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Sex and age-class differences in calls of Iberian red deer during the rut: reversed sex dimorphism of pitch and contrasting roars from farmed and wild stags

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Abstract Stag rutting calls differ among subspecies of red deer *Cervus elaphus*. Studying sex-, age-, and subspecies-related vocal variation may highlight the forces driving this evolution. This study presents the first bioacoustical comparison of oral calls produced during the rut by Iberian red deer *Cervus elaphus hispanicus* stags, hinds and calves and compares the acoustics of nasal and oral calls of hinds and calves. Also, it provides the first comparison of rutting roars between farmed and wild stags. Call maximum and mean fundamental frequencies (f0max and f0mean) were higher in farmed than in wild stags. Moreover, hinds had lower f0max and f0mean compared with both farmed and wild stags. The call minimum fundamental frequency (f0min) was indistinguishable between all groups of adults, irrespective of sex, farming and nasal versus oral vocal emission. In calves, but not in hinds, oral calls had higher f0max and f0mean compared with nasal calls. The higher fundamental frequencies in farmed as compared with wild stags may have resulted from emotional arousal due to human presence at recordings or from the higher body conditions of stags reared under a farm. The comparison of our results with previously published data on various subspecies of red deer suggests that there are different pathways of vocal ontogeny in eastern and western branches of *Cervus elaphus* and that the acoustics of stag and hind calls are more similar within the various subspecies than they are among the subspecies.

Keywords Ungulate · Rutting roars · Nasal and oral calls · Age differences · Sex differences · *Cervus elaphus*

Introduction

Red deer (*Cervus elaphus*) stags use rutting calls for deterring rival male deers and for attracting receptive female deer (Reby et al. 2005; Charlton et al. 2007; Frey et al. 2012), whereas hinds and calves use their contact calls for mother–offspring communication (Vankova et al. 1997; Kidjo et al. 2008). Roars of red deer are a striking example of sexually selected male-specific vocalization that are important predictors of mating success in both wild and captive stags (Clutton-Brock and Albon 1979; McPherson and Chenoweth 2012).

At the same time, hinds and calves produce contact calls both in rut (Kidjo et al. 2008) and non-rut periods (Vankova et al. 1997; Teichroeb et al. 2013). Although stag, hind and calf calls serve different functions and are considered as different call types, a recent review suggests that all these call types are designed to attract conspecifics (Lingle et al. 2012). The characteristics of these vocalizations can be compared to

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better understand the acoustic mechanisms underlying vocal communication among conspecifics.

Genetic studies suggest that *C. elaphus* originated in Middle Asia and then slowly spread to Siberia, Northern America, and Europe (Mahmut et al. 2002; Ludt et al. 2004; Kuznetsova et al. 2012). This global geographic radiation resulted in a continuum of subspecies or recent species, differing in patterns of stag rutting calls (Canadian elk *C.e. canadensis* or *C. canadensis*, Struhsaker 1968; Roosevelt elk *C.e. roosevelti*, Bowyer and Kitchen 1987; Rocky Mountain elk *C.e. nelsoni*, Feighny et al. 2006; Frey and Riede 2013; Siberian elk *C.e. sibiricus*, Nikol'skii 2011; Volodin et al. 2013b; Bactrian red deer *C.e. bactrianus*, Nikol'skii et al. 1979; Volodin et al. 2013a; Scottish red deer *C.e. scoticus*, Long et al. 1998; Reby and McComb 2003; Corsican red deer *C.e. corsicanus*, Kidjo et al. 2008; and Iberian red deer *C.e. hispanicus*, Frey et al. 2012; Passilongo et al. 2013). Examining the differences among mating calls made by subspecies of *C. elaphus* may provide insight into the evolution of vocal communication in mammalian species (e.g. Campbell et al. 2010).

From a practical perspective, distinctive patterns of rutting calls may be useful in game and conservation biology, as a non-invasive tool for discriminating subspecies (Nikol'skii et al. 1979; Frey et al. 2012). During the Late Pleistocene glacial maximum (25–12 thousand years ago), European red deer remained only in few isolates, and one of them was the Iberian/Southern France refuge (Sommer et al. 2008; Zachos and Hartl 2011). Modern Western European subspecies that radiated from this isolate cannot be distinguished from the primordial Iberian red deer or from each other by mitochondrial DNA markers (i.e. mitochondrial cytochrome b and D-loop; Zachos and Hartl 2011). The probable reason is that the time frame of divergence (~10 thousand years) is too small to have lead to a genetic discrimination in mtDNA (Ludt et al. 2004). Also, overlapping morphological and biometrical traits complicate discriminating subspecies. For instance, Scottish and Iberian stags are neither distinguishable by size of their mandibles (Azorit et al. 2003) nor by body masses (Frey et al. 2012). At the same time, strongly differing stag rutting roars allow discrimination between these subspecies (Reby and McComb 2003; Frey et al. 2012).

Acoustic differences mainly result from differences in sizes of sound-producing structures, represented in mammals by the vocal folds in the larynx, and by the vocal tract, involving the pharyngeal, nasal and oral cavities. In accordance to the source-filter framework (Fant 1960), the call fundamental frequency (f0), generated by the larynx, is filtered subsequently by the cavities of the vocal tract, selectively accentuating certain resonance frequencies (formants) and attenuating anti-resonances. The fundamental frequency and formants are inversely related to the sizes of the sound-producing structures: larger larynges produce lower fundamental frequencies, and longer vocal tracts produce lower formants (Morton 1977; Fitch and Hauser 2002; Taylor and Reby 2010). Other factors, potentially affecting vocal variables of *C. elaphus*, are the viscoelasticity of the vocal folds (Riede et al. 2010), the degree of retraction of the larynx (Frey and Riede 2013), the source-filter coupling (Titze and Riede 2010; Volodin et al. 2013a, b, c), nasal versus oral vocal output (Richardson et al. 1983; Efremova et al. 2011; Volodin et al. 2011, 2013c), and prominent tongue protrusion, which is a characteristic exclusively for rutting calls of Iberian stags (Frey et al. 2012). Additionally, the degree of emotional arousal can affect call structure (Charlton and Reby 2011; Briefer 2012). Captivity and domestication per se can also influence mammalian vocalization (Gogoleva et al. 2009, 2013; Monticelli and Ades 2011). However, vocalizations from farmed and free-ranging animals have not been directly compared for any ungulate.

Scottish, Corsican and Iberian stags produce their rutting calls in bouts that include from one to a few roars, emitted via a widely opened mouth (Reby and McComb 2003; Kidjo et al. 2008; Frey et al. 2012). The longest and the highest-frequency roars in their bouts are either common roars, with clearly visible f0 and harmonics, or harsh roars, where the f0 is invisible for most of the roar (Reby and McComb 2003; Frey et al. 2012). The f0 can only be measured in common roars, which are more common than harsh roars in Iberian stags (89 % and 11 %, respectively, Frey et al. 2012).

Effects of nasal versus oral vocal emission on the acoustics are still unknown for cervids; however, studies with a few bovid species and in African elephants *Loxodonta africana* revealed higher fundamental frequency in the oral compared with the nasal calls (Volodin et al. 2009, 2011, 2013c; Sebe et al. 2010; Efremova et al. 2011; Stoeger et al. 2012). Red deer stags produce rutting roars through an open mouth (Reby and McComb 2003; Frey et al. 2012), and therefore, the effect of nasal versus oral vocal emission on the acoustic variables cannot be studied in their rutting calls. At the same time, these effects can be investigated for nasally and orally produced contact calls of hinds and calves (Richardson et al. 1983).

In most mammals, females and juveniles are smaller than adult males, so the smaller juvenile and female larynges with correspondingly shorter vocal folds should produce higher fundamental frequencies (Fitch and Hauser 2002), with few exceptions, related both to acoustical and body size dimorphism (Matrosova et al. 2007, 2011). In vocalizations of red deer hinds, f0 was found either higher (Kidjo et al. 2008) or the same (Feighny et al. 2006) as in males of the same subspecies. Calf vocalizations have been described for Rocky Mountain elk, Middle European red deer *C.e. hippelaphus* and Corsican red deer (Vankova and Malek 1997; Feighny 2005; Kidjo et al. 2008). For hinds and calves of Iberian red deer, neither oral nor nasal vocalizations have been investigated to date.

This study compares the acoustics of Iberian red deer stags, hinds and calves during the rut period. The rut period is optimal for a comparative study of this nature because stags are mostly silent during the rest of year. In Spain, the rut period lasts from August to November and peaks in mid-September to early October (Gaspar-López et al. 2010). Since the peak of the calving season in Spain takes place from May to June (Landete-Castillejos et al. 2000, 2001), during the rut in September, calves are 3–4 months old. At this age, they still retain vocal contact with their mothers as they still rely on them for milk and social support (Landete-Castillejos et al. 2000, 2001). The objectives of this study were to (1) compare the acoustics of stags, hinds and calves, (2) estimate the effect of nasal versus oral vocal emission in hinds and calves and (3) compare roar acoustics between farmed and wild stags.

Materials and methods

Study sites, subjects and dates of recordings

Calls of farmed stags, hinds and calves were recorded 1–9 September 2010 at the farm of University of Castilla-La Mancha (Albacete, Spain, 38°57′10″N, 1°47′00″W, 690 m a.s.l.). The population originated in 1994 from 15 male and 50 female pure Iberian deer from a nearby Las Dehesas public game reserve in Alpera (Albacete) and from Cabaneros National Park (Toledo). The animals used in this study were born and kept in a 10,000-m^2 enclosure on an irrigated pasture. They were fed ad libitum a diet of barley straw and meal from barley, alfalfa, oats and sugar beets (Landete-Castillejos et al. 2003).

Calls from three individually identified stags, sitting separately from each other in their home enclosures together with their harems, were recorded daily between 08:00 and 20:00. Calls of hinds and their 3- to 4-month calves were recorded from two herds each containing 20–25 females and 20–25 offspring. During recordings, hinds and calves were separated by a wire mesh, and we were able to distinguish between hind and calf calls because they originated from different directions. More than half of the hinds and calves of each herd vocalized, so calls were recorded from at least 20 hinds and from at least 20 calves. During recordings, the individual identity of hinds and calves and the sex identity of calves were undetermined, as many animals emitted calls together from different parts of an enclosure. In total, we made 7.4 h of recordings from stags (3.8, 1.9 and 1.7 h for individual stags) and 3.7 h of recordings from many different individual hinds and calves. All animals were weighed with Mettler-Toledo ID1 scales (Mettler-Toledo S.A.E., Barcelona, Spain) as part of routine farm management (Landete-Castillejos et al. 2001) on the 6 September 2010, during the period of acoustic recordings.

For acoustic recordings of stags (48 kHz, 16 bit), we used a solid-state recorder Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with a AKG-C1000S cardioid electret condenser microphone (AKG-Acoustics GmbH, Vienna, Austria). For hind and calf recordings, we used a Sennheiser K6-ME66 cardioid electret condenser microphone (Sennheiser electronic, Wedemark, Germany) with the same recorder. The distance from the hand-held microphone to the animals was 5–35 m. Audio recordings were made with synchronous video, using a digital camcorder Panasonic HDC-HS100 (Panasonic Corp., Kadoma, Japan). However, as many animals called simultaneously, most calling animals were out of focus of the camcorder.

Calls of wild stags were recorded 11 September–23 October 2009 at the Parque Natural de la Sierra Norte de Sevilla (Andalucía, Spain, 37°47′32″N, 6°04′46″W) in open woodlands of oaks (*Quercus ilex* and *Q. suber*). Calls were recorded from an unknown number of free-ranging stags using a stationary automated recording system Song Meter SM1 (Wildlife Acoustic Inc., Concord, MA, USA) with a setting of 3 min/h, 24 h/day (22.05 kHz, 16-bit). In total, 999 files each of 3-min duration provided 50 h of recordings. The distance from the microphone to animals was unknown, as the recordings were made in the absence of humans. However, these recordings provided for analysis of calls are of excellent quality that should be produced in the vicinity of the microphone and at very low background noise, so all acoustic variables could be reliably measured.

Call samples

Within the recordings of stag rutting calls, we identified call bouts (Fig. 1). The bout could be represented either by single roar or by a sequence of a few roars (Reby and McComb 2003; Kidjo et al. 2008; Frey et al. 2012). Within bouts, we selected the longest call for analysis. All calls selected for acoustic analyses were common roars, as harsh roars occurred very rarely in our call samples. For each farmed stag, we selected the longest roars from 100 bouts, taken uniformly over the period of data collection. For wild stags, we analysed all roars that were produced at a high signal-to-noise ratio and were not disrupted by wind. These roars were taken from the total of 80 sound files, evenly distributed among recordings collected between 18 September and 18 October, what reduced the possibility of over-representation of certain individuals. In total, we selected for analyses 586 common roars, 300 from farmed stags and 286 from wild stags.

Calls were identified to be from hinds or calves based on the video clips, the strong pitch differences, and the comments of observers that were made during the recordings. To discriminate between nasally and orally emitted calls of the hinds and calves, we used observer comments, the video clips, ‘nasal’ quality of sound and difference in call energy distribution (Fig. 2). We analysed all available calls with high signal-to-noise ratios that were not disrupted by wind or the calls of other animals. To decrease over-representation of calls from

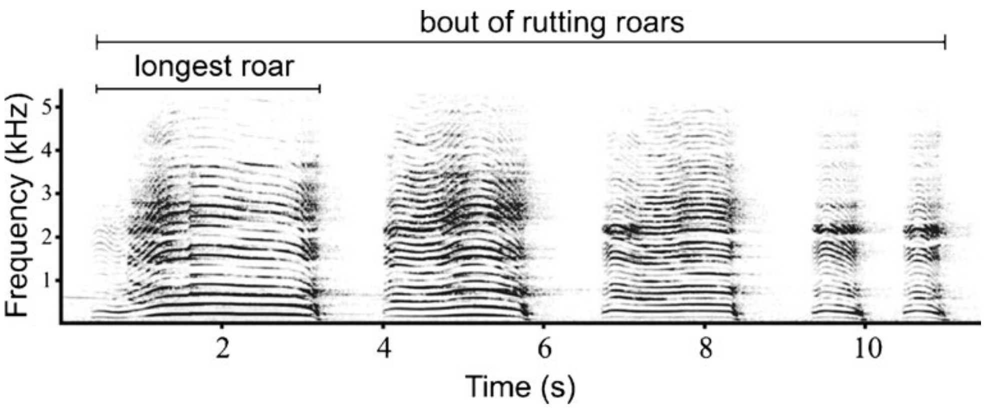


Fig. 1 Spectrogram of a bout of five oral calls (common roars) of a wild Iberian red deer stag. The first roar, which is also the longest roar of this bout, starts nasally, but at approximately 0.8 s the stag opens its mouth. From this point the visibility of the upper harmonics is greatly improved, as sound is less absorbed by nasal epithelium and is freely emitted through the opened mouth. Spectrograms were created with an 11,025-Hz sampling frequency, Hamming window, FFT 1,024 points, frame 50 % and overlap 93.75 %

certain individuals, we selected only one call per series and never analysed similar looking calls produced in series. In total, we selected for analyses 335 hind calls (81 oral and 254 nasal) and 157 calf calls (101 oral and 56 nasal).

Acoustic analyses

Acoustic analyses were conducted in the same way for stags, hinds and calves. Before the analysis, the calls were downsampled to 11,025 Hz. We measured the duration of each call on the screen with the standard marker cursor in the spectrogram window (Hamming window, FFT 1024 points, frame 50 % and overlap 96.87 %) by using Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). Measurements were exported automatically to Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

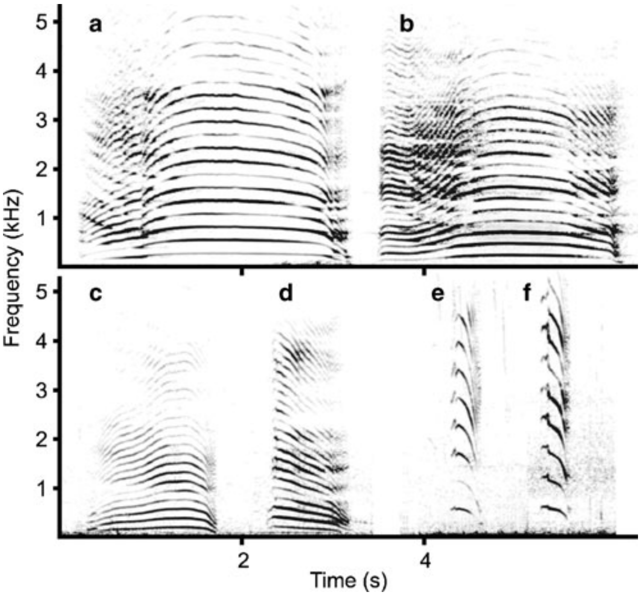


Fig. 2 Spectrogram illustrating calls of different sex and age-classes of Iberian red deer during rut. Common roars of **a** farmed stag, **b** wild unmarked stag. Contact calls of farmed female **c** nasal and **d** oral. Contact calls of farmed juvenile **e** nasal and **f** oral. Initial parts of common roars reveal formants, descending due to elongation of stag vocal tract because of retraction of the larynx. The descending formants cross the harmonics of the fundamental frequency, increasing from the beginning to the middle of the roars. In central parts of common roars the formants become invisible as they either fall between harmonics, where vocal energy is lacking, or coincide with harmonic bands and become undistinguishable from them. For spectrogram settings see Fig. 1

We measured f0 variables in the stag calls according to the method of Reby and McComb (2003) by using Praat DSP package v. 5.2.07 (Boersma and Weenink 2009). The f0 contour was extracted by using a cross-correlation algorithm (to Pitch (cc) command in Praat). The time step in the analysis was 0.05 s, and the lower and upper limits of the expected range of f0 were 60 and 360 Hz, respectively. The lower limit was set at 60 Hz, as the f0 in this part of the call spectrum was indistinguishable from the background noise owing to the very low energy flow of the calls of stags. Besides, a preliminary visual analysis of the spectrograms showed that the lower limit of 60 Hz was lower than the minimum f0 for most of the stag calls. Spurious values and octave jumps in the f0 contour were corrected manually on the basis of the spectrograms. We extracted the f0 contour in the hind and calf calls with the same method but with distinctive settings: the time steps were 0.01 s for hinds and 0.005 s for calves, the lower and upper limits of the f0 range were 75–400 Hz for hinds and 100–800 Hz for calves. Values of minimum f0 (f0min), maximum f0 (f0max), average f0 (f0mean) and the depth of frequency modulation f0 (Δf0) were taken automatically by using Pitch info command in the Pitch edit window.

We did not measure formants, as they can only be measured either in low-frequency calls with closely spaced harmonics or in noisy calls (e.g. harsh roars), where the sound energy is dispersed over the call spectrum (Taylor and Reby 2010; Frey et al. 2012; Teichroeb et al. 2013). In Iberian stags, the average distance between neighboring formants is 228–247 Hz (Frey et al. 2012; Passilongo et al. 2013), which coincides with the distance between the neighboring harmonics of f0 (Table 1). So, in common roars in this study, formant frequencies could not be measured because either they fell between the harmonics and were thus invisible or they were undistinguishable from the harmonics because they coincided with them in frequency (Fig. 2). By the same reason (widely spaced harmonics), it was impossible to measure formants in calls of calves and in most calls of hinds.

Statistical analyses

Statistical analyses were made with STATISTICA, v. 6.0 (StatSoft, Tulsa, OK, USA); all means are given as mean± 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 ($P>0.05$), except call duration. For this latter case, we log-transformed the call duration values to meet the assumptions of parametric statistical tests. We used a two-way factorial ANOVA with a Tukey honestly significant difference (HSD) test to assess whether acoustics differed between nasal and oral calls of hinds and calves. We used a one-way ANOVA with a Tukey HSD test to assess if acoustics of oral calls differed among wild stags, farmed stags, farmed hinds, or farmed calves. Individuals were unidentified from automated and herd recordings and thus could not be included as a random factor.

Results

Nasal and oral calls

Stag calls included only orally produced rutting roars, although many of the roars had a short nasal onset (Fig. 1). Hinds and calves produced their contact calls either through their noses or through their mouths (Fig. 2). From hinds, we recorded more nasal than oral contact calls (75.8 %, $N=335$), whereas from calves more oral than nasal calls were recorded (64.3 %, $N=157$).

In calves, all f0 variables of oral calls were significantly higher than those of nasal calls (Tukey test, $P<0.001$ in all cases; Fig. 3; Table 1). In contrast, in hinds the f0min and f0mean did not differ between oral and nasal calls ($P=0.99$ and $P=0.20$, respectively), whereas the f0max and ∆f0 were marginally higher in oral than in nasal calls ($P=0.051$ and $P=0.06$, respectively). We did not find significant differences in the duration between nasal and oral calls of calves ($P=0.51$; Fig. 3; Table 1), whereas in hinds the duration of oral calls was significantly higher than those of nasal calls ($P<0.001$).

Calls of farmed stags, hinds and calves

Body mass was 235.5±15.0 kg, $N=3$ for stags, 95.2±13.3 kg, $N=55$ for hinds, and 33.2±6.1 kg, $N=56$ for calves. For oral calls, the f0max and f0mean were significantly higher in farmed stags than in hinds (Tukey test, $P<0.001$ for both variables), whereas the f0min did not differ between sexes ($P=0.99$; Fig. 3; Table 1). The ∆f0 was significantly higher in farmed stags than in hinds ($P<0.001$). The duration was significantly longer in farmed stags than in hinds ($P<0.001$). For calf oral calls, the f0max, f0mean and f0min were significantly higher than those of hinds or farmed stags ($P<0.001$ in all cases). The duration was significantly shorter in calf calls compared with hind or farmed stag calls ($P<0.001$ in both cases; Fig. 3; Table 1).

For nasal calls, the f0max, f0mean and f0min were significantly higher in calves than those in hinds (Tukey test, $P<0.001$ in all cases, Fig. 3; Table 1). The call duration was shorter in calves than those in hinds ($P<0.001$).

Calls of farmed and wild animals

The f0max and f0mean of common roars were significantly higher in farmed compared with wild stags (Tukey test,

Table 1 Descriptive statistics (mean±SD) for calls of hinds, calves and stags of Iberian red deer and ANOVA results for comparison of acoustics between animal groups and between nasal and oral calls (only for hinds and calves)

Group	Calls	*N*	Duration, s	f0mean, Hz	f0min, Hz	f0max, Hz	∆f0, Hz
Hinds	Nasal	254	0.65±0.25	166.0±24.7	102.2±15.2	194.8±34.5	92.7±33.0
	Oral	81	0.79±0.31	175.9±25.6	102.6±18.8	208.9±37.0	106.3±35.3
Calves	Nasal	56	0.42±0.20	396.3±58.3	267.5±45.6	466.8±62.9	199.3±53.6
	Oral	101	0.36±0.01	480.0±46.1	305.3±49.5	567.6±52.6	262.3±60.5
Stags	Farmed	300	2.53±0.44	215.7±27.3	102.9±19.0	270.2±38.4	167.3±38.3
	Wild	286	2.49±0.53	189.2±25.8	107.1±23.6	223.5±27.1	116.4±26.7
Comparison between animal groups			$F_{3,767}=940.6$	$F_{3,767}=2649.2$	$F_{3,767}=1665.8$	$F_{3,767}=2378.9$	$F_{3,767}=419.9$
Comparison between nasal and oral calls			$F_{1,488}=15.3$	$F_{1,488}=102.3$	$F_{1,488}=34.7$	$F_{1,488}=92.0$	$F_{1,488}=30.0$

N number of calls, *Duration* call duration, *f0mean*mean fundamental frequency, *f0min* minimum fundamental frequency, *f0max* maximum fundamental frequency, *∆f0* depth of fundamental frequency modulation, *Nasal* nasal contact calls, *Oral* oral contact calls, *Farmed* oral common roars of farmed stags, *Wild* oral common roars of free-ranging stags

All ANOVA effects are significant, $P<0.001$ for all comparisons

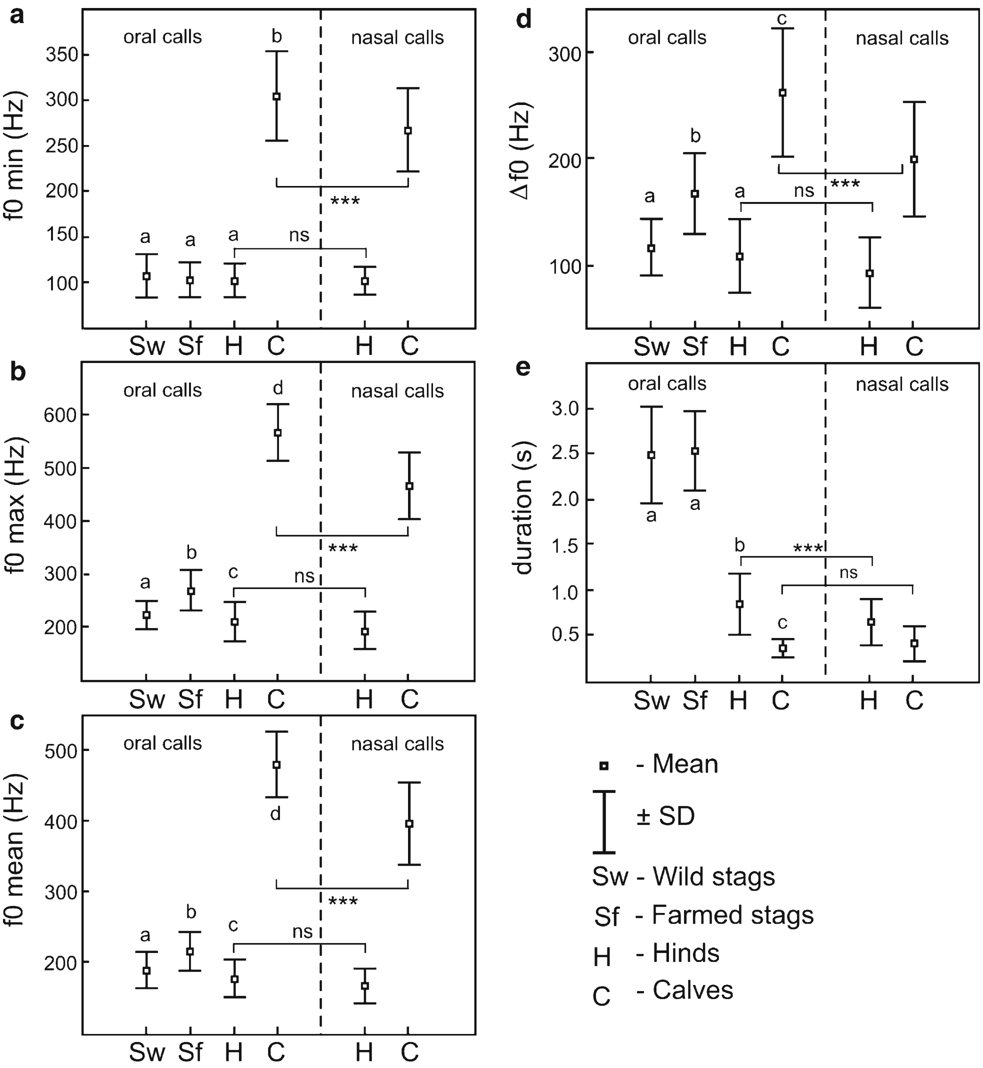


Fig. 3 Values (mean±SD) of acoustic variables for oral and nasal calls of wild stags (*Sw*), farmed stags (*Sf*), hinds (*H*) and calves (*C*). **a** Minimum fundamental frequency, **b** maximum fundamental frequency, **c** mean fundamental frequency, **d** depth of fundamental frequency modulation and **e** call duration. Results for comparison of acoustics between animal groups (ANOVA with Tukey HSD test) are given with *letters*; means sharing the *same letter* are not significantly different. Results for comparison of acoustics between nasal and oral calls (ANOVA with Tukey HSD test) are given with *brackets*, where ****P*<0.001 and *ns* non-significant

P<0.001 in both cases), whereas the f0min did not differ significantly between farmed and wild stags (*P*=0.21; Fig. 3; Table 1). Consistently, the Δf0 was significantly greater in farmed than in wild stags (*P*<0.001). Call duration did not differ significantly between farmed and wild stags (*P*=0.59).

Similar to the case of farmed stags, the f0max and f0mean were significantly higher in wild stags than in farmed hinds (Tukey test, *P*<0.01 in both cases; Fig. 3; Table 1), and the f0min did not differ between sexes (*P*=0.53). However, the Δf0 did not differ significantly between wild stags and farmed hinds (*P*=0.15). The call duration was significantly shorter in farmed hinds than in wild stags (*P*<0.001).

Discussion

This study revealed a reversed sex dimorphism of call fundamental frequency in Iberian red deer with lower f0max and f0mean in hinds than in stags. The calls of farmed stags had higher f0max and f0mean than wild stags. The f0min did not differ between sexes or between farmed and wild stags. The calls of calves were higher in f0max, f0mean and f0min than calls of adults. Oral calls were higher in f0max and f0mean than nasal calls in calves, but not in hinds. The call duration was shortest in calves, intermediate in hinds, and longest in stags.

Our results support previous reports demonstrating the lack of a relationship between body size and fundamental frequency in *C. elaphus*. Corsican stags, with an average body mass of 88 kg produce roars with an f0mean of 40 Hz (Kidjo et al. 2008), whereas larger Scottish and Iberian stags, both with an average body mass of 125 kg, produce roars with an f0mean of 107 Hz (Reby and McComb 2003) and of 189 Hz (Frey et al. 2012 and this study), respectively. The heavier Rocky Mountain elk (331–403 kg, Bender et al. 2003) and Siberian elk (265–416 kg, Fedosenko 1980) produce rutting calls with f0max over 1000 Hz (Feighny et al. 2006; Nikol'skii 2011; Frey and Riede 2013; Volodin et al. 2013a, 2013b). This discrepancy between body size and f0 is unusual for mammals, for which the common rule suggests that larger animals should produce calls with lower f0 (Morton 1977, Fitch and Hauser 2002; Matrosova et al. 2007). Possible hypotheses explaining this pattern focus on the greater elasticity of the vocal folds of elk (Riede and Titze 2008; Riede et al. 2010) and differences in mechanisms for vocal productions in elk, such as source-filter coupling (Titze and Riede 2010; Volodin et al. 2013a, b, c) and air flow through their narrowed larynx (Frey and Riede 2013).

The results of this study suggest that the discrepancy between f0 and body mass occurs also between sexes within the Iberian subspecies of red deer, at least in the rut period. Vocal sex dimorphism showed an inverse relation with the prominent sex differences in body mass in our sample (236 kg for farmed stags compared with 96 kg for farmed hinds). Nevertheless, while f0max and f0mean differed significantly between sexes, the f0min was similar between sexes, and the ranges of fundamental frequency of stags and hinds overlapped (103–270 Hz in stags and of 103–212 Hz in hinds). This overlap is consistent with the fact that size of the larynx is similar in male and female Iberian red deer (Frey et al. 2012).

In addition to the Iberian red deer, close ranges of fundamental frequency between sexes have been reported for the Corsican red deer (of 32–52 Hz in stags and 64–103 Hz in hinds, Kidjo et al. 2008) and for the Rocky Mountain elk (over 1.5 kHz in either sex, Feighny et al. 2006). However, unlike the Iberian red deer, Corsican red deer show straight vocal sex dimorphism, i.e. higher-frequency calls in hinds as compared with stags. At the same time, the Rocky Mountain elk have no sex dimorphism in f0. Therefore, these three subspecies demonstrate a spectrum of straight, reversed and lacked vocal sex dimorphism. However, in all the three subspecies, male and female values of fundamental frequency are closer within than between subspecies (Reby et al. 2010). Figure 4 illustrates this pattern by example spectrograms of stag and hind calls of a few other subspecies of *C. elaphus.*

The similarity of fundamental frequency between sexes in this study and in other subspecies (Fig. 4) suggests that the fundamental frequencies of stag and hind calls are more similar within subspecies than they are among subspecies. Stag rutting calls experience strong sexual selection (Clutton-Brock and Albon 1979; Reby and McComb 2003; Reby et al. 2005; Charlton et al. 2007), whereas hind vocalizations mediate intrasexual competition for resources (Feighny et al. 2006; Ceacero et al. 2012), breeding opportunities (Bebié and McElligott 2006; Bro-Jørgensen 2007; Clutton-Brock 2009) or are used for rejecting undesirable males (Bowyer et al. 2011). Alternately, the similar fundamental frequencies of stags and hinds within subspecies may be the outcome of other factors affecting vocalization of both sexes simultaneously, such as gene drift or environmental conditions (Campbell et al. 2010).

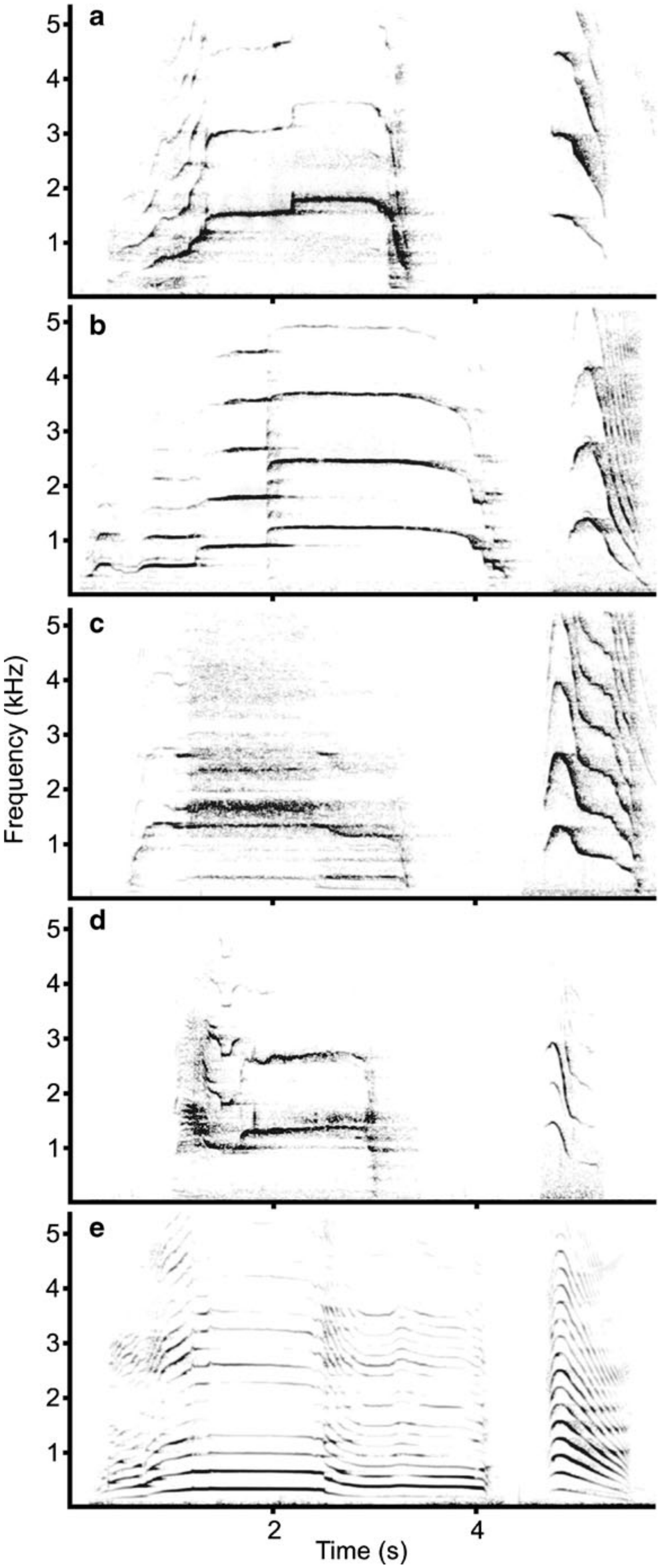
Rutting calls of wild and farmed Iberian stags were similar in duration and f0min, but f0max and f0mean were higher in farmed than in wild stags. Our data support previous findings (Frey et al. 2012) that Iberian stag roars are of high frequency compared with other European subspecies, the Scottish and the Corsican (Long et al. 1998; Reby and McComb 2003; Kidjo et al. 2008). In addition, in Iberian stags, the longest roars of bouts were also the highest in frequency (Frey et al. 2012). The high f0 advertises muscular strength and endurance (Titze and Riede 2010). Thus, Iberian stags may try to achieve maximum possible f0 due to sexual selection for high-frequency vocalization. The well-fed farmed stags had higher body mass (236 kg, see also Gaspar-López et al. 2010) than free-ranging stags (125 kg: Frey et al. 2012) suggesting that the farmed stags were in better condition compared with their wild counterparts. This likely explains why farmed stags were capable of producing higher-frequency main roars without shortening the duration of their calls.

Another explanation is that the higher f0mean and f0max of calls of the farmed stags could be related to their higher emotional arousal (Watts and Stookey 1999; Manteuffel et al. 2004; Briefer 2012). Our field recordings were made using automated equipment in the absence of people, whereas the farmed stags could see people and threatened them during acoustic recordings. In captivity, the close vicinity of people may increase the emotional arousal of animals, resulting in shifts to higher-frequency vocalization (Gogoleva et al. 2010; Briefer 2012). The increase of f0max has also been shown for rut groans of fallow deer bucks in the vicinity of females and actively vocalizing rival males (Charlton and Reby 2011).

Our study expands the knowledge of acoustic variation in red deer and confirms that the upper limit of f0 of stag roars exceeds 300 Hz (Long et al. 1998; Favaretto et al. 2006; Frey et al. 2012; Bocci et al. 2013; Passilongo et al. 2013; this study) rather than the ranges of 70–160 Hz (Reby et al. 2010). In this study, rutting calls of wild stags recorded in 2009 at the Parque Natural de la Sierra Norte de Sevilla were surprisingly similar in all variables of fundamental frequency with rutting calls of wild stags, recorded in 2007 in three other localities in southern Spain, separated by a distance of 100–150 km from our study site (Frey et al. 2012; f0min 107 Hz versus 107 Hz; f0max 224 Hz versus 223 Hz; f0mean 189 Hz versus 186 Hz). However, they were somewhat longer in duration (2.49 versus 1.96 s). We conclude therefore that the high fundamental frequency of rutting calls is an essential acoustic trait of free-ranging Iberian red deer. In farmed stags this trait is becoming even more prominent due to their higher ambient level of arousal.

This provides good acoustic indices for discriminating between Iberian and other stags sharing the same mitochondrial A haplotype group (Frey et al. 2012). In conservation and game biology of red deer, discrimination between subspecies is crucial because of multiple translocations and hybridization

Fig. 4 Spectrograms of stag (*left*) and hind (*right*) oral calls during the rut, illustrating similar fundamental frequency values between the sexes within subspecies of *Cervus elaphus*: **a** *C.e. canadensis* (Tierpark Berlin, Germany), **b** *C.e. sibiricus* (Saint Petersburg Zoo, Russia), **c** *C.e. nannodes* (Tierpark Berlin, Germany), **d** *C.e. bactrianus* (nature, North Uzbekistan) and **e** *C.e. hispanicus* (Albacete farm, Spain). For spectrogram settings see Fig. 1



of subspecies across Europe in the past (Zachos and Hartl 2011). However, further study is necessary to test the potential of acoustic traits for separating between other subspecies of red deer.

In calves, nasal calls had lower fundamental frequencies as compared with oral calls, whereas in hinds, there was no difference in the fundamental frequencies of nasal and oral calls. Thus, in Iberian red deer, calling through the nose significantly affected only vocalization of calves. Nasal calls have also been shown to have a lower fundamental frequency than oral calls for young and adult female saiga *Saiga tatarica* (Volodin et al. 2009, 2013c), young goitred gazelles *Gazella subgutturosa* (Efremova et al. 2011; Volodin et al. 2011), adult sheep *Ovis aries* (Sebe et al. 2010) and young African elephants (Stoeger et al. 2012). In adult male saigas, sexual selection favours the nasal rutting calls due to their lower formants compared with the oral calls (Frey et al. 2007). At the same time, in young goitred gazelles, oral calls are favoured when the calf became detached from the mother because they are louder and more individualistic compared with their nasal calls (Volodin et al. 2011).

The calls of Iberian calves were higher than the calls of adults in all frequency variables. Compared with calves of other European subspecies, the calls of Iberian calves, which were 3–4 months old in this study, were lower than in smaller-sized 4-month Corsican calves (Kidjo et al. 2008) and than 2–4 days old Middle-European calves (Vankova and Malek 1997). However, the calves of all European subspecies have lower frequencies than the adults of Siberian and American subspecies (Struhsaker 1968; Feighny et al. 2006; see also Fig. 4). This suggests different pathways in the vocal ontogeny of fundamental frequency, directed towards lower-frequency calls in European subspecies and towards higher-frequency calls in Siberian and American subspecies (Feighny 2005). Further study of the vocal ontogeny of different subspecies of *C. elaphus* is required to test this hypothesis.

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