Honest signaling in domestic piglets (Sus scrofa domesticus): vocal allometry and the information content of grunt calls

Maxime Garcia¹*, Marianne Wondrak²,³, Ludwig Huber²,³ and W. Tecumseh Fitch¹

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 a caller’s traits 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 et al., 2007), age (Charlton et al., 2009a; Reby and McComb, 2003), individual identity (Charlton et al., 2011a, 2009b; Reby et al., 1998; Robisson et al., 1993), sex (Charlton et al., 2009a; Vignal and Kelley, 2007), 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), hormone levels (Charlton et al., 2011a, 2009b; Reby and McComb, 2003), 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, 2000c; 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 (hereafter \( F_0 \)), 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 \( F_0 \) (Morton, 1977). This suggestion seems plausible based on the anatomical–physical description of sound production: \( F_0 \) 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 \( F_0 \). However, this acoustic feature has been shown to poorly reflect thecaller’s body size in various mammalian species (Lass and Brown, 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 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 length of the volume of air within the vocal tract enhance 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 (VTL). Formant-related features have been shown to be a good indicator of body size in multiple species (Charlton et al., 2011b, 2009a; Fitch, 1997; Harris et al., 2006; Reby and McComb, 2003). Even when particular adaptations have led to an exaggerated VTL (Fitch and Reby, 2001), formant characteristics can still correlate with VTL 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 VTL 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 was previously thought to be uniquely human (Lieberman, 1984), it has recently been reported in non-human primates (chimpanzee; Nishimura et al., 2003), artiodactyls
Data collection

Piglets were born on 20 June 2015 (litters B and Z) and 22 June 2015 (litter R). Data collection 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). Body weight (BW) curves from the previous generation were used to evaluate variation in growth rate and select appropriate dates to capture the measurement series. The first three series covered the pre-weaning period, when the piglets’ BW increase was not linear, whereas the fourth series occurred after weaning when the piglets’ BW 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, ±100 g accuracy) as soon as some of the piglets weighed more than 10 kg (series 3–4).

Acoustic recordings

Vocalizations were recorded 10 cm to 1.5 m away from the subjects with a Sennheiser ME-66 directional microphone (frequency response, 40–20,000 Hz ±2.5 dB; Sennheiser Electronic GmbH & Co. KG, Wedemark, Germany) powered by an 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 (Stroud, UK) WS 7 Kit for Shotgun Microphones. 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 1 day prior to 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, as these common low-frequency calls highlight formants better than squeals. For the first series, grunt vocalizations were elicited by preventing the piglets from exiting 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 to 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 reward was used in addition to 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 a hand-made piece of fabric for the following three series (to avoid discomfort as piglets grew older and heavier). Mid-sagittal 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. Because of time and logistic constraints, half of the individuals (N=10) were radiographed in series 2, and the other half in series 3 (piglet selection was based on BW distribution, chosen to span a measurement range representative of the entire group).

**Data analysis**

**Acoustic measures**

All acoustical analyses were made in Praat (P. Boersma and D. Weenink 2014: http://www.praat.org/). Based on both visual inspection of spectrograms and listening, only high-quality grunts (i.e. those deemed to have a high enough signal-to-noise ratio and inspection of spectrograms and listening, only high-quality grunts D. Weenink 2014: http://www.praat.org/). Based on both visual analysis constraints, half of the individuals (N=10) were radiographed on the digital radiograph (1: certain, 2: intermediate, 3: unclear), providing a way of accounting 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.

All measurements from radiographs were made using ImageJ (v2.0.0-rc-15-1.49k). DICOM files were loaded in ImageJ, 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, as 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 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 with 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 analyses, all parameter units were chosen to avoid scaling issues (all frequency parameters are expressed in kHz, length parameters are in cm and weight is 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 VTL component (eigenvalue=2.96, explaining 98.7% of the variance), the other grouping $F_1$ and $F_2$ into one ‘formant’ or Fn component (eigenvalue=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. $\Delta F$ was maintained as an individual measurement as it represents a relative measure of $F_1$ and $F_2$ 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 BW on VTL), anatomical–acoustical correlations (testing the effect of VTL on formant characteristics) and acoustic allometry (testing the effect of BW 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 models (GLMMs). Models were computed including non-intercorrelated 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 BW 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 versus null model, excluding the fixed effect for which significance was being tested; following Winter, 2013). Provided residuals were normally distributed, this model was considered to be validated. Otherwise, a GLMM 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 model 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 the quality of one of the three measurements was ranked as low with ‘3’. Data was prepared in SPSS Statistics (v21.0) and statistical analyses were conducted using SPSS and R (http://www.R-project.org/) with the R-package lme4 (Bates et al., 2015). Two-tailed $P$-values are reported with the significance level set at 0.05.

**Ethical note**
All procedures were approved by the institutional ethics committee in accordance with GSP guidelines and national legislation (ref. 12/07/97).

**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 intercorrelated ($P<0.001$).
Table 1. Spearman correlation coefficients between the anatomical and acoustic variables measured in the study

|       | F1       | F2       | ΔF       | Fn       | Skull    | Nasal tract | Oral tract | VTL       | BW       | log BW    |
|-------|----------|----------|----------|----------|----------|-------------|------------|-----------|----------|-----------|
| 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   | -0.936      | 1.000       | 0.974     | 0.990    | 0.955     |
| ΔF    | 0.861    | 0.967    | 1.000    | 0.919    | -0.884   | -0.932      | 0.974       | 1.000     | 0.980    | 0.994     |
| Fn    | 0.987    | 0.985    | 0.919    | 1.000    | -0.936   | -0.932      | 0.974       | 1.000     | 0.990    | 0.954     |
| Skull | -0.922   | -0.935   | -0.884   | -0.936   | 1.000    | 0.974       | 0.990       | 0.993     | 0.964    | 0.964     |
| Nasal tract | -0.917 | -0.928 | -0.877 | -0.932 | 0.974 | 1.000 | 0.990 | 0.993 | 0.964 | 0.964 |
| Oral tract | -0.926 | -0.940 | -0.892 | -0.943 | 0.978 | 0.990 | 0.990 | 0.993 | 1.000 | 1.000 |
| VTL   | -0.931   | -0.942   | -0.890   | -0.946   | 0.978    | 0.990       | 0.990       | 0.993     | 1.000    | 1.000     |
| BW    | -0.954   | -0.955   | -0.891   | -0.963   | 0.955    | 0.954       | 0.955       | 0.955     | 0.964    | 1.000     |
| log BW | -0.954   | -0.955   | -0.891   | -0.963   | 0.955    | 0.954       | 0.955       | 0.955     | 0.964    | 1.000     |

F1 and F2, first and second formant; ΔF, formant spacing; Fn, PCA component on F1 and F2; PCA, principal component analysis; VTL, vocal tract length; BW, body weight.

All correlations are significant at the P<0.001 level.

for all correlations; Table 1). Overall, the two components resulting from the PCA 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 log10 of body weight (hereafter, log BW)], 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 with Fn, ΔF showed less strong correlations with both BW 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 BW was used rather than BW because volume is proportional to the cube of a linear dimension (BW was the only variable log-transformed as the relationships between log BW and VTL and between log BW and Fn appeared to be linear after visual inspection).

Anatomical dependencies: BW predicts VTL

Because log BW and VTL were strongly and positively correlated (r=0.964, P<0.001; Fig. 3A), we further examined the dependence of VTL on log BW with linear models. log BW, 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 model were calculated, either specifying random slopes for the by-Individual and by-Series effect of log BW, or only for the by-Series effect of log BW (based on visual inspection of the data prior to running the analysis; see Table S2 for initial model composition). After stepwise removal of the fixed effects based on a decrease in AIC scores, the best-fitting model was a GLMM (because the residuals from the LMM were non-normally distributed) with a gamma distribution and an inverse link function, including only log BW as fixed effect and random slope only for the by-Series effect of log BW (Table 2). We thus found that BW was the only significant predictor of VTL (N=60; predictions not back-transformed: \(\hat{\beta}=-1.515, \text{s.e.m.}=0.48, r^2=0.318, P=0.002\)), excluding an effect of sexual dimorphism on this relationship. Inspection of the initial GLMM confirmed the selection of our final model, as neither sex nor litter effects were significant (P>0.9). 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 the growth of the oral tract is dependent on BW entirely with no additional significant effects of Sex or Age.

Acoustical dependencies: VTL 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; \(\hat{\beta}=-0.574, \text{s.e.m.}=0.15, r^2=0.395, P=0.006\)) of Fn (Table 2), including when Age is controlled for instead of Series. Likelihood ratio

![Fig. 3. Bivariate plots illustrating intercorrelations of body weight, VTL and formants.](image-url)
tests on initial models excluding one main factor at a time (Winter, 2013) confirmed the selection for our final model, as neither sex nor litter effects were significant (respectively, $P<0.9$ and $P>0.8$). This analysis shows that the observed decrease in formant frequencies with body size (Table 1) depends only on the increase in VTL; again, no sex differences were significant.

**Acoustical allometry: BW predicts formants**

Finally, looking at acoustic allometric correlations, $F_n$ depended strongly and negatively upon log BW ($r=-0.963$, $P<0.001$; Fig. 3C), as expected based on the previous two correlations. Following the same procedure for model selection (see Table S2 for initial model), the best-fitting model for this analysis only included a significant effect of log BW ($N=77$; $\beta=-2.191$, s.e.m.$=0.42$, $t=-5.178$, $P<0.001$) on $F_n$ (Table 2). This was again confirmed by likelihood ratio tests on initial models, showing non-significant effects of sex ($P>0.6$) and litter ($P>0.7$). 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 BW, via the intervening variable of VTL, with no additional significant dependence upon age, litter or sex.

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

**Predictive relationships between VTLs and formants**

To evaluate the fit between measured formant frequencies and those predicted for a simple uniform tube closed at one end and open at the other, we compared predicted and measured $F_1$ and $F_2$ values. From each average individual $F_1$ (Table S3A–C) and $F_2$ (Table S4A–C), the predicted VTL was calculated based on the following equations:

$$F_1 = c/4L,$$

$$F_2 = 3c/4L,$$

where $c$ is the approximate speed of sound in the warm, moist air of a mammalian vocal tract (350 m s$^{-1}$) and $L$ is the length of the supralaryngeal tract when considered as a half-open resonant tube (Titze, 1994). Wilcoxon signed-rank tests indicated that the measured nasal tract length and oral tract length were significantly different from the predicted VTL calculated from $F_1$ (nasal tract length: $Z=-6.018$, $P<0.001$; oral tract length: $Z=-6.567$, $P<0.001$) and $F_2$ (nasal tract length: $Z=-6.567$, $P<0.001$; oral tract length: $Z=-6.567$, $P<0.001$).

Most of our nasal measurements were shorter than predicted from $F_1$ ($N=49/57$) and all were shorter than predicted from $F_2$ ($N=57/57$); all of our oral measurements were shorter than predicted from $F_1$ and $F_2$. Although highly correlated, measured nasal and oral tract length also significantly differed, and nasal tract length was always longer than oral tract length ($F_1$: $Z=-6.567$, $P<0.001$; $F_2$: $Z=-6.567$, $P<0.001$). Thus, although apparently underestimating VTL, our measured nasal tract length was consistently closer to the VTL predicted from $F_1$ and $F_2$ than our measured oral tract length (based on the differences between expected values and oral tract measurements: $F_1$: $Z=-6.567$, $P<0.001$; $F_2$ (paired sample t-test): $t_{56}=-35.23$, $P<0.001$).

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 VTL 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 small but consistent underestimate. From digital images of a cross-section of a domestic piglet (W.T.F., unpublished data), we estimated this distance and calculated the resulting increase in VTL. 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 by 8.15% and 9.68%, respectively (see corrected nasal and oral tract length, Tables S3 and S4) and ran the above analyses again. Nonetheless, as before, the corrected measurements differed from VTL predicted from $F_1$ (corrected nasal tract length: $Z=-4.024$, $P<0.001$; corrected oral tract length: $Z=-6.567$, $P<0.001$) and $F_2$ (corrected nasal tract length: $Z=-6.567$, $P<0.001$; corrected oral tract length: $Z=-6.567$, $P<0.001$). Most of the corrected nasal measurements were still shorter than predicted ($F_1$: $N=42/57$; $F_2$: $N=59/57$) and all corrected oral measurements were shorter than predicted from $F_1$ and $F_2$. Corrected nasal tract length was always longer than corrected oral tract length ($F_1$: $Z=-6.567$, $P<0.001$; $F_2$: $Z=-6.567$, $P<0.001$) and thus also closer than corrected oral tract length to the predictions from $F_1$ ($Z=-6.567$, $P<0.001$) and $F_2$ ($t_{56}=-32.52$, $P<0.001$).

**DISCUSSION**

While acoustic cues to adult male quality have been shown to vary over time (see Briefer et al., 2010), the data collected in this study represent, to our knowledge, the first attempt at a longitudinal investigation of acoustic allometry. We found that formants measured in grunt vocalizations provide a reliable cue to body size (assessed by BW) in growing domestic piglets. The very strong correlations between 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 BW 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. Grunt formants could therefore provide relevant information to listeners, provided that

### Table 2. Details of the best-fitting models for each of the main three analyses

| Analysis                  | Type       | Final model formula                                                                 | AIC  | $\beta$   | s.e.m. | $t$  | $P$   |
|---------------------------|------------|-------------------------------------------------------------------------------------|------|-----------|--------|------|-------|
| Anatomical                | GLMM       | VTL=−log BW+(1|Individual|)+(1+log BW|Series)           | 140.5| −1.51     | 0.48   | −3.16| 0.002*|
| Anatomical–acoustical     | LMM        | $F_n$−VTL+(1+VTL|Individual|)+(1+VTL|Series)        | 49.2 | −0.57     | 0.15   | −3.96| 0.006*|
| Acoustic allometry        | LMM        | $F_n$−log BW+(1+log BW|Individual|)+(1+log BW|Series)        | 10.6 | −2.19     | 0.42   | −5.18| <0.001*|

Best-fitting models were obtained after model reduction based on inspection of the Akaike information criterion (AIC) scores. *Significant $P$-values.
these acoustic cues to body size are perceived and used by conspecifics.

**On the origin of formant frequencies within grunts**

Estimations of VTL based on $F_1$ and $F_2$ (Tables S3 and S4) were invariably closer to the measured nasal tract length than to the measured oral tract length. Measured nasal and oral tract length were always shorter than predicted by $F_2$ (Table S4A–C). Regarding the VTL predicted by $F_1$, measured nasal tract length was shorter than predicted in most individuals ($N=49/57$), 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 grunts by a vocalizing piglet (Fitch, 2000b).

The fact that predicted VTLs do not perfectly match nasal tract measurements can be explained by several factors. First, our calculations and predictions for expected VTL were based on a quarter-wave resonance tube model, which assumes a closed end (at the glottis) and an open end (the mouth for the oral tract, the nostrils for the nasal tract; Titze, 1994). This does not take into account the changing cross-sectional area (or ‘shape’) of the vocal tract, which is also important in determining formant frequencies and could partly explain the difference between observed and expected VTLs. However, we expect the effect of vocal tract shape to be negligible based on these and previous X-ray observations (Fitch, 2000a,b); furthermore, the effect, if present, would equally concern the nasal and oral airways and thus does not modify our analysis and conclusion. Second, VTL measurements were made down to the base of the epiglottis, which was clearly visible in our radiographs. However, according to the source-filter theory of voice production (Fant, 1960), sound is produced by the vibrating vocal folds (whose vibration rate defines $F_0$ 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, 2000b) and larynx position could thus descend during vocalization (and thus contain lower formants) when piglets produce grunts compared with when they remain silent (which was typically the case during radiographs). Finally, in a few cases nasal tract length was longer than predicted by $F_1$: this could also be explained by laryngeal mobility and our experimental setup. Although we tried to keep piglets as calm as possible while proceeding with radiographs, in some cases piglets produced squeals while being scanned. Squeals in the domestic pig are very loud calls, which involve retracting the larynx down from the nasopharyngeal region (Fitch, 2000b), in turn leading to a fully extended supralaryngeal tract. Measurements of radiographs of the VTL in this configuration would therefore exceed 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 related, and it has been suggested that while individual formants could provide information regarding VTL, 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, in contrast, 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, 2009a; Fischer et al., 2004; Fitch, 1997; Reby and McComb, 2003; Sanvito et al., 2007). In the present study, information redundancy was low because we were only able to measure the first two 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 as a result of higher sound absorbance from nasal cavities (Fitch, 2000b) or the generation of antiformants by the closed mouth cavity (Kurowski and Blumstein, 1987), explaining why only two 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 the vocal repertoires of both domestic pigs (Kiley, 1972; Tallet et al., 2013) and wild boars (Garcia et al., 2016; Klingholz et al., 1979). Unlike squeals, the acoustic characteristics of grunts make them particularly well suited for highlighting formants because of their low $F_0$ (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 with formants from calls of other mammalian 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 ability in mammals on both 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 individuals, 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 in 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 in which the formants are shifted to simulate different phenotypes, would be necessary to test this prediction.

In several mammalian 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), the 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 would be produced from nasal grunts than expected from grunts 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 ("snores") 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 the production mechanisms of this vocalization.

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 to be 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 cooperation 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 typical of the socio-communicative system characterizing this species where cues to individuality could exist, as such recognition relies on vocal communication in other mammalian species (Briefe and McElligott, 2011; Charrier et al., 2001; Fischer, 2004; Insley, 2001). Previous work on domestic pigs indeed 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). Together with our results, this suggests that grunts formants have the potential for carrying multiple messages, as seen in other mammals [rhesus macaques (Fitch, 1997; Rendall, 1996), koalas (Charlton et al., 2012, 2011a)]. Again, playback studies would be required to test this hypothesis.

In conclusion, our results show that formants in domestic piglet grunts are a reliable indicator of body size throughout piglet development. These acoustic cues are available and would in theory be useful to the receiver in various contexts such as sexual selection and agonistic interactions. However, whether information related to vocal tract filtering is perceived and used by conspecifics, including in the case of multi-message signaling, 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 pig grunts by their conspecifics. This would in turn provide additional insight regarding the selective pressures, such as sexual selection and/or 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, wild boars, still exist and remain both widespread and relatively accessible.

Acknowledgements
We are especially grateful to Ariane Veit for her essential contribution to the diverse data acquisition. We are thankful to Larissa Kawasaki, Sarah Deventer, Martina Schleiss and Andrius Pasukonis for their help with data collection. We thank Nadja Kavck for figure editing, Riccardo Hofer for advice on the experimental design and Christian Tanczos for X-ray capture. Finally, we thank Benjamin D. Charlton and an anonymous reviewer for valuable comments on the manuscript.

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. helped design the study, provided daily care to the animals and helped collect weight and radiographic data; L.H. provided the research facility and helped design the study; W.T.F. helped conceive and design the study and data analysis, and helped write the manuscript. All authors gave final approval for publication.

Funding
This research was funded by the University of Vienna (Universität Wien; M.G. and 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’). Deposited in PMC for immediate release.

Supplementary information
Supplementary information available online at http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.138255/-/DC1

References
Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. J. Stat. Soft. 67, 1-48.
Boughman, J. W. (1997). Greater spear-nosed bats give group-distinguishing calls. Behav. Ecol. Sociobiol. 40, 61-70.
Bradbury, J. W. and Vehrencamp, S. L. (1998). Principles of Animal Communication. Sunderland, MA: Sinauer Associates.
Briefer, E. F. (2012). Vocal expression of emotions in mammals: mechanisms of production and evidence. J. Zool. 288, 1-20.
Briefer, E. and McElligott, A. G. (2011). Mutual mother-offspring vocal recognition in an ungulate hider species (Capra hircus). Anim. Cogn. 14, 585-598.
Briefer, E., Vannoni, E. and McElligott, A. G. (2010). Quality prevails over identity in the sexually selected vocalisations of an ageing mammal. BMC Biol. 8, 35.
Carranza, J. (1996). Sexual selection for male body mass and the evolution of litter size in mammals. Am. Nat. 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., 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, 2721-2732.
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, 3414-3422.
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, 71-74.
Charlton, B. D., Ellis, W. A. H., Larkin, R. and Fitch, W. T. (2012). Perception of size-related formant information in male koalas (Phascolarctos cinereus). Anim. Cogn. 15, 999-1006.
Charrier, I., Mathévon, N. and Jouventin, P. (2001). Mother’s voice recognition by seal pups. Nature 412, 873.
Clutton-Brock, T. (2009). Sexual selection in females. Anim. Behav. 77, 3-11.
Clutton-Brock, T. H., Albion, 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, 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, 1065-1068.
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, 160-163.
Fant, G. (1960). Acoustic Theory of Speech Production. The Hague: Mouton.
Favaro, G., 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. Sci. Rep. 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. (1994). Lack of correlation between body size and frequency of acquisition by nonhuman primates: acoustic, physiologic, and functional constraints on “honest” advertisement. Am. J. Prim. 37, 191-219.

Fitch, W. T. and Reby, D. (2001). The descended larynx is not uniquely human. Proc. Biol. Sci. 268, 1669-1675.

Frey, R. and Gebler, A. (1989). The larynx of roaring and non-roaring cats. J. Acoust. Soc. Am. 85, 717-736.

Frey, R., Volodin, I. and Volodina, E. (2007). A nose that roars: anatomical specializations and behavioural features of rutting male saiga. J. Anat. 211, 717-736.

Gaier, U., Klingholz, F. and Meynhardt, H. (2003). The highly specialized vocal tract of the male 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. 142, 528-542.

Hedrick, A. V. and Temelese, E. J. (1989). The evolution of sexual dimorphism in anuran calls. Trends Ecol. Evol. 4, 136-138.

Illmann, G., Schrader, L. and S., (2003). The evolution of speech: a comparative review. J. Exp. Biol. 216, 1913-1921 doi:10.1242/jeb.138255

Klingholz, F. and Meynhardt, H. (2003). Acoustical mother-offspring recognition in pigs (Sus Scrofa domesticus). 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.

Janssen, 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.

Kenselaar, P. (2002). The Ethology of Domestic Animals: An Introductory Text. Wallingford, Oxfordshire: 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 Saiga tatarica (Pallas, 1777-Mammalia, Bovidae). J. Anat. 203, 451-471.

Kroetz, M., Zhang, J., B., Eric, V. and Lavoué, K. (1999). Social stimulation modulates the use of the ‘A’ phrase in male canary songs. Behaviour 136, 1235-1334.

Kuworski, K. and Blumstein, S. E. (1987). Acoustic properties for place of articulation in nasal consonants. J. Acoust. Soc. Am. 81, 1917-1927.

Lass, N. J. and Brown, W. S. (1978). Correlational study of speakers' heights, weights, body surface areas, and speaking fundamental frequencies. J. Acoust. Soc. Am. 63, 1218-1220.

Lieberman, P. (1984). The Biology and Evolution of Language. Harvard: Harvard University Press.

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. 46 (ed. M. Naguib, L. Barrett, H. J. Brockmann, S. Healy, J. C. Mitani, T. J. Roper and W. S. Leigh), pp. 281-310. Oxford: Academic Press.

Masatuka, 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.

Meynhardt, H. (1990). Schwarzwild-Report: Mein Leben Unter Wildschweinen. Melsungen: Neumann.