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The Anatomy of Vocal Divergence in North American Elk and European Red Deer

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Abstract

Loud and frequent vocalizations play an important role in courtship behavior in *Cervus* species. European red deer (*Cervus elaphus*) produce low-pitched calls, whereas North American elk (*Cervus canadensis*) produce high-pitched calls, which is remarkable for one of the biggest land mammals. Both species engage their vocal organs in elaborate maneuvers but the precise mechanism is unknown. Vocal organs were compared by macroscopic and microscopic dissection. The larynx is sexually dimorphic in red deer but not in elk. The laryngeal lumen is more constricted in elk, and narrows further during ontogeny. Several elements of the hyoid skeleton and two of four vocal tract segments are longer in red deer than in elk allowing greater vocal tract expansion and elongation. We conclude that elk submit the larynx and vocal tract to much higher tension than red deer, whereby, enormously stressed vocal folds of reduced effective length create a high resistance glottal source. The narrow, high impedance laryngeal vestibulum matches glottal and vocal tract impedance allowing maximum power transfer. In red deer longer and relaxed vocal folds create a less resistant glottal source and a wider vestibulum matches the low glottal impedance to the vocal tract, thereby also ensuring maximum power transfer.

Keywords

vocal production; acoustic communication; vocal folds; lamina propria; larynx; vocal tract; hyoid

INTRODUCTION

Size-dependent relations predict that smaller animals with smaller vocal organs produce sounds with a higher pitch, that is, fundamental frequency (F0) and formants (vocal tract filter frequencies) are larger in smaller animals. Body size can explain some vocal variability in many species (e.g., mammals: Tembrock, 1996; Fletcher, 2004; birds: Ryan and Brenowitz, 1985), but the F0 range a species can produce is often larger than a small range around the expected frequency. There are also dramatic exceptions. The Rocky Mountain elk (*Cervus elaphus nelsoni*; Erxleben, 1777; hereafter “elk”) at a maximum body mass of 450–500 kg is one of the largest land mammals. Male rutting calls have a F0 of 2 kHz and higher (Struhsaker, 1968; Fig. 1). No vocal performer with similar abilities can be

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found in this size class among terrestrial animals. Even most humans with their considerably smaller vocal anatomy have difficulties to reach the 2 kHz range (e.g., Titze, 1989). The European red deer (*Cervus elaphus*; Linnaeus, 1758; hereafter “red deer”) is a closely related but smaller species than the elk (Geist, 1998 and Köhler 1982). Yet, males produce calls with a F0 between 60 and 200 Hz (Clutton-Brock and Albon, 1979; Fig. 1). The anatomy of this divergent vocal evolution in both species is mostly unknown, but the high F0 vocalization in elk presumably requires high vocal fold tension (Titze and Riede, 2010). In almost all mammals the evolution of vocal organs is not understood, partly owing to the fact that most parts of the vocal organs do not fossilize (e.g., Veselka et al., 2010). Comparative research provides an avenue to a better understanding, and Cervinae are an excellent model as they comprise species and subspecies with widely differing vocal characteristics (Fig. 1).

Vocal production requires the control of movement in the larynx, the vocal tract (i.e., oral and pharyngeal cavity), and the respiratory system. Laryngeal features determining F0 include effective vocal fold length, vocal fold mechanical properties, and the biomechanics of the laryngeal cartilage framework, that is, the adjustable assemblage of five laryngeal cartilages. Vocal folds of adult male elk are 30 mm long, and the thyroarytenoid muscle consists of at least three portions potentially facilitating a reduction of vocal fold length (Riede and Titze, 2008). Tensile tests of vocal folds demonstrated species-specific viscoelastic properties (Riede and Titze, 2008; Riede et al., 2010), but it is little understood how the laryngeal cartilage framework supports vocal fold movements. Vocal tract geometry, which has not been investigated in elk, could affect call production, for example, by nonlinear source-filter coupling enhancing the driving pressures of the vocal folds (e.g., Titze, 2002, 2008; cf. Volodin et al., 2012). The interaction between the sound source and the vocal tract filter is controlled by the length and cross-sectional areas of the supralaryngeal vocal tract (Titze, 2008). The low formant frequencies in red deer calls are supported by a very flexible vocal tract length, which is based on an elastic connection between the hyoid skeleton and larynx (Fitch and Reby, 2001; Reby and McComb, 2003; Frey et al., 2012). So far, the anatomy of the red deer’s larynx has received little attention (Köhler, 1982, Frey et al., 2012).

Vocal adaptations and their acoustic effect can be dramatic, as the sexual dimorphism of larynx size in various species demonstrates (e.g., Zeller, 1984; Frey and Riede, 2003). However, minor morphological adaptations may also facilitate a large increase in vocal complexity (e.g., in frogs: Ryan and Drewes, 1990). This investigation of the vocal anatomy of the elk and a comparison with the red deer aims at understanding the evolution of diverging vocal adaptations in two mammals.

METHODS

We used video and acoustic recordings of free-ranging animals, macroscopic and microscopic dissection, and mechanical tests to investigate the larynx and vocal tract of North American elk and European red deer to identify adaptations for their respective, and very different vocal repertoires. Seven elk head–neck specimens (five males, two females) and 28 larynx specimens (eight males, 20 females; various ages) were retrieved from hunter harvested animals submitted to the Colorado Division of Wildlife’s chronic wasting disease surveillance program between 2006 and 2011. Two red deer head–neck specimens were purchased from a farm in Wisconsin. Further tissue samples (thyrohyoid ligaments) were collected from animals of the same farm. Two red deer head–neck specimens became available from the Tierpark Berlin, Germany (Frey et al., 2012). Incisors (I1 or I2) were collected from all elk specimens older than two years, for age determination. The teeth were processed by Matson’s Laboratory for examination of annuli in the cementum of incisor root

tips. Age estimates were performed and provided by the laboratory (Hamlin et al., 2000). Animals two years old and younger were aged by eruption wear. Ages of the red deer specimens were either known or estimated from antlers or tooth wear.

Elk larynges and histological preparations are stored at the University of Utah collection. The German red deer samples are stored at the collections of the Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany.

Two elk head–neck specimens were midsagittally cut to determine the length of various vocal tract segments. Five elk and four red deer head–neck specimens were subjected to macroscopic dissection and photographic documentation while being submerged in water. This technique considerably facilitates the diagnosis of structural details. Photographic documentation was done with a Nikon D70s digital camera (Nikon Corp., Tokyo, Japan). Subsequently, the images were transferred to a personal computer and processed with imaging software (Adobe Photoshop 5.5 and CS4). Dissections focused on the following points and questions: a) resting shape portions of the supraglottal vocal tract; b) strap muscle architecture; c) dimensions and structure of the larynx, the laryngeal cartilages, the intrinsic laryngeal muscles, and the vocal folds; d) proportions of the pharynx, the pharyngeal muscles, and the surrounding tissues; and e) the hyoid apparatus, the hyoid muscles and the structure, flexibility and histological structure of the thyrohyoid ligament.

Larynges were dissected from eight males and 20 females elk, and 25 linear measures were taken from the laryngeal cartilage framework (Fig. 2, Supporting Information Table S1, Supporting Information Figure S1). Length, histology, and tensile properties of the thyrohyoid ligament were compared between elk and red deer. Two ligament specimens from each species were measured in relaxed length and then fixed (with 5% strain) in 10% buffered formalin phosphate (SF100-4; Fisher Scientific) for five days. Longitudinal and cross sections (5 μm thickness) were stained with haematoxylin-eosin for a general overview, Masson's Trichrome (TRI) as collagen fiber stain, Elastica-Van Gieson as elastic fiber stain or alcian blue (AB) stain (pH 2.5), before and after digestion procedure with bovine testicular hyaluronidase (2 h at 37°C), for mucopolysaccharides and glycosaminoglycans. Incubation with bovine testicular hyaluronidase increases specificity for various acid mucosubstances of the AB stain.

To avoid significant post mortem effects on mechanical properties (e.g., Chan and Titze, 2004), thyrohyoid ligaments were collected from fresh specimens (five specimen from each species, older than four years of age). Elk and red deer tissue was collected within 48 (elk) and 2 h (red deer), respectively, after death. Tissue was maintained at 4°C from the time of death until the experiment which was performed within 2 h after the collection. Force–elongation data were recorded by a dual-mode servo-control lever system (Aurora Scientific Model 305B, Aurora, ON, Canada; resolution 1 μm and 0.3 mN). The setup and stress calculations were the same as in previous studies (Riede and Titze, 2008; Riede et al., 2010, Riede, 2010). Briefly, elongation was applied in a longitudinal direction followed by a release to the original length. A controlled sinusoidal displacement was applied so that the ligament was stretched and released 15 times at a rate of 1 Hz. The force and elongation signals were sampled at 1 kHz (Windaq Model DI722, DATAQ Instruments; Akron, OH). Tensile strain (ϵ) was calculated as a specimen's length change divided by its original mounting length. Tensile stress (σ) is defined as the ratio between the applied longitudinal force (F in N) and the cross-sectional area (A_0 in m^2) of the specimen. Assuming tissue incompressibility and uniform specimen cross-sectional area with roughly cylindrical geometry, the cross-sectional area is calculated as a function of tissue mass, length, and density. Time-varying cross-sectional area allows calculation of the tensile stress (σ in Pa). The stress–strain relationship was modeled with a combination of linear and exponential

models. Stiffness (i.e., a material's ability to withstand tensile strain) is Young's modulus. Young's modulus was calculated by determining the first derivative of the stress–strain model. Young's modulus is almost constant to about 40% and thereafter increases with increasing strain. Therefore, only Young's moduli for strains between 40 and 100% are presented. Hysteresis is an additional measure of a tissue's flexibility and ease of return to its original length after strain. Hysteresis describes the energy lost as heat when the stretched ligament returns to its original length. It was estimated as the difference between the area under the curves of the loading and the unloading phase of the 1 Hz-sinusoidal stress–strain response.

Results are presented in three parts. First, we describe the anatomy of the larynx (cartilaginous framework and muscles) and the mechanism to elongate and tension vocal folds. Second, the anatomy of the vocal tract is described focusing on functional differences between elk and red deer. Third, we provide data on the major components that enable the mobility of the larynx: the hyoid skeleton including the thyrohyoid ligament. In this article, we focus on functionally relevant aspects of the anatomy but additional anatomical description is presented as Supporting Information, to which references are made at appropriate places.

RESULTS

The Larynx

Laryngeal cartilaginous skeleton—The laryngeal cartilaginous framework consisted of thyroid, cricoid, two arytenoid cartilages, and the epiglottis (Supporting Information Fig. S1). The thyroid cartilage consisted of left and right lamina, and two sets of cornua which projected from the dorsal aspect of the cartilage. The rostral cornua connected the larynx to the hyoid skeleton by means of the thyrohyoid ligaments. The rostral cornua were longer in elk than in red deer (Supporting Information Table S1). The caudal cornua formed joints with the cricoid cartilage (Supporting Information Fig. S2a; Fig. 2). The laminae of the thyroid cartilage fused ventrally at a certain angle. Interestingly, this angle decreased in elk with age by more than 10° (Fig. 3). The angle was larger and seemed to remain larger in red deer in both sexes (Supporting Information Table S1). In the elk, the reduction of the angle implied an increasing constriction of the laryngeal lumen during ontogeny. The pronounced and repeated contractions of the caudal sphincters of the pharynx during vocalizations constrict the larynx (almost like an “elastic stocking”). Over the years, this will tend to reduce the angle between the thyroid laminae, increasingly stabilized by calcification and ossification. However, this ontogenetic trend requires additional confirmation for older ages of male elk.

The cricoid cartilage formed a complete ring, consisting of a dorsal plate (lamina) and a ventral arcus. The dorso-ventral inner diameter was smaller in elk (Supporting Information Table S1). The caudal end of the lamina was flexible and embedded in the connective tissue between the dorsal endpoints of the rostral three dorsally open tracheal rings. This, together with the flexible connections between the tracheal rings, allowed a rostradorsal tilting of the cricoid cartilage (Fig. 4).

Size differences between males and females in laryngeal dimensions could not be explained by overall body size differences. Body sizes between males and females differ by about 30% in both species (male elk: 400 kg; female elk: 300 kg; male red deer: 200 kg; female red deer: 150 kg). Sex ratios for linear laryngeal measures ranged between 0 and 18% in elk but always exceeded 23% in red deer (Supporting Information Table S1). Laryngeal cartilages were 3–4 times heavier in male than in female red deer, but the difference was only 12–18%

in elk (Supporting Information Table S1). Although based on a small sample size, this suggests a more pronounced sexual dimorphism in red deer than in elk.

Laryngeal intrinsic muscles—The structure of the intrinsic laryngeal muscles was basically the same in both species. There were five intrinsic muscles (Fig. 2). The fibers of the cricothyroid muscle ran from ventrocaudally to rostr dorsally. They originated from the lateral side of the cricoid ring and terminated on the caudal edge and inner surface of the thyroid lamina (Fig. 2). Some dorsal fibers also terminated on the ventral surface of the caudal horn of the thyroid cartilage. A contraction of the cricothyroid muscle would cause either a ventrocaudal tilting of the thyroid cartilage about the cricothyroid joint or a rostr dorsally tilting of the cricoid cartilage (Fig. 4).

The large thyroarytenoid muscle coursed ventro dorsally (Fig. 2). There was a caudal portion that ran inside the vocal fold and a rostral portion that ran in the lateral wall of the vestibulum and extended on the base of the epiglottis. The caudal portion consisted of two parts that diverged dorsally. One portion ran between the internal surface of the ventral angle of the thyroid cartilage and the vocal process of the arytenoid cartilage (Fig. 5). The other portion (*Musculus thyroarytenoideus, pars foveolaris*) branched off at about mid-length of the vocal fold and terminated onto a foveola, that is, a small indentation, of the vocal ligament (Figs. 2, 5). A contraction of this foveolar portion pulls laterally on the vocal process. This generates a compression force alongside the vocal fold and, additionally, a medial rotation of the vocal process reducing the glottal gap and/or increasing adduction between left and right vocal fold.

Three additional intrinsic muscles regulate larynx configuration. The lateral cricoarytenoid muscle runs between the lateral surface of the cricoid ring and the muscular process of the arytenoid cartilage (Fig. 2). Its contraction causes an abduction of the vocal folds. The dorsal cricoarytenoid muscle originated from the dorsal lamina of the cricoid and terminated on the muscular process of the arytenoid cartilage (Fig. 2). Its contraction pulls the arytenoid cartilage dorsally or arrests it by prevention of ventral tilting. Accordingly, it is an important antagonist to the caudal portion of the thyroarytenoid muscle. The transverse arytenoid muscle bridged the left and right arytenoid cartilage dorsally and inserted on the left and right muscular processes (Fig. 2). Its contraction adducts the vocal folds.

Regulation of vocal fold length and tension—The vocal folds were positioned between the thyroid cartilage and the vocal process of the arytenoid cartilage. In a first approximation, vocal folds behave like a string. Oscillation frequency depends on length and tension (tissue stress). Vocal folds consist of viscoelastic material causing tissue stress to increase exponentially at larger strains (>20%). An increase in vocal fold length can be achieved by either of two mechanisms. First, a ventrocaudal tilting of the thyroid cartilage is caused by the cricothyroid muscle. (Fig. 4b, Arrow 1). The arytenoid cartilages remain arrested or are pulled dorsally by the dorsal cricoarytenoid muscle (Fig. 4b, Arrow 2). Second, a contraction of the cricothyroid muscle is associated with a rostr dorsally rotation of the cricoid cartilage about the cricothyroid articulation (Fig. 4c; Arrow 1), and a contraction of the rostral portion of the large thyroarytenoid muscle. This causes the arytenoid cartilage to rotate about the cricoarytenoid articulation, thereby pulling the corniculate process ventrally and the vocal process dorsally (Fig. 4c; Arrow 2). More detail about the two mechanisms is provided in Supporting Information Figure 2. One or the other of the two mechanisms can strain the elk's vocal folds to a length of about 40 mm. Tissue stress will increase (Riede and Titze, 2008), but due to its length and physical constraints, the vocal folds are unlikely to produce the highest F0 rates (Titze and Riede 2010). We hypothesize that, while the vocal folds are maximally strained in elk, adductive forces reduce the effective length of the vocal folds. As the adductive forces are most effective at the ventral

and dorsal ends of the vocal folds, this will expose a considerably smaller mid-membranous portion of the vocal folds responding to the passing air flow.

Vocal Tract

Using natural landmarks, the supraglottal vocal tracts of elk and red deer were divided into four segments (Fig. 6; Supporting Information S1): 1) the oral cavity (hereafter “oral cavity”) from the lips to the palatoglossal arcs; 2) the isthmus of the fauces (hereafter “isthmus faucium”) connecting the oral cavity with the oral portion of the pharynx at the level of the palatoglossal arcs and the last molar teeth; 3) the pharynx (hereafter “pharynx”), between the level of the last molar teeth and the larynx; and 4) the laryngeal vestibulum (hereafter “vestibulum”), between the entrance of the larynx and the vocal folds. The relative lengths of three of the four segments differed between male elk and male red deer. Oral cavities were 300 (elk) and 230 (red deer) mm long. The isthmus faucium was short (about 30 mm in both species) and narrow. The pharynx measured 100 mm in male elk and 150 mm in male red deer. The vestibulum measured 50 mm in length in male elk and 60 mm in male red deer. The elk’s vestibulum is not only shorter but also smaller in cross sectional area.

Despite the neck mane of elk and red deer, video sequences suggested a lesser degree of larynx displacement in elk than in red deer. This was confirmed by a different structure of the hyoid apparatus and a dramatically shorter thyrohyoid ligament in elk (see below). Reconstructions revealed an influence of this on the amount of vocal fold tension (Fig. 7). The available space along the ventral neck region was narrow. Larynx and pharynx in their resting positions were aligned with the longitudinal axis of the rostral neck portion of the trachea. However, depending on the distance by which the larynx was retracted, different forces will act on the laryngeal framework.

In elk, the larynx seemed to be retracted not further than the level of C4, approximately (Fig. 7A). The shorter distance of larynx retraction (compared to red deer) prevented a pronounced angulation between pharynx and trachea. Thereby, pharynx and trachea were kept in line with the main direction of the caudal pull of the strap muscles and this allowed synergistic action of the strap muscles and the cricothyroid muscle. As a consequence, the cricothyroid gap is narrowed, adding to the high tension acting on the vocal folds.

In red deer, larynx and trachea were maximally retracted and pulled close to the thoracic aperture (Fig. 7B). Hence, the caudal curvature of the neck inevitably leads to angulation, ventral bending, and stretch on the larynx and caused a widening of the cricothyroid gap. As a result, the tension of the cricothyroid ligament increased thereby countering the effect of the cricothyroid muscle and relaxing the vocal fold. Thus, in red deer a synergistic effect of the cricothyroid and the strap muscles is considerably reduced and production of the low F₀ is assumed to occur with more relaxed vocal folds than in elk.

Different caudal displacement of the larynx will also affect the activity of the caudal pharyngeal constrictors, that is, the thyro- and cricopharyngeus muscles. The thyropharyngeus muscle enwrapped the thyroid cartilage almost completely. In red deer, maximal retraction of the larynx entailed maximal extension of the caudal pharyngeal constrictors, which, therefore, would contract less effectively at the same time. In contrast, less pronounced larynx retraction in the elk leave substantial contraction power to the caudal pharyngeal constrictors even during larynx retraction. As a consequence, strong contraction of the thyropharyngeus muscle during each call can aid in constricting the vestibulum in elk, but not in red deer, by approaching epiglottis and corniculate processes, thereby repeatedly decreasing the angle between the thyroid laminae. The permanent reduction of the angle

between left and right thyroid lamina during ontogeny was in accordance with this mechanism.

A dorsoventral constriction of the lumen of the vestibulum can be achieved by a contraction of the rostral portion of the thyroarytenoid muscle as its rostral fibres originate laterally from the base of the epiglottis and terminate on the arytenoid cartilage. Altogether there were three mechanisms affecting vestibulum width and vocal fold adduction: 1) the caudal pharyngeal constrictors constrict the vestibulum along its entire circumference and assist in vocal fold adduction, 2) the thyroarytenoid muscle constricts the vestibulum dorsoventrally and adducts the vocal folds, and 3) the transverse arytenoid muscle adducts the vocal folds.

Hyoid Skeleton and thyrohyoid Ligament

The hyoid skeleton in association with the hyoid muscles and the thyrohyoid ligament accommodated the caudal displacement of the larynx during vocalization. The hyoid skeleton consisted of five paired elements, which were ventrally connected by the unpaired basihyoid (Fig. 8). Several elements of the hyoid skeleton were shorter in elk (Fig. 8; Supporting Information Table S2). The particularly short epihyoid in elk will cause a smaller dorsoventral diameter of the rostral portion of the pharynx both in the resting state and during caudal displacement of the larynx. The long epihyoid in red deer contributed to the greater caudal displacement of the larynx in this species and increased the expansibility of the pharynx (Fig. 7).

The thyrohyoid ligament was twice as long in red deer than in elk (Supporting Information Table S3) ($N_{1,2} = 5$, $T = 11.9$; $P < 0.001$). The ligaments in both species contained elastin and collagen fibers as well as adipocytes (Fig. 9). The cross sectional area of 50 elastic fibers was measured in each of two specimens. Elastic fibers were thicker in elk ($13.3 \pm 4.0 \mu\text{m}^2$) than in red deer ($8.3 \pm 2.1 \mu\text{m}^2$) (Supporting Information Fig. S4) ($N_{1,2} = 50$; $t = 11.0$, $P < 0.001$) suggesting greater elastic recoil force in elk. The red deer ligament contained more fat cells, which are organized in big clusters along the entire ligament (Fig. 9O, P). In elk, they were fewer and entirely missing in some aspects of the ligament, where fibrous proteins were densely packed (Fig. 9G, H). We found striated muscle fibers in the thyrohyoid ligament in both elk specimens (Supplementary Fig. S5) but not in the red deer specimens. Muscle fibers could assist in actively increasing tensile strength during larynx retraction and/or facilitate shortening of the ligament to its original length after calling/laryngeal caudal displacement.

The stress–strain curves of ligaments from both species showed exponential stress increase at strains larger than 40% (Supporting Information Fig. S6). Although stress in red deer ligaments were larger at strains higher than about 60% (Supporting Information Fig. S6), the stress was not very large (below 200 kPa at 100% strain in both species). Hysteresis was significantly larger in the red deer's ligament ($N_{1,2} = 5$, $T = 2.4$; $P < 0.05$; Supporting Information Table S3). This suggests that more elastic energy was lost as heat when the red deer ligament was stretched and released. Together with the thicker elastic fibers, this supports the hypothesis that in elk elastic properties contribute more to restoring the original length of the ligament than in red deer.

DISCUSSION

A common feature of rutting calls from both species is the high amplitude. Vocalizations of elk and red deer can be heard over several kilometers in optimal conditions (Geist, 1998). To achieve maximum power output from the lips, it is necessary to maximize the power transfer from the sound source, the larynx and through the vocal tract. We suggest that this is achieved differently in both species. In the following, we will present a model of vocal

production for elk and red deer based on current anatomical findings and the maximum power transfer theorem applied to voice production developed by Titze (2002).

Several differences in the vocal anatomy between elk and red deer exist (Table 1). In red deer, the lower larynx resting position, the greater length of epihyoid bone and thyrohyoid ligament, and the sexual dimorphism of the larynx point to the ability of males to produce low fundamental and formant frequencies. Those results confirm and extend earlier studies on red deer (Clutton-Brock and Albon, 1979; Köhler, 1982; Fitch and Reby, 2001; Reby and McComb, 2003; Frey et al., 2012). The red deer's long and much less tensed vocal folds during vocalization entail a low glottal resistance. For maximum power transfer, a low glottal resistance requires a vocal tract with low impedance. The large glottal flow, that is, air passing through the glottis during each vocal fold oscillation cycle, is best transferred through an unobstructed wide vocal tract. The red deer's vocal tract during a call is probably wider than that of the elk (Fig. 7). The male larynx is large and, compared to elk, becomes less constricted by pharyngeal constrictors. Furthermore, the long epihyoids allow a wide expansion of the pharynx.

In elk, maximally strained vocal folds with decreased effective lengths due to three adductive mechanisms, create a high resistance glottal source. A high glottal resistance with much smaller glottal flow requires a narrow vocal tract with a high impedance for maximum power transfer, preventing loss of acoustic energy. The small glottal flow would simply dissipate much of the acoustic energy if released into a wide vocal tract. The vestibulum functions as an impedance matching device. It couples the high impedance of the glottis to a relatively low impedance of the oral cavity, vocal tract and the environment. The necessary constriction of the vestibulum is achieved by strongly contracting caudal pharyngeal constrictors. The elk's shorter thyrohyoid ligament impedes maximal retraction of the larynx down to the thoracic aperture. Furthermore, the lower hysteresis suggests that elastic properties (recoil potential) are larger in elk contributing to a faster restoration of the original length. The muscles in the elk's thyrohyoid ligaments provide an additional active retraction mechanism helping in maintaining a larger tension and faster repositioning of the larynx. The epihyoid is short and therefore the rostral pharynx is less expandable in elk than in red deer.

In summary, both species displace their larynx caudally but to a different degree. While red deer keep their intrinsic laryngeal musculature rather relaxed, elk submit vocal tract and larynx to high tension, ultimately achieving high vocal fold tension and a shorter effective vocal fold length. Simultaneously to these different settings of the larynx, optimal conditions for power transfer are generated in both species by adjusting the width of the supralaryngeal vocal tract.

Selection for Low or High Pitch

Elk and red deer share a common ancestor in Asia between 1.2 and 3.5 million years ago (Fig. 1; e.g., Polzehl and Strobeck, 1998, 2002; Kuwayama and Ozawa, 2000; Mahmut et al., 2002; Ludt et al., 2004; Pitra et al., 2004). The “ancestral vocal anatomy” may have facilitated the production of sounds with low and intermediate F0 ranges (approx. 150–300 Hz) and somewhat lowered resonance frequencies. Current results suggest that despite large vocal differences, anatomical differences need only be moderate. The suggested model for producing the elk's bugle calls combines an increase of vocal fold tension, a simultaneous reduction of effective vocal fold length and the vestibulum serving as an impedance matching device. Recent Asian species, including Sika deer, Bactrian red deer, and Siberian elk, are able to produce high F0 vocalizations (e.g., Minami and Kawamichi, 1992; Long et al., 1998). The elk is actually not the only extreme vocal performer, although the combination of large body size and F0 above 2 kHz seems unique. Furthermore, descriptions

of vocal repertoires of hybrids between European red deer and elk or Sika deer (e.g., Caughley, 1971; Long et al., 1998) suggest that such hybrids are capable of producing high and low F0 vocalizations.

Despite the flexibility in vocal production, our model also suggests, that the specialization of vocal behavior in elk and red deer is associated with constraints rendering both vocal types mutually exclusive. The low larynx resting position and the long and floppy thyrohyoid ligament support the production of low resonance frequencies in red deer. In addition, the maximal caudal displacement of the larynx creates an unfavorable condition for vocal fold length changes. Vocal folds remain more relaxed and produce sound with a lower oscillation rate in red deer than in elk. Female red deer prefer the lowest male resonance frequency calls, as shown in a series of playback experiments (Taylor and Reby, 2010). However, within a red deer's F0 range between 60 and 200 Hz, females prefer calls with higher fundamental frequencies (Reby et al., 2010). This could reflect a selection for males capable of challenging the biomechanical difficulties between two vocal extremes. Such behavioral data and our anatomical data indicate that although morphological prerequisites (flexible laryngeal placement and vocal fold length variation) affect the sound source and the vocal tract filter differently, they remain linked. On the one hand, a filter-related "vocal extreme" (maximal caudal displacement of the larynx in red deer) cannot evolve without setting new boundaries to source-related acoustic properties, that is, F0 probably maxes out at 200–300 Hz. On the other hand, source-related extremes (high F0 calls in elk), cannot evolve without setting boundaries to vocal tract-related acoustic properties. Formants in elk are either difficult to perceive because there is little acoustic energy available in the respective frequency ranges, and/or formants cannot be as low as in red deer because caudal displacement is limited to the level of C4.

The anatomical differences in elk and red deer are moderate. They would not allow to predict the elk's high pitched voice if looked at in isolation and with a purely size-dependent F0 determination in mind. Our model suggests that several moderate morphological adaptations added up to ultimately generating a large behavioral effect. Studies on the relation between call characters and vocal anatomy in other taxa obtained similar results. For example, four species within the frog species group, *Physalaemus pustulosus*, produce a "whine" call component. Only one species adds a second call component ("chucks"), which is generated by a large fibrous mass attached to the vocal fold (Ryan and Drewes, 1990; Gridi-Papp et al., 2006). The fibrous mass is present in all four species, but it is larger and specifically connected in the one producing whines and chucks, suggesting a) that changes in the morphology were simple to effectively add to sound production and b) that species not producing the second call component, nevertheless possess the potential (Ryan and Drewes, 1990). The presence of anatomical predispositions can more easily facilitate the evolution towards a complex vocal pattern.

In summary, current data suggest that extreme F0 ranges need not be associated with dramatic size changes, in either body size or the size of the vocal organ as in some species (e.g., Zeller, 1984; Frey and Riede, 2003). The relation between F0 and size can be uncoupled by functional specialization which requires only moderate anatomical adaptations. However, there are limitations to the functional interpretation of anatomical differences. Vocalization is a highly dynamical process and future research, including physical and computational modeling, will further test the proposed model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Adit. Lar	laryngeal entrance
Ang styloh	angle of stylohyoid
Arccart. Cric	arch of cricoid cartilage
Art cricaryt	cricoarytenoid articulation
Art. cricithyr	cricothyroid articulation
Basih	basihyoid
Cart. aryt	arytenoid cartilage
Cart. cric	cricoid cartilage
Cart. thyr	thyroid cartilage
Cart. thyroh	thyrohyoid cartilage
Cav. infrglott	infraglottic cavity
Ceratoh	ceratohyoid
Cont. M. cricaryt. Lat	contour of lateral cricoarytenoid muscle
Cont. M. cricithyr	contour of cricothyroid muscle
Cornu rostr	rostral horn of the thyroid cartilage
Cornu caud	caudal horn of the thyroid cartilage
Epigl	epiglottis
Epih	epihyoid
F0	fundamental frequency
Fov. lig. voc	foveola of the vocal ligament
Lam. cart. Cric	lamina of cricoid cartilage
Lig. Cricithyr	cricothyroid ligament
Lig. Voc	vocal ligament
M. aryt. transv	transverse arytenoid muscle
M. cricaryt. dors	dorsal cricoarytenoid muscle
M. cricaryt. lat	lateral cricoarytenoid muscle
M. cricithyr	cricothyroid muscle
M. sternthyr	sternothyroid muscle
M. thyroh	thyrohyoid muscle
M. thyraryt	thyroarytenoid muscle
M. thyraryt. p. fov	foveolar portion of the thyroarytenoid muscle
Oesoph	esophagus
Palat. Mol	soft palate

Plic. Voc	vocal fold
Proc. Corn	corniculate process
Proc. Musc	muscular process of the arytenoid cartilage
Proc. Med	medial process
Proc. Voc	vocal process of the arytenoid cartilage
Rec. pirif	piriform recessus
Styloh	stylohyoid
Trach	trachea
Tympoh	tympanohyoid
Thyroh	thyrohyoid
Vest. Lar	laryngeal vestibulum

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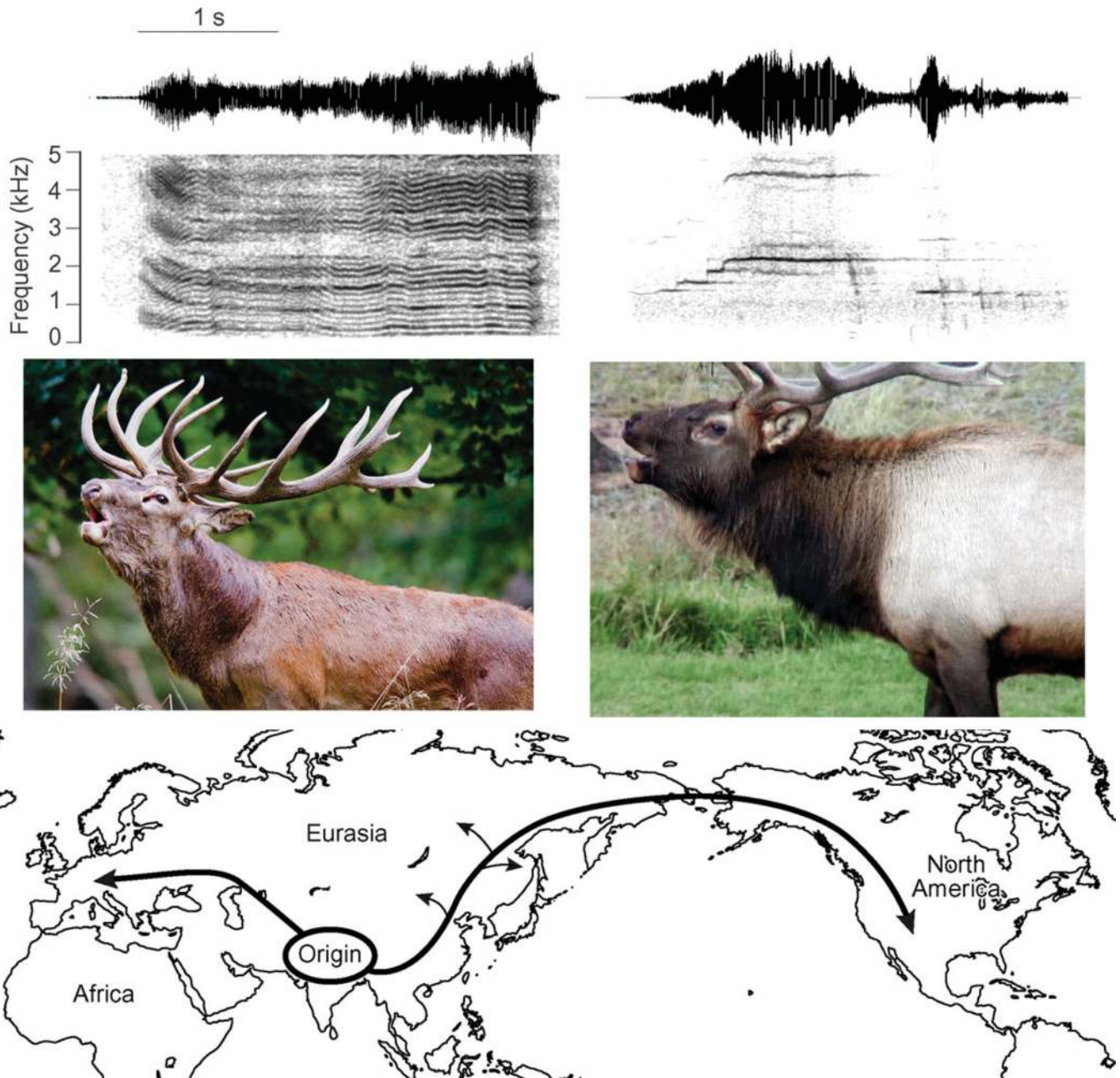


Fig. 1. Vocal divergence in the genus *Cervus*. The F0 of mating calls of the North American elk and the European red deer differs by three octaves representing an unprecedented challenge to vocal anatomy. **Top two panels:** Oscillogram and spectrograms of male red deer and elk calls. The F0 of red deer calls ranges between 60 and 200 Hz, while that of elk can be as high as 2.3 kHz. **Middle:** Photos of red deer and elk during vocalization. **Bottom panel:** Phylogeographic and molecular phylogenetic studies (Polziehn and Strobeck, 1998, 2002; Kuwayama and Ozawa, 2000; Mahmut et al., 2002; Ludt et al., 2004; Pitra et al., 2004) agree that the genus *Cervus* originated in Asia from where ancestor species migrated west, establishing red deer populations in different parts of Europe. Some ancestors migrated east founding current Asian species. They also passed the Bering bridge into North America,

where the biggest recent representative of *Cervus*, the North American elk or Wapiti, lives. The western populations/species produce all low-pitched calls (e.g., Cap et al., 2008; Frey et al., 2012), while the eastern migrants including many species in Asia, produce intermediate or high-pitched calls.

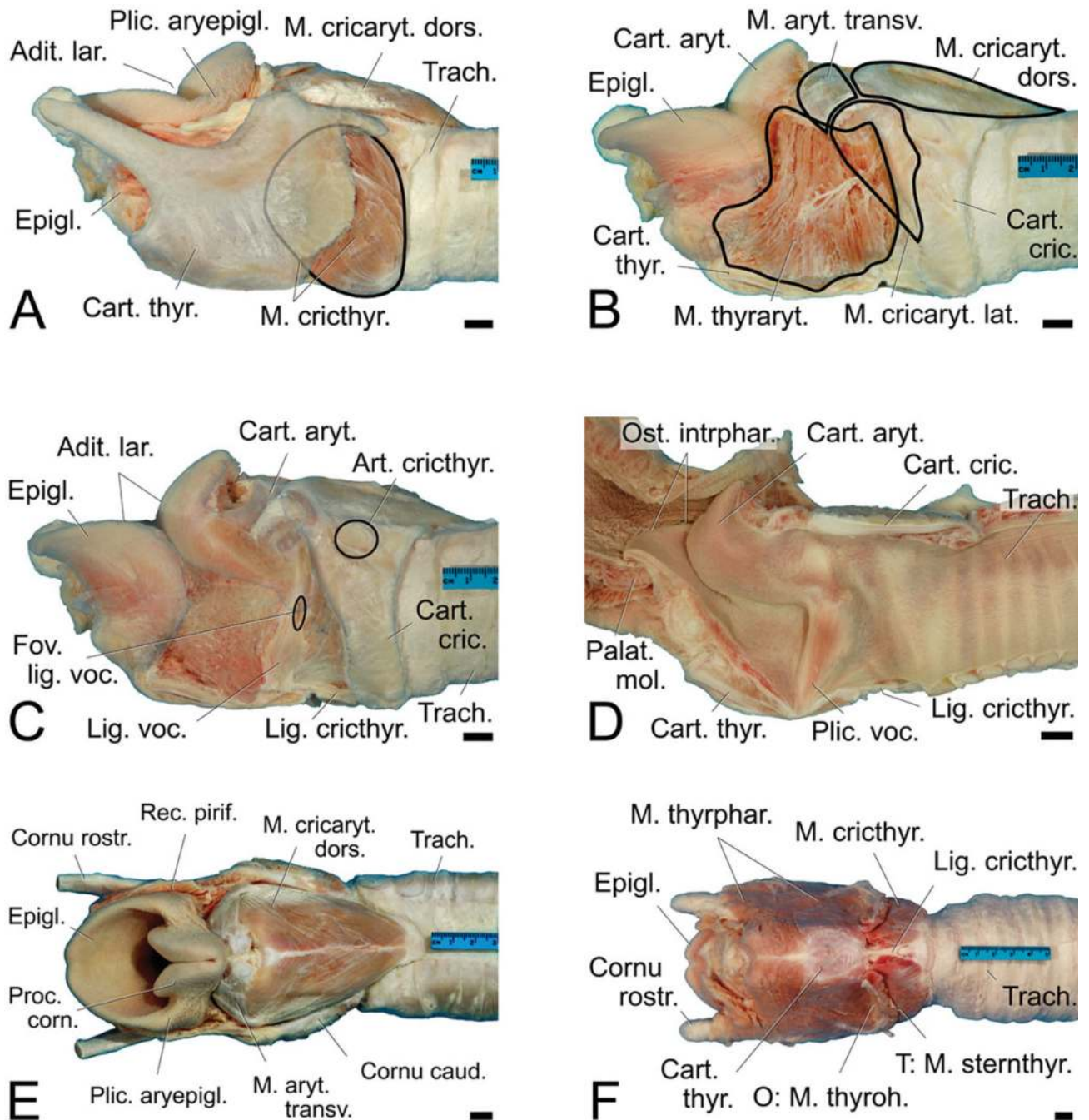


Fig. 2.

Adult male larynx of North American elk. **A:** In situ-position of the thyroid cartilage. The cricothyroid muscle (contour indicated) originates laterally from the cricoid cartilage and terminates caudomedially on the thyroid cartilage, **B:** Contours of the lateral cricoarytenoid muscle and dorsal cricoarytenoid muscle and large dimensions of thyroarytenoid muscle. **C:** Thyroarytenoid muscle removed to expose the underlying vocal ligament and its foveola. **D:** Medial aspect of arytenoid cartilage and vocal fold. The triangular space rostral to (left of) the vocal fold is the vestibulum of the larynx (not labelled). **E:** Entrance to the larynx surrounded by the epiglottis, the aryepiglottic folds, and the arytenoid cartilages. Position

and dimensions of left and right dorsal cricoarytenoid muscle. **F**: Except a small circular medioventral area, the larynx is completely enclosed by the thyropharyngeal muscle, one of the caudal constrictor muscles of the pharynx. **A–C** Dissection in layers, proceeding from lateral to medial, left lateral view; **D** Larynx mediosagittally sectioned, medial view of right half; **E** Dorsal view; **F** Ventral view of entire larynx.

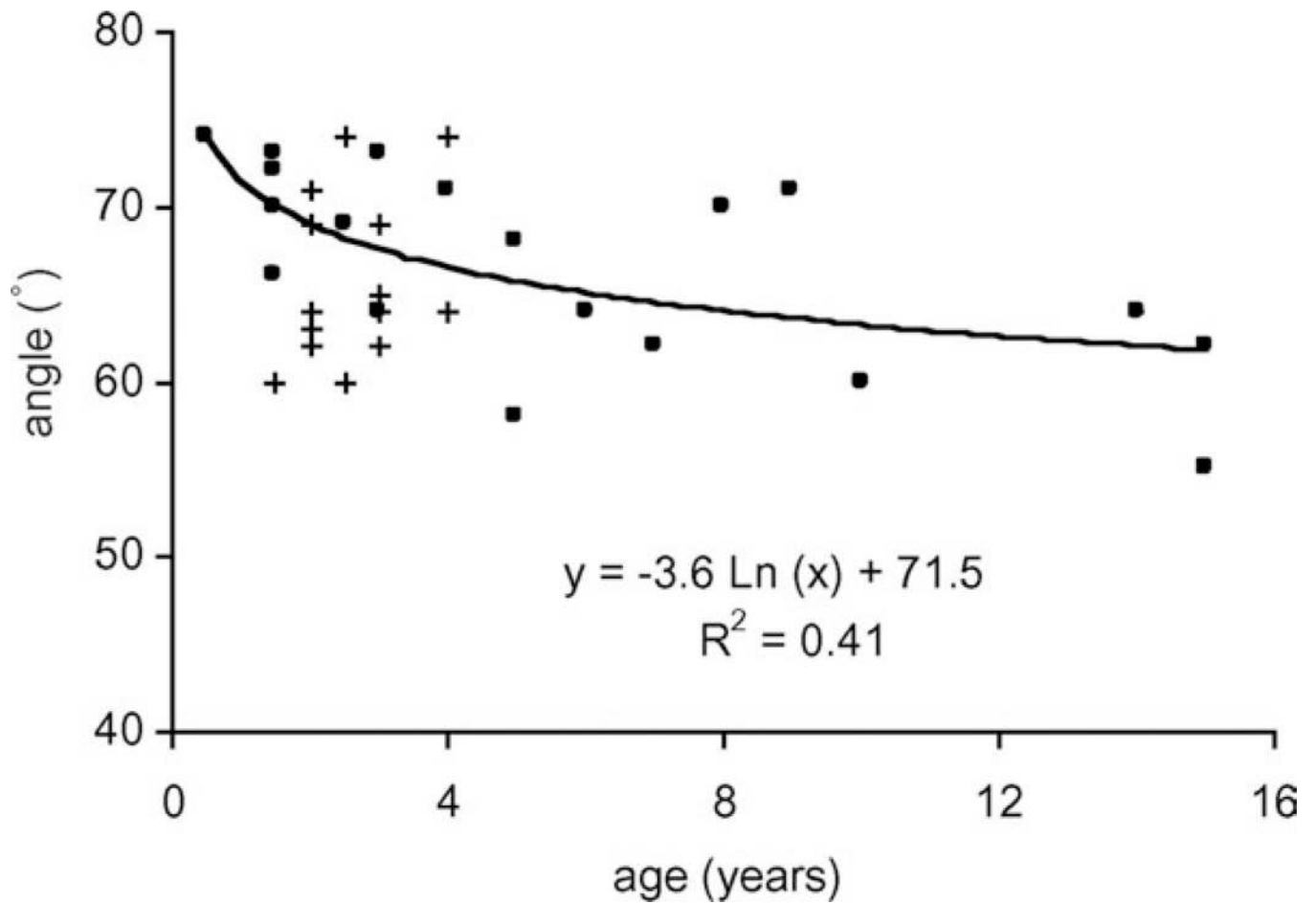


Fig. 3. The angle between left and right lamina of the thyroid cartilage decreases with age in elk. Regression function was calculated only for females owing to lack of data on older males. Females (closed circles) and males (pluses). Female elk produce similar call types and sometimes at comparable rates, as males.

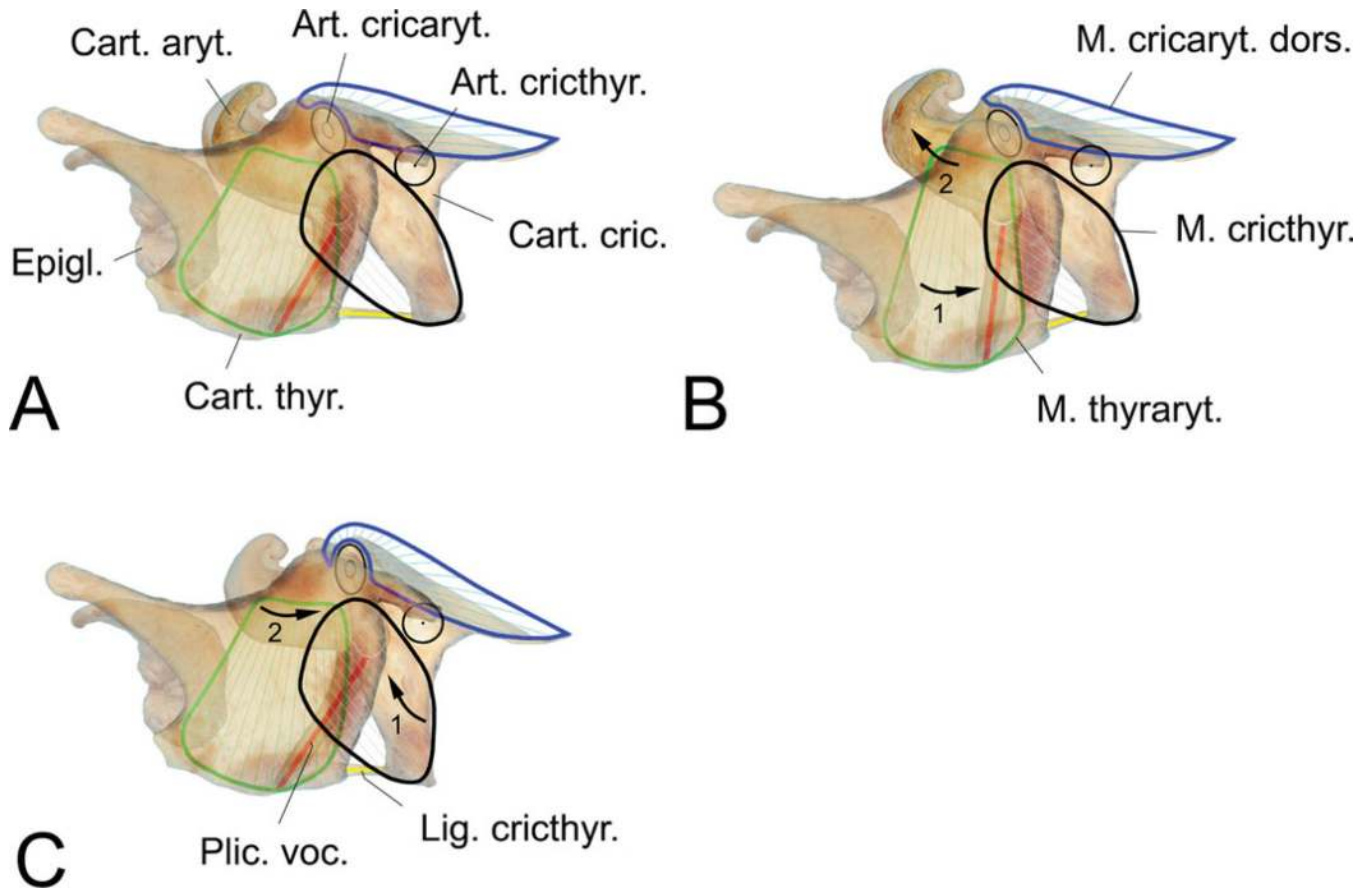


Fig. 4.

Vocal folds can be tensioned by two mechanisms. **A:** Resting position of laryngeal framework and vocal folds. **B:** Mode of vocal fold elongation facilitated by a contraction of the cricothyroid muscle which causes the thyroid cartilage to move ventrally and caudally. Thereby, the ventral end of the vocal fold is also moved ventrally and caudally. When the thyroid cartilage and the cricoid cartilage approach each other, they close the cricothyroid gap. A simultaneous contraction of the dorsal cricoarytenoid muscle keeps the arytenoid cartilage in place or pulls it dorsally (arrow 2). Important here is a rotation of the thyroid cartilage around the cricothyroid joint (arrow 1). **C:** Nickel et al. (2004) suggested an alternative mode of vocal fold elongation. Contraction of the cricothyroid muscle causes the cricoid cartilage to move rostrally and dorsally around the cricothyroid joint (arrow 1). The simultaneous contraction of the rostral portion of the thyroarytenoid muscle (a homologue of the *M. ventricularis*) causes a ventrocaudal movement of the arytenoid cartilage (arrow 2). Thereby, the dorsal end of the vocal fold is moved dorsally and caudally.

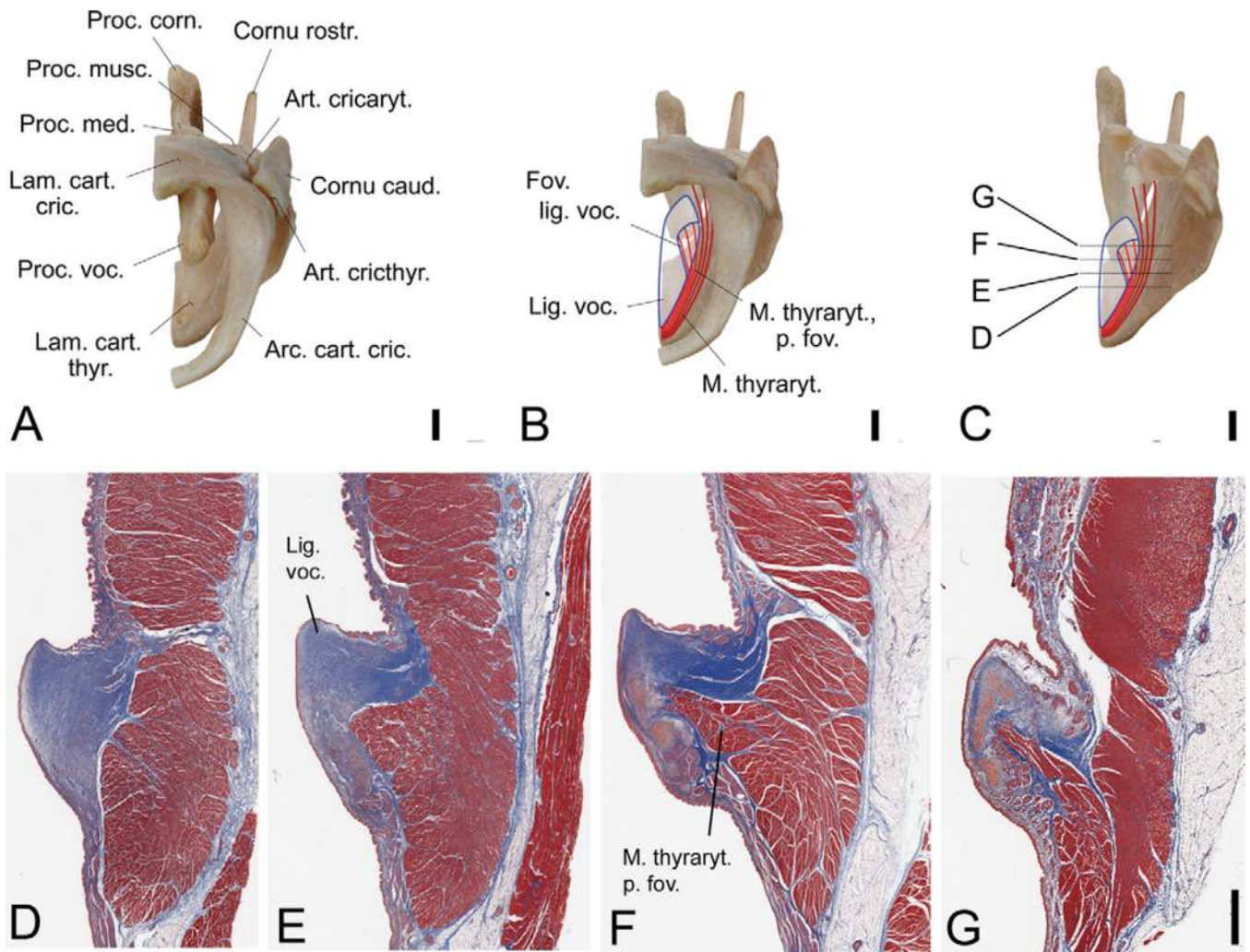


Fig. 5. The pars foveolaris of the thyroarytenoid muscle helps to shorten the effective length of the vocal fold. **A:** Caudal view of the right half of the laryngeal cartilage framework. The vocal ligament and the thyroarytenoid muscle are schematically added to the cartilage framework in **B**. Histological cross sections of the vocal fold at various positions from ventral to dorsal are shown in **D–G**. The positions of the cross sections are indicated in **C**. Sections were stained with Masson's trichrome. The large vocal ligament is blue. Scale bars: 10 mm.

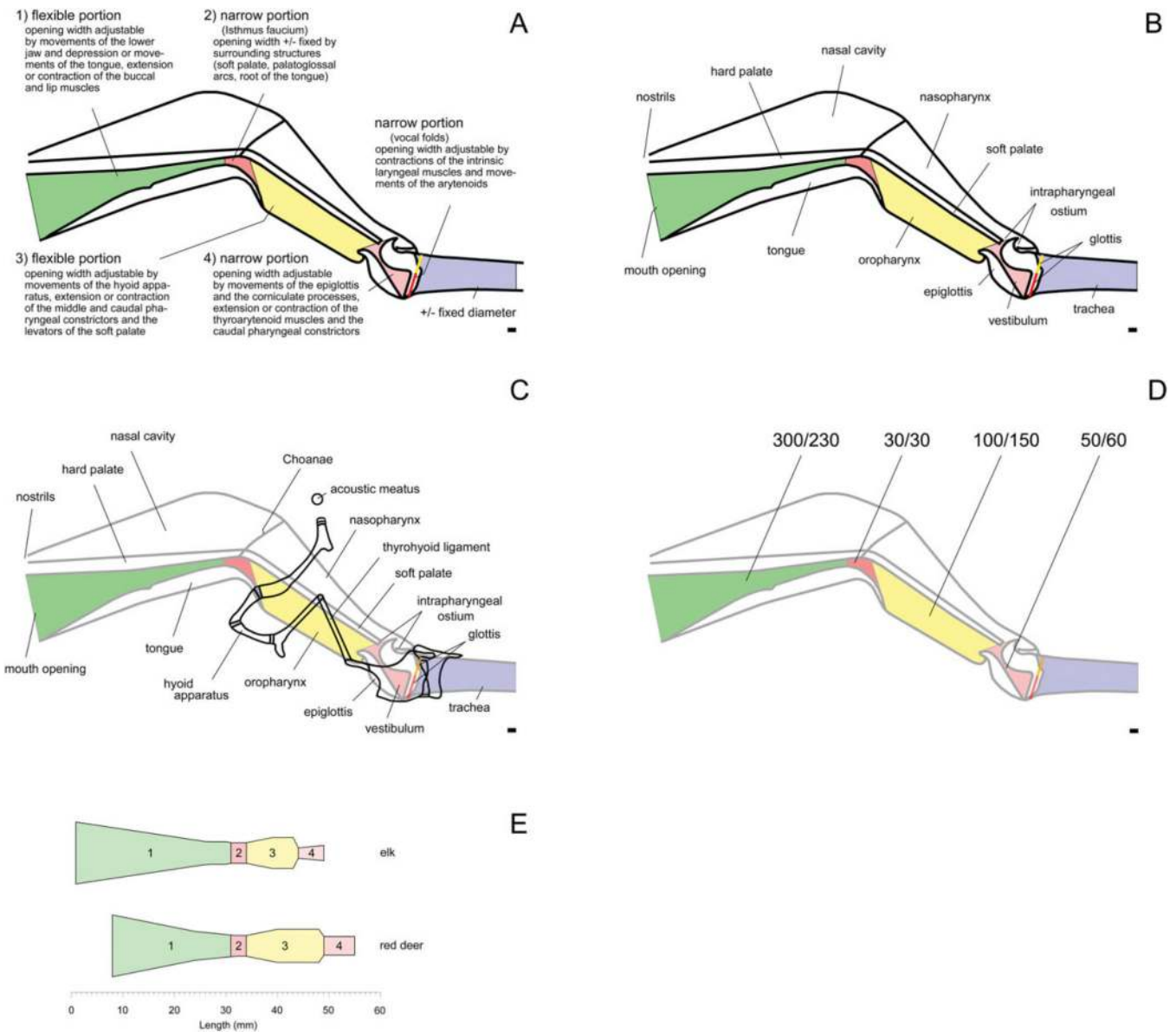


Fig. 6. The vocal tracts of elk and red deer can be separated in four sections: 1) oral cavity, 2) isthmus faucium, 3) pharynx, and 4) vestibulum laryngis. **A:** Schematic of the four sections with explanations of the main features. **B:** Same schematic of the vocal tract with the key boundary structures indicated. **C:** Same schematic of the vocal tract with an overlay of the hyoid skeleton. **D:** Approximate lengths (in mm) of the four segments in elk and red deer. **E:** Dimensions of the four segments of the vocal tract in both species relative to the isthmus faucium. Scale bars in A–D: 10 mm.

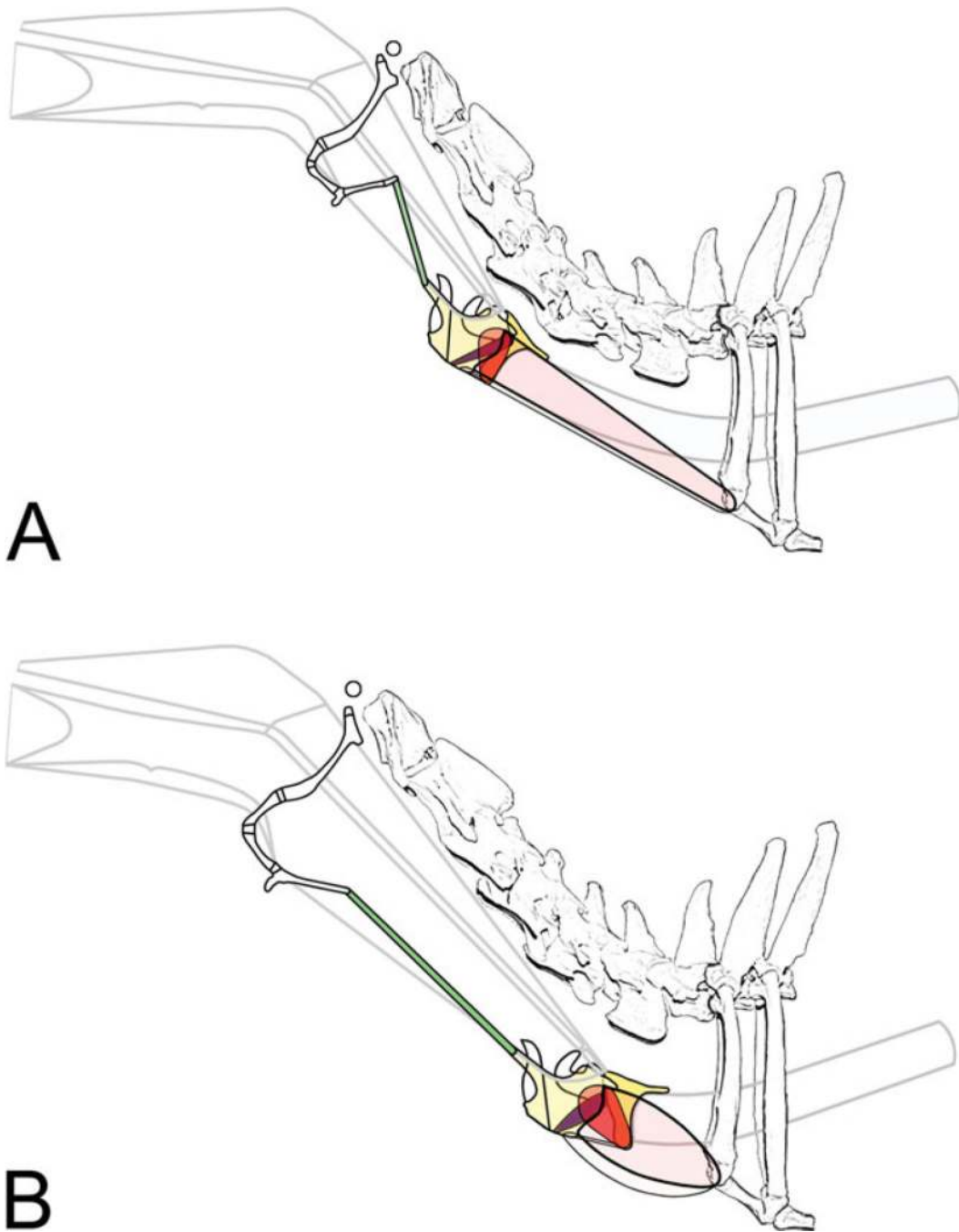


Fig. 7. The caudal displacement of the larynx is more limited in elk (**A**) than in red deer (**B**). The lower resting position and maximal caudal displacement of the larynx in red deer is facilitated by the longer thyrohyoid ligament, the longer pharynx and the longer epihyoid.

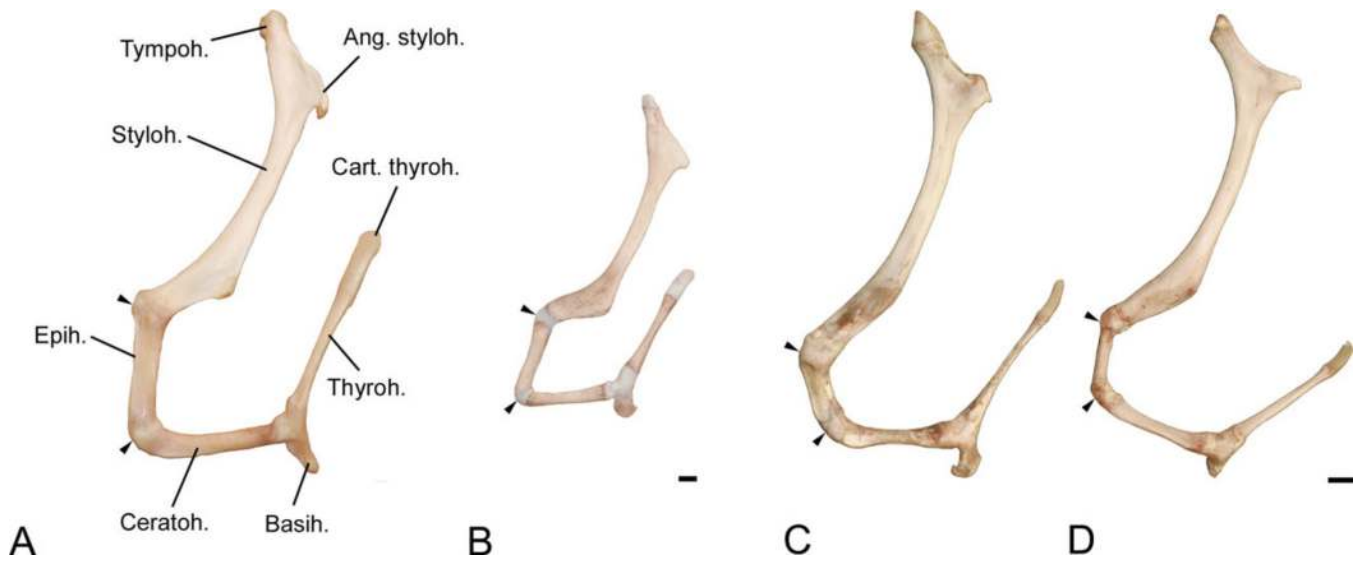


Fig. 8.
 The epiphyoid of the hyoid skeleton is longer in male (A) and female (B) Red deer than in North American elk (male: C, female: D). The arrows indicate end points of the epiphyoid bone, which is shorter in elk. The ceratohyoid and the thyrohyoid are also longer in an old Red deer male than in elk (Supporting Information Table S2). Scale bars (for red deer in B, for elk in D): 10 mm.

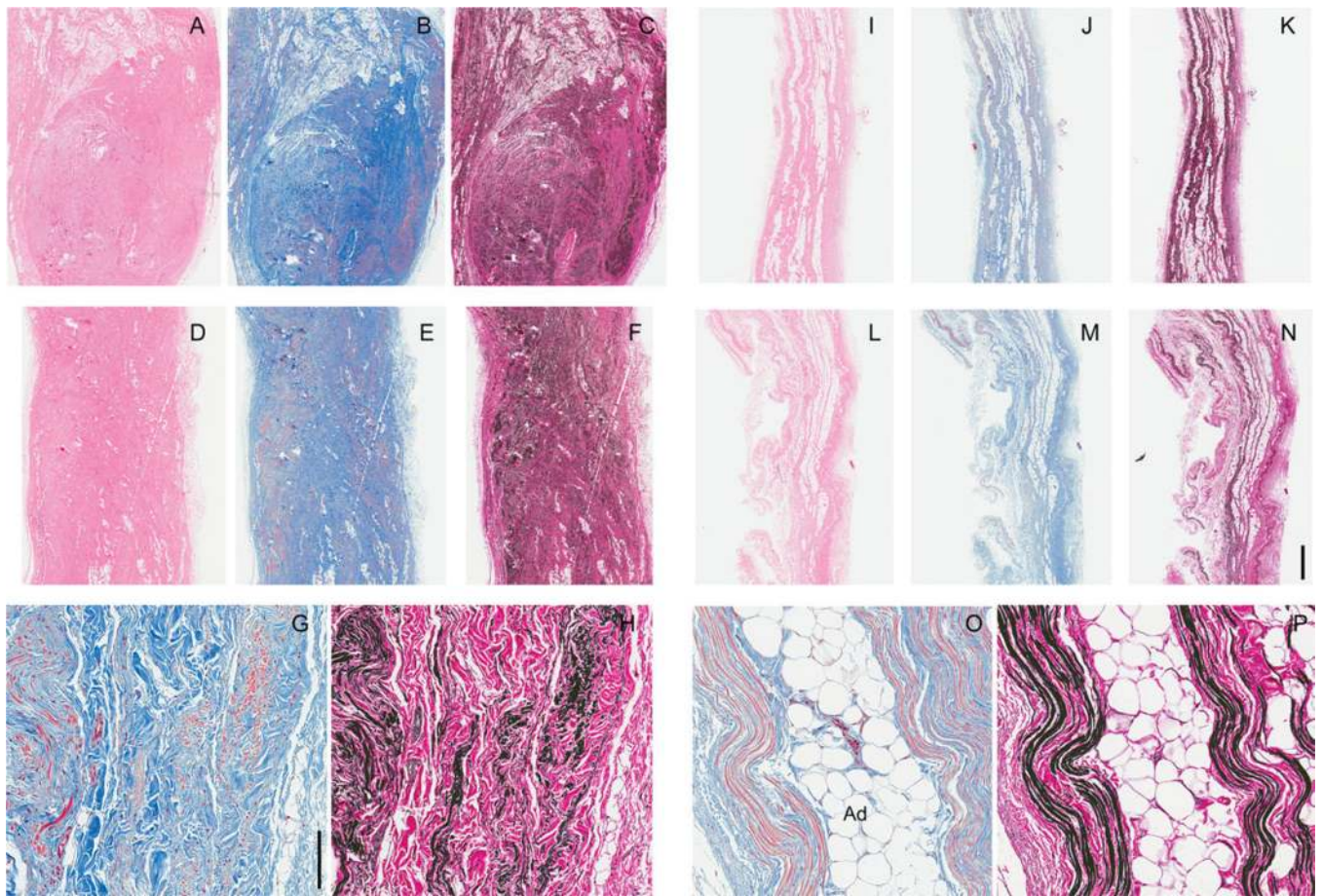


Fig. 9. Longitudinal sections through two samples of the thyrohyoid ligament of elk (**A–H**) and red deer (**I–P**) show that tissue is composed of collagen and elastin fibers as well as adipocytes ('Ad') (A, D, I, L: Haematoxylin and eosin stain, B, E, G, J, M, O: Masson's trichrome collagen stain, C, F, H, K, N, P: Elastica van Gieson stain; bar in N indicates 1 mm distance in A–F and I–N; bar in G indicates 100 μ m in G, H, O, P). The red deer thyrohyoid ligament contains more adipocytes. Fat cells in elk are clustered in small pockets, sometimes sections show only few single fat cells. In the red deer ligament, the fat cells are organized in larger clusters which 'pervade' the ligament along its entire length.

TABLE 1

Summary of differences in vocal anatomy between Rocky mountain elk and European red deer, reviewed from various studies

Anatomical structure	Species difference
Vocal fold	(1) Vocal fold stiffness is a little higher in elk in the high-strain region than in other species (Riede and Titze, 2008; Titze and Riede, 2010; Riede et al., 2010). Data in red deer missing.
Larynx	(2) The laryngeal vestibulum is shorter in elk than in red deer (This study). (3) The rostral cornua of thyroid cartilage are longer in elk (Köhler, 1982). (4) Angle between thyroid laminae is smaller in elk and decreases with age in elk (This study). (5) Sexual dimorphism in red deer but not in elk (This study).
Hyoid skeleton	(6) The epihyoid is shorter but thicker in elk, thereby (a) the extended hyoid framework is shorter and (b) the pharynx area is less expanded in elk (This study).
Ligamentum thyrohyoideum	(7) The thyrohyoid ligament is longer and less stiff in red deer, thereby (a) enabling a lower larynx resting position than in elk and (b) allowing a greater laryngeal descent during vocalization (Fitch and Reby, 2001; Reby and McComb, 2003; Frey et al., 2012; This study). (8) In elk, the thyrohyoid ligament contains muscle fibers, the elastic fibers are thicker (This study). (9) In red deer, the thyrohyoid ligament contains more fat cells (This study).
Vocal tract	(10) The resting position of the larynx is lower in red deer, thereby contributing to a relatively longer vocal tract (Fitch and Reby, 2001; Reby and McComb, 2003; Frey et al., 2012; This study). (11) The male elk's laryngeal vestibulum is shorter and can probably be constricted more in elk (This study).