee after a call was produced, this vocalization was contextually labeled as an 'alarm' call. Similarly, a call that caused the group to stop its ongoing activity and freeze for a moment was labeled 'alert/nervous' call; calls produced when individuals were being physically attacked, typically by a larger individual, were labeled 'attacked' calls; vocalizations characterized by a particular call-sequence produced by individuals being chased by another group member were labeled 'chased' calls; calls produced in apparently neutral situations, when animals were foraging at a short distance from each other and/or when they were at rest were labeled 'contact' calls; calls produced by individuals being threatened by larger individuals but which managed to escape physical contact were labeled 'scared/threatened' calls; finally, calls produced by individuals being threatened by larger individuals but which would initiate a snout-to-head contact with the larger individual (around the jaw/cheek area, instead of escape) were labeled 'submissive' calls.

Data analysis

Extraction of acoustic parameters

Sound files were initially inspected for quality and vocalizations produced in the presence of background noise such as wind or anthropogenic activities, other group members running or chewing loudly, other species' sounds (chorusing frogs, singing birds), or excessive echo (in the case of the recordings made in the indoor pen) were not analyzed. Sound files of acceptable recording quality were retained, and annotated with basic call categories (see below and Figure 1), sex/age and behavioral context using the 'Annotate: To TextGrid' function within Praat (Boersma & Weenink 2014). This process led to the selection of 746 high-quality calls suitable for spectral analysis.

Following descriptions made in previous studies (Kiley 1972; Klingholz et al. 1979; Schrader & Todt 1998), an initial rough perceptual classification of these calls was conducted by author MG after visual inspection of the spectrograms while listening to the recordings. This resulted in the initial identification of five acoustically based categories of calls: grunts, squeals, grunt-squeals, barks, and trumpets – see all but barks in Fig 1 (barks were eventually removed from the dataset in this study – see below for further details).

Basic call categories

Klingholz et al. (1979) divided wild boar vocalizations into ten call types distributed over three categories (see Introduction). While relying on these three categories to assess the calls recorded in this study, we deemed their subdivision into ten call types too subjective to be followed consistently. For example, what they defined as 'voiced' and 'unvoiced' sounds (or respectively grunts and squeals/screams) are perceptually very distinct, while the perceptual distinction between adult 'defense', 'fight' and 'hunger' calls (all considered as different subtypes of the 'unvoiced' category) was ambiguous.

Grunts (see Fig 1) are pulsatile, low frequency sounds whose main spectral energy is below 2 kHz. The time delay between pulses generally suggests fundamental frequencies well below 100 Hz, with rare maxima up to 150 Hz. As such, grunts essentially correspond to the 'common grunt' described by Kiley (1972), indicating that 'the fry are well represented and largely spaced' (where 'fry' is used to indicate the individual pulses of the sound).

Squeals (see Fig 1) contain energy in a broader frequency range (contrary to grunts, some energy is clearly visible up to 8 kHz). They typically lack the pulsed structure described above, which makes grunts and squeals perceptually highly distinctive. Most often, squeals sound noisy or harsh, although they can also vary in structure and sometimes contain nearly periodic segments. The broad acoustic definition we use to describe squeals includes multiple contextual call types described by Klingholz et al. (1979; namely 'fear', 'complaint', 'defense', 'fight', 'isolation' and 'hunger' calls); again we avoided any assessment relying mainly on behavioral context at this stage.

Cases where both a grunt and a squeal were observed within a single call led us to define an intermediate call category: grunt-squeals (Kiley, 1972; see Fig 1) are defined here as a mixture between grunts and squeals with both a pulsatile structure and broadband energy, sometimes being a temporal concatenation of a grunt and a squeal with a progressive transition from one to the other, and other times sounding as if both a grunt and a squeal are being produced at the same time.

Barks (Kiley, 1972) were isolated, short, high-intensity vocalizations that are generally non-harmonic and are usually produced with an abrupt onset, making them harder to discriminate from squeals.

Finally, trumpets (see Fig 1) are harmonic calls, with a high fundamental frequency (ranging from 200 to 400kHz and generally lacking energy above 5 kHz) that, contrary to other call types, gave the impression of being produced nasally and with very low intensity (almost impossible to record in EP1 and EP2 since animals were not close enough to the microphone; almost all trumpets analyzed here were recorded in the indoor pen). Of the five call types that were defined in this study, the trumpet was the only type that did not easily fit into the earlier classification based

on the intensity, frequency (Klingholz et al. 1979) and ‘tonality’ (Kiley 1972) information provided in previous research.

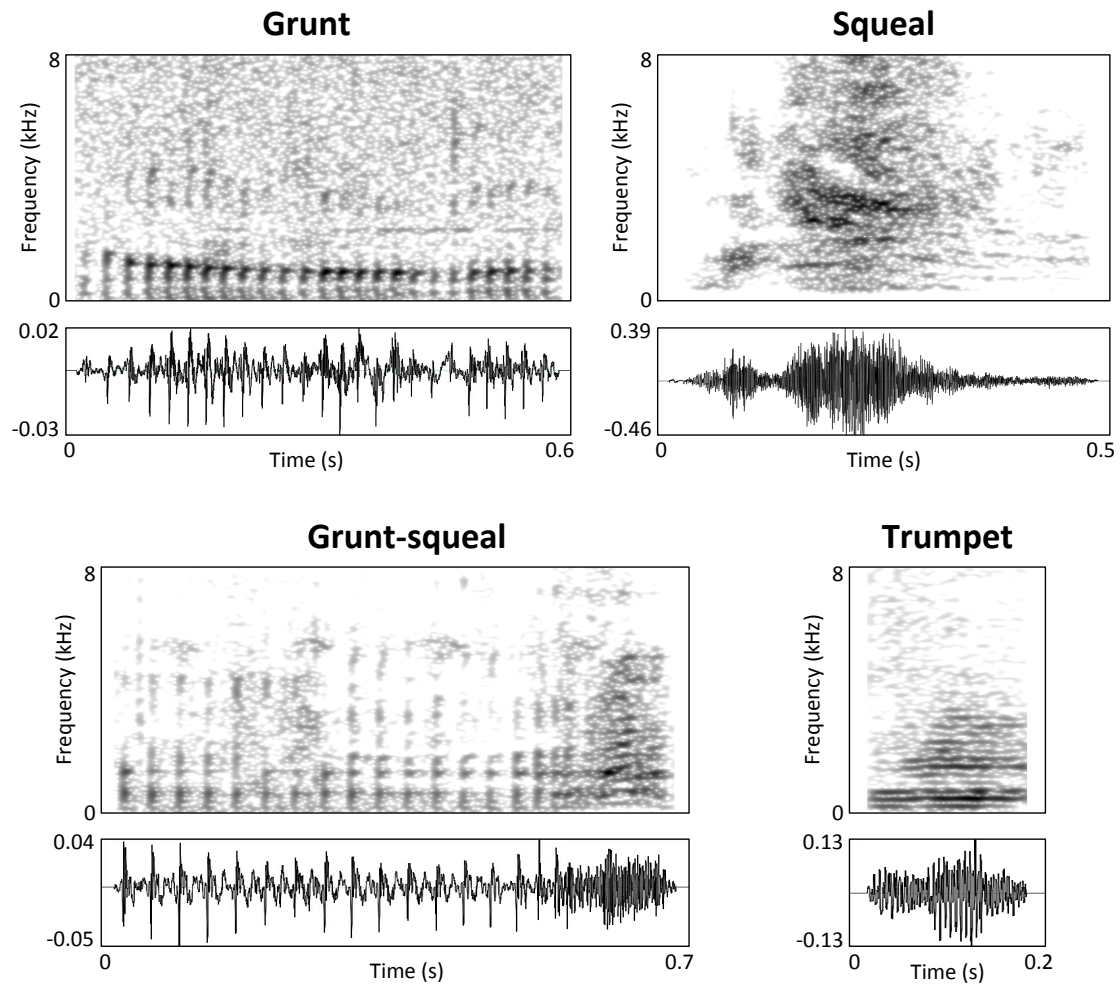


Figure 1. Narrow-band spectrograms and acoustic waveforms of the four call types identified from our recordings: grunt, squeal, grunt-squeal and trumpet. The spectrograms were generated in Praat 5.4.01 using the following settings: Gaussian window shape; time steps: 1000; frequency steps: 250; frequency range: 0–8000 Hz; window length: 0.015 s; dynamic range: 40 dB.

Nineteen acoustic parameters were extracted from each call included in our analysis (see parameter definitions in the Electronic Supplementary Material –

hereafter ESM– Table 1). Call duration (DUR) and spectral energy quartiles (Q25, Q50 and Q75) were extracted using a custom-built Praat script written by David Reby. The remainder of the acoustical analysis was conducted using the MIR Toolbox 1.4.1 (Lartillot et al. 2008) in Matlab (V. 7.11.0.284, R2010b, The MathWorks, Inc., Natick, US), using an analysis window of 1024 samples (~21 ms at a sampling rate of 48000 Hz) with a 50% overlap between adjacent analysis windows. Each consecutive portion of the acoustic signal was multiplied by a Hamming window before calculating a 1024-point discrete Fourier transform. The following parameters were extracted for each analysis window: mean dominant frequency (DF), spectral centroid (SC), spectral entropy (ENT), coefficient of variation of the root-mean-square of the amplitude (CVA), spectral flatness (FLAT), spectral flux (SF) and zero crossing rate (ZC). For several parameters, the following additional features were calculated: means of the differentials between consecutive window frames, which provide information regarding the positive or negative trend of a parameter over the duration of a vocalization; maximum differentials, which provide information regarding the rate of change of a parameter, and standard deviations, which provide information regarding the overall variation within a parameter. This resulted in the extraction of the following parameters: mean differential of the dominant frequency (DDF), maximum differential of the dominant frequency (MDDF), standard deviation of the dominant frequency (STDF), mean differential of the normalized root-mean-square (DRMS), standard deviation of the normalized root-mean-square (STRMS), mean differential of the spectral flux (DSF), maximum differential of the spectral flux (MDSF), standard deviation of the spectral flux (STSF).

Other frequently examined acoustic parameters, such as fundamental frequency or formants (as well as related parameters, such as the harmonics-to-noise ratio [HNR] or Mel-Frequency Cepstral Coefficients [MFCC]) were not included because they could not be accurately measured in all vocalizations.

Selection of the sample

Since the acoustic components of a vocalization depend on the physical characteristics of both the sound source and the vocal tract, it is likely that the parameters extracted here vary as a function of the age (because older adults were larger and thus are expected to have longer vocal tracts and vocal folds). Accordingly, variability introduced by combining calls from older adults with calls from one-year-old adults could potentially prevent the identification of call types on the basis of acoustic parameters. To circumvent this issue and to use comparable cases, vocalizations from older adults were excluded, given that calls produced by one-year-old adults represented the main part of the dataset (642 calls out of 746).

In order to establish a representative sample of the group's vocal repertoire, calls had to be collected from as many individuals as possible. Experience gathered from daily observations indicated that animals came to the feeder location in relatively large groups (often above 15, up to about 70 individuals) and that at these times, most animals would vocalize. Furthermore, although individual identification could not be performed during recordings at night, behavior at the feeder location typically involved high numbers of interactions and calls coming from diverse spatial locations (with respect to the experimenter, MG), indicating the presence of multiple vocalizing individuals. Together, these facts suggest that the recordings analyzed are representative of the target group's vocal repertoire.

A random sample of ten calls from each call type was selected for a blind evaluation (i.e., disregarding any information on the behavioral context in which they were produced) to determine if knowledge of the context influenced call type classification. Following this procedure, it was found that barks could not be reliably classified without knowledge of the behavioral context, and upon re-listening barks were often classified as other call types (grunts, squeals, or grunt-squeals). Consequently, the 26 bark recordings were also removed from the dataset, leaving a total of 616 calls to be used for further analysis. Of these 616 calls, the context could be determined in 96% of the cases. In all cases, calls were from individuals of the

same age class, from which approximately 2% were females, 14% were males and 83% were unspecified gender.

Statistical analysis

In order to assess the validity of the initial perceptual classification, made after listening to the recordings and visually inspecting the sound spectrograms, two series of statistical validations were run. First a multinomial logistic regression was performed to identify the set of parameters that best fit the perceptual classification. Based on the selected parameters, a hierarchical cluster analysis was conducted (see below), the output of which was compared with the perceptual classification. Statistical analyses were conducted in SPSS Statistics (V. 21.0) and in Matlab. Two-tailed p -values are reported and significance levels were set at 0.05.

The statistical distribution for each of the 19 extracted parameters was tested for normality using Shapiro-Wilk tests in conjunction with inspection of Q-Q plots. Significant deviations from normality were observed for all parameters (all p s < 0.05). This prevented use of a discriminant function analysis to test the validity of the perceptual classification, and suggested the use of a Multinomial Logistic Regression (MLR) model instead.

Prior to running the MLR models, all parameters were normalized to z-scores to obtain unit-free parameters, and data were weighted according to frequency to avoid biases that could have been introduced by the fact that each call type class included a different number of cases. Data weighting was done following a uniform prior distribution that stipulated an equal prior probability for all four call types. This prevented the MLR algorithm fitting the data with an equation that mostly depended on the most frequent call types. The multicollinearity of the 19 measured variables was also assessed by computing a Pearson's correlation matrix. For each pair of parameters with a correlation higher than 0.7, one of the two parameters was removed. The choice of the parameter to remove was determined by: (1) whether a parameter was derived from another feature, in which case the derived parameter was removed (e.g. mean spectral flux was selected over standard

deviation of the spectral flux); this step led to the exclusion of STDF, STSF and MDSF; and (2) which parameter had higher correlations with other variables, in which case the parameter with the higher number of correlations was excluded (this step led to the exclusion of Q50, Q75, ZC, SC and ENT). This procedure thus resulted in 11 parameters being retained for the MLR: DUR, Q25, DF, DDF, MDDF, CVA, DRMS, STRMS, FLAT, SF and DSF. In this group, one correlation greater than 0.7 remained between DF and Q25. Since no obvious reason led to the choice of one of these parameters over the other, two different MLR models including 10 parameters were run, one with DF but excluding Q25 (DF regression) and one with Q25 but excluding DF (Q25 regression). The MLR models used forward stepwise entry to test for the contribution of each acoustic parameter to separating perceptually-determined call types (the dependent categorical variable: grunt, squeal, trumpet or grunt-squeal). The Bayesian Information Criterion (BIC) was used to determine the best-fitting model. BIC was preferred over the Akaike Information Criterion since it more severely penalizes an increase in the number of parameters obtained in the final model, and is considered more appropriate for inferential questions like those addressed here.

Following application of the MLR, a hierarchical cluster analysis (HCA) was conducted using the parameters retained in the best MLR model, in order to compare the perceptual classification to a classification produced by a more objective, 'hands-off' approach. The HCA was performed using Ward's method (Ward 1963), with Euclidean distances (Szekely & Rizzo 2005), no normalization (since the data were already z-score transformed) and a clustering range of 2-4 clusters. In order to evaluate how the resulting clusters compared with the perceptual classification, the Hubert-Arabie adjusted Rand Index was calculated following Warrens (2008). This procedure assesses the similarities and differences between two classifications, generating a contingency table from which Cohen's Kappa can be computed.

For cross-validation purposes, the same MLR followed by the HCA procedure was also conducted on a subsample of the dataset, with an equal number of calls in each call type (53, the minimum number of cases in one group; in groups with an

initial sample size of more than 53, cases were randomly selected). The only difference in this cross-validation is that no weighting needed to be applied to the calls in the dataset because the selected groups were equal in size.

Results

Perceptual classification: 616 high-quality calls were used to establish a perceptual classification scheme for the wild boar vocalizations recorded, based on previous research, and leading to the designation of four different classes (see Methods): grunts, squeals, trumpets and grunt-squeals (see Fig 1). Grunts and squeals were the most common call types, while contact and threat situations were the most common contexts in which vocalizations were produced (see Figure 2, ESM: Figure 1,2; Table 2,3).

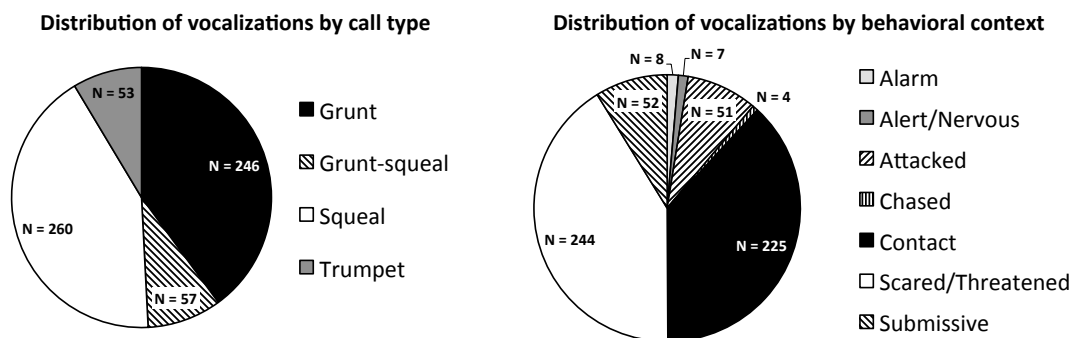


Figure 2. The distribution of recorded and analyzed vocalizations, as a function of call types and of behavioral contexts.

Multinomial Logistic Regressions: In the 'DF regression' model, the best fit was obtained for the final model including the following 6 predictor variables, sorted from most to least significant: DF, DUR, SF, FLAT, MDDF and DSF ($\chi^2(18) = 968.91$, $p < 0.001$). This final model had the lowest BIC and shows an overall classification agreement of 77.6 % with our perceptual classification (meaning that in 77.6% of the cases, the MLR algorithm placed the calls within the same category

as what was predicted by our perceptual classification; see details ESM Table 4). In the 'Q25 regression' model, the best fit was obtained for the final model including the following 4 predictor variables, sorted from most to least significant: Q25, DUR, SF, FLAT ($\chi^2(12) = 1018.58$, $p < 0.001$). This final model had the lowest BIC and shows an overall classification agreement of 80.2 % with our perceptual classification (ESM Table 4). This second model was judged to be superior to the first model because it showed a higher classification accuracy (improving the classification of squeals and trumpets (respectively by 5.8 % and 5.6 %), while losing only 0.8 % of correct classification for grunts; this is likely due to the fact that DF is more susceptible to artifacts than Q25, which accounts for a range of frequencies rather than a single value), while using fewer predictor variables and with a higher χ^2 . Additionally, the agreement percentage between this model and the perceptual classification was significantly above chance (25% since data were weighted; $Z = 31.66$, $p < 0.001$; calculated following (Titus et al. 1984). These four acoustic parameters - Q25, DUR, SF, and FLAT – were thus retained for subsequent cluster analysis (Figure 3).

Cross-validation of this second model based on a randomly selected subsample with equal numbers of calls in each category (53 for each call type, resulting in a total of 212 calls) showed very similar results. Starting with the same set of 10 variables the final model built on this subsample again showed the best fit for Q25, DUR, SF, FLAT ($\chi^2(12) = 353.76$, $p < 0.001$), with an overall classification agreement of 81.6% with our perceptual classification, and calls distributed between categories in similar proportions to the full sample model (see ESM Table 4).

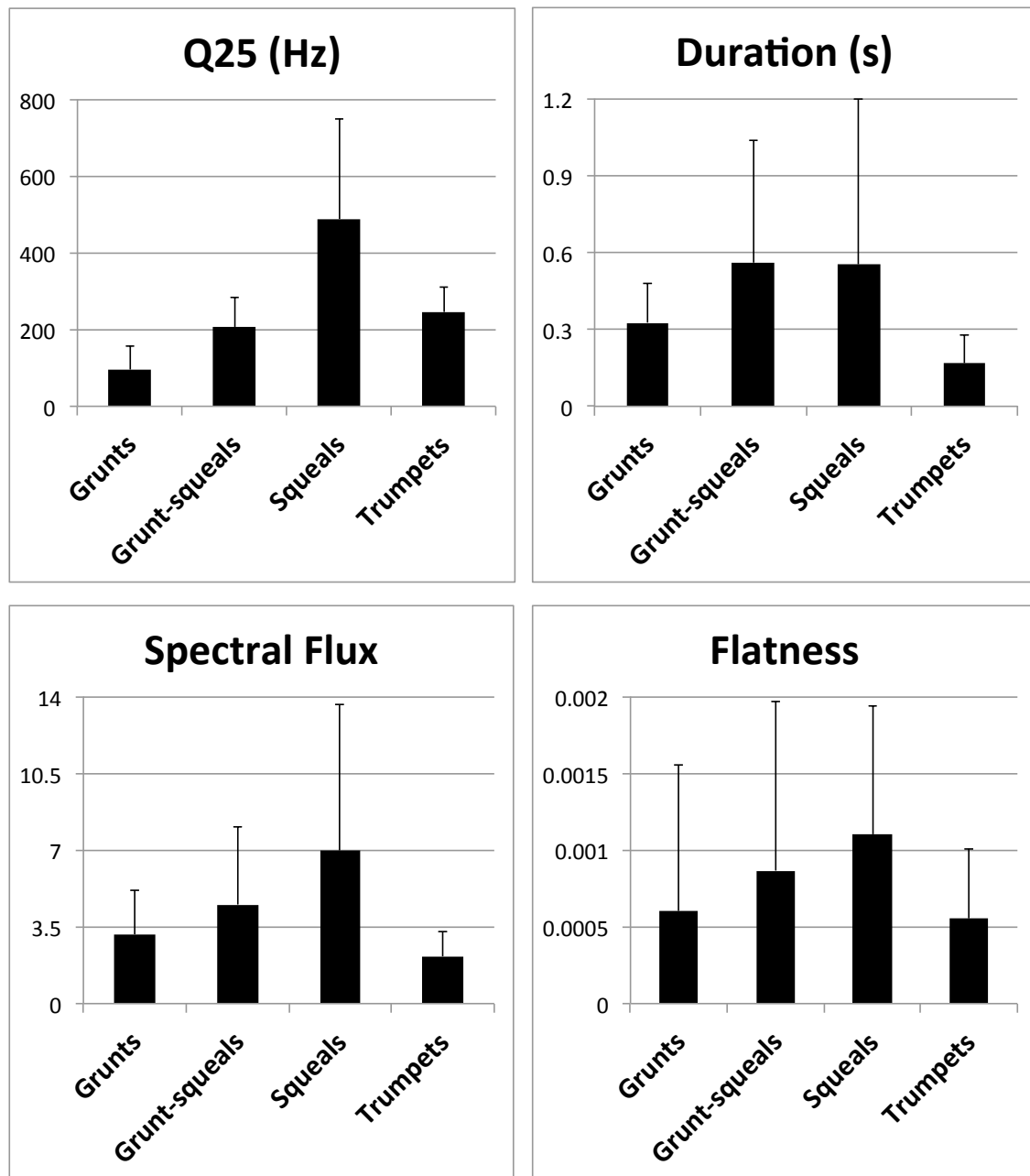


Figure 3. For each acoustical parameter retained from our MLR classification, mean values ($\pm$ SD) are displayed for the four call types identified.

Hierarchical Cluster Analysis: The hierarchical cluster analysis clustered calls into 2, 3 and 4 clusters. The agreement with the perceptual classification is represented by Cohen's κ values, which were 0.33, 0.25 and 0.23 for 2, 3 and 4 clusters respectively. Even though these values indicated a modest amount of

agreement between the initial perceptual classification and the HCA classification, all were highly significant, suggesting that the observed levels of agreement were not accidental (2 clusters: $z (\kappa/\sigma) = 128.74, p < 0.001$; 3 clusters: $z (\kappa/\sigma) = 105.51, p < 0.001$; 4 clusters: $z (\kappa/\sigma) = 100.84, p < 0.001$). The best agreement (suggested by the highest Cohen's κ) between the initial and the HCA classification was obtained for the 2-cluster solution. For this solution, the first cluster included 84.2% of the squeals, 14.6% of the grunts, 13.2% of the trumpets and 57.9% of the grunt-squeals, whereas the second cluster included 15.8% of the squeals, 85.4% of the grunts, 86.8% of the trumpets and 42.1% of the grunt-squeals. Figure 3 and ESM Figure 3 support this cluster analysis, separating in particular grunts and squeals, with grunt-squeals placed as intermediate between those.

Discussion

Proposed classification and graded vs. continuous vocal signaling: In this study we present and statistically assess a four-call-type classification scheme for the wild boar vocal repertoire, based on a homogenous sample of high-quality recordings of vocalizations from a semi-free ranging population. We used information from previous studies examining domestic pig (Kiley 1972) and wild boar (Klingholz et al. 1979) repertoires as a starting point, but avoided biases potentially introduced by human perception (Range & Fischer 2004), i.e. the knowledge of the behavioral context in which vocalizations were produced. Like other recent analyses of domestic pig vocalizations (Tallet et al. 2013), we made an initial perceptual classification based on the existing literature and on the vocalizations alone, and then verified our classification scheme using statistical methods and objective acoustic properties. A comparison of the MLR classification with our perceptual classification performed on the same data set showed 80.2% agreement (ESM Table 4), suggesting that the four perceptually-defined call types (grunts, squeals, grunt-squeals, and trumpets) correspond to objectively measurable differences in wild boar vocalizations. In addition, the MLR classification identified four acoustic

parameters, quartile Q25, duration, spectral flux and spectral flatness (respectively Q25, DUR, SF and FLAT) as the best parameters to discriminate among the four call categories.

However, our results leave space for a certain degree of flexibility in the way we can categorize call types, similarly to previous findings in domestic pigs (Kiley, 1972, Tallet et al., 2013) and suggestions for wild boars (Klingholz et al., 1979). On the one hand, the classification we propose was robust when Q25, DUR, SF and FLAT were used in combination, and the separation between call types appeared distinct for instance when considering the opposition between grunts and squeals (see Fig 3 and ESM Fig 3) or the highly distinguishable trumpet calls (94.3% agreement between the perceptual and the MLR classification, see ESM Table 4). On the other hand, considerable overlap was observed when the average values of Q25, DUR, SF and FLAT were compared across call types (see Fig 3), indicating transitions from one call type to another, i.e. the grunt-squeals representing an acoustic intermediate between grunts and squeals. This interpretation is supported by the fact that grunt-squeals were less well classified in the MLR model (64.9%) and mixed between the two clusters in the HCA (57.9 % of grunt-squeals pertaining to the cluster containing most of the squeals and 42.1% in the cluster containing most of the grunts; see also Fig 3 and ESM Fig 3).

More generally, these results raise the question of acoustic continuity and illustrate the ambiguity that can emerge from interpreting a species' vocal production and/or perception in a categorical manner, a topic which has been widely discussed (e.g., Marler 1975; Marler 1976; Morton 1982; Cheney & Seyfarth 1990; Volodina 2000; Boisseau 2005; Stoeger-Horwath et al. 2007; Nair et al. 2009; Fitch 2010). Marler (1975) proposed that the evolution of graded and discrete signals depends on a species' habitat and social structure. In particular, graded acoustic signals have been suggested to prevail in species living in open habitats with frequent close range interactions occurring between conspecifics (Marler 1976; Keenan et al. 2013; Manser et al. 2014). Accordingly to this hypothesis, the highly social lifestyle of wild boars (Meynhardt 1990) predicts continuous acoustic

variation, while the fact that they often occupy dense, closed habitats (Wilson & Mittermeier 2011) predicts discrete call types. We found evidence supporting both of Marler's predictions in our vocal repertoire analysis, including continuous and discrete components. One important caveat common to all study species, however, is that humans do not necessarily perceive sounds like the animals themselves (Range & Fischer 2004), and while an acoustic continuum is present at the production level, conspecific receivers may perceive discrete calls (Byrne 1982; Slocombe et al. 2009). Here we suggest that avoiding the consideration of contextual framework may enhance objectivity and reduce biases while establishing distinct call categories, but that one should not neglect the potential existence of vocal intermediates, both in terms of the acoustic structure on the production side and of the animal's perception. The only way to thoroughly investigate a species vocal communication at this level is to run playback experiments testing the subtle acoustic variations, in particular if conducted in combination with signal processing and re-synthesis techniques (Reby et al. 2005; Fitch & Fritz 2006; Charlton et al. 2012; Garcia et al. 2013).

Animal welfare and domestication: From a more applied and practical perspective, the fact that grunt-squeals have an intermediate acoustical structure between grunts and squeals may also have implications for the field of animal welfare. Inspection of the relation of grunt-squeals to behavioral context shows that 96.6% of grunt-squeals occurred in situations that were likely experienced by the animals as negatively valenced (being scared/threatened, requiring submission, attacked), although they rarely occurred in situations likely to evoke pain (Tallet et al. 2013), since being attacked only represented 1.8% of these cases (see ESM Fig 2 and Table 3). Instead, they were more likely to be produced at intermediate levels of negativity (scared/threatened: 82.5%; submissive: 12.3%; see ESM Table 3). Thus, as suggested in domestic pigs (Kiley 1972; Schrader & Todt 1998), in addition to representing a transition between grunts and squeals acoustic structures, grunt-squeals may also represent an intermediate expression of negative emotional valence. Since grunt-squeals in this study were classified with 64.9% accuracy by

the MLR model, we provide some support for the possibility of reliable automatic identification and therefore the opportunity to alleviate suffering before animals reach higher/maximal levels of discomfort and/or suffering, such as described in various studies (Weary et al. 1998; Taylor et al. 2001; Marx et al. 2003; Hillmann et al. 2004; Puppe et al. 2005; Döpjan et al. 2008; Leidig et al. 2009). These results advocate the overall importance of acoustic studies in the field of animal welfare, especially those investigating the vocal correlates of emotion (Briefer 2012) for improving the care given to farm animals (see for example von Borell et al. (2009)).

From a more evolutionary point of view, the question of the effect of domestication on vocal production arises from our study in comparison to the domestic pig. The trumpet reported in this study does not seem to exist in the domestic pig repertoire (Tallet et al. 2013). Like most grunts, trumpets were typically used as contact calls. However, trumpets comprise different frequency content than grunts. The use of contact calls with different frequency content in wild boars could function as an adaptation to communicate in diverse acoustic environments (low and high frequencies show different propagation properties in different habitats; (Marten & Marler 1977) and/or may modulate the ability of conspecifics/predators to localize the vocalizer (the directionality of sound varies depending on frequency content; Richards & Wiley 1980). While the data necessary to test these possibilities is not presently available, they might explain why domestic pigs, which are not under the same selective pressures as wild boars, do not seem to produce trumpets.

Potential effects of domestication on vocal behavior are also apparent in other species. Adult dogs, for example, show increased barking compared to adult wolves (Yin S 2002), and adult domestic cats, unlike undomesticated cats, often continue to produce vocalizations typically only made by kittens, such as purring or meowing (Bradshaw & Cameron-Beaumont 2000). Thus, farm animals and pets represent ideal targets for investigating the conservation of emotional vocal indicators throughout evolution or the changes in repertoire use that a species could undergo, because of the modification of different factors such as basic needs, social interactions or environment quality. Further research is greatly needed to

obtain a clear understanding of the selective forces affecting vocal communication that are generated by the domestication process.

Limits and prospects: Our conclusions are subject to some limitations. First, we do not claim to have exhaustively assessed the complete wild boar repertoire. Some specific call types may have been missed, e.g., no male advertisement calls (Klingholz et al. 1979) were recorded despite having been observed on previous occasions at our field site (MG, personal observation). Second, we may have overestimated the apparent continuity of the acoustic signals in our analysis due to a methodological issue generally found in current acoustic research: the fact that each recording necessarily contains some residual background noise may mask some information and might increase the apparent similarity between sounds that would appear more different under perfect conditions. Apparent gradation could also be caused by the different frequency response of the microphone depending on the recording angle, even though we believe this effect is negligible due to the high variability of the calls.

Finally, our study species may employ acoustic parameters that are not easily perceived by humans (Range & Fischer 2004) or that we did not measure. In particular, since periodicity and fundamental frequency could not be consistently measured for all calls, they were not examined here, despite being used to differentiate call-types in other species (Volodina 2000; Leong et al. 2003; Stoeger-Horwath et al. 2007; Baotic et al. 2014). Another potentially important parameter, absolute sound intensity, was not included here because it would require an amplitude calibration based on measured emitter-microphone distances, which are difficult to measure accurately or consistently in the field (especially at night). However, perceptually, MG noted that a very low intensity level was typical of trumpet calls and those grunts produced as contact calls. It is not unlikely that amplitude, if measured correctly, could aid in call type discrimination.

One last acoustic parameter neglected here is formant frequencies, which could not be consistently extracted from the recorded vocalizations but is potentially important. Formant spacing and formant frequencies have indeed been

shown to carry information regarding individuality (Reby et al. 2006; Vannoni & McElligott 2007) as well as size-related attributes of the emitter in a variety of mammalian species (Fitch 1997; Reby & McComb 2003; Sanvito et al. 2007; Vannoni & McElligott 2008; Charlton et al. 2009; Charlton et al. 2011).

Conclusions: The repertoire presented here is the most objective comprehensive analysis to date of the common wild boar vocalizations, and should thus serve as a solid foundation for subsequent analyses and extensions. The issues we raised concerning the search for objectivity and acoustic continuity may benefit other studies investigating animal vocal repertoires. Additionally, the relationships we have emphasized to animal welfare and the effect of domestication should be taken into consideration to introduce new approaches, for both applied and evolution-oriented acoustic research.

Acknowledgements

We confirm that none of the authors has a conflict of interest to declare. Animal capture and housing at Société Eric Pradat (Ets. Pradat) were in accordance with the legislative requirements of the French prefecture de l'Indre. As the present study consisted solely of behavioral observations and non-invasive acoustic recordings there were no negative animal welfare implications. During the study, we adhered to the 'Guidelines for the treatment of animals in behavioural research and teaching' (Anim. Behav., 2006, 71, 245–253). The described protocol was reviewed and approved by the Ethical Review Committee of the University of Vienna (Ethics approval number 2015-004). We are grateful to the Ets. Pradat for providing full access to their facilities. We thank the European Science Foundation for funding MG throughout the field season ('Advances in Farm Animal Genomic Resources' Research Networking Programme, Exchange Grant 4547). C.T.H. was supported by the institutional fund of Palacký University Olomouc, Czech Republic. We would also like to thank Jinook Oh and David Reby for helping with programming. Finally we

thank Marta Manser and two anonymous reviewers for their insightful comments and criticisms on the manuscript.

References

- Algers, B. 1993: Nursing in pigs: communicating needs and distributing resources. *J. Anim. Sci.* **71**, 2826-2831.
- Arnold, K. & Zuberbühler, K. 2008: Meaningful call combinations in a non-human primate. *Curr. Biol.* **18**, R202-R203.
- Baotic, A., Stoeger, A. S., Li, D., Tang, C. & Charlton, B. D. 2014: The vocal repertoire of infant giant pandas (*Ailuropoda melanoleuca*). *Bioacoustics* **23**, 15-28.
- Boersma, P. & Weenink, D. 2014: Praat: doing phonetics by computer [Computer program]. Version 5.3.12 edn, University of Amsterdam, The Netherlands, Retrieved from <http://www.praat.org/>.
- Boisseau, O. 2005: Quantifying the acoustic repertoire of a population: The vocalizations of free-ranging bottlenose dolphins in Fiordland, New Zealand. *The Journal of the Acoustical Society of America* **117**, 2318.
- Bouchet, H., Pellier, A. S., Blois-Heulin, C. & Lemasson, A. 2010: Sex differences in the vocal repertoire of adult red-capped mangabeys (*Cercocebus torquatus*): A multi-level acoustic analysis. *Am. J. Primatol.* **72**, 360-375.
- Bradbury, J. W. & Vehrencamp, S. L. 1998: Principles of Animal Communication. Sinauer Associates, Sunderland, MA.
- Bradshaw, J. W. S. & Cameron-Beaumont, C. L. 2000: The signalling repertoire of the domestic cat and its undomesticated relatives. In: *The Domestic Cat: The Biology of Its Behaviour*. (Turner, D. C. & Bateson, P. P. G., eds). Cambridge University Press, New York. pp. 68-93.
- Briefer, E. F. 2012: Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1-20.
- Byrne, R. W. 1982: Primate Vocalisations: Structural and Functional Approaches to Understanding. *Behaviour* **80**, 241-258.
- Charlton, B. D., Ellis, W. A., Larkin, R. & Fitch, W. T. 2012: Perception of size-related formant information in male koalas (*Phascolarctos cinereus*). *Anim. Cogn.* **15**, 999-1006.

- Charlton, B. D., Ellis, W. A., McKinnon, A. J., Cowin, G. J., Brumm, J., Nilsson, K. & 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.
- Charlton, B. D., Zhihe, Z. & Snyder, R. J. 2009: The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim. Behav.* **78**, 893-898.
- Cheney, D. L. & Seyfarth, R. M. 1990: *How Monkeys See the World: Inside the Mind of Another Species*. University of Chicago Press, Chicago.
- Düpjan, S., Schön, P.-C., Puppe, B., Tuchscherer, A. & Manteuffel, G. 2008: Differential vocal responses to physical and mental stressors in domestic pigs (*Sus scrofa*). *Appl. Anim. Behav. Sci.* **114**, 105-115.
- Fischer, J. & Hammerschmidt, K. 2002: An overview of the barbary macaque, *Macaca sylvanus*, vocal repertoire. *Folia Primatol. (Basel)* **73**, 32-45.
- 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.
- Fitch, W. T. 2010: *The Evolution of Language*. Cambridge University Press, Cambridge.
- Fitch, W. T. & Fritz, J. B. 2006: Rhesus macaques spontaneously perceive formants in conspecific vocalizations. *The Journal of the Acoustical Society of America* **120**, 2132.
- Garcia, M., Charlton, B. D., Wyman, M. T., Fitch, W. T. & Reby, D. 2013: Do Red Deer Stags (*Cervus elaphus*) Use Roar Fundamental Frequency (F0) to Assess Rivals? *PLoS ONE* **8**, e83946.
- Gingras, B. & Fitch, W. T. 2013: A three-parameter model for classifying anurans into four genera based on advertisement calls. *J. Acoust. Soc. Am.* **133**, 547-559.
- Hauser, M. D. 1997: *The evolution of communication*. MIT Press, Cambridge.
- Hillmann, E., Mayer, C., Schön, P.-C., Puppe, B. & Schrader, L. 2004: Vocalisation of domestic pigs (*Sus scrofa domestica*) as an indicator for their adaptation towards ambient temperatures. *Appl. Anim. Behav. Sci.* **89**, 195-206.

- Illmann, G., Neuhauserová, K., Pokorná, Z., Chaloupková, H. & Šimečková, M. 2008: Maternal responsiveness of sows towards piglet's screams during the first 24h postpartum. *Appl. Anim. Behav. Sci.* **112**, 248-259.
- Illmann, G., Schrader, L., Špinka, M. & Šustr, P. 2002: Acoustical Mother-Offspring Recognition in Pigs (*Sus Scrofa domestica*). *Behaviour* **139**, 487-505.
- Keenan, S., Lemasson, A. & Zuberbühler, K. 2013: Graded or discrete? A quantitative analysis of Campbell's monkey alarm calls. *Anim. Behav.* **85**, 109-118.
- Kiley, M. 1972: The Vocalizations of Ungulates, their Causation and Function. *Z. Tierpsychol.* **31**, 171-222.
- Klingholz, F. & Meynhardt, H. 1979: Lautinventare der Säugetiere — diskret oder kontinuierlich? *Z. Tierpsychol.* **50**, 250-264.
- Klingholz, F., Siegert, C. & Meynhardt, H. 1979: Die akustische Kommunikation des Europäischen Wildschweines (*Sus scrofa* L.). *Der Zoologische Garten N. F.* **49**, 277-303.
- Lartillot, O., Toivainen, P. & Eerola, T. 2008: A Matlab Toolbox for Music Information Retrieval. In: *Data Analysis, Machine Learning and Applications*. (Preisach, C., Burkhardt, H., Schmidt-Thieme, L. & Decker, R., eds). Springer Berlin Heidelberg. pp. 261-268.
- Leidig, M. S., Hertrampf, B., Failing, K., Schumann, A. & Reiner, G. 2009: Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour. *Appl. Anim. Behav. Sci.* **116**, 174-178.
- Lemasson, A. & Hausberger, M. 2011: Acoustic variability and social significance of calls in female Campbell's monkeys (*Cercopithecus campbelli campbelli*). *J. Acoust. Soc. Am.* **129**, 3341-3352.
- Leong, K. M., Ortolani, A., Burks, K. D., Mellen, J. D. & Savage, A. 2003: Quantifying Acoustic and Temporal Characteristics of Vocalizations for a Group of Captive African Elephants *Loxodonta Africana*. *Bioacoustics* **13**, 213-231.
- Manser, M. B., Jansen, D. A. W. A. M., Graw, B., Hollén, L. I., Bousquet, C. A. H., Furrer, R. D. & le Roux, A. 2014: Chapter Six - Vocal Complexity in Meerkats and Other Mongoose Species. In: *Adv. Study Behav.* (Naguib, M., Barrett H., L.,

- Brockmann, J., Healy, S., Mitani, J. C., Roper, T. J. & Leigh, W. S., eds). Academic Press, Oxford. pp. 281-310.
- Manteuffel, G., Puppe, B. & Schön, P. C. 2004: Vocalization of farm animals as a measure of welfare. *Appl. Anim. Behav. Sci.* **88**, 163-182.
- Marchant, J. N., Whittaker, X. & Broom, D. M. 2001: Vocalisations of the adult female domestic pig during a standard human approach test and their relationships with behavioural and heart rate measures. *Appl. Anim. Behav. Sci.* **72**, 23-39.
- Marler, P. 1975: On the origin of speech from animal sounds. In: *The Role of Speech in Language* Ed. by J. F. Kavanagh & J. Cutting, Cambridge, Massachusetts: MIT Press. pp. 11: 37.
- Marler, P. 1976: Social organization, communication and graded signals: The chimpanzee and the gorilla. In: *Growing points in ethology*. (Bateson, P. P. G. & Hinde, R. A., eds). Cambridge U Press, Oxford, England.
- Marler, P. 1977: The evolution of communication. In: *How Animals Communicate*. (Sebeok, T. A., ed). Indiana Univ. Press, Bloomington, IN. pp. 45-70.
- Marten, K. & Marler, P. 1977: Sound transmission and its significance for animal vocalization. *Behav. Ecol. Sociobiol.* **2**, 271-290.
- Marx, G., Horn, T., Thielebein, J., Knubel, B. & von Borell, E. 2003: Analysis of pain-related vocalization in young pigs. *Journal of Sound and Vibration* **266**, 687-698.
- McGlone, J. J. 1985: A quantitative ethogram of aggressive and submissive behaviors in recently regrouped pigs. *J. Anim. Sci.* **61**, 559-565.
- Melisova, M., Illmann, G., Chaloupkova, H. & Bozdechova, B. 2014: Sow postural changes, responsiveness to piglet screams, and their impact on piglet mortality in pens and crates. *J. Anim. Sci.* **92**, 3064-3072.
- Meynhardt, H. 1990: *Schwarzwild-Report: mein Leben unter Wildschweinen*. Neumann, Melsungen.
- Morton, E. S. 1982: Grading, discreteness, redundancy, and motivation-structural rules. In: *Acoustic communication in birds*. (Kroodsma, D. E., Miller E.H. & Ouellet, H. (Eds.)). New York: Academic Press. pp. 182-212

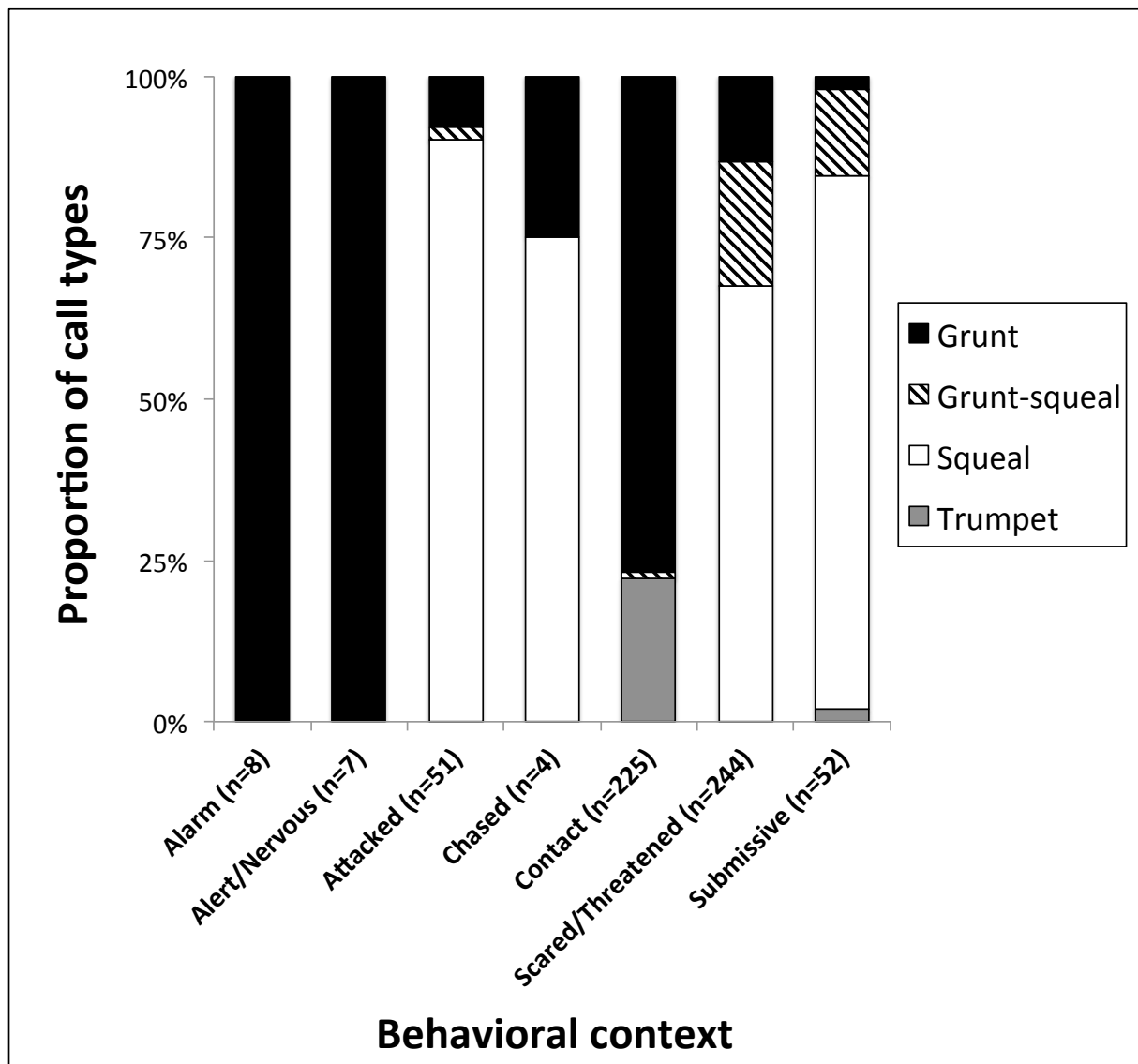
- Nair, S., Balakrishnan, R., Seelamantula, C. S. & Sukumar, R. 2009: Vocalizations of wild Asian elephants (*Elephas maximus*): structural classification and social context. *J. Acoust. Soc. Am.* **126**, 2768-2778.
- Oczak, M., Ismayilova, G., Costa, A., Viazzi, S., Sonoda, L. T., Fels, M., Bahr, C., Hartung, J., Guarino, M., Berckmans, D. & Vranken, E. 2013: Analysis of aggressive behaviours of pigs by automatic video recordings. *Comput. Electron. Agric.* **99**, 209-217.
- Puppe, B., Schön, P. C., Tuchscherer, A. & Manteuffel, G. 2005: Castration-induced vocalisation in domestic piglets, *Sus scrofa*: Complex and specific alterations of the vocal quality. *Appl. Anim. Behav. Sci.* **95**, 67-78.
- Range, F. & Fischer, J. 2004: Vocal Repertoire of Sooty Mangabeys (*Cercocebus torquatus atys*) in the Taï National Park. *Ethology* **110**, 301-321.
- Reby, D., André-Obrecht, R. g., Galinier, A., Farinas, J. & Cargnelutti, B. 2006: Cepstral coefficients and hidden Markov models reveal idiosyncratic voice characteristics in red deer (*Cervus elaphus*) stags. *J. Acoust. Soc. Am.* **120**, 4080.
- Reby, D. & 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.
- Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T. & Clutton-Brock, T. 2005: Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proc. R. Soc. Lond. B* **272**, 941-947.
- Richards, D. G. & Wiley, R. H. 1980: Reverberations and Amplitude Fluctuations in the Propagation of Sound in a Forest: Implications for Animal Communication. *The American Naturalist* **115**, 381-399.
- Rothschild, M. F., Ruvinsky, A. & International, C. A. B. 2011: The Genetics of the Pig. CABI, Wallingford.
- Rowell, T. E. & Hinde, R. A. 1962: Vocal communication by the rhesus monkey (*Macaca mulatta*). *Proc. Zool. Soc. Lond.* **138**, 279-294.
- Sanvito, S., Galimberti, F. & Miller, E. H. 2007: Vocal signalling of male southern elephant seals is honest but imprecise. *Anim. Behav.* **73**, 287-299.

- Scandura, M., Iacolina, L. & Apollonio, M. 2011: Genetic diversity in the European wild boar *Sus scrofa*: phylogeography, population structure and wild x domestic hybridization. *Mammal. Rev.* **41**, 125-137.
- Scandura, M., Iacolina, L., Crestanello, B., Pecchioli, E., Di Benedetto, M. F., Russo, V., Davoli, R., Apollonio, M. & Bertorelle, G. 2008: Ancient vs. recent processes as factors shaping the genetic variation of the European wild boar: are the effects of the last glaciation still detectable? *Mol. Ecol.* **17**, 1745-1762.
- Schön, P.-C., Puppe, B., Gromyko, T. & Manteuffel, G. 1999: Common Features and Individual Differences in Nurse Grunting of Domestic Pigs (*Sus scrofa*): A Multi-Parametric Analysis. *Behaviour* **136**, 49-66.
- Schrader, L. & Todt, D. 1998: Vocal Quality is Correlated with Levels of Stress Hormones in Domestic Pigs. *Ethology* **104**, 859-876.
- Slocombe, K. E., Townsend, S. W. & Zuberbuhler, K. 2009: Wild chimpanzees (*Pan troglodytes schweinfurthii*) distinguish between different scream types: evidence from a playback study. *Anim. Cogn.* **12**, 441-449.
- Stoeger-Horwath, A. S., Stoeger, S., Schwammer, H. M. & Kratochvil, H. 2007: Call repertoire of infant African elephants: first insights into the early vocal ontogeny. *J. Acoust. Soc. Am.* **121**, 3922-3931.
- Szekely, G. J. & Rizzo, M. L. 2005: Hierarchical Clustering via Joint Between-Within Distances: Extending Ward's Minimum Variance Method. *J. Classif.* **22**, 151-183.
- Tallet, C., Linhart, P., Policht, R., Hammerschmidt, K., Simecek, P., Kratinova, P. & Spinka, M. 2013: Encoding of situations in the vocal repertoire of piglets (*Sus scrofa*): a comparison of discrete and graded classifications. *PLoS ONE* **8**, e71841.
- Taylor, A. A., Weary, D. M., Lessard, M. & Braithwaite, L. 2001: Behavioural responses of piglets to castration: the effect of piglet age. *Appl. Anim. Behav. Sci.* **73**, 35-43.
- Titus, K., Mosher, J. A. & Byron, K. W. 1984: Chance-corrected Classification for Use in Discriminant Analysis: Ecological Applications. *Am. Midl. Nat.* **111**, 1-7.

- Vannoni, E. & McElligott, A. G. 2007: Individual Acoustic Variation in Fallow Deer (*Dama dama*) Common and Harsh Groans: A Source-Filter Theory Perspective. *Ethology* **113**, 223-234.
- Vannoni, E. & McElligott, A. G. 2008: Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS ONE* **3**, e3113.
- Volodina, E. V. 2000: Vocal repertoire of the cheetah *Acinonyx jubatus* (Carnivora, Felidae) in captivity: sound structures and their potential for estimating the state of adult animals. *Zoologicheskii-Zhurnal* **79**, 833-843.
- von Borell, E., Bunger, B., Schmidt, T. & Horn, T. 2009: Vocal-type classification as a tool to identify stress in piglets under on-farm conditions. *Anim. Welf.* **18**, 407-416.
- Ward, J. H., Jr. 1963: Hierarchical Grouping to Optimize an Objective Function. *Journal of the American Statistical Association* **58**, 236-244.
- Warrens, M. J. 2008: On the Equivalence of Cohen's Kappa and the Hubert-Arabie Adjusted Rand Index. *J. Classif.* **25**, 177-183.
- Weary, D. M., Braithwaite, L. A. & Fraser, D. 1998: Vocal response to pain in piglets. *Appl. Anim. Behav. Sci.* **56**, 161-172.
- Whittemore, C. T. & Kyriazakis, I. 2006: Whittemore's Science and Practice of Pig Production. Wiley-Blackwell Eds, Oxford.
- Wilson, D. E. & Mittermeier, R. A. 2011: Handbook of the Mammals of the World: Hoofed mammals. Lynx Edicions, Barcelona.
- Yin S. 2002: A New Perspective on Barking in Dogs (*Canis familiaris*). *J. Comp. Psychol.* **116**, 189-193.
- Zuberbühler, K. 2000: Interspecies semantic communication in two forest primates. *Proc. R. Soc. Lond. B* **267**, 713-718.
- Zuberbühler, K., Noë, R. & Seyfarth, R. M. 1997: Diana monkey long-distance calls: messages for conspecifics and predators. *Anim. Behav.* **53**, 589-604.

Parameter extracted	Definition of the parameter extracted
1. Duration (DUR)	Duration of the vocalization, in seconds
2. Quartile Q25 (Q25)	Frequency in the spectrum below which 25% of the energy in the signal is comprised, in hertz
3. Quartile Q50 (Q50)	Frequency in the spectrum below which 50% of the energy in the signal is comprised, in hertz
4. Quartile Q75 (Q75)	Frequency in the spectrum below which 75% of the energy in the signal is comprised, in hertz
5. Mean dominant frequency (DF)	Point of maximal amplitude along the frequency spectrum (here mean is calculated from DF obtained in each analysis window), in hertz
6. Mean differential of the dominant frequency (DDF)	Mean difference between points of maximal amplitude along the frequency spectrum from 2 consecutive analysis windows, in hertz
7. Maximum differential of the dominant frequency (MDDF)	Maximum difference between points of maximal amplitude along the frequency spectrum from 2 consecutive analysis windows, in hertz
8. Standard deviation of the dominant frequency (STDF)	Standard deviation of the points of maximal amplitude along the frequency spectrum (here obtained from all analysis windows within a sound file), in hertz
9. Spectral centroid (SC)	Weighted mean by amplitude of the frequencies present in the audio signal (here mean is calculated from SC obtained in each analysis window), in hertz
10. Spectral entropy (SE)	Measurement assessing the regularity of the spectrum by returning the relative Shannon entropy of the input spectrum, no unit
11. Coefficient of variation of the root-mean-square of the amplitude (CVA)	Standard deviation of the root-mean-square of the amplitude divided by the true mean, no unit
12. Mean differential of the normalized root-mean-square (DRMS)	Mean difference between the normalized root-mean-square from 2 consecutive analysis windows, no unit
13. Standard deviation of the normalized root-mean-square (STRMS)	Standard deviation of the normalized root-mean-square (here obtained from all analysis windows within a sound file), no unit
14. Spectral flatness (FLAT)	Ratio between the geometric mean and the arithmetic mean (here mean is calculated from FLAT obtained in each analysis window); indicates whether a frequency spectrum is smooth ("flat") or spiky (contains definite peaks), no unit
15. Spectralflux (SF)	Distance between the spectra of successive frames (here Euclidean distances were used and mean is calculated from SF obtained in each analysis window), no unit
16. Mean differential of the spectral flux (DSF)	Mean difference of the distances between the spectra of successive frames, no unit
17. Maximum differential of the spectral flux (MDSF)	Maximum difference of the distance between the spectra of successive frames, no unit
18. Standard deviation of the spectral flux (STSF)	Standard deviation of the distance between the spectra of successive frames (here obtained from all analysis windows within a sound file), no unit
19. Zero-crossing (ZC)	Rate at which the amplitude of the audio signal changes from positive to negative and vice-versa (here mean is calculated from ZC obtained in each analysis window), no unit

ESM Table 1. Parameters extracted from audio files and used in the statistical analysis, together with their definitions (see also Gingras and Fitch, 2013).



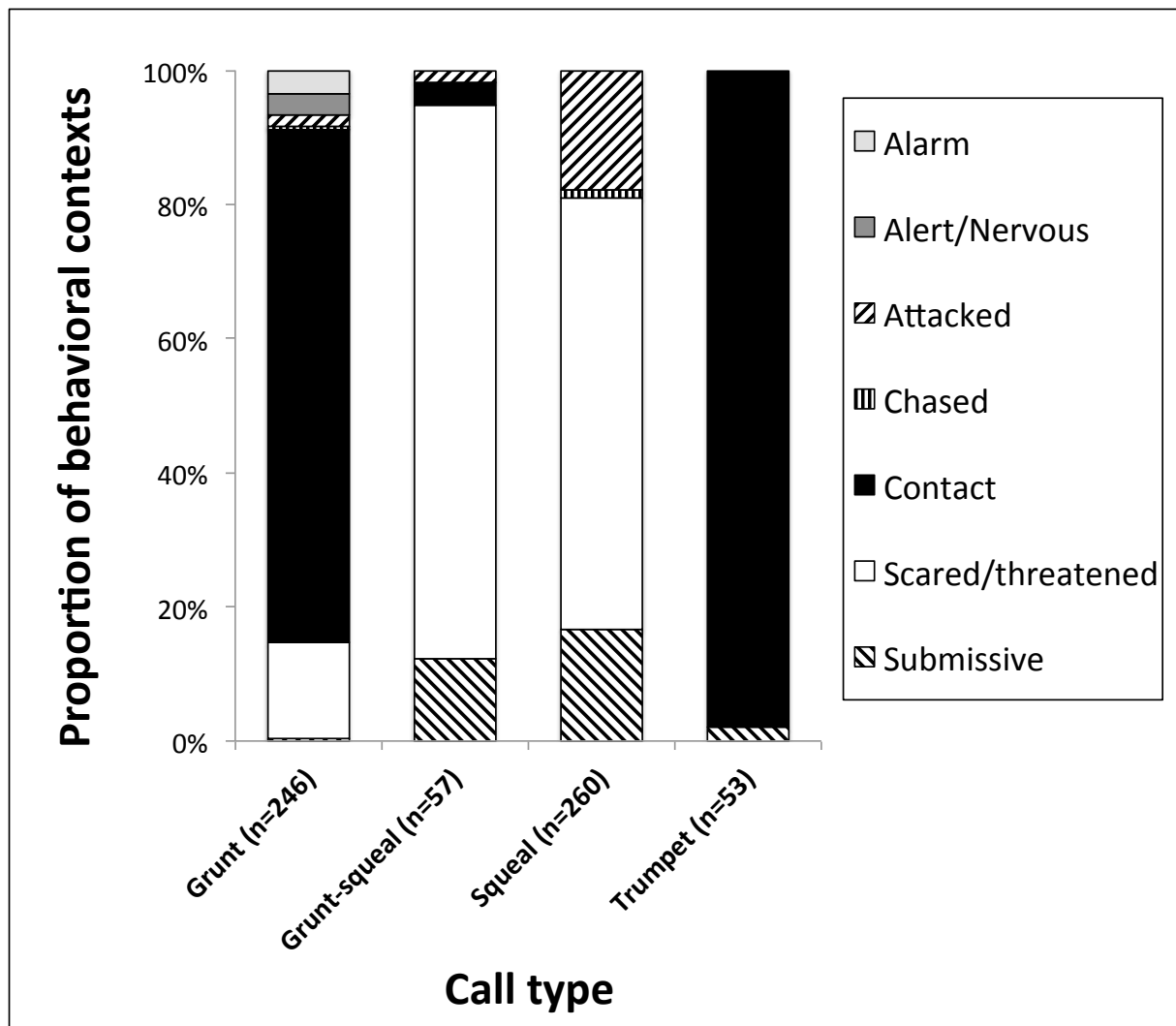
ESM Figure 1. Proportion of call types associated with each behavioral context (calls for which the context of emission is unknown are not represented here; see ESM Table 2 for detailed values).

Short description: All of the alarm and alert/nervous calls and most of the contact calls were grunts. The rest of the contact calls were mostly trumpets. The majority of the calls produced in negative contexts (e.g., scared/threatened, submission, attacked; see descriptions in Material and Methods; and see also McGlone 1985; Weng et al. 1998; Oczak et al. 2013) were squeals and grunt-squeals.

Chapter 1 | Supplementary Material

ESM Table 2. Proportion of call types associated with each behavioral context.

Behavioral context		Alarm	Alert/ Nervous	Attacked	Chased	Contact	Scared/ Threatened	Submissive	Unknown
Call type		8 (100%)	7 (100%)	4 (7.8%)	1 (25%)	173 (76.9%)	32 (13.1%)	1 (1.9%)	20 (80%)
Grunt		0 (0%)	0 (0%)	46 (90.2%)	3 (75%)	0 (0%)	165 (67.6%)	43 (82.7%)	3 (12%)
Squeal		0 (0%)	0 (0%)	0 (0%)	0 (0%)	50 (22.2%)	0 (0%)	1 (1.9%)	2 (8%)
Trumpet		0 (0%)	0 (0%)	1 (2%)	0 (0%)	2 (0.9%)	47 (19.3%)	7 (13.5%)	0 (0%)
Grunt-squeal		8 (100%)	7 (100%)	51 (100%)	4 (100%)	225 (100%)	244 (100%)	52 (100%)	25 (100%)
Sum									



ESM Figure 2. Proportion of behavioral contexts associated with each call type (calls for which the context of emission is unknown are not represented; see ESM Table 3 for detailed values).

Short description: Whereas squeals were used across several negative contexts (being scared/threatened, during submission and when being attacked), most of the trumpets were contact calls and most of the grunt-squeals were scared/threatened calls. Grunts were used across all identified contexts, although predominantly as contact calls. Thus call types varied considerably in their context-specificity.

Chapter 1 | Supplementary Material

ESM Table 3. Proportion of behavioral contexts associated with each call type.

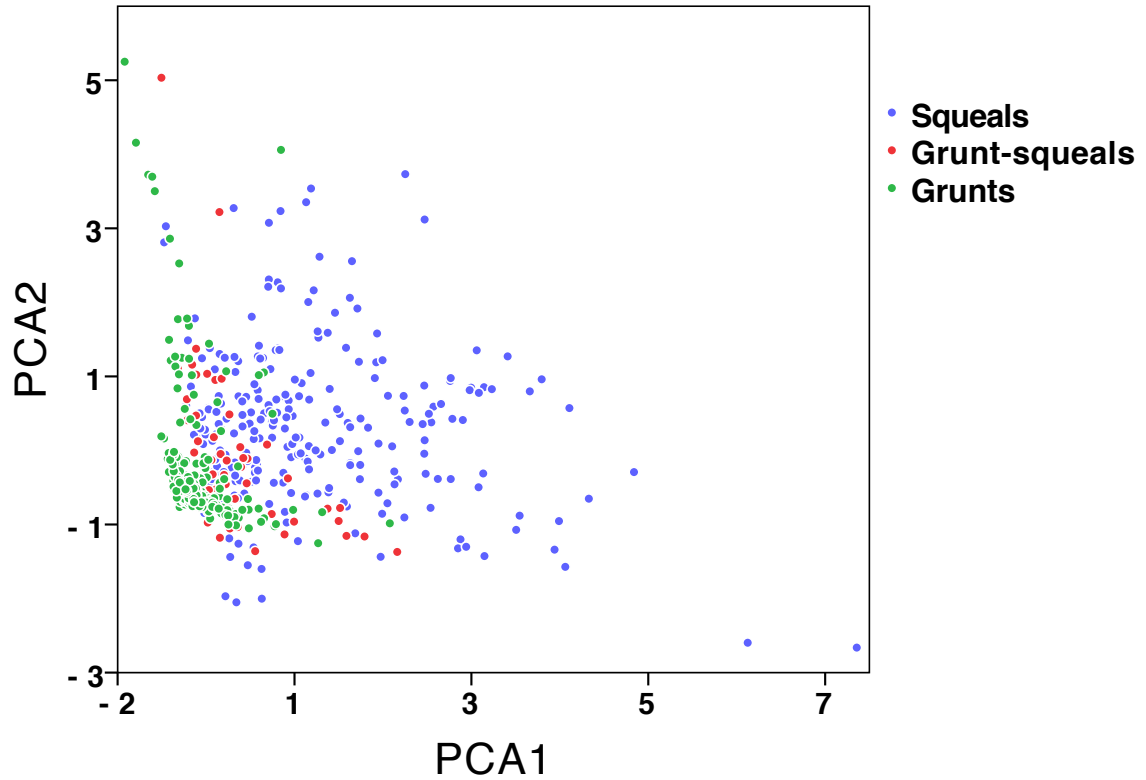
Behavioral context Call type	Alarm	Alert/ Nervous	Attacked	Chased	Contact	Scared/ Threatened	Submissive	Unknown	Sum
Grunt	8 (3.3%)	7 (2.8%)	4 (1.6%)	1 (0.4%)	173 (70.3%)	32 (13%)	1 (0.4%)	20 (8.1%)	246 (100%)
Squeal	0 (0%)	0 (0%)	46 (17.7%)	3 (1.2%)	0 (0%)	165 (63.5%)	43 (16.5%)	3 (1.2%)	260 (100%)
Trumpet	0 (0%)	0 (0%)	0 (0%)	0 (0%)	50 (94.3%)	0 (0%)	1 (1.9%)	2 (3.8%)	53 (100%)
Grunt-squeal	0 (0%)	0 (0%)	1 (1.8%)	0 (0%)	2 (3.5%)	47 (82.5%)	7 (12.3%)	0 (0%)	57 (100%)

Chapter 1 | Supplementary Material

ESM Table 4. Classification agreement between perceptual classification and MLR models.

MLR model Call type	DF Regression (N = 616)	Q25 Regression (N = 616)	Cross-validation (N = 212)
Squeals	66.9 %	72.7 %	73.6 %
Grunts	89.8 %	89 %	88.7 %
Grunt-squeals	64.9 %	64.9 %	69.8 %
Trumpets	88.7 %	94.3 %	94.3 %
Overall	77.6 %	80.2 %	81.6 %

Call distribution based on a 4-variable PCA



ESM Figure 3. The two principal components (PCA1 and PCA2) resulting from a PCA run on the four variables retained from the best MLR model (Q25, DUR, SF and FLAT), illustrating grunt-squeals' acoustic structure as an intermediate between grunts and squeals.

References:

- Gingras, B. & Fitch, W. T. 2013: A three-parameter model for classifying anurans into four genera based on advertisement calls. *J. Acoust. Soc. Am.* 133, 547-559.
- McGlone, J. J. 1985: A quantitative ethogram of aggressive and submissive behaviors in recently regrouped pigs. *J. Anim. Sci.* **61**, 559-565.
- Weng, R. C., Edwards, S. A. & English, P. R. 1998: Behaviour, social interactions and lesion scores of group-housed sows in relation to floor space allowance. *Appl. Anim. Behav. Sci.* **59**, 307-316.
- Oczak, M., Ismayilova, G., Costa, A., Viazzi, S., Sonoda, L. T., Fels, M., Bahr, C., Hartung, J., Guarino, M., Berckmans, D. & Vranken, E. 2013: Analysis of aggressive behaviours of pigs by automatic video recordings. *Comput. Electron. Agric.* **99**, 209-217.

Chapter 2 |

Acoustic recordings and anatomical measurements:

Identifying the potential information content
encoded within acoustic signals

This chapter is an article submitted to a peer-reviewed journal.

Citation: **Garcia M**, Wondrak M, Huber L & Fitch WT. *Submitted*. Honest signaling in domestic pigs (*Sus scrofa*): vocal allometry and the information content of grunt calls. *Journal of Experimental Biology*

Title: Honest signaling in domestic pigs (*Sus scrofa*): vocal allometry and the information content of grunt calls

Running title: Vocal allometry in the domestic pig

Authors: Maxime Garcia§, Marianne Wondrak#*, Ludwig Huber#* & W. Tecumseh Fitch§

§Department of Cognitive Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.

Comparative Cognition, Messerli Research Institute, University of Veterinary Medicine Vienna, Medical University of Vienna, University of Vienna, Veterinaerplatz 1, 1210 Vienna, Austria

* Haidlhof Research Station, Bad Vöslau, Austria

Corresponding author: maxime.garcia@univie.ac.at ; phone: +431427776160

Key words: Domestic pig; Acoustic allometry; Honest signaling; Formants; Grunt; Vocal communication

Summary statement: This study provides the first case of longitudinal investigation of acoustic allometry. It relies on complementary analyses and relates to the evolution of honest signaling in mammals.

Abstract

The information content of acoustic signals is a central topic in mammal vocal communication research. Body size is one form of information that can be encoded in calls. Acoustic allometry aims to identify the vocal correlates of body size within the vocalizations of a given species, and formants have proved a useful acoustic cue in this context. We conducted a longitudinal investigation of acoustic allometry in the domestic pig (*Sus scrofa*), asking whether formants of grunt vocalizations provide information concerning the caller's body size over time. We recorded grunts from 20 Kune Kune piglets, measured their vocal tract length by means of radiographs (X-rays) and weighed them on four occasions. Controlling for effects of age and sex, we found that body weight strongly predicts vocal tract length, which in turn determines formant frequencies. We conclude that grunt formant frequencies could allow domestic pigs to assess a signaler's body size as it grows. We also found indications of individual specificity within grunt formants. Further research using playback experiments is needed to determine the role of formants in domestic pig communication.

Introduction

Identifying the type of information conveyed by animal acoustic signals is a central research focus in the field of bioacoustics (Bradbury and Vehrencamp, 1998). Studies conducted on different model species have shown that diverse information concerning traits of a caller may be encoded within the acoustic signal it produces. Vocalizations may thus allow receivers to evaluate many relevant attributes of the caller including body size (Charlton et al., 2009a; Pitcher et al., 2012; Reby and McComb, 2003; Vannoni and McElligott, 2008), sex (Charlton et al., 2009a; Vignal and Kelley, 2007), age (Charlton et al., 2009a; Reby and McComb, 2003), individual identity (Charlton et al., 2011a; Charlton et al., 2009b; Reby et al., 1998; Robisson et al., 1993), group membership (Boughman, 1997; Randall et al., 2005), geographical origin (Catchpole and Armanda, 1993), motivational state (Kreutzer et al., 1999), physical condition (Wyman et al., 2008), hormonal levels (Charlton et al., 2011c; Koren and Geffen, 2008) and emotional state (Briefer, 2012).

Among these topics, the particular study of ‘acoustic allometry’ has recently emerged, focusing on identifying the vocal correlates of a caller’s body size (Fitch, 2000b; Reby and McComb, 2003; Rendall et al., 2005). Because body size has a fundamental influence on animal ecology (Peters, 1983), physiology (Taylor et al., 1982) and social behavior (Clutton-Brock et al., 1979; Ryan, 1980), accurate acoustic cues to body size should be biologically relevant, and not only perceived but also interpreted and utilized by receivers.

In birds and mammals, early work suggested that fundamental frequency (F0 hereafter), a key component in many acoustic signals, might be negatively correlated with body size, and thus that an impression of bigger size would be conveyed by a lower F0 (Morton, 1977). This suggestion seems plausible based on the anatomical-physical description of sound production: F0 corresponds to the rate of vibration of the vocal folds, and longer, thicker vocal folds vibrate at a lower rate (Titze, 1994). If vocal fold length correlated with body size, it would thus be possible to predict a caller’s body size based on F0. However, this acoustic feature has been shown to poorly reflect caller’s body size in various mammalian species (Lass, 1978;

Masataka, 1994; Pfefferle et al., 2007; Rendall et al., 2005), probably due to the absence of strict anatomical constraints on the size of the larynx, which can thus grow with a relative independence from overall body size (Fitch and Hauser, 1995).

Unlike the laryngeal structures, the dimensions of the supralaryngeal vocal tract (hereafter simply “vocal tract”) are often more closely linked to those of the rest of the body (Fitch and Hauser, 1995). The shape and the length of the volume of air within the vocal tract enhances certain resonant frequencies, called formants, and both formants and formant spacing (the mean frequency spacing between consecutive formants) are inversely correlated with vocal tract length. Formant-related features have been shown to be a good indicator of body size in multiple species (Charlton et al., 2011b; Charlton et al., 2009a; Fitch, 1997; Harris et al., 2006; Reby and McComb, 2003). Even when particular adaptations have led to an exaggerated vocal tract length (Fitch and Reby, 2001), formant characteristics can still correlate with vocal tract length and remain a robust and honest indicator of body size within the species because all individuals are subject to the same physical limits imposed by body size (Reby and McComb, 2003).

Research investigating acoustic allometry typically involves cross-sectional studies, sampling a specific group of subjects at a fixed point in time (Evans et al., 2006; Fitch, 1997; Hauser, 1993; Rendall et al., 2005; Riede and Fitch, 1999). For example, a cross-sectional study conducted on humans (Fitch and Giedd, 1999) looked at vocal allometry at different life stages (childhood, puberty and adulthood) and showed that key differences between vocal tract length in males and females arose at puberty, caused by a male-specific laryngeal descent. Although a descended larynx is not typically found in mammals and has been thought to be uniquely human (Lieberman, 1984), it has been reported in some artiodactyls (red and fallow deer (Fitch and Reby, 2001), Mongolian gazelle (Frey and Gebler, 2003)) and some carnivores (lion, tiger, jaguar, leopard and snow leopard (Hast, 1989; Weissengruber et al., 2002)). Additionally, cineradiographic observations on several mammal species have shown that the larynx is more mobile than previously thought (Fitch, 2000a). Allometric relationships between body size and formants should be affected by larynx descent, whether it occurs at a given point in life or while an

animal is vocalizing. However, the importance of acoustic allometry in relation with vocal ontogeny and laryngeal descent/position remains little explored.

In this context, domestic pigs (*Sus scrofa domesticus*) represent an ideal model species to examine acoustic allometry, because they are extremely vocal and social, and produce abundant low frequency and relatively broadband grunts (Kiley, 1972) (ideal for formant salience (Fitch and Hauser, 1995)). Within a pig group, size and dominance status are normally strongly correlated (Jensen, 2002), so if cues to body size are present in pig grunts, they should thus be highly relevant for receivers. Previous work indicates that grunts could allow sows to recognize their own offspring (Illmann et al., 2002), and that formant-related acoustic features potentially allow piglets to recognize sows individually, based on their nursing grunts (Schön et al., 1999). In summary, pig grunts, and especially their formants, may serve as a multi-message signal, providing cues both to body size and individual identity.

In the present study, we investigated acoustic allometry longitudinally in domestic pigs from the Kune Kune breed as they grew, making multiple measurements of the same individuals at different life stages. To our knowledge, this is the first longitudinal acoustic allometry study. We captured radiographs (X-rays) of awake piglets and collected weight data and acoustic recordings of grunts as they aged in order to quantify the anatomical-acoustical correlations relevant to allometric relationships, focusing on formants. As cineradiography data previously collected on a domestic piglet showed only a slight variation of the larynx position while emitting grunts (as opposed to piglet screams, which typically involve laryngeal retraction) (Fitch, 2000a), we expected a close relationship between vocal tract length and overall body size, and we predicted that formant characteristics in grunts would provide reliable information regarding the caller's body size in this species. We discuss our findings in relation to the domestic pig's complex communication system, and consider the potential selective advantages regarding cue extraction in multi-message signals for the receiver.

Material and methods

Study site and animals

The subjects were 20 Kune Kune piglets (*Sus scrofa domesticus*) from 3 different litters (litter B: N=7 (3 females); litter R: N=6 (4 females); litter Z: N=7 (2 females)) at the Haidlhof Research Station in Bad Vöslau, Austria. Subjects were between 8 and 131 days old during the course of the study. They were housed in semi-natural free ranging conditions in an 8 ha pasture and a forested patch where 5 A-shaped huts, a muddy wallow and the water supply were located. The animals had continuous free access to pasture and forest where they spent the nights or found shelter. The pigs lived together in a stable natural social structure, consisting of sounders of three sows and their offspring of two consecutive years, altogether 41 pigs (22 females). The subjects of this study were the youngest three litters. Animals were fully habituated to humans (high number of interactions on a daily basis) and had *ad libitum* water and grass to graze. Additionally, they were fed daily with a diverse mixture of fruits, vegetables, bread and grain.

Data collection

Piglets were born on June 20th 2015 (litters B and Z) and June 22nd 2015 (litter R). Data collection for some or all of the piglets occurred on four different occasions (hereafter “series”), namely when piglets were on average 9, 43, 72 and 130 days old (weaning occurred at about 80 days). Weight curves from the previous generation were used to select appropriate dates to capture the measurement series. The first three series covered the pre-weaning period, when piglets’ weight increase was not linear, whereas the fourth series occurred after weaning when piglets’ weight increase was stable over time. All piglets were weighed on each of the four series with a My Weigh WR-12K Washdown Scale (reading accuracy: ± 20 g) when they were less than 10 Kg (series 1-2), and later with a Soehnle 7858

Veterinary scale (reading accuracy: $\pm 100\text{g}$ accuracy) as soon as some of the piglets weighed more than 10Kg (series 3-4).

Acoustic recordings

Vocalizations were recorded with a Sennheiser ME-66 directional microphone (frequency response 40-20000 Hz ± 2.5 dB; Sennheiser Electronic GmbH & Co. KG, Wedemark Germany) powered by a LR6 battery, and connected to a Zoom H4N digital recorder (48 kHz sampling frequency and 16-bit quantization; Zoom Corporation, Tokyo, Japan). These recordings were stored as uncompressed WAV files. For shock and wind-noise reduction, the microphone was mounted on a Rycote Modular Windshield WS 7 Kit for Shotgun Microphones (Rycote Microphone Windshields Ltd, Stroud, UK). Recordings were carried out in a sheltered hut regularly used by the animals, which provided ideal recording conditions (minimal wind and background noise). All 20 individuals were led individually to the hut and had their calls recorded on each of the four series. Recordings were obtained either on the same day, or one day prior or following radiograph collection; time constraints prevented collection of both types of data in a single day.

The typical vocalizations recorded from piglets were grunts, since these low frequency calls highlight formants better than squeals. For the first series, grunt vocalizations were elicited by preventing the piglets from going out of the hut (blocking the way with the experimenter's hand) or by holding them briefly (which at first elicited squeals, followed by grunts upon their return on the floor). Once piglets were old enough to feed on solid food (from the second series onwards), food was presented as a stimulus to which piglets would produce grunts. This food bait was used on top of the daily food supply and the *ad libitum* grazing possibility provided by the pasture (no food restriction was imposed, and only the piglets' preference for particular foods was utilized to obtain recordings of grunts, which were then rewarded by several food items during a given recording series).

Radiographs

Animals were placed in a restrainer, made of Plexiglas for the first series and hand-made piece of fabric for the following three series (to avoid discomfort as piglets grew older and heavier). Midsagittal radiographs of the head and neck region were made with a mobile digital x-ray system, using a full bridge inverter (Physia Gamma light AD 100/120) with different acquisition settings depending on animal size and tissue thickness (series 1: 64 kV, 2.8 mA; series 2: 68 kV, 3.2 mA; series 3: 68 kV, 3.6 mA; series 4: 74 kV, 3.2 mA). Scaling was automatically recorded on the digital radiograph imaging plates used for image capture. All 20 individuals were radiographed on the first and last series. Due to time and logistic constraints, one half of the individuals (N=10) were radiographed on series 2, and the other half on series 3 (piglet selection was based on weight distribution aiming to have these measurements representative of the entire group).

Data analysis

Acoustic measures

All acoustical analyses were made in Praat (Boersma and Weenink, 2014). Using visual inspection of spectrograms and listening, only high quality grunts (i.e. those deemed to have a high enough signal-to-noise ratio and visible formants) were annotated with 'Individual' and 'Series' using the 'Annotate: To TextGrid' function. Special attention was paid to identify true grunts clearly, as opposed to 'grunt-squeals' which have quite different acoustic characteristics (Garcia et al., In press). Annotated grunts were extracted and average formants values were retrieved from each call via a custom Praat script (M.G.) that used linear predictive coding (LPC) via the 'LPC: To Formants (Burg)' function and allowed editing the formant contour via the 'Down to FormantGrid' function. Formant editing allowed us to remove sections to which Praat automatically attributed a formant value to background noise although the section actually lacked vocalization. Our analysis

parameters differed across series and were based on visual inspection of the spectrograms (window of analysis: 0.025s; time step: 0.00625s (one fourth of window length); maximum number of formants: series1=3, series2=4, series3=4, series4=2, maximum formant frequencies, series1=4500Hz, series2=4500Hz, series3=4000Hz, series4=1500Hz). These input settings were adjusted so that formants 1 (F1) and 2 (F2) could be distinctly identified and extracted for each series (Fig. 1).

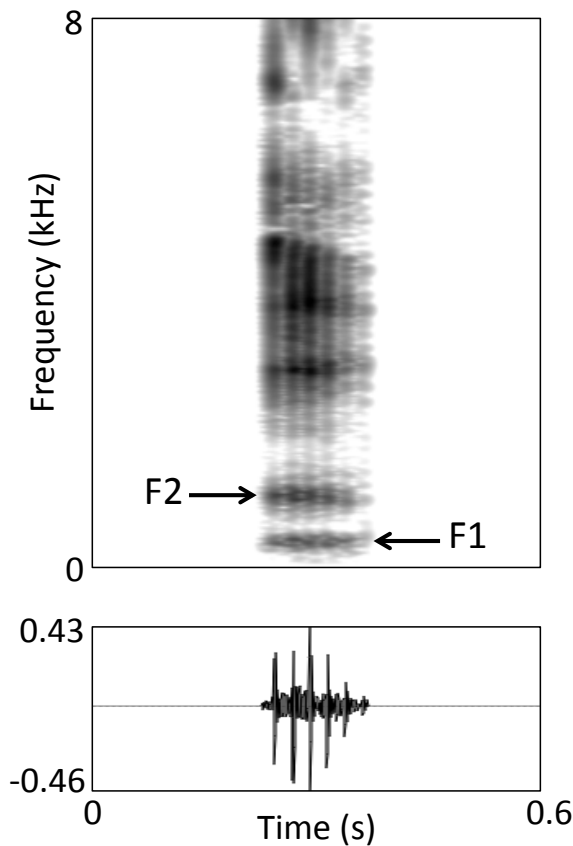


Fig. 1: Spectrogram of a grunt (individual: Baldur; fourth series) showing its first 2 formants. In most cases, formants higher than F2 could not be clearly distinguished. Visualization settings: view range, 0-8 kHz; window length, 0.04s; time steps, 700, frequency steps, 250, Gaussian window; dynamic range = 40 dB.

Higher formants were not extracted since they could not reliably be clearly identified, for two reasons. First higher formants did not appear to be consistently as well defined as F1 and F2. Second, tracking accuracy for higher formants appeared to be affected by slight vocal tract adjustments (both by potentially changing formant contours and spacing, and/or by introducing ‘nasal zeros’ or ‘antiformants’, such as seen in humans (Kurowski and Blumstein, 1987). Ultimately

we retained 5 grunts per series and per individual from which we extracted F1 and F2 and calculated the average F1, F2 (Supplementary Material – S –, Table S1A-D) and formant spacing (DeltaF hereafter) for each individual within each series. Whenever more than 5 calls per individual and per series were available, we performed a second, stricter quality assessment and if this was not sufficient to winnow the sample down to five, we made a random selection of 5 calls among the remaining highest quality files. Overall, only 3 individuals in series 1 did not have enough good quality recordings to reach the criterion of 5 calls; these cases were therefore excluded from the analysis.

Radiographic measurements

Vocal tract length was measured from lateral radiographs obtained from piglets (Table S1A-D). For each radiograph, 3 types of measurements were carried out based on several cranial and soft-tissue landmarks (see illustrations and definitions Fig. 2): the first measurement, skull length, is based on traditional skull morphometry and corresponds to the distance between the “prosthion” (‘P’) and the “basion” (‘B’). The two other measurements of vocal tract length aim to evaluate the piglets’ airway length anterior to the larynx (following the path of sound emitted from the vocal folds). Here, nasal tract length corresponds to the distance between the tip of the snout (‘S’, defined as the projection from the nasal airway onto a line connecting the two apexes of the piglet snout: see ‘apical line’ Fig. 2C) following the upper jaw dorsally and then the airway down to the base of the epiglottis (‘E’) within the larynx, which marks a clear sharp inflexion point in the airway between the pharynx and the tracheal portion of the airway. Oral tract length corresponds to the distance between the “lower incision” (‘I’) following dorsally the teeth of the lower jaw and then the airway down to the same ‘E’.

In order to account for the uncertainty sometimes caused by low absorbance and scan blurriness (due to slight animal movements during radiograph capture), a quality assessment was made for each radiograph (1: certain, 2: intermediate, 3:

unclear), providing a way to easily search for potential outliers and/or errors in the later statistical analysis.

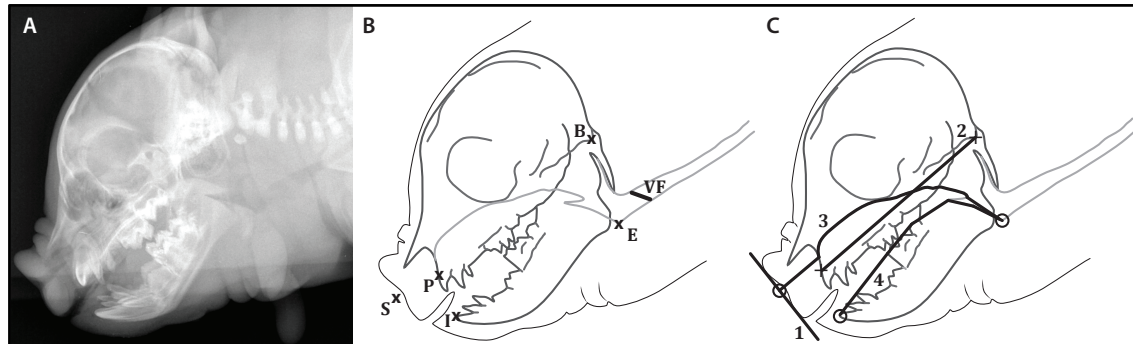


Fig. 2. Illustration of the measurements obtained from landmarks placed on radiographic images.

A) Radiograph of a domestic piglet (individual: Bolero; first series).

B) Landmarks used to measure vocal tract length from radiographs. P: Prosthion, most anterior portion of the maxilla between the incisor roots; I: Incision, located at the incisal level of the lower central incisors; B: Basion, the midline anterior margin of the foramen magnum; E, base of the epiglottis; S: projection from the nasal airway onto the snout apical line (see Fig. 3C); VF: position of the vocal folds as estimated from anatomical data. 'I' was chosen over the lower jaw equivalent of prosthion because the latter point could not always be identified. 'E' was chosen over the location of the vocal folds themselves, since they were rarely clearly observed on radiographs because of the low absorbance difference between soft and calcified tissues in these young animals (although their expected position is indicated Fig. 2B, based on anatomical images of sectioned piglet heads (Fitch, *unpublished data*).

C) Illustration of the measurement taken from radiographs; 1: apical line; 2: proxy of skull length (straight-line distance between P and B); 3: nasal tract length (segmented line between S and E following upper jaw dorsally); 4: oral tract length (segmented line between I and E following dorsally the teeth of the lower jaw).

All measurements from radiographs were made using Image J (version 2.0.0-rc-15-1.49k). DICOM files were loaded in Image J, fine-scaled based on DICOM

metadata, adjusted for optimal visualization of the landmarks and measurements were made on segments (PB) or segmented lines (SE and IE). A second measurement session, blind to the first session, was conducted on 10% of the data (based on a random selection excluding the scans labeled with 'quality 3' during the first session, since the quality bias is taken into account by the statistical analysis _ see below). This resulted in an overall agreement of 99.9% (Pearson's $r = 0.9993$), illustrating the reliability of this measurement procedure. The accuracy of the measurements was very high: the mean absolute measurement error ranged from 0.046 mm to 2.48 mm (mean = 0.8 mm) and represented between 0.03% and 1.6% (mean = 0.6%) of the overall length, which is negligible compared to the average variation found between individuals of the same age (coefficient of variation ranging from 4.2% to 9.5%) and between series (coefficient of variation ranging from 10.3% to 36.2%).

Statistical analysis

Prior to running analyses, all parameter units were chosen to avoid scaling issues (all frequency parameters expressed in kHz, length parameters in cm and weight in Kg). Data normality was assessed using a Shapiro-Wilk test; afterwards pairwise correlations were computed. Principal component analyses (PCA) were run on groups of variables that were highly correlated and thus redundant with respect to the acoustic and anatomical measurements. Two different PCA were run, one grouping skull, nasal tract and oral tract length into a single 'vocal tract length' or VTL component (eigen value = 2.96, explaining 98.7 % of the variance), the other grouping F1 and F2 into one 'Formant' or Fn component (eigen value = 1.93, explaining 96.8 % of the variance). VTL and Fn components were also assessed for normality and then correlations among all variables were computed. DeltaF was maintained as an individual measurement since it represents a relative measure of F1 and F2 variation and could give insight into how evenly/differently formants change through time.

Three types of analysis were conducted, respectively on purely anatomical correlations (testing the effect of body weight on VTL), anatomical-acoustical correlations (testing the effect of VTL on formant characteristics) and acoustic allometry (testing the effect of body weight on formant characteristics).

To evaluate statistical significance and relative predictive power, data were analyzed by means of model selection using Linear Mixed Models (LMMs) with Restricted Maximum Likelihood estimation (REML) and/or generalized linear mixed model (GLMMs). Models were computed including non-inter-correlated fixed effects and random effect intercepts. Based on visual inspection of the data, models were also run including random slopes for the effect of the main factor of interest (VTL for the anatomical-acoustical dependency, and body weight for the anatomical and acoustic allometry dependencies). Our model selection procedure followed a stepwise removal of fixed effects, evaluating a decrease in Akaike Information Criterion (AIC) scores (corresponding to an improvement of the model), to reach the best model with the lowest AIC. Statistical significance of the final models was evaluated using likelihood ratio tests (final model vs. null model: excluding the fixed effect which significance was being tested, following Winter (2013). Provided residuals were normally distributed, this model was considered to be validated. Otherwise, a generalized linear mixed model fitting the dependent data distribution was computed, including the same fixed and random effects/slopes as in the LMM (see Table S2 for details on initial models composition).

To control for the effect of potentially significant errors in the measurements, the same overall analysis was conducted on a reduced sample, excluding the cases in which one of the 3 measurements' quality was ranked as low with '3'. Data was prepared in SPSS Statistics (V. 21.0) and statistical analyses were conducted using SPSS and R (RCoreTeam, 2015) with the R-package lme4 (Bates et al., 2014). Two-tailed p-values are reported with significance levels set at 0.05.

Results

Examination of normality revealed that all variables measured were non-normally distributed. Therefore, non-parametric Spearman rank correlations were computed, which showed that all measured variables were significantly inter-correlated ($p < 0.001$ for all correlations; Table 1). Overall, the two components resulting from the PCAs have higher correlations with other variables than variables singled out from the components (e.g. Fn correlates better than F1 and F2 with VTL and Log_{10} of body weight (Log Wt hereafter)), justifying the use of the PCA variables. Because we were generally interested in determining the predictability of one variable by another, and because when compared to Fn, DeltaF showed less strong correlations with both weight and VTL (Table 1), Fn was the only frequency-related variable retained for further analysis (moreover, formant dispersion is usually based on an average of more than three formants, and cannot be appropriately calculated here as only F1 and F2 could be clearly distinguished). Finally, Log Wt was used rather than body weight because volume is proportional to the cube of a linear dimension (body weight was the only variable log-transformed as the relationships between Log Wt and VTL and between Log Wt and Fn appeared to be linear after visual inspection).

	F1	F2	DeltaF	Fn	Skull	Nasal Tract	Oral Tract	VTL	Weight	Log Wt
F1	1.000	0.954	0.861	0.987	-0.922	-0.917	-0.926	-0.931	-0.954	-0.954
F2	0.954	1.000	0.967	0.985	-0.935	-0.928	-0.940	-0.942	-0.955	-0.955
DeltaF	0.861	0.967	1.000	0.919	-0.884	-0.877	-0.892	-0.890	-0.891	-0.891
Fn	0.987	0.985	0.919	1.000	-0.936	-0.932	-0.943	-0.946	-0.963	-0.963
Skull	-0.922	-0.935	-0.884	-0.936	1.000	0.974	0.978	0.990	0.955	0.955
Nasal Tract	-0.917	-0.928	-0.877	-0.932	0.974	1.000	0.980	0.990	0.954	0.954
Oral Tract	-0.926	-0.940	-0.892	-0.943	0.978	0.980	1.000	0.993	0.955	0.955
VTL	-0.931	-0.942	-0.890	-0.946	0.990	0.990	0.993	1.000	0.964	0.964
Weight	-0.954	-0.955	-0.891	-0.963	0.955	0.954	0.955	0.964	1.000	1.000
Log Wt	-0.954	-0.955	-0.891	-0.963	0.955	0.954	0.955	0.964	1.000	1.000

Table 1: Spearman correlation coefficients between the anatomical and acoustic variables measured in the study. All correlations are significant at the $p < 0.001$ level.

Anatomical Dependencies: Body Weight Predicts Vocal Tract Length

Because Log Wt and VTL were strongly and positively correlated ($r = 0.964$, $p < 0.001$; Fig. 3A), we further examined the dependence of VTL on Log Wt with linear models. Log Wt, Litter (B, R or Z) and Sex (male or female) were entered as fixed effects whereas Individual and Series (1, 2, 3 or 4) were entered as random effects. Two types of models were calculated, either specifying random slopes for the by-Individual and by-Series effect of Log Wt, or only for the by-Series effect of Log Wt (based on visual inspection of the data prior to running the analysis; see initial model composition Table S2). After stepwise removal of the fixed effects based on a decrease in AIC scores, the best fitting model was a generalized linear mixed model (because the residuals from the linear mixed model were non-normally distributed) with a gamma distribution and an inverse link function, including only Log Wt as fixed effect and random slope only for the by-Series effect of Log Wt (Table 2). We thus found that body weight was the only significant predictor of vocal tract length ($N = 60$; predictions not back transformed: $\beta = -1.515$, $SE = 0.48$, $t = -3.158$, $P < 0.002$), and thus excluding an effect of sexual dimorphism on this relationship.

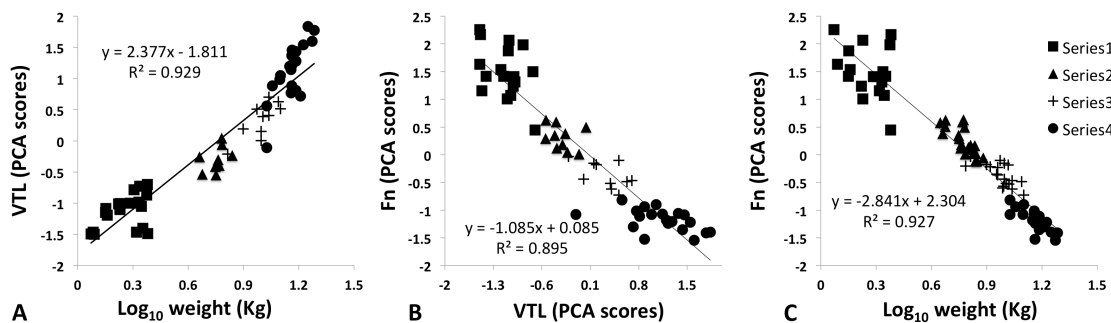


Fig. 3: Bivariate plots illustrating inter-correlations of body weight (Log Wt, in Kg), vocal tract length (VTL, PCA scores from a PCA on 'Skull length', 'Nasal tract

length' and 'Oral tract length') **and formants** (Fn, PCA scores from a PCA on Formant1 and Formant2). A: N = 60; B: N = 57; C: N = 77.

The same analysis was conducted controlling for Age instead of Series and produced the same final model (which is not surprising considering that series number increased in time and was tightly linked to age). Because this study is a longitudinal sampling of the same individuals, our analysis shows that in domestic pigs, the growth of the vocal tract is dependent on body weight entirely with no additional significant effects of Sex or Age.

Acoustical Dependencies: Vocal Tract Length Predicts Formants

VTL and Fn were strongly negatively correlated ($r = -0.946$, $p < 0.001$; Fig. 3B), as predicted based on acoustic principles, and we thus further examined the dependence of Fn on VTL (an anatomical-to-acoustic relationship) in a similar way to the previous analysis (Table S2). Our best fitting model revealed that VTL is the only significant determinant (N = 57; $\beta = -0.574$, SE = 0.15, $t = -3.955$, $P = 0.006$) of Fn (Table 2), including when Age is controlled for instead of Series. This analysis shows that the observed decrease in formant frequencies with body size (Table 1) depends only on the increase in vocal tract length; again no sex differences were significant.

Acoustical Allometry: Body Weight Predicts Formants

Finally, looking at acoustic allometric correlations, Fn depended strongly and negatively upon Log Wt ($r = -0.963$, $p < 0.001$; Fig. 3C), as expected based on the previous two correlations). Following the same procedure for model selection (see initial model Table S2), the best fitting model now only included a significant effect of Log Wt (N= 77; $\beta = -2.191$, SE = 0.42, $t = -5.178$, $P < 0.001$) on Fn (Table 2). As for the two previous analyses, replacing Series by Age yielded the same final

model. This result therefore shows that formants are tightly determined by body weight, via the intervening variable of vocal tract length, with no additional significant dependence upon age or sex.

Analysis	Type	Final model formula	AIC	β	SE	t	p
Anatomical	GLMM	VTL~ Log Wt + (1 Individual)+(1+Log Wt Series)	140.5	-1.51	0.48	-3.16	0.002*
Anatomical-acoustical	LMM	Fn~ VTL + (1+VTL Individual)+(1+VTL Series)	49.2	-0.57	0.15	-3.96	0.006*
Acoustic allometry	LMM	Fn~ Log Wt + (1+Log Wt Individual)+(1+Log Wt Series)	10.6	-2.19	0.42	-5.18	<0.001*

Table 2: Details of the best fitting models (obtained after model reduction based on inspection of the AIC scores) for each of the main three analyses.

These anatomical and anatomical-acoustical analyses were run a second time, removing all cases where VTL measurements based on the radiographs included at least one uncertain measurement (quality labeled with ‘3’). While AIC scores and significance values differed slightly from the main analyses, all best fitting models were the same, indicating that potentially lower-accuracy measurements involving greater uncertainty did not affect the fundamental relationships found in the analyses reported above.

Predictive relationships between vocal tract lengths and formants

To evaluate the fit between measured formant frequencies and those predicted for a simple uniform tube, we compared predicted and measured F1 and F2 values. From each average individual F1 (Table S3A-C) and F2 (Table S4A-C), the predicted vocal tract length was calculated based on the following equations:

$$F1 = c/4L \quad (\text{Eqn. 1})$$

$$F2 = 3c/4L \quad (\text{Eqn. 2})$$

where c is the approximate speed of sound in the warm, moist air of a mammalian vocal tract (350 m/s) and L is the length of the supralaryngeal tract when considered as a half-open resonant tube (Titze, 1994). Wilcoxon signed-ranks tests

indicated that the measured nasal tract length and vocal tract length were significantly different from the predicted VTL calculated from F1 (nasal tract length: $Z = -6.106$, $p < .001$; oral tract length: $Z = -6.680$, $p < 0.001$) and F2 (nasal tract length: $Z = -6.680$, $p < 0.001$; oral tract length: $Z = -6.680$, $p < 0.001$). Most of our measurements were shorter than predicted from F1 ($N=51/59$) and all were shorter than predicted from F2 ($N= 59/59$). Although highly correlated, measured nasal and oral tract length also significantly differed, and nasal tract length was always longer than oral tract length (F1: $Z = -6.680$, $p < 0.001$; F2: $Z = -6.680$, $p < 0.001$). Thus, although apparently underestimating vocal tract length, our measured nasal tract length was consistently closer to the vocal tract length predicted from F1 and F2 than our measured oral tract length (based on the differences between expected values and nasal or oral tract measurements: F1: $Z = -6.680$, $p < 0.001$; F2 (paired sample t-test): $t(58) = -36.39$, $p < 0.001$).

Because the vocal folds were not visible in our radiographs, our tracing of nasal and oral tracts stopped at the base of the epiglottis ('E'), and the full vocal tract length was thus not included. Specifically, the distance between 'E' and the vocal folds ('VF', taken at their mid-point) was not included in our measurements, which thus represent a consistent underestimate. From digital images of a cross-sectional section of a domestic piglet (Fitch, *unpublished data*), we estimated this distance and calculated the resulting increase in vocal tract length. The distance 'E-VF' represented respectively 8.15% and 9.68% of the nasal and oral tract length stopping at 'E'.

In order to compensate for this additional portion of the vocal tract, we therefore increased our measured nasal and oral tract lengths respectively by 8.15% and 9.68% (see corrected nasal and oral tract length, Tables S3, S4) and ran the above analyses again. Nonetheless, as before, the corrected measurements differed from vocal tract length predicted from F1 (corrected nasal tract length: $Z = -4.031$, $p < 0.001$; corrected oral tract length: $Z = -6.680$, $p < 0.001$) and F2 (corrected nasal tract length: $Z = -4.031$, $p < 0.001$; corrected oral tract length: $Z = -6.680$, $p < 0.001$) with most of the corrected measurements still being shorter than predicted (F1: $N=43/59$; F2: $N= 59/59$). Nasal tract length was always longer than

oral tract length (F1: $Z = -6.680$, $p < 0.001$; F2: $Z = -6.680$, $p < 0.001$) and thus also closer than oral tract length to the predictions from F1 ($Z = -6.680$, $p < 0.001$) and F2 ($t(58) = -33.57$, $p < 0.001$).

Formants and Individuality

Because formants have been suggested to provide information on the caller's identity in pigs (Illmann et al., 2002; Schön et al., 1999), we investigated whether F1 and F2 could convey information about either individual or litter identity. In each of the four measurement series, using either individual or litter as a unit, we calculated the potential for individual coding (PIC, defined as the ratio between inter-individual and intra-individual variability (Lengagne et al., 1997; Robisson et al., 1993)) of F1 and F2. For each series, F1 and F2, we first calculated the intra-individual (CV_{intra} ; based on the five F1 and F2 values obtained for each individual unit; based on the six or seven F1 and F2 values in each litter unit) and inter-individual coefficients of variation (CV_{inter} ; based on individual or litter F1 or F2 values within a series), using the following equation:

$$CV = (1 + 1/4N) * (SD * 100 / \bar{X}) \quad (\text{Eqn. 3})$$

where N is the sample size, SD is the standard deviation, and $\bar{X}$ is the mean of the sample (Sokal and Rohlf, 1995). The potential for individual coding (PIC) is then calculated for each series by dividing the CV_{inter} by the mean of the CV_{intra} :

$$PIC = (CV_{inter} / \overline{CV_{intra}}), \quad (\text{Eqn. 4})$$

A PIC value greater than 1 indicates that the parameter is more variable between than within individuals and therefore has potential for coding individual identity (Charrier et al., 2001a; Garcia et al., 2012; Lengagne et al., 1997; Padilla de la Torre et al., 2015; Robisson et al., 1993). While F1 showed a PIC above 1 only for

the fourth series, F2 had PIC values superior to 1 in all series but the first (Table 3), showing that, at least after the first series, F2 provides a potential indicator of individuality. Using litter as our comparative unit instead of individual, only one PIC value was above 1, namely for F1 in the first series. This suggests that when piglets are about 9 days old (series 1), F1 may contain a cue to litter identity. Because this was not the focus of the present paper and because of our low sample size (regarding the number of grunts per individual), no further analysis (such as discriminant function analysis) on individual/litter identity was carried out. Nonetheless, these data are clearly consistent with the hypothesis that formants, especially the second formant, could provide an acoustic indication of individual identity in our sample.

Variable	Series 1	Series 2	Series 3	Series 4
Litter identity				
F1	1.13	0.51	0.22	0.29
F2	0.54	0.28	0.28	0.46
Individual identity				
F1	0.99	0.87	1	1.38
F2	0.93	1.14	1.34	1.55

Table 3. Assessment of the potential for individual coding of formants F1 and F2 for individual identity and litter identity.

Discussion

The data collected in this study represent, to our knowledge, the first attempt at a longitudinal investigation of acoustic allometry. We show that formants measured in grunt vocalizations provide a reliable cue to body size (assessed by body weight) in growing domestic pigs. The very strong correlations between vocal tract length (VTL), formants and body size (Table 1; Fig. 3), together with the predictive models that we have computed (Table 2), leave little doubt that formants

contain accurate information regarding body size, because increasing body weight strongly predicts increasing VTL, which in turn predicts decreasing formant frequencies. Crucially, by resampling the same individuals on four occasions and controlling for age and sex, we could disentangle the specific roles of these parameters in pig vocal allometry. We found that formant frequencies were predicted by body size rather than age, and found no suggestions of potential acoustic sexual dimorphism, or vocal tract modification specifically dependent on age in this species and stage of development. Examination of the potential for individual coding additionally suggested that grunt formants could provide several types of information to listeners, provided that acoustic cues to body size and individuality are perceived and used by conspecifics.

On the origin of formant frequencies within grunts

Estimations of VTL based on F1 and F2 (Tables S3, S4) were invariably closer to the measured nasal tract length as compared to the measured oral tract length. Measured nasal and oral tract length were always shorter than predicted by F2 (Table S4A-C). Regarding the VTL predicted by F1, measured nasal tract length was shorter than predicted in most individuals ($N = 51/59$), while measured oral tract length was always shorter than predicted (Table S3A-C). We therefore suggest that grunts for our sample were mostly produced nasally, in accordance with previous cineradiographic observations of a vocalizing piglet (Fitch, 2000a).

The fact that predicted vocal tract lengths do not perfectly match nasal tract measurements can be explained by several factors. Vocal tract length measurements were made down to the base of the epiglottis. However, according to the source-filter theory of voice production (Fant, 1960), sound is produced by the vibrating vocal folds (whose vibration rate defines the fundamental frequency, F_0) and then filtered by the supralaryngeal tract (enhancing formants). When correcting our initial measurements for the missing distance between the base of the epiglottis and the vocal folds, we reached similar conclusions, with measurements still typically shorter than predicted. Another potential reason is that laryngeal position in

domestic pigs is not as static as previously thought (Fitch, 2000a) and larynx position could thus slightly lower (and thus contain lower formants) when piglets produce grunts as compared to when they remain silent (which was typically the case during radiographs). Finally, in a few cases nasal tract length was longer than predicted by F1: this could also be explained by laryngeal mobility and our experimental setup. Although we tried to keep piglets' arousal to a minimum while proceeding with radiographs, in some cases piglets produced squeals while being scanned. Squeals in the domestic pig are very loud calls, which appear to involve retracting the larynx down from the nasopharyngeal region (Fitch, 2000a) which in turn leads to a fully extended supralaryngeal tract. Measurements of radiographs of the vocal tract length in this configuration would therefore exceed that characteristic of a grunt call and could explain these isolated observations.

It should be noted that in this study we investigated how formants, instead of formant dispersion, predict body size. These measures are of course intimately connected, and it has been suggested that while individual formants could provide information regarding vocal tract length, they are more liable to uncontrolled variability due either to movements or to deviations from the uniform tube assumption (Fitch, 1997; Owren et al., 1997); formant dispersion on the other hand relies on the redundancy of formant spacing pattern and is thus expected to be more robust (Fitch, 1997). As a result, rather than focusing on individual formant measurements (Owren et al., 1997), most studies investigating formant-related characteristics in mammal vocal communication have used some variant of formant dispersion (Charlton et al., 2011b; Charlton et al., 2009a; Fischer et al., 2004; Fitch, 1997; Reby and McComb, 2003; Sanvito et al., 2007). In the present study, information redundancy was nonexistent because we were only able to measure the first 2 formants consistently. Furthermore, the grunts extracted from our labeling were chosen to be as stable and consistent as possible, minimizing the problem raised by formant variability through time. Finally, because grunts appeared to be produced nasally, acoustic attenuation could have occurred due to higher sound absorbance from nasal cavities (Fitch, 2000a) or the generation of antiformants by

the closed mouth cavity (Kurowski and Blumstein, 1987), explaining why only 2 formants were clearly distinguishable.

Selection pressures and grunt-specific cues

Previous work has shown that two main call types, grunts and squeals, could be consistently identified while investigating both the vocal repertoires of domestic pigs (Kiley, 1972; Tallet et al., 2013) and wild boars (Garcia et al., In press; Klingholz et al., 1979). Unlike squeals, the acoustic characteristics of grunts make them particularly well suited for highlighting formants due to their low F0 (Fitch and Hauser, 1995; Ryalls and Lieberman, 1982), even though the nasal production typical of this call type might slightly impair our ability to track formants compared to other mammals species (Charlton et al., 2011b; Reby and McComb, 2003).

Grunts are produced across various contexts in which extracting information about the caller might prove beneficial to the receiver. Grunts are for instance produced by male domestic (Kiley, 1972) and wild (Meynhardt, 1990) boars as a courtship display, and as an alarm signal in female wild boars (Klingholz and Meynhardt, 1979; Klingholz et al., 1979). It has been shown in various taxa that body size often plays a major role in sexual selection (Carranza, 1996; Clutton-Brock, 2009; Clutton-Brock et al., 2006; Hedrick and Temeles, 1989; Ryan, 1985), and body size influences resource holding potential and fighting abilities in mammals both on a within-species and a between-species level (Clutton-Brock et al., 1979; Morton, 1977; Persson, 1985), including in domestic pigs (Jensen, 2002). Advertising body size in such contexts may be beneficial for large animals, and the results of the current study suggest that, presumably originating in wild boar vocalizations, the domestic pig grunt can provide a cue to the signaler's body size. Furthermore, retrieval of this information should be biologically relevant to conspecifics (both in sexual competition and agonistic group encounters, as documented by (Meynhardt, 1990)), which suggests that pigs should both perceive and attend to formants in conspecific vocalizations. Playback experiments,

preferably using resynthesized grunts with computer-controlled formant frequencies, would be necessary to test this prediction.

In several mammal species, the selective pressures on body size advertisement appear to have led to specific vocal tract adaptations that allow exaggeration of the acoustic impression of body size via formant lowering. Some examples include laryngeal retraction down to the sternum (Fitch and Reby, 2001) or possibly even into the thoracic chamber (Charlton et al., 2011b), presence and inflation of vocal air sacs (Harris et al., 2006) and rostral extension of a nasal vestibulum (Frey et al., 2007). Our results combined with previous radiographic observations strongly suggest that domestic pig grunts are produced nasally. Because measured nasal tract length was consistently longer than measured oral tract length, this implies that lower formants are expected from nasal grunts than expected from grunts if they were produced orally, potentially indicating a mild form of body size exaggeration.

We note a previous speculation that the sound source in at least some grunts could be a dorsal velar closure (“snoring”) rather than vocal fold vibrations (Klingholz et al., 1979). We know of no data relevant to this speculation. Whether such a non-standard production mechanism would have an effect on formants in the context of size exaggeration would require further in-depth investigation of this vocalization’s production mechanisms.

In addition to the agonistic or courtship contexts mentioned above, grunts are also used more generally as contact calls, noticeably occurring during foraging and nursing events in domestic pigs (Kiley, 1972) and wild boars (Klingholz et al., 1979). In both of these contexts, individuality appears as another type of potentially useful acoustic information. It has indeed been shown in several species that contact calls contain cues to individual identity (Favaro et al., 2015; Jansen et al., 2012; Müller and Manser, 2008; Shapiro, 2009; Townsend et al., 2010). In meerkats and banded mongooses for instance, individual-specific information is used by conspecifics during foraging for vigilance and coordination purposes (for a review see Manser et al. (2014)). Given the strong similarities with the social and vocal

communication system found in pigs and meerkats and banded mongooses (also highly social and vocal mammals (Manser et al., 2014)), it is reasonable to suggest that cues to individual identity might be perceived and used by other conspecifics in domestic pigs. Parent-offspring recognition is another situation where cues to individuality could exist, since such recognition relies on vocal communication in other mammal species (Briefer and McElligott, 2011; Charrier et al., 2001b; Fischer, 2004; Insley, 2001). Previous work on domestic pigs reported that grunts produced during nursing allowed litter discrimination by sows (Illmann et al., 2002) and suggested mother recognition by piglets based on formant-related acoustic features (Schön et al., 1999). Our data lend support to the idea that formants may provide individual-specific information (Table 3): we found that F1 could potentially encode litter identity when piglets were 8 to 10 days old, but only at this age (similarly, piglets in the (Illmann et al., 2002) study were 9 days *post partum*). Furthermore, we documented the potential for individual coding by F2 within 3 of the 4 series examined (with PIC values above 1, ranging from 1.14 to 1.55). Together, these data suggest that grunts produced in very early life stages contain a cue to litter identity only (allowing for sows to discriminate their own litter (Illmann et al., 2002)), and that a more stable cue to individual identity may emerge later on (potentially allowing piglets to recognize their mother (Schön et al., 1999) or siblings). This hypothesis is further supported by the decrease (after series 1; Table 3) in PIC values for F1 regarding litter identity, and the gradual increase (from series 1 to 4; Table 3) in PIC values for F2 regarding individual identity. While these data are in line with previous published findings, it must be noted that (1) other signaling channels can play a role in this context (olfaction was suggested to mediate piglet individual recognition by sows early *post partum* (Maletínská et al., 2002)); (2) we were limited in the number of acoustic features that we could measure. Other acoustic variables, alone or in combination, could play a role in conveying litter and individual information later or earlier than what we found based on F1 and F2. Together with the literature cited above, these results overall lead us to suggest that a formant-based cue to identity could be contained in domestic pig grunts, and that the socio-communicative system characterizing this species provides an

appropriate context for such information to be perceived and used by conspecifics. Again, playback studies would allow us to test this hypothesis.

In conclusion, our results show that formants in the domestic pig grunt are a reliable indicator of body size throughout piglet development, and that individual and litter identity may represent additional information conveyed by this acoustic feature. Grunt formants therefore seem to have the potential for carrying multiple messages, as seen in other mammals (rhesus macaques (Fitch, 1997; Rendall, 1996), koalas (Charlton et al., 2012; Charlton et al., 2011a)). These acoustic cues are available and would in theory be useful to the receiver in various contexts ranging from sexual selection to group cohesion and parent-offspring interactions. However, whether or not information related to vocal tract filtering is perceived and used by conspecifics remains unknown. Future research involving playback experiments combined with formant manipulation and signal re-synthesis should improve our understanding of the mechanisms involved in perception and interpretation of domestic pigs grunts by their conspecifics. This would in turn provide additional insights regarding the selective pressures, such as sexual selection and size exaggeration, acting upon this species communication system. Because domestic pigs are common, highly vocal, and easy to work with, they provide excellent potential as a study species for future bioacoustics research, especially given that their wild progenitors still exist and remain both widespread and relatively accessible.

List of symbols and abbreviations

AIC: Akaike Information Criterion

B: basion

DeltaF: formant spacing

E: base of the epiglottis

Fn: PCA component on F1 and F2

F1: first formant

F2: second formant

I: incision

Log Wt: base 10 logarithm of body weight

P: prosthion

PIC: potential for individual coding

S: intersection between nasal tract and apical segment of a piglet snout

VF: vocal folds

VTL: vocal tract length (PCA component on skull length, nasal tract length and oral tract length)

Acknowledgements

We are especially grateful to Ariane Veit for her essential contribution to the diverse data acquisition. We are thankful to Larissa Kawasch, Sarah Deventer, Martina Schiestl and Andrius Pasukonis for their occasional help with data collection. We thank Nadja Kavcik for figure editing, Riccardo Hofer for advice on the experimental design and Christian Tanczos for X-ray capture.

Competing interests

The authors declare no competing or financial interests.

Author contributions

M.G. designed the study, collected acoustic, weight and radiographic data, processed and analyzed the data, created the figures and wrote the manuscript; M.W. designed the study, provided daily care to the animals and collected weight and radiographic data; L.H. provided the research facility and helped designing the study; W.T.F. helped conceive and design the study and data analysis, and helped writing the manuscript. All authors gave final approval for publication.

Funding

This research was funded by the University of Vienna (M.G. & W.T.F). Financial support to L.H. and M.W. came from the Messerli Foundation (project grant: “Socio-cognitive abilities of domestic pigs, their ethical implications, and indicators of well-being”).

References

- Bates, D., Maechler, M., Bolker, B. and Walker, S.** (2014). *lme4: Linear mixed-effects models using Eigen and S4*.
- Boersma, P. and Weenink, D.** (2014). Praat: doing phonetics by computer [Computer program]. University of Amsterdam, The Netherlands, Retrieved from <http://www.praat.org/>.
- Boughman, J. W.** (1997). Greater spear-nosed bats give group-distinctive calls. *Behav. Ecol. Sociobiol.* **40**, 61-70.
- Bradbury, J. W. and Vehrencamp, S. L.** (1998). Principles of Animal Communication: Sinauer Associates.
- Briefer, E. and McElligott, A. G.** (2011). Mutual mother-offspring vocal recognition in an ungulate ruminant species (*Capra hircus*). *Anim. Cogn.* **14**, 585-98.
- Briefer, E. F.** (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1-20.
- Carranza, J.** (1996). Sexual Selection for Male Body Mass and the Evolution of Litter Size in Mammals. *The American Naturalist* **148**, 81-100.
- Catchpole, C. K. and Armanda, R.** (1993). Song Sharing and Local Dialects in a Population of the European Wren *Troglodytes troglodytes*. *Behaviour* **125**, 67-78.
- Charlton, B. D., Ellis, W. A., Larkin, R. and Fitch, W. T.** (2012). Perception of size-related formant information in male koalas (*Phascolarctos cinereus*). *Anim. Cogn.* **15**, 999-1006.
- Charlton, B. D., Ellis, W. A., McKinnon, A. J., Brumm, J., Nilsson, K. and Fitch, W. T.** (2011a). Perception of male caller identity in Koalas (*Phascolarctos cinereus*): acoustic analysis and playback experiments. *PLoS ONE* **6**, e20329.

Charlton, B. D., Ellis, W. A., McKinnon, A. J., Cowin, G. J., Brumm, J., Nilsson, K. and Fitch, W. T. (2011b). Cues to body size in the formant spacing of male koala (*Phascolarctos cinereus*) bellows: honesty in an exaggerated trait. *J. Exp. Biol.* **214**, 3414-22.

Charlton, B. D., Keating, J. L., Kersey, D., Rengui, L., Huang, Y. and Swaisgood, R. R. (2011c). Vocal cues to male androgen levels in giant pandas. *Biol. Lett.* **7**, 71-4.

Charlton, B. D., Zhihe, Z. and Snyder, R. J. (2009a). The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim. Behav.* **78**, 893-898.

Charlton, B. D., Zhihe, Z. and Snyder, R. J. (2009b). Vocal cues to identity and relatedness in giant pandas (*Ailuropoda melanoleuca*). *J. Acoust. Soc. Am.* **126**, 2721-32.

Charrier, I., Jouventin, P., Mathevon, N. and Aubin, T. (2001a). Individual identity coding depends on call type in the South Polar skua *Catharacta maccormicki*. *Polar Biol.* **24**, 378-382.

Charrier, I., Mathevon, N. and Jouventin, P. (2001b). Mother's voice recognition by seal pups. *Nature* **412**, 873-873.

Clutton-Brock, T. (2009). Sexual selection in females. *Anim. Behav.* **77**, 3-11.

Clutton-Brock, T. H., Albon, S. D., Gibson, R. M. and Guinness, F. E. (1979). The logical stag: Adaptive aspects of fighting in red deer (*Cervus elaphus* L.). *Anim. Behav.* **27**, Part 1, 211-225.

Clutton-Brock, T. H., Hodge, S. J., Spong, G., Russell, A. F., Jordan, N. R., Bennett, N. C., Sharpe, L. L. and Manser, M. B. (2006). Intrasexual competition and sexual selection in cooperative mammals. *Nature* **444**, 1065-8.

Evans, S., Neave, N. and Wakelin, D. (2006). Relationships between vocal characteristics and body size and shape in human males: an evolutionary explanation for a deep male voice. *Biol. Psychol.* **72**, 160-3.

Fant, G. (1960). Acoustic theory of speech production. The Hague: Mouton.

Favaro, L., Gamba, M., Alfieri, C., Pessani, D. and McElligott, A. G. (2015). Vocal individuality cues in the African penguin (*Spheniscus demersus*): a source-filter theory approach. *Scientific reports* **5**, 17255.

Fischer, J. (2004). Emergence of individual recognition in young macaques. *Anim. Behav.* **67**, 655-661.

Fischer, J., Kitchen, D. M., Seyfarth, R. M. and Cheney, D. L. (2004). Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. *Behav. Ecol. Sociobiol.* **56**, 140-148.

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.

Fitch, W. T. (2000a). The phonetic potential of nonhuman vocal tracts: comparative cineradiographic observations of vocalizing animals. *Phonetica* **57**, 205-18.

Fitch, W. T. (2000b). Skull dimensions in relation to body size in nonhuman mammals: The causal bases for acoustic allometry. *Zoology* **103**, 40-58.

Fitch, W. T. and Giedd, J. (1999). Morphology and development of the human vocal tract: a study using magnetic resonance imaging. *J. Acoust. Soc. Am.* **106**, 1511-22.

Fitch, W. T. and Hauser, M. D. (1995). Vocal production in nonhuman primates: acoustics, physiology, and functional constraints on "honest" advertisement. *Am J Prim* **37**.

Fitch, W. T. and Reby, D. (2001). The descended larynx is not uniquely human. *Proc. Biol. Sci.* **268**, 1669-75.

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.

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.

Garcia, M., Bowling, D., Gingras, B., Herbst, C., Böckle, M., Locatelli, Y. and Fitch, W. (In press). Structural classification of Wild Boar (*Sus scrofa*) vocalizations. *Ethology*.

Garcia, M., Charrier, I., Rendall, D. and Iwaniuk, A. N. (2012). Temporal and Spectral Analyses Reveal Individual Variation in a Non-Vocal Acoustic Display: The Drumming Display of the Ruffed Grouse (*Bonasa umbellus*, L.). *Ethology* **118**, 292-301.

Harris, T. R., Fitch, W. T., Goldstein, L. M. and Fashing, P. J. (2006). Black and White Colobus Monkey (*Colobus guereza*) Roars as a Source of Both Honest and Exaggerated Information About Body Mass. *Ethology* **112**, 911-920.

Hast, M. H. (1989). The larynx of roaring and non-roaring cats. *J. Anat.* **163**, 117-121.

Hauser, M. D. (1993). The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *Am. Nat.*, 528-542.

Hedrick, A. V. and Temeles, E. J. (1989). The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends Ecol. Evol.* **4**, 136-138.

Illmann, G., Schrader, L., Špinka, M. and Šustr, P. (2002). Acoustical Mother-Offspring Recognition in Pigs (*Sus Scrofa domestica*). *Behaviour* **139**, 487-505.

Insley, S. J. (2001). Mother-Offspring vocal recognition in northern fur seals is mutual but asymmetrical. *Anim. Behav.* **61**, 129-137.

Jansen, D. A., Cant, M. A. and Manser, M. B. (2012). Segmental concatenation of individual signatures and context cues in banded mongoose (*Mungos mungo*) close calls. *BMC Biol.* **10**, 97.

Jensen, P. (2002). The Ethology of Domestic Animals: An Introductory Text: CABI Pub.

Kiley, M. (1972). The Vocalizations of Ungulates, their Causation and Function. *Z. Tierpsychol.* **31**, 171-222.

Klingholz, F. and Meynhardt, H. (1979). Lautinventare der Säugetiere — diskret oder kontinuierlich? *Z. Tierpsychol.* **50**, 250-264.

Klingholz, F., Siegert, C. and Meynhardt, H. (1979). Die akustische Kommunikation des Europäischen Wildschweines (*Sus scrofa* L.). *Der Zoologische Garten N. F.* **49**, 277-303.

- Koren, L. and Geffen, E.** (2008). Complex call in male rock hyrax (*Procavia capensis*): a multi-information distributing channel. *Behav. Ecol. Sociobiol.* **63**, 581-590.
- Kreutzer, M., Irina, B., Eric, V. and Lazoura, K.** (1999). Social Stimulation Modulates the Use of the 'A' Phrase in Male Canary Songs. *Behaviour* **136**, 1325-1334.
- Kurowski, K. and Blumstein, S. E.** (1987). Acoustic properties for place of articulation in nasal consonants. *J. Acoust. Soc. Am.* **81**, 1917-27.
- Lass, N. J.** (1978). Correlational study of speakers' heights, weights, body surface areas, and speaking fundamental frequencies. *J. Acoust. Soc. Am.* **63**, 1218-20.
- Lengagne, T., Lauga, J. and Jouventin, P.** (1997). A method of independent time and frequency decomposition of bioacoustic signals: inter-individual recognition in four species of penguins. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie* **320**, 885-891.
- Lieberman, P.** (1984). *The Biology and Evolution of Language*: Harvard University Press.
- Maletínská, J., Marek, M., Víchová, J. and Stěhulová, I.** (2002). Individual Recognition of Piglets by Sows in the Early Post-Partum Period. *Behaviour* **139**, 975-991.
- Manser, M. B., Jansen, D. A. W. A. M., Graw, B., Hollén, L. I., Bousquet, C. A. H., Furrer, R. D. and le Roux, A.** (2014). Chapter Six - Vocal Complexity in Meerkats and Other Mongoose Species. In *Adv. Study Behav.*, vol. Volume 46 eds. M. Naguib L. Barrett H. J. Brockmann S. Healy J. C. Mitani T. J. Roper and W. S. Leigh), pp. 281-310: Academic Press.
- Masataka, N.** (1994). Lack of correlation between body size and frequency of vocalizations in young female Japanese macaques (*Macaca fuscata*). *Folia Primatol. (Basel)* **63**, 115-8.
- Meynhardt, H.** (1990). *Schwarzwild-Report: mein Leben unter Wildschweinen*: Neumann.
- Morton, E. S.** (1977). On the Occurrence and Significance of Motivation-Structural Rules in Some Bird and Mammal Sounds. *The American Naturalist* **111**, 855-869.
- Müller, C. A. and Manser, M. B.** (2008). Mutual recognition of pups and providers in the cooperatively breeding banded mongoose. *Anim. Behav.* **75**, 1683-1692.
- Owren, M. J., Seyfarth, R. M. and Cheney, D. L.** (1997). The acoustic features of vowel-like grunt calls in chacma baboons (*Papio cyncephalus ursinus*): Implications for production processes and functions. *J. Acoust. Soc. Am.* **101**, 2951-2963.
- Padilla de la Torre, M., Briefer, E. F., Reader, T. and McElligott, A. G.** (2015). Acoustic analysis of cattle (*Bos taurus*) mother-offspring contact calls from a source-filter theory perspective. *Appl. Anim. Behav. Sci.* **163**, 58-68.
- Persson, L.** (1985). Asymmetrical Competition: Are Larger Animals Competitively Superior? *The American Naturalist* **126**, 261-266.

- Peters, R. H.** (1983). The ecological implications of body size. . New York: Cambridge University Press.
- Pfefferle, D., West, P. M., Grinnell, J., Packer, C. and Fischer, J.** (2007). Do acoustic features of lion, *Panthera leo*, roars reflect sex and male condition? *J. Acoust. Soc. Am.* **121**, 3947-53.
- Pitcher, B. J., Harcourt, R. G. and Charrier, I.** (2012). Individual identity encoding and environmental constraints in vocal recognition of pups by Australian sea lion mothers. *Anim. Behav.* **83**, 681-690.
- Randall, J. A., McCowan, B., Collins, K. C., Hooper, S. L. and Rogovin, K.** (2005). Alarm signals of the great gerbil: Acoustic variation by predator context, sex, age, individual, and family group. *J. Acoust. Soc. Am.* **118**, 2706.
- RCoreTeam.** (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Reby, D., Joachim, J., Lauga, J., Lek, S. and Aulagnier, S.** (1998). Individuality in the groans of fallow deer (*Dama dama*) bucks. *J. Zool.* **245**, 79-84.
- 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.
- Rendall, C. A.** (1996). Social communication and vocal recognition in free-ranging rhesus monkeys (*Macaca mulatta*). University of California, Davis.
- Rendall, D., Kollias, S., Ney, C. and Lloyd, P.** (2005). Pitch (F0) and formant profiles of human vowels and vowel-like baboon grunts: The role of vocalizer body size and voice-acoustic allometry. *J. Acoust. Soc. Am.* **117**, 944.
- Riede, T. and Fitch, T.** (1999). Vocal tract length and acoustics of vocalization in the domestic dog (*Canis familiaris*). *J. Exp. Biol.* **202**, 2859-67.
- Robisson, P., Aubin, T. and Bremond, J.-C.** (1993). Individuality in the Voice of the Emperor Penguin *Aptenodytes forsteri*: Adaptation to a Noisy Environment. *Ethology* **94**, 279-290.
- Ryalls, J. H. and Lieberman, P.** (1982). Fundamental frequency and vowel perception. *J. Acoust. Soc. Am.* **72**, 1631-1634.
- Ryan, M. J.** (1980). Female mate choice in a neotropical frog. *Science* **209**, 523-525.
- Ryan, M. J.** (1985). The Tungara Frog: A Study in Sexual Selection and Communication: University of Chicago Press.
- Sanvito, S., Galimberti, F. and Miller, E. H.** (2007). Vocal signalling of male southern elephant seals is honest but imprecise. *Anim. Behav.* **73**, 287-299.
- Schön, P.-C., Puppe, B., Gromyko, T. and Manteuffel, G.** (1999). Common Features and Individual Differences in Nurse Grunting of Domestic Pigs (*Sus scrofa*): A Multi-Parametric Analysis. *Behaviour* **136**, 49-66.
- Shapiro, A. D.** (2009). Recognition of individuals within the social group: signature vocalizations. In *Handbook of Mammalian Vocalization*, (ed. S. M. Brudzynski), pp. 495-504. Oxford: Academic Press.
- Sokal, R. R. and Rohlf, F. J.** (1995). Biometry (3rd Edition). New-York: W. H. Freeman.

Tallet, C., Linhart, P., Policht, R., Hammerschmidt, K., Simecek, P., Kratinova, P. and Spinka, M. (2013). Encoding of situations in the vocal repertoire of piglets (*Sus scrofa*): a comparison of discrete and graded classifications. *PLoS ONE* **8**, e71841.

Taylor, C. R., Heglund, N. C. and Maloiy, G. M. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 1-21.

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

Townsend, S. W., Hollén, L. I. and Manser, M. B. (2010). Meerkat close calls encode group-specific signatures, but receivers fail to discriminate. *Anim. Behav.* **80**, 133-138.

Vannoni, E. and McElligott, A. G. (2008). Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS ONE* **3**, e3113.

Vignal, C. and Kelley, D. (2007). Significance of temporal and spectral acoustic cues for sexual recognition in *Xenopus laevis*. *Proc. R. Soc. Lond. B* **274**, 479-488.

Weissengruber, G., Forstenpointner, G., Peters, G., Kübber - Heiss, A. and Fitch, W. (2002). Hyoid apparatus and pharynx in the lion (*Panthera leo*), jaguar (*Panthera onca*), tiger (*Panthera tigris*), cheetah (*Acinonyx jubatus*) and domestic cat (*Felis silvestris f. catus*). *J. Anat.* **201**, 195-209.

Winter, B. (2013). Linear models and linear mixed effects models in R with linguistic applications. [<http://arxiv.org/pdf/1308.5499.pdf>].

Wyman, M. T., Mooring, M. S., McCowan, B., Penedo, M. C. T. and Hart, L. A. (2008). Amplitude of bison bellows reflects male quality, physical condition and motivation. *Anim. Behav.* **76**, 1625-1639.

Chapter 2 | Supplementary Material

Table S1. Raw data including general information on the subjects, acoustic and X-ray measurements. A- First series; B- Second series; C- Third series; D- Fourth series.

S1A-

General information				Acoustic analysis			X-ray measurements			Quality assessment		
Individual	Sex	Series	Age (days)	Weight (kg)	Formant1	Formant2	Skull (Prosthion-Basion)	Nasal tract (mm)	Vocal tract (mm)	Skull	Nasal	Oral
Baldur	Male	1	10	1.66	664.13	1758.26	83.646	118.407	85.450	1	1	1
Barbarossa	Male	1	10	2.08	678.93	1674.67	70.370	103.969	75.661	1	2	2
Belana	Female	1	10	2.17	691.50	1848.78	87.075	126.535	98.720	1	1	1
Bernadette	Female	1	10	2.21	712.28	1551.88	83.790	113.029	87.138	1	1	2
Blossom	Female	1	10	2.25	748.02	1673.72	69.322	105.853	80.934	1	2	1
Bolero	Male	1	10	2.145	668.20	1791.82	83.309	116.864	89.742	1	1	1
Bruno	Male	1	10	2.39	605.67	1422.14	88.168	127.514	99.057	1	1	1
Zafran	Male	1	10	2.37	797.26	1896.05	84.752	122.310	92.816	1	2	2
Zardo	Male	1	10	1.93	701.00	1775.36	80.920	118.665	89.612	1	2	2
Zeppelin	Male	1	10	2.4	790.74	2019.54	71.725	101.736	74.171	1	2	2
Zeus	Male	1	10	2.015			84.767	128.892	94.219	1	1	2
Zirbe	Female	1	10	1.69	670.68	1606.16	78.266	113.658	88.911	1	2	1
Zita	Female	1	10	1.18	869.05	1897.06	72.303	100.085	74.344	1	2	1
Zoltan	Male	1	10	1.7	745.44	1844.74	83.518	115.299	89.242	1	2	1
Radischen	Female	1	8	1.413	819.39	1512.40	80.051	111.853	84.004	1	2	1
Radomir	Male	1	8	1.445	806.21	1613.57	81.159	111.600	78.969	1	2	1
Raya	Female	1	8	1.69	799.02	1940.02	80.327	113.472	88.876	1	2	1
Ronon	Male	1	8	1.21	694.89	1929.09	72.139	103.986	73.246	1	2	2
Rosine	Female	1	8	1.23	727.74	1848.02	71.444	99.234	76.170	1	1	1
Rubina	Female	1	8	1.42	833.53	1749.00	81.080	113.958	86.511	1	3	2

Chapter 2 | Supplementary Material

S1B-

General information				Weight (Kg)	Acoustic analysis		X-ray measurements			Quality assessment		
Individual	Sex	Series	Age (days)		Formant1	Formant2	Skull (Prosthion- Basion)	Nasal tract (mm)	Vocal tract (mm)	Skull	Nasal	Oral
Baldur	Male	2	44	4.58	560.96	1486.08	91.435	148.730	113.324	1	3	2
Barbarossa	Male	2	44	5.79	556.34	1381.19	98.059	137.683	111.972	1	1	1
Belana	Female	2	44	5.64	555.17	1447.78	92.622	128.234	106.202	1	2	1
Bernadette	Female	2	44	6.35	566.69	1303.74						
Blossom	Female	2	44	6.49	553.56	1378.20						
Bolero	Male	2	44	7.03	503.05	1321.98						
Bruno	Male	2	44	7.62	510.18	1340.08						
Zafran	Male	2	44	6.88	521.85	1375.07	102.712	134.194	115.952	2	3	2
Zardoz	Male	2	44	5.59	555.23	1481.89	98.692	130.438	108.513	1	2	1
Zeppelin	Male	2	44	6.07	551.17	1290.92	107.662	139.401	119.893	1	1	1
Zeus	Male	2	44	6.32	557.55	1347.55						
Zirbe	Female	2	44	4.44	609.48	1494.06						
Zita	Female	2	44	4.75	650.02	1426.11	83.325	138.541	108.679	2	3	3
Zoltan	Male	2	44	4.73	569.06	1542.14						
Radischen	Female	2	42	5.94	626.76	1484.93						
Radomir	Male	2	42	6.89	531.56	1257.17						
Raya	Female	2	42	6.83	542.62	1397.85						
Ronon	Male	2	42	6.03	614.86	1432.39	109.204	145.293	121.290	1	2	1
Rosine	Female	2	42	5.73	530.36	1399.55	98.896	135.883	105.523	1	1	2
Rubina	Female	2	42	5.87	621.42	1471.98	98.228	133.135	107.981	1	2	1

S1C-

General information				Acoustic analysis		X-ray measurements			Quality assessment			
Individual	Sex	Series	Age (days)	Weight (kg)	Formant1	Formant2	Skull (Prosthion-Basion)	Nasal tract (mm)	Vocal tract (mm)	Skull	Nasal	Oral
Baldur	Male	3	73	6.1	494.42	1289.94						
Barbarossa	Male	3	73	9	536.76	1106.86						
Belana	Female	3	73	9.1	490.24	1207.15						
Bernadette	Female	3	73	9.9	493.73	1153.83	109.183	147.655	115.642	1	1	2
Blossom	Female	3	73	9.9	548.48	1198.46	110.630	157.838	116.943	1	2	2
Bolero	Male	3	73	10.9	482.02	1162.49	122.682	171.397	137.686	3	3	3
Bruno	Male	3	73	12.3	457.79	1208.22	122.216	173.353	129.544	1	2	2
Zafran	Male	3	73	10.5	454.94	1188.22						
Zardo	Male	3	73	8.3	493.64	1285.93						
Zeppelin	Male	3	73	9.7	467.72	1117.74						
Zeus	Male	3	73	10.9	480.74	1077.87	111.333	163.997	133.015	1	2	1
Zirbe	Female	3	73	6.5	532.97	1304.85	98.162	138.069	119.632	1	3	2
Zita	Female	3	73	7	524.78	1305.11						
Zoltan	Male	3	73	7.9	493.51	1306.05	108.382	150.513	130.552	1	1	1
Radischen	Female	3	71	9.4	532.11	1266.10	116.832	164.515	134.877	1	2	1
Radomir	Male	3	71	12.6	443.96	1094.49	118.680	161.182	135.940	1	1	1
Raya	Female	3	71	10.1	441.40	1224.28	116.096	155.489	133.885	1	2	1
Ronon	Male	3	71	10.4	517.49	1247.94						
Rosine	Female	3	71	9.8	457.27	1159.31						
Rubina	Female	3	71	9.1	525.61	1204.32						

S1D-

General information				Acoustic analysis		X-ray measurements			Quality assessment			
Individual	Sex	Series	Age (days)	Weight (kg)	Formant1	Formant2	Skull (Prosthion-Basion)	Nasal tract (mm)	Vocal tract (mm)	Skull	Nasal	Oral
Baldur	Male	4	131	11.4	408.56	1052.32	125.728	171.480	150.461	1	1	1
Barbarossa	Male	4	131	14.1	392.93	942.50	133.951	181.848	157.785	1	2	2
Belana	Female	4	131	14.4	425.60	934.07	130.850	181.462	157.357	1	1	1
Bernadette	Female	4	131	15.3	392.02	986.49	122.974	176.080	142.707	2	3	3
Blossom	Female	4	131	14.5	400.89	998.46	139.894	188.397	159.600	1	1	1
Bolero	Male	4	131	16.8	404.59	894.81	143.516	193.793	164.872	1	1	1
Bruno	Male	4	131	19.2	360.94	878.60	144.303	205.770	172.869	1	2	2
Zafran	Male	4	131	16.2	366.00	931.81	120.092	171.646	142.473	3	3	3
Zardož	Male	4	131	12.6	433.10	1016.43	134.348	171.495	154.420	1	1	1
Zeppelin	Male	4	131	15.3	371.46	890.85	142.507	186.494	163.508	1	1	1
Zeus	Male	4	131	17.8	360.02	891.22	151.286	206.186	168.956	1	2	2
Zirbe	Female	4	131	10.6	454.78	1016.59	112.709	163.600	145.414	2	1	1
Zita	Female	4	131	10.6	387.59	1017.10	104.430	141.312	117.729	2	3	3
Zoltan	Male	4	131	12.5	379.46	1032.51	124.723	180.392	151.988	1	1	1
Radischen	Female	4	129	14.6	406.22	969.82	141.477	189.400	164.369	1	1	1
Radomir	Male	4	129	18.7	345.89	833.80	146.427	188.054	172.062	1	2	1
Raya	Female	4	129	14.5	340.73	858.49	129.170	169.083	148.241	1	1	1
Ronon	Male	4	129	15.3	384.00	949.79	134.231	184.834	162.115	1	2	1
Rosine	Female	4	129	14.6	399.55	896.01	134.539	180.046	161.344	1	2	2
Rubina	Female	4	129	14.4	414.67	993.89	119.579	172.965	146.201	2	3	1

Chapter 2 | Supplementary Material

Table S2. Detailed composition of the initial models used in the analyses conducted on anatomical correlations, anatomical-acoustical correlations and acoustic allometry.

Analysis	Type	Predicted variable	Fixed effect	Random effect	Effect for which random slope is created
Anatomical dependence	LMM	Vocal Tract Length (VTL)	Weight (Log Wt) + Sex + Litter	Individual + Series	Weight (Log Wt)
Anatomical-acoustical dependence	LMM	Formant frequencies (Fn)	Vocal tract length (VTL) + Sex + Litter	Individual + Series	Vocal Tract Length (VTL)
Acoustic allometry	LMM	Formant frequencies (Fn)	Weight (Log Wt) + Sex + Litter	Individual + Series	Weight (Log Wt)

Chapter 2 | Supplementary Material

Table S3. Difference between expected vocal tract length (based on first formant value) and measured (both corrected and non-corrected) nasal and oral tract length. A – First series; B – Second and third series; C – Fourth series.

S3A-

Series	Individual	F1 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected Nasal tract length (+8.15%) (mm)	Measured Oral tract length (mm)	Corrected Oral tract length (+9.68%) (mm)	Difference Nasal/Expected (mm)	Difference Nasal/Expected (%)	Difference Corrected Nasal/Expected (%)	Difference Oral/Expected (mm)	Difference Oral/Expected (%)	Difference Corrected Oral/Expected (mm)	Difference Corrected Oral/Expected (%)
1	Baldur	664.13	131.752	118.407	128.057	85.450	93.722	13.345	10.13	3.695	46.302	35.14	38.030	28.87
1	Barbarossa	678.93	128.880	103.969	112.442	75.661	82.985	24.911	19.33	16.437	53.219	41.29	45.895	35.61
1	Belana	691.50	126.537	126.535	136.848	98.720	108.276	0.002	< 0.01	-10.311	27.817	21.98	18.261	14.43
1	Bernadette	712.28	122.846	113.029	122.241	87.138	95.573	9.817	7.99	0.605	35.708	29.07	27.273	22.20
1	Blossom	748.02	116.975	105.853	114.480	80.934	88.768	11.122	9.51	2.495	36.041	30.81	28.207	24.11
1	Bolero	668.20	130.948	116.864	126.388	89.742	98.429	14.084	10.76	4.560	41.206	31.47	32.519	24.83
1	Bruno	605.67	144.468	127.514	137.906	99.057	108.646	16.954	11.74	6.562	45.411	31.43	35.822	24.80
1	Zafran	797.26	109.751	122.310	132.278	92.816	101.801	-12.559	-11.44	-22.528	16.935	15.43	7.950	7.24
1	Zardoz	701.00	124.822	118.665	128.336	89.612	98.286	6.157	4.93	-3.514	35.210	28.21	26.536	21.26
1	Zeppelin	790.74	110.656	101.736	110.027	74.171	81.351	8.920	8.06	0.629	36.485	32.97	29.306	26.48
1	Zeus			128.892	139.397	94.219	103.339							
1	Zirbe	670.68	130.465	113.658	122.921	88.911	97.518	16.807	12.88	7.544	41.554	31.85	32.947	25.25
1	Zita	869.05	100.685	100.085	108.242	74.344	81.540	0.600	0.60	-7.557	26.341	26.16	19.145	19.01
1	Zoltan	745.44	117.380	115.299	124.696	89.242	97.881	2.081	1.77	-7.316	28.138	23.97	19.499	16.61
1	Radischen	819.39	106.787	111.853	120.969	84.004	92.136	-5.066	-4.74	-14.182	22.783	21.34	14.652	13.72
1	Radomir	806.21	108.532	111.600	120.695	78.969	86.613	-3.068	-2.83	-12.163	29.563	27.24	21.919	20.20
1	Raya	799.02	109.509	113.472	122.720	88.876	97.479	-3.963	-3.62	-13.211	20.633	18.84	12.030	10.99
1	Ronon	694.89	125.919	103.986	112.461	73.246	80.336	21.933	17.42	13.459	52.673	41.83	45.583	36.20
1	Rosine	727.74	120.236	99.234	107.322	76.170	83.543	21.002	17.47	12.914	44.066	36.65	36.693	30.52
1	Rubina	833.53	104.975	113.958	123.246	86.511	94.885	-8.983	-8.56	-18.271	18.464	17.59	10.090	9.61
Mean		738.09	119.585	113.346	122.584	85.390	93.655	7.058	5.63	-2.113	34.660	28.59	26.440	21.68
SD		70.62	11.485	8.927	9.655	8.000	8.775	10.990	9.16	11.227	11.066	7.49	11.324	8.22
Min		605.67	100.685	99.234	107.322	73.246	80.336	-12.559	-11.44	-22.528	16.935	15.43	7.950	7.24
Max		869.05	144.468	128.892	139.397	99.057	108.646	24.911	19.33	16.437	53.219	41.83	45.895	36.20

Chapter 2 | Supplementary Material

S3B-

Series	Individual	F1 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected (+8.15%) Nasal tract length (mm)	Corrected (+9.68%) Oral tract length (mm)	Difference Nasal / Expected (mm)	Difference Nasal / Expected (%)	Difference Corrected Nasal / Expected (mm)	Difference Corrected Nasal / Expected (%)	Difference Oral / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral / Expected (mm)	Difference Corrected Oral / Expected (%)
2	Baldur	560.96	155.982	148.730	160.851	124.294	7.252	4.65	-4.869	-3.12	42.658	27.35	31.688	20.32
2	Barbarossa	556.34	157.279	137.683	148.904	122.811	19.596	12.46	8.375	5.32	45.307	28.81	34.468	21.92
2	Belana	555.17	157.608	128.234	138.685	116.482	29.374	18.64	18.923	12.01	51.406	32.62	41.126	26.09
2	Zafran	521.85	167.672	134.194	145.131	127.176	33.478	19.97	22.541	13.44	51.720	30.85	40.495	24.15
2	Zardo	555.23	157.593	130.438	141.069	119.017	27.155	17.23	16.525	10.49	49.080	31.14	38.576	24.48
2	Zeppelin	551.17	158.753	139.401	150.762	131.499	19.352	12.19	7.991	5.03	38.860	24.48	27.254	17.17
2	Zita	650.02	134.612	138.541	149.832	119.199	-3.929	-2.92	-15.220	-11.31	25.933	19.27	15.413	11.45
2	Ronon	614.86	142.308	145.293	157.134	133.031	-2.985	-2.10	-14.827	-10.42	21.018	14.77	9.277	6.52
2	Rosine	530.36	164.981	135.883	146.957	115.738	29.098	17.64	18.023	10.92	59.458	36.04	49.243	29.85
2	Rubina	621.42	140.807	133.135	143.986	118.434	7.672	5.45	-3.179	-2.26	32.826	23.31	22.373	15.89
Mean		571.74	153.759	137.153	148.331	122.768	16.606	10.32	5.428	3.01	41.827	26.86	30.991	19.78
SD		42.14	10.836	6.313	6.828	6.127	13.761	8.54	14.098	9.24	12.208	6.48	12.466	7.11
Min		521.85	134.612	128.234	138.685	115.738	-3.929	-2.92	-15.220	-11.31	21.018	14.77	9.277	6.52
Max		650.02	167.672	148.730	160.851	133.031	33.478	19.97	22.541	13.44	59.458	36.04	49.243	29.85
3	Bernadette	493.73	177.223	147.655	159.689	126.836	29.568	16.68	17.534	9.89	61.581	34.75	50.387	28.43
3	Blossom	548.48	159.532	157.838	170.702	128.263	1.694	1.06	-11.170	-7.00	42.589	26.70	31.269	19.60
3	Bolero	482.02	181.528	171.397	185.366	151.014	10.131	5.58	-3.838	-2.11	43.842	24.15	30.514	16.81
3	Bruno	457.79	191.135	173.353	187.481	142.084	17.782	9.30	3.654	1.91	61.591	32.22	49.051	25.66
3	Zeus	480.74	182.010	163.997	177.363	145.891	18.013	9.90	4.647	2.55	48.995	26.92	36.119	19.84
3	Zirbe	532.97	164.174	138.069	149.322	131.212	26.105	15.90	14.853	9.05	44.542	27.13	32.962	20.08
3	Zoltan	493.51	177.301	150.513	162.780	143.189	26.788	15.11	14.521	8.19	46.749	26.37	34.112	19.24
3	Radischen	532.11	164.439	164.515	177.923	147.933	-0.076	-0.05	-13.484	-8.20	29.562	17.98	16.506	10.04
3	Radomir	443.96	197.091	161.182	174.318	149.099	35.909	18.22	22.772	11.55	61.151	31.03	47.992	24.35
3	Raya	441.40	198.234	155.489	168.161	146.845	42.745	21.56	30.073	15.17	64.349	32.46	51.389	25.92
Mean		490.67	179.267	158.401	171.310	141.237	20.866	11.33	7.956	4.10	50.495	27.97	38.030	21.00
SD		37.61	13.647	10.897	11.785	9.044	14.072	7.38	14.473	7.98	11.295	4.88	11.363	5.35
Min		441.40	159.532	138.069	149.322	126.836	-0.076	-0.05	-13.484	-8.20	29.562	17.98	16.506	10.04
Max		548.48	198.234	173.353	187.481	151.014	42.745	21.56	30.073	15.17	64.349	34.75	51.389	28.43

Chapter 2 | Supplementary Material

S3C-

Series	Individual	F1 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected (+8.15%) Nasal tract length (mm)	Measured Oral tract length (mm)	Corrected (+9.68%) Oral tract length (mm)	Difference Nasal / Expected (%)	Difference Nasal / Expected (mm)	Difference Nasal / Expected (mm)	Difference Nasal / Expected (%)	Difference Oral / Expected (%)	Difference Oral / Expected (mm)	Difference Corrected Oral / Expected (%)	Difference Corrected Oral / Expected (mm)
4	Baldur	408.56	214.167	171.480	185.456	150.461	165.026	19.93	42.687	28.712	13.41	63.706	29.75	49.142	22.95
4	Barbarossa	392.93	222.688	181.848	196.669	157.785	173.059	18.34	40.840	26.019	11.68	64.903	29.15	49.629	22.29
4	Belana	425.60	205.590	181.462	196.251	157.357	172.589	11.74	24.128	9.339	4.54	48.233	23.46	33.001	16.05
4	Bernadette	392.02	223.204	176.080	190.431	142.707	156.521	21.11	47.124	32.773	14.68	80.497	36.06	66.683	29.88
4	Blossom	400.89	218.266	188.397	203.751	159.600	175.049	13.68	29.869	14.515	6.65	58.666	26.88	43.217	19.80
4	Bolero	404.59	216.270	193.793	209.587	164.872	180.832	10.39	22.477	6.682	3.09	51.398	23.77	35.438	16.39
4	Bruno	360.94	242.426	205.770	222.540	172.869	189.603	15.12	36.656	19.885	8.20	69.557	28.69	52.823	21.79
4	Zafran	366.00	239.073	171.646	185.635	142.473	156.264	28.20	67.427	53.438	22.35	96.600	40.41	82.809	34.64
4	Zardoz	433.10	202.030	171.495	185.472	154.420	169.368	15.11	30.535	16.558	8.20	47.610	23.57	32.662	16.17
4	Zeppelin	371.46	235.557	186.494	201.693	163.508	179.336	20.83	49.063	33.863	14.38	72.049	30.59	56.221	23.87
4	Zeus	360.02	243.043	206.186	222.990	168.956	185.311	15.16	36.857	20.053	8.25	74.087	30.48	57.732	23.75
4	Zirbe	454.78	192.402	163.600	176.933	145.414	159.490	14.97	28.802	15.468	8.04	46.988	24.42	32.911	17.11
4	Zita	387.59	225.757	141.312	152.829	117.729	129.125	37.41	84.445	72.928	32.30	108.028	47.85	96.631	42.80
4	Zoltan	379.46	230.593	180.392	195.094	151.988	166.700	21.77	50.201	35.499	15.39	78.605	34.09	63.892	27.71
4	Radischen	406.22	215.402	189.400	204.836	164.369	180.280	12.07	26.002	10.566	4.91	51.033	23.69	35.122	16.31
4	Radomir	345.89	252.967	188.054	203.380	172.062	188.718	25.66	64.913	49.587	19.60	80.905	31.98	64.250	25.40
4	Raya	340.73	256.801	169.083	182.863	148.241	162.591	34.16	87.718	73.938	28.79	108.560	42.27	94.210	36.69
4	Ronon	384.00	227.867	184.834	199.898	162.115	177.808	18.89	43.033	27.969	12.27	65.752	28.86	50.060	21.97
4	Rosine	399.55	218.994	180.046	194.720	161.344	176.962	17.78	38.948	24.274	11.08	57.650	26.32	42.032	19.19
4	Rubina	414.67	211.009	172.965	187.062	146.201	160.353	18.03	38.044	23.948	11.35	64.808	30.71	50.656	24.01
Mean		391.45	224.705	180.217	194.905	155.224	170.249	19.52	44.488	29.801	12.96	69.482	30.65	54.456	23.94
SD		29.13	16.716	14.507	15.689	12.834	14.076	7.17	18.602	19.236	7.75	18.583	6.68	19.215	7.33
Min		340.73	192.402	141.312	152.829	117.729	129.125	10.39	22.477	6.682	3.09	46.988	23.46	32.662	16.05
Max		454.78	256.801	206.186	222.990	172.869	189.603	37.41	87.718	73.938	32.30	108.560	47.85	96.631	42.80
Mean		550.45	171.127	147.113	159.103	120.322	131.969	12.21	23.705	11.690	4.83	50.363	28.89	38.673	22.01
SD		152.53	45.860	30.233	32.697	30.649	33.616	9.77	21.466	20.208	10.61	20.333	6.65	18.710	7.29
Min		340.73	100.685	99.234	107.322	73.246	80.336	-11.44	-12.559	-22.528	-20.53	16.935	14.77	7.950	6.52
Max		869.05	256.801	206.186	222.990	172.869	189.603	37.41	87.718	73.938	32.30	108.560	47.85	96.631	42.80

Chapter 2 | Supplementary Material

Table S4. Difference between expected vocal tract length (based on second formant value) and measured (both corrected and non-corrected) nasal and oral tract length. A – First series; B – Second and third series; C – Fourth series.

S4A-

Series	Individual	F2 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected Nasal tract length (+8.15%) (mm)	Measured Oral tract length (mm)	Corrected Oral tract length (+9.68%) (mm)	Difference Nasal / Expected (mm)	Difference Nasal / Expected (%)	Difference Nasal / Expected (mm)	Difference Nasal / Expected (%)	Difference Oral / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral / Expected (mm)	Difference Corrected Oral / Expected (%)
1	Baldur	1758.26	149.295	118.407	128.057	85.450	93.722	30.888	20.69	21.238	14.23	63.845	42.76	55.574	37.22
1	Barbarossa	1674.67	156.747	103.969	112.442	75.661	82.985	52.778	33.67	44.305	28.27	81.086	51.73	73.762	47.06
1	Belana	1848.78	141.986	126.535	136.848	98.720	108.276	15.451	10.88	5.138	3.62	43.266	30.47	33.710	23.74
1	Bernadette	1551.88	169.149	113.029	122.241	87.138	95.573	56.120	33.18	46.909	27.73	82.011	48.48	73.576	43.50
1	Blossom	1673.72	156.836	105.853	114.480	80.934	88.768	50.983	32.51	42.356	27.01	75.902	48.40	68.068	43.40
1	Bolero	1791.82	146.499	116.864	126.388	89.742	98.429	29.635	20.23	20.111	13.73	56.757	38.74	48.070	32.81
1	Bruno	1422.14	184.581	127.514	137.906	99.057	108.646	57.067	30.92	46.674	25.29	85.524	46.33	75.935	41.14
1	Zafran	1896.05	138.446	122.310	132.278	92.816	101.801	16.136	11.66	6.168	4.45	45.630	32.96	36.645	26.47
1	Zardoz	1775.36	147.857	118.665	128.336	89.612	98.286	29.192	19.74	19.521	13.20	58.245	39.39	49.571	33.53
1	Zeppelin	2019.54	129.980	101.736	110.027	74.171	81.351	28.244	21.73	19.953	15.35	55.809	42.94	48.629	37.41
1	Zeus			128.892	139.397	94.219	103.339								
1	Zirbe	1606.16	163.433	113.658	122.921	88.911	97.518	49.775	30.46	40.512	24.79	74.522	45.60	65.916	40.33
1	Zita	1897.06	138.372	100.085	108.242	74.344	81.540	38.287	27.67	30.130	21.77	64.028	46.27	56.832	41.07
1	Zoltan	1844.74	142.297	115.299	124.696	89.242	97.881	26.998	18.97	17.601	12.37	53.055	37.28	44.416	31.21
1	Radschen	1512.40	173.566	111.853	120.969	84.004	92.136	61.713	35.56	52.597	30.30	89.562	51.60	81.430	46.92
1	Radomir	1613.57	162.683	111.600	120.695	78.969	86.613	51.083	31.40	41.988	25.81	83.714	51.46	76.070	46.76
1	Raya	1940.02	135.308	113.472	122.720	88.876	97.479	21.836	16.14	12.588	9.30	46.432	34.32	37.829	27.96
1	Ronon	1923.09	136.074	103.986	112.461	73.246	80.336	32.088	23.58	23.614	17.35	62.828	46.17	55.738	40.96
1	Rosine	1848.02	142.044	99.234	107.322	76.170	83.543	42.810	30.14	34.722	24.44	65.874	46.38	58.501	41.18
1	Rubina	1749.00	150.086	113.958	123.246	86.511	94.885	36.128	24.07	26.841	17.88	63.575	42.36	55.201	36.78
Mean		1755.38	150.802	113.346	122.584	85.390	93.655	38.274	24.90	29.103	18.78	65.877	43.35	57.656	37.87
SD		161.25	14.573	8.927	9.655	8.000	8.775	14.285	7.54	14.476	8.16	14.261	6.35	14.463	6.97
Min		1422.14	129.980	99.234	107.322	73.246	80.336	15.451	10.88	5.138	3.62	43.266	30.47	33.710	23.74
Max		2019.54	184.581	128.892	139.397	99.057	108.646	61.713	35.56	52.597	30.30	89.562	51.73	81.430	47.06

Chapter 2 | Supplementary Material

S4B-

Series	Individual	F2 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected (+8.15%) Nasal tract length (mm)	Measured Oral tract length (mm)	Corrected (+9.68%) Oral tract length (mm)	Difference Nasal / Expected (%)	Difference Corrected Nasal/ Expected (mm)	Difference Corrected Nasal/ Expected (%)	Difference Oral / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral/ Expected (mm)	Difference Corrected Oral/ Expected (%)
2	Baldur	1486.08	176.639	148.730	160.851	113.324	124.294	27.909	15.788	8.94	63.315	35.84	52.346	29.63
2	Barbarossa	1381.19	190.054	137.683	148.904	111.972	122.811	52.371	41.150	21.65	78.082	41.08	67.243	35.38
2	Belana	1447.78	181.313	128.234	138.685	106.202	116.482	53.079	42.627	23.51	75.111	41.43	64.830	35.76
2	Zafran	1375.07	190.899	134.194	145.131	115.952	127.176	56.705	45.768	23.98	74.947	39.26	63.723	33.38
2	Zardoz	1481.89	177.139	130.438	141.069	108.513	119.017	46.701	36.070	20.36	68.626	38.74	58.122	32.81
2	Zeppelin	1290.92	203.343	139.401	150.762	119.893	131.499	63.942	52.581	25.86	83.450	41.04	71.844	35.33
2	Zita	1426.11	184.067	138.541	149.832	108.679	119.199	45.526	34.235	18.60	75.388	40.96	64.868	35.24
2	Ronon	1432.39	183.260	145.293	157.134	121.290	133.031	37.967	26.125	14.26	61.970	33.82	50.229	27.41
2	Rosine	1399.55	187.560	135.883	146.957	105.523	115.738	51.677	40.603	21.65	82.037	43.74	71.823	38.29
2	Rubina	1471.98	178.331	133.135	143.986	107.981	118.434	45.196	34.346	19.26	70.350	39.45	59.898	33.59
Mean		1419.30	185.260	137.153	148.331	111.993	122.768	48.107	36.929	19.81	73.328	39.54	62.492	33.68
SD		60.06	8.140	6.313	6.828	5.586	6.127	10.045	10.356	5.01	7.236	2.89	7.370	3.17
Min		1290.92	176.639	128.234	138.685	105.523	115.738	27.909	15.788	8.94	61.970	33.82	50.229	27.41
Max		1486.08	203.343	148.730	160.851	121.290	133.031	63.942	52.581	25.86	83.450	43.74	71.844	38.29
3	Bernadette	1153.83	227.504	147.655	159.689	115.642	126.836	79.849	67.815	29.81	111.862	49.17	100.668	44.25
3	Blossom	1198.46	219.030	157.838	170.702	116.943	128.263	61.192	48.328	22.06	102.087	46.61	90.767	41.44
3	Bolero	1162.49	225.809	171.397	185.366	137.686	151.014	54.412	40.443	17.91	88.123	39.03	74.795	33.12
3	Bruno	1208.22	217.261	173.353	187.481	129.544	142.084	43.908	29.780	13.71	87.717	40.37	75.177	34.60
3	Zeus	1077.87	243.536	163.997	177.363	133.015	145.891	79.539	66.173	27.17	110.521	45.38	97.645	40.09
3	Zirbe	1304.85	201.172	138.069	149.322	119.632	131.212	63.103	51.851	25.77	81.540	40.53	69.960	34.78
3	Zoltan	1306.05	200.988	150.513	162.780	130.552	143.189	50.475	38.208	19.01	70.436	35.04	57.798	28.76
3	Radischen	1266.10	207.330	164.515	177.923	134.877	147.933	42.815	29.407	14.18	72.453	34.95	59.397	28.65
3	Radomir	1094.49	239.838	161.182	174.318	135.940	149.099	78.656	65.519	27.32	103.898	43.32	90.739	37.83
3	Raya	1224.28	214.411	155.489	168.161	133.885	146.845	58.922	46.250	21.57	80.526	37.56	67.566	31.51
Mean		1199.66	219.688	158.401	171.310	128.772	141.237	61.287	48.378	21.85	90.916	41.20	78.451	35.50
SD		79.48	14.730	10.897	11.785	8.245	9.044	14.125	14.442	5.63	15.241	4.84	15.524	5.31
Min		1077.87	200.988	138.069	149.322	115.642	126.836	42.815	29.407	13.71	70.436	34.95	57.798	28.65
Max		1306.05	243.536	173.353	187.481	137.686	151.014	79.849	67.815	29.81	111.862	49.17	100.668	44.25

Chapter 2 | Supplementary Material

S4C-

Series	Individual	F2 (Hz)	Expected vocal tract length (mm)	Measured Nasal tract length (mm)	Corrected Nasal tract length (+8.15%) (mm)	Measured Oral tract length (mm)	Corrected Oral tract length (+9.68%) (mm)	Difference Nasal / Expected (%)	Difference Corrected Nasal / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral / Expected (mm)	Difference Nasal / Expected (%)	Difference Corrected Nasal / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral / Expected (mm)	Difference Nasal / Expected (%)	Difference Corrected Nasal / Expected (mm)	Difference Oral / Expected (%)	Difference Corrected Oral / Expected (mm)
4	Baldu	1052.32	249.450	171.480	185.456	150.461	165.026	77.970	31.26	63.994	25.65	98.989	39.68	84.424	33.84	98.989	39.68	84.424	33.84
4	Barbarossa	942.50	278.514	181.848	196.669	157.785	173.059	96.666	34.71	81.846	29.39	120.729	43.35	105.456	37.86	120.729	43.35	105.456	37.86
4	Belana	934.07	281.029	181.462	196.251	157.357	172.589	99.567	35.43	84.778	30.17	123.672	44.01	108.440	38.59	123.672	44.01	108.440	38.59
4	Bernadette	986.49	266.094	176.080	190.431	142.707	156.521	90.014	33.83	75.663	28.43	123.387	46.37	109.573	41.18	123.387	46.37	109.573	41.18
4	Blossom	998.46	262.904	188.397	203.751	159.600	175.049	74.507	28.34	59.152	22.50	103.304	39.29	87.855	33.42	103.304	39.29	87.855	33.42
4	Bolero	894.81	293.358	193.793	209.587	164.872	180.832	99.565	33.94	83.771	28.56	128.486	43.80	112.526	38.36	128.486	43.80	112.526	38.36
4	Bruno	878.60	298.771	205.770	222.540	172.869	189.603	93.001	31.13	76.231	25.51	125.902	42.14	109.168	36.54	125.902	42.14	109.168	36.54
4	Zafran	931.81	281.711	171.646	185.635	142.473	156.264	110.065	39.07	96.075	34.10	139.238	49.43	125.446	44.53	139.238	49.43	125.446	44.53
4	Zardo	1016.43	258.256	171.495	185.472	154.420	169.368	86.761	33.59	72.784	28.18	103.836	40.21	88.888	34.42	103.836	40.21	88.888	34.42
4	Zeppelin	890.85	294.662	186.494	201.693	163.508	179.336	108.168	36.71	92.969	31.55	131.154	44.51	115.327	39.14	131.154	44.51	115.327	39.14
4	Zeus	891.22	294.540	206.186	222.990	168.956	185.311	88.354	30.00	71.549	24.29	125.584	42.64	109.229	37.08	125.584	42.64	109.229	37.08
4	Zirbe	1016.59	258.215	163.600	176.933	145.414	159.490	94.615	36.64	81.282	31.48	112.801	43.68	98.725	38.23	112.801	43.68	98.725	38.23
4	Zita	1017.10	258.086	141.312	152.829	117.729	129.125	116.774	45.25	105.257	40.78	140.357	54.38	128.960	49.97	140.357	54.38	128.960	49.97
4	Zoltan	1032.51	254.234	180.392	195.094	151.988	166.700	73.842	29.04	59.140	23.26	102.246	40.22	87.534	34.43	102.246	40.22	87.534	34.43
4	Radischen	969.82	270.668	189.400	204.836	164.369	180.280	81.268	30.03	65.832	24.32	106.299	39.27	90.388	33.39	106.299	39.27	90.388	33.39
4	Radomir	833.80	314.825	188.054	203.380	172.062	188.718	126.771	40.27	111.445	35.40	142.763	45.35	126.108	40.06	142.763	45.35	126.108	40.06
4	Raya	858.49	305.771	169.083	182.863	148.241	162.591	136.688	44.70	122.907	40.20	157.530	51.52	143.180	46.83	157.530	51.52	143.180	46.83
4	Ronon	949.79	276.378	184.834	199.898	162.115	177.808	91.544	33.12	76.480	27.67	114.263	41.34	98.570	35.67	114.263	41.34	98.570	35.67
4	Rosine	896.01	292.966	180.046	194.720	161.344	176.962	112.920	38.54	98.246	33.53	131.622	44.93	116.004	39.60	131.622	44.93	116.004	39.60
4	Rubina	993.89	264.115	172.965	187.062	146.201	160.353	91.150	34.51	77.053	29.17	117.914	44.64	103.761	39.29	117.914	44.64	103.761	39.29
Mean		949.28	277.727	180.217	194.905	155.224	170.249	97.510	35.01	82.823	29.71	122.504	44.04	107.478	38.62	122.504	44.04	107.478	38.62
SD		63.72	18.874	14.507	15.689	12.834	14.076	16.820	4.75	17.190	5.14	15.530	4.02	15.733	4.41	15.530	4.02	15.733	4.41
Min		833.80	249.450	141.312	152.829	117.729	129.125	73.842	28.34	59.140	22.50	98.989	39.27	84.424	33.39	98.989	39.27	84.424	33.39
Max		1052.32	314.825	206.186	222.990	172.869	189.603	136.688	45.25	122.907	40.78	157.530	54.38	143.180	49.97	157.530	54.38	143.180	49.97
Mean		1330.97	211.344	147.113	159.103	120.322	131.969	63.921	28.97	51.907	23.18	90.579	42.57	78.889	37.01	90.579	42.57	78.889	37.01
SD		352.85	55.463	30.233	32.697	30.649	33.616	29.180	7.25	27.415	7.84	28.088	5.06	25.841	5.55	28.088	5.06	25.841	5.55
Min		833.80	129.980	99.234	107.322	73.246	80.336	15.451	10.88	5.138	3.62	43.266	30.47	33.710	23.74	43.266	30.47	33.710	23.74
Max		2019.54	314.825	206.186	222.990	172.869	189.603	136.688	45.25	122.907	40.78	157.530	54.38	143.180	49.97	157.530	54.38	143.180	49.97

Chapter 3 |

Playback experiments and signal re-synthesis:

Exploring the perceptual and behavioral role of specific acoustic features in red deer vocal communication

This chapter is an article published in a peer-reviewed journal.

Citation: **Garcia M**, Charlton BD, Wyman MT, Fitch WT & Reby D. 2013. Do red deer stags (*Cervus elaphus*) use roar fundamental frequency (F0) to assess rivals? PLoS ONE 8(12): e83946.

Do Red Deer Stags (*Cervus elaphus*) Use Roar Fundamental Frequency (F0) to Assess Rivals?

Maxime Garcia¹, Benjamin D. Charlton², Megan T. Wyman², W. Tecumseh Fitch¹, David Reby^{2*}

¹ Department of Cognitive Biology, University of Vienna, Vienna, Austria, ² School of Psychology, University of Sussex, Brighton, United Kingdom

Abstract

It is well established that in humans, male voices are disproportionately lower pitched than female voices, and recent studies suggest that this dimorphism in fundamental frequency (F0) results from both intrasexual (male competition) and intersexual (female mate choice) selection for lower pitched voices in men. However, comparative investigations indicate that sexual dimorphism in F0 is not universal in terrestrial mammals. In the highly polygynous and sexually dimorphic Scottish red deer *Cervus elaphus scoticus*, more successful males give sexually-selected calls (roars) with higher minimum F0s, suggesting that high, rather than low F0s advertise quality in this subspecies. While playback experiments demonstrated that oestrous females prefer higher pitched roars, the potential role of roar F0 in male competition remains untested. Here we examined the response of rutting red deer stags to playbacks of re-synthesized male roars with different median F0s. Our results show that stags' responses (latencies and durations of attention, vocal and approach responses) were not affected by the F0 of the roar. This suggests that intrasexual selection is unlikely to strongly influence the evolution of roar F0 in Scottish red deer stags, and illustrates how the F0 of terrestrial mammal vocal sexual signals may be subject to different selection pressures across species. Further investigations on species characterized by different F0 profiles are needed to provide a comparative background for evolutionary interpretations of sex differences in mammalian vocalizations.

Citation: Garcia M, Charlton BD, Wyman MT, Fitch WT, Reby D (2013) Do Red Deer Stags (*Cervus elaphus*) Use Roar Fundamental Frequency (F0) to Assess Rivals? PLoS ONE 8(12): e83946. doi:10.1371/journal.pone.0083946

Editor: Antje Engelhardt, German Primate Centre, Germany

Received: June 11, 2013; **Accepted:** November 8, 2013; **Published:** December 30, 2013

Copyright: © 2013 Garcia et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding organization: University of Vienna. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: David Reby serves as an editor for this journal. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: reby@sussex.ac.uk

Introduction

A key objective of research in animal vocal communication is to identify the origin, nature and function of the information contained in acoustic signals, in order to understand how selection pressures have shaped their evolution [1]. Sexually-selected male calls are displays typically given during the reproductive period [2]. In vertebrates, these calls are often multi-component signals [3,4], which encode information on long term, static (size [5–11]; sex [9,12]; identity [13–17]) or shorter term, dynamic (arousal, 'motivational' state [18–20]; hormonal levels [8,21,22]; physical condition [20,23]) attributes of callers. Studies of these calls in terrestrial mammals indicate that this information may be used in both male competition and/or female mate choice contexts in a wide range of species (baboon: [24], bison: [11], fallow deer: [7], orangutans: [25]) with a handful of experimental studies actually demonstrating such a function (koala: [26], red deer: [27–29]).

The generalization of the source-filter theory of voice production [30] to non-human vocal signals [31–33] has led to considerable advances in our understanding of the acoustic structure of mammalian calls (reviewed by Taylor & Reby, [34]). According to this theory, voiced vocalizations result from a two-step production process. First, a source signal (the glottal wave) is generated by vibrations of the vocal folds in the larynx. When these vibrations are periodic, their rate determines the fundamental frequency (F0), and the perceived pitch of the radiated

vocalization. This glottal wave subsequently travels through the supra-laryngeal cavities of the vocal tract that act as a filter, adding broadband resonance frequencies or 'formants' to the spectral envelope of the emitted vocalization. Because F0 and formants are produced independently, and both subject to various biomechanical constraints, both parameters have the potential to carry reliable information about the signaler [34,35].

The function of formants in sexually-selected male calls has recently received considerable attention, with studies identifying negative correlations between formant frequency spacing (a measure of formant scaling in the frequency domain) and body size in marsupials (koala: [10]), ungulates (red deer: [5], fallow deer: [7], bison: [11]), carnivores (elephant seals: [6], giant panda: [9]), and primates (macaques: [33], colobus monkeys: [36]), due to the allometric relationship between formants, vocal tract length (VTL), and overall body size [33,35]. In contrast, research on the determination and function of F0 variation is less conclusive. A negative correlation between body size and mean F0 is expected across a wide range of mammal species (e.g. [37,38]) because animals with larger and heavier vocal folds should produce calls with lower F0 [39]. However F0 is typically poorly related, or not related at all, to body size variation within multiple species of adult mammals (baboons: [40], fallow deer: [7], humans: [41], Japanese macaques: [42], lions: [43], red deer: [5]; but see [9,44,45]).

Deer have proved to be a very useful model for testing hypotheses on the variation and function of spectral components

in sexually-selected male vocalizations. During the breeding season (or rut), red deer (*Cervus elaphus*) stags give loud, conspicuous roars [46]. While roaring, stags pull their larynx down towards the sternum, thereby extending their vocal tract and lowering the formants (or vocal tract resonances) of their roars [47,48]. The minimum formant frequency values and spacing, achieved when the larynx is fully retracted to the sternum and the vocal tract fully extended, provide reliable cues to body size [5,47] which are attended to by both male and female conspecifics: resynthesized roars with formants indicative of larger individuals elicit stronger responses from potential male rivals [27] and are preferred by oestrous females [49].

Although F0 is a highly salient and individually distinctive feature of roars [50], it is unlikely to function as an index of body size, because neither vocal fold length nor F0 are correlated with body weight in adult stags [5,48]. However recent playback experiments indicate that red deer hinds prefer male roars with relatively high F0 [29]. While the communicative value of F0 in red deer roars remains unclear, it has been suggested that it may function as an index of subglottal pressure [51], with high F0s associated with increased activity, arousal or superior physical condition [5,52,53].

In order to investigate the hypothesis that roar F0 plays such a role in the context of male-male competition in red deer, we conducted playback experiments testing the reaction of harem-holding adult male red deer to re-synthesized stimuli with different median F0s, in their natural environment, mimicking the intrusion of an unfamiliar adult male in the close vicinity. More specifically, we examined the behavioural response of stags to playbacks of roaring stimuli with median F0s of 70 Hz, 100 Hz, 130 Hz or 160 Hz. We predicted that stags would respond more strongly to roars characterized by higher F0 that may indicate more highly motivated, threatening opponents. While the effect of F0 on perceived attractiveness in male human voice has been thoroughly investigated using experimental approaches [54–56], to our knowledge the current study is the first playback experiment to investigate the potential function of F0 in the context of male competition in a non-human mammal.

Materials and Methods

Authorizations and Approvals

Permissions to run experiments and park off roads within Richmond Park were granted by Simon Richards, Park Superintendent of the Royal Parks of London. The playback protocol used in this experiment was reviewed and approved by the Ethical Review Committee of the University of Sussex (UoS ERC Reby/Wyman 20/10/09).

Study Site and Animals

Playback experiments were conducted at Richmond Park, London, UK, during the 2012 autumn breeding season (from October 1st until October 19th), where 8 resident red deer stags served as subjects. The red deer population at Richmond Park is semi-captive and culled on a yearly basis.

Playback Stimuli

Red deer stags produce two distinct types of roars during the mating season: common roars and harsh roars [5]. The most frequent type, the common roar, is defined by a mostly tonal structure and slow amplitude onsets and offsets, as well as a pronounced formant modulation as the vocal tract is lengthened during the course of the vocalization [47]. Harsh roars are less frequent, and typically given after intense herding of females and/

or during vocal contests with other males [5,48]. They are defined by deterministic chaos (non-periodic vibrations of the vocal folds), abrupt amplitude onsets and offsets and reduced formant modulation as the vocal tract is fully extended before and throughout the vocalization [5]. Because harsh roars typically do not have a discernible fundamental frequency [5], common roars were used as stimuli in our playback experiments.

The common roars that were used to create the playback stimuli were recorded by DR from 7 farmed adult Scottish red deer stags (*Cervus elaphus scoticus*) at Redon experimental farm in France and in New Zealand farms. These stags were comparable in body size to adult stags found in Richmond Park, and were unfamiliar to the tested stags. Our stimuli were resynthesized using the Pitch Synchronous Overlap and Add (PSOLA) [57] algorithm in Praat (version 5.3.12, [58]). PSOLA enables the independent modification of the fundamental frequency (F0) whilst leaving all other acoustic features unchanged (see Fig. 1). The median F0 of the roar stimuli was set at 70 Hz, 100 Hz, 130 Hz and 160 Hz using the “change gender” command in Praat and the following settings: pitch floor = 30 Hz, pitch ceiling = 300 Hz, formant shift = 1, pitch range factor = 1 and duration factor = 1. These values cover the range of F0 observed in farmed and free-ranging populations of Scottish red deer stags (see [5]; range = 66 Hz to 168 Hz, average = 112 Hz) and have already been shown to elicit preferences in oestrous hinds in previous experiments [29]. After the re-synthesis procedure, the maximum amplitude of each bout was normalized to 99% peak (using Audacity version 2.0.1).

A total of 28 different playback sequences were created (4 fundamental frequency variants for each of the 7 exemplar stags). Each playback sequence consisted of two roar bouts (based on exemplars produced by the same stag), separated by 20 seconds of silence. Bouts were composed of 1 to 3 different roars (average $\pm$ SD = 2.06 ± 0.56) and thus lasted between 2.06 and 8.72 seconds (average $\pm$ SD = 4.39 ± 1.51 s), representing the natural variation of this parameter [5].

Playback Design and Procedure

Each of the 8 focal stags were presented with all 4 F0 variants from the same exemplar, resulting in a total of 32 playback experiments (i.e. a total of four playbacks per stag). Using re-synthesis enables us to present individual stags with several F0 variants from each of the exemplars, ensuring that only F0 varies between the four stimuli presented to one given stag. It also enables us to present different exemplars to different focal stags, thereby maximizing the external validity of our observations by ensuring that our stimuli cover the natural variability of roars. In other words, re-synthesis preserves independence between F0, the parameter of interest, and other untouched acoustic parameters (e.g., duration, formant frequency, F0 contour, intensity contour etc.). The variation in these unmodified parameters is fixed (and presumably inter-dependent) within subjects, and represents the natural variation between subjects. Presentation order was alternated using a Latin square design. One of the exemplars was used twice in the experiment (played to two different stags). To prevent stags from habituating to the playback procedure, sequences were played back a minimal of 2 hours apart, and a maximum of 3 sequences were presented to each stag on a given day (a level of encounters consistent with the size and density of the red deer population at Richmond Park, where stags are likely to interact with several intruders within a day during the rut – pers. obs.).

Playback trials were conducted throughout the day when a stable harem was located (defined as a mature stag and at least 4 hinds (number of hinds average $\pm$ SD = 17.63 ± 9.62)). The focal

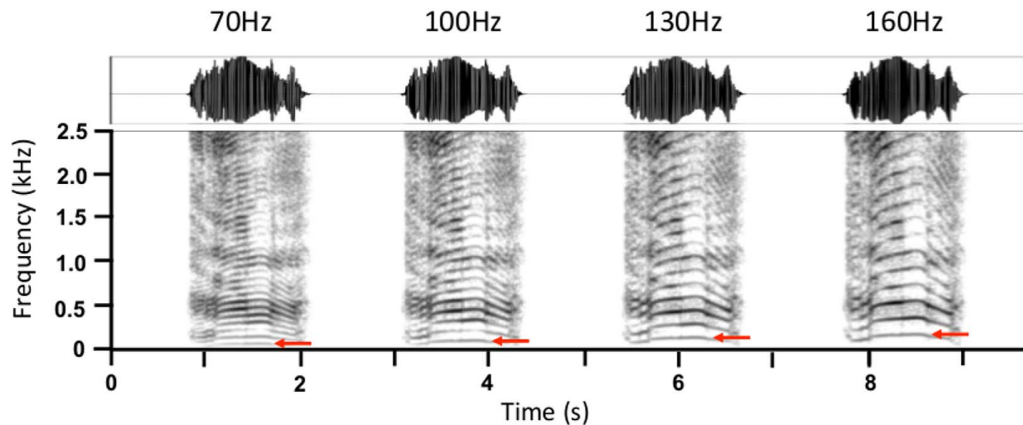


Figure 1. Spectrograms of resynthesized roars from one of the male exemplars showing the four F0 variants. F0 (indicated by the red arrow) was re-scaled to median values of 70 Hz, 100 Hz, 130 Hz and 160 Hz. All other acoustic parameters (duration, intensity contour, amplitude, formant frequencies etc.) remained unchanged.
doi:10.1371/journal.pone.0083946.g001

stag had to display an overall behaviour typical of the rutting period (e.g., some roaring activity, herding and defense against other males). Playback trials were initiated when the harem holder was not engaged in a direct interaction with another stag, and not disrupted by new hinds entering the harem at least for the 15 minutes preceding the playback sequence. The experimenter placed a loudspeaker (Anchor Audio Liberty 6000HIC) 40–70 m away from the stag at an amplitude of 98 dB, measured at 1 m away from the source. The loudspeaker was connected to a MacBook Pro (Mac OS X, version 10.7.4) using a 50-m co-axial cable. This allowed the experimenter to initiate playback sequences while standing perpendicular to the “Stag-Loudspeaker” axis, and in doing so, minimize disturbance around the speaker position. The focal stag’s response was recorded using tripod-mounted Sony (HDR TG3) or Canon (LEGRIA FS200) video camcorders, from the beginning of the sequence until 5 minutes after the last bout terminated.

Behavioural and Statistical Analyses

The video sequences were analyzed frame-by-frame (frame = 0.04 s) using Gamebreaker v7.5.5 software (SportsTec, Sydney), starting when the first bout was initiated, until 5 minutes after the second bout was terminated. To quantify the behavioural responses of subjects to our stimuli we measured the number of common roars (CR), number of harsh roars (HR), time spent looking towards the speaker (LK), time spent walking towards the speaker (WK), latency to look towards the speaker (LTLK), and latency to roar back (LTR).

Behaviours were characterized as “oriented towards the loudspeaker” when the direction (looking/walking) was judged to be at a maximum of 30 degrees from the stag-loudspeaker axis. The behavioural responses were measured by MG. An independent observer double-coded 10% of the trials. The inter-observer agreement of 100% confirmed the reliability of the coding procedure.

Because the response variables were not normally distributed (Kolmogorov-Smirnov test: $P < 0.05$), with the exception of CR ($P = 0.181$), we used non-parametric Friedman tests (with exact statistics, appropriate to our sample size [59]) to examine the effect of the F0 variant and order of presentation on each of the dependent variables characterizing the stag’s behavioural response. P-values were corrected for multiple testing following

Benjamini & Hochberg [60]. All the statistical tests were computed using SPSS v.19, significance levels were set at $p = 0.05$ and two-tailed statistics are reported.

Results

During the playback experiments, males typically interrupted their current behaviour by looking (100%; $n = 32/32$ trials) and roaring back (94%; $n = 30/32$ trials) at the loudspeaker. Stags also moved towards the loudspeaker in half of the trials (50%; $n = 16/32$). Raw scores for all the response variables across the 32 playback trials are reported in Table S1. Friedman comparisons with Benjamini-Hochberg correction for multiple testing showed that the effect of presentation order was non significant for all the tested variables (all $P > 0.05$), indicating that stags did not significantly habituate across the presentation of the four F0 variants. Friedman comparisons with Benjamini-Hochberg correction for multiple testing showed that the F0 variant did not have a significant effect on any of the response variables (with corrected p-values: CR: Chi-Square ($N = 8$, $df = 3$) = 2.520, $P = 1$; HR: Chi-Square ($N = 8$, $df = 3$) = 1.056, $P = 0.974$; LK: Chi-Square ($N = 8$, $df = 3$) = 0.750, $P = 0.890$; WK: Chi-Square ($N = 8$, $df = 3$) = 1.544, $P = 1$; LTLK: Chi-Square ($N = 8$, $df = 3$) = 9.150, $P = 0.132$; LTR: Chi-Square ($N = 6$, $df = 3$) = 1.400, $P = 1$; see Fig. 2). Finally, a separate test confirmed that F0 variant did not have an effect on the cumulated number of Common Roars and Harsh Roars (CR+HR: (Chi-Square ($N = 8$, $df = 3$) = 0.5, $P = 0.930$)).

Discussion

We found that the strength of the agonistic response of free-ranging adult Scottish red deer stags to playbacks of re-synthesized roars was not affected by the F0 of the roar. Neither the vocal response (latency and number of roars) nor the approach response (walking towards speaker) nor the stag’s attention (latency to look and looking duration) differed between F0 variants. This is in contrast with similar experiments testing the effect of formant frequencies in red deer stags, where roars with lower formant frequencies (indicating larger individuals) provoked stronger responses from focal stags [27]. It is important to acknowledge that our results are based on observations involving a relatively small number of focal stags, raising the possibility that our design

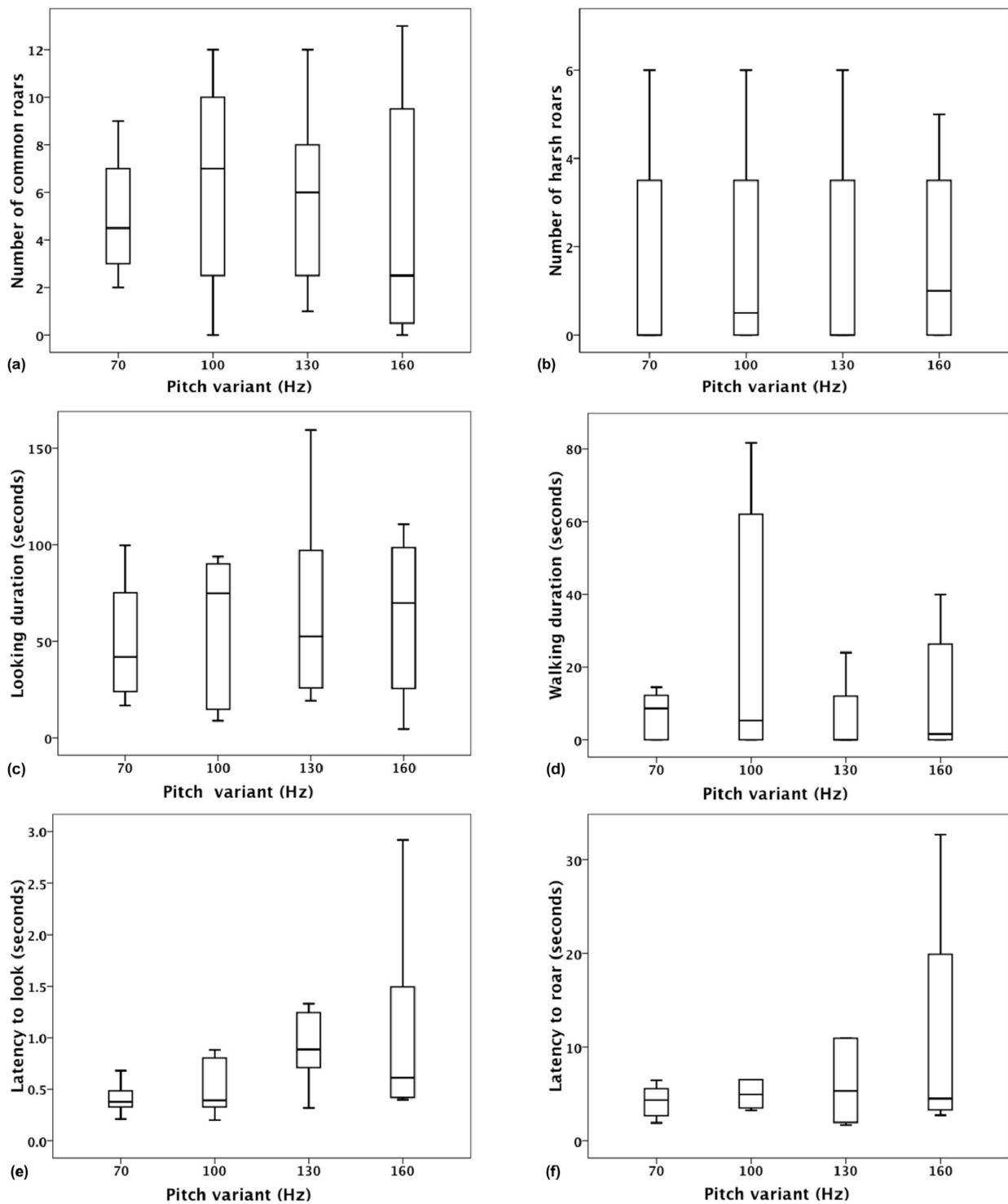


Figure 2. Behavioural responses of focal stags to playback experiments. Boxplots (with first, second (median), third quartiles, and range; outliers are not represented) illustrating the effect of F0 variant on the key behavioural variables characterizing the stag's response to playbacks (Friedman test, p-values adjusted following Benjamini-Hochberg correction; $N = 8$); number of common (A) and harsh (B) roars, time spent looking (C) and walking (D) towards the loudspeaker, latency to look at the speaker (E) and latency to roar back after stimulus presentation (F).
doi:10.1371/journal.pone.0083946.g002

may lack statistical power to highlight very small-sized effects. However we did not identify any noticeable trend on any of the response variables across the F0 variants (with the exception of

latency to look, which was significant ($P = 0.022$) prior to correction for multiple testing). While further investigations involving a larger sample size may be required to investigate the

possibility of very small effects of pitch variation on stag's reaction, the present results enable us to exclude a strong role for roar F0 in determining the dynamic of male agonistic interactions. This observation is consistent with anatomical and acoustical data in this species: in adult male Scottish red deer, neither vocal fold length [48] nor F0 [5] are correlated with adult body size, a key factor in determining the outcome of male contests [61]. Previous studies that have investigated the role of roaring during intrasexual competition have shown that stags assess the fighting ability of their opponents using roaring rate [62] as well as body size using the roar's vocal tract resonances (formants) [27]. Our results show that F0 in the roars of intruding red deer stags does not play an equivalent role.

Studies of acoustic variation and playback experiments have confirmed the role of F0 as an index of body size in male competition in several groups of vertebrates (frogs [63,64], birds [65] and mammals [9,44]). However, male bullfrogs (*Rana catesbeiana*) do not respond differently to F0 variation in rival male vocalizations despite strong negative correlations between F0, body size and associated fighting ability [66]. In contrast, in many mammal species, including humans, F0 is not significantly correlated with adult body size (baboons: [40], fallow deer: [7], humans: [41], Japanese macaques: [42], crested macaques: [67], lions: [43], red deer: [5], koalas: [10]). However, F0 appears to convey useful non-size information across a wide range of vertebrate species: established dominance in fallow deer [7] and crested macaques [67], perceived dominance in humans [68–70], hormonal state in humans [71] and zebra finches [72], and resource-holding potential in several species of birds [73,74].

In addition, human salivary testosterone is a negative predictor of F0 in males [71,75], and males with lower pitch voices are perceived as more physically and socially dominant [69], more attractive [54–56] and better leaders [76], corroborating observations showing that human males with lower F0 appear to have higher mating [56] and reproductive [77] success. The positive effect of testosterone on vocal fold length (sheep: [78], humans: [75]), which consequently lowers F0 [39], is likely to be a key underlying cause of the negative correlation between maleness-related traits and F0.

A direct consequence of sexual selection for a lower F0 in male vocalizations is that in many species, including humans, males have a disproportionately lower F0 than females (baboons: [79], fallow deer: [15], lions: [43], humans: [80]). In contrast, in Scottish red deer, there is no sexual dimorphism in call F0 between males and females [29]. In fact, after correcting for body-size differences between the sexes, male red deer may have shorter vocal folds and higher pitched vocalizations than expected for their size, relative to females (Reby, unpublished data). This suggests that, unlike antler strength, which is positively correlated with testosterone levels [81], vocal fold length and F0 may be independent of androgen levels in Scottish red deer. These observations are consistent with the documented positive correlation that exists between male roaring minimum F0 and reproductive success in this subspecies [5]. Moreover, while experiments investigating responses to F0 variants in perioestrous females failed to identify differential responses [82,83], a study carried out on oestrous females highlighted their preference for higher pitched roars [29]. Besides, harems are very unstable [46,84] and females often leave their current harem to visit other males when in oestrus [84]. Altogether, this suggests that in Scottish red deer, the relatively high F0 of male roars may be a consequence of intersexual, rather than intrasexual, selection.

More generally, polygynous deer are characterized by strong interspecific variation in the F0 of male sexually-selected

vocalizations [48], which is clearly independent of interspecific variation in body size. Fallow deer (*Dama dama*), Corsican deer (*Cervus elaphus corsicanus*), and Japanese sika deer (*Cervus nippon nippon*), three polygynous species smaller in size relative to red deer, illustrate both interspecific and intraspecific F0 variation: fallow deer males have a large descended larynx and produce an almost infrasonic low pitched groan, with lower F0 (F0mean = 28.2 Hz, [15]) than females (F0mean = 365 Hz, [85]), both sexes of Corsican deer produce very low-pitched calls (F0mean = 40.1 Hz for male calls and 86.7 Hz for female calls, [86]), and male sika deer produce a high-pitched whistle with higher mean F0 than females (mean F0 = 1187 Hz for males and 968 Hz for females, [87]). This variation, both in terms of range, and in terms of the direction of sex dimorphism, suggests that very different selection pressures operate on the fundamental frequency of sexually-selected calls in polygynous deer.

It has been proposed that high F0 calls may be indices of physical capacity [5,52,53]. Indeed the production of higher frequencies may require higher subglottal pressure [51,88] and stronger muscular contraction [53], and might therefore provide relevant information on the caller's condition. High frequency signals have also been correlated with improved glottal efficiency and radiation [53], which could in turn improve the active space of the vocalization and consequently increase mating opportunities for males that produce them. Nevertheless, a high fundamental frequency will reduce the density of the harmonics sampling the formant envelope, decreasing the salience of the formant structure [89], potentially affecting the ability of the signal to broadcast size-related information in formant frequencies [83]. Finally, independently of its causes, it is unclear how selection for high F0 in male vocal sexual signals is compatible with a function of F0 as an index of androgen levels in the same species.

In conclusion, our results have shown that the strength of Scottish red deer stags' agonistic responses to roars of simulated intruders is not affected by the F0 of the re-synthesized roar. This suggests that F0 does not play a role in red deer male competition (at least in this subspecies) and that in the male-male context, size-related formant variation is the key information conveyed in the acoustic structure of roars. In order to better understand how sexual selection operates on the different spectral components of vocal sexual signals, leading to the extraordinary acoustic diversity observed in polygynous deer, future work should involve the playback of re-synthesized vocalizations investigating the propagation properties of source vs. filter components as well as their effect on male and female listeners in a wider range of species. Such insight would provide a very useful background for understanding the roles and evolutionary origins of these strongly sexually dimorphic components of the human voice.

Acknowledgments

We thank the Royal Parks of London and Simon Richards for granting access to the deer and John Bartram, head Gamekeeper at Richmond Park, for facilitating our observations. We also thank Solène Derville for helping with double-coding the videos.

Supporting Information

Table S1 Independent and dependent variables for all 32 playback trials.

(PDF)

Author Contributions

Conceived and designed the experiments: DR MG MTW. Performed the experiments: MG. Analyzed the data: MG DR. Wrote the paper: DR MG BDC MTW WTF.

References

- Bradbury JW, Vehrencamp SL (1998) Principles of Animal Communication: Sinauer.
- Andersson B (1994) Sexual Selection: Princeton University Press.
- Candolin U (2003) The use of multiple cues in mate choice. *Biol Rev* 78: 575–595.
- Richardson C, Lengagne T (2010) Multiple signals and male spacing affect female preference at cocktail parties in treefrogs. *Proc Roy Soc B* 277: 1247–1252.
- Reby D, 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.
- Sanvito S, Galimberti F, Miller EH (2007) Vocal signalling of male southern elephant seals is honest but imprecise. *Anim Behav* 73: 287–299.
- Vannoni E, McElligott AG (2008) Low Frequency Groans Indicate Larger and More Dominant Fallow Deer (*Dama dama*) Males. *PLoS One* 3: e3113.
- Koren L, Gefen E (2009) Complex call in male rock hyrax (*Procavia capensis*): a multi-information distributing channel. *Behav Ecol Sociobiol* 63: 581–590.
- Charlton BD, Zhihe Z, Snyder RJ (2009) The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim Behav* 78: 893–898.
- Charlton BD, Ellis WA, McKinnon AJ, Cowin GJ, Brumm J, et al. (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.
- Wyman MT, Mooring MS, McCowan B, Penedo MCT, Reby D, et al. (2012) Acoustic cues to size and quality in the vocalizations of male North American bison, *Bison bison*. *Anim Behav* 84: 1381–1391.
- Vignal C, Kelley D (2007) Significance of temporal and spectral acoustic cues for sexual recognition in *Xenopus laevis*. *Proc Roy Soc B* 274: 479–488.
- Reby D, Joachim J, Lauga J, Lek S, Aulagnier S (1998) Individuality in the groans of fallow deer (*Dama dama*) bucks. *J Zool* 245: 79–84.
- McComb K, Reby D, Baker L, Moss C, Sayialel S (2003) Long-distance communication of acoustic cues to social identity in African elephants. *Anim Behav* 65: 317–329.
- Vannoni E, McElligott AG (2007) Individual Acoustic Variation in Fallow Deer (*Dama dama*) Common and Harsh Groans: A Source-Filter Theory Perspective. *Ethology* 113: 223–234.
- Charlton BD, Zhihe Z, Snyder RJ (2009) Vocal cues to identity and relatedness in giant pandas (*Ailuropoda melanoleuca*). *J Acoust Soc Am* 126: 2721–2732.
- Charlton BD, Ellis WAH, McKinnon AJ, Brumm J, Nilsson K, et al. (2011) Perception of Male Caller Identity in Koalas (*Phascolarctos cinereus*): Acoustic Analysis and Playback Experiments. *PLoS One* 6: e20329.
- Kreutzer MB, I.; Vallet, E.; Kiosseva, L. (1999) Social stimulation modulates the use of the 'A' phrase in male canary songs. *Behaviour* 136: 1325–1334.
- Burgdorf J, Kroes RA, Moskal JR, Pfau JG, Brudzynski SM, et al. (2008) Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. *J Comp Psychol* 122: 357–367.
- Wyman MT, Mooring MS, McCowan B, Penedo MCT, Hart LA (2008) Amplitude of bison bellows reflects male quality, physical condition and motivation. *Anim Behav* 76: 1625–1639.
- Charlton BD, Keating JL, Kersey D, Rengui L, Huang Y, et al. (2011) Vocal cues to male androgen levels in giant pandas. *Biol Lett* 7: 71–74.
- Charlton BD, Swaisgood RR, Zhihe Z, Snyder RJ (2012) Giant pandas attend to androgen-related variation in male bleats. *Behav Ecol Sociobiol* 66: 969–974.
- Martin-Vivaldi M, Palomino J, Soler M (1998) Song structure in the Hoopoe (*Upupa epops*) – Strophe length reflects male condition. *J Ornithol* 139: 287–296.
- Rendall D, Owren MJ, Weerts E, Hienz RD (2004) Sex differences in the acoustic structure of vowel-like grunt vocalizations in baboons and their perceptual discrimination by baboon listeners. *J Acoust Soc Am* 115: 411.
- Mitani JC (1985) Sexual selection and adult male orangutan long calls. *Anim Behav* 33: 272–283.
- Charlton BD, Ellis WAH, Brumm J, Nilsson K, Fitch WT (2012) Female koalas prefer bellows in which lower formants indicate larger males. *Anim Behav* 84: 1565–1571.
- Reby D, McComb K, Cargnelli B, Darwin C, Fitch WT, et al. (2005) Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proc Roy Soc B* 272: 941–947.
- Charlton BD, Reby D, McComb K (2007) Female perception of size-related formant shifts in red deer, *Cervus elaphus*. *Anim Behav* 74: 707–714.
- Reby D, Charlton BD, Locatelli Y, McComb K (2010) Oestrous red deer hinds prefer male roars with higher fundamental frequencies. *Proc Roy Soc B* 277: 2747–2753.
- Fant G (1970) Acoustic Theory of Speech Production: De Gruyter.
- Owren MJ (1990) Acoustic classification of alarm calls by vervet monkeys (*Cercopithecus aethiops*) and humans (*Homo sapiens*): I. Natural calls. *J Comp Psychol* 104: 20–28.
- Fitch WT (2002) Comparative Vocal Production and the Evolution of Speech: Reinterpreting the Descent of the Larynx. In: Alison Wray e, editor. *The Transition to Language*: Oxford: Oxford University Press. 21–45.
- Fitch WT (1997) Vocal tract length and formant frequency dispersion correlate with body size in rhesus macaques. *J Acoust Soc Am* 102: 1213–1222.
- Taylor AM, Reby D (2010) The contribution of source-filter theory to mammal vocal communication research. *J Zool* 280: 221–236.
- Fitch WT (2000) The evolution of speech: a comparative review. *Trends Cogn Sci* 4: 258–267.
- Harris TR, Fitch WT, Goldstein LM, Fashing PJ (2006) Black and White Colobus Monkey (*Colobus guereza*) Roars as a Source of Both Honest and Exaggerated Information About Body Mass. *Ethology* 112: 911–920.
- Hauser MD (1993) The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *Am Nat* 142: 528–542.
- Herbst CT, Stoeger AS, Frey R, Lohscheller J, Titze IR, et al. (2012) How low can you go? Physical production mechanism of elephant infrasonic vocalizations. *Science* 337: 595–599.
- Titze IR (1994) Principles of voice production: Prentice Hall.
- Rendall D, Kollias S, Ney C, Lloyd P (2005) Pitch (F0) and formant profiles of human vowels and vowel-like baboon grunts: The role of vocalizer body size and voice-acoustic allometry. *J Acoust Soc Am* 117: 944.
- Lass NJ, Brown WS (1978) Correlational study of speakers' heights, weights, body surface areas, and speaking fundamental frequencies. *J Acoust Soc Am* 63: 1218–1220.
- Masataka N (1994) Lack of correlation between body size and frequency of vocalizations in young female Japanese macaques (*Macaca fuscata*). *Folia Primatol* 63: 115–118.
- Pfefferle D, West PM, Grinnell J, Packer C, Fischer J (2007) Do acoustic features of lion, *Panthera leo*, roars reflect sex and male condition? *J Acoust Soc Am* 121: 3947–3953.
- Pfefferle D, Fischer J (2006) Sounds and size: identification of acoustic variables that reflect body size in hamadryas baboons, *Papio hamadryas*. *Anim Behav* 72: 43–51.
- Evans S, Neave N, Wakelin D (2006) Relationships between vocal characteristics and body size and shape in human males: an evolutionary explanation for a deep male voice. *Biol Psychol* 72: 160–163.
- Clutton-Brock TH, Guinness FE, Albon SD (1982) Red Deer: Behavior and Ecology of Two Sexes: University of Chicago Press.
- Fitch WT, Reby D (2001) The descended larynx is not uniquely human. *Proc Roy Soc B* 268: 1669–1675.
- Reby D, McComb K (2003) Vocal Communication and Reproduction in Deer. In: Slater P, Rosenblatt J, Roper T, Snowdon C, Naguib M, editors. *Adv Stud Behav*. San Diego: Academic Press Inc. 231–264.
- Charlton BD, Reby D, McComb K (2007) Female red deer prefer the roars of larger males. *Biol Lett* 3: 382–385.
- Reby D, André-Obrecht Rg, Galinier A, Farinas J, Cargnelli B (2006) Cepstral coefficients and hidden Markov models reveal idiosyncratic voice characteristics in red deer (*Cervus elaphus*) stags. *J Acoust Soc Am* 120: 4080.
- Titze IR (1989) On the relation between subglottal pressure and fundamental frequency in phonation. *J Acoust Soc Am* 85: 901–906.
- Fischer J, Kitchen DM, Seyfarth RM, Cheney DL (2004) Baboon loud calls advertise male quality: acoustic features and their relation to rank, age, and exhaustion. *Behav Ecol Sociobiol* 56: 140–148.
- Titze IR, Riede T (2010) A Cervid Vocal Fold Model Suggests Greater Glottal Efficiency in Calling at High Frequencies. *PLoS Comput Biol* 6: e1000897.
- Collins SA (2000) Men's voices and women's choices. *Anim Behav* 60: 773–780.
- Feinberg DR, Jones BC, Little AC, Burt DM, Perrett DI (2005) Manipulations of fundamental and formant frequencies influence the attractiveness of human male voices. *Anim Behav* 69: 561–568.
- Puts DA (2005) Mating context and menstrual phase affect women's preferences for male voice pitch. *Evol Hum Behav* 26: 388–397.
- Moulines E, Charpentier F (1990) Pitch-synchronous waveform processing techniques for text-to-speech synthesis using diphones. *Speech Commun* 9: 453–467.
- Boersma PW, D. (2012) Praat: Doing phonetics by computer (Version 5.3.12) [Computer program]. Retrieved from <http://www.praat.org/>. University of Amsterdam, The Netherlands.
- Mundry R, Fischer J (1998) Use of statistical programs for nonparametric tests of small samples often leads to incorrect P values: examples from Animal Behaviour. *Anim Behav* 56: 256–259.
- Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Ser B Stat Methodol* 57: 289–300.

61. Lindenfors P, Gittleman JL, Jones KE (2007) Sexual size dimorphism in mammals. Sex, size and gender roles: evolutionary studies of sexual size dimorphism: 16–26.
62. Clutton-Brock TH, Albon SD (1979) The roaring of red deer and the evolution of honest advertisement. *Behaviour* 69: 145–169.
63. Wagner W, Jr. (1989) Fighting, assessment, and frequency alteration in Blanchard's cricket frog. *Behav Ecol Sociobiol* 25: 429–436.
64. Bee MAP, S. A.; Owen, P C. (1999) Size assessment in simulated territorial encounters between male green frogs (*Rana clamitans*). *Behav Ecol Sociobiol* 45: 177–184.
65. Hardouin LA, Reby D, Bavoux C, Burneleau G, Bretagnolle V (2007) Communication of male quality in owl hoots. *Am Nat* 169: 552–562.
66. Bee MA (2002) Territorial male bullfrogs (*Rana catesbeiana*) do not assess fighting ability based on size-related variation in acoustic signals. *Behav Ecol* 13: 109–124.
67. Neumann C, Assahad G, Hammerschmidt K, Perwitasari-Farajallah D, Engelhardt A (2010) Loud calls in male crested macaques, *Macaca nigra*: a signal of dominance in a tolerant species. *Anim Behav* 79: 187–193.
68. Puts DA, Gaulin SJC, Verdolini K (2006) Dominance and the evolution of sexual dimorphism in human voice pitch. *Evol Hum Behav* 27: 283–296.
69. Puts DA, Hodges CR, Cárdenas RA, Gaulin SJC (2007) Men's voices as dominance signals: vocal fundamental and formant frequencies influence dominance attributions among men. *Evol Hum Behav* 28: 340–344.
70. Wolff SE, Puts DA (2010) Vocal masculinity is a robust dominance signal in men. *Behav Ecol Sociobiol* 64: 1673–1683.
71. Evans S, Neave N, Wakelin D, Hamilton C (2008) The relationship between testosterone and vocal frequencies in human males. *Physiol Behav* 93: 783–788.
72. Cynx J, Bean NJ, Rossman I (2005) Testosterone implants alter the frequency range of zebra finch songs. *Horm Behav* 47: 446–451.
73. ten Cate C, Slabbekoorn H, Ballintijn MR (2002) Birdsong and male–male competition: Causes and consequences of vocal variability in the collared dove (*Streptopelia decaocto*). *Adv Stud Behav* 31: 31–75.
74. Hardouin LA, Bretagnolle V, Tabel P, Bavoux C, Burneleau G, et al. (2009) Acoustic cues to reproductive success in male owl hoots. *Anim Behav* 78: 907–913.
75. Dabbs Jr JM, Mallinger A (1999) High testosterone levels predict low voice pitch among men. *Pers Individ Differ* 27: 801–804.
76. Tigue CC, Borak DJ, O'Connor JJM, Schandl C, Feinberg DR (2012) Voice pitch influences voting behavior. *Evol Hum Behav* 33: 210–216.
77. Apicella CL, Feinberg DR, Marlowe FW (2007) Voice pitch predicts reproductive success in male hunter-gatherers. *Biol Lett* 3: 682–684.
78. Beckford NS, Rood SR, Schaid D, Schanbacher B (1985) Androgen stimulation and laryngeal development. *Ann Otol Rhinol Laryngol* 94: 634–640.
79. Fischer J, Hammerschmidt K, Cheney DL, Seyfarth RM (2002) Acoustic features of male baboon loud calls: Influences of context, age, and individuality. *J Acoust Soc Am* 111: 1465.
80. Titze IR (1988) The physics of small-amplitude oscillation of the vocal folds. *J Acoust Soc Am* 83: 1536–1552.
81. Malo AF, Roldan ER, Garde JJ, Soler AJ, Vicente J, et al. (2009) What does testosterone do for red deer males? *Proc Roy Soc B* 276: 971–980.
82. McComb KE (1991) Female choice for high roaring rates in red deer, *Cervus elaphus*. *Anim Behav* 41: 79–88.
83. Charlton BD, Reby D, McComb K (2008) Effect of combined source (F0) and filter (formant) variation on red deer hind responses to male roars. *J Acoust Soc Am* 123: 2936–2943.
84. Stopher KV, Nussey DH, Clutton-Brock TH, Guinness F, Morris A, et al. (2011) The red deer rut revisited: female excursions but no evidence females move to mate with preferred males. *Behav Ecol* 22: 808–818.
85. Torriani MVG, Vannoni E, McElligott AG (2006) Mother-Young Recognition in an Ungulate Hider Species: A Unidirectional Process. *Am Nat* 168: 412–420.
86. Kidjo N, Cargnelutti B, Charlton BD, Wilson C, Reby D (2008) Vocal behaviour in the endangered Corsican Deer, description and phylogenetic implications. *Bioacoustics* 18: 159–181.
87. Minami MK, T. (1992) Vocal repertoires and classification of the sika deer *Cervus nippon*. *J Mamm Soc Jpn* 17: 71–94.
88. Ishizaka K, Flanagan JL (1972) Synthesis of voiced sounds from a two-mass model of the vocal cords. *Bell Syst Tech J* 51: 1233–1268.
89. Ryalls JH, Lieberman P (1982) Fundamental frequency and vowel perception. *J Acoust Soc Am* 72: 1631–1634.

Chapter 3 | Supplementary material: Table S1

Focal Stag	Exemplar stag	F0 (Hz)	Order	Nb hinds/harem	CR	HR	LK (s)	WK (s)	LTLK (s)	LTR (s)
4	E3	70	3	20	5	0	20.17	0	0.32	3.73
4	E3	100	4	24	8	0	11.98	0	0.20	4.53
4	E3	130	1	28	9	0	75.99	0	1.33	1.70
4	E3	160	2	35	6	0	67.63	14.76	0.43	3.31
5	E1	70	1	11	9	0	50.58	12.72	0.34	1.95
5	E1	100	2	11	12	1	93.8	10.53	0.31	9.03
5	E1	130	3	10	12	0	25.99	0	0.73	6.46
5	E1	160	4	18	0	0	71.8	1.72	0.47	
9	Yellow991	70	3	25	3	0	47.36	11.68	0.49	43.19
9	Yellow991	100	4	30	0	0	8.84	0	0.39	
9	Yellow991	130	1	25	1	0	25.8	0	1.16	30.30
9	Yellow991	160	2	40	0	2	23.52	0	0.40	7.77
11	E2	70	4	11	2	1	251.44	14.48	0.21	5.02
11	E2	100	1	4	8	3	78.99	47.37	0.88	3.52
11	E2	130	2	5	4	1	19.36	0	1.04	3.53
11	E2	160	3	15	2	0	27.7	0	1.64	32.67
13	Blue328	70	2	19	4	6	27.84	6.72	0.68	1.92
13	Blue328	100	3	18	1	0	17.72	0	25.10	5.35
13	Blue328	130	4	19	1	0	29.2	0	0.32	1.98
13	Blue328	160	1	20	25	17	230.75	37.89	2.92	5.28
15	Red109	70	4	30	9	6	99.68	10.6	0.48	2.67
15	Red109	100	1	15	6	6	86.52	81.72	0.35	6.55
15	Red109	130	2	11	7	6	159.33	30.47	0.70	7.11
15	Red109	160	3	36	1	1	4.62	0	0.76	2.72
16	E4	70	2	10	3	0	16.88	0	0.36	5.56
16	E4	100	3	10	4	0	70.69	0	0.40	17.31
16	E4	130	4	8	7	6	91.67	24.01	0.72	10.95
16	E4	160	1	15	13	1	110.6	1.52	1.35	3.75
17	E1	70	4	20	5	0	36.72	0	0.40	6.48
17	E1	100	1	7	12	4	286	76.84	0.73	3.25
17	E1	130	2	7	5	0	102.32	0	2.83	31.91
17	E1	160	3	7	3	5	86.4	39.96	0.41	19.89

Chapter 4 |

Playback experiments:

Exploring the role of different call types in red
deer vocal communication

This chapter is an article published in a peer-reviewed journal.

Citation: **Garcia M**, Wyman MT, Charlton BD, Fitch WT & Reby D. 2014.
Response of red deer stags (*Cervus elaphus*) to playback of harsh versus
common roars. *Naturwissenschaften* 101(10): 851-4.

Response of red deer stags (*Cervus elaphus*) to playback of harsh versus common roars

Maxime Garcia · Megan T. Wyman · Benjamin D. Charlton ·
W. Tecumseh Fitch · David Reby

Received: 6 May 2014 / Revised: 8 July 2014 / Accepted: 23 July 2014 / Published online: 14 August 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Red deer stags (*Cervus elaphus*) give two distinct types of roars during the breeding season, the “common roar” and the “harsh roar.” Harsh roars are more frequent during contexts of intense competition, and characterized by a set of features that increase their perceptual salience, suggesting that they signal heightened arousal. While common roars have been shown to encode size information and mediate both male competition and female choice, to our knowledge, the specific function of harsh roars during male competition has not yet been studied. Here, we investigate the hypothesis that the specific structure of male harsh roars signals high arousal to competitors. We contrast the behavioral responses of free ranging, harem-holding stags to the playback of harsh roars from an unfamiliar competitor with their response to the playback of common roars from the same animal. We show that males react less strongly to sequences of harsh roars than to sequences of common roars, possibly because they are reluctant to escalate conflicts with highly motivated and threatening unfamiliar males in the absence of visual information. While future work should investigate the response of stags to harsh roars from familiar opponents, our observations

remain consistent with the hypothesis that harsh roars may signal motivation during male competition, and illustrate how intrasexual selection can contribute to the diversification of male vocal signals.

Keywords Red deer · Vocal communication · Sexual signals · Deterministic chaos · Nonlinear phenomena

Introduction

A key objective of research in bioacoustics is to understand the evolutionary processes responsible for the diversification of vocal repertoires (Clemens et al. 2005). The extraordinary interspecific diversity of the sexually selected vocalizations of polygynous deer species (*Cervinae*) makes this family an ideal model for studying these processes (Reby and McComb 2003b; Vannoni and McElligott 2007; Wyman et al. 2011, 2014; Yen et al. 2013). With their conspicuous, abundant, and sophisticated vocal displays, male Scottish red deer (*Cervus elaphus scoticus*) have proved a particularly productive model for investigating how sexual selection drives the evolution of vocal signals (Charlton et al. 2007; Reby and McComb 2003a, b; Reby et al. 2005). During the breeding season, stags produce two distinct types of roars, the common roar and the harsh roar (Reby and McComb 2003a). Common roars are characterized by slow amplitude onset and offset, a mostly harmonic structure, and modulation of both fundamental frequency (F0) and formant frequencies (vocal tract resonances) over the course of the calls (Fitch and Reby 2001; Fig. 1). Previous research on red deer has demonstrated that the fundamental frequency of common roars affects female mating decisions, with oestrous hinds preferring higher-pitched roars (Reby et al. 2010), but does not influence male response during intrasexual contests (Garcia et al. 2013). Additional studies carried out using common roars have also

Communicated by: Sven Thatje

Electronic supplementary material The online version of this article (doi:10.1007/s00114-014-1217-8) contains supplementary material, which is available to authorized users.

M. Garcia · W. Tecumseh Fitch
Department of Cognitive Biology, University of Vienna,
Althanstrasse 14, 1090 Vienna, Austria

M. T. Wyman · D. Reby (✉)
School of Psychology, University of Sussex, Brighton BN1 9QH,
UK
e-mail: reby@sussex.ac.uk

B. D. Charlton
School of Biology and Environmental Science, Science Centre West,
University College Dublin (UCD), Belfield, Dublin 4, Ireland

established that formants constitute a reliable cue to body size (with lower formant frequencies indicating larger body size, (Reby and McComb 2003a)), and that size-related variation in formant frequencies affects both male behavior during intrasexual competition (Reby et al. 2005) and female behavior in mate choice contexts (Charlton et al. 2007).

Harsh roars are qualitatively different from common roars (Reby et al. 2005, see Fig. 1). They are characterized by abrupt amplitude onsets and offsets (giving bouts of harsh roars a staccato quality), by low and stable formant frequencies (reflecting the fact that the stag fully extends its vocal tract before vocalization starts) (Reby and McComb 2003a), and most distinctively, by a noisy, aperiodic quality (due to deterministic chaos, a type of nonlinear phenomenon caused by irregular vibrations of the vocal folds (Wilden et al. 1998)). This noisy, aperiodic quality of red deer harsh roars should increase the perceptual salience of size information in these calls, and is also likely to make the calls more evocative to receivers (as seen in marmots (Blumstein and Récapet 2009) and meerkats (Townsend and Manser 2011) alarm calls). Furthermore, males typically deliver harsh roars during contexts of intense competition (Reby and McComb 2003b; Reby et al. 2005), suggesting that they are likely to signal a caller's high motivational state/arousal level during male contests. Finally, while oestrous red deer hinds are not differentially attracted by loudspeakers broadcasting harsh roars over those playing common roars (Charlton et al. 2014), they pay more attention to harsh roars as well as to common roars played after a bout of harsh roar (Reby and Charlton 2012).

Here, in order to test the hypothesis that harsh roars communicate high arousal during male contests, we contrast the response of harem-holding males to playback sequences of roars simulating the intrusion of an unfamiliar male and containing either two bouts of common roars or two bouts of harsh roars. We predict that, given their different acoustic properties, these two roar types should elicit different responses from harem-holding male listeners.

Material and methods

Playback experiments

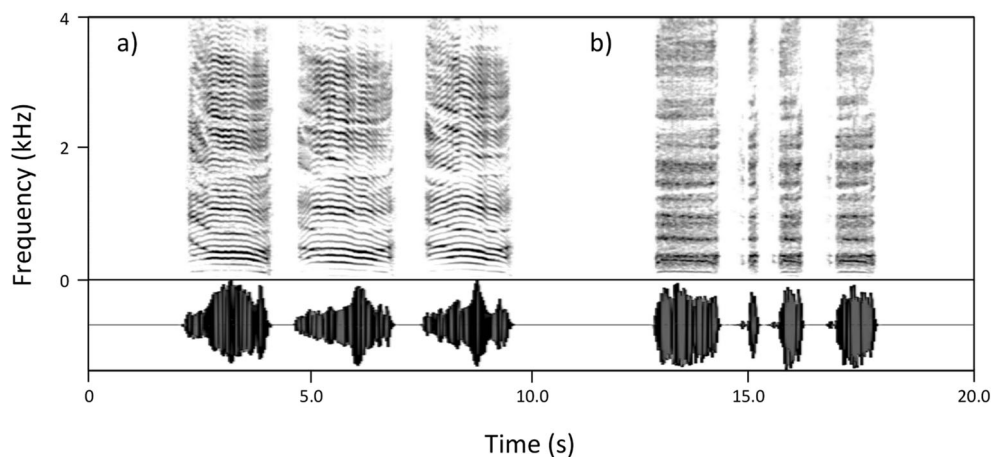
The playback stimuli were created using roars from seven unfamiliar exemplar Scottish red deer stags recorded by DR. For each of these 7 exemplar stags, we created 1 sequence of common roars and 1 sequence of harsh roars, resulting in a total of 14 sequences. All sequences were edited using Praat (Boersma and Weenink 2012) and consisted of two bouts of either common roars or harsh roars separated by a 20-s silence interval.

Playback trials were performed on 12 stags, which were presented with both playback variants (from the same unfamiliar exemplar) separated by a minimum of 1 h (to prevent habituation to the playback procedure), and the order of presentation of the two variants (common roars or harsh roars) was alternated between the stags. The stags' responses were video-recorded. Additional information about the stimuli preparation and playback design are reported in the [Electronic supplementary material](#).

Behavioral and statistical analyses

Video sequences were analyzed frame-by-frame (frame = 0.04 s) using Gamebreaker v7.5.5 software (Sportstec, Sydney). MG analyzed the videos and 10 % of the trials were double coded by an independent observer, resulting in an overall agreement of 99.4 % (Pearson's $r=0.997$). In order to quantify stags' behavioral responses, we performed a principal component analysis (PCA) on four response variables: number of common roars (CR), and harsh roars (HR) elicited, time spent looking towards the speaker (LK), and latency to roar back (LTR). The effect of the playback condition (common roar sequence vs. harsh roar sequence) on male behavioral response was then tested using a linear mixed model. All statistical tests were computed using SPSS v.19, significance

Fig. 1 Spectrogram of **a** one bout of common roars, and **b** one bout of harsh roars



levels were set at $p=0.05$ and two-tailed statistics are reported. Further details of the statistical procedures are presented in the [Electronic supplementary material](#).

Results

Both roar types elicited strong, clear responses: over the 24 playback trials, males looked towards the loudspeaker in 100 % of trials, and roared back in 92 % of trials. Box plots in Fig. 2 show the scores obtained for the different behavioral variables, for each playback condition. We report raw scores for all the response variables across the 24 playback trials and detailed statistical output in the [Electronic supplementary material](#).

The principal component analysis returned one factor (PC1) exceeding Kaiser's criterion (eigenvalue=2.572), which explained 64.29 % of the variance in our dataset. All four response variables "CR," "HR," "LK," and "LTR" were strongly loaded (respective scores of 0.905, 0.885, 0.871, and -0.459) onto PC1, which clearly represents the intensity of a stag's behavioral response. PC1 scores were normally distributed (Shapiro-Wilk, $p=0.789$) and were therefore used in the subsequent linear mixed model (LMM) analysis.

The LMM analysis performed on PC1 revealed that there was a significant effect of the roar type variant ($F_{1, 8,3}=6.664$, $p=0.032$): mean PC1 scores were lower in response to harsh roar sequences than to the common roar sequences (see Fig. 2), indicating that stags responded less strongly to the harsh roar condition.

Discussion

In this study, harem holding red deer stags responded both to playbacks of unfamiliar common roars and to playbacks of unfamiliar harsh roars by directing their attention towards the speaker and roaring back, indicating that they perceived the playback as a potential threat. However, stags responded slightly but significantly less strongly to playbacks of harsh roar sequences than to playbacks of common roar sequences, indicating that stags perceive the differences between the two call types, and suggesting that they may convey different information.

In red deer male contests, common roars have been shown to allow recipients to assess caller body size through formant frequencies (Reby and McComb 2003a; Reby et al. 2005). Harsh roars are less frequent than common roars and have been reported after intense intrasexual or intersexual interactions (Reby and McComb 2003b; Reby et al. 2005). Because harsh roars are characterized by deterministic chaos, abrupt onsets and offsets, and very low vocal tract resonances, it has been suggested that they may be more costly to produce than common roars (Reby and Charlton 2012). This led us to assume that they may signal heightened arousal and mediate escalation in agonistic interactions. We therefore predicted that harem holding males would respond differently to harsh roars and common roars.

The observation that stags responded less strongly to harsh roar sequences may at first indicate that, contrary to our assumptions, harsh roars are less threatening than common roars and therefore trigger a relatively weaker response from harem holding stags. This is, however, rather unlikely, as harsh roars are typically given during more intense interactions, and in

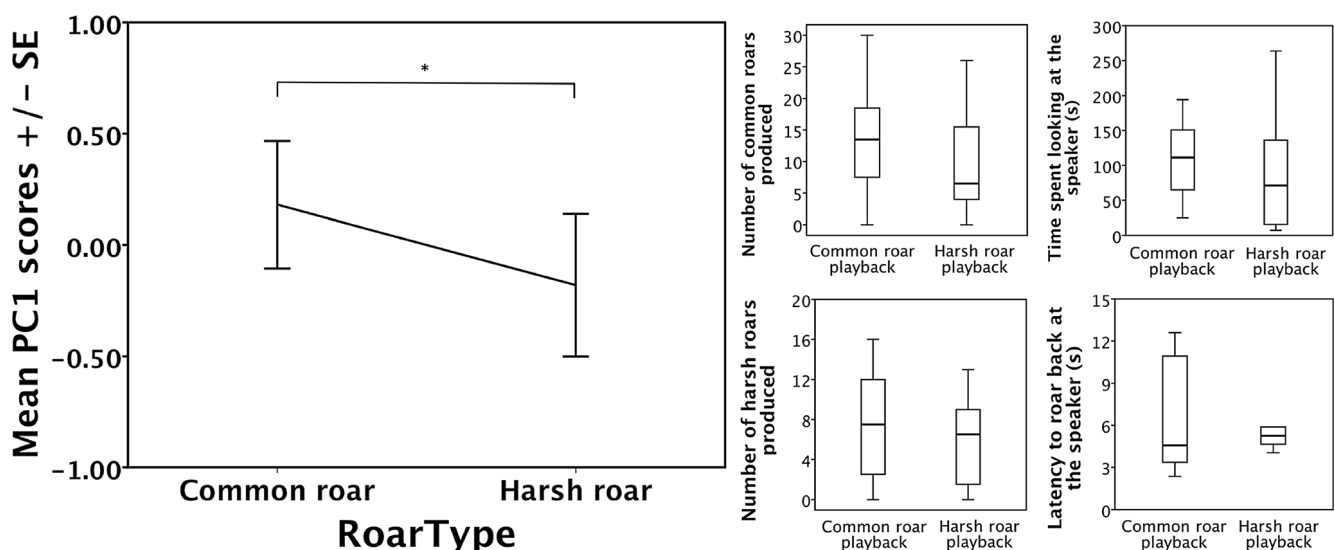


Fig. 2 The effect of roar type on male behavioral response (mean PC1 scores $\pm$ SE) is presented in the main frame. * $p < 0.05$. Box plots on the right show the separate behavioral responses to playback sequences of "common roars" and "harsh roars"; outliers are not represented on the box plots

response to more threatening individuals (Reby and McComb 2003b; Reby et al. 2005). Alternatively, our observations may derive from the specific design of our experimental protocol, where stags hear a vocalization from an unfamiliar male, but fail to visually confirm its presence. Stags may also be surprised to hear harsh roars from a male in the absence of an initial roaring exchange. In these circumstances, harem-holding stags may benefit from avoiding escalating a direct conflict with an unfamiliar, highly aroused individual. This is consistent with previous playbacks of common roars conducted on red deer stags showing that males reacted slightly less strongly to playback variants simulating very large adults than to playback variants simulating medium-sized adults (Reby et al. 2005). Our results are also consistent with a recent study on koalas showing that males were slower to reply to playbacks of bellows simulating larger individuals, possibly reflecting their reluctance to engage in vocal exchanges with larger males that represent more dangerous rivals (Charlton et al. 2013).

A previous investigation of the function of harsh roars during intersexual communication (Reby and Charlton 2012) has shown that female red deer pay more attention to harsh roars than to common roars, adding to several recent studies suggesting that one function of nonlinear phenomena including deterministic chaos is to generate unpredictability and prevent habituation in listeners (Blumstein and Récapet 2009; Fitch et al. 2002; Townsend and Manser 2011). Furthermore, a recent study using a two-speaker mate choice experiment (Charlton et al. 2014) revealed that female red deer do not prefer individuals producing harsh roars, a result consistent with the observation that all males are able to produce harsh roars, and that these may function as a dishabituation signal and/or as a dynamic index of motivation rather than a fixed index of quality.

The present playback experiments confirm that harsh roars play a role in male vocal contests, but further playback experiments are needed to disentangle the function of the different acoustic characteristics that distinguish harsh roars from common roars. While the results of our experiment are consistent with the hypothesis that harsh roars could function to signal heightened arousal, additional playbacks are required to clarify their specific function, in particular in the context of escalating contests between familiar, rather than unfamiliar, males.

Acknowledgments We thank the Royal Parks of London and Simon Richards for granting access to the deer and John Bartram, head Gamekeeper at Richmond Park, for facilitating our observations. We also thank Solène Derville for helping with double-coding the videos. Finally, we thank four anonymous referees for their very insightful comments on an earlier version of this manuscript. B. D. C. was financially supported by a Leverhulme Trust Early Career Fellowship. W. T. F. was financially supported by an ERC Advanced Grant 230604 SOMACCA.

References

- Blumstein DT, Récapet C (2009) The sound of arousal: the addition of novel non-linearities increases responsiveness in marmot alarm calls. *Ethology* 115:1074–1081. doi:10.1111/j.1439-0310.2009.01691.x
- Boersma P, Weenink D (2012) Praat: doing phonetics by computer (Version 5.3.12) [Computer program]. University of Amsterdam, The Netherlands, Retrieved from <http://www.praat.org/>
- Charlton BD, Reby D, McComb K (2007) Female red deer prefer the roars of larger males. *Biol Lett* 3:382–385. doi:10.1098/rsbl.2007.0244
- Charlton BD, Whisson DA, Reby D (2013) Free-ranging male koalas use size-related variation in formant frequencies to assess rival males. *PLoS One* 8:e70279. doi:10.1371/journal.pone.0070279
- Charlton BD, Wyman MT, Locatelli Y, Fitch WT, Reby D (2014) Do red deer hinds prefer stags that produce harsh roars in mate choice contexts? *J Zool* 293:57–62. doi:10.1111/jzo.12120
- Clemins PJ, Johnson MT, Leong KM, Savage A (2005) Automatic classification and speaker identification of African elephant (*Loxodonta africana*) vocalizations. *J Acoust Soc Am* 117:956–963
- Fitch WT, Reby D (2001) The descended larynx is not uniquely human. *Proc Roy Soc B* 268:1669–1675. doi:10.1098/rspb.2001.1704
- Fitch WT, Neubauer J, Herzog H (2002) Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. *Anim Behav* 63:407–418. doi:10.1006/anbe.2001.1912
- Garcia M, Charlton BD, Wyman MT, Fitch WT, Reby D (2013) Do red deer stags (*Cervus elaphus*) use roar fundamental frequency (F0) to assess rivals? *PLoS One* 8:e83946. doi:10.1371/journal.pone.0083946
- Reby D, Charlton BD (2012) Attention grabbing in red deer sexual calls. *Anim Cogn* 15:265–270. doi:10.1007/s10071-011-0451-0
- Reby D, McComb K (2003a) Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Anim Behav* 65:519–530. doi:10.1006/anbe.2003.2078
- Reby D, McComb K (2003b) Vocal communication and reproduction in deer. In: Slater P, Rossenblatt J, Roper T, Snowdon C, Naguib M (eds) *Advances in the study of behavior*, vol Volume 33. Academic Press Inc, San Diego, pp 231–264. doi:10.1016/S0065-3454(03)33005-0
- Reby D, McComb K, Cargnelutti B, Darwin C, Fitch WT, Clutton-Brock T (2005) Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proc Roy Soc B* 272:941–947. doi:10.1098/rspb.2004.2954
- Reby D, Charlton BD, Locatelli Y, McComb K (2010) Oestrous red deer hinds prefer male roars with higher fundamental frequencies. *Proc Roy Soc B* 277:2747–2753. doi:10.1098/rspb.2010.0467
- Townsend SW, Manser MB (2011) The function of nonlinear phenomena in meerkat alarm calls. *Biol Lett* 7:47–49. doi:10.1098/rsbl.2010.0537
- Vannoni E, McElligott AG (2007) Individual acoustic variation in fallow deer (*Dama dama*) common and harsh groans: a source-filter theory perspective. *Ethology* 113:223–234. doi:10.1111/j.1439-0310.2006.01323.x
- Wilden I, Herzog H, Peters G, Tembrock G (1998) Subharmonics, biphonation, and deterministic chaos in mammal vocalization. *Bioacoustics* 9:171–196. doi:10.1080/09524622.1998.9753394
- Wyman MT, Charlton BD, Locatelli Y, Reby D (2011) Variability of female responses to conspecific vs. heterospecific male mating calls in polygynous deer: an open door to hybridization? *PLoS One* 6:e23296
- Wyman MT, Locatelli Y, Charlton BD, Reby D (2014) No preference in female sika deer for conspecific over heterospecific male sexual calls in a mate choice context. *J Zool* 293:92–99. doi:10.1111/jzo.12123
- Yen SC, Shieh BS, Wang YT, Wang Y (2013) Rutting vocalizations of Formosan sika deer *Cervus nippon taiouanus*-acoustic structure, seasonal and diurnal variations, and individuality. *Zool Sci* 30:1025–1031

Chapter 4 | Supplementary material

Playback experiments

The recordings used to create our stimuli were obtained by DR in Redon experimental farm in France (Reby et al. 2001; Reby et al. 2006) and in New-Zealand farms (Reby & Charlton 2012). The exemplar stags were well-fed farm animals comparable in size and unfamiliar to the tested subjects.

Playback trials were performed during the breeding season of October 2012, in Richmond Park, London, to stags that were actively holding harems and displaying normal breeding behaviour (which occurs during daytime in this park as sport hunting is banned in the Park). A population of about 300 red deer roams freely in this area of approximately one thousand hectares. Encounters between stags within this population are frequent, since harem holders can be defeated and loose their harem throughout the breeding season (personal observations).

Common roar bouts were composed of one to three different roars (average $\pm$ SD = 2.29 ± 0.73) and lasted between 2.07 and 7.1 seconds (average $\pm$ SD = 4.46 ± 1.50 s) and harsh roar bouts were composed of three to ten different roars (average $\pm$ SD = 6.50 ± 2.10) and lasted between 2.19 and 6.73 seconds (average $\pm$ SD = 4.07 ± 1.50 s), both reflecting these displays natural variation (Reby & McComb 2003). For five out of the seven exemplar stags, only one bout of harsh roars (out of two bouts used in our sequences) was available. Therefore, for eight out of the twelve stags we tested, the harsh roar playback trial involved a sequence composed of the repetition of the same harsh roar bout. Bouts within common roar sequences were all different. The potential effect of bout repetition within a sequence of harsh roars was controlled for in our statistical analyses (see ESM: statistical analysis).

As we had seven exemplar stags and twelve focal stags, five exemplar stags were used on two different stags. This repetition of the exemplar was also taken into consideration as a random factor in the later LMM analysis.

Chapter 4 | Supplementary material

After generating playback sequences, the maximum amplitude of each bout was normalized to 99% of peak amplitude with Audacity (version 2.0.1; Effect: Normalize; Remove any DC offset (center on 0.0 vertically); Normalize maximum amplitude to “-1dB”), so that playback stimuli preserved a natural within-bout amplitude variation.

A stable harem was defined for a harem holder which was mature and with at least five hinds (number of hinds, average $\pm$ SD = 14.08 ± 5.70). Assessment of a typical breeding period behaviour involved observing some roaring activity, herding and defense against other males. Playback trials were initiated when the harem holder was not engaged in a direct interaction with another stag (experiments were only conducted when no other stags were in the vicinity, and when neighboring stags were at least 100m away), and not disrupted by new hinds entering or leaving the harem in the 15 minutes preceding the playback. The size of harems varied between playback trials, as hinds frequently move between harems. The experimenter then placed a loudspeaker (Anchor Audio Liberty 6000HIC) 40-70m away from the stag at an amplitude of 98 dB, measured at 1m away from the source. The loudspeaker was directed at the focal stag and connected to a MacBook Pro (Mac OS X, version 10.7.4) using a 50-m co-axial cable. The experimenter initiated playback sequences while standing perpendicular to the “stag to loudspeaker” axis, minimizing disturbance around the speaker position. The focal stag’s response was recorded using tripod-mounted Sony (HDR TG3) or Canon (LEGRIA FS200) video camcorders, from the beginning of the sequence until 5 min after the last bout terminated. In order to prevent habituation, no more than two playbacks were performed on the same animal in a single day, with at least one-hour interval. A maximum of 8 days separated the two complementary playback sequences, as males had to fulfill the criterion of holding a harem of at least 5 hinds.

Chapter 4 | Supplementary material

Statistical analysis

In order to quantify the behavioral responses of subjects to our stimuli using uncorrelated variables, we performed a principal component analysis based on a correlation matrix including all our behavioural variables (number of common roars (CR), number of harsh roars (HR), time spent looking towards the speaker (LK), and latency to roar back at the speaker (LTR)).

Principal components were estimated using Varimax Rotation, missing values were excluded listwise and components with eigenvalues >1 were kept for further processing. Extracted principal components were checked for normality (using Shapiro-Wilk test) for later use in the Linear Mixed Model analysis.

The effect of bout repetition within harsh roars playback sequence was tested for and showed no significant level. This parameter was therefore left out of subsequent analysis.

In order to test for the effect of the roar type on the behavioural response (PC1), we ran a linear mixed model with roar type: 'common roar' vs. 'harsh roar' variant) as a fixed factor and, the identity of the 'Exemplar stag' and the identity of the 'Focal Stag' as random factors. We used a Linear Mixed Model, with Restricted Maximum Likelihood estimation (REML) and Compound Symmetry covariance type. Estimated Marginal Means were computed for each roar type variant and with only one paired comparison performed, no specific correction was used and significance level was set at $p=0.05$.

Our model also tested for a fixed effect of sequence presentation order, as well as for the interaction between 'roar type' and 'presentation order'. None of these effects affected significantly the stag's responses (see ESM: Summary of statistical output). However non-significant, they remained included within our model, as removing them did not improve the quality of our model (Akaike's information criterion (AIC) scores did not become significantly smaller while removing either or both of them).

Chapter 4 | Supplementary material

Raw data table

Stagtest	Exemplar	Roar type	Order	Bout repetition	Nb of hinds	CR	HR	LK (s)	LTR (s)
1	E4	CR	1	0	14	12	0	24.64	10.02
1	E4	HR	2	0	6	0	0	17.32	
2	E4	CR	2	0	7	13	9	133.49	10.07
2	E4	HR	1	0	10	7	5	81.58	4.65
3	E2	CR	2	0	10	30	34	194.28	3.12
3	E2	HR	1	0	8	23	22	263.55	5.88
4	Red109	CR	1	0	25	15	6	124.76	4.11
4	Red109	HR	2	1	20	5	6	10.19	5.57
5	Blue328	CR	1	0	10	23	6	149.24	28.08
5	Blue328	HR	2	1	15	16	3	60.53	5.83
6	Yellow991	CR	2	0	11	14	9	152.16	3.36
6	Yellow991	HR	1	1	15	15	7	233.86	4.92
9	Blue503	CR	2	0	25	0	0	46.79	
9	Blue503	HR	1	1	17	6	7	14.03	6.59
10	Green066	CR	2	0	15	5	5	89.57	12.59
10	Green066	HR	1	1	5	3	0	60.13	70
11	E2	CR	1	0	15	10	15	82.79	3.82
11	E2	HR	2	0	14	6	11	100.75	4.88
12	Green066	CR	1	0	18	20	16	167.72	5.02
12	Green066	HR	2	1	8	26	13	167.8	4.04
13	Red109	CR	2	0	22	17	9	97.77	2.35
13	Red109	HR	1	1	20	13	7	104.59	1.58
16	Yellow991	CR	1	0	11	2	0	25.03	10.95
16	Yellow991	HR	2	1	17	2	0	7.15	12.57

Chapter 4 | Supplementary material

Summary of statistical analysis output

Principal component analysis: loading scores of the four behavioral variables measured in the study on a single principal component (PC1).

Stagtest	CR	HR	LK (s)	LTR (s)	PC1 Scores
1	12	0	24.64	10.02	-0.79919
1	0	0	17.32		
2	13	9	133.49	10.07	0.14777
2	7	5	81.58	4.65	-0.46687
3	30	34	194.28	3.12	2.34748
3	23	22	263.55	5.88	1.81451
4	15	6	124.76	4.11	0.1375
4	5	6	10.19	5.57	-0.85969
5	23	6	149.24	28.08	0.30915
5	16	3	60.53	5.83	-0.27307
6	14	9	152.16	3.36	0.3622
6	15	7	233.86	4.92	0.68614
9	0	0	46.79		
9	6	7	14.03	6.59	-0.76698
10	5	5	89.57	12.59	-0.61431
10	3	0	60.13	70	-1.76277
11	10	15	82.79	3.82	0.11343
11	6	11	100.75	4.88	-0.1632
12	20	16	167.72	5.02	0.98125
12	26	13	167.8	4.04	1.12666
13	17	9	97.77	2.35	0.24916
13	13	7	104.59	1.58	0.02899
16	2	0	25.03	10.95	-1.24691
16	2	0	7.15	12.57	-1.35126

Component matrix	
	Component 1
CR	0.905
HR	0.885
LK	0.871
LTR	-0.459

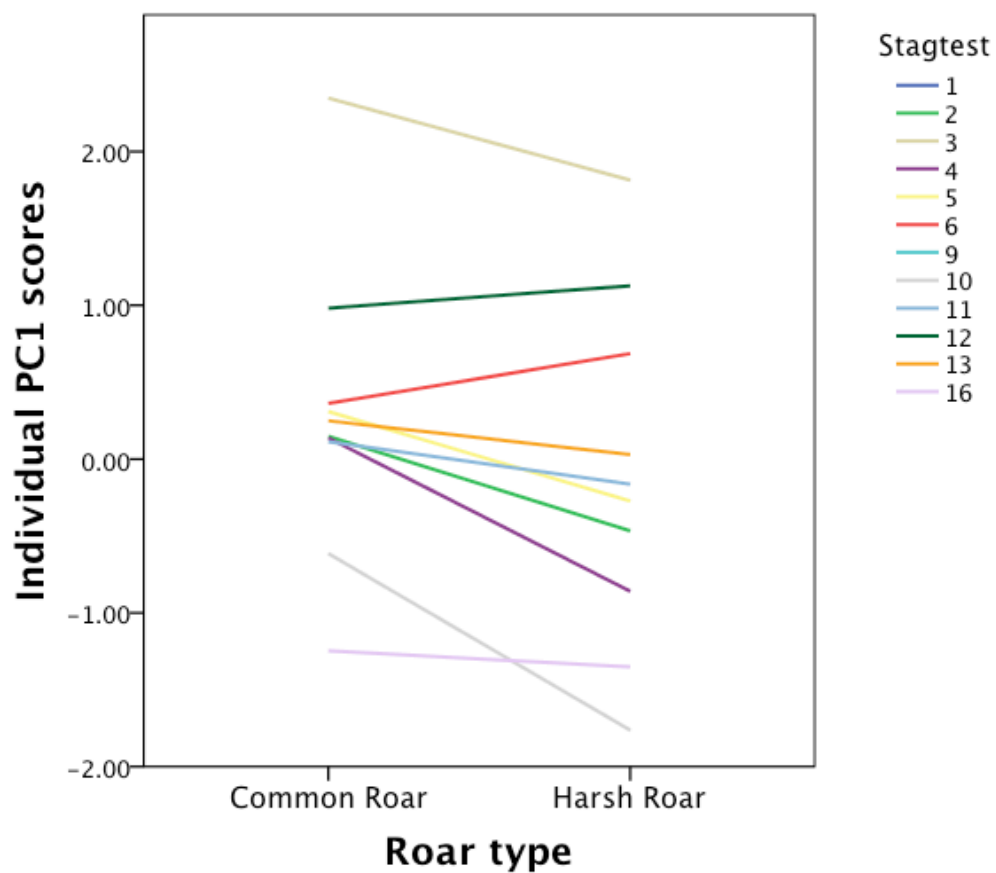
Chapter 4 | Supplementary material

LMM estimates of fixed effects on stags' behavioral response (represented as PC1).

Term	Estimate	SE	df	<i>F</i> value	<i>P</i> value
Intercept	-0.430	0.423	11.924	0.035	0.854
Roar type	0.807	0.599	11.924	6.664	0.032
Order	0.352	0.592	11.511	0.123	0.735
Roar Type * Order	-0.813	1.143	10.083	0.506	0.493

LMM model: REML (Restricted Maximum Likelihood estimation). The estimated effect size and standard error (SE) from LMM are given, with *F* values and *P* values based on type III sums of squares. The model includes 2 random factors: The 'exemplar stag' identity and the 'focal stag' identity.

Individual stag responses to playbacks



Individual PC1 scores (mean scores are represented in the main manuscript in Figure2). Lines for stags 1 and 9 do not appear on the graph as they were excluded list-wise in the principal component analysis.

References

Reby D, Hewison M, Izquierdo M, Pépin D. 2001 Red Deer (*Cervus elaphus*) Hinds Discriminate Between the Roars of Their Current Harem-Holder Stag and Those of Neighbouring Stags. *Ethology* 107(10), 951-959. (doi:10.1046/j.1439-0310.2001.00732.x).

Reby D, André-Obrecht Rg, Galinier A, Farinas J, Cargnelutti B. 2006 Cepstral coefficients and hidden Markov models reveal idiosyncratic voice characteristics in red deer (*Cervus elaphus*) stags. *J. Acoust. Soc. Am.* 120(6), 4080. (doi:10.1121/1.2358006).

Reby D, Charlton BD. 2012 Attention grabbing in red deer sexual calls. *Anim. Cogn.* 15(2), 265-270. (doi:10.1007/s10071-011-0451-0).

Reby D, McComb K. 2003 Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. *Anim. Behav.* 65(3), 519-530. (doi:10.1006/anbe.2003.2078).

Chapter 5 |

Excised larynx experiments:

Empirically examining the connection between
vocal production mechanisms and acoustic structure

This chapter is an early stage manuscript in preparation for a peer-reviewed journal. As data acquisition is still ongoing, the presented results are considered preliminary but noteworthy enough to be included in this dissertation

Citation: **Garcia M**, Herbst CT, Bowling DL, Dunn JC & Fitch WT. *In Prep*. A comparative study of vocal production in primates using excised larynx experiments: determinants of F0.

A comparative study of vocal production in primates using excised larynx experiments: determinants of F0.

Maxime Garcia^a, Christian T. Herbst^b, Daniel L. Bowling^a, Jacob C. Dunn^c and W. Tecumseh Fitch^a

^a Department of Cognitive Biology, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria.

^b Voice Research Lab, Department of Biophysics, Faculty of Science, Palacký University, Olomouc, Czech Republic

^c Division of Biological Anthropology, University of Cambridge, Pembroke Street, Cambridge CB2 3QG, UK

Abstract

In the study of animal vocal communication, identifying how acoustic signals could be constrained by physical attributes of the signaler has received considerable attention. In mammals in particular, including primates, fundamental frequency (F0) was initially suggested to correlate with the body size of the caller. This assumption was challenged latter as studies showed F0 to be a poor predictor of body size within a given species. In this comparative study including several primate species, we conducted excised larynx experiments on specimens for which the size of the laryngeal vocal folds and that of the entire body was known. With matched acoustic and anatomical data from the same specimens, we investigated whether the minimum F0 (minF0) produced by the sound source was likely to be determined by the body size and/or vocal fold length of the emitter. Supporting previous research, we found that body size is a poor predictor of minF0; instead, vocal fold length strongly predicts minF0. We discuss the implications of this result in light of the diversity found in primate vocalizations and vocal morphology.

Introduction

Our understanding of vocal production in mammal communication systems has greatly benefited from the source-filter theory (Fitch and Hauser, 1995; Taylor and Reby, 2010). According to this theory, originally designed for human speech (Fant, 1960; Titze, 1994) and later applied to mammals, a sound source is produced by the vibrating vocal folds within the larynx (determining the fundamental frequency of the acoustic signal, hereafter F0) which then propagates through the vocal tract that filters out some frequencies and enhances others (the formants). The connection between anatomical constraints on sound production and cues to physical attributes of the signaler has initiated research investigating among other things how an individual's body size could be reflected by the F0 of its vocalizations (Davies and Halliday, 1978; Morton, 1977; Ohala, 1984). The prediction of a body size/F0 correlation relies on 2 assumptions: 1) the size of the vocal folds is directly determined by the size of the larynx, itself determined by body size, and 2) the size of the vocal folds directly determines the signal F0. The latter condition has been verified, with longer focal folds generating lower F0s (Titze, 1994). However, because larynx size is often not constrained by its surrounding structures (Fitch and Hauser, 1995), the former assumption has been challenged with many studies showing a poor correlation between body size and F0 within a given species (Lass, 1978; Masataka, 1994; McComb, 1991; Pfefferle et al., 2007).

Research on primate vocal production has also followed this trend. A meta-analysis conducted by Hauser (Hauser, 1993) concluded that 'larger species produce relatively lower-pitched vocalizations than smaller species'. In this study, the methodology applied to assess "frequency" pooled various types of measurements together, potentially reducing the interpretability of the results. The term 'frequency' included manual measurements of both the dominant frequency (hereafter DF) and F0, depending on whether the signal was atonal or tonal. These are 2 different parameters: while F0 reflects the rate of vibration of the vocal folds, DF corresponds to the point of maximal amplitude along the frequency spectrum

(see e.g. Gingras and Fitch, 2013) and could reflect F0, filtering characteristics of the vocal tract or higher harmonics. This conflation could easily modify the quantitative estimation of the relationship between frequency and body size. This is supported by recent results showing that body size correlates better with F0 than DF while considering a wide range of vocalizations from multiple carnivore and primate species (Bowling et al., *In Prep.*). Second, the frequency Hauser attributed to a species resulted from an average over the frequency range obtained from a number of call types of a species repertoire. This number was limited to call types available in published spectrograms at the time of the study, hence potentially omitting vocal displays that could have influenced the results in either direction.

Besides vocal fold length, F0 strongly depends on at least two other parameters: subglottal pressure (hereafter P_{sub}) and vocal fold tension (Titze, 1994). An increase in P_{sub} (determined by the air pressure coming from the lungs) typically leads to an increase in F0; an increase in the tension applied on the vocal folds has similar effects: as vocal folds are stretched, mainly by the action of the cricothyroid muscle, the more tension there is, the stiffer they get and the higher the F0 produced (Titze, 1994). Looking at the minimum F0 (hereafter minF0) that an individual is able to produce, which should be obtained with minimal P_{sub} and tension on the vocal folds, therefore appears to be the appropriate standardized approach to determine whether F0 is a good indicator of body size. Although Hauser (1993) also found negative correlations between body size and minF0 and between body size and maximum F0 (maxF0), neither P_{sub} nor vocal fold tension could be experimentally controlled for in the acoustic signals recorded from the various studies combined in his analysis.

Some of our recent work revisits the approach used by Hauser (1993) and suggests that F0 directly reflects laryngeal size rather than body size in primates and carnivores (Bowling et al., *In Prep.*). In the present comparative study, we take an in-depth look at the physical and physiological determinants of primate F0. We investigate specifically how well minF0 is predicted by vocal fold length and body size in multiple primate species. Following an anatomical estimation of vocal fold

length using CT-scans of excised laryngeal specimens, we phonate these same specimens with an excised larynx system under standardized conditions of Psub and vocal fold tension. Because larynx size and body size are not necessarily related and based on the reasoning above, we predict that a measure of vocal fold length, rather than body size, will best predict the minF0 of acoustic signals. Although the possible decoupling of larynx and body size has been previously discussed in primate vocal production (Fitch and Hauser, 1995; Fitch and Hauser, 2002), this study is the first empirical test of this assumption with matching anatomical and acoustical measurements. Given the extreme diversity of primate vocal signals and vocal anatomy (see Brown, 2002; Fischer, 2002; Fitch, 2002; Fitch and Hauser, 1995; Hammerschmidt and Fischer, 2008 for reviews on various aspects of primate vocal production), we expect this approach to yield additional insights on factors influencing vocal production in this taxon. In particular, we could directly examine the possibility that vibrating tissue other than the vocal folds (e.g. epiglottis or ventricular folds) influence or wholly determine F0.

Material and methods

Data collection

Anatomical specimens

As part of the specimen acquisition program at the National Museum of Scotland, the remains of deceased European zoo animals are regularly collected and processed. Specimen body length was measured as the distance between the ischium of the pelvis and the top of the skull. Larynges from cadavers of 30 different primate species were excised, then frozen and stored at -20° Celsius before being shipped to the Department of Cognitive Biology, University of Vienna. Each larynx was then thawed, cleaned, inspected, photographed and measured in preparation

for X-ray CT scanning, after which they were frozen again and stored at -20° Celsius.

CT scans

Depending on the size of the larynx specimen, 2 different scanning procedures were applied: 5 larynges were scanned using micro CT and 25 larynges using normal (macro) CT (each larynx from a different species). All CT scans were performed at the University of Veterinary Medicine Vienna. Macro-CT scans were made using a Siemens Somatom Emotion multislice helical CT-scanner (Siemens AG, Munich, Germany), and Micro-CT scans were made using an Xradia microXCT-400 (0.4x lens; Carl Zeiss X-ray Microscopy, Pleasanton, CA). For macro-CT scans, specimens were positioned in ventral recumbancy on X-ray-transparent styrofoam plates and scanned frozen. Scanning parameters varied with specimen size, with 110 kV source voltage and 80 mA intensity being used for smaller specimens and 130kV and 94 mA being used for larger specimens. Reconstructed image slices measured 512 x 512 pixels. Depending on specimen size, voxel resolution of the reconstructed volumes varied between 238 and 340 μm in the X and Y planes, and between 200 and 500 μm in the Z plane. Due to the small specimen sizes and longer scanning times, micro-CT specimens were thawed prior to scanning and mounted vertically inside sealed Falcon tubes, the bottoms of which had been filled with phosphate-buffered saline to prevent specimen dehydration. Specimens were scanned at 40keV source voltage and 200 μA intensity. Projections were recorded with 1s exposure time (camera binning = 4) and an angular increment of 0.25°. Reconstructed slices measured 512x512 pixel. Depending on specimen size, voxel resolution of reconstructed volumes varied between 30 μm^3 and 35 μm^3 .

Excised larynx experiments

The choice of specimens to study further was based on the data available and our final selection did not include specimens for which data on body length or on vocal fold length were missing (see Table1).

Family	Species	Common Name	CT scan	Sex	Specimen body length (cm)	Vocal fold length (mm)	Min F0 (Hz)
Atelidae	Alouatta caraya	Black howler	macro	f	57	57.76	35.5
Atelidae	Alouatta Sara	Bolivian red howler	macro	m	65.5	64.40	25.42
Atelidae	<i>Ateles fusciceps</i>	<i>Black-headed spider monkey</i>	<i>macro</i>	<i>f</i>	<i>34.5</i>	<i>16.64</i>	
Callitrichidae	<i>Cebuella pygmaea niveiventris</i>	<i>Pygmy marmoset</i>	<i>micro</i>	<i>f</i>	<i>15.5</i>	<i>4.84</i>	
Cercopithecidae	<i>Cercopithecus neglectus</i>	<i>De Brazza's monkey</i>	<i>macro</i>	<i>m</i>	<i>60.8</i>	<i>23.99</i>	
Cercopithecidae	<i>Chlorocebus pygerythrus</i>	<i>Vervet monkey</i>	<i>macro</i>	<i>u</i>	<i>51.1</i>	<i>15.06</i>	
Cercopithecidae	<i>Colobus guereza</i>	<i>Mantled guereza</i>	<i>macro</i>	<i>f</i>	<i>56.5</i>	<i>21.77</i>	
Hominidae	<i>Gorilla gorilla gorilla</i>	<i>Western Gorilla</i>	<i>macro</i>	<i>f</i>	<i>94</i>	<i>35.40</i>	
Lemuridae	<i>Lemur catta</i>	<i>Ring-tailed lemur</i>	<i>macro</i>	<i>m</i>	<i>43.7</i>	<i>13.84</i>	
Callitrichidae	<i>Leontopithecus chrysomelas</i>	<i>Golden-headed lion tamarin</i>	<i>micro</i>	<i>m</i>	<i>25</i>	<i>6.67</i>	
Cercopithecidae	Macaca fuscata	Japanese macaque	macro	f	72.6	17.15	91.49
Cercopithecidae	Macaca silenus	Lion-tailed macaque	macro	f	53	15.70	123.81
Cercopithecidae	Macaca sylvanus	Barbary macaque	macro	f	59	14.87	185.85
Cercopithecidae	<i>Mandrillus leucophaeus</i>	<i>Drill</i>	<i>macro</i>	<i>m</i>	<i>85.5</i>	<i>21.19</i>	
Cercopithecidae	<i>Mandrillus sphinx</i>	<i>Mandrill</i>	<i>macro</i>	<i>m</i>	<i>83.5</i>	<i>20.25</i>	
Hylotidae	<i>Nomascus leucogenys</i>	<i>White-cheeked gibbon</i>	<i>macro</i>	<i>u</i>	<i>49.2</i>	<i>20.03</i>	
Hominidae	<i>Pan troglodytes</i>	<i>Chimpanzee</i>	<i>macro</i>	<i>f</i>	<i>98</i>	<i>38.25</i>	
Cercopithecidae	Papio hamadryas	Hamadryas baboon	macro	m	78	25.05	63.46
Pitheciidae	<i>Pithecia pithecia</i>	<i>White-faced saki</i>	<i>macro</i>	<i>u</i>	<i>39.5</i>	<i>13.90</i>	
Callitrichidae	<i>Saguinus bicolor bicolor</i>	<i>Pied tamarin</i>	<i>micro</i>	<i>f</i>	<i>22</i>	<i>6.90</i>	
Callitrichidae	<i>Saguinus oedipus</i>	<i>Cotton-top tamarin</i>	<i>micro</i>	<i>m</i>	<i>21</i>	<i>6.00</i>	
Cebidae	<i>Sapajus Apella</i>	<i>Brown capuchin</i>	<i>macro</i>	<i>f</i>	<i>41.5</i>	<i>11.36</i>	
Hylotidae	<i>Symphalangus syndactylus</i>	<i>Siamang</i>	<i>macro</i>	<i>m</i>	<i>68.5</i>	<i>29.47</i>	
Lemuridae	<i>Varecia rubra</i>	<i>Red-ruffed lemur</i>	<i>macro</i>	<i>f</i>	<i>56.5</i>	<i>18.10</i>	
Lemuridae	<i>Varecia variegata</i>	<i>Black-and-white ruffed lemur</i>	<i>macro</i>	<i>f</i>	<i>50</i>	<i>17.86</i>	

Table 1. Primate species for which data was available both for body length (from anatomical measurements) and vocal fold length (proxy estimated from CT-scan measurements). Bold: species for which phonation from an excised larynx is

available to date. *Italic*: species intended to be run to have an accurate representation of primate families and sizes. Normal font: species to potentially add for sampling purposes.

Specimens used to date (in bold in Table1) were slowly thawed, then prepared (i.e. removal of excess tissue and tracheal rings) and mounted on a vertical subglottic tube. The tube diameter was adjusted depending on the specimen size so to form an airtight seal with the trachea. Larynx stability and support were ensured by adjustable customized plastic support structures placed on either side and anteriorly to the larynx (built using LEGO blocks). Phonation was obtained by pushing warm (ca. 37° Celsius) and humid (100%) air through the larynx. Vocal folds were adducted by 2 manual micromanipulators (Warzhauser MM33 with tilting system). For standardization purposes, adduction degree was fixed when phonation could be reliably induced with airflow and reached a chest register (assessed by ear and via EGG examination by the experimenters). Psub was controlled with the "ELLApp" software (written in Python by Christian T. Herbst, Dpt. of Cognitive Biology, University of Vienna). Acoustic, EGG and SPL signals were acquired, calibrated and stored with ELLApp, respectively using a DPA 4061 omnidirectional microphone positioned at a known distance from the vocal folds, a Glottal Enterprises EG 2-1000 two-channel electroglottograph (lower cutoff-frequency 2 Hz) and a NL-52 RION SPL-meter (placed 30cm away from the vocal folds; 'dB C' weighting). Pressure sweeps were applied to the mounted larynx (15s increase followed by 15s decrease; lower margin below phonation threshold pressure (PTP henceforth); upper margin varied depending on the size of the specimen but aimed at avoiding damage to the vibrating source). Two pressure sweeps were run both with and without the epiglottis covering the airway (4 sweeps in total). Finally, throughout the experiments, larynges were kept hydrated with saline solution (0.9% NaCl).

Data analysis

Anatomical measurements: CT scans

Both macro and micro CT data were analyzed in AMIRA (version 5.6.0). Because soft tissue geometry was difficult to visualize in CT, the aim was to obtain clear 3D visualization of the cartilages (meaning, in this study, hyoid bone, thyroid, cricoid and arytenoid cartilages). After creating an “isosurface” model of these cartilages, vocal fold length was estimated based on measurement landmarks placed on a sagittal plan cutting the cricoid and thyroid cartilages (Figure 1).

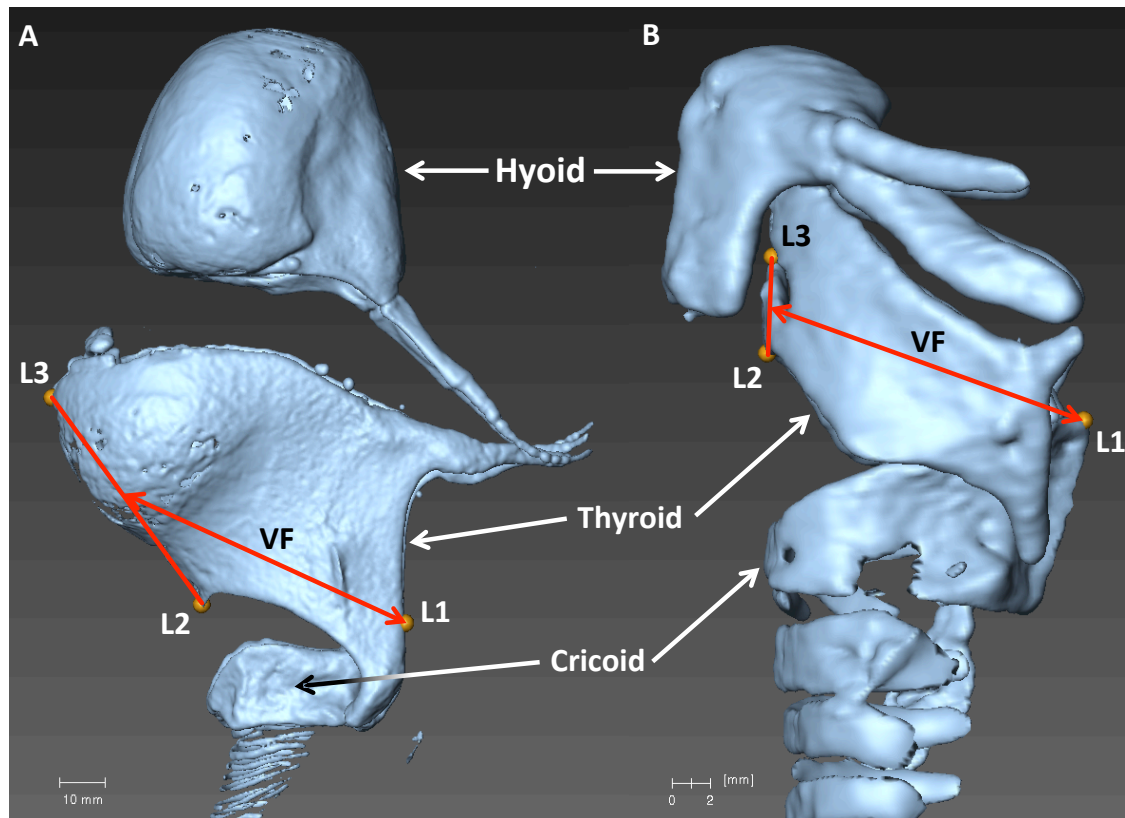


Figure 1. Isosurface of large and small laryngeal specimens (A: *Alouatta sara*; B: *Macaca fuscata*) showing the landmarks used to estimate vocal fold length. L1: Dorsal apical cricoid; L2: Ventral basal thyroid; L3: Ventral apical thyroid. VF: segment used as a proxy for vocal fold length.

The most dorsal point of the vocal folds was defined at the apex of the cricoid cartilage (used as a proxy for the vocal processes of the arytenoids). The most ventral possible attachment point of the vocal folds was defined as the midline distance between the apex and the base of the thyroid cartilage (as the thyroid attachment of the vocal fold could not be seen directly).

Signal analysis: Excised larynx experiments

Analysis of acquired signals was conducted using Praat (Boersma and Weenink, 2014) and ELLApp. After synchronization of the various input signals (implemented within ELLApp), EGG signals were annotated in Praat and F0 was extracted with appropriately adjusted settings (Praat function ‘Analyze periodicity: To Pitch (ac)...’). Settings were adjusted both relying on visual inspection of the spectrograms (non-periodic regimes were not considered) and of the waveform (in order to discriminate periodic signals from subharmonics). The pitch object created by F0 extraction was then edited manually in order to minimize pitch-tracking errors induced by automatic F0 extraction. As a result, we removed from the minF0 analysis all parts of the signals characterized by a non-periodic oscillation of the vocal folds, as well as those where Praat’s automatically calculated F0 was in obvious contradiction with the signal analyzed from the waveform (from which the period of oscillation, hence the F0, can be obtained and compared to the attributed F0). MinF0 was queried based on the remaining annotated sections, using the praat ‘Get minimum...’ function.

Additionally, to investigate the relationship between F0 and Psub, time and Psub data were extracted from data arrays, keeping only the corresponding segments to those annotated with F0. Because larynges had been frozen, the details of the relationship between Psub and F0 might have been altered; additionally, the pressure sweeps applied were not designed to investigate the full range of Psub that could be applied to the vocal folds. Therefore, only a qualitative description is made regarding the connection between these two parameters.

Statistics

Data normality was assessed using Shapiro-Wilk tests and appropriate pairwise correlations were computed on the two following regressions: minF0 vs. body size and minF0 vs. vocal fold length. Computations were done using R (RCoreTeam, 2015).

Results

Anatomical correlates of F0

Vocal fold length data did not follow a normal distribution, but body length and minF0 were normal (values are reported Table 1). After log-transformation, all data met normality; data were thus log-transformed and Pearson product-moment correlations were computed on the following regressions: minF0 vs. body length and minF0 vs. vocal fold length. F0 correlated significantly and negatively with vocal fold length ($r = -0.96$, $p = 0.002$) whereas no significant correlation was found with body length ($r = -0.21$, $p = 0.69$) (Figure 2).

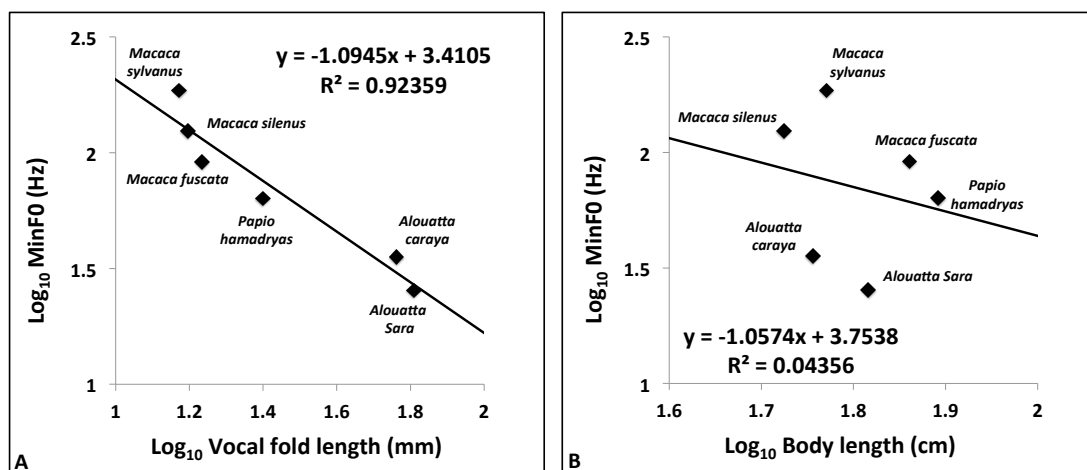


Figure 2. Bivariate plots illustrating correlations between vocal fold length and minF0 (panel A) and between body length and minF0 (panel B).

This is unsurprising given the inclusion of two howler monkey species (which have a greatly enlarged larynx) in our current sample. For 3 out of the 6 species investigated, minF0 was obtained when the epiglottis rested over the airway (*Macaca sylvanus*, *Alouatta caraya*, *Macaca fuscata*), the other half when the epiglottis was pulled out of the airway (*Papio hamadryas*, *Macaca silenus*, *Alouatta sara*). Covering of the airway by the epiglottis therefore did not appear to play a key role in determining minF0.

Qualitative description of vocal fold vibrations as a function of Psub

In most of the recordings we obtained, including the test runs, we observed a positive relationship between Psub and F0 (e.g. Figure 3A). Additionally, in most of these cases, hysteresis was present, meaning that there is a dependency of a given vibratory mode on the previous mode. A direct result of this phenomenon is that for a given input Psub, different F0 values could be obtained (Figure 3B, e.g. at Psub = 35 mBars). In a few cases, a clear dichotomy could be observed with sections of phonation for which Psub was negatively related to F0 and others showing a positive relationship (Figure 3B).

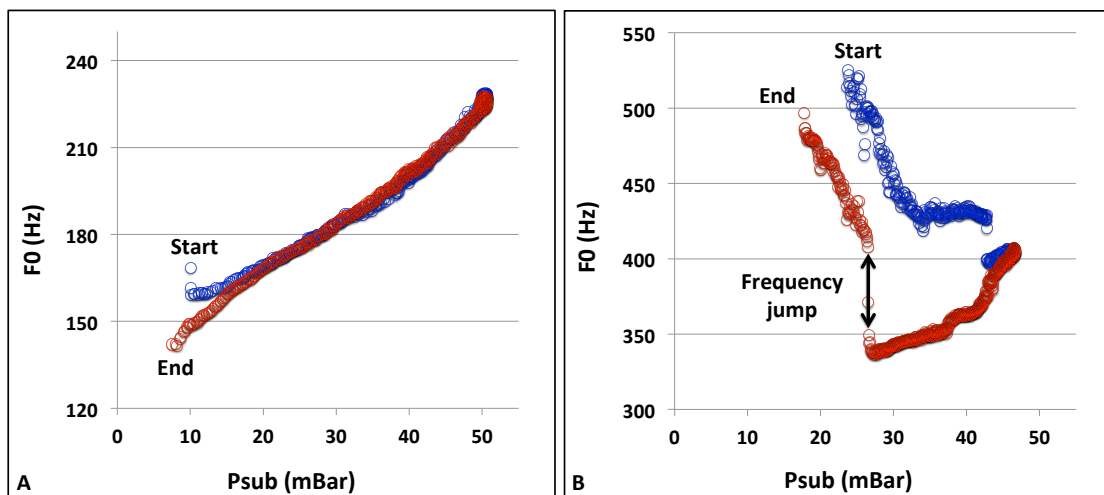


Figure 3. Bivariate plots illustrating the different types of relationships obtained between Psub and F0. Blue: Increasing Psub; Red: decreasing Psub. Panel A shows

an example of a positive and relatively linear relationship (Species: *Papio hamadryas*, recording ID: 3308.2) while the relationship in panel B shows an assortment of different phenomena (hysteresis, frequency jumps and sections of either positive or negative relationships; Species: *Macaca sylvanus*, recording ID: 3336.1).

Finally, gaps in F0 variation can be seen (Figure 3B, e.g. at ca. 28 mBars a frequency jump occurs while Psub decreases; another might occur at ca. 42 mBars when Psub increases), reflecting abrupt changes in the vibratory regimes of the vocal folds (which are reminiscent of register breaks in the human voice (van den Berg, 1968)). This last observation was verified by visual inspection of the spectrograms and evaluation of the corresponding praat pitch object (which had to be continuous, because non-annotated sections could have led to artifactual frequency jumps).

Discussion

We found that, at least within the primate species investigated to date in this study, body length is a poor predictor of the minimum fundamental frequency attainable by a given larynx. In contrast, vocal fold length strongly predicted minF0. This result clearly highlights the lack of correlation between the length of the vocal folds and that of the body and therefore confirms the potential for decoupling that exists between primate larynx size and body size.

Although F0 correlates relatively well with body size when considering a wide range of mammalian species (Dunn et al., 2015; Herbst et al., 2012), this correlation is typically weak or nonexistent at the within species level ((Lass, 1978; Masataka, 1994; Pfefferle et al., 2007; Rendall et al., 2005; Vannoni and McElligott, 2008) but see (Charlton et al., 2009; Pfefferle and Fischer, 2006)). Our results show that F0 is poorly predicted by individual body size across primate species, instead reflecting laryngeal anatomy, in particular vocal fold length. This suggests strong

variations of the laryngeal anatomy among different species, independently from body size. The two species of howler monkeys (*Alouatta spp.*) included in this study illustrate this clearly, with extremely enlarged hyoid apparatus and larynx (Schon, 1971; Schön Ybarra, 1988) and very low frequency vocalizations (Dunn et al., 2015; Fitch and Hauser, 2002; Kitchen, 2004).

Unlike F0, formant-related features have been shown to strongly predict an individual's body size in various mammalian species (Charlton et al., 2011; Charlton et al., 2009; Fitch, 1997; Garcia et al., *Submitted*; Harris et al., 2006; Reby and McComb, 2003). The enlarged larynx of howler monkeys contains an air sac, which potentially acts as a resonance chamber and could constitute an impedance-matching system to transmit low frequencies into the environment more efficiently or lower formant frequencies, thus exaggerating the size impression conveyed to conspecifics (Fitch and Hauser, 1995). Playback experiments will be necessary to test whether conspecifics attend to these cues and disentangle the roles of F0 and formants in these species. Because the *Alouatta* enlarged thyroid could constrain both vocal fold length (which are ventrally attached to the thyroid) and the size of the air sac, it is possible that both F0 and/or formants could provide a cue to individual body size within howler species.

Although vocal fold oscillation can be induced by active contraction of the vocal fold musculature (the so-called “active” theory of phonation, which applies to cat purring (Remmers and Gautier, 1972)) phonation is typically generated by the airflow passing through the glottis (the myoelastic-aerodynamic theory (MEAD; van den Berg, 1958)). Because of physiological limitations on the rate of muscular contraction, active phonation is only expected below rates of 40 Hz (Fitch, 2002; Hirose et al., 1969), unless it results from the contraction of superfast laryngeal muscles, which can contract up to 180 times a second in bats (Elemans et al., 2011). We found that, for the two howler species investigated, the MEAD principle is sufficient to generate frequencies below 40Hz. This suggests that active contraction of the laryngeal muscles is not necessary to produce the low-frequency calls typical of howler species. In order to definitely rule out the implication of active phonation

however, further investigation of the laryngeal musculature and neurophysiological evidence will be necessary, since, to our knowledge, such data is not yet available.

Supporting previous studies (Briefer, 2012; Scherer, 1986), the positive relation between P_{sub} and F_0 , commonly observed in our experiments, suggests that F_0 could provide a cue to the internal state and arousal of the caller. Higher F_0 would require higher P_{sub} , reflecting e.g. stronger or faster respiratory muscle contraction, which could in turn depend on the emotional or affective state of the caller (Scherer, 1986). The various other patterns in the data we gathered on the P_{sub} - F_0 relationship illustrate one way that larynx experiments can contribute to deepen our understanding of both vocal production mechanisms and the control of vocal production in primates. Because our experimental protocol was not specifically designed to answer such questions (both in terms of adequacy and sample size, and potentially relative to the freezing process), the following reflection is speculative and intends to produce hypotheses testable in future research. This underscores how further work based on excised larynges could benefit the field of primate, and more generally mammal, vocal communication research.

The transitions between vibratory regimes observed in some of the F_0 - P_{sub} curves in our study might provide insight into behavioral aspects of vocal usage in primates (see Fischer, 2002 for an interesting review on this topic). Our observations suggest a behaviorally-relevant potential usage of the nonlinearities inherent to the primate vocal production apparatus. Brown and colleagues conducted excised larynx experiments with squirrel monkeys (Brown et al., 2003) and found that, for a given P_{sub} , the vocal output of the system varied rapidly and abruptly from one regime to another, without apparent predictability. As a result, Brown suggested that acoustic fluctuation within call sequences produced by animals in their natural habitat might not be necessarily controlled (Brown, 2002). The common opinion and available research on primate vocal communication is that vocalizations are largely innate, while subtle changes in vocal production and limited control are possible (see Fischer, 2002; Hammerschmidt and Fischer, 2008

for reviews). Accordingly, from our observations it seems extremely unlikely that an individual would have substantial control over vocal nonlinearities themselves (these phenomena are typically avoided by adult humans (Fitch, 2002)), but it might learn to use these nonlinearities in a functional way, for instance in generating rapidly varied vocal sequences (Brown, 2002) or by ‘vocalizing at the edge’ (Riede et al., 2007).

The hysteresis effect that we commonly observed provides additional evidence that a low-level control of vocal parameters such as F0 might occur in some cases. This could contribute to the understanding of the puzzling vocal accommodation found in primates (Fischer, 2002; Hammerschmidt and Fischer, 2008). Coo calls in Japanese macaques (*macaca fuscata*) typically represent an upward-downward frequency sweep and Sugiura (Sugiura, 1998) showed that subjects exposed to playback stimuli produced response calls that match the frequency range of the broadcast call, compared to those they usually produce. If hysteresis occurs in the same ways *in vivo* and *in vitro*, a certain degree of control over F0 (anyways suggested by the author) is likely to be involved. In-depth investigation of regime transitions and hysteresis needs to be carried out both via excised larynx and acoustic playback experiments to elucidate the role of nonlinear phenomena (see Fitch et al., 2002; Wilden et al., 1998 for suggestions) in order to understand their use in vocal repertoires (see e.g. Reby and Charlton, 2012; Townsend and Manser, 2011).

The negative relationship between Psub and F0 that we sometimes observed in our data suggests that additional parameters might influence F0. Vocal membranes (or vocal lips) are thin, lightweight upward extensions of the main bulk of the vocal folds present in various primate species (Mergell et al., 1999; Schön Ybarra, 1995). They have been suggested to enable the production of high frequency vocalizations due to their low mass (Fitch, 2002; Fitch and Hauser, 1995; Fitch and Hauser, 2002; Mergell et al., 1999; Schön Ybarra, 1995) and could help explain our occasional observations. As Psub increases, more of these membranes begin vibrating, thus decreasing F0 until the main bulk of the vocal folds is recruited for phonation, then exhibiting a positive relationship between Psub and F0. Inspection

of the EGG signals supports our hypothesis: as P_{sub} increases and F_0 decreases, the amplitude of the EGG signal increases as well but is nevertheless lower than when both P_{sub} and F_0 increase, and *vice versa*. However, we have no direct evidence for this and the role of vocal fold mass on F_0 has been recently challenged (Titze, 2011). Further research investigating the detailed biomechanics of vocal fold oscillation will be necessary to shed light on this question. It will also be of interest to investigate how the ontogeny of the vocal apparatus can interact with the development of vocal behavior as individuals gain more experience with vocal production and usage (see Fischer, 2002; Hammerschmidt and Fischer, 2008 for insightful perspective on vocal ontogeny in primates).

Finally, conclusions drawn from excised larynx experiments seem intrinsically related to the source characteristics of vocal production. Because of the diversity found in primate vocal signals in terms of F_0 and vocal tract length and shape, a combination of F_0 and vocal tract that would favor interactions between the sound source and the vocal tract (Titze, 1994) is likely to occur (so-called “feedback systems” (Sundberg, 1991)). While excised larynx experiments obviously cannot provide information on the vocal tract characteristics, they might establish a basis for identifying the conditions under which such a phenomenon could occur.

Ultimately, this study demonstrates that, at least in the primate species investigated here, body size does not predict F_0 , which instead appears to be mainly determined by the length of the vocal folds. This conclusion directly emphasizes the large anatomical variation that can be found in primate vocal production systems. The hypotheses raised in our discussion offer a promising picture of how excised larynx experiments can potentially contribute to the understanding of the production mechanisms and functions of primate and mammal vocal communication, and call for an increased application of this methodology in bioacoustics research.

References

- Boersma, P. and Weenink, D.** (2014). Praat: doing phonetics by computer [Computer program]. University of Amsterdam, The Netherlands, Retrieved from <http://www.praat.org/>.
- Bowling, D., Garcia, M., Dunn, J., Ruprecht, R., Stewart, A., Hanke, G., Heinz-Frommolt, K., S., H., Dengg, S., Gumpenberger, M. et al.** (*In Prep.*). Body size, the larynx and vocalization.
- Briefer, E. F.** (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1-20.
- Brown, C., H.** (2002). Ecological and Physiological Constraints for Primate Vocal Communication. In *Primate Audition: Ethology and Neurobiology*: CRC Press.
- 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.
- 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**, 3414-22.
- Charlton, B. D., Zhihe, Z. and Snyder, R. J.** (2009). The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim. Behav.* **78**, 893-898.
- Davies, N. B. and Halliday, T. R.** (1978). Deep croaks and fighting assessment in toads *Bufo bufo*.
- Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., Dengg, S., Fitch, W. T. and Knapp, L. A.** (2015). Evolutionary Trade-Off between Vocal Tract and Testes Dimensions in Howler Monkeys. *Curr. Biol.* **25**, 2839-44.
- Elemans, C. P. H., Mead, A. F., Jakobsen, L. and Ratcliffe, J. M.** (2011). Superfast Muscles Set Maximum Call Rate in Echolocating Bats. *Science* **333**, 1885-1888.
- Fant, G.** (1960). Acoustic theory of speech production. The Hague: Mouton.
- Fischer, J.** (2002). Developmental Modifications in the Vocal Behavior of Non-Human Primates. In *Primate Audition: Ethology and Neurobiology*: CRC Press.
- 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.
- Fitch, W. T.** (2002). Primate Vocal Production and Its Implications for Auditory Research. In *Primate Audition: Ethology and Neurobiology*: CRC Press.
- Fitch, W. T. and Hauser, M. D.** (1995). Vocal production in nonhuman primates: acoustics, physiology, and functional constraints on "honest" advertisement. *Am J Prim* **37**.
- Fitch, W. T. and Hauser, M. D.** (2002). Unpacking "Honesty": Vertebrate Vocal Production and the Evolution of Acoustic Signals. In *Acoustic Communication*, (ed. A. M. Ed. Simmons, Fay, R. R. & Popper, A. N.), pp. 65-137. New York: Springer.

Fitch, W. T., Neubauer, J. and Herzel, H. (2002). Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. *Anim. Behav.* **63**, 407-418.

Garcia, M., Wondrak, M., Huber, L. and Fitch, W. T. (Submitted). Honest signaling in domestic pigs (*Sus scrofa*): vocal allometry and the information content of grunt calls *J. Exp. Biol.*

Gingras, B. and Fitch, W. T. (2013). A three-parameter model for classifying anurans into four genera based on advertisement calls. *J. Acoust. Soc. Am.* **133**, 547-59.

Hammerschmidt, K. and Fischer, J. (2008). Constraints in primate vocal production. In *The evolution of communicative creativity: From fixed signals to contextual flexibility*, pp. 93-119: The MIT Press.

Harris, T. R., Fitch, W. T., Goldstein, L. M. and Fashing, P. J. (2006). Black and White Colobus Monkey (*Colobus guereza*) Roars as a Source of Both Honest and Exaggerated Information About Body Mass. *Ethology* **112**, 911-920.

Hauser, M. D. (1993). The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *Am. Nat.*, 528-542.

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.

Hirose, H., Ushijima, T., Kobayashi, T. and Sawashima, M. (1969). An experimental study of the contraction properties of the laryngeal muscles in the cat. *Ann. Otol. Rhinol. Laryngol.* **78**, 297-306.

Kitchen, D. M. (2004). Alpha male black howler monkey responses to loud calls: effect of numeric odds, male companion behaviour and reproductive investment. *Anim. Behav.* **67**, 125-139.

Lass, N. J. (1978). Correlational study of speakers' heights, weights, body surface areas, and speaking fundamental frequencies. *J. Acoust. Soc. Am.* **63**, 1218-20.

Masataka, N. (1994). Lack of correlation between body size and frequency of vocalizations in young female Japanese macaques (*Macaca fuscata*). *Folia Primatol. (Basel)* **63**, 115-8.

McComb, K. E. (1991). Female choice for high roaring rates in red deer, *Cervus elaphus*. *Anim. Behav.* **41**, 79-88.

Mergell, P., Fitch, W. T. and Herzel, H. (1999). Modeling the role of nonhuman vocal membranes in phonation. *J. Acoust. Soc. Am.* **105**, 2020-8.

Morton, E. S. (1977). On the Occurrence and Significance of Motivation-Structural Rules in Some Bird and Mammal Sounds. *Am. Nat.* **111**, 855-869.

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

Pfefferle, D. and Fischer, J. (2006). Sounds and size: identification of acoustic variables that reflect body size in hamadryas baboons, *Papio hamadryas*. *Anim. Behav.* **72**, 43-51.

Pfefferle, D., West, P. M., Grinnell, J., Packer, C. and Fischer, J. (2007). Do acoustic features of lion, *Panthera leo*, roars reflect sex and male condition? *J. Acoust. Soc. Am.* **121**, 3947-53.

RCoreTeam. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.

Reby, D. and Charlton, B. D. (2012). Attention grabbing in red deer sexual calls. *Anim. Cogn.* **15**, 265-70.

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.

Remmers, J. E. and Gautier, H. (1972). Neural and mechanical mechanisms of feline purring. *Respir. Physiol.* **16**, 351-361.

Rendall, D., Kollias, S., Ney, C. and Lloyd, P. (2005). Pitch (F0) and formant profiles of human vowels and vowel-like baboon grunts: The role of vocalizer body size and voice-acoustic allometry. *J. Acoust. Soc. Am.* **117**, 944.

Riede, T., Arcadi, A. C. and Owren, M. J. (2007). Nonlinear acoustics in the pant hoots of common chimpanzees (*Pan troglodytes*): Vocalizing at the edge. *J. Acoust. Soc. Am.* **121**, 1758.

Scherer, K. R. (1986). Vocal affect expression: a review and a model for future research. *Psychol. Bull.* **99**, 143-65.

Schon, M. A. (1971). The anatomy of the resonating mechanism in howling monkeys. *Folia Primatol. (Basel)* **15**, 117-32.

Schön Ybarra, M. (1988). Morphological adaptations for loud phonation in the vocal organ of howling monkeys. *Primate Rep* **22**, 19-24.

Schön Ybarra, M. A. (1995). A Comparative Approach to the Non-Human Primate Vocal Tract: Implications for Sound Production. In *Current Topics in Primate Vocal Communication*, eds. E. Zimmermann J. D. Newman and U. Jürgens), pp. 185-198. Boston, MA: Springer US.

Sugiura, H. (1998). Matching of acoustic features during the vocal exchange of coo calls by Japanese macaques. *Anim. Behav.* **55**, 673-687.

Sundberg, J. (1991). *The Science of Musical Sounds*: Academic Press.

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

Titze, I. R. (1994). *Principles of Voice Production*: Prentice Hall.

Titze, I. R. (2011). Vocal fold mass is not a useful quantity for describing F0 in vocalization. *J. Speech Lang. Hear. Res.* **54**, 520-2.

Townsend, S. W. and Manser, M. B. (2011). The function of nonlinear phenomena in meerkat alarm calls. *Biol. Lett.* **7**, 47-9.

van den Berg, J. (1958). Myoelastic-Aerodynamic Theory of Voice Production. *J. Speech Lang. Hear. Res.* **1**, 227-244.

van den Berg, J. (1968). Register problems. *Ann. N. Y. Acad. Sci.* **155**, 129-134.

Vannoni, E. and McElligott, A. G. (2008). Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS ONE* **3**, e3113.

Wilden, I., Herzel, H., Peters, G. and Tembrock, G. (1998). Subharmonics, Biphonation, and Deterministic Chaos in Mammal Vocalization. *Bioacoustics* **9**, 171-196.

General discussion

In this dissertation I have provided a broad overview of some of the questions and methodologies currently applied in mammal vocal communication research. Using the source filter theory as a foundation, I adopt both proximate and ultimate approaches to investigate how vocal signals are produced and constrained, and how they are perceived and interpreted by conspecifics. Throughout the several studies composing this thesis, I hope to contribute to ongoing discussions regarding recurrent topics in bioacoustics such as acoustic gradation and the general allometric rules that apply on the mammalian vocal apparatus. I also extend our understanding of how sexual selection pressures can shape vocal signal diversity, particularly considering that functional decisions of the receiver are reciprocally related to modifications of the sender's vocal output (either induced via direct behavioral or physiological feedback, or via anatomical changes on an evolutionary scale).

Playback experiments (Fischer 1998; Keenan et al. 2013) have demonstrated that what animals perceive from a conspecific's vocalization does not necessarily correspond to what the human ear distinguishes. This is probably because while continuous in terms of acoustic structure, calls could appear discrete to the receiver based on additional factors such as the context in which they occur (Fischer et al. 1995; Keenan et al. 2013; Price et al. 2015). Our examination of the wild boar (*Sus scrofa*) vocal repertoire illustrates this ambiguity regarding acoustic gradation. While paying attention only to the acoustic structure of their vocal signals, the production of the various call types composing the repertoire appears gradual, whereas the extremes of this acoustic continuum seem rather discrete in both their structure and the situations they are mostly associated with. Acoustic continuity is an issue perennially discussed with respect to mammal vocal signals (Marler 1976; Murray et al. 1998; Volodina 2000; Manser 2001; Boisseau 2005; Stoeger-Horwath et al. 2007; Keenan et al. 2013), and recent work provides new and insightful methods that will help to establish a common and solid comparative basis for the study of vocal repertoires

in multiple species (Wadewitz et al. 2015). Nevertheless, most study species that rely on vocal communication have their own species-specific communicative background (Dooling et al. 1992), with potentially different production characteristics and functions. In-depth understanding of a given vocal communication system therefore requires the integrative approach described in the general introduction and illustrated in the current thesis.

On the mechanisms of vocal production

Documenting the ways in which acoustic signals are constrained and determined by the physical characteristics of the vocal apparatus is a key component of this approach. After the source-filter theory defined the role of the larynx (with vocal folds as sound source) and that of the vocal tract (acting as a bank of bandpass filters), early research emphasized how F0 could be limited by body size and could thus provide an accurate cue to this trait of the caller (Morton 1977; Davies & Halliday 1978; Ohala 1983; Hauser 1993). This hypothesis was based on the assumption that larger animals have larger larynges and thus longer and thicker vocal folds, which have been shown to produce lower F0 (Titze 1994). While this assumption may generally hold across species (Hauser 1993; Herbst et al. 2012; Dunn et al. 2015), larynx growth is not particularly constrained by the dimensions of the rest of the body (Fitch & Hauser 1995). Therefore, within a given mammalian species, F0 is often poorly related to adult body size (Lass 1978; Masataka 1994; Reby & McComb 2003; Rendall et al. 2005; Pfefferle et al. 2007; Vannoni & McElligott 2008). In the comparative study presented in chapter 5, we investigated this relationship across various primate species and extend our understanding of vocal allometry within mammals. Using acoustic recordings from excised larynx experiments and anatomical measurements from CT scans of the same specimens, we show that vocal fold length, rather than body size, predicts F0 in our sample of primates. Our data show that this applies as well to species presenting extreme laryngeal modifications, and demonstrate why these species fall out of the standard 'body

size vs. F0' regression (see also Dunn et al. 2015 for an example with howler monkeys, *Alouatta spp.*). Additionally, chapter 5 confirms the relationship that exists between subglottal pressure and fundamental frequency, at least in the primate species investigated in this thesis. Although interesting phenomena could be seen that suggest a more complicated relationship than previously thought, we observed that F0 generally increases with subglottal pressure for a given laryngeal configuration (determined in our experiments by a steady adduction of the vocal folds, which has been shown to be an important factor in initiating phonation (Titze 1994)). Our data do not provide direct insight into the physiological conditions experienced by an animal, as would an *in vivo* assessment, and they would for instance benefit from complementary measurements such as heart rate monitoring (Briefer et al. 2015) or stress hormones levels (Schrader & Todt 1998). Nevertheless, they provide a window into the signaler's internal state, based on the existing connection between subglottal pressure, emotional state and muscular contraction of the laryngeal and respiratory systems (Scherer 1986; Briefer 2012). Overall, the study emphasizes how source-related acoustic features of the signaler's vocalizations (more specifically F0 in these cases) can convey various types of information to the receiver.

Because the supra-laryngeal portion of the vocal tract is much more confined by the surrounding anatomical structures like the skull (Fitch & Hauser 1995; Fitch 2000a; Fitch 2000b), and because the length of the vocal tract mainly determines its formants, formants are more likely to provide reliable information about body size in mammals than is F0 (Fitch & Hauser 1995). Accordingly, formants and/or formant spacing have been shown to negatively correlate with body size in many mammalian species (chacma baboons (Owren et al. 1997), colobus monkeys (Harris et al. 2006), domestic dogs (Riede & Fitch 1999), fallow deer (Vannoni & McElligott 2008), giant pandas (Charlton et al. 2009), koalas (Charlton et al. 2011), red deer (Reby & McComb 2003), rhesus macaques (Fitch 1997) and southern elephant seals (Sanvito et al. 2007) – which is not exhaustive). The study we presented in chapter 2 allows us to add the domestic pig (*Sus scrofa domesticus*) to this list, as we show through acoustic, weight and X-ray measurements that, as pigs grow, overall body size is positively correlated

with vocal tract length and negatively correlated with formant frequencies. Even in species presenting extreme modifications of the vocal tract (which can mainly be interpreted as vocal tract elongation (Fitch & Hauser 2002)), size information conveyed by formants or formant spacing at a within-species level remains relevant. Male red deer (*Cervus elaphus*) for instance have specific anatomical adaptations that allow them to retract their larynx down to the sternum while producing mating calls (Fitch & Reby 2001). Although they sound larger than other species of the same size that did not evolve similar adaptations, assessment of body size by conspecifics is still possible (Reby et al. 2005; Charlton et al. 2007a; Charlton et al. 2007b) because the anatomical limitations imposed on vocal tract length are the same for all individuals within the same species (Fitch & Hauser 2002; Reby & McComb 2003). Other types of vocal tract modifications exists such as air sacs and extended trunks (see Fitch & Hauser 2002; Taylor & Reby 2010 for brief reviews) and the second chapter of this thesis suggests a similar mechanism in domestic pigs. Because grunts appear to be nasal vocalizations, this could represent a mild case of size exaggeration (since the longer nasal tract is characterized by lower formants in comparison to what would be expected if grunts were produced orally), which would nonetheless provide honest size information to conspecifics that have similar anatomical constraints.

It should be noted in this discussion that, while I focus on body size to illustrate how to unravel the potential information content of mammalian vocalizations, source- and filter-related acoustic parameters potentially provide cues to various other traits of the signaler. For example our study on domestic pigs also suggests that formants might carry vocal signatures of individual identity to conspecifics. Therefore one needs to adjust the orientation of a study based on the parameters examined and the issues being addressed.

On the function of vocalizations

The above outline of the proximate ways to study the mechanisms underlying mammal vocalizations demonstrates that exploring how acoustic signals are produced and constrained can provide insights into the potential information encrypted within acoustic signals. However, testing the perception and function of calls types and acoustic features is essential to validate predictions or assumptions and to reveal how animals actually perceive and use the information made available to them.

Studying the neural processing mechanisms induced by acoustic stimuli is one way to provide an deeper understanding of the reaction of the receiver to vocal signals (Simmons 2003). Similarly, behavioral analyses of the responses obtained through acoustic playback experiments (broadcasting natural or synthetic signals to animals) are highly informative (McGregor & Division 1992; Simmons 2003) and constitute a typical example of how scientists test the function of acoustic signals in many animal taxa (e.g. in birds (Kroodsma 1976; Kiefer et al. 2011), insects (Walker 1957; Clemens et al. 2014), fish (Tavolga 1958; McKibben & Bass 2001), amphibians (Ryan 1980; Amézquita et al. 2011), mammals (Espmark 1971; Manser et al. 2002; Charrier et al. 2010; Clay & Zuberbuhler 2011) and reptiles (Vergne & Mathevon 2008)). It is beyond the scope of this thesis to attempt to summarize all the methodological concerns (e.g.: pseudoreplication (Catchpole 1989; McGregor & Division 1992; Kroodsma et al. 2001; Wiley 2003)) and paradigms (e.g.: habituation/dishabituation (Eimas et al. 1971; Cheney & Seyfarth 1988; Rendall et al. 1996; Fischer 1998)) used in acoustic playback experiments.

The 3rd and 4th chapters of this thesis provide examples of playback experiments that investigate the meaning of acoustic signals, using the framework of the source-filter theory and focusing on red deer. We rely on well-established prior research on this model species, which revealed that formants and fundamental frequency are relevant acoustic features within inter- and intra-sexual selection contexts (Reby et al. 2001; Reby et al. 2005; Charlton et al. 2007a; Charlton et al. 2007b; Charlton et al. 2008; Reby et al. 2010; Reby &

Charlton 2012). This species provided an excellent example to understand the selective forces that lead to size exaggeration, as stags can elongate their vocal tract by laryngeal retraction, which lowers the formants in their calls, giving an impression of larger body size (Fitch & Reby 2001; Reby & McComb 2003). In chapter 3, we build on this previous knowledge and use playback experiments and signal re-synthesis to demonstrate that, unlike oestrous females (Reby et al. 2010), male red deer do not seem to attend to F0 in their assessment of other males. In chapter 4, we test the discrimination abilities of male red deer and show that, like females in this case (Reby & Charlton 2012), stags can differentiate between two different types of roars (common and harsh) and that harsh roars potentially provide the opponent with a cue to the motivational level of the signaler. This chapter also emphasizes the need for further research on nonlinear phenomena (NLP henceforth), among which we find deterministic chaos, the form of NLP characterizing the harsh roar. NLP are common features of mammal vocal signals (Fitch et al. 2002) that can affect both F0 (e.g.: deterministic chaos is created by irregular vibrations of the vocal folds (Wilden et al. 1998)) and formants (e.g.: deterministic chaos creates harsh, broadband frequency sounds (Wilden et al. 1998), which highlight formants better (Fitch & Hauser 1995)), and can carry various roles (Wilden et al. 1998; Fitch et al. 2002; Blumstein & Récapet 2009; Townsend & Manser 2011; Reby & Charlton 2012). With our studies, we thereby illustrate at the same time how to question and increase our knowledge about the role of acoustic features that are prominent in the source-filter theory. Our results also highlight how sexual selection pressures and honest signaling can influence acoustic signal structure and usage of various vocalizations, through the evolutionary feedback loop generated by functional decisions of the receiver (Taylor & Reby 2010).

It must be noted that the context considered above (sexual selection) is representative but not the only evolutionary pressure that can be investigated within the functional approach to mammal vocal communication. Other factors such as environment (Ey & Fischer 2009), predators (Robinson 1980) or sociality (Freeberg et al. 2012) can also create evolutionary feedback loops affecting acoustic structure or call usage (Maciej et al. 2013; Manser et al. 2014). In regards to the habitat type for instance, with propagation experiments one can

functionally test how acoustic stimuli are tuned to specific purposes such as sound localization (Waser 1977) or transmission (Mathevon et al. 1997).

In summary, using various model species (wild boar, domestic pig, red deer and a wide range of primate species) and methodological tools (X-rays, CT-scans, excised larynx experiments, anatomical measurement, field playback experiments and acoustic recordings), this thesis shows how we can improve our understanding of mammal vocal communication by pursuing both proximate and ultimate questions. The results clearly illustrate the need to apply an integrative analysis to any particular study species, trying to understand the connections between internal state, mechanical constraints, acoustic structure, functional meaning and associated response behaviors of mammal vocalizations. Such a multi-faceted approach can help support inferences regarding the evolutionary pressures that drove the emergence of vocalization, and shaped vocal communication systems.

I believe that conducting research while bearing in mind these objectives will benefit future comparative work both within and between taxa – see Elemans et al. 2015 as an example comparing sound production mechanisms in birds and mammals. As outlined by some of the perspectives raised in our studies, such research will support topics of growing interest in bioacoustics like nonlinear phenomena and vocal correlates of emotion, and may further serve in applications to animal welfare and conservation. Finally bioacoustic investigations can help gaining insight into evolutionary questions such as the effect of domestication on vocal communication systems or even the emergence of spoken language in our own species.

References

- Amézquita, A., Flechas, S. V., Lima, A. P., Gasser, H. & Hödl, W. 2011: Acoustic interference and recognition space within a complex assemblage of dendrobatid frogs. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 17058-17063.
- Blumstein, D. T. & Récapet, C. 2009: The Sound of Arousal: The Addition of Novel Non-linearities Increases Responsiveness in Marmot Alarm Calls. *Ethology* **115**, 1074-1081.
- Boisseau, O. 2005: Quantifying the acoustic repertoire of a population: The vocalizations of free-ranging bottlenose dolphins in Fiordland, New Zealand. *J. Acoust. Soc. Am.* **117**, 2318.
- Briefer, E. F. 2012: Vocal expression of emotions in mammals: mechanisms of production and evidence. *J. Zool.* **288**, 1-20.
- Briefer, E. F., Tettamanti, F. & McElligott, A. G. 2015: Emotions in goats: mapping physiological, behavioural and vocal profiles. *Anim. Behav.* **99**, 131-143.
- Catchpole, C. K. 1989: Pseudoreplication and external validity: Playback experiments in avian bioacoustics. *Trends Ecol. Evol.* **4**, 286-287.
- Charlton, B. D., Ellis, W. A., McKinnon, A. J., Cowin, G. J., Brumm, J., Nilsson, K. & 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.
- Charlton, B. D., Reby, D. & McComb, K. 2007a: Female perception of size-related formant shifts in red deer, *Cervus elaphus*. *Anim. Behav.* **74**, 707-714.
- Charlton, B. D., Reby, D. & McComb, K. 2007b: Female red deer prefer the roars of larger males. *Biol. Lett.* **3**, 382-385.
- Charlton, B. D., Reby, D. & McComb, K. 2008: Effect of combined source (F0) and filter (formant) variation on red deer hind responses to male roars. *J. Acoust. Soc. Am.* **123**, 2936-2943.
- Charlton, B. D., Zhihe, Z. & Snyder, R. J. 2009: The information content of giant panda, *Ailuropoda melanoleuca*, bleats: acoustic cues to sex, age and size. *Anim. Behav.* **78**, 893-898.
- Charrier, I., Aubin, T. & Mathevon, N. 2010: Mother-calf vocal communication in Atlantic walrus: a first field experimental study. *Anim. Cogn.* **13**, 471-482.
- Cheney, D. L. & Seyfarth, R. M. 1988: Assessment of meaning and the detection of unreliable signals by vervet monkeys. *Anim. Behav.* **36**, 477-486.
- Clay, Z. & Zuberbuhler, K. 2011: Bonobos extract meaning from call sequences. *PLoS ONE* **6**, e18786.
- Clemens, J., Krämer, S. & Ronacher, B. 2014: Asymmetrical integration of sensory information during mating decisions in grasshoppers. *Proceedings of the National Academy of Sciences* **111**, 16562-16567.
- Davies, N. B. & Halliday, T. R. 1978: Deep croaks and fighting assessment in toads *Bufo bufo*.
- Dooling, R. J., Brown, S. D., Klump, G. M. & Okanoya, K. 1992: Auditory perception of conspecific and heterospecific vocalizations in birds: evidence for special processes. *J. Comp. Psychol.* **106**, 20-28.
- Dunn, J. C., Halenar, L. B., Davies, T. G., Cristobal-Azkarate, J., Reby, D., Sykes, D., Dengg, S., Fitch, W. T. & Knapp, L. A. 2015: Evolutionary Trade-Off between

- Vocal Tract and Testes Dimensions in Howler Monkeys. *Curr. Biol.* **25**, 2839-2844.
- Eimas, P. D., Siqueland, E. R., Jusczyk, P. & Vigorito, J. 1971: Speech Perception in Infants. *Science* **171**, 303-306.
- Elemans, C. P., Rasmussen, J. H., Herbst, C. T., During, D. N., Zollinger, S. A., Brumm, H., Srivastava, K., Svane, N., Ding, M., Larsen, O. N., Sober, S. J. & Svec, J. G. 2015: Universal mechanisms of sound production and control in birds and mammals. *Nat. Commun.* **6**, 8978.
- Espmark, Y. 1971: Individual recognition by voice in reindeer mother-young relationship. Field observations and playback experiments. *Behaviour* **40**, 295-301.
- Ey, E. & Fischer, J. 2009: The "Acoustic Adaptation Hypothesis"—a Review of the Evidence from Birds, Anurans and Mammals. *Bioacoustics* **19**, 21-48.
- Fischer, J. 1998: Barbary macaques categorize shrill barks into two call types. *Anim. Behav.* **55**, 799-807.
- Fischer, J., Hammerschmidt, K. & Todt, D. 1995: Factors Affecting Acoustic Variation in Barbary-macaque (*Macaca sylvanus*) Disturbance Calls. *Ethology* **101**, 51-66.
- 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.
- Fitch, W. T.. 2000a: The phonetic potential of nonhuman vocal tracts: comparative cineradiographic observations of vocalizing animals. *Phonetica* **57**, 205-218.
- Fitch, W. T.. 2000b: Skull dimensions in relation to body size in nonhuman mammals: The causal bases for acoustic allometry. *Zoology* **103**, 40-58.
- Fitch, W. T. & Hauser, M. D. 1995: Vocal production in nonhuman primates: acoustics, physiology, and functional constraints on "honest" advertisement. *Am J Prim* **37**.
- Fitch, W. T. & Hauser, M. D.. 2002: Unpacking "Honesty": Vertebrate Vocal Production and the Evolution of Acoustic Signals. In: *Acoustic Communication*. (Ed. Simmons, A. M., Fay, R. R. & Popper, A. N.), ed). Springer, New York. pp. 65-137.
- Fitch, W. T., Neubauer, J. & Herzel, H. 2002: Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. *Anim. Behav.* **63**, 407-418.
- Fitch, W. T. & Reby, D. 2001: The descended larynx is not uniquely human. *Proc. Biol. Sci.* **268**, 1669-1675.
- Freeberg, T. M., Dunbar, R. I. & Ord, T. J. 2012: Social complexity as a proximate and ultimate factor in communicative complexity. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **367**, 1785-1801.
- Harris, T. R., Fitch, W. T., Goldstein, L. M. & Fashing, P. J. 2006: Black and White Colobus Monkey (*Colobus guereza*) Roars as a Source of Both Honest and Exaggerated Information About Body Mass. *Ethology* **112**, 911-920.
- Hauser, M. D. 1993: The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *Am. Nat.*, 528-542.
- Herbst, C. T., Stoeger, A. S., Frey, R., Lohscheller, J., Titze, I. R., Gumpenberger, M. & Fitch, W. T. 2012: How Low Can You Go? Physical Production Mechanism of Elephant Infrasonic Vocalizations. *Science* **337**, 595-599.

- Keenan, S., Lemasson, A. & Zuberbühler, K. 2013: Graded or discrete? A quantitative analysis of Campbell's monkey alarm calls. *Anim. Behav.* **85**, 109-118.
- Kiefer, S., Scharff, C. & Kipper, S. 2011: Does age matter in song bird vocal interactions? Results from interactive playback experiments. *Front Zool* **8**, 1-8.
- Kroodsma, D. E. 1976: Reproductive development in a female songbird: differential stimulation by quality of male song. *Science* **192**, 574-575.
- Kroodsma, D. E., Byers, B. E., Goodale, E., Johnson, S. & Liu, W.-C. 2001: Pseudoreplication in playback experiments, revisited a decade later. *Anim. Behav.* **61**, 1029-1033.
- Lass, N. J. 1978: Correlational study of speakers' heights, weights, body surface areas, and speaking fundamental frequencies. *J. Acoust. Soc. Am.* **63**, 1218-1220.
- Maciej, P., Ndao, I., Hammerschmidt, K. & Fischer, J. 2013: Vocal communication in a complex multi-level society: constrained acoustic structure and flexible call usage in Guinea baboons. *Front Zool* **10**, 58-58.
- Manser, M. B. 2001: The acoustic structure of suricates' alarm calls varies with predator type and the level of response urgency. *Proc. Biol. Sci.* **268**, 2315-2324.
- Manser, M. B., Jansen, D. A. W. A. M., Graw, B., Hollén, L. I., Bousquet, C. A. H., Furrer, R. D. & le Roux, A. 2014: Chapter Six - Vocal Complexity in Meerkats and Other Mongoose Species. In: *Adv. Study Behav.* (Naguib, M., Barrett H., L., Brockmann, J., Healy, S., Mitani, J. C., Roper, T. J. & Leigh, W. S., eds). Academic Press, Oxford. pp. 281-310.
- Manser, M. B., Seyfarth, R. M. & Cheney, D. L. 2002: Suricate alarm calls signal predator class and urgency. *Trends in Cognitive Sciences* **6**, 55-57.
- Marler, P. 1976: Social organization, communication and graded signals: The chimpanzee and the gorilla. In: *Growing points in ethology.* (Bateson, P. P. G. & Hinde, R. A., eds). Cambridge U Press, Oxford, England.
- Masataka, N. 1994: Lack of correlation between body size and frequency of vocalizations in young female Japanese macaques (*Macaca fuscata*). *Folia Primatol. (Basel)* **63**, 115-118.
- Mathevon, N., Aubin, T. & Brémond, J.-C. 1997: Propagation of bird acoustic signals: comparative study of starling and blackbird distress calls. *Comptes Rendus de l'Académie des Sciences - Series III - Sciences de la Vie* **320**, 869-876.
- McGregor, P. K. & Division, N. A. T. O. S. A. 1992: Playback and studies of animal communication. Plenum Press.
- McKibben, J. R. & Bass, A. H. 2001: Effects of temporal envelope modulation on acoustic signal recognition in a vocal fish, the plainfin midshipman. *J. Acoust. Soc. Am.* **109**, 2934.
- Morton, E. S. 1977: On the Occurrence and Significance of Motivation-Structural Rules in Some Bird and Mammal Sounds. *Am. Nat.* **111**, 855-869.
- Murray, S. O., Mercado, E. & Roitblat, H. L. 1998: Characterizing the graded structure of false killer whale (*Pseudorca crassidens*) vocalizations. *J. Acoust. Soc. Am.* **104**, 1679-1688.
- Ohala, J. J. 1983: Cross-language use of pitch: an ethological view. *Phonetica* **40**, 1-18.

- Owren, M. J., Seyfarth, R. M. & Cheney, D. L. 1997: The acoustic features of vowel-like grunt calls in chacma baboons (*Papio cyncephalus ursinus*): Implications for production processes and functions. *J. Acoust. Soc. Am.* **101**, 2951-2963.
- Pfefferle, D., West, P. M., Grinnell, J., Packer, C. & Fischer, J. 2007: Do acoustic features of lion, *Panthera leo*, roars reflect sex and male condition? *J. Acoust. Soc. Am.* **121**, 3947-3953.
- Price, T., Wadewitz, P., Cheney, D., Seyfarth, R., Hammerschmidt, K. & Fischer, J. 2015: Vervets revisited: A quantitative analysis of alarm call structure and context specificity. *Scientific reports* **5**, 13220.
- Reby, D. & Charlton, B. D. 2012: Attention grabbing in red deer sexual calls. *Anim. Cogn.* **15**, 265-270.
- Reby, D., Charlton, B. D., Locatelli, Y. & McComb, K. 2010: Oestrous red deer hinds prefer male roars with higher fundamental frequencies. *Proc. R. Soc. Lond. B* **277**, 2747-2753.
- Reby, D., Hewison, M., Izquierdo, M. & Pepin, D. 2001: Red Deer (*Cervus elaphus*) Hinds Discriminate Between the Roars of Their Current Harem-Holder Stag and Those of Neighbouring Stags. *Ethology* **107**, 951-959.
- Reby, D. & 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.
- Reby, D., McComb, K., Cargnelutti, B., Darwin, C., Fitch, W. T. & Clutton-Brock, T. 2005: Red deer stags use formants as assessment cues during intrasexual agonistic interactions. *Proc. R. Soc. Lond. B* **272**, 941-947.
- Rendall, D., Kollias, S., Ney, C. & Lloyd, P. 2005: Pitch (F0) and formant profiles of human vowels and vowel-like baboon grunts: The role of vocalizer body size and voice-acoustic allometry. *J. Acoust. Soc. Am.* **117**, 944.
- Rendall, D., Rodman, P. S. & Emond, R. E. 1996: Vocal recognition of individuals and kin in free-ranging rhesus monkeys. *Anim. Behav.* **51**, 1007-1015.
- Riede, T. & Fitch, T. 1999: Vocal tract length and acoustics of vocalization in the domestic dog (*Canis familiaris*). *J. Exp. Biol.* **202**, 2859-2867.
- Robinson, S. R. 1980: Antipredator behaviour and predator recognition in Belding's ground squirrels. *Anim. Behav.* **28**, 840-852.
- Ryan, M. J. 1980: Female mate choice in a neotropical frog. *Science* **209**, 523-525.
- Sanvito, S., Galimberti, F. & Miller, E. H. 2007: Vocal signalling of male southern elephant seals is honest but imprecise. *Anim. Behav.* **73**, 287-299.
- Scherer, K. R. 1986: Vocal affect expression: a review and a model for future research. *Psychol. Bull.* **99**, 143-165.
- Schrader, L. & Todt, D. 1998: Vocal Quality is Correlated with Levels of Stress Hormones in Domestic Pigs. *Ethology* **104**, 859-876.
- Simmons, A. 2003: Perspectives and Progress in Animal Acoustic Communication. In: *Acoustic Communication*. (Simmons, A., Fay, R. & Popper, A., eds). Springer New York. pp. 1-14.
- Stoeger-Horwath, A. S., Stoeger, S., Schwammer, H. M. & Kratochvil, H. 2007: Call repertoire of infant African elephants: first insights into the early vocal ontogeny. *J. Acoust. Soc. Am.* **121**, 3922-3931.
- Tavolga, W. N. 1958: The Significance of Underwater Sounds Produced by Males of the Gobiid Fish, *Bathygobius soporator*. *Physiol. Zool.* **31**, 259-271.

- Taylor, A. M. & Reby, D. 2010: The contribution of source-filter theory to mammal vocal communication research. *J. Zool.* **280**, 221-236.
- Titze, I. R. 1994: *Principles of Voice Production*. Prentice Hall.
- Townsend, S. W. & Manser, M. B. 2011: The function of nonlinear phenomena in meerkat alarm calls. *Biol. Lett.* **7**, 47-49.
- Vannoni, E. & McElligott, A. G. 2008: Low frequency groans indicate larger and more dominant fallow deer (*Dama dama*) males. *PLoS ONE* **3**, e3113.
- Vergne, A. L. & Mathevon, N. 2008: Crocodile egg sounds signal hatching time. *Curr. Biol.* **18**, R513-514.
- Volodina, E. V. 2000: Vocal repertoire of the cheetah *Acinonyx jubatus* (Carnivora, Felidae) in captivity: sound structures and their potential for estimating the state of adult animals. *Zoologicheskii-Zhurnal* **79**, 833-843.
- Wadewitz, P., Hammerschmidt, K., Battaglia, D., Witt, A., Wolf, F. & Fischer, J. 2015: Characterizing Vocal Repertoires--Hard vs. Soft Classification Approaches. *PLoS ONE* **10**, e0125785.
- Walker, T. J. 1957: Specificity in the Response of Female Tree Crickets (Orthoptera, Gryllidae, Oecanthinae) to Calling Songs of the Males. *Ann. Entomol. Soc. Am.* **50**, 626-636.
- Waser, P. M. 1977: Sound Localization by Monkeys: A Field Experiment. *Behav. Ecol. Sociobiol.* **2**, 427-431.
- Wilden, I., Herzel, H., Peters, G. & Tembrock, G. 1998: Subharmonics, Biphonation, and Deterministic Chaos in Mammal Vocalization. *Bioacoustics* **9**, 171-196.
- Wiley, R. H. 2003: Is there an ideal behavioural experiment? *Anim. Behav.* **66**, 585-588.

Publication overview and author contribution

Published

Garcia M, Wyman MT, Charlton BD, Fitch WT & Reby D. 2014. Response of red deer stags (*Cervus elaphus*) to playback of harsh versus common roars. *Naturwissenschaften* 101(10): 851-4.

Garcia M, Charlton BD, Wyman MT, Fitch WT & Reby D. 2013. Do red deer stags (*Cervus elaphus*) use roar fundamental frequency (F0) to assess rivals? *PLoS ONE* 8(12): e83946.

In press

Garcia M, Bowling DL, Gingras B, Herbst CT, Böckle M, Locatelli, Y & Fitch WT. *In Press*. Structural classification of Wild Boar (*Sus scrofa*) vocalizations. *Ethology*.

Hampala V, **Garcia M**, Svec JG, Scherer RC & Herbst CT. *In Press*. Relationship between the Electroglottographic Signal and Vocal Fold Contact Area. *Journal of Voice*.

Submitted

Garcia M, Wondrak M, Huber L & Fitch WT. *Submitted*. Honest signaling in domestic pigs (*Sus scrofa*): vocal allometry and the information content of grunt calls. *Journal of Experimental Biology*.

In prep

Bowling DL, **Garcia M**, Dunn JC, Ruprecht R, Stewart A, Hanke G, Heinz-Frommolt K, Handschuhe S, Dengg S, Gumpenberger M & Fitch WT. Body size, the larynx and vocalization.

Herbst CT. Hampala V, **Garcia M**, Hofer R & Švec JG. Hemi-laryngeal setup for excised larynx experiments. *Journal of visualized experiments*.

Garcia M, Herbst CT, Bowling DL, Dunn JC & Fitch WT. A comparative study of vocal production in primates using excised larynx experiments: determinants of F0.

Curriculum Vitae

Maxime GARCIA

<i>Personal information</i>	<i>Contact</i>
Age : 28 Born 05.12.1987 in Brest, France Gender: Male Nationality: French	Department of Cognitive Biology Faculty of Life Sciences, University of Vienna Althanstrasse 14, 1090 Vienna, Austria ☎ (+43) 680 55 05 341 maxime.garcia@ymail.com

<i>Research Interests</i>
Vocal communication, Ethology, Behavioral biology, Conservation

<i>Education</i>	
2012 - present	PhD candidate – Biology, Dpt. of Cognitive Biology, University of Vienna. Supervisor: Prof. Dr. WT Fitch . Graduation scheduled April 20th 2016 .
2010-2011	Graduation Master 2 (University Paris-Sud XI – France). High Honors – Cell Signaling and Neurosciences, specialization Neurosciences.
2008-2009	Graduation Master 1 (University Paris XIII – France). Honors – Ethology.
2005-2008	Graduation Bachelor degree (University Rennes 1 – France). High Honors – Biology of Organisms.
2005	Graduation high school (Brest - France). High Honors – Earth and Life Sciences – English European Section.

<i>Work experience</i>	
October – November 2014	Teaching assistant: Bioacoustics I, University of Vienna
January – June 2011 University Paris Sud XI, France / University of Lethbridge, Canada	Research internship (Master’s): Characteristics and functions of drumming: a non vocal acoustic signal in a territorial species, the Ruffed Grouse (<i>Bonasa umbellus</i>). Supervisors: Dr. T Aubin, Dr. I Charrier & Dr. A Iwaniuk
May – June 2010 Volunteer internship CNRS – CNPS UMR 8195.	Research internship: Study of the acoustic interactions and their variations in a territorial oscine species, the Winter Wren (<i>Troglodytes troglodytes</i>). Supervisor: Dr. T Aubin
April – May 2009 University Paris XIII / Animal reserve Orlu, Ariège	Research internship (Master’s): Behavioural responses of a Canadian Wolfpack (<i>Canis lupus lycaon</i>) to olfactory stimuli of different interest (Territoriality, Feeding, Predation). Supervisor: M Berthelon, Director “La Maison des Loups”

Grants & media	
Grants:	- August 2015: Dean's Travel Grant, University of Vienna: 1410 € - August 2014: International Society for Behavioural Ecology travel Award: 1500 \$ - January 2014: European Science Foundation Exchange Grant: 6050 € - 2008/2009: Bourse au mérite – French Government: 1800 €
Media:	2012: Grouse have signature drumming styles. New Scientist, issue 2848.

Language skills (other than French)	
English & Spanish:	Fluent
German:	Basics

Publications/Communications	
Publications:	Garcia M, Wondrak M, Huber L & Fitch WT. <i>Submitted</i>. Honest signaling in domestic pigs (<i>Sus scrofa</i>): vocal allometry and the information content of grunt calls. Journal of Experimental Biology. Garcia M, Bowling DL, Gingras B, Herbst CT, Böckle M, Locatelli, Y & Fitch WT. <i>In Press</i>. Structural classification of Wild Boar (<i>Sus scrofa</i>) vocalizations. Ethology. - Hampala V, Garcia M, Svec JG, Scherer RC & Herbst CT. <i>In Press</i>. Relationship between the Electroglottographic Signal and Vocal Fold Contact Area. Journal of Voice. Garcia M, Wyman MT, Charlton BD, Fitch WT & Reby D. 2014. Response of red deer stags (<i>Cervus elaphus</i>) to playback of harsh versus common roars. Naturwissenschaften 101(10): 851-4. Garcia M, Charlton BD, Wyman MT, Fitch WT & Reby D. 2013. Do red deer stags (<i>Cervus elaphus</i>) use roar fundamental frequency (F0) to assess rivals? PLoS ONE 8(12): e83946. Garcia M, Charrier I. & Iwaniuk AN. 2012. Directionality of the Ruffed Grouse (<i>Bonasa umbellus</i>, L.) drumming display. The Condor 114(3): 500-506. Garcia M, Charrier I, Rendall D & Iwaniuk AN. 2012. Temporal and spectral analyses reveal individual variation in a non-vocal acoustic display: the wingbeat drumming display of the Ruffed Grouse (<i>Bonasa umbellus</i>, L.). Ethology 118(3): 292-301.
Poster presentation:	XXV International Bioacoustics Council (IBAC) 2015, Murnau, Germany, 7-12.09.2015. Acoustic allometry in 91 mammals: body size, larynx size and call frequency. 15th International Society for Behavioral Ecology congress (ISBE) 2014, NYC, USA, 31.07-05.08.2014. Red deer stags (<i>Cervus elaphus</i>) keep a low profile when facing noisy opponents. Canadian Society for Ecology and Evolution (CSEE) 2011, Banff, Canada, 12-15.05.2011. Bioacoustic analyses reveal individual variation among male Ruffed Grouse (<i>Bonasa umbellus</i>) drumming displays.

Oral communication:

Behaviour 2015. Cairns, Australia, 09-14.08.2015. Structural classification of Wild Boar (*Sus scrofa*) vocalizations.

2nd European student conference on Behaviour and Cognition 2015. Zurich, Switzerland, 05-06.03.2015. Responses of red deer stags (*Cervus elaphus*) to playbacks of harsh vs. common roars.

COGCOM student workshop 2015. Vienna, Austria 02-03.07.2015. Mechanisms and functions of vocal communication in mammals.

VII European Conference on Behavioural Biology (ECBB) 2014, Prague, Czech Republic, 17-20.07.2014. Call classification of the complex Wild Boar (*Sus scrofa*) vocalization system.

10th Ecology and Behaviour meeting (EB) 2014, Montpellier, France, 12-16.05.2014. Do red deer stags use roar pitch to assess rivals?

9th topical meeting of the Ethological Society (“Function and Mechanisms of Animal Behaviour”) 2014, Tutzing, Germany, 06-08.02.2014. Red deer stags keep a low profile when facing noisy opponents.

Pan-European Voice Conference (PEVOC) 2013, Prague, Czech Republic, 21-24.08.2013. Call classification design of the Wild Boar (*Sus scrofa*) complex vocalization system.

International Ethological Conference (IEC) 2013, Newcastle UK, 4-8.08.2013. Do red deer stags use roar pitch to assess rivals?

Workshops:

COGCOM student workshop 2015. Vienna, Austria, 02-04.07.2015.

European Academy of Voice Tutorial 2013, Prague, Czech Republic, 21.08.2013.

Comparative Cognition, 2013, St-Andrews, UK, 03.08.2013.

Scientific skills

- | | |
|--|--|
| - Pack Microsoft Office | - Imaging softwares: Amira, ImageJ, Gimp |
| - Signal analysis: Avisoft, Goldwave, Praat | - Acoustics and Video data collection and analysis |
| - Cartography: Mapsource. | - Sampling of biological material (tissues/organs) |
| - Statistics: SPSS, R | - Authorization to manipulate Liquid Nitrogen |
| - Programming: basics in Python, R, Praat and Matlab | |

Zusammenfassung

Die Erforschung von Kommunikationssystemen ist ein essentieller Punkt für das Verständnis von Verhalten, Ökologie und Evolution von Tieren. Vor allem bei Säugetieren wurde der Erforschung der Lautkommunikation beträchtliches Augenmerk geschenkt, beginnend mit rein beschreibenden Ansätzen, bis hin zur Anwendung der Theorie von Quelle und Filter in der menschlichen Sprachproduktion (source-filter theory) bei tierischen Lautäußerungen. Dieser Ansatz hat entscheidende Fortschritte für die Erforschung und Interpretation der Funktion von Lautäußerungen gebracht. Nichtsdestotrotz wurde der Erforschung der Mechanismen der Lautproduktion – bei denen es sich um einen wichtigen Aspekt der Lautkommunikation handelt – und ihrer biologischen Relevanz weit weniger Aufmerksamkeit geschenkt (obwohl sie in der Physik als myoelastisch-aerodynamischer Prozess (MEAD theory) gut beschrieben sind).

Aufbauend auf die Quelle/Filter-Theorie zeige ich in dieser Dissertation auf, wie aktuelle Methoden und Erkenntnisse über Lautäußerungen bei Säugetieren in diesem Feld zur Anwendung gebracht werden können – sowohl durch die Erforschung des Lautrepertoires einer bis jetzt unerforschten Tierart, als auch durch die detaillierte Entschlüsselung der Lautäußerungen einer bereits gut beschriebenen Model-Tierart. Danach befasse ich mich eingehend mit der Verbindung zwischen der Produktion von Lauten und den resultierenden Lautäußerungen und zeige, wie anatomische Beschränkungen auf der Ebene der Lautquelle das Kommunikationssystem einer Tierart formen können. Insbesondere studiere ich die Zusammenhänge zwischen physisch-anatomischen Eigenschaften des Lautproduzenten und den entsprechenden akustischen Ergebnissen. Abschließend untersuche ich diese Zusammenhänge experimentell an herauspräparierten Kehlköpfen, wodurch sich einzigartige Erkenntnisse über die Lauterzeugung gewinnen lassen.

Zusammengenommen bieten die Studien, die Teil dieser Dissertation sind, einen facettenreichen Überblick über die Zusammenhänge zwischen verschiedenen Aspekten der Lautkommunikation bei Säugetieren. Sie schaffen

eine Grundlage für zukünftige Forschung, die es sich zum Ziel gesetzt hat, gleichzeitig die Funktionen und die Mechanismen der Produktion von akustischen Signalen bei Tieren zu untersuchen – einschließlich jener Fälle, wo evolutionäre Zwänge zu einer Spezialisierung des Lautapparats und damit verbundenen Lautäußerungen geführt haben.

Abstract

The study of communication systems is a central issue in the understanding of animal behavior, ecology and evolution. In mammals in particular, vocal communication has drawn considerable attention, starting with purely descriptive approaches, until more recently when the source-filter theory was applied from human speech to mammal acoustic signals. This framework has allowed fundamental progress regarding the interpretation and investigation of the function of vocalizations. Nonetheless, the mechanisms of sound production, an entire branch of vocal communication, have largely been neglected in terms of their biological relevance (the physics being relatively well explicated by the myoelastic-aerodynamic (MEAD) theory).

Relying on the source-filter theory, in this dissertation I highlight how current methodologies and knowledge about mammalian vocal communication are implemented in the field, either while initiating the study of a new species' vocal repertoire or while decoding in-depth some characteristics of an already well-known model species. I then delve into the connection between sound production and vocal output, investigating how anatomical constraints at the level of the sound source can shape a species vocal communication system. More specifically, I study the relationships between physical-anatomical traits of the signaler and their acoustic correlates. Finally, I empirically examine these relationships by means of excised larynx experiments, which provide a unique insight on sound generation.

The studies collected in this thesis provide a multi-faceted overview of the connections that exist between the various components of mammal vocal communication. They establish the foundation for future research intended to explore in parallel the functions and the production mechanisms of animal acoustic signals, including those particular cases where evolutionary pressures have led to specialization of the vocal apparatus and associated vocal displays.