

Bull bellows and bugles: a remarkable convergence of low and high-frequency vocalizations between male domestic cattle *Bos taurus* and the rutting calls of Siberian and North American wapiti

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ABSTRACT

Whereas low-frequency bellows (below 200 Hz) have been investigated in detail in both male and female domestic cattle (*Bos taurus*), male high-frequency bugle calls (over 800 Hz) have not been described so far in any large bovid species. In this study, high-frequency bugles and low-frequency bellows were recorded from three crossbred free-ranging domestic cattle bulls and analysed spectrographically. The maximum fundamental frequency of bellows was 113.6 Hz, whereas the maximum fundamental frequency of bugles was 958.4 Hz, ranging from 801 to 1125 Hz in different males. These amazingly high fundamental frequencies of bull bugles are comparable with those reported for Siberian wapiti *Cervus elaphus sibiricus*, but lower than reported values for some subspecies of North American wapiti *Cervus canadensis*. The similarity with both Siberian and North American wapiti was also observed in the production of bull biphonic bugles with two fundamental frequencies: the low and the high one. We suggest that bugles of domestic cattle bulls provide an excellent model for comparative research with cervid bugles concerning the mechanism of vocal production and the underlying anatomical and behavioural adaptations.

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Introduction

Many large ruminants are very vocal and use an acoustic channel of communication in various behavioural contexts, including vocalizations during the rut (Feighny et al. 2006; Frey et al. 2012; Wyman et al. 2012; Volodin et al. 2016), contact calls in groups, primarily to support cohesion between mother and young (Padilla de la Torre et al. 2015; Sibiryakova et al. 2015), during painful procedures (Watts and Stookey 1999), in relation to arousal (Hall et al. 1988; Watts and Stookey 1999; Weary and Chua 2000; Yeon et al. 2006) or when disturbed (Long et al. 1998; Volodin, Volodina, Frey, Maymanakova 2013). Usually, large

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bovid species produce low-frequency calls with fundamental frequencies below 300 Hz: American bison *Bison bison* (Gunderson and Mahan 1980), giraffe *Giraffa camelopardalis* (Baotic et al. 2015); domestic cattle *Bos taurus* (Hall et al. 1988; Barfield et al. 1994; Watts and Stookey 1999; Padilla de la Torre et al. 2015).

At the same time, large cervid species are capable of producing a wide range of fundamental frequencies. High-frequency bugles with fundamental frequencies over 1000 Hz are well-known for male and female Siberian wapiti *Cervus elaphus sibiricus* (Volodin, Volodina, Frey, Maymanakova 2013; Volodin et al. 2016) and North American wapiti *C. canadensis* (Feighny et al. 2006). Rutting calls of other Asian and American subspecies of *C. elaphus* and *C. canadensis* range in fundamental frequency between 600 and 2500 Hz (Struhsaker 1968; Bowyer and Kitchen 1987; Frey and Riede 2013; Volodin, Volodina, et al. 2015). In contrast, European subspecies of red deer *Cervus elaphus* (both males and females) commonly produce low-frequency calls between 40 and 380 Hz (Long et al. 1998; Reby and McComb 2003; Kidjo et al. 2008; Frey et al. 2012; Bocci et al. 2013; Passilongo et al. 2013; Sibiryakova et al. 2015; Volodin, Matrosova, et al. 2015). A few Asian and American subspecies of *C. elaphus* and *C. canadensis* produce biphonic calls comprising both a high and a low fundamental frequency (Nicol'skii et al. 1979; Volodin, Volodina, Frey, Carranza, et al. 2013; Volodin, Volodina, Frey, Maymanakova 2013; Reby et al. 2016).

Production of the wide range of fundamental frequencies by the same larynx was experimentally tested using physical cervid larynx models (Titze and Riede 2010), excised cervid vocal folds (Riede and Titze 2008; Riede et al. 2010) and excised cervid larynges (Herbst 2014). The explaining hypothesis for producing high-frequency bugles by airflow through a strongly muscle-compressed larynx (Frey and Riede 2013) has been supported by comparisons of the acoustic structure of stag rutting bugles with flute acoustics (Volodin, Volodina, Frey, Maymanakova 2013; Reby et al. 2016). A further hypothesis based on non-linear source-filter coupling (Titze and Riede 2010) has been supported by observation of this phenomenon *in vivo* in Iberian red deer *C. e. hispanicus* (Volodin, Volodina, Frey, Carranza, et al. 2013). A recent hypothesis considers participation of the vocal tract and soft palate in the simultaneous production of high and low fundamental frequencies (Reby et al. 2016). Notwithstanding this, the morphological and physiological basis for producing the high fundamental frequency in bugles still remain under debate, as different structures may be potentially involved in the production of high-frequency bugling calls and biphonic calls (Frey and Riede 2013; Reby et al. 2016).

Despite the very different acoustics and sounding of roars and bugles, the laryngeal morphologies are very similar in bugling and roaring cervids (Titze and Riede 2010; Frey and Riede 2013). Retraction of the larynx, representing one of the most remarkable parts of stag vocal behaviour during production of rutting calls, is apparently less prominent in the bugling than in the roaring cervids (Frey and Riede 2013). Whereas the laryngeal morphology of domestic cattle only slightly differs from that of both bugling and roaring cervids (cattle: Nickel et al. 1987; Budras and Wünsche 2002; cervids: Köhler 1982; Frey et al. 2012; Frey and Riede 2013; Titze and Riede 2010; Reby et al. 2016) the vocal apparatus of domestic cattle does not allow pronounced retraction of the larynx as a thyrohyoid ligament is lacking (Saber and Hofmann 1985; Nickel et al. 1987; Budras and Wünsche 2002). So far, high-frequency bugles have not been reported for bovids.

In this study, we conduct a bioacoustical analysis of the high-frequency bugles and the low-frequency bellows produced by the same three subject domestic cattle bulls. We report

a remarkable similarity between bovid and cervid bugles in terms of their general spectral pattern and their acoustics, primarily fundamental frequency.

Methods

Study sites, subjects and dates of recordings

Low-frequency calls (bellows) and high-frequency calls (bugles) of three individually identified mature crossbred Brahman × European cattle bulls (photos are available in Supplementary Figure 1) were recorded between 24 and 27 May 2015 and 15 January–7 February 2016 at the Okambara Ranch near the Bush Camp watering place, Namibia (22.69°S, 18.21°E, 1500 m a.s.l.). The cattle population on this ranch, a few dozen cows, calves and a few mature bulls, originated from an unknown number of European (*Bos taurus taurus*) and zebu-like Brahman cattle (*Bos taurus indicus*) breeds, imported from different countries since the end of the XIX century. The animals used in this study were born and kept in a 15,000 hectare ranch on a non-irrigated pasture. They were provided with water at watering places as well as with supplementary hay during the dry seasons and with salt *ad libitum*.

The subject bulls attended herds of free-ranging cows. The bulls vocalized during interactions when different herds were temporarily mixing close to the watering place. Calls were recorded at daylight between 14:00 and 20:00. For acoustic recordings (48 kHz, 16 bit), we used a solid state recorder Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with an AKG-C1000S (AKG-Acoustics GmbH, Vienna, Austria) or a Sennheiser K6-ME66 (Sennheiser electronic, Wedemark, Germany) cardioid electret condenser microphone. Distance from the microphone to the animals varied from 20 to 100 m.

Call samples

During 10 recording sessions (four in 2015 and six in 2016), we recorded in total 354 bellows and 22 bugles from the three subject bulls: 272 bellows and 10 bugles from Bull 1, 65 bellows and 9 bugles from Bull 2, 17 bellows and 3 bugles from Bull 3. All the 22 bugles recorded from the three subject bulls and 29 bellows (10 bellows from Bull 1, 10 bellows from Bull 2 and 9 bellows from Bull 3) were included in the acoustic analyses. The 29 bellows were selected among bellows with a high signal-to-noise ratio and not disrupted by wind. To decrease pseudoreplication, for each individual we selected bellows from different recording sessions or took bellows that were separated by other calls within sessions.

Acoustic analyses

Before the analyses, the calls were downsampled to 6000 Hz for better frequency resolution and high-pass filtered at 50 Hz to reduce the low-frequency background noise. We used two different sets of acoustic variables: one for the low-frequency bellows and another for the high-frequency bugles, respectively. The acoustic variables were measured using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany) and Praat DSP package v. 5.2.07 (Boersma and Weenink 2013). Measurements were exported automatically to Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The measured variables are

commonly used in studies of bellows in bovids (e.g. Padilla de la Torre et al. 2015) and bugles in cervids (e.g. Reby et al. 2016; Volodin et al. 2016).

For the bellows, we measured the duration of each call manually on the screen with the reticule cursor in the Avisoft spectrogram window (Hamming window, FFT = Fast Fourier Transform 1024 points, frame 50% and overlap 96.87%, frequency resolution 5 Hz, temporal resolution 5.3 ms). Then we performed manual measurements on the screen with the standard marker cursor of the initial (f_{01} beg), maximum (f_{01} max) and end (f_{01} end) fundamental frequencies of each bellow. The minimum fundamental frequency (f_{01} min) was selected as the least value among the f_{01} beg and f_{01} end values. The depth of f_{01} modulation (Δf_{01}) was calculated as the difference between f_{01} max and f_{01} min. In addition, we registered the presence in the bellows of non-linear vocal phenomena: deterministic chaos and subharmonics (Wilden et al. 1998; Frey et al. 2012).

Additionally, we extracted the f_{01} contour using a cross-correlation algorithm (to Pitch (cc) command) in Praat. The time step in the analysis was 0.05 s; the lower and upper limits of the f_{01} range were 50–300 Hz. Spurious values and octave jumps in the f_{01} contour were corrected manually on the basis of the spectrograms (Reby and McComb 2003; Reby et al. 2005). Values of f_{01} max and average f_{01} of a call (f_{01} mean) were taken automatically using the Pitch info command in the Pitch edit window.

For the bugles, we measured the duration of each call, the duration of the bugle fundamental frequency (g_0) rises to the plateau (dur rise), the duration of the plateau (dur plateau) and the duration of the g_0 falls towards the end of the call (dur fall) manually on the screen with the reticule cursor in the spectrogram window (Hamming window, FFT = Fast Fourier Transform 1024 points, frame 50% and overlap 96.87%) using Avisoft (Figure 1). We also recorded the presence of breaks in the contour of g_0 and measured the duration of these breaks (dur break) (Figures 1 and 2). Then we performed manual measurements on the screen with the standard marker cursor of the initial (g_0 beg), maximum (g_0 max) and end (g_0 end) high fundamental frequencies of each bugle (Figure 1). For each bugle, we selected the least value between g_0 beg and g_0 end values as the value of the minimum fundamental frequency (g_0 min) and calculated the depth of frequency modulation g_0 ($\Delta g_0 = g_0$ max – g_0 min).

For a subset of biphonic bugles (13 of 22), i.e. for bugles containing a second low fundamental frequency (f_{02}) along the high fundamental frequency g_0 , we measured the maximum (f_{02} max) and minimum (f_{02} min) values of this low fundamental frequency as the difference between the values of the high fundamental frequency or its harmonics (g_0 , $2g_0$) and the values of frequency bands representing the linear combinations of g_0 and f_{02} (Wilden et al. 1998; Volodin and Volodina 2002; Frey et al. 2016; Reby et al. 2016) in the single power spectrum of Avisoft (Figure 3). In each biphonic bugle, we calculated g_0/f_{02} as the ratio of g_0 max over f_{02} max to show the absence of harmonic relations between g_0 and f_{02} as a reliable indicator of biphonation (Wilden et al. 1998; Volodin and Volodina 2002; Reby et al. 2016).

Additionally, we extracted the g_0 contour using a cross-correlation algorithm (to Pitch (cc) command) in Praat. The time step in the analysis was 0.05 s; the lower and upper limits of the g_0 range were 50–1300 Hz. Spurious values and octave jumps in the g_0 contour were deleted manually on the basis of the spectrograms (Reby et al. 2005). Values of g_0 max and average g_0 of a call (g_0 mean) were taken automatically by using the Pitch info command in the Pitch edit window.

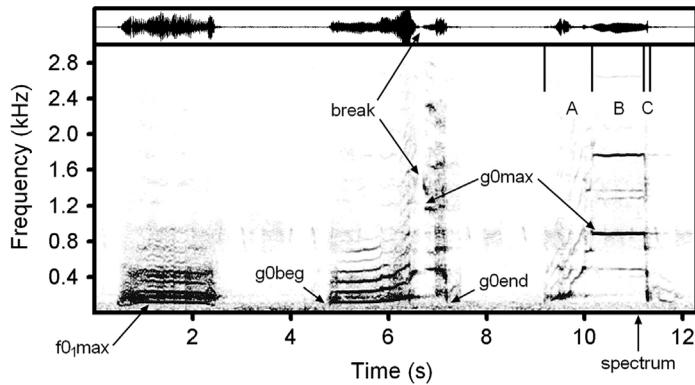


Figure 1. Spectrogram (below) and wave-form (above) of a natural call sequence of Bull 1, comprising one bellow (left) and two bugles (middle and right). In the bellow, the fundamental frequency $f_{0,1}$ is visible as well as deterministic chaos and subharmonics. In the first bugle, the fundamental frequency g_0 rises steadily, then the g_0 contour is interrupted by a break. After that g_0 reaches its maximum and then rapidly falls. In the second bugle, the fundamental frequency g_0 rises rapidly without breaks, then reaches a plateau and afterwards rapidly falls towards the end of the bugle.

Designations: $f_{0,max}$ = the maximum fundamental frequency of the bellow; g_{0beg} = the initial fundamental frequency of the bugle; g_{0max} = the maximum fundamental frequency of the bugle; g_{0end} = the final fundamental frequency of the bugle; break = the break of the g_0 contour; A = dur rise = the duration of the g_0 rise before reaching the plateau; B = dur plateau = the duration of the plateau; C = dur fall = the duration of the g_0 fall after the plateau; spectrum = the position of the 5.3-ms segment that was used for creation of the single power spectrum shown in Figure 3(a). The spectrogram was created with a Hamming window, 6000 Hz sampling rate, FFT 1024 points, frame 50%, and overlap 93.75%. Calls are available in Supplementary Audio 1.

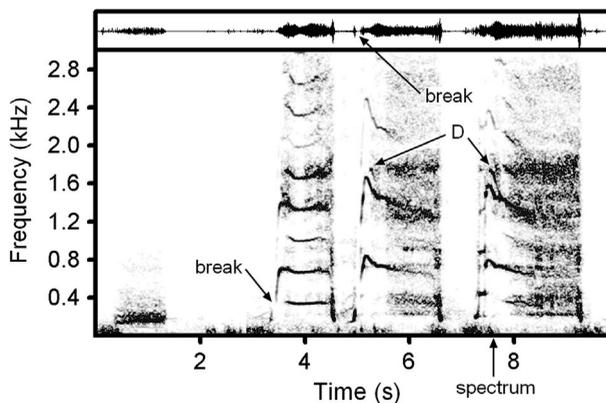


Figure 2. Spectrogram (below) and wave-form (above) of a natural call sequence of Bull 2, comprising one bellow (left) and three bugles (right). In the bellow, the low fundamental frequency ($f_{0,1}$) is visible only at call onset and thereafter is hidden in deterministic chaos. In all three bugles, the high fundamental frequency (g_0) rises rapidly. In the first and in the second bugles, the breaks in the g_0 contour are visible. In the second and in the third bugles, the additional frequency bands, representing the linear combinations of g_0 and $f_{0,2}$, are visible.

Designations: break = the break of the g_0 contour; D = the linear combination of g_0 and $f_{0,2}$ ($2g_0 + f_{0,2}$); spectrum = the position of the 5.3-ms segment that was used for creating the single power spectrum in Figure 3(b). The spectrogram was created with a Hamming window, 6000 Hz sampling rate, FFT 1024 points, frame 50%, and overlap 93.75%. Calls are available in Supplementary Audio 2.

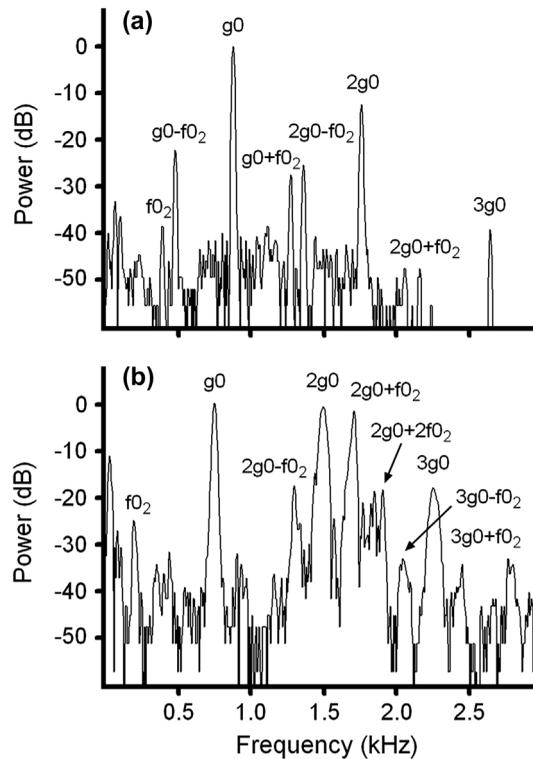


Figure 3. Power spectra of two 5.3-ms time segments indicated by arrows and “spectrum” in Figures 1 (a) and 2(b).

Note: The power peaks represent high fundamental frequency (g_0), harmonics of g_0 ($2g_0$, $3g_0$), low fundamental frequency (f_{0_2}), and linear combinations of g_0 and f_{0_2} .

Two different methods of measuring f_{0_1} max in bellows and g_{0 max in bugles (one using Avisoft and another using Praat) applied to the same calls, resulted in very similar values. Coefficients of correlation, calculated separately for 29 bellows and for 22 bugles, yielded 0.978 and 0.999 ($R^2 = 0.956$ and 0.998), respectively. Thus, for subsequent acoustic analyses both methods (Avisoft and Praat) were applicable. We decided to use the f_{0_1} and g_0 values measured with Avisoft.

Statistical analyses

Statistical analyses were made with STATISTICA, v. 6.0 (StatSoft, Tulsa, OK, USA); all means are given as mean \pm SD. Significance levels were set at 0.05, and two-tailed probability values are reported. A Kolmogorov–Smirnov test showed that the distribution of all acoustic parameters did not depart from normality ($p > 0.05$). We used a one-way ANOVA with a Tukey honest significant difference (HSD) test to estimate whether the acoustics differed between individual bulls. We used a two-way ANOVA for comparison of acoustic variables between bellows and bugles, with individual identity as fixed factor.

Results

All three subject bulls provided both low-frequency bellows and high-frequency bugles. The bugles occurred occasionally, intercalated in long series of bellows, without any noticeable additional stimuli specifically provoking bugling phonation.

Bull bellows were low-frequency tonal calls of average duration 1.45 s, ranging from 0.73 to 3.19 s (Table 1). The average maximum fundamental frequency of the bellows was 113.6 Hz, (ranging from 84.5 to 158.0 Hz). The depth of frequency modulation was small, ranging from 11.0 to 60.0 Hz for the total sample taken from the three bulls. Non-linear phenomena (deterministic chaos and subharmonics) covered more 50% call duration in 13 of the 29 bellows. Among individuals, bellows were similar by their patterns and the acoustics: $f_{0,mean}$, $f_{0,max}$ and $f_{0,beg}$ did not differ among the three bulls (Table 1). However, the bellow duration was significantly longer in Bull 1 compared to Bulls 2 and 3, the $f_{0,min} = f_{0,end}$ was significantly higher, and the $\Delta f_{0,1}$ was significantly lower in Bull 2 in comparison with Bull 1 (Table 1).

Bull bugles were high-frequency tonal calls of average duration 1.69 s, ranging from 0.76 to 2.44 s (Table 2, Figures 1 and 2). In most bugles (20 of 22) the g_0 contour initially

Table 1. Values (mean \pm SD) of the acoustic variables of the low-frequency bellows of the three subject bulls and ANOVA results of their comparison among individuals.

Call parameters	Male 1, $n=10$	Male 2, $n=10$	Male 3, $n=9$	Total, $n=29$	ANOVA results
duration (s)	1.88 \pm 0.70 ^a	1.26 \pm 0.40 ^b	1.17 \pm 0.28 ^b	1.45 \pm 0.58	$F_{2,26} = 5.85, p < 0.01$
$f_{0,mean}$ (Hz)	106.1 \pm 10.8	110.4 \pm 29.8	102.4 \pm 7.3	106.4 \pm 18.7	$F_{2,26} = 0.42, p = 0.66$
$f_{0,max}$ (Hz)	112.0 \pm 8.6	114.8 \pm 30.6	114.0 \pm 9.5	113.6 \pm 18.8	$F_{2,26} = 0.05, p = 0.95$
$f_{0,min}$ (Hz)	66.8 \pm 8.8 ^a	89.6 \pm 22.3 ^b	77.8 \pm 11.8 ^{a,b}	78.1 \pm 17.8	$F_{2,26} = 5.38, p = 0.01$
$f_{0,beg}$ (Hz)	89.0 \pm 16.8	94.6 \pm 19.5	82.1 \pm 14.5	88.8 \pm 17.3	$F_{2,26} = 1.25, p = 0.30$
$f_{0,end}$ (Hz)	67.3 \pm 9.1 ^a	97.5 \pm 31.1 ^b	80.4 \pm 11.2 ^{a,b}	81.8 \pm 23.2	$F_{2,26} = 5.70, p < 0.01$
$\Delta f_{0,1}$ (Hz)	45.2 \pm 10.1 ^a	25.2 \pm 9.8 ^b	36.2 \pm 13.9 ^{a,b}	35.5 \pm 13.8	$F_{2,26} = 7.87, p < 0.01$

Note: Different superscripts indicate which acoustic variables differed significantly between individuals ($p < 0.05$, Tukey *post hoc* test).

Designations: n = number of calls; duration = bellow duration; $f_{0,mean}$ = mean fundamental frequency; $f_{0,max}$ = maximum fundamental frequency; $f_{0,min}$ = minimum fundamental frequency; $f_{0,beg}$ = initial fundamental frequency; $f_{0,end}$ = end fundamental frequency; $\Delta f_{0,1}$ = depth of $f_{0,1}$ modulation.

Table 2. Values (mean \pm SD) of the acoustic variables of the high-frequency bugles of the three subject bulls and ANOVA results of their comparison among individuals.

Call parameters	Male 1, $n=10$	Male 2, $n=9$	Male 3, $n=3$	Total, $n=22$	ANOVA results
duration (s)	1.66 \pm 0.52	1.74 \pm 0.31	1.65 \pm 0.77	1.69 \pm 0.46	$F_{2,19} = 0.07, p = 0.93$
dur rise (s)	1.00 \pm 0.49 ^a	0.41 \pm 0.20 ^b	0.88 \pm 0.56 ^{a,b}	0.75 \pm 0.48	$F_{2,17} = 4.72, p = 0.02$
dur plateau (s)	0.52 \pm 0.33 ^a	1.24 \pm 0.39 ^b	0.26 \pm 0.06 ^a	0.78 \pm 0.52	$F_{2,19} = 14.7, p < 0.001$
dur fall (s)	0.12 \pm 0.05 ^a	0.13 \pm 0.02 ^a	0.30 \pm 0.08 ^b	0.15 \pm 0.07	$F_{2,19} = 18.3, p < 0.001$
g_{0mean} (Hz)	490.8 \pm 199.3	603.6 \pm 96.2	383.0 \pm 101.4	522.3 \pm 166.2	$F_{2,19} = 2.68, p = 0.09$
g_{0max} (Hz)	1050.2 \pm 144.0 ^a	801.0 \pm 114.4 ^b	1124.7 \pm 51.2 ^a	958.4 \pm 180.8	$F_{2,19} = 12.5, p < 0.001$
g_{0min} (Hz)	120.8 \pm 23.2	133.6 \pm 20.7	101.0 \pm 9.2	123.3 \pm 22.8	$F_{2,19} = 2.81, p = 0.09$
g_{0beg} (Hz)	124.4 \pm 31.1	133.5 \pm 25.7	101.0 \pm 9.2	124.6 \pm 28.0	$F_{2,17} = 1.56, p = 0.24$
g_{0end} (Hz)	143.6 \pm 16.7 ^a	146.6 \pm 11.1 ^a	314.0 \pm 81.1 ^b	168.0 \pm 65.7	$F_{2,19} = 42.2, p < 0.001$
Δg_0 (Hz)	906.6 \pm 148.0 ^a	654.4 \pm 111.1 ^b	810.7 \pm 104.9 ^{a,b}	790.4 \pm 171.9	$F_{2,19} = 9.05, p = 0.002$

Note: Different superscripts indicate which acoustic variables differed significantly between individuals ($p < 0.05$, Tukey *post hoc* test).

Designations: n = number of calls; duration = bugle duration; dur rise = the duration of the g_0 rise before reaching the plateau; dur plateau = the duration of the plateau; dur fall = the duration of the g_0 fall after the plateau; g_{0mean} = mean fundamental frequency; g_{0max} = maximum fundamental frequency; g_{0min} = minimum fundamental frequency; g_{0beg} = initial fundamental frequency; g_{0end} = end fundamental frequency; Δg_0 = depth of g_0 modulation.

rose, then reached a plateau and afterwards fell rapidly towards the end of a call. However, in two bugles (one of Bull 1 and another of Bull 2), the rising phase was lacking so that the bugle started with the high frequency corresponding to g_0 plateau (1183 and 966 Hz, respectively; for these two bugles the dur rise and g_0 beg variables could not be measured). The average g_0 max of the bugles was 958.4 Hz, ranging from 662 to 1183 Hz (Table 2), whereas the average g_0 min was only 123.3 Hz. This resulted in a very deep frequency modulation of g_0 (Table 2).

Although the duration of the bugles did not differ significantly between the three subject bulls, the contour of g_0 differed substantially between individuals (Table 2). In Bulls 1 and 3, the fundamental frequency rose slowly resulting in a prolonged increase of g_0 and large dur rise values, whereas in Bull 2, the g_0 rose rapidly, so that the duration of the plateau comprised more than half of bugle duration (Figures 1 and 2). The g_0 max was significantly lower in Bull 2 in comparison with Bulls 1 and 3, and the depth of frequency modulation was significantly lower in comparison with Bull 1 (Table 2).

Breaks in the contour of g_0 occurred in 14 of the 22 bugles (in 5 bugles of Bull 1, in 6 bugles of Bull 2 and in 3 bugles of Bull 3) and during the g_0 rise in all cases (Figures 1 and 2). The break duration was 0.17 ± 0.11 s on average and ranged from 0.05 to 0.44 s. The second low fundamental frequency f_{0_2} occurred in 13 of the 22 bugles (in 7 bugles of Bull 1, in 4 bugles of Bull 2 and in 2 bugles of Bull 3), during the plateau of g_0 in all cases (Figures 1 and 2). In these biphonic bugles, the average f_{0_2} max value was 217.5 ± 80.3 Hz, ranging from 140 to 395 Hz; the average f_{0_2} min value was 200.6 ± 81.3 Hz, ranging from 100 to 395 Hz. In all biphonic bugles, the calculated g_0/f_{0_2} ratio was 4.90 ± 1.43 on average and ranged from 2.24 to 7.48. In all biphonic bugles, these numbers were not integer multiples of f_{0_2} , thereby confirming the biphonic nature of these calls, i.e. the presence of two independent fundamental frequencies in their spectra (Figure 3).

Comparison of the acoustics between bellows and bugles showed that durations did not differ between these two call types ($F_{1,47} = 1.57$, $p = 0.22$). However, the initial and final fundamental frequencies were substantially lower in f_{0_1} beg and f_{0_1} end of bellows than in g_0 beg and g_0 end of bugles ($F_{1,45} = 27.8$, $p < 0.001$ and $F_{1,47} = 62.0$, $p < 0.001$ respectively). Consistently, the f_{0_1} max of the bellows was significantly lower than the f_{0_2} max of the biphonic bugles ($F_{1,38} = 42.9$, $p < 0.001$).

Discussion

This is the first study describing high-frequency bugles of domestic cattle bulls. The same individuals were capable of producing both low-frequency bellows and high-frequency bugles within the same call series. The fundamental frequencies were 4–6 times higher in the bugles than in the bellows. Among ruminants, this wide vocal variation in call fundamental frequency was known so far only in cervids: sika deer *Cervus nippon* (Minami and Kawamichi 1992; Long et al. 1998; Wyman et al. 2016), red deer *C. elaphus* (Volodin, Volodina, Frey, Carranza, et al. 2013) and wapiti or elk *C. canadensis* (Frey and Riede 2013; Volodin, Volodina, Frey, Carranza, et al. 2013; Reby et al. 2016).

The low-frequency bellows of our subject bulls were very similar in their acoustics to those of domestic cattle bull and cow bellows, reported earlier. They were comparable in duration but lower in fundamental frequency than bull bellows of the Chillingham cattle breed (duration = 1.5 s, f_{0} max = 150–170 Hz, Hall et al. 1988), bellows of yearling bulls

and cows during sham branding (duration = 1.5 s, $f_{0\max}$ = 142 Hz, Watts and Stookey 1999), bellows of adult cows during feeding anticipation and in oestrus (duration = 1.85–1.88 s, $f_{0\max}$ = 215–222 Hz, Yeon et al. 2006) and at separation from their offspring (duration = 1.11–1.41 s, f_0 = 121–206 Hz, Weary and Chua 2000). At the same time, the bull bellows of this study were longer in duration and slightly higher in fundamental frequency than the bellows of crossbred domestic cows, either kept in stables with their offspring (duration = 0.53–0.84 s, f_0 = 88–127 Hz, Weary and Chua 2000) or free-ranging (duration = 1.3 s, $f_{0\max}$ = 85 Hz, Padilla de la Torre et al. 2015). Bellows of our subject bulls were slightly shorter and lower in fundamental frequency than bellows of American bison bulls (duration = 2.05 s, $f_{0\max}$ = 230 Hz, Gunderson and Mahan 1980).

The high-frequency bugles of our subject bulls did not resemble any reported vocalizations of domestic cattle, including those produced at high arousal (Hall et al. 1988; Watts and Stookey 1999; Weary and Chua 2000; Yeon et al. 2006). However, they were strongly reminiscent of the bugling rutting calls of male wapiti, either North American (Feighny et al. 2006; Reby et al. 2016), or especially Siberian wapiti (Volodin, Volodina, Frey, Maymanakova 2013; Volodin et al. 2016). As in wapiti, bugles of our subject bulls commonly started at a low fundamental frequency, which then rose stepwise up to a maximum. The maximum fundamental frequency reached a plateau and then rapidly fell towards the end of the bugle. However, bull bugles were much shorter in duration and lower in the maximum fundamental frequency than in Siberian wapiti (duration = 3.04–3.07 s, $g_{0\max}$ = 1200–1230 Hz, Volodin, Volodina, Frey, Maymanakova 2013; Volodin et al. 2016) or North American wapiti (duration = 2.37–2.80 s, $g_{0\max}$ = 2080–2824 Hz, Feighny et al. 2006; Reby et al. 2016).

Probably, the stepwise rise of the bugle high fundamental frequency and the breaks in its contour, observed in domestic bulls, may result from non-linear interaction between sound source (the time-varying glottal airflow in the larynx) and filter (vocal tract resonance frequencies) at the crossing of the vocal tract resonances (formants) by the bugle fundamental frequency (Maxfield et al. 2016; Reby et al. 2016). Non-linear source-filter interaction may also result in maintaining the high fundamental frequency at the level of one of the formant frequencies, thereby producing the fundamental frequency plateau, as was modelled by Titze and Riede (2010), observed *in vivo* in Iberian red deer (Volodin, Volodina, Frey, Carranza, et al. 2013), and experimentally confirmed for humans emitting similar to bugles “glide” vocalizations with artificially elongated vocal tracts (Maxfield et al. 2016).

As in wapiti bugles (Volodin, Volodina, Frey, Maymanakova 2013; Reby et al. 2016), some bugles of our subject bulls contained a second low fundamental frequency (f_{0_2}), whose values were close to that of the low-frequency bull bellows. In North American wapiti, the values of the second low fundamental frequency ($f_{0_2\max}$ = 193 Hz, $f_{0_2\min}$ = 107 Hz, Reby et al. 2016) were close to the values of the low-frequency rutting roars of the Iberian red deer ($f_{0_1\max}$ = 207–224 Hz, $f_{0_1\min}$ = 107–121 Hz, Frey et al. 2012; Passilongo et al. 2013; Volodin, Matrosova, et al. 2015) and Central European red deer *C. e. hippelaphus* ($f_{0_1\max}$ = 274 Hz, $f_{0_1\min}$ = 150 Hz, Bocci et al. 2013).

Although domestic bulls have a typical ruminant larynx (Nickel et al. 1987; Budras and Wünsche 2002), not pronouncedly different from the larynx of red deer and wapiti (Köhler 1982; Frey et al. 2012; Frey and Riede 2013; Reby et al. 2016), vocal behaviour during the emission of the bugles was apparently different. Whereas bugle production in red deer and wapiti occurs simultaneously with pronounced retraction of the larynx (Fitch and Reby 2001; Frey et al. 2012; Frey and Riede 2013; Reby et al. 2016), we did not observe this

specific behaviour during the bugling of domestic cattle bulls. Their hyoid apparatus does not include an extensible thyrohyoid ligament of appreciable length (Nickel et al. 1987; Budras and Wünsche 2002) similar to that involved in the retraction of the larynx in red deer and wapiti (Fitch and Reby 2001; Frey et al. 2012; Frey and Riede 2013). In addition, there is no reported evidence of larynx retraction in sika deer during the high-frequency rutting calls (moans and howls) with an onset frequency exceeding 2000 Hz (Minami and Kawamichi 1992; Long et al. 1998). Other cervid and bovid species that possess an extensible thyrohyoid ligament and retract the larynx during their rutting calls, as fallow deer *Dama dama* (McElligott et al. 2006), Mongolian gazelle *Procapra gutturosa* (Frey et al. 2008a, 2008b) and goitred gazelle *Gazella subgutturosa* (Frey et al. 2011; Efremova et al. 2016) produce only low-frequency vocalizations, very dissimilar to bugles. Therefore, it appears that this anatomical adaptation (an extensible thyrohyoid ligament) as well as the behavioural adaptation (retraction of the larynx) are not critically necessary for producing the bugles and evolved for different vocal or non-vocal functions.

The elasticity of the vocal folds of North American wapiti is much higher than that of non-bugling mule deer (*Odocoileus hemionus*) (Riede et al. 2010). Correspondingly, Brahman zebu-like cattle may differ from other cattle breeds in terms of vocal fold elasticity. Further investigation of larynx structure and the elasticity of the vocal folds in domestic cattle are necessary.

The lack of a direct relation between size and structure of the larynx and call fundamental frequency in mammals (reviews: Riede and Brown 2013; Titze et al. 2016) was previously discussed in application to vocalization in different subspecies of red deer (Volodin, Matrosova, et al. 2015) and also of North American and Siberian wapiti, in which adults and newborns produce undistinguishable fundamental frequencies (Feighny 2005; Volodin et al. 2016). Consistently with wapiti, undistinguishable fundamental frequencies were also reported for mother and young European crossbred domestic cattle (Padilla de la Torre et al. 2015). This surprising coincidence further confirms the unusual convergence of vocal production between wapiti and domestic cattle.

In conclusion, we document another ruminant species capable of producing call types that differ 4–6 times in fundamental frequency. This opens the new doors for comparative anatomical, behavioural, bioacoustical and physiological studies with bugling cervids for highlighting the mechanism of production of extremely high fundamental frequencies by animals with massive larynges. Physiological experiments with live vocalizing animals are necessary, including the possibility of a direct control of vocal production, as was done previously in a few species of large mammals (Fitch 2000), big brown bats *Eptesicus fuscus* (Fattu and Suthers 1981), human 4 kHz whistles (Tsai et al. 2008, 2009) and in many species of birds (e.g. Larsen and Goller 2002; Zollinger and Suthers 2004; Ohms et al. 2012). Domestic bulls may serve as a good experimental model for studying the production of high-frequency calls. Compared to red deer and other deer species, domestic cattle are readily available and accustomed to people, so can be trained and used for physiological experiments including vocalization.

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Supplementary Figure 1. Photo of three subject bulls.



Bull 1



Bull 2



Bull 3