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Rutting roars in native Pannonian red deer of Southern Hungary and the evidence of acoustic divergence of male sexual vocalization between Eastern and Western European red deer (*Cervus elaphus*)



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ABSTRACT

The acoustics of male rutting roars, aside from genetic markers, are useful tools for characterization of populations and subspecies of red deer Cervus elaphus. This study of rutting mature male Pannonian red deer from Southern Hungary presents a description of the calling posture, a graphical reconstruction of the oral vocal tract length during rutting roar production and a spectrographic analyses of 1740 bouts containing a total of 5535 rutting roars. In addition, this study provides the first direct comparison of the bouts and main (=longest) rutting roars between Pannonian and Iberian red deer stags, representative of the Western and Eastern lineages of European red deer. The bouts of the Pannonian stags comprised 1-15 roars per bout; 24.37% were single-roar bouts and 23.68% were two-roar bouts. The duration of the main roars within bouts ranged from 0.52 s to 4.60 s, $1.13 \pm 0.50 \text{ s}$ on average. Main common roars (66.3% of the 1740 main roars), were longer than harsh roars $(1.27 \pm 0.55 \text{ s} \text{ vs} 0.87 \pm 0.25 \text{ s})$ and higher in maximum fundamental frequency (179 ± 61 Hz vs 147 ± 54 Hz). In multi-roar bouts, main harsh roars were first roars in 47.4%, intermediate roars in 19.2% and last roars in 18.8% of the bouts. Bout structure and the acoustics of main roars in the Pannonian stags differed from those in the Iberian stags and in stags from other populations of Cervus elaphus. These results support the power of rutting vocalizations for consideration as an additional tool for discriminating populations and subspecies within Cervus elaphus. © 2018 Deutsche Gesellschaft für Säugetierkunde. Published by Elsevier GmbH. All rights reserved.

Introduction

In red deer *Cervus elaphus*, rutting calls represent an important part of male rutting behaviour (Clutton-Brock et al., 1987; Reby and McComb, 2003; Frey et al., 2012) and decisively contribute to male reproductive success (Reby and McComb, 2003). During the rut, red deer stags vocalize to attract females and to compete with other stags for female harems (Clutton-Brock and Albon, 1979). Rutting vocal displays shift the ovulation in hinds to earlier dates (McComb, 1987), and avoid unnecessary combats and energy losses in establishing rank order between competing males (Clutton-Brock and Albon, 1979; Reby and McComb, 2003; Reby et al., 2005) by advertising male quality acoustically (Bowyer and Kitchen, 1987; Clutton-Brock and Albon, 1979; Reby and McComb, 2003). Male rutting roars differ between European subspecies of red deer: Scottish red deer *Cervus elaphus scoticus* (Reby and McComb, 2003), Iberian red deer *C.e. hispanicus* (Frey et al., 2012; Passilongo et al., 2013; Volodin et al., 2015a), Corsican red deer *C.e. corsicanus* (Kidjo et al., 2008), Mesola red deer *C.e. italicus* (Della Libera et al., 2015).

For Central European red deer, data on the acoustics of male roars are available from a translocated Alpine population in Eastern Italy (Bocci et al., 2013) whose status as *C.e. hippelaphus* has been validated by genetic data (Skog et al., 2009; Lorenzini and Garofalo, 2015). Data on the acoustics of male roars are also available for the formerly Central European (Austrian–Hungarian) stock of red deer introduced to Argentina (Hurtado et al., 2012), whose status as *C.e. hippelaphus* has not yet been validated by genetic data.

Male red deer produce rutting roars in bouts that include one to several calls (Reby and McComb, 2003; Kidjo et al., 2008; Frey et al., 2012; Passilongo et al., 2013; Golosova et al., 2017). The longest roars within bouts are termed main roars (Reby and McComb, 2003;

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Frey et al., 2012). Vocal cues to subspecies can be analyzed at two hierarchical levels: at the level of bouts and at the level of particular roars within bouts.

At the level of particular roars within bouts, the main acoustic cues to subspecies are call fundamental frequency (f0) and formants. According to the source-filter theory of voice production, the f0 of mammalian vocalizations is generated by oscillation cycles of the vocal folds (the source) and then acoustically filtered by the vocal tract (the filter), which selectively accentuates and attenuates certain frequencies, formants and anti-resonances, respectively (Fant, 1960; Titze, 1994). In red deer, males emit rutting roars via a widely opened mouth, so that the length of the oral vocal tract predominantly determines the formants of these roars (Reby and McComb, 2003; Frey et al., 2012). The oral vocal tract of the European red deer has been evolutionarily elongated by a permanently descended larynx resting position (Fitch and Reby, 2001; Frey et al., 2012; Frey and Riede, 2013). In addition, male European red deer retract the larynx down towards the sternum during their rutting calls (Fitch and Reby, 2001; Reby and McComb, 2003; Frey et al., 2012). This retraction results in an additional, temporary vocal tract elongation and, accordingly, in an additional, call-synchronous lowering of the formants, which are clearly visible in spectrograms of the low-frequency roars of most European subspecies of red deer (Reby and McComb, 2003; Kidjo et al., 2008; Passilongo et al., 2013). In terms of acoustic structure, the roars of European red deer can be subdivided into common roars with a clearly visible f0 and its harmonics, and harsh roars, where f0 is masked by deterministic chaos and subharmonics for most part of call duration (Reby and McComb, 2003; Frey et al., 2012; Passilongo et al., 2013).

Compared to Asian subspecies of *Cervus elaphus*, e.g. *C.e. bactrianus* (Volodin et al., 2013a), *C.e. sibiricus* (Volodin et al., 2016; Golosova et al., 2017) and *C.e. xanthopygus* (Volodin et al., 2015b), which produce high-frequency bugles with a maximum f0 of 660–1360 Hz depending on the subspecies, the European subspecies of *Cervus elaphus* produce low-frequency roars with a maximum f0 of 52–274 Hz depending on the subspecies. Among European subspecies, the maximum f0 of the rutting roars is 52 Hz in *C.e. corsicanus* (Kidjo et al., 2008), 91 Hz in *C.e. italicus* (Della Libera et al., 2015), 137–210 Hz in *C.e. scoticus* (Long et al., 1998; Reby and McComb, 2003), 207–270 Hz in *C.e. hispanicus* (Frey et al., 2012; Passilongo et al., 2013; Volodin et al., 2013).

The vocal features of a certain subspecies are constant across different populations, e.g. in Iberian red deer *C.e. hispanicus* (Frey et al., 2012; Passilongo et al., 2013; Volodin et al., 2015a) and Siberian wapiti *C.e. sibiricus* (Volodin et al., 2013b; Golosova et al., 2017). Therefore, bioacoustical analysis represents a powerful tool for defining and validating subspecies of red deer, provided they were not subjected to the introgression of genes from other subspecies as a result of multiple anthropogenic translocations of red deer over historical times (Zachos and Hartl, 2011; Zachos et al., 2016; Frantz et al., 2017). Although subspecies-specific rutting vocalizations do not provide reliable reproductive barriers against hybridization (Long et al., 1998; Nussey et al., 2006; Wyman et al., 2016), they may serve for the affiliation of native populations of *Cervus elaphus* with a particular subspecies (Frey et al., 2012; Volodin et al., 2015a, b; Golosova et al., 2017).

Subspecies-level taxonomic ranks of many native European populations of red deer have been confirmed by both genetic studies (Skog et al., 2009; Zachos and Hartl, 2011, Zachos et al., 2016) and bioacoustical studies of male rutting roars (Frey et al., 2012; Volodin et al., 2015a; Passilongo et al., 2013; Della Libera et al., 2015). Accordingly to archeological excavations, red deer is native for the Carpathian Basin, as forms similar to current red deer have been present continuously since at least 100 thousand years (Szunyoghy, 1963). The distribution area of *Cervus elaphus* hippelaphus is considered to be South-East and Central Europe, isolated from the Western subspecies by the Alps and Carpathians (Szunyoghy, 1963). The estimated total population number in Hungary in 2018 was 111,450 individuals (OVA, 2018). Red deer are most abundant in the South Transdanubian region (which includes the study site) in forest-agricultural habitats with broadleaved (dominated by *Quercus robur*) forests and crop fields (maize, cereals, rape). Supplementary feeding and game field cultivation are widely applied in this area.

Different Hungarian populations of red deer are poorly investigated from taxonomical point of view. Definitions of the Hungarian red deer populations are mainly based on early enzyme analyses (Hartl et al., 1990). Available limited genetic analyses classify samples from Hungarian red deer to mitochondrial DNA C haplogroup (Skog et al., 2009). Other available genetic data either do not provide precise coordinates for the sites of data collection (Markov et al., 2015) or report DNA sequence data from a single individual, potentially belonging to Pannonian red deer (Frank et al., 2016). By morphological features, wild-living Pannonian red deer of Southern Hungary and Croatia were considered either as a native population of *C.e. hippelaphus* or as a separate subspecies within *Cervus elaphus, C.e. pannoniensis* (Banwell 1998a, 1998b, 2002).

Pannonian red deer is large-sized (body mass up to 454 kg in stags and up to 175 kg (130 kg on average) in hinds); the coat is lighter and the ears are larger than in other European red deer populations, e.g. those living in Western Hungary, on the foothills of the Alps, close to the Austrian border; the antlers are nearly as large as in wapiti although their shape is that of European red deer (Banwell 1998a, 1998b and own observations of the authors). Studying the acoustics of the rutting roars of Pannonian red deer can serve as a basis for supporting or rejecting its status as a separate taxonomic unit (Banwell, 1998a; Zachos, 2018) and for further comparative studies of yet unstudied populations of red deer.

Data about the acoustic structure of male roars of C.e. hippelaphus, the subspecies considered closest to C.e. pannoniensis, are limited to measurements of few acoustic variables of male rutting roars from the translocated Alpine population introduced to Eastern Italy (Bocci et al., 2013) and to the translocated Austrian-Hungarian population introduced to Argentina (Hurtado et al., 2012). So far, detailed data on the acoustic structure of both roaring bouts and main (= longest) roars within bouts are only available for Iberian red deer C.e. hispanicus (Frey et al., 2012; Passilongo et al., 2013) and, to some extent, also for Siberian wapiti C.e. sibiricus (Golosova et al., 2017). Consequently, at present only the data on the acoustic structure of male rutting roars in Iberian red deer are suited for a comparative statistical analysis among the European subspecies of red deer. Therefore, the aim of this study was to investigate the acoustic structure of the bouts and main roars in native wild-living rutting male Pannonian red deer in Southern Hungary and to statistically compare the obtained results with corresponding data of rutting male Iberian red deer, obtained in the context of a preceding study (Frey et al., 2012).

Material and methods

Site, animals and dates of work

Audio and video recordings of vocalizations and rutting behaviour of unmarked wild-living mature male Pannonian red deer were conducted in the Inner-Somogy landscape, near the city of Nagyatád (46.067 N, 17.492 E) during the peak of the rut and highest vocal activity, from 9 September to 6 October 2015. Study site was a native unfenced habitat of Pannonian red deer. The area is a forest-crop field mosaic habitat, with 37% forest cover. In addition to natural feeding resources (trees, shrubs, herbs), abundant supplementary food, available for the study animals on special feeding fields with corn and sunflower mix, attracts both male and female Pannonian red deer and promotes a very high density of rutting stags in the area. In the study site, mature males during the rut are subjected to regular legal hunting. The 12,800 ha hunting area belongs to the Lábod Forestry of the SEFAG Forest Share Holding State Company, with an annual hunting bag of 25 red deer stags (A. Náhlik, personal communication).

As up to 25 mature rutting stags were legally shot during the period of data collection, the rotation of rutting males on the territory of the study site can be considered very high. This enables the automated recording of the rutting calls of many males (Volodin et al., 2015b, 2016; Golosova et al., 2017) with a low probability of repeated recording of the same males over the rutting period. For instance, nine stags were shot over approximately one week during the peak of the rut close to the position of the automated recording system for data collection. The ensuing high rotation of rutting males at the recording sites can be expected to thoroughly decrease potential pseudoreplications by repeatedly recording the same individual. However, a small amount of pseudoreplication might have occurred. For comparison of the acoustic structure of rutting roar bouts and of the main roars within bouts between Pannonian and Iberian red deer stags, we used data of acoustic measurements conducted in a similar preceding study on Iberian red deer (Frey et al., 2012).

Data collection

From 9 to 12 September 2015, two observers conducted preliminary observations plus audio and video recordings of calling stags using hand-held audio and video equipment. These preliminary observations were conducted from two hunting towers (4-5 m high), separated by a distance of about 1 km, during morning (05:00-08:00) and evening (17:00-20:00) hours, coinciding with the periods of highest diurnal vocal activity of rutting Pannonian red deer stags. For the manual audio recordings (48 kHz, 16 bit), we used solid state recorders Marantz PMD-660 (D&M Professional, Kanagawa, Japan) with Sennheiser K6-ME66 cardioid electret condenser microphones (Sennheiser electronic, Wedemark, Germany). For the video recordings, we used two Canon PowerShot SX50HS camcorders (Canon U.S.A., Melville, Ney York). The distance of the animals to the microphone and to the camera varied from 50 to 200 m. Video recordings of male vocal behaviour and audio recordings of rutting roars were made of about 20 rutting males in total. The manual video recordings were used for selecting video single frames for description of the vocal posture and for graphical reconstruction of the oral vocal tract length during rutting roar production. The obtained oral vocal tract lengths of the stags served to establish the settings for measuring the call formant frequencies by using the Praat DSP package (Boersma and Weenink, 2013). The preliminary manual acoustic recordings were conducted for correctly assigning the automatically recorded roars to red deer species by ear and by spectrographic representations. In addition, the manual acoustic recordings served to select the most appropriate site for mounting the automated system for acoustic recordings.

The automated audio recordings (22.05 kHz, 16 bit, stereo) of Pannonian red deer stag rutting calls were collected from 9 September to 6 October 2015 with the automated recording system Song Meter SM2+ (Wildlife-Acoustics Inc., Maynard, MA, USA). The automated recording system was mounted on a pillar about 5 m above ground, at a site of active rut between two feeding fields. The recording system was equipped with two omnidirectional microphones, fixed horizontally at 180° to each other. The recording system was set at maximum possible sensitivity, so, it potentially collected rutting roars within a radius of about 0.5 km around the

device. The automated recordings in the absence of people provided high-quality recordings of the rutting roars, as the stags mostly vocalized at a close distance (within 100 m) to the recording device.

The acoustic recording (22.05 kHz, 16 bit, stereo) was scheduled from 18:00 to 21:00 and from 05:00 to 08:00, each 9 min of recording followed by 1 min pause (the minimum possible pause for cutting the recording into separate files), and from 21.00 to 05.00, each 9 min of recording followed by 21 min pause. Each 9min recording was stored as a wav-file. This storage mode was selected for file management purposes, to avoid the inconvenience of working with very long acoustic files. Thus, for each 24-h period the system collected fifty-two 9-min audio files. In total, we collected 210.6 h of recordings in 1404 audio files, each file of 9-min duration.

Acoustic analyses

For the acoustic analyses, we used only the automatically recorded calls, as these calls were of better quality than those manually recorded. Only high quality calls with clearly visible spectral structure and not superimposed by wind or other noises were used for the acoustical analyses. The high-quality bouts were selected evenly throughout the rutting period. Before analysis, the calls were downsampled to 11.025 kHz for better frequency resolution.

Earlier reports showed that the rutting calls of European red deer stags include call types differing in the acoustic structure: long common roars, short common roars, harsh roars and grunt roars, produced in bouts, as well as chase barks, produced in series, and single barks (Reby and McComb, 2003; Kidjo et al., 2008; Frey et al., 2012; Passilongo et al., 2013). For the acoustic analyses, we took only bouts consisting of roars, whereas the rarely occurring barks were not analyzed. A call sequence was registered as a bout only when we were sure that all calls of the sequence came from the same animal and did not contain concurrently produced calls of other stags. The concurrently produced calls of other stags are commonly well visible as overlapping bands in the spectrogram. Bouts with two or more single roars were termed multi-roar bouts. Bouts containing only one roar were termed single-roar bouts. In total, we analyzed 1740 roaring bouts containing 5535 individual roars (Fig. 1).

For each bout, we selected the longest roars within bouts to analyze them separately as 'main roars' of the bouts (Frey et al., 2012). For the main roars of multi-roar bouts, we scored their position within a bout and classified them as first main roars, last main roars and intermediate main roars. The roars in single-roar bouts were treated as the main roars of these bouts. Main roars were additionally classified as either common roars, with a clearly visible f0 and its harmonics, or harsh roars, without a clearly visible f0 (Reby and McComb, 2003; Kidjo et al., 2008; Frey et al., 2012). In addition, we selected the highest-frequency roar in each bout irrespective of whether it was a main roar or a different roar. We scored each main roar for presence of nonlinear phenomena: deterministic chaos or subharmonics (Wilden et al., 1998; Fitch et al., 2002). Sections of these nonlinear phenomena may comprise up to 50% of the duration of common roars and from 50 to 100% of the duration of harsh roars (Fig. 1; Reby and McComb, 2003; Frey et al., 2012).

For each of the 5535 roars, we measured the duration 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). We further measured the maximum fundamental frequency (f0max) for 5372 of the 5535 roars with the harmonic cursor from the power spectrum created in the 100 ms section of the f0-maximum area of the roar. For 163 roars, the f0max was not measured, because f0 could not be tracked in these roars.



Fig. 1. Spectrogram (below) and wave-form (above) of natural roaring bouts of rutting Pannonian red deer stags (Supplementary Audio 1). (a) An eight-roar bout; the first five roars are harsh roars, the remaining three are common roars; the main (longest) roar is in third position. (b) A single-roar bout with a main harsh roar. (c) A single-roar bout with a main common roar. (d) A two-roar bout with the main common roar in first position. (e) A two-roar bout with the main common roar in first position; the second half of the main roar displays a section with source-filter interaction (coupling). Descending formants are well visible at the beginning of all roars on the spectrogram. The spectrogram was created at 11.025 kHz sampling frequency, Hamming window, FFT 1024, frame 50%, overlap 93.75%.

All measurements were exported automatically to Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

The measuring of formants was only possible in 211 main harsh roars containing long sections of deterministic chaos, revealing the formants, whereas in harsh roars with shorter sections of deterministic chaos, formants could not be tracked. In the 211 harsh roars, we measured the first eight formants (F1-F8) using Linear Predictive Coding (LPC) with Praat. The formants were measured in those call parts exhibiting clearly visible formants, produced with a fully retracted larynx and maximally extended vocal tract, i.e. where formant tracks reached their minimum values and were nearly horizontal. The oral vocal tract length, estimated from reconstructions in video single frames of Pannonian stag calling posture (see below), served to establish the settings for LPC. The selected LPC parameters for creating the formant tracks were: Burg analysis. time step 0.05 s; window analysis 0.1 s; 8-9 formants and maximum formant frequency 1800-2000 Hz. Positions of the formants were verified by superposition on the spectrogram. In each roar, we analysed the formants in the call section, taking as many as possible (up to 15) sequential point values separated by a time interval of 0.05 s. Point values of the formant tracks were extracted, exported to Excel where the value of each formant of a given call was calculated as the average value of all the extracted point values of the track.

We used two different methods for calculating the minimum formant dispersion (dF), to select the method providing the closest possible accordance with the graphical oral vocal tract length estimates in the video single frames. Following Riede and Fitch (1999), we calculated the dF as dF = (F8-F1)/7 for each main harsh roar (n = 211), by applying the model of a straight uniform tube closed

at one end. Following Reby and McComb (2003), we calculated the dF for the average formant values of the 211 main harsh roars by using linear regression. Then, for each method, the maximum vocal tract length achieved by stags during the roars was calculated by the equation: vocal tract length = c/2 dF, where c is the speed of sound in air, approximated as 350 ms⁻¹ (Fitch and Reby, 2001; Reby and McComb, 2003).

Stag calling posture and reconstruction of the oral vocal tract length

The relative vocal tract lengths of calling stags were estimated in video single frames by using externally visible landmarks (Fig. 2A, B): a) the lips mark the rostral end of the oral vocal tract; b) the hyoid prominence, visible only at maximal extension of the vocal tract, marks the position of the basihyoid, i.e. the most ventral element of the hyoid apparatus, located ventral to the oropharynx in the throat region; c) the laryngeal bulge indicates the position of the larynx, its most protruding part representing the laryngeal prominence, where the vocal folds attach to the inner ventral surface of the thyroid cartilage. Therefore, the apex of the laryngeal bulge marks the caudal beginning of the vocal tract. The vocal tract line for graphically ascertaining the oral vocal tract length was drawn along its assumed topographic course between the two endpoints. The course of the oral vocal tract relative to the ventral neck, throat and head region is known from the dissection and reconstruction of specimens of other red deer subspecies (Frey et al., 2012 and R. Frey unpublished data) and by comparison with the anatomy of domestic ruminants in textbooks (Nickel et al., 2004). For reconstruction of the approximate course and length of the oral vocal tract and



Fig. 2. Estimation of the oral vocal tract length in a video single frame pair of a Pannonian red deer stag. (A) Resting position of the larynx and vocal tract. (B) Maximal retraction of the larynx and maximal extension of the vocal tract during a roar. Blue line - skull basal length. Turquoise line – rostral edge of large foramen at the level of the paracondylar process. Upper left red line - rostral edge of the lips; lower right red line - assumed position of the vocal folds close to the laryngeal prominence, i.e. the most protruding point of the externally visible laryngeal bulge. Yellow line - oral vocal tract. The inserted skull is that of a Scottish red deer stag photographed in lateral view (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

owing to the lack of Pannonian red deer skull photographs in good lateral view, we graphically inserted the laterally viewed skull and lower jaw of a Scottish red deer skull (*C.e. scoticus*) to the head and neck contour of an also laterally viewed Pannonian red deer stag. This skull of the Scottish red deer stag was taken from the Morphological collection of the IZW, Berlin. To get relative estimates of both the resting oral vocal tract length and the maximally extended oral vocal tract length we used video single frame pairs of the same stag – one for the resting position of the larynx. The single frames were selected for showing the required features in an approximately 90° lateral view of the individual to avoid foreshortening of the depicted structures as far as possible. Subsequently, the oral vocal tract line was reconstructed in these single frame pairs by using the mentioned landmarks (Fig. 2A, B). This procedure was executed in

three single frame pairs selected from videos taken of three different Pannonian red deer stags during the rut. Single video frames were analyzed by means of Adobe Premiere Pro, 1.5 AOO License ALL software (Adobe Systems Inc., San Jose, CA, USA).

Absolute oral vocal tract length estimation required introduction of an absolute measure into the body contours and relative reconstructions done so far. We achieved this by measuring the skull basal length in a series of 11 skulls of mature adult male Pannonian red deer, all more than 5 years old. These stags had been legally culled at the study site during previous rutting periods. Basal length was measured ventrally, from the tip of the incisive bone (Os incisivum, premaxillary) to the rostral edge of the large foramen (Foramen magnum) by using a tape with 1 mm precision. In lateral view of the skull, the rostral edge of the large foramen corresponds to the level of the paracondylar process (Processus paracondylaris). Therefore, the distance from the tip of the incisive bone rostrally to the paracondylar process of the inserted and graphically size-adjusted Scottish red deer skull caudally, served to establish an absolute measure for estimation of the oral vocal tract length. The lower jaw was adjusted to fit the size of the inserted skull. It helped in correct reconstruction of the vocal tract line at closed mouth (relaxed vocal tract) and at open-mouth calling (maximally extended vocal tract). The length of the inserted skull basal length in the video stills was measured with a ruler with 1 mm precision. This measurement was executed three times and the average value of the three measurements was calculated. The length of the oral vocal tract was measured directly on a 27inch screen with an appropriate string measure. This measurement was also executed three times - for the relaxed state and for the maximally extended state, respectively. To account for potential body size differences calculating the ratios between the skull basal lengths measured on the screen and the smallest (372 mm), the mean (395 mm) and the largest (417 mm) skull basal lengths of the Pannonian red deer skulls produced three scaling factors. By multiplying the measured relative oral vocal tract length with these scaling factors, we obtained a lower, a mean and an upper limit for the absolute oral vocal tract resting length and for the callsynchronous maximally extended length in the three investigated male individuals. In the third male, we additionally estimated the maximally extended oral vocal tract length in two different single frames, in which the relative size of this male differed. Despite this difference, a close match of the estimated oral vocal tract length was achieved, demonstrating the validity of the applied method.

The estimation of vocal tract length in video single frame pairs without inherent absolute measure is a preliminary method for getting an idea of the real vocal tract length in video- and acoustically recorded red deer stags in which the larynx is externally visible and from which cadavers for measurements of vocal tract length are not available. This indirect method is subjected to several errors. First, the video of a stag might not have been taken from a perfect 90° lateral position. Any deviation from this ideal position and the resulting oblique view/reduced angle will lead to a foreshortening of the depicted structures and, thus, to an underestimation of the vocal tract length. Secondly, maximal retraction in a selected video might not be the maximal possible retraction that a stag can actually attain. This will also lead to an underestimation of the vocal tract length. Thirdly, the insertion of the skull into the body contours of a video single frame might not be perfect and, thus, lead to over- or underestimation of the vocal tract length. Fourthly, the insertion of the laterally photographed skull of a different subspecies might introduce a minor error owing to different skull proportions. As appropriately photographed skulls of Pannonian red deer (90° lateral view) were not available, we used the skull of a Scottish red deer for establishing an absolute measure (skull basal length) in the selected video frames.

Table 1

Oral vocal tract length (mm) estimated in video single frame pairs of three vocalizing mature Pannonian red deer stags for establishing the settings for measuring the formants of the rutting roars. Estimates were done for the resting position of the larynx with a relaxed vocal tract and for the maximally retracted position of the larynx with a maximally extended vocal tract. Designations: Skull basal length = Skull basal length in video single frame, mm; Scaling factor1: 10 mm in the skull photo = x mm in nature, basis 372 mm; Scaling factor2: 10 mm in the skull photo = x mm in nature, basis 395 mm; Scaling factor3: 10 mm in the skull photo = x mm in nature, basis 417 mm; Oral vtl = length of the oral vocal tract in video single frame, mm.

Oral vocal tract state	Stag No	Skull basal length	Scaling factor1 (372)	Scaling factor2 (395)	Scaling factor3 (417)	Oral vtl	Lower limit vtl (372)	Mean vtl (395)	Upper limit vtl (417)
Resting	1	102	36.471	38.725	40.882	150	547.1	580.9	613.2
Extended	1	102	36.471	38.725	40.882	190	692.9	735.8	776.8
Resting	2	97	38.351	40.722	42.990	142	544.6	578.3	610.5
Extended	2	97	38.351	40.722	42.990	202	774.7	822.5	868.4
Resting	3	103	36.117	38.350	40.485	152	549.0	582.9	615.4
Extended1	3	103	36.117	38.350	40.485	195	704.3	747.8	789.5
Extended2	3	93	40.000	42.473	44.839	176	704.0	747.5	790.1

Statistical analyses

Statistical analyses were made with STATISTICA, v. 8.0 (StatSoft, Tulsa, OK, USA). Means are given as mean \pm SD, all tests were twotailed, and differences were considered significant whenever p < 0.05. Distributions of 115 measured parameter values of 160 distributions did not depart from normality (Kolmogorov-Smirnov test, p > 0.05). As ANOVA is relatively robust to departures from normality (Dillon and Goldstein, 1984), this was not an obstacle to the application of the parametric tests.

We used Student *t*-test to compare the acoustics between main common and main harsh roars. We applied a one-way ANOVA with Tukey HSD test separately for main common and main harsh roars to compare the acoustics of the roars that differed in position within a bout and to compare the acoustics of the main roars depending on the number of roars in a bout. In addition, we used Student *t*test and χ^2 test, to compare the acoustics of main roars between Pannonian and Iberian red deer stags.

Results

Oral vocal tract length estimation

The resting oral vocal tract length (Table 1) was about 581 mm (lower limit 547 mm–upper limit 613 mm) in stag 1, about 578 mm (lower limit 545 mm–upper limit 610 mm) in stag 2 and about 583 mm (lower limit 549–upper limit 615 mm) in stag 3. The maximally extended oral vocal tract length was about 736 mm (lower limit 693 mm–upper limit 777 mm) in stag 1, about 823 mm (lower limit 775 mm–upper limit 868 mm) in stag 2 and about 748 mm (lower limit 704 mm–upper limit 789 mm) in stag 3 (Table 1).

Acoustics

Roaring bouts (n = 1740) consisted of 1–15 individual roars (3.18 ± 2.17 on average); single-roar bouts comprised 24.37% and two-roar bouts 23.68% of the total number. The total number of multi-roar bouts, i.e. bouts consisting of two and more roars, was n = 1316. Main roars were in first position in 31.61%, in last position in 34.42% and in intermediate position in 33.97% of the multi-roar bouts (Fig. 1). The duration of the main roars within all bouts (n = 1740), i.e. including the single-roar bouts, ranged from 0.47 s to 4.60 s (1.13 ± 0.50 s on average) and f0max ranged from 63 Hz to 584 Hz (168.3 ± 60.5 Hz on average).

In a total of 1740 main roars, 1154 (66.3%) were common roars and 586 (33.7%) were harsh roars. Main common roars were significantly longer than main harsh roars (1.27 ± 0.55 s vs 0.87 ± 0.25 s, t = 16.63, df = 1738, p < 0.001) and higher in f0max (178.7 ± 60.9 Hz vs 147.2 ± 53.9 Hz, t = 10.46, df = 1717, p < 0.001). Compared to main common roars, main harsh roars occurred more often in single-roar bouts (218 of 424, 51.4%) than in multi-roar bouts (368 of 1316, 28.0%). In multi-roar bouts (*n* = 1316), main harsh roars were more often in first position (197 of 416, 47.4%) than in intermediate position (86 of 447, 19.2%) or in last position (85 of 453, 18.8%), whereas the intermediate or last positions were mostly occupied by main common roars.

The distribution of roar durations (for a pooled sample of main and other roars, n = 5535 roars) displayed two peaks, the first one at 0.1–0.2 s and the second one at 0.7–0.8 s, separated by a depression at 0.3–0.4 s (Fig. 3). The distribution of main roar durations (n = 1740 main roars) displayed one peak, at 0.8–0.9 s. The minimum value for main roar duration (0.47 s) exceeded the value at the depression in the pooled sample of roars. The first peak comprised roars shorter than 0.4 s, evidently representing either short common roars or grunt roars in the terminology of Passilongo et al. (2013).

In the overall set of 1740 bouts, comprising both single-roar and multi-roar bouts, the longest (=main) roars were also the highest in f0max in 996 (57.2%) of the bouts. In the subset of 1154 main common roars, 585 (50.7%) roars were also the highest in f0max within bouts, whereas in the subset of 586 main harsh roars, 411 (70.1%) roars were also the highest in f0max within bouts. In the subset of 1316 multi-roar bouts, main roars were also the highest in f0max in 572 (43.5%) of the bouts.

For main common roars, position within a bout influenced the f0max but not the duration (Table 2). The f0max was significantly higher in single-roar bouts or main roars in first position than in main roars in intermediate or last position (Table 2). In the multi-roar bouts, the number of roars per bout influenced only the f0max ($F_{8,939} = 18.81, p < 0.001$) but not the duration ($F_{8,939} = 1.53, p = 0.14$) of the main common roars. The f0max was significantly higher in two-roar bouts (199.9±65.1 Hz) than in bouts with more roars, ranging from 172.4 ± 46.3 Hz in three-roar bouts to 140.4 ± 37.5 Hz in nine-roar bouts.

For main harsh roars, position within a bout influenced both f0max and duration (Table 3). The f0max was significantly lower in single-roar bouts than in any main roars within multi-roar bouts (Table 3). Duration was shortest in single-roar bouts and longest in intermediate roars of multi-roar bouts (Table 3). In multi-roar bouts, the number of roars per bout influenced only duration ($F_{8,359} = 3.75$, p < 0.001) but not f0max ($F_{8,359} = 0.57$, p = 0.80) of the main harsh roars. The duration of main harsh roars was significantly shorter in both two-roar bouts (0.86 ± 0.18 s) and three-roar bouts (0.91 ± 0.31 s) than in either eight-roar bouts (1.22 ± 0.45 s) or ten-roar bouts (1.35 ± 0.26 s).

For those main harsh roars in which formants were measured (n = 211), we compared the values of the first eight formants (F1-F8) regarding their position within bouts (Table 4). Position within a bout only affected the values of F1, F2 and F4 formants (Table 4). The F1 was significantly higher in single-roar bouts and in the first roars of multi-roar bouts compared to intermediate or last roars of multi-roar bouts. The F2 was significantly higher in single-roar



Fig. 3. Distribution of the male Pannonian red deer rutting roars according to their durations; n = 5535 roars from 1740 bouts.

Table 2

Values (mean \pm SD) of the duration and the maximum fundamental frequency (f0max) of the main common roars of male Pannonian red deer in single–roar bouts and in different positions within multi–roar bouts, and one–way ANOVA results for their comparison. The same superscripts indicate which values did not differ significantly (p > 0.05, Tukey HSD test).

Acoustic	Total	Single-roar bouts	Multi-roar bouts (n=	ANOVA			
able	(n = 1154)	(n = 206)	First roar (<i>n</i> = 219)	Intermediate roar (n=361)	Last roar (<i>n</i> = 368)		
Duration, s f0max, Hz	$\begin{array}{c} 1.27 \pm 0.55 \\ 179 \pm 61 \end{array}$	$\begin{array}{c} 1.28 \pm 0.66 \\ 219 \pm 75 \ ^{a} \end{array}$	$\begin{array}{c} 1.29 \pm 0.55 \\ 206 \pm 68 \ ^{a} \end{array}$	$\begin{array}{c} 1.24 \pm 0.52 \\ 156 \pm 37 \ ^{b} \end{array}$	$\begin{array}{c} 1.27 \pm 0.50 \\ 162 \pm 48 \ ^{b} \end{array}$	$F_{3,1150} = 0.26, p = 0.85$ $F_{3,1150} = 84.8, p < 0.001$	

Table 3

Values (mean \pm SD) of the duration and the maximum fundamental frequency (f0max) of the main harsh roars of male Pannonian red deer in single-roar bouts and in different positions within multi-roar bouts, and one-way ANOVA results for their comparison. The same superscripts indicate which values did not differ significantly (p > 0.05, Tukey HSD test).

Acoustic	Total	Single-roar bouts	Multi-roar bouts (n=	ANOVA			
able	(n = 586)	(n=218)	First roar (<i>n</i> = 197)	Intermediate roar (n = 86)	Last roar (<i>n</i> = 85)		
Duration, s f0max, Hz	$\begin{array}{c} 0.87 \pm 0.25 \\ 147 \pm 54 \end{array}$	$\begin{array}{c} 0.80 \pm 0.18 \ ^{a} \\ 132 \pm 55 \ ^{a} \end{array}$	$\begin{array}{c} 0.92 \pm 0.26^{b,c} \\ 156 \pm 56^{\ b} \end{array}$	$\begin{array}{c} 0.97 \pm 0.32 \ ^{c} \\ 158 \pm 38 \ ^{b} \end{array}$	$0.84 \pm 0.23^{a,b} \\ 156 \pm 51^{\ b}$	$F_{3,582} = 14.0, p < 0.001$ $F_{3,582} = 10.1, p < 0.001$	

Table 4

Values (mean \pm SD) for the first eight formants (F1-F8) of the main harsh roars (n = 211) of male Pannonian red deer in single–roar bouts and in different positions within multi–roar bouts, and one–way ANOVA results for their comparison. The same superscripts indicate which values did not differ significantly (p > 0.05, Tukey HSD test).

Acoustic	Total	Single-roar bouts	Multi-roar bouts (n = 1	ANOVA		
able	roars (<i>n</i> =211)	(<i>n</i> =85)	First roar $(n = 62)$ Intermediate roar $(n = 33)$		Last roar $(n = 31)$	hitovit
F1, Hz F2, Hz F3, Hz F4, Hz F5, Hz F6, Hz	$\begin{array}{c} 225\pm 32 \\ 402\pm 34 \\ 628\pm 84 \\ 835\pm 50 \\ 1093\pm 119 \\ 1387\pm 108 \end{array}$	$\begin{array}{c} 231\pm 32\ ^{a} \\ 410\pm 34\ ^{a} \\ 630\pm 85 \\ 844\pm 51\ ^{a} \\ 1101\pm 118 \\ 1381\pm 108 \end{array}$	$\begin{array}{c} 233 \pm 33 \ ^{a} \\ 400 \pm 33 \ ^{a,b} \\ 632 \pm 87 \\ 840 \pm 53 \ ^{a,b} \\ 1097 \pm 126 \\ 1392 \pm 121 \end{array}$	$\begin{array}{c} 209 \pm 31 \\ 387 \pm 35 \\ 629 \pm 80 \\ 820 \pm 43 \\ 1093 \pm 114 \\ 1397 \pm 106 \end{array}$	$\begin{array}{c} 211\pm 24\ ^{b}\\ 400\pm 29\ ^{a,b}\\ 616\pm 84\\ 819\pm 44\ ^{b}\\ 1063\pm 113\\ 1383\pm 88 \end{array}$	$\begin{array}{l} F_{3,207} = 7.36, p < 0.001 \\ F_{3,207} = 3.74, p = 0.01 \\ F_{3,207} = 0.26, p = 0.85 \\ F_{3,207} = 3.31, p = 0.02 \\ F_{3,207} = 0.79, p = 0.50 \\ F_{3,207} = 0.22, p = 0.88 \end{array}$
F7, Hz F8, Hz dF, Hz	$\begin{array}{c} 1567 \pm 73 \\ 1714 \pm 71 \\ 219.3 \pm 12.3 \end{array}$	$\begin{array}{c} 1560\pm76\\ 1712\pm69\\ 217.6\pm11.5 \end{array}$	$\begin{array}{c} 1576 \pm 76 \\ 1718 \pm 64 \\ 220.9 \pm 12.3 \end{array}$	$\begin{array}{c} 1579 \pm 53 \\ 1727 \pm 79 \\ 222.9 \pm 12.8 \end{array}$	$\begin{array}{c} 1555\pm 78 \\ 1698\pm 80 \\ 216.7\pm 13.3 \end{array}$	$F_{3,207} = 1.18, p = 0.32$ $F_{3,207} = 0.72, p = 0.54$ $F_{3,207} = 2.28, p = 0.08$

bouts and the first and last roars of multi-roar bouts than in intermediate roars, and F4 was significantly higher in single-roar bouts than in the last roars of multi-roar bouts (Table 4). In multi-roar bouts, there was no effect of the number of roars per bout on the formant values: F1 ($F_{5,120} = 1.96$, p = 0.09), F2 ($F_{5,120} = 1.28$, p = 0.28), F3 ($F_{5,120} = 0.34$, p = 0.89), F4 ($F_{5,120} = 0.22$, p = 0.95), F5 ($F_{5,120} = 0.40$, p = 0.85), F6 ($F_{5,120} = 1.41$, p = 0.22), F7 ($F_{5,120} = 0.99$, p = 0.43), F8 ($F_{5,120} = 1.88$, p = 0.11). The distances between the neighboring formants of roars were uneven (in order of increasing distance): F7 - F8 (147 Hz), F1 - F2 (177 Hz), F6 - F7 (180 Hz), F5 - F6 (294 Hz), F4 - F5 (257 Hz), suggesting non-uniformity of the vocal tract (Table 4).

Formant dispersion of the main harsh roars, calculated by the method of Riede and Fitch (1999), was 219.3 Hz. This formant dispersion provided an estimated maximum oral vocal tract length of 798.1 mm, which is between the mean and upper limit of the extended vocal tract length estimates obtained by our video single

frame reconstructions (Table 1). Formant dispersion was neither affected by roar position within a bout (Table 4) nor by the number of roars per bout ($F_{5,120} = 1.40$, p = 0.23).

Formant dispersion of the main harsh roars, calculated by the method of Reby and McComb (2003) comprised 239.96 Hz. This formant dispersion provided an estimated maximum oral vocal tract length of 729.3 mm, which is close to the lower limit of the extended vocal tract length estimates obtained by our video single frame reconstructions (Fig. 4; Table 1). Thus, the linear regression method provided different values compared to the methods where dF was calculated via the difference between formants as dF = (F8-F1)/7.

We compared bout variables and the acoustics of the main roars that were measured in this study in Pannonian red deer stags with the corresponding acoustic values of Iberian red deer stags measured in a previous study (Frey et al., 2012). Compared to Iberian red deer stags, the Pannonian stags had a significantly larger num-

Table 5

Comparison of acoustic variables of main rutting roars (Student *t*-test) and of percentages of main common and main harsh roars (χ^2 test) between Pannonian red deer stags (measured in this study) and Iberian red deer stags (measured in Frey et al., 2012).

Acoustic variable	Pannonian red deer	Iberian red deer	Student <i>t</i> -test; χ^2 test
Roars per bout	3.18 ± 2.17	2.11 ± 1.71	<i>t</i> = 8.25, <i>df</i> = 2884, <i>p</i> < 0.001
All main roars			
Duration, s	1.13 ± 0.50	1.90 ± 0.50	<i>t</i> = 40.45, <i>df</i> = 2884, <i>p</i> < 0.001
f0max, Hz	168.3 ± 60.5	224.1 ± 33.8	<i>t</i> = 23.50, <i>df</i> = 2456, <i>p</i> < 0.001
Main common roars	66.3%	89.1%	χ2 = 191.73, <i>p</i> < 0.001
Duration, s	1.27 ± 0.55	1.88 ± 0.50	<i>t</i> = 27.31, <i>df</i> = 2173, <i>p</i> < 0.001
f0max, Hz	178.7 ± 60.9	222.7 ± 34.5	<i>t</i> = 17.02, <i>df</i> = 1809, <i>p</i> < 0.001
Main harsh roars	33.7%	10.9%	$\chi^2 = 191.73, p < 0.001$
Duration, s	0.87 ± 0.25	2.12 ± 0.49	<i>t</i> = 41.56, <i>df</i> = 709, <i>p</i> < 0.001
f0max, Hz	147.2 ± 53.9	235.7 ± 28.7	<i>t</i> = 14.56, <i>df</i> = 645, <i>p</i> < 0.001
Main roar position within multi-roar bouts			
First roar	31.6%	49.9%	$\chi^2 = 66.76, p < 0.001$
Intermediate roar	34.0%	20.5%	$\chi^2 = 41.58, p < 0.001$
Last roar	34.4%	29.7%	$\chi^2 = 4.73, p = 0.03$



Fig. 4. Estimation of the minimum formant dispersion (dF) for average formant values of the main harsh roars of male Pannonian red deer (*n* = 211) by using linear regression according to **Reby and McComb** (2003). Central points show the means of the first eight formants (F1-F8), whiskers show the SD.

ber of roars per bout, a larger percent of main harsh roars and a larger number of main roars in either intermediate or last position within multi-roar bouts (Table 5). The duration of the main roars was significantly shorter and the f0max of the main roars was significantly lower in the Pannonian than in the Iberian red deer stags (Table 5).

Among a total of 5535 roars of the Pannonian red deer stags, 19 roars contained sections with source-filter coupling (Fig. 1), similar to the source-filter coupling observed in one Iberian red deer stag (Volodin et al., 2013a). Among the 19 roars of Pannonian red deer stags containing sections with source-filter coupling, 11 were main roars. The duration of the roars with source-filter coupling ranged from 0.03 s to 1.40 s (mean = 0.77 ± 0.35 s); the f0max (coinciding with the f0max of the coupling part) ranged from 338.0 to 583.6 kHz (mean = 485.9 ± 73.7 kHz). The number of roars in bouts containing roars with source-filter coupling, ranged from 1 to 10 roars per bout; the roars with source-filter coupling were always either in first position (15 of the 19 roars) or in second position (4 of the 19 roars) within a bout.



Fig. 5. Two acoustic variables of stag rutting roars (duration and maximum fundamental frequency) across European subspecies of red deer: 1. McComb, 1988; 2. Long et al., 1998; 3. Reby and McComb, 2003; 4. Kidjo et al., 2008; 5. Frey et al., 2012; 6. Passilongo et al., 2013; 7. Bocci et al., 2013; 8. Volodin et al., 2015a; 9. Della Libera et al., 2015; 10. Hurtado et al., 2012, Austrian–Hungarian stock from the mountains of the Alps and the Carpathian Mountains, introduced to Argentina in the beginning of the XXth century; 11. This study.

Discussion

This study presents a detailed spectrographic analysis of the Pannonian red deer stag rutting vocalizations. The obtained acoustical data might be applicable to the entire population of Pannonian red deer, as the analyzed acoustic recordings should include the roars of many individuals as a consequence of the substantial rotation of harem males at the recording site.

The first direct comparison between the acoustics of the rutting roars of native Pannonian and Iberian red deer stags *C.e. hispanicus* provided in this study, revealed strong differences at both hierarchical levels: at the level of bouts and at the level of particular roars within bouts. Most pronounced differences of bout structure between Pannonian stags and Iberian stags were the number of roars per bout and the ratio of harsh to common roars (Table 5). Additionally, the main roars of the Pannonian stags were shorter and lower in fundamental frequency than those of the Iberian stags (Table 5; Fig. 5).

We also compared stag rutting roars of the Pannonian red deer with stag roars of other European subspecies, regarding fundamental frequency and duration (Fig. 5, Table A1 in Appendix A). Overall, the acoustics of stag rutting roars in the European subspecies of red deer fit well to the respective three haplogroups of mitochondrial DNA (A, B, and C), which, in their order, fit to the three refugia, where red deer survived during the Last European Glaciation (Skog et al., 2009; Zachos and Hartl, 2011). The roars of male Corsican red deer *C.e. corsicanus* (haplogroup B, Skog et al., 2009; Doan et al., 2017) are the lowest in maximum fundamental frequency and are well distinguishable from the roars of both the Mesola red deer stags *C.e. italicus* (lineage intermediate between A and C haplogroups, Zachos et al., 2014) and from the roars of the Scottish and the Iberian stags (haplogroup A, Skog et al., 2009) (Fig. 5).

In the Pannonian stags, the roars are very short (1.13 s). This closely resembles the roar duration of *C.e. hippelaphus* stags from the Eastern Alps (1.18 s, Bocci et al., 2013) and of stags of the Austrian–Hungarian stock introduced to Argentina (1.07 s, Hurtado et al., 2012). At the same time, the maximum fundamental frequency of the Pannonian stags (168 Hz, this study) is comparable only with those of the Austrian–Hungarian stock introduced to Argentina (132 Hz, Hurtado et al., 2012), but is substantially lower than that of the *C.e. hippelaphus* stags from the Eastern Alps (274 Hz, Bocci et al., 2013).

Thus, the similarly short duration of the roars of the Pannonian stags and of the *C.e. hippelaphus* stags (both of haplogroup C) separates both of them from all other subspecies (Fig. 5). Yet, additionally, the Pannonian and the Eastern Alpine stags from Italy are separated by substantial differences in the maximum fundamental frequency, whereas the maximum fundamental frequency of the Pannonian stags (168 Hz, this study) closely resembles some data for the *C.e. scoticus* stags (159 Hz, McComb, 1988).

We found that two different methods for calculating formant dispersion (and the corresponding vocal tract length), formant differences (Riede and Fitch, 1999) and linear regression (Reby and McComb, 2003), provided clearly different values. One proximate reason for this disagreement might come from the linear regression method as F1 lay above and F8 below the regression line (Fig. 4).

We compared the calculated maximally extended oral vocal tract lengths, achieved during production of the rutting roars, across populations of red deer (Table A1 in Appendix A). As the values of minimum formant dispersion and the corresponding vocal tract lengths depend on the calculation method (result of this study), all vocal tract lengths used for comparison, were calculated using the linear regression method of Reby and McComb (2003). The estimated oral vocal tract lengths at maximal extension during roars were 686 mm for the Corsican stags (Kidjo et al., 2008), 726 mm for the Mesola stags (Della Libera et al., 2015), 719 mm for the Scottish stags (Reby and McComb, 2003), 767 mm for the Iberian stags, in one study (Frey et al., 2012) and 713 mm in another study (Passilongo et al., 2013), 716 mm in the stags of the Austrian-Hungarian stock from the Alps and the Carpathian Mountains, which were also introduced to Argentina (Hurtado et al., 2012), and 729 mm in the Pannonian stags (this study).

As evidenced by Table A1 in Appendix A, the calculated values of vocal tract lengths were very close for all the stags, despite pronounced differences in body size, e.g. Mesola stags 91 kg (Mattioli and Ferretti, 2014), Scottish stags 125 kg (Reby and McComb, 2003) and Pannonian stags over 200 kg (Horn and Sugár, 1992; Banwell, 1998a). Exceptions were the short vocal tract lengths in the Corsican stags that are the smallest of the studied red deer at 88 kg (Kidjo et al., 2008), and the large difference of the calculated vocal tract lengths for the Iberian stags (125 kg) (Carranza et al., 2004), obtained in two studies (Frey et al., 2012; Passilongo et al., 2013). We conclude, therefore, that the acoustic-based calculation of the minimum formant dispersion and of the maximally extended vocal tract length during production of the rutting roars are less reliable features for describing populations of red deer than the combination of frequency and temporal features of the rutting roars.

This lack of correlation between vocal tract length and body weight across subspecies (this study) was unexpected and appears to be inconsistent with the findings by Reby and McComb (2003) for stags within the Scottish subspecies C.e. scoticus. At the moment we can only speculate about the possible reasons for the unexpected close similarity of vocal tract lengths, calculated on the basis of minimum formant dispersion in red deer males of very different body weights. Perhaps, head size and neck size vary less than body size among the males of different red deer subspecies because they have to carry large antlers, which are used in aggressive encounters between males during the rut. This might exert a selective pressure towards longer necks and larger heads in the smaller subspecies for effective use of the antlers in fighting. Some insight might come from the stags of the Corsican subspecies, which, judging from photographs and videos, have rather large heads and necks. Corsican stags have an average body weight of 88 kg and a vocal tract length of 686 mm (Kidjo et al., 2008). Compared with Pannonian stags, assuming an average body weight of 200 kg and a vocal tract length of 729 mm (Table A1 in Appendix A), the body weight of the Corsican stags is 55% whereas the vocal tract length is 94% that of the Pannonian stags. Possibly, the correlation of calculated vocal tract lengths with body weight holds within one particular subspecies but not across the males of different European red deer subspecies.

The F1 values of the Pannonian red deer stag rutting roars were higher than the values predicted by the uniform tube model (Fig. 4). Higher than expected F1 values were also reported for the oral rutting roars of Scottish and Iberian red deer stags (Reby and McComb, 2003; Frey et al., 2012). And a higher than expected F1 occurs in other species of ruminants, e.g. in the nasal rutting roars of male saiga *Saiga tatarica* (Frey et al., 2007b), in the oral contact calls of mother and young saiga (Volodin et al., 2014; Sibiryakova et al., 2017), in the oral rutting roars and growls of male goitred gazelles *Gazella subgutturosa* (Frey et al., 2011) and in the oral rutting belows of male American bison *Bison bison* (Wyman et al., 2012).

Deviations from the predicted linear regression might indicate the inexactness of the underlying uniform tube model. Mammalian vocal tracts, particularly in the head region, do not correspond to a uniform tube. There are narrow passages, e.g. the fauces, and wider passages, e.g. the funnel-like oral cavity when the mouth is opened for call production. Therefore, deviations of particular formant values from the expected values do not come as a surprise. To achieve a better fit between measured and calculated formant values, the vocal tract model for calculation of the formants would have to be more closely adjusted to the natural shape of a mammalian vocal tract (or, even better, to that of the investigated vocal tract) by a more realistic mathematical depiction. However, this would require the difficult collection of vocal tract cross-sectional data and it would render the calculations more complicated than in the simple uniform tube model (Reby et al., 2018).

A true elevated F1 might result from extra air volumes connected to the vocal tract, e.g., the nasal cavities during oral calls (Fitch, 2006), a laryngeal air sac as, e.g., in reindeer (Frey et al., 2007a) or lateral nasal pouches as in male saigas (Frey et al., 2007b). In experiments, such volumes significantly shifted the position of the first formant, whereas the upper formants remained weakly affected (Riede et al., 2008). As there are no air sacs, ventricles or pouches in red deer, an elevation of F1 might indicate an involvement of the nasal cavities in the production of the presumed purely oral roars, as in male fallow deer *Dama dama* (Reby et al., 2018).

Source-filter coupling in the European red deer was previously found in a single roar of an Iberian red deer stag among 2928 roars without source-filter coupling (Volodin et al., 2013a). In the Pannonian stags of this study, we found a slightly higher proportion of such roars: 19 roars from a total of 5535 roars (0.34%). In the Pannonian red deer stags, most roars with source-filter coupling occupied the first position within a bout. Probably, when a stag starts vocalizing at high amplitude and retracts the larynx, formants decrease and the fundamental frequency might coincide with a formant frequency. Source-filter coupling arises when the sound source starts producing the fundamental frequency at the same frequency as a formant. Then, the sound amplitude of the roar increases strongly, suggesting a resonance effect (Titze, 2008; Volodin et al., 2013a).

Whereas source-filter coupling represents a very rare phenomenon in the European red deer (Volodin et al., 2013a; and this study), in the American wapiti Cervus canadensis and in the Siberian wapiti Cervus elaphus sibiricus source-filter coupling represents a usual phenomenon in stag rutting bugles (Volodin et al., 2013b; Reby et al., 2016; Golosova et al., 2017). However, in the Pannonian red deer stags, as in other European subspecies of red deer, the rutting roars lack biphonation (calls with two independent fundamental frequencies, Wilden et al., 1998), which are characteristic of many rutting bugles of wapiti (Volodin et al., 2013a,b; Reby et al., 2016; Golosova et al., 2017). Therefore, in an evolutionary perspective, the European subspecies of red deer might have lost the upper fundamental frequency and biphonation but retained the mechanism of source-filter coupling (Volodin et al., 2013a). However, such roars with source-filter coupling occur very rarely and unpredictably and, most probably, have no functional load, thus representing a kind of vocal rudiment, retained after simplification of the complex rutting calls of the central Asian ancestors (Mahmut et al., 2002; Ludt et al., 2004; Volodin et al., 2013a). The second fundamental frequency, regularly occurring in the rutting bugles of the American wapiti (Reby et al., 2016) and Siberian wapiti (Golosova et al., 2017), might also be such sort of vocal rudiment.

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Appendix A

Table A1

European populations of red deer (*Cervus elaphus*) subspecies, in which two variables of stag rutting roars (duration and maximum fundamental frequency = f0max) and the oral vocal tract length at maximal extension during stag rutting roars (vtl) have been measured or estimated bioacoustically.

N in Fig. !	5 Subspecies of red deer	Locality	Coordinates	Population	duration (s) f0max (Hz)	estimated vtl (mm)	weight (kg)	Reference
1	C.e. scoticus	The Island of Rhum, Inner Hebrides, Scotland. UK		wild	2.06	159	-	125 (Reby and McComb, 2003)	McComb (1988) [*]
2	C.e. scoticus	Killarney National Park, Co. Kerry, Ireland	52.05 N, 09.53 W	' wild	2.3	210	-	125 (Reby and McComb, 2003)	Long et al. (1998)
3	C.e. scoticus	The Island of Rhum, Inner Hebrides, Scotland, UK		wild	1.9**	137	719	125	Reby and McComb (2003)
4	C.e. corsicanus	Quenza enclosure near the Bavella needles, Casabianda enclosure in national reserve in the Casabianda penitentiary, Corsica, France		captive	1.8	52	686	88	Kidjo et al. (2008)
5	C.e. hispanicus	Doñana National Park and three fincas in Andalucia. Spain	37.02 N, 06.43W	wild and semi-captive	1.96	223	767	125 (Carranza et al., 2004)	Frey et al. (2012)
6	C.e. hispanicus	Doñana National Park, Andalucia, Spain		wild	1.83	207	713	125 (Carranza et al., 2004)	Passilongo et al. (2013)
7	C.e. hippelaphus	Natural Park of Paneveggio-Pale di San Martino, North-Eastern Italian Alps, Italy	46.50 N, 12.00 E	wild	1.18	274	-	-	Bocci et al. (2013)
8	C.e. hispanicus	Parque Natural de la Sierra Norte de Sevilla, Andalucía, Spain	37.79 N, 06.08W	wild	2.49	224	-	125 (Carranza et al., 2004)	Volodin et al. (2015a)
9	C.e. italicus	Bosco della Mesola Nature Reserve, Italy		wild	1.5	91	726	91 (Mattioli and Ferretti, 2014)	Della Libera et al. (2015)
10	C.e. hippelaphus	Nahuel Huapi National Reserve, Peninsula Huemul, Argentina		wild	1.07	132	716	_	Hurtado et al. (2012)
11	C.e. pannoniensis	Inner-Somogy, Nagyatád, Hungary	46.07 N, 17.49 E	wild	1.13	168	729	over 200 (Horn and Sugár, 1992)	d This study

* The mean value was calculated from 28 mean values of the acoustic variables of the roars of 12 stags 5 to 13 years old.

** From Table 1 in Kidjo et al. (2008).

*** Austrian-Hungarian stock from the Alps and the Carpathian Mountains, introduced to Argentina in the beginning of the XXth century (Hurtado et al., 2012).

Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.mambio.2018.10.009.

References

- Banwell, D.B., 1998a. The Pannonians *Cervus elaphus pannoniensis* a rare apart. Deer 10 (5), 275–277.
- Banwell, D.B., 1998b. Identification of the Pannonian, or Danubian, red deer. A maraloid–Cervus elaphus pannoniensis. Deer 10 (8), 495–497.
- Banwell, D.B., 2002. In defence of the Pannonian Cervus elaphus pannoniensis. Deer 12 (3), 198–203.
- Bocci, A., Telford, M., Laiolo, P., 2013. Determinants of the acoustic behaviour of red deer during breeding in a wild alpine population, and implications for species survey. Ethol. Ecol. Evol. 25, 52–69.
- Boersma, P., Weenink, D., retrieved 2 June 2013 from 2013. Praat: Doing Phonetics by Computer. Version 5.3.51. http://www.praat.org/.
- Bowyer, T.R., Kitchen, D.W., 1987. Sex and age-class differences in vocalization of Roosevelt elk during rut. Am. Midl. Nat. 118, 225–235.
- Carranza, J., Alarcos, S., Sanchez-Prieto, C.B., Valencia, J., Mateos, C., 2004. Disposable-soma senescence mediated by sexual selection in an ungulate. Nature 432, 215–218.
- Clutton-Brock, T.H., Albon, S.D., 1979. The roaring of red deer and the evolution of honest advertising. Behaviour 69, 145–170.
- Della Libera, M., Passilongo, D., Reby, D., 2015. The acoustics of male rutting roars in the endangered population of Mesola red deer *Cervus elaphus italicus*. Mammal. Biol. 80, 395–400.
- Dillon, W.R., Goldstein, M., 1984. Multivariate Analysis: Methods and Applications. Wiley, New York.
- Doan, K., Zachos, F.E., Wilkens, B., Vigne, J.-D., Piotrowska, N., Stanković, A., Jędrzejewska, B., Stefaniak, K., Niedziałkowska, M., 2017. Phylogeography of the Tyrrhenian red deer (*Cervus elaphus corsicanus*) resolved using ancient DNA of radiocarbon-dated subfossils. Sci. Rep. 7, 2331, http://dx.doi.org/10.1038/ s41598-017-02359-y.
- Fant, G., 1960. Acoustic Theory of Speech Production. Mouton & Co, The Hague, Netherlands.
- Fitch, W.T., 2006. Production of vocalizations in mammals. In: Brown, K. (Ed.),
- Encyclopedia of Language and Linguistics. Elsevier, Oxford, pp. 115–121. Fitch, W.T., Reby, D., 2001. The descended larynx is not uniquely human. Proc. R. Soc. Lond. B 268, 1669–1675.
- Fitch, W.T., Neubauer, J., Herzel, H., 2002. Calls out of chaos: the adaptive significance of nonlinear phenomena in mammalian vocal production. Anim. Behav. 63, 407–418.
- Frank, K., Barta, E., Bana, N.Á., Nagy, J., Horn, P., Orosz, L., Stéger, V., 2016. Complete mitochondrial genome sequence of a Hungarian red deer (*Cervus elaphus pannoniensis*) from high-throughput sequencing data and its phylogenetic position within the family Cervidae. Acta. Biol. Hung. 67, 133–147.
- Frantz, A.C., Zachos, F.E., Bertouille, S., Eloy, M.-C., Colyn, M., Flamand, M.-C., 2017. Using genetic tools to estimate the prevalence of non-native red deer (*Cervus elaphus*) in a Western European population. Ecol. Evol. 7 (19), 7650–7660.
- Frey, R., Riede, T., 2013. The anatomy of vocal divergence in North American elk and European red deer. J. Morphol. 274, 307–319.
 Frey, R., Gebler, A., Fritsch, G., Nygren, K., Weissengruber, G.E., 2007a. Nordic rattle:
- Frey, R., Gebler, A., Fritsch, G., Nygren, K., Weissengruber, G.E., 2007a. Nordic rattle: the hoarse vocalization and the inflatable laryngeal air sac of reindeer (*Rangifer tarandus*). J. Anat. 210, 131–159.
- Frey, R., Volodin, I.A., Volodina, E.V., 2007b. A nose that roars: anatomical specializations and behavioural features of rutting male saiga. J. Anat. 211, 717–736.
- Frey, R., Volodin, I.A., Volodina, E.V., Soldatova, N.V., Juldaschev, E.T., 2011. Descended and mobile larynx, vocal tract elongation and rutting roars in male goitred gazelles (*Gazella subgutturosa* Güldenstaedt, 1780). J. Anat. 218, 566–585.
- Frey, R., Volodin, I., Volodina, E., Carranza, J., Torres-Porras, J., 2012. Vocal anatomy, tongue protrusion behaviour and the acoustics of rutting roars in free-ranging Iberian red deer stags (*Cervus elaphus hispanicus*). J. Anat. 220, 271–292.
- Golosova, O.S., Volodin, I.A., Isaeva, I.L., Volodina, E.V., 2017. Effects of free-ranging, semi-captive and captive management on the acoustics of male rutting calls in Siberian wapiti *Cervus elaphus sibiricus*. Mammal Res. 62, 387–396.
- Hartl, G.B., Willing, R., Lang, G., Klein, F., Köller, J., 1990. Genetic variability and differentiation in red deer (*Cervus elaphus* L) of Central Europe. Genet. Sel. Evol. 22, 289–306.
- Horn, P., Sugár, L., 1992. Hungarian red deer and its potential performance as a farm deer. In: Brown, R.D. (Ed.), The Biology of Deer. Springer, New York, NY, pp. 222–222.
- Hurtado, A.M., Smith-Flueck, J.M., Black-Decima, P., 2012. Comparison of vocalisations of introduced European red deer stags (*Cervus elaphus*) in north-western Patagonia (Argentina) with native European populations. Anim. Prod. Sci. 52, 714–719.
- Kidjo, N., Cargnelutti, B., Charlton, B.D., Wilson, C., Reby, D., 2008. Vocal behaviour in the endangered Corsican deer: description and phylogenetic implications. Bioacoustics 18, 159–181.

- Long, A.M., Moore, N.P., Hayden, T.J., 1998. Vocalizations in red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), and red × sika hybrids. J. Zool. Lond. 224, 123–134.
- Lorenzini, R., Garofalo, L., 2015. Insights into the evolutionary history of *Cervus* (Cervidae, tribe Cervini) based on Bayesian analysis of mitochondrial marker sequences, with first indications for a new species. J. Zool. Syst. Evol. Res. 53, 340–349.
- Ludt, C.J., Schroeder, W., Rottmann, O., Kuehn, R., 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). Mol. Phylogenet. Evol. 31, 1064–1083.
- Mahmut, H., Masuda, R., Onuma, M., Takahashi, M., Nagata, J., Suzuki, M., Ohtaishi, N., 2002. Molecular phylogeography of the red deer (*Cervus elaphus*) populations in Xinjiang of China: Comparison with other Asian, European, and North American populations. Zool. Sci. 19, 485–495.
- Markov, G.G., Kuznetsova, M.V., Danilkin, A.A., Kholodova, M.V., Sugár, L., Heltai, M., 2015. Genetic diversity of the red deer (*Cervus elphus* L.) in Hungary revealed by cytochrome b gene. Acta Zool. Bulg. 67, 11–17.
- Mattioli, S., Ferretti, F., 2014. Morphometric characterization of Mesola red deer Cervus elaphus italicus (Mammalia: Cervidae). Ital. J. Zool. 81, 144–154.
- McComb, K., 1987. Roaring by red deer stags advances date of oestrus in hinds. Nature 330, 648–649.
- McComb, K.E., PhD thesis, 1988. Roaring and Reproduction in Red Deer (*Cervus elaphus*). University of Cambridge, 104 p.
- Nickel, R., Schummer, A., Seiferle, E., 2004. Lehrbuch der Anatomie der Haustiere. Band II Eingeweide. 9. unveränderte Auflage. Parey, Hamburg, Berlin.
- Nussey, D.H., Pemberton, J., Donald, A., Kruuk, L.E.B., 2006. Genetic consequences of human management in an introduced island population of red deer (*Cervus elaphus*). Heredity 97, 56–65.
- OVA, 2018. The National Game Management Database. http://www.vvt.gau.hu/ adattar/index-en.html.
- Passilongo, D., Reby, D., Carranza, J., Apollonio, M., 2013. Roaring high and low: composition and possible functions of the Iberian stag's vocal repertoire. PLoS One 8, e63841, http://dx.doi.org/10.1371/journal.pone.0063841c.
- Reby, D., McComb, K., 2003. Anatomical constraints generate honesty: acoustic cues to age and weight in the roars of red deer stags. Anim. Behav. 65, 519–530.
- Reby, D., McComb, K., Cargnelutti, B., Darwin, C.J., Fitch, W.T., Clutton-Brock, T.H., 2005. Red deer stags use formants as assessment cues during intra-sexual agonistic interactions. Proc. R. Soc. Lond. B 272, 941–947.
- Reby, D., Wyman, M.T., Frey, R., Passilongo, D., Gilbert, J., Locatelli, Y., Charlton, B.D., 2016. Evidence of biphonation and source–filter interactions in the bugles of male North American wapiti (*Cervus canadensis*). J. Exp. Biol. 219, 1224–1236.
- Reby, D., Wyman, M.T., Frey, R., Charlton, B.D., Dalmont, J., Gilbert, J., 2018. Vocal tract modelling in fallow deer: are male groans nasalized? J. Exp. Biol. 221, http://dx.doi.org/10.1242/jeb.179416, jeb.179416.
- Riede, T., Fitch, T., 1999. Vocal tract length and acoustics of vocalization in the domestic dog (*Canis familiaris*). J. Exp. Biol. 202, 2859–2867.
- Riede, T., Tokuda, I.T., Munger, J.B., Thomson, S.L., 2008. Mammalian laryngeal air sacs add variability to the vocal tract impedance: physical and computational modeling. J. Acoust. Soc. Am. 124, 634–647.
- Sibiryakova, O.V., Volodin, I.A., Frey, R., Zuther, S., Kisebaev, T.B., Salemgareev, A.R., Volodina, E.V., 2017. Remarkable vocal identity in wild-living mother and neonate saiga antelopes: a specialization for breeding in huge aggregations? Sci. Nat. 104, 11, http://dx.doi.org/10.1007/s00114-017-1433-0.
- Skog, A., Zachos, F.E., Rueness, E.K., Feulner, P.G.D., Mysterud, A., Langvatn, R., Lorenzini, R., Hmwe, S.S., Lehoczky, I., Hartl, G.B., Stenseth, N.C., Jakobsen, K.S., 2009. Phylogeography of red deer (*Cervus elaphus*) in Europe. J. Biogeogr. 36, 66–77.
- Szunyoghy, J., Doctoral Thesis 1963. A magyarországi szarvas (The Hungarian Deer). Magyar Természettudományi Múzeum (Hungarian Museum of Natural History), Budapest, 193 pp. [in Hungarian].

Titze, I., 1994. Principles of Voice Production. Prentice Hall, Englewood Cliffs, New Jersey.

- Titze, I.R., 2008. Nonlinear source-filter coupling in phonation: theory. J. Acoust. Soc. Am. 123, 2733–2749.
- Volodin, I., Volodina, E., Frey, R., Carranza, J., Torres-Porras, J., 2013a. Spectrographic analysis points to source-filter coupling in rutting roars of Iberian red deer. Acta Ethol. 16, 57–63.
- Volodin, I.A., Volodina, E.V., Frey, R., Maymanakova, I.L., 2013b. Vocal activity and acoustic structure of the rutting calls of Siberian wapiti (*Cervus elaphus sibiricus*) and their imitation with a hunting luring instrument. Russ. J. Theriol. 12, 99–106.
- Volodin, I.A., Sibiryakova, O.V., Kokshunova, L.E., Frey, R., Volodina, E.V., 2014. Nasal and oral calls in mother and young trunk-nosed saiga antelopes, *Saiga tatarica*. Bioacoustics 23, 79–98.
- Volodin, I., Matrosova, V., Volodina, E., Garcia, A.J., Gallego, L., Márquez, R., Llusia, D., Beltrán, J.F., Landete-Castillejos, T., 2015a. Sex and age-class differences in calls of Iberian red deer during rut: reversed sex dimorphism of pitch and contrasting roars from farmed and wild stags. Acta Ethol. 18, 19–29.
- Volodin, I.A., Volodina, E.V., Sibiryakova, O.V., Naidenko, S.V., Hernandez-Blanco, J.A., Litvinov, M.N., Rozhnov, V.V., 2015b. Vocal activity and the acoustic structure of rutting calls in red deer in the Russian Far East. Dokl. Biol. Sci. 462, 144–147.
- Volodin, I.A., Volodina, E.V., Golosova, O.S., 2016. Automated monitoring of vocal rutting activity in red deer (*Cervus elaphus*). Russ. J. Theriol. 15, 91–99.
- Wilden, I., Herzel, H., Peters, G., Tembrock, G., 1998. Subharmonics, biphonation, and deterministic chaos in mammal vocalization. Bioacoustics 9, 171–196.

- Wyman, M.T., Mooring, M.S., McCowan, B., Penedo, M.C.T., Reby, D., Hart, L.A., 2012. Acoustic cues to size and quality in the vocalizations of male North American bison, *Bison bison*. Anim. Behav. 84, 1381–1391.
- Wyman, M.T., Locatelli, Y., Charlton, B.D., Reby, D., 2016. Female sexual preferences toward conspecific and hybrid male mating calls in two species of polygynous deer, *Cervus elaphus* and *C. nippon*. Evo. Biol. 43, 227–241.
- Zachos, F.E., Hartl, G.B., 2011. Phylogeography, population genetics and conservation of the European red deer *Cervus elaphus*. Mammal. Rev. 41, 138–150.
- Zachos, F.E., Mattioli, S., Ferretti, F., Lorenzini, R., 2014. The unique Mesola red deer of Italy: taxonomic recognition (*Cervus elaphus italicus* nova ssp., Cervidae) would endorse conservation. Ital. J. Zool. 81, 136–143.
- Zachos, F.E., Frantz, A.C., Kuehn, R., Bertouille, S., Colyn, M., Niedziałkowska, M., Pérez-González, J., Skog, A., Sprěm, N., Flamand, M.C., 2016. Genetic structure and effective population sizes in European red deer (*Cervus elaphus*) at a continental scale: insights from microsatellite DNA. J. Heredity 107, 318–326.
- Zachos, F.E., 2018. Mammals and meaningful taxonomic units: the debate about species concepts and conservation. Mamm. Rev. 48, 153–159.