

Vocal Tract Morphology Determines Species-Specific Features in Vocal Signals of Lemurs (*Eulemur*)

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Abstract The source-filter theory describes vocal production as a two-stage process involving the generation of a sound source, with its own spectral structure, which is then filtered by the resonant properties of the vocal tract. This theory has been successfully applied to the study of animal vocal signals since the 1990s. As an extension, models reproducing vocal tract resonance can be used to reproduce formant patterns and to understand the role of vocal tract filtering in nonhuman vocalizations. We studied three congeneric lemur species —*Eulemur fulvus*, *E. macaco*, *E. rubriventer*— using morphological measurements to build computational models of the vocal tract to estimate formants, and acoustic analysis to measure formants from natural calls. We focused on call types emitted through the nose, without apparent articulation. On the basis of anatomical measurements, we modeled the vocal tract of each species as a series of concatenated tubes, with a cross-sectional area that changed along the tract to approximate the morphology of the larynx, the nasopharyngeal cavity, the nasal chambers, and the nostrils. For each species, we calculated the resonance frequencies in 2500 randomly generated vocal tracts, in which we simulated intraspecific length and size variation. Formant location and spacing showed significant species-specific differences determined by the length of the vocal tract. We then measured formants of a set of nasal vocalizations (“grunts”) recorded from captive lemurs of the same species. We found species-specific differences in the natural calls. This is the first evidence that morphology of the vocal tract is relevant in generating filter-related acoustic cues that potentially provide receivers with information about the species of the emitter.

Keywords *Eulemur* · Formant · Modeling · Strepsirrhini · Vocalization

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Introduction

In both human and nonhuman primates, the sound generation process starts with the adduction of the vocal folds. The vocal fold closure blocks the flow of air from the lungs, resulting in an increased subglottal pressure. When the pressure becomes greater than the resistance provided by the vocal folds, they open again. If the pressure conditions are steady, the vocal cords will continue to open and close in a quasiperiodic way. The frequency of puffs of air passing through the glottis determines the fundamental frequency (F₀) of the laryngeal source. The column of air then vibrates into the supralaryngeal vocal tract, which consists of both the oral and nasal airways. In a successive independent event, the vocal tract serves as a filter, which suppresses the passage of sound energy at certain frequencies, leaving others unchanged. Those frequencies at which the energy remains at its maximum, sustained by the supralaryngeal vocal tract, are called formants. The frequency value of formants is determined in part by the length, the shape, and the volume of the vocal tract. The vocal tract can be altered to a certain degree, into a variety of shapes, by means of the articulators (Titze 1994). Articulation of the mandible, mouth opening, or a change in the relative position of the lips may lead to variation in the length and the shape of the vocal tract, generating a shift in the frequencies of formants in the output signal. The interpretation of phonation as a two-event process is postulated by the source-filter theory of voice production (Fant 1960), wherein the larynx is the source of the vocal signal and the vocal tract, as mentioned previously, acts as a filter.

In animals, formants reflect the characteristics, e.g., length, shape, of the cavities of the anatomical tubes (Taylor and Reby 2010) and can be studied to investigate differences between species (Gamba and Giacoma 2005), or to describe variation in the configurations of the vocal tract during the emission of different call types (Gamba and Giacoma 2007). Formants also correlate with vocal tract length in mammals (*Canis familiaris*: Riede and Fitch 1999; *Cervus elaphus*: Reby and McComb 2003) and may represent an indexical cue (Ghazanfar *et al.* 2007) for receivers, thus providing information related to the physical characteristics of the emitter.

The recognition of source and filter components as codeterminants of the acoustic features of the human voice has enabled the development of mathematical models to simulate vocal production. An extension of the source-filter theory to the interpretation of nonhuman vocal phonation (Gamba *et al.* 2012) is the application of computational models to describe the phonation processes of animals (Lieberman *et al.* 1969). Mammalian vocal tracts during vocalization resemble flared (or uniform) tubes and formants are evenly spaced (Shipley *et al.* 1991).

A computational model of the vocal tract is a mathematical representation of the luminal areas of the supralaryngeal tract with the boundaries of the internal organs and tissues. Two studies have used computational models to show that spectral prominences of nonhuman primate vocalizations were formants (*Cercopithecus diana*: Riede *et al.* 2005; *Varecia variegata*: Gamba and Giacoma 2006). However, the explanatory potential of applying models of vocal production is limited by the lack of knowledge concerning the anatomy and dynamics of vocal tracts (de Boer and Fitch 2010).

Nasal vocalizations are common in mammal species in various social and environmental circumstances (Efremova *et al.* 2011; Fitch 2006; Frey *et al.* 2007; Volodin

et al. 2011). Primates use nasal calls, and they are particularly common in forest environments (Gautier and Gautier-Hion 1982). For example, lemurs use sounds resonating in the nasal tract extensively (Colquhoun 1997; Gamba and Giacoma 2005; Gamba *et al.* 2012; Gosset *et al.* 2001; Macedonia and Stanger 1994).

The most common nasal vocalization in true lemurs (*Eulemur* spp.) is the grunt, which is emitted in various situations, including during group movements in the forest. Grunts are low-pitched calls of variable duration (Gamba and Giacoma 2005, 2006; Gamba *et al.* 2012). From previous observations we know that closed-mouth grunts in lemurs are given with the larynx engaged in the nasal airways, and air resonates through the nasal tract (M. Gamba *et al. pers. obs.*). We also observed the expulsion of mucus from the nostrils and that lemurs were able to produce grunts with food in the mouth, keeping it slightly open, and making the sound through the nose (M. Gamba *et al. pers. obs.*). When we visually compared spectrograms of grunts emitted in this circumstance with those given with a closed or open mouth, we found the same formant pattern as for closed-mouth grunts. Thus, we can hypothesize that lemurs are capable of the formation of a strong velar-lingual seal and can direct an exhalatory air stream exclusively through the nasal tract.

Focusing attention on the filtering process, we aimed to investigate the species-specific potential of nasal calls in three lemur species using formant analysis and computational models. We investigated the impact of the species-specific vocal tract morphology by characterizing the formants of nasal grunts in three congeneric species: *Eulemur fulvus*, *E. macaco*, and *E. rubriventer*. We 1) examined whether models are effective in reproducing species-specific formant patterns within and across the species, and then analyzed the relationship between formants and length of the vocal tract in each species. We 2) hypothesized that formants simulated from the computational models differ across species and correlate with length. We then conducted an acoustic analysis of grunts emitted by lemurs through the nasal airways to 3) show whether the formants of grunts vary across species. Finally, starting from the fact that mammalian vocal tracts resemble flared tubes during vocalization, we 4) hypothesized that higher formants appear as odd-numbered multiples of the first formant and that all formants are evenly spaced.

Materials and Methods

Focal Species

All of the focal species are medium-sized lemurs. The common brown lemur (*Eulemur fulvus*) has a head–body length of 43–50 cm and a tail length of 41–51 cm (Mittermeier *et al.* 2010). Body mass ranges from 2.0 to 3.1 kg (Mittermeier *et al.* 2010; M. Gamba *pers. obs.*). *Eulemur macaco* has a head–body length of 39–45 cm and a tail length of 51–65 cm. Body mass is 1.8–2.3 kg (M. Gamba *pers. obs.*; Terranova and Coffman 1997). *Eulemur rubriventer* has a head–body length of 35–40 cm and a tail length of 43–53 cm. Body mass is 1.6–2.4 kg (Glander *et al.* 1992; Terranova and Coffman 1997).

Morphology and Vocal Tract Modeling

We studied dead specimens in the collection at the Parc Botanique et Zoologique Tsimbazaza (Antananarivo, Madagascar). All specimens were adult and frozen shortly after death. We studied a single specimen for each species. The specimens were: an adult male *Eulemur rubriventer* (greatest skull length=85.65 mm), an adult female *E. fulvus* (greatest skull length=94.34 mm), and an adult female *E. macaco* (greatest skull length=98.78 mm).

Despite the obvious limitations due to the fact that investigation of cadaver structures cannot provide dynamic representations and the specimens may have decreased flexibility, the use of vocal tract casts of cadavers can be effective in describing cavity structures (Riede *et al.* 2006) and is usually feasible in developing countries such as Madagascar (Gamba and Giacoma 2006). We generated the cast by cutting the trachea 3–4 cm below the hyoid bone and then closing the tracheal tube by means of surgical clamps. We injected high-speed catalyzed silicon rubber (Blue Star Silicones Rhodorsil® RTV) that yielded a flexible cast, which we extracted with minimum effort from the mouth without opening the skull. After extraction, all fresh casts consisted of a single piece. We carefully investigated the areas around the larynx and we found that none of the study specimens had vocal air sacs.

We then measured the vocal tract silicon cast to build the computational model. All length and volume measurements of the cast were taken with a Mitutoyo digital caliper (accurate to 0.01 mm) with a 3.5-mm increment from the vocal folds toward the nostrils.

To create a vocal-tract model, we sampled the cross-sectional area function of the vocal tract, from the glottal constriction to the nostrils, such that the tract consisted of many cylindrical segments of equal lengths but different cross-sectional areas. The vocal tract area function represents the input of a customized version of VTAR modeling software (Zhang and Espy-Wilson 2004; Zhou *et al.* 2004), which is capable of simulating complex frequency-domain vocal tract models and calculates their acoustic response. As we studied the potential of nasal tract morphology in providing species-specific cues, we did not consider resonance in the oral tract or quantify articulation because vocal cavities are generally fixed during vocal production. In fact, in nonhuman primates, the column of air coming up from the lungs and passing the glottis then resonates either solely in the oral or solely in the nasal tract (Fitch 2000, 2006).

Body size variation may affect vocal tract length and shape. In the literature, we found very little evidence of variation in the length of primate vocal tracts, which usually fell between 2 % and 16 %. Most of those data are for humans (Xue and Hao 2006), but vocal tract length variation in rhesus macaques (*Macaca mulatta*) is 15 % (Fitch 1997). Body size measurements and vocal tract shape and length in *Lemur catta* show intraspecific variation of 15 % (Gamba 2006). Thus, we assumed that length and shape of a vocal tract segment could potentially increase or decrease 15 % from the original cast measurements. We used the *random* module (a set of classes and functions in python3) to generate random cross-sectional areas within this range of variation. We then randomized vocal tract segments within their sagittal position and generated vocal tract models for each species. During this process we varied the cross-sectional area of any cylinder independently to simulate the vocal tracts of a

virtual population of 2500 lemurs. This number was large enough to estimate variability within a wild population, as we calculated that a single lemur in the Malagasy rain forest may encounter 2000–2500 other lemurs, of any species, in its entire life. For each model we calculated the acoustic response in terms of frequency of the first four formants (F1–4).

Sound Recording and Analysis

We recorded vocalizations from 16 captive groups of lemurs, composed of 2–7 individuals (males:females), in the following zoos: Parco Natura Viva – Garda Zoological Park [Bussolengo, Italy, 2 *Eulemur rubriventer* (1:1), 9 *E. macaco* from 2 groups (6:3), 4 *E. fulvus* (2:2)] Mulhouse Zoo [France; 14 *E. rubriventer* from 3 groups (9:5), 9 *E. macaco* from 2 groups (5:4)], Parc Botanique et Zoologique de Tsimbazaza [Madagascar; 5 *E. rubriventer* from 2 groups (3:2), 8 *E. macaco* from 2 groups (5:3), 18 *E. fulvus* from 6 groups (10:8)], Banham Zoo [U.K.; 4 *E. rubriventer* (2:2), 2 *E. macaco* (1:1)], Linton Zoo [UK; 3 *E. macaco* (2:1), 3 *E. fulvus* (2:1)], Twycross Zoo [UK; 4 *E. rubriventer* (2:2)], Köln Zoo [Germany; 5 *E. rubriventer* (3:2), 2 *E. fulvus* (1:1)]. All focal groups received daily diets consisting mainly of fruits and vegetables, and water *ad libitum*. All subjects were kept on a natural light/dark daily cycle. All subjects were treated in accordance with Animal Care guidelines and international regulations. We collected data between June 1999 and May 2005. We sampled a total of 34 *Eulemur rubriventer* (20:14), 31 *E. macaco* (19:12), and 27 *E. fulvus* (15:12).

We conducted recordings with Sony TCD-D100 and TCD-D8 digital tape recorders, a Sennheiser microphone ME88, and Sony EMS-907 microphones. Nasal calls occurred when the lemurs were standing or moving. Video clips showed that they produced these calls through the nose with the mouth closed. To increase the number of vocalizations recorded, we used both all-occurrence and focal animal sampling methods (Altmann 1974). We digitized recordings at a sample rate of 44.1 kHz (16-bit resolution, mono format) using Praat (Boersma 2001). We selected all nasal vocalizations whose recording quality allowed acoustic analysis and saved them in separate files. The total sample of selected vocalizations comprised 1762 adult brown lemur calls, 1237 adult black lemur calls, and 4673 adult red-bellied lemur calls. We undertook this acoustic analysis for a comparison with the modeling approach, and wanted the results to be as general as possible so as to be able to compare their variability with the results we could obtain from the models of the vocal tracts we analyzed. We do not examine the effects of sex (because the models were from one male and two female lemurs), age, or other individual features of the caller on calls (Gamba and Giacoma 2007, 2008; Gamba *et al.* 2012), but instead provide a broader view of species-specific vocalization traits.

We studied formants (F1, F2, F3, F4) using linear predictive coding (LPC; Markel and Gray 1976). LPC is based on the assumption that the vocal signal is produced by a buzz generated at the glottis. The signal then passes through the vocal tract (throat, mouth, and nose) representing the tube, characterized by its resonances, which produces the formants. LPC estimates the frequency values corresponding to each formant. Because vocal signals usually vary over time, we conducted this process along all the signal frames and then calculated mean formants. Depending on the acoustic characteristics of the vocalization we used a window length of 0.04–0.06 s.

Because the position and number of formants vary with vocal tract length, we detected 5–7 formants depending on the vocal type. We used two methods to check the formants predicted by LPC. First, we superimposed formant analyses over the signal spectrogram. Second, we overlaid autocorrelation-based LPC spectra on independently derived FFT spectra of the same frames. The frequency range to measure up to F4 was 7000–8000 Hz, depending on the species, but we set the maximum formant to 12 000 Hz to ensure that we found a fit for higher formants too. We determined the formant pattern using a step-by-step monitored process, in which the operator could interrupt the analysis and modify the analysis parameters. We used a Praat script to automate file opening and editing and measurement saving. There is very little information on how to measure formant bandwidth in animal calls, so we did not examine this variable.

Because our aim was to investigate the relationship between species-specific acoustic properties and vocal tract morphology, we did not investigate call context or caller identity, nor did we measure sound variables other than formants (Fig. 1).

Statistical Analysis

We calculated coefficients of variation within species (CV_s) and between species (CV_b). We transformed formants derived from the models and measured from natural calls logarithmically and compared them using analysis of variance (ANOVA), in which data were normal, and the Kruskal-Wallis test, in which they were not normal. We used Tukey's test for *post hoc* analysis of ANOVA. To evaluate the effect of vocal tract length and volume we used a general linear model (GLM) in PASW Statistics 18 (IBM) using log-transformed formants as dependent variables, species as a fixed factor and log-transformed volume and length as covariates. We randomly resampled the original data set into smaller sets of *ca.* 25 % and repeated our analyses on these subsets to validate the predictive accuracy of the tests, which could be masked or magnified with a larger data set. We used two-step clustering to quantify the grouping

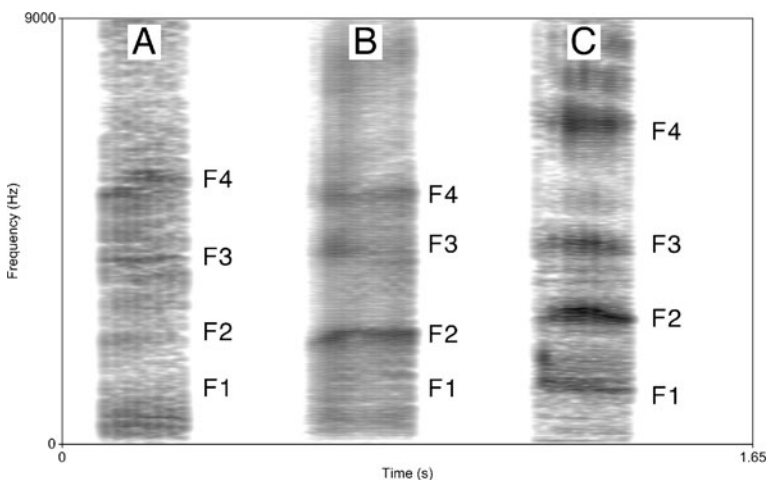


Fig. 1 Nasal grunts emitted by lemurs: *Eulemur fulvus* (a), *Eulemur macaco* (b), and *Eulemur rubriventer* (c). All spectrograms were generated in Praat (University of Amsterdam) using the following settings: view range, 0–9000 Hz; window length, 0.05 s; dynamic range, 50 dB.

power of model formants and of formants measured from vocalizations. We used two external criteria to estimate clustering quality: the Silhouette method (a. S.; Rousseeuw 1987) and Purity (Zhao and Karypis 2001).

Results

Vocal Tract Modeling

The vocal apparatus of *Eulemur* is not attached to the cranium and exhibits the same structure previously identified in other primate species (Gautier 1971; Harrison 1995). Viewed in the midsagittal plane, the vocal tracts were orientated horizontally in all the species. However, there were clear differences between species in cross-sectional area function (Fig. 2). Total tract length was 84.0 mm in *Eulemur rubriventer*, 94.5 mm in *E. macaco*, and 98.0 mm in *E. fulvus*, corresponding to computational models consisting of 24, 27, and 28 concatenated tubes of 3.5 mm each, respectively (Fig. 2).

Apart from F1, formants predicted from the computational models showed differences ranging from 10.6 % to 21.5 % depending on the species considered (Table I). Higher formants were lower in *Eulemur fulvus* and higher in *E. rubriventer*, as they were inversely scaled to the length and volume of the vocal tract model. For F1, the cumulative coefficient of variation within species was higher than between species (Table I). For all other variables, CV_b was greater than CV_s : about 7 times for F2 and

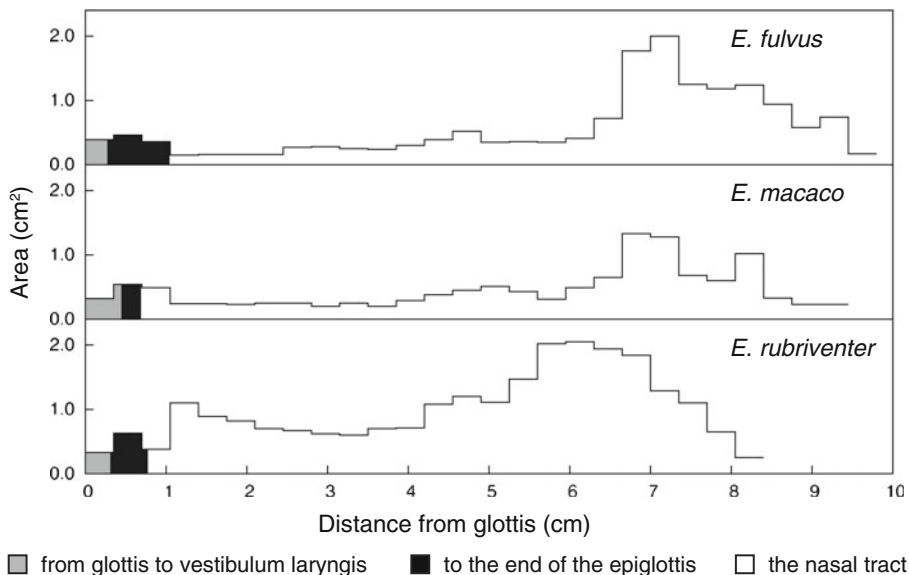


Fig. 2 Vocal tract area functions as estimated from the original casts. The larynx and the nasal tract are modeled as a succession of tubes, from the glottis to the nostrils. The image shows the concatenation of tubes along the vocal tract length. Each tube has a cross-sectional area as shown by its vertical extent on the y-axis and a constant length of 0.35 cm (x-axis). We then randomized tubes reduced and increased by 15 % of their original length and area, taking into account their mid-sagittal position.

Table 1 Mean and standard deviation of first (F1), second (F2), third (F3), and fourth formant (F4) for three species of *Eulemur* and in total, for both models and vocalizations

	Species	N	F1 (Hz)	F2 (Hz)	F3 (Hz)	F4 (Hz)
Models	<i>E. fulvus</i>	2500	1041±25	1923±41	3774±84	6363±132
	<i>E. macaco</i>	2500	1031±20	2088±46	4247±96	6648±151
	<i>E. rubriventer</i>	2500	1007±26	2490±55	5502±119	7645±169
	Total	7500	1026±28	2167±243	4508±736	6885±570
	CV _s		2.3	2.2	2.2	2.2
	CV _b		1.8	14.6	21.5	10.6
Vocalizations	<i>E. fulvus</i>	1762	1031±121	2008±258	3794±260	6253±490
	<i>E. macaco</i>	1237	1383±112	2017±115	5007±141	5862±411
	<i>E. rubriventer</i>	4673	873±50	2788±105	5436±136	6495±419
	Total	7672	991±201	2484±410	4990±692	6337±492
	CV _s		8.5	7.4	4.0	7.1
	CV _b		23.8	19.7	17.9	5.1

We calculated intra- (CV_s) and interspecies (CV_b) coefficients of variation for all of the acoustic parameters considered in the analysis (Sokal and Rohlf 1995)

5 times for F4. F3 showed the highest potential for species recognition coding, as its CV_b was about 10 times greater than CV_s.

Formants calculated from the vocal tract models differed significantly between species both when using the full data set ($N=7500$; ANOVA, F1, $F=1362.69$, $df=7499$, $P<0.001$; F2, $F=91476.04$, $df=7499$, $P<0.001$; F3, $F=189849.09$, $df=7497$, $P<0.001$; F4, $df=7499$, $F=48168.60$, $P<0.001$) and when using random subsets comprising ca. 25 % of the data (Kruskal Wallis test, F1, $\chi^2=492.56\pm 202.32$, $df=1870$, $P<0.001$; F2, $F=22617.11\pm 963.23$, $df=1870$, $P<0.001$; F3, $F=47304.62\pm 1726.01$, $df=1870$, $P<0.001$; F4, $\chi^2=1630.97\pm 658.78$, $df=1870$, $P<0.001$). *Post hoc* tests showed highly significant differences between all species for all variables ($P<0.001$).

Species accounted for substantial variation in all formants (GLM; F 199.69–11,156.32, $df=2$, $P<0.001$). The main effect of length was significant for all formants (F 1944.76–14,560.58, $df=2$, $P<0.001$). The interaction between species and length was not significant for F1 ($F=0.55$, $df=2$, $P=0.575$) and F4 ($F=2.45$, $df=2$, $P=0.087$), but was significant for F2 ($F=8.06$, $df=2$, $P<0.001$) and F3 ($F=7.34$, $df=2$, $P<0.001$).

We found a similar pattern when we tested each species separately. All formants correlate negatively with length in *Eulemur fulvus* (F1, $R^2_{adj}=0.318$, $df=2498$, $P<0.001$; F2, $R^2_{adj}=0.484$, $df=2498$, $P<0.001$; F3, $R^2_{adj}=0.493$, $df=2498$, $P<0.001$; F4, $R^2_{adj}=0.567$, $df=2498$, $P<0.001$), *E. macaco* (F1, $R^2_{adj}=0.471$, $df=2498$, $P<0.001$; F2, $R^2_{adj}=0.535$, $df=2498$, $P<0.001$; F3, $R^2_{adj}=0.523$, $df=2498$, $P<0.001$; F4, $R^2_{adj}=0.537$, $df=2498$, $P<0.001$), and *E. rubriventer* (F1, $R^2_{adj}=0.327$, $df=2498$, $P<0.001$; F2, $R^2_{adj}=0.550$, $df=2498$, $P<0.001$; F3, $R^2_{adj}=0.612$, $df=2498$, $P<0.001$; F4, $R^2_{adj}=0.599$, $df=2498$, $P<0.001$). All formant values decreased as model length increased, even across species. *Eulemur rubriventer* exhibited a distinct plot whereas *E. macaco* and *E. fulvus* had overlapping distributions (Fig. 3). We found high values

of both a. S. and Purity for all formants (F1, a. S. = 0.6, Purity=0.950; F2, a. S. = 0.8, Purity=0.976; F3, a. S. = 0.8, Purity=0.995; F4, a. S. = 0.7, Purity=0.915).

Sound Analysis

Formants measured from recorded vocalizations differed significantly between species both when analyzed using the complete data set (total $N=7672$; Kruskal-Wallis test, F1, $\chi^2=4547.70$, $df=7670$, $P<0.001$; F2, $\chi^2=5428.96$, $df=7670$, $P<0.001$; F3, $\chi^2=5785.92$, $df=7670$, $P<0.001$; F4, $\chi^2=1651.69$, $df=7670$, $P<0.001$) and when tested on 50 random subsets comprising *ca.* 25 % of the data set (F1, $\chi^2=1125.95\pm 34.05$, $df=1912$, $P<0.001$; F2, $\chi^2=1354.81\pm 29.34$, $df=1912$, $P<0.001$; F3, $\chi^2=1440.91\pm 34.06$, $df=1912$, $P<0.001$; F4, $\chi^2=415.23\pm 33.43$, $df=1912$, $P<0.001$). As an example of the grouping power of formants, we plotted F2 against F3 (Fig. 4) and found high values of clustering evaluation indexes for both models (a. S. = 0.9, Purity=0.997) and natural formants (a. S.=0.8, Purity=0.997).

Discussion

We found that generalized computational models built from the specimens we dissected could reasonably reproduce formants measured from the living lemurs. Our results support our first hypothesis: in general, formants predicted from the models fitted well with those measured from nasal grunts. In *Eulemur fulvus*, all modeled formants were

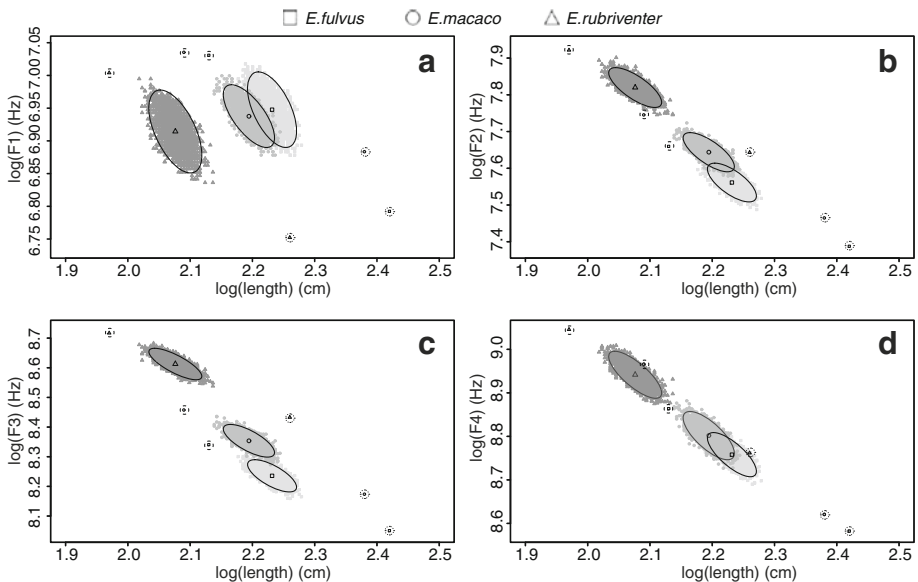
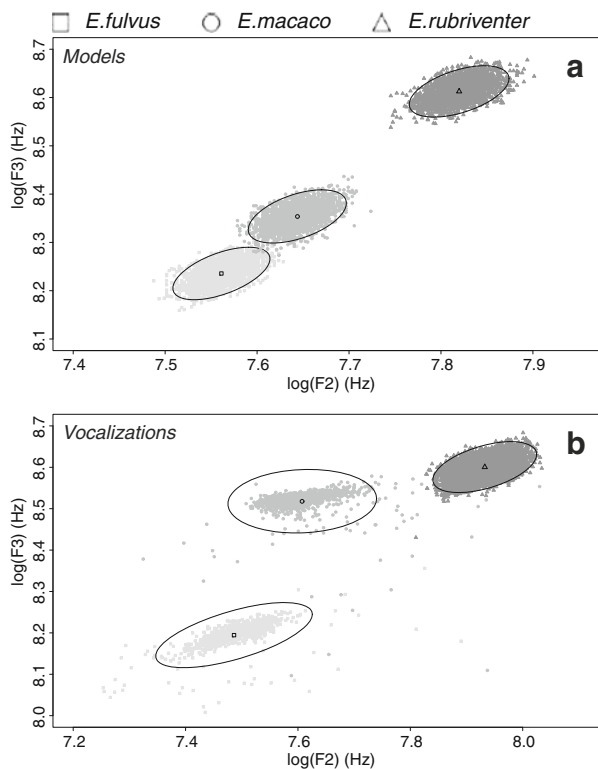


Fig. 3 Log-transformed formant values of F1 (a), F2 (b), F3 (c), and F4 (d) against log-transformed length of the corresponding vocal tract model. We plotted the normal-probability contours at 95 % using the *dataEllipse* function of the *car* library in R (R Development Core Team 2008). Circles mark formants generated from models in which all cross-sectional areas and all tube lengths were reduced (dashed lines) or increased by 15 % (dotted lines).

Fig. 4 Log-transformed formant values of F2 against F3 for the results of vocal tract models (a) and measurements of natural vocalizations (b). We plotted the normal-probability contours at 95 % using the *dataEllipse* function of the *car* library in R (R Development Core Team 2008).



within the range of variation of those measured in the natural nasal grunts. In *Eulemur rubriventer*, modeled formants were a mean of <10 % different from the natural grunts, while in *E. macaco* the difference was *ca.* 15 %. In *Eulemur macaco*, we modeled F2 within the natural range of formant variation, but there were problems with F1, which was 25 % different from the natural range. These differences may occur for several reasons. We may have analyzed a cadaver that was not representative of its species. Alternatively, the area of the nasal passages may have been reduced by the reduction of mucous membranes in the cadaver. However, it is unclear why this would have occurred in *Eulemur macaco* and not in the other focal specimens.

Our findings suggest that there is a strong correlation within species between vocal tract length and the position of formants, in agreement with previous studies on various other mammal species (Fitch 1997; Reby and McComb 2003; Riede and Fitch 1999). This suggests that, as in humans, differences in the formant pattern of nonhuman primates may provide primary cues to body size (Fitch 1997) and individuality (Gamba *et al.* 2012; Rendall *et al.* 1998), and could be used to differentiate among vocal types (Gamba and Giacoma 2007).

Our data support our second hypothesis: formants simulated from the computational models differ across species and correlate with vocal tract length. Resonance in lemurs' nasal cavities produces species-specific F1, which vary according to vocal tract length with a similar trend across species. F2, F3, and F4 are also species-specific and change in a different way across the species, in line with changes in the length of the nasal tract. This is the first evidence that resonance in the nasal tract

generates acoustic cues that potentially provide receivers with information about the species of the emitter. The models differed from recorded vocalizations because they showed considerable interspecific variation in F2, F3, and F4 and corresponding lower intraspecific variation. The recorded vocalizations also showed high intraspecific coefficients of variation for F1, possibly caused by interspecific morphological differences not identified in the focal specimens and thus not properly modeled in this article. The tissues in the walls of the vocal tract may absorb energy, particularly at low frequencies (Riede and Fitch 1999).

This combined use of modeling and acoustic analysis demonstrated that the morphology of the larynx and the nasal airways is relevant in determining differences in formant position and formant spacing in lemurs. We provide direct evidence that there is a basis for species-specific recognition in lemur nasal calls, also showing that prominent spectral features are the result of vocal tract filtering. Our findings demonstrate that vocal cues discriminate species, in agreement with previous studies on the oral “meows” of felids (Nicastrò 2004). Indirectly, our data support the hypothesis that formants can be perceived spontaneously in Strepsirrhini, as demonstrated in macaques (Fitch and Fritz 2006). It is interesting to evaluate our results in relation to the perceptual abilities of lemurs. Studies of the hearing abilities of *Eulemur fulvus* and *E. macaco* suggest that the perceptual sensibility of these two species is optimal at frequencies between 2000 and 15 000–20 000 Hz (Mitchell *et al.* 1970), thus in the range of formants F2–F4 that we investigated in this study.

Our data support our third hypothesis, that formants measured from the grunts differ across species. The ability of baboons (*Papio anubis*) to discriminate changes in formant structure is roughly comparable to that observed for humans (Hienz *et al.* 2004). Between species we found that formants vary within a range of 15 % (for F2 and F3 in the natural calls, see Table 1), which should provide information about the species of the emitter to lemur receivers. Within species, variation of *ca.* 8 % should be perceivable for lemurs to assess the conspecificity of an emitter. This suggests that the ability of lemurs to discriminate formant changes may not be as fine-scaled as it is in humans (Kewley-Port and Watson 1994), but is roughly similar.

Our data do not support our fourth hypothesis. Lemur nasal tract cannot be modeled using uniform tube models and resulting formants are not evenly spaced. Formants were not uniformly distributed in lemurs. This is not the first evidence of nonuniform vocal tract shapes in nonhuman primates (Fitch 2000; Riede *et al.* 2005), but it is particularly interesting because it is in agreement with results for *Varecia variegata* (Gamba and Giacoma 2006). The role of F1 is particularly important, as in most species F2 is around twice F1, while F3 and F4 are around twice and three times F2, respectively. In agreement with previous studies (Riede and Fitch 1999), we found that F1 provided much less reliable indication of vocal tract length and that higher formants are better candidates for providing this information to conspecifics. This fits well with evidence from lemur studies in which higher formants (F2, F3, F4) provided more information on individual (Gamba and Giacoma 2007; Gamba *et al.* 2012) and sex differences (Gamba and Giacoma 2007).

Social and nonsocial interactions are heavily influenced by the characteristics of the interacting animals. Vocalizations may provide cues to sex, age, size, hierarchical position, kinship, and identity of the actors (Aubin and Jouventin 2002; Aubin *et al.* 2007; Blumstein and Munos 2005). Our sound analyses showed that the degree of

differences in the nasal vocalizations among the three species may serve as indexical cues to species and lead to species recognition.

Our findings should be interpreted carefully because they are based on a single individual from each species (one male and two females), although a study of a male *Eulemur macaco* (Negus 1924) matches our findings for that species. Our unpublished data for other species seem to suggest that vocal tract length, but not necessarily shape and volume, varies with body size. The methods we present should be validated on a larger scale, perhaps using primates from which it is easier to collect data, e.g., large captive colonies. Future studies should also employ multiple casts of different individuals for each species to simulate variation in a wild population. Studies of a wider set of vocal tracts and additional species would be of great interest to facilitate understanding of the evolution of lemur communication and of the importance of acoustic cues in species differentiation in lemurs.

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