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RESEARCH ARTICLE

The functional morphology of male courtship displays in the Pectoral Sandpiper (*Calidris melanotos*)

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ABSTRACT

The Pectoral Sandpiper (*Calidris melanotos*) is one of a few highly polygynous shorebirds with strong sexual size dimorphism. The vocal part of the male courtship display has received some attention, but how the sound is generated is largely unknown. To fill this gap, we analyzed video and sound recordings collected on the breeding grounds at Barrow, Alaska, USA. The anatomy of 2 males was investigated by macroscopic and histological dissection. Synchronized wing movements and a closed beak accompany hooting calls during flight displays. Courtship vocalizations on the ground include stereotypic beak and hyoid movements. We found a symmetric bipartite syrinx with songbird-like adduction and abduction mechanisms. Lateral and medial labia consisted of homogeneous extracellular matrix containing collagen fibers, which were only loosely organized, few elastin fibers, and a high proportion of hyaluronan. The upper vocal tract includes the trachea and an inflatable esophagus supported by thick and heavy skin over the ventral neck region. A highly organized network of fat and collagen makes this skin region relatively thick but also stretchable and robust. The hyoid skeleton was not distinctly different from that of pigeons, a group that also uses esophagus inflation to produce their characteristic sounds. These data lay a foundation for understanding the acoustic properties of the vocal signals used in territorial and courtship contexts.

Keywords: hyoid skeleton, Scolopacidae, vocal communication

La morphologie fonctionnelle de la parade nuptiale du mâle chez *Calidris melanotos*

RÉSUMÉ

Calidris melanotos est l'un des rares limicoles fortement polygynes avec un dimorphisme sexuel marqué de la taille. La partie vocale de la parade nuptiale du mâle a reçu une certaine attention, mais beaucoup de choses concernant la façon dont le son est généré demeurent spéculatives. Pour combler cette lacune, nous avons analysé des vidéos et des enregistrements sonores recueillis sur les sites de reproduction à Barrow, en Alaska. L'anatomie de deux mâles a été examinée par dissection macroscopique et histologique. Des mouvements synchronisés des ailes et un bec fermé accompagnent des hullements au cours des vols nuptiaux. Les vocalisations lors des parades au sol incluent des mouvements stéréotypés du bec et des os hyoïdes. Nous avons trouvé un syrinx bipartite symétrique avec des mécanismes d'adduction et d'abduction rappelant ceux des oiseaux chanteurs. Les membranes externes et internes consistaient en une matrice extracellulaire homogène contenant des fibres de collagène peu organisées, peu de fibres d'élastine et une grande proportion d'hyaluronane. Le conduit vocal supérieur comprend la trachée ainsi qu'un œsophage gonflable supporté par la peau épaisse et lourde couvrant la région ventrale du cou. Un réseau très organisé de graisses et de collagène rend cette région cutanée relativement épaisse mais également extensible et robuste. Le squelette hyoïde n'était pas distinctement différent de celui des pigeons, un groupe qui utilise également le gonflement de l'œsophage pour produire des sons caractéristiques. Ces données constituent une base pour la compréhension des propriétés acoustiques des signaux vocaux utilisés dans des contextes territoriaux et de parade nuptiale.

Mots-clés: communication vocale, Scolopacidae, squelette hyoïde

INTRODUCTION

The combination of visual and acoustic courtship signals is a hallmark of many complex mating displays and is particularly spectacular in promiscuous species (e.g.,

Darwin 1871). A vivid example is the elaborate courtship display of the male Pectoral Sandpiper (Scolopacidae: *Calidris melanotos*; Pitelka 1959, Pitelka et al. 1974, Myers 1982, Nelson 1884). Males produce “hooting” calls while performing a ritualized flight pattern, either along the

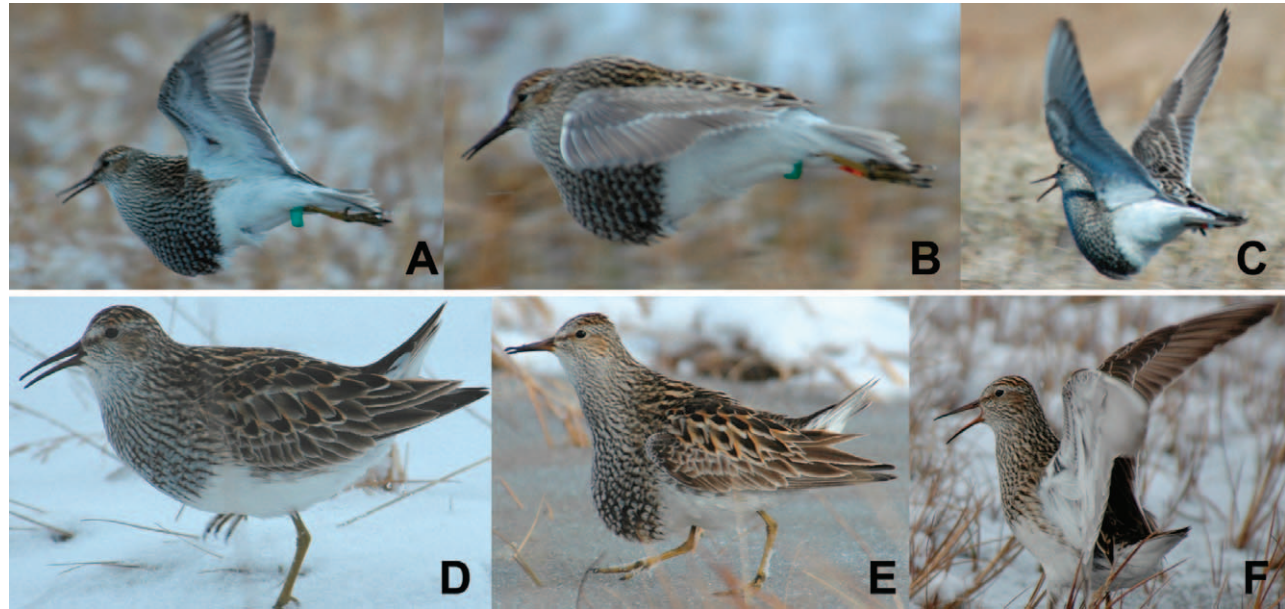


FIGURE 1. Hooting calls produced in flight, and courtship vocalizations given on the ground. (A–C) In flight, cycles of sound production are often, but not always, coupled with wing beats. (A) Beak almost closed, presumably right after inhalation. (B) Sound production with closed beak (apart from the tip) during the phase of downbeat of wings. (C) Presumed inhalation after sound production with wings up. (D–F) Courtship on the ground consists of 3 phases. Phase 1: (D) The male generates a repeated call (spectrogram shown in Figure 8B), which can last ≤ 2 min; some males sit and call, presumably to entice an interested female to approach them, and some males walk toward the female while giving this call. Phase 2: (E) The full display, performed only in close proximity to the female, consists of low-pitched gurgling sounds (spectrogram shown in Figure 8C), which are generated with a fully inflated throat; note the closed beak with the tip open as in B; this phase often lasts for 10–30 s, and normally females fly off after having seen this. Phase 3: (F) A very noisy sound is given while the male typically opens one or both wings, often flapping in a way that is reminiscent of similar behavior during copulation, but still standing ~ 30 cm behind the female; this can last for ≤ 1 min, after which copulation may occur.

border of their territory (Myers 1982) or low, over a female hidden in the tundra vegetation (Figure 1A–1C). Males also produce a different acoustic signal together with a visual display while on the ground near a female (Figure 1D, 1E; Myers 1982, Lesku et al. 2012). Here, we report on the anatomical basis of 2 striking features of this multimodal display: the extension of the neck region (“throat sac”) and the vocal “hooting” signal.

The Pectoral Sandpiper’s mating system is unusual among the subfamily *Calidridinae*. Shorebirds (Charadriiformes: ~ 350 species—sandpipers, plovers, gulls, and allies) demonstrate a large diversity of mating systems (Székely and Reynolds 1995, Reynolds and Székely 1997, Bokony et al. 2003, Thomas et al. 2007). The Pectoral Sandpiper breeds in the Arctic and can be categorized as highly polygynous (Farmer et al. 2013) and as displaying on an exploded lek (Emlen and Oring 1977). The Pectoral Sandpiper shows strong sexual dimorphism, as is typical for species in which strong sexual selection on elaborate male displays occurs through male–male competition or female choice. Males defend territories and actively pursue and court females 24 h a day, but females do not necessarily forage or nest on the territory (Lesku et al.

2012, Farmer et al. 2013). Females build the nest, incubate the eggs, and brood and guard the hatchlings without any male assistance. No persistent bond is formed between the male and female.

The vocal part of the male courtship display has received some attention, but how it is generated remains largely unknown (Farmer et al. 2013). The courtship call in flight is a low-pitched hooting call, and the calls are uttered in long bouts (Miller et al. 1988) with an extended neck region (“throat sac”) (Figure 1A–1C). The “throat sac” is also extended during parts of the courtship on the ground (Figure 1D, 1E), but the vocalizations are very different (Lesku et al. 2012). The anatomical basis of the temporal neck extension has been debated (Nelson 1884, Sutton 1932, Pitelka 1959, Portenko 1968), but it resembles what has been described as esophagus inflation in pigeons (Columbidae; Riede et al. 2004). The gas volume inside the esophagus increases when air flows through the syrinx in the expiratory direction. To sustain this inflation, the beak and nares must be closed to provide a tight seal (cf. Riede et al. 2004). Although the syrinx anatomy of some Charadriiformes has been studied (King 1989, Brown and Ward 1990), the syrinx of the Pectoral Sandpiper has not

been investigated, and it is unclear how much it contributes to the characteristic low-pitched aerial vocalization.

METHODS

Vocalizations were recorded with a Sennheiser ME67 directional microphone and a Microtrack II, M-Audio solid-state recorder near Barrow, Alaska, USA (71°18'N, 156°44'W). Using an acoustic analysis of hoot and courtship vocalizations, we determined fundamental frequency range and spectral emphasis using PRAAT version 5.2.12 (see Acknowledgments).

Video recordings became available through cameraman Ralf Schiecke (Doclights, Hamburg, Germany). Two sequences of hoot vocalization during flight and 2 sequences during ground displays were recorded at 240 frames s^{-1} , with a playing speed of 25 frames s^{-1} . One video of a phase 1 courtship display was recorded at 33 frames s^{-1} . Video analysis was performed with MaxTraq version 2.12b (Innovision Systems, Columbiaville, Michigan, USA). Distances were computed from the coordinates of 4 manually placed points (tips of upper and lower beak, distal tip of left wing, and shoulder joint) in each video frame. Wing movement was estimated as the distance between the wingtip and a horizontal line through the shoulder joint (Figure 2; [Supplemental Material Video 1](#)). Beak gape was estimated as the distance between the 2 beak markers. Videos also revealed movements of the hyoid skeleton. When the lateral elements of the hyoid skeleton ("cornua") moved laterally, they generated a bulging of the neck area ([Supplemental Material Video 2](#)). The presence or absence of this lateral bulging was marked in each video frame.

Two male specimens of Pectoral Sandpipers were collected during the peak period of male display in June 2011, at the breeding site in Barrow (Lesku et al. 2012). The 2 specimens were subjected to macroscopic dissection and photographic documentation while being submersed in water. This technique facilitates the discerning of structural details. Photographic documentation was done with a Canon XTi digital camera. Dissections focused on the proportions of the oral, pharyngeal, and esophageal cavity and surrounding tissues, the hyoid skeleton, the beak, and the histological structure of the syrinx.

Labia in the syrinx contain cellular components (fibroblasts) and extracellular matrix (elastin, collagen, and hyaluronan). All 3 matrix components determine viscoelastic properties, or how the respective tissue responds to deformation (oscillations of labia or stretching of the skin). Syrinx and ventral neck-skin specimens were fixed in 10% buffered formalin phosphate (SF100-4; Fisher Scientific) for 1 wk. Serial coronal sections of the syrinx and skin cross sections (5 μm thick) were stained with

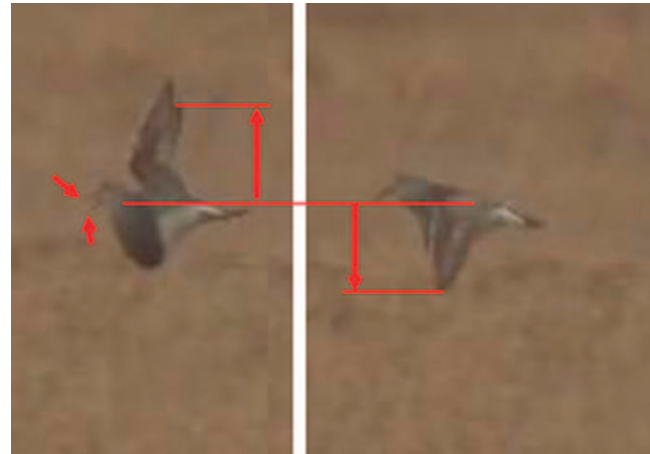


FIGURE 2. Wing movement was measured as the distance between the wingtip of the left wing and a horizontal line through the shoulder joint (images were taken from [Supplemental Material Video 1](#)). Beak gape was measured as distance between the tips of the upper and lower mandible (small arrows in left image).

haematoxylin–eosin for a general overview, Masson's Trichrome (TRI) for collagen fiber stain, Elastica–Van Gieson (EVG) for elastic fiber stain, or alcian blue (AB) stain (pH 2.5) for mucopolysaccharides and glycosaminoglycans. We also performed a digestion procedure with bovine testicular hyaluronidase (2 hr at 37°C) in combination with a subsequent AB stain. Incubation with bovine testicular hyaluronidase increases specificity for various acid mucosubstances in the AB stain. If hyaluronan is a major component of the mucosubstances, the AB stain is destroyed. All stains were performed in conjunction with control stains on human tissue in order to confirm positive stains for the respective material (liver for TRI, artery for EVG, and umbilical cord for AB). Staining was conducted at the HSC Cores Research Facility at the University of Utah (Salt Lake City, Utah, USA). Stained sections were digitized with a ScanScope CS2 slide scanner (Aperio, Vista, California, USA).

RESULTS

Sound and Video Analysis

Acoustic analysis of the vocalizations used in courtship showed a fundamental frequency range from 170 to 600 Hz. In hoot calls given during flight, the fundamental frequency (F_0) appears to be between 170 and 220 Hz (Figure 3), and the second harmonic ($2F_0$) is emphasized. An alternative interpretation that there are sidebands at exactly half of the fundamental frequency cannot be ruled out, but it seems unlikely without an apparent modulation at this frequency.

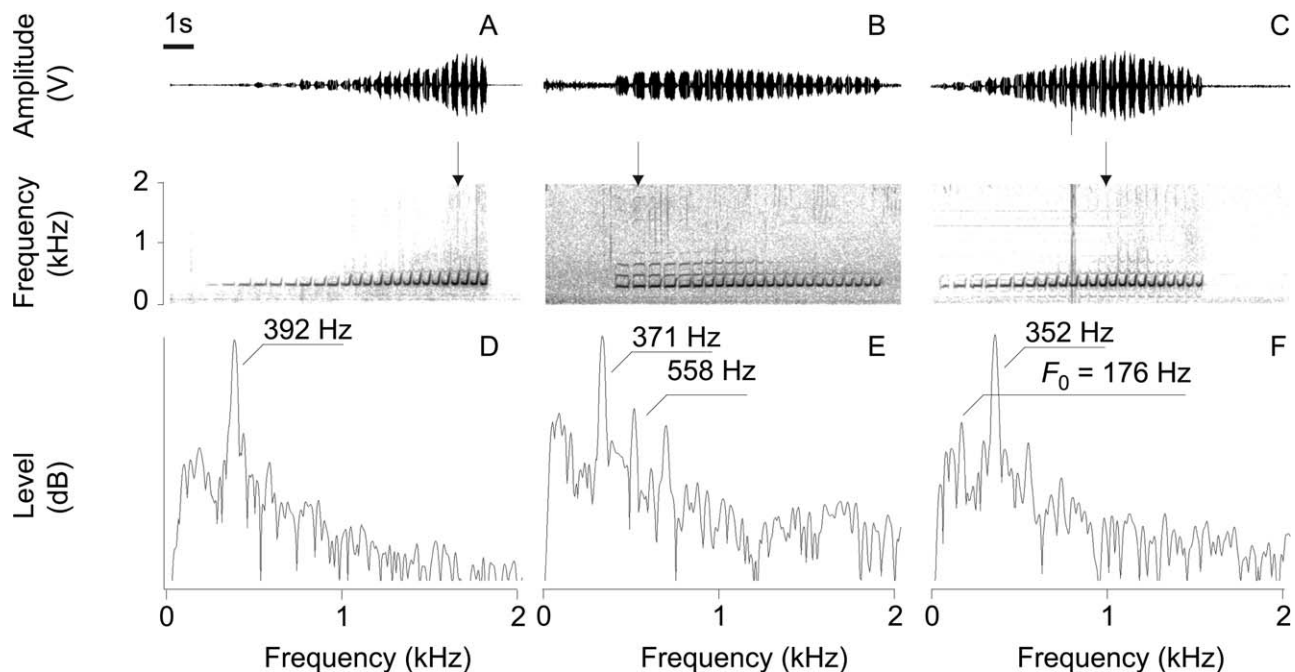


FIGURE 3. Three examples of hoot call series produced in flight. **(A)** Sometimes only the second emphasized harmonic is noticeable in the spectrogram; arrow indicates the time point around which the power spectrum **(D)** was generated. **(B)** The second (371 Hz), third (558 Hz), and fourth harmonics (743 Hz) can be detected; arrow indicates the time point of the power spectrum **(E)**. **(C)** The fundamental frequency (176 Hz) is detectable; arrow indicates the time point of the power spectrum **(F)**.

Video analysis suggests that multiple wing movements are associated with each hoot vocalization. Two large and one smaller wing movement occur with every beak opening (Figure 4). At the end of the second large wing movement, the beak was opened for 62 and 91 ms, respectively, which is similar to the duration of the pauses between 2 hoot calls (76 ± 2.6 ms; averaged means measured in 4 hooting sequences from 4 different birds). We assume that the beak opening coincides with an inspiration. The function of the half wingbeat is not clear. It could be part of a visual signal or simply a stabilizing movement before the next hoot call. Every inhalation is accompanied by a rapid upward movement of the head and downward movement of the throat. This, in combination with the bouncing throat sac, could disturb the bird's balance, requiring adjustment with an additional, smaller wingbeat.

Hoot calls are typically given at a rate of $2\text{--}4\text{ s}^{-1}$, in series of 10–20 calls (Figure 3; also see Pitelka 1959). Call rates are fastest in the center or toward the end of the hooting series, reaching an average rate of 3.9 hoots s^{-1} ($n = 576$ recordings from 79 color-banded males). A hoot series contains, on average, 16 hoot calls but can contain ≤ 50 calls.

Courtship vocalization sequences given on the ground consist of 3 phases that transition into each other. One such transition between 2 phases is shown in Figure 5. F_0

in such courtship vocalizations ranges between 400 and 600 Hz. Different phases are characterized by an emphasis on different harmonics (Figure 5C). Video analysis of a phase 1 sequence shows that sounds are produced with open beak and slightly extended throat sac (Figure 6). Before and after this calling sequence, the throat sac was more inflated, which suggests that the partly inflated throat sac during phase 1 functions as a visual signal rather than serving an acoustic filter function. Interestingly, beak and hyoid skeleton are moved synchronously. As x-ray studies in doves have shown, the esophagus is actively inflated prior to vocalization (Riede et al. 2004). It remains partially extended even when the beak opens and internal pressure equalizes with ambient pressure. A full deflation requires contraction of skin musculature.

The emphasis in the frequency range between 2,000 and 3,000 Hz (Figure 5E) is probably also produced with open beak, whereas the singular emphasis on the range around F_0 (500 Hz) is probably produced with closed beak and inflated esophagus (Figures 1E and 5F). In summary, throat inflation associated with a closed beak is required during phase 2 and serves an acoustic filter function (such as during hoot calling). During phases 1 and 3, the throat sac remains partially inflated and may serve mostly a visual function.

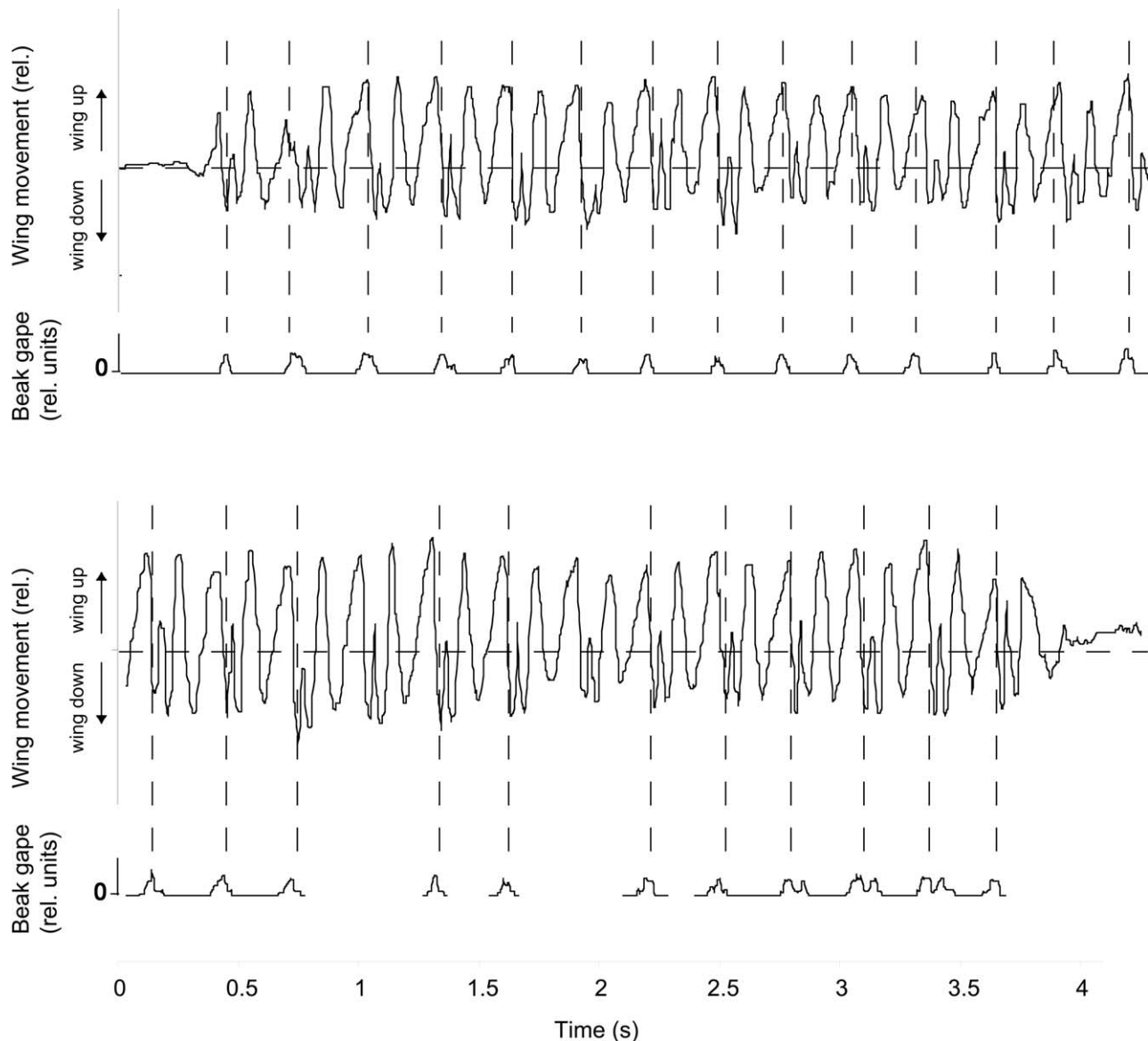


FIGURE 4. Wing movement and beak gape in 2 videos. The bird in the first clip is gliding at first, whereas the bird in the second clip transitions into a gliding flight at the end. Two large and one small wing movement are associated with each beak opening. The beak opening probably indicates the break between 2 hoot calls when the bird inhales. The second bird opens its beak twice during the last 4 cycles.

Morphology

The dissection revealed that the esophagus is the only inflatable structure in the head and neck area (Figure 7). No pouches or other extensions could be identified in the oropharyngeal area, the esophagus, or the trachea. The esophagus of both specimens could be injected with dental cast to a volume of 50 mL (Figure 7). However, it is unlikely that sandpipers inflate their esophagus much beyond 30 mL, because the trachea became compressed at volumes greater than that, which would prevent airflow and, thus, further inflation and syringeal sound production.

The skin overlying this area of the esophagus was thicker than in dorsal areas and below the neck because of large amounts of fat embedded into connective tissue. Strands of striated muscle were embedded in the composure of fat and connective tissue. A thick subepithelial layer of collagen fibers was also present, probably providing tensile stability (Figure 8). Fat cells were encased in pockets made of collagen fibers.

The hyoid skeleton, which helps to facilitate tongue movement, is composed of the basihyale, urohyale, pairwise ceratobranchiale, and epibranchiale (Figure 9). The

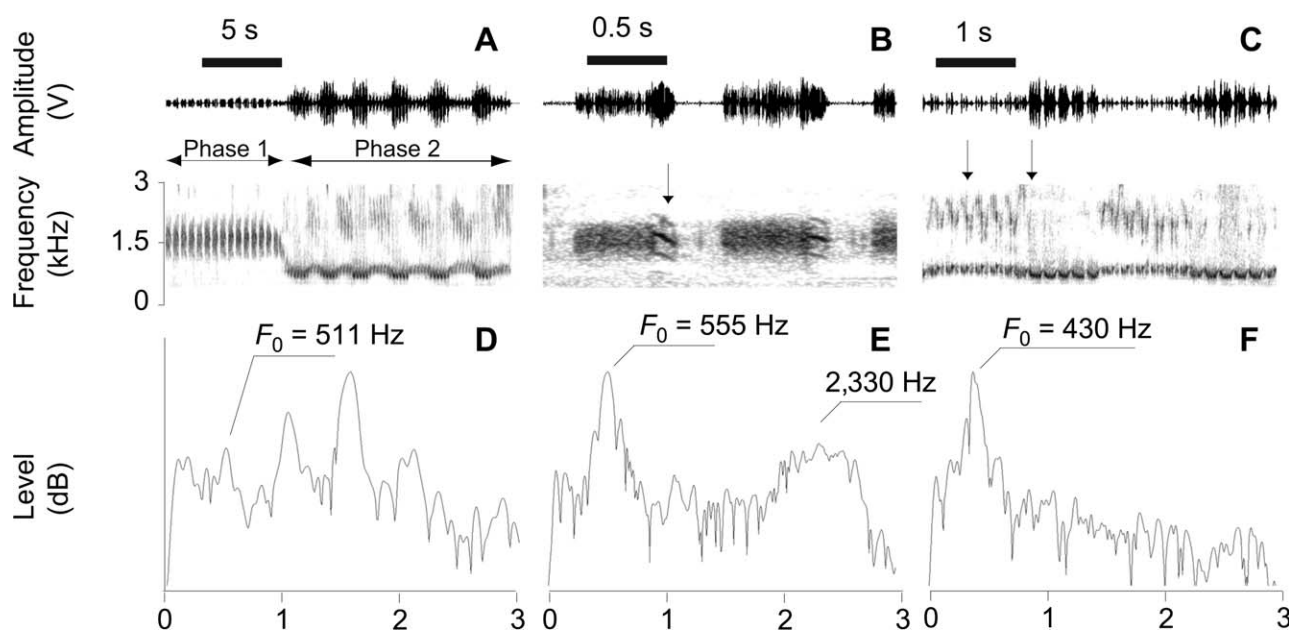


FIGURE 5. Courtship vocalization given on the ground. (A) Transition between 2 phases of a courtship vocalization. (B) Two calls of the first phase (see Figure 1D); arrow indicates the time point around which the power spectrum (D) was generated. Fundamental frequency is 511 Hz, and the third harmonic ($3 F_0$) is most emphasized. The harmonic element is preceded by a noisy element with most spectral energy around 1,500 Hz. (C) A segment of the second phase (see Figure 1E); note the alternating spectral pattern with a dual emphasis at the first arrow (power spectrum, E) and a single emphasis around the second arrow (power spectrum, F). Fundamental frequency is slightly higher at the first arrow than at the second arrow. (A–C) The top trace is the time waveform, and the bottom trace is the spectrogram.

most prominent difference from a columbiform hyoid is the longer paraglossale.

The tracheae of the 2 birds measured 82 and 88 mm, respectively, between larynx and tracheal bifurcation (similar to previous measurements by Audubon 1842). The bronchial syrinx of the sandpiper is a bipartite vocal organ with a high degree of symmetry between both sides (Figure 10). A pessulus-like structure is formed by 2 thin parallel beams that bridge the tracheal lumen between the ventral and dorsal wall. A medial membrane (composed of a medial labium and medial tympaniform membrane in songbirds) is 70–200 μm thick. It spans from the pessulus cranially to the 5th bronchial ring rostrally. It consists of collagen, elastin, hyaluronan, some fibroblasts sandwiched between epithelia facing the interclavicular air sac medially and the bronchial lumen laterally. The lateral labia are very large on both sides. Their tissue appears to be homogeneous and is composed of collagen, elastin, and hyaluronan. No interbronchial ligament was found.

The tracheolateral muscle (TLM) runs laterally on the trachea and attaches via a narrow tendon to the third bronchial half ring (A3) (Figure 11). The attachment to the tympanum or the first bronchial half ring is described as the ancestral condition (King 1989:123), indicating a somewhat derived condition in the Pectoral Sandpiper. King (1989) suggests that this condition in which the TLM

moves its insertion caudally—as in the Pied Plover (*Vanellus cayanus*), another species within Charadriiformes—could represent an intermediate stage in the evolution or differentiation of an extrinsic to an intrinsic syringeal muscle from a caudal portion of the TLM. The TLM attachment in Pectoral Sandpipers resembles the pattern found in songbirds, in which the dorsal and ventral tracheobronchial muscles attach to and cause a rotation of A3 either to adduct or abduct the lateral labia (e.g., Larsen and Goller 2002). The exact function in Pectoral Sandpipers remains to be investigated, but it is most likely that TLM contraction abducts the lateral labium.

DISCUSSION

The morphological findings are summarized in Figure 12. Esophagus inflation is the most likely mechanism that causes the neck extension (“throat sac”) during the hoot calls in Pectoral Sandpipers. The highly flexible tissue characteristics of the esophagus wall and overlying skin facilitate the extension. Similar morphology has been described in Columbidae, which use esophagus inflation to produce their calls (Riede et al. 2004). The sandpiper syrinx is a bipartite symmetric sound source. Lateral labia are large homogeneous tissue masses consisting of extracellular matrix that contains fibrillar proteins such

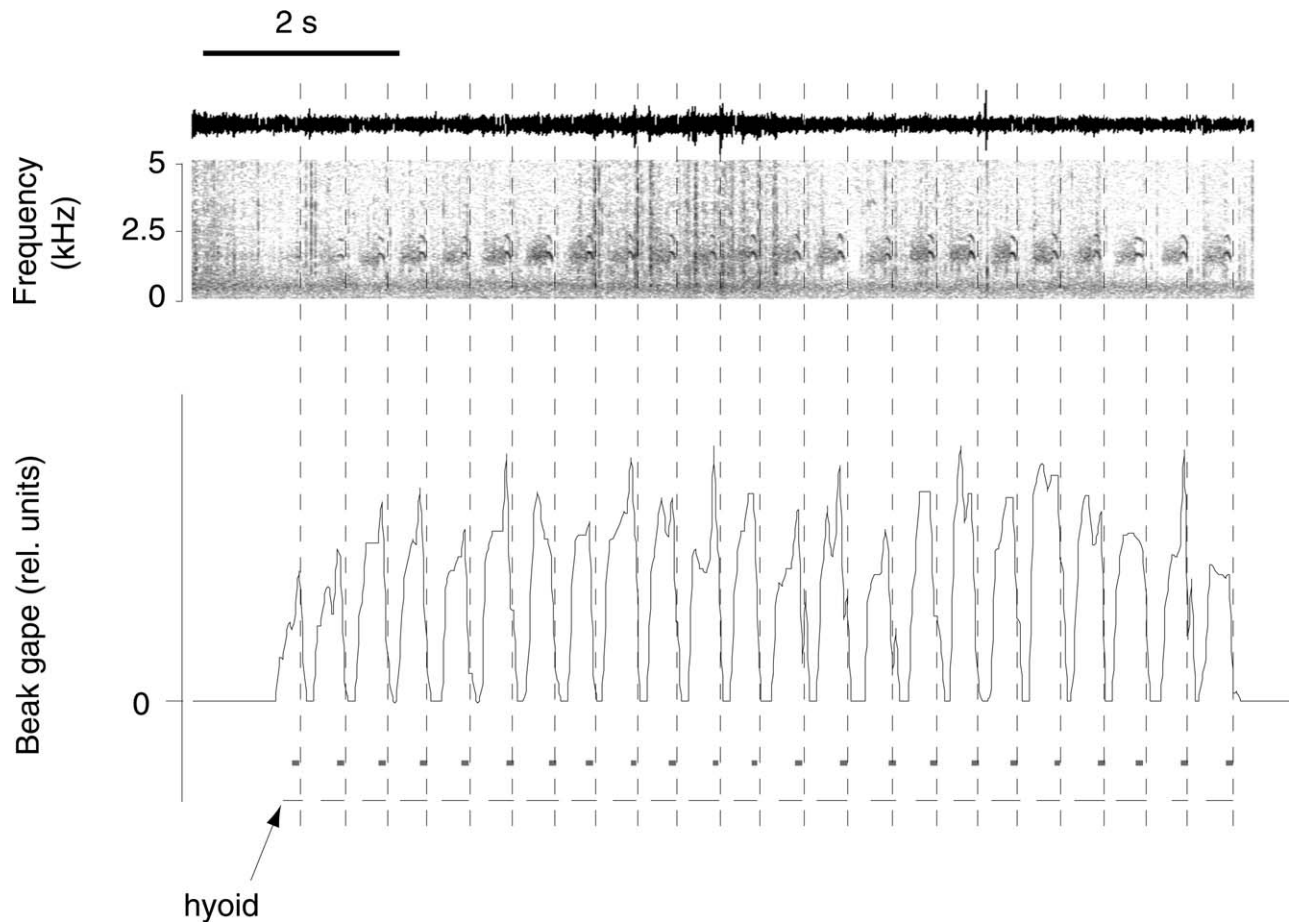


FIGURE 6. Beak and hyoid movement during phase 1 courtship vocalization. Beak gape is large during sound production. Sound production is also associated with a lateral move of the cornua (i.e. hyoid skeleton; small horizontal dashes indicate duration of lateral movement; see [Supplemental Material Video 2](#)) causing a large oropharyngeal–esophageal cavity. Each call consists of a noisy segment followed by a harmonic segment ($F_0 \sim 500$ Hz; compare Figure 5B). Red dots indicate the duration of the harmonic segment. Below the time waveform on top is the spectrogram, followed by a trace of beak gape. Maximum beak gape was on the order of 5 cm, but no reference measure was available.

as elastin and collagen as well as amorphous substances such as hyaluronan. The labia composition is not different from that in songbirds (Riede and Goller 2010a). The homogeneous one-layered morphology of the labial extracellular matrix, associated with a fundamental frequency range of <1 kHz, corresponds well with the predictive relationship between labial morphology and a species' fundamental frequency range found across songbirds (Riede and Goller 2014). Labia are probably set into vibration by a passing airstream. Muscle activity adducts the labia into prepharyngeal position, and their viscoelastic properties and the driving aerodynamic forces determine their oscillation rate and thereby the acoustic output (Riede and Goller 2010b). It is likely that the large homogeneous labia support the production of the low-fundamental-frequency sounds. Related species of similar size are reported to produce mostly higher-pitched sounds (Miller 1992, Miller and

Baker 2009), and we would expect different labial morphology.

The adaptation of the geometry of the upper vocal tract is well-known in birds (Riede and Goller 2010b) and is achieved either by movements of the hyoid skeleton (Riede et al. 2006, 2013, Riede and Suthers 2009) or by esophagus inflation (Riede et al. 2004, Dantzker and Bradbury 2006), or, in the case of the Pectoral Sandpiper, by a combination of both. Initially, the esophagus is most likely inflated prior to vocalization by 1 to 3 expiratory movements and closed beak and nares, as has been found in doves. The anatomical challenge to inflate the esophagus is twofold. First, a tight seal rostral to the glottis must ensure that expired air is collected in the esophagus and not leaking through the beak. The larynx can engage with the palate to form a tight seal (Zweers et al. 1981, Zweers 1982, Zweers and Berkhoudt 1986). Second, the pharynx, the esophagus,

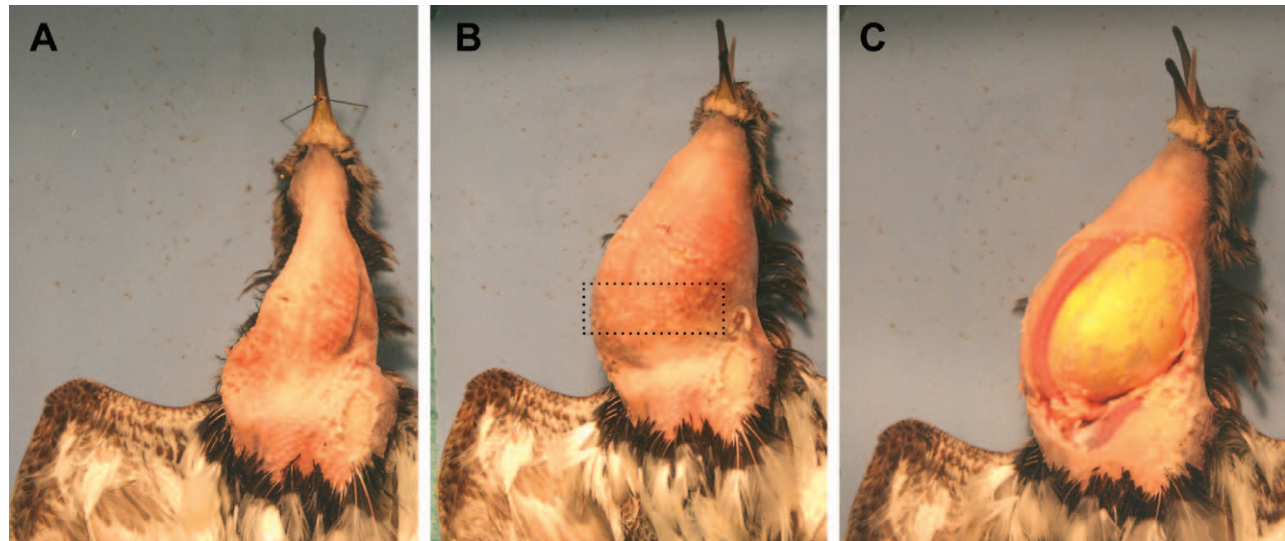


FIGURE 7. Inflating the esophagus with yellow cast. **(A)** Ventral view of the head-and-neck region after removing feathers and before casting material was injected. **(B)** Casting material was injected through the beak. **(C)** Skin removed after casting material was injected. The trachea bends on the right side around the filled esophageal throat sac. The dotted rectangle in **B** indicates the location of the skin sample in Figure 8.

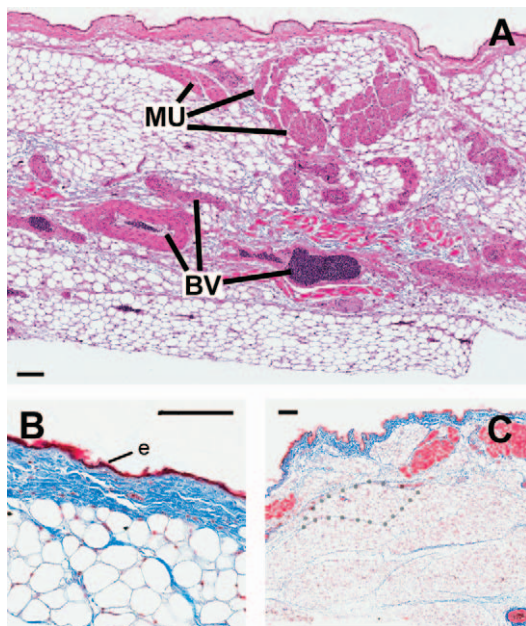


FIGURE 8. Histological cross-sections of skin from the lower ventral neck area, which becomes inflated during vocalization. **(A)** Hematoxylin and eosin stain of a ventral neck-skin cross section indicates multiple strands of striated muscle (MU) and many blood vessels (BV). **(B, C)** Masson's trichrome stain of ventral neck skin. **(B)** Below the stratified epithelium (e) is a thick and dense layer of collagen (all blue stain in **(B)** and **(C)**) above large pockets of fat cells (large white spaces of 20–50 μm diameter). **(C)** Fat cells are clustered in large pockets surrounded by blue-stained collagen fibers. One such pocket is outlined with gray dots. Reference lines in all 3 images indicate 100 μm . For the location of this skin sample, see Figure 7.

and the overlying skin must be sufficiently elastic and flexible to allow expansion.

Dynamics of Vocal Production and Inflation Behavior

Inflatable upper-vocal-tract structures associated with vocal production exist in many vertebrate taxa, not just in birds (e.g., frogs: Ryan 2001, Gridi-Papp 2008; mammals: Frey et al. 2007, Riede et al. 2008). Esophagus inflation in birds is used during vocal behavior by species from different families (e.g., Neunzig 1921, Chapin 1922, Niethammer 1961, Dantzker et al. 1999, Riede et al. 2004), which may indicate independent evolution of this feature. The inflated esophagus and sound radiation through the skin of the neck generate a strong filter effect (Fletcher et al. 2004, Riede et al. 2004). The stretched skin acts as a band-pass filter eliminating higher and lower spectral energy, thereby limiting energy to one harmonic (Fletcher et al. 2004). The tracheal tube is an initial resonant structure, and a subsequent filter effect is facilitated through the action of the extended hyoid and the inflated esophageal sac. The filter effect of this sac is only moderately affected by the degree of inflation.

In order to be engaged to act as a filter and acoustic coupling device, the esophagus must be inflated prior to vocalization; considering the large volume, this inflation must take several respiratory cycles. Once inflated, escape of air must be prevented by keeping the beak closed. Behavioral observations suggest that during a display flight, the esophagus is cyclically inflated and deflated (Pitelka 1959, Farmer et al. 2013). Presumably, opening the

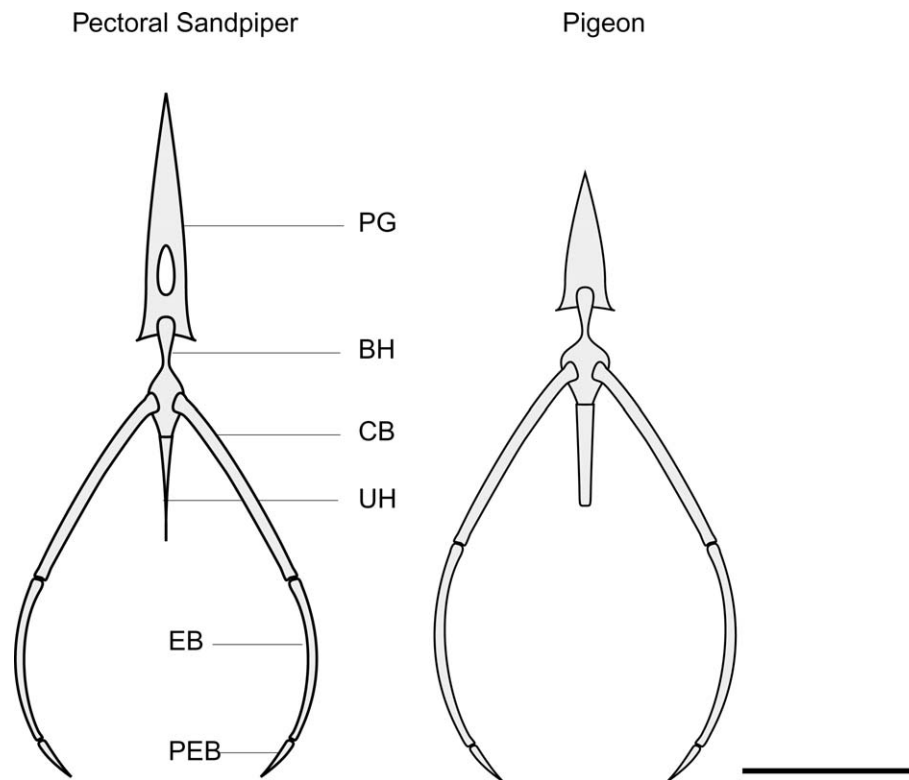


FIGURE 9. Schematic of the hyoid skeleton of a male Pectoral Sandpiper and a male Rock Pigeon drawn to scale from a dissected hyoid skeleton of the respective species. Reference bar is 1 cm. Abbreviations: PG = paraglossale, BH = basihyale, CB = ceratobranchiale, UH = urohyale, EB = epibranchiale, and PEB = cartilaginous process of epibranchiale (cartilago epibrachialis).

beak and/or disengaging the larynx from the palate for breathing causes a passive deflation through recoil of the esophagus wall and overlying skin, though not completely. At this point, in order to maintain an inflated esophagus for the next cycle, active deflation through muscles is unlikely. Ring Doves, for example, require 3 to 5 respiratory cycles to inflate the esophagus, and the inflated esophagus deflates by $\sim 30\%$ within 1 s if the seal is opened (T. Riede personal observation).

If the esophageal pouch were filled with fully oxygenated air, and this air could be moved to the lung, 30 mL would sustain flight for ~ 8.4 s, assuming a flight metabolic rate of $8 \text{ mL O}_2 \text{ s}^{-1} \cdot \text{kg}^{-1}$ and a body mass of 94 g (e.g., Torre-Bueno and Larochelle 1978, Bundle et al. 2007). Therefore, it is not surprising that repeated inflation and deflation occur during a display flight, in which the hooting phase lasts, on average, 4 to 5 s. The throat is slowly inflated once before the hooting starts, then the throat sac swings forth and back with each hoot, and finally the throat is deflated again after the end of the hooting. Hoot calls are synchronously produced with wing beats (Pitelka et al. 1974, Miller et al. 1988, and video analysis in present study). The beak is rhythmically opened during the hooting as well as during the courtship vocalization

(Figures 4 and 6). We assume that in hooting, the opening coincides with the short period of inhalation between hoots.

Esophagus Inflation

In doves, F_0 and vocal-sac resonance match (Riede et al. 2004), whereas the sandpiper vocal tract (trachea, larynx, and vocal sac) emphasizes higher harmonics (i.e. frequencies of 350–2,500 Hz, but rarely F_0 ; exception in Figure 5C). Sound is radiated from the inflated esophagus and overlying skin. Free skin—either on the entire neck, as in the Ostrich (*Struthio camelus*) and frigatebirds (*Fregata* spp.), or as smaller sacs, as in the Greater Sage-Grouse (*Centrocercus urophasianus*)—can generate specific radiation patterns around the vocalizing male. The specific radiation patterns can perhaps be advantageous in “targeting/addressing” a female in the vicinity (Dantzker et al. 1999). The inflation is often associated with an important and obvious visual signal, colorful skin or feathers in the throat area (frigatebirds and Greater Sage-Grouse), elaborate body movement (head bobbing in pigeons and doves), or a highly stereotyped flight pattern (sandpipers; Miller 1983a, 1983b). The rhythmic increase in neck size in less colorful species (e.g., Pectoral

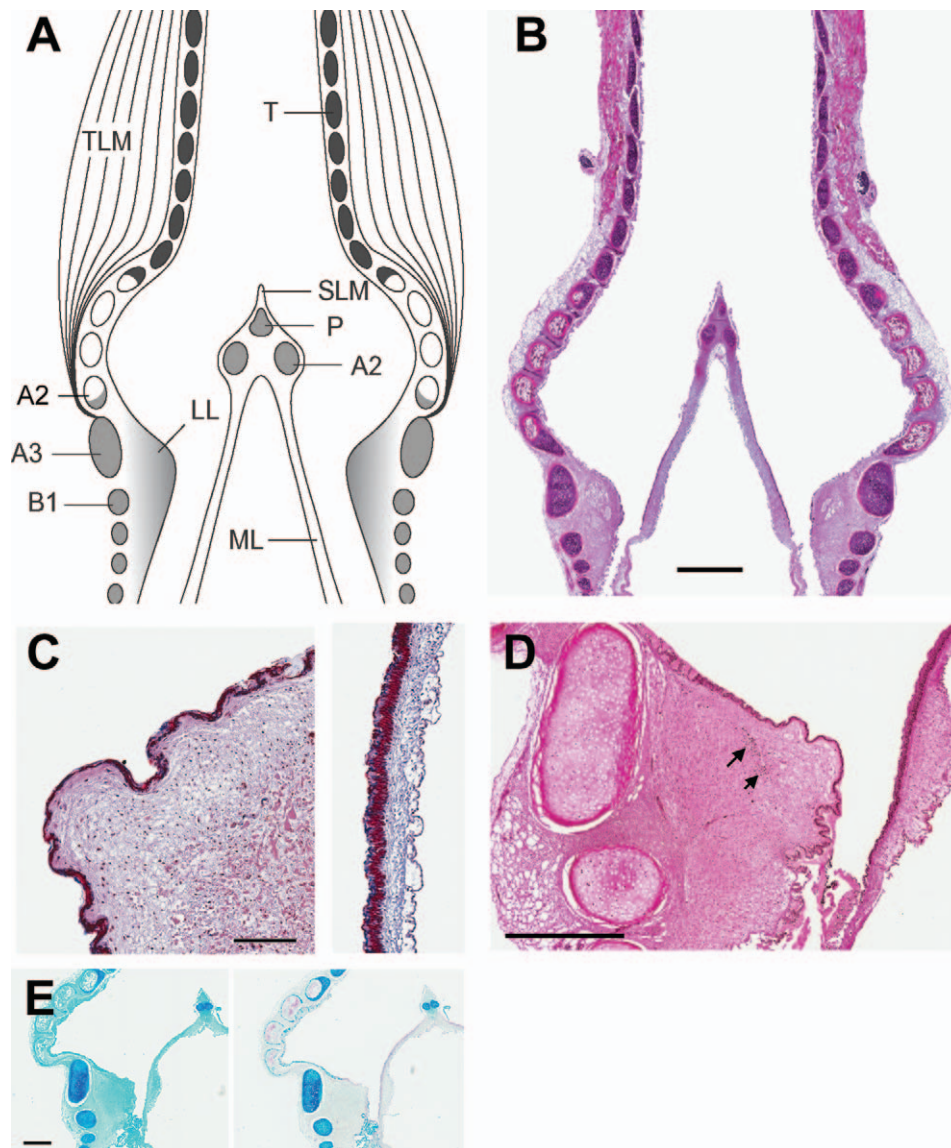


FIGURE 10. Schematic and hematoxylin- and eosin-stained cross section of the syrinx of a male Pectoral Sandpiper (**A**, **B**), and Masson's trichrome (**C**), elastica-van-Giesson (**D**), and alcian blue stain (**E**) of lateral and medial labia. The blue or bluish stain in (**C**) indicates that much of the extracellular matrix in the lateral and medial labia consists of collagen fibers. Arrows in (**D**) indicate a cluster of elastic fiber cross sections (black stain). In (**E**), 2 subsequent sections stained with alcian blue are shown before (left) and after (right) hyaluronidase treatment. The disappearance of the bright blue stain in the right image indicates that hyaluronan is present and was digested by the hyaluronidase treatment. Abbreviations: TLM = tracheolateral muscle, T = trachea, SLM = semilunar membrane, P = pessulus, A2 = second bronchial half ring, A3 = third bronchial half ring, B1 = first bronchial ring, ML = medial labium, and LL = lateral labium.

Sandpiper, pigeons, and doves) may serve to potentiate the advertisement signal and is evaluated by females or by other males.

Sexual selection acts through male–male competition and female choice (Darwin 1871), and sometimes these mechanisms favor different traits. Hoots are given in male–male competition: Males may hoot while flying in parallel next to each other or, more often, while flying

alone along territory boundaries. Hoots are also produced in a context that suggests advertisement to females (W. Forstmeier and B. Kempenaers personal observation). A male's long hooting flights appear to be specifically targeted to females sitting on the ground. The male performs 2 or 3 hooting flights, during which it passes just a few centimeters over the female's head before landing and approaching the female. During courtship (Figure 1),



FIGURE 11. Mechanism to abduct and adduct the lateral labia by rotating the third bronchial half ring. The location of the high-magnification image of the tracheolateral muscle (TLM) and the second (A2) and third (A3) bronchial half rings is indicated in the inset of a schematic cross section of a syrinx (for comparison, see Figure 10) in the lower left corner. The arrows point to the ligamentous attachment of the TLM to the third bronchial half ring. Reference bar is 100 μm .

males perform multiple “phases” during which higher-pitched vocalizations (phases 1 and 3) as well as deep gurgling sounds (phase 2) are produced. Calls of phase 1 and phase 2 are illustrated in Figure 8. The use of low-pitched sounds in both male–male competition and advertisement to females strongly suggests that both

components (intrasexual and intersexual selection) contribute to the evolution of this extreme signal.

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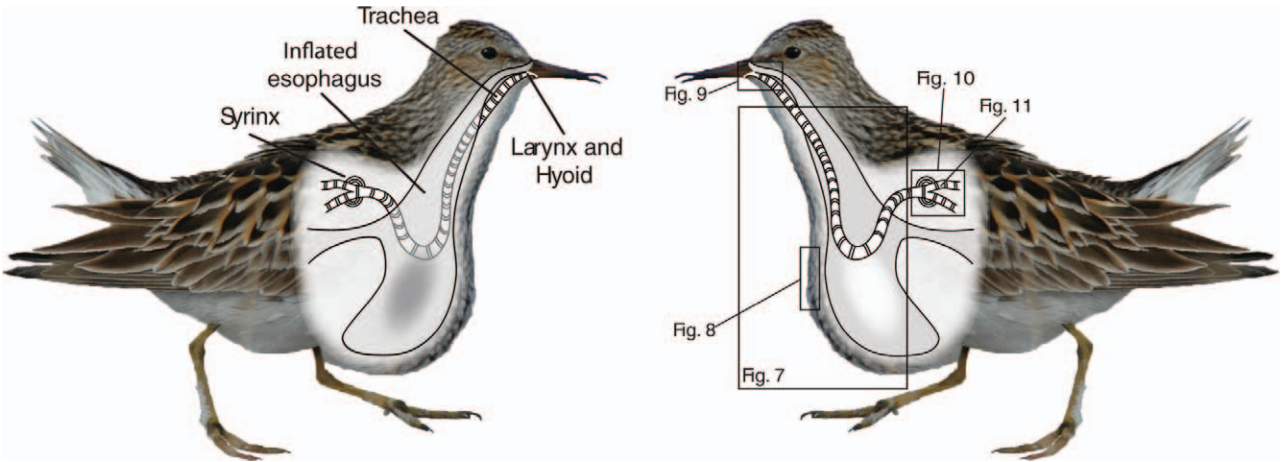


FIGURE 12. Schematic illustration of the vocal apparatus of the Pectoral Sandpiper, viewed from the left and the right, demonstrating an inflated esophagus. Locations of structures explained in detail in Figures 7–11 are indicated by outlines. Art credit: Lotte Schlicht

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SUPPLEMENTAL MATERIAL VIDEO 1. A male Pectoral Sandpiper produces hoot calls during flight (high-speed video recording at 240 frames s^{-1} , with a playing speed of 25 frames s^{-1}). The tip of the left wing was marked with a white dot. The wing-movement analysis is presented in Figure 4. Video credit: Ralf Schiecke/Doclights

SUPPLEMENTAL MATERIAL VIDEO 2. A male Pectoral Sandpiper produces a phase 1 courtship display (video recorded at 33 frames s^{-1}). The beak-movement analysis is presented in Figure 6. Note how the hyoid movement caused a lateral bulging of the neck area. Video credit: Ralf Schiecke/Doclights