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Cite this article: Pasch B, Tokuda IT, Riede T. 2017 Grasshopper mice employ distinct vocal production mechanisms in different social contexts. *Proc. R. Soc. B* **284**: 20171158. http://dx.doi.org/10.1098/rspb.2017.1158

Received: 25 May 2017
Accepted: 20 June 2017

Subject Category:
Behaviour

Subject Areas:
behaviour, biomechanics, physiology

Keywords:
bioacoustics, source-filter theory, vocal production

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Electronic supplementary material is available online at http://dx.doi.org/10.6084/m9.figshare.c.3820792.

THE ROYAL SOCIETY
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Grasshopper mice employ distinct vocal production mechanisms in different social contexts

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Functional changes in vocal organ morphology and motor control facilitate the evolution of acoustic signal diversity. Although many rodents produce vocalizations in a variety of social contexts, few studies have explored the underlying production mechanisms. Here, we describe mechanisms of audible and ultrasonic vocalizations (USVs) produced by grasshopper mice (genus *Onychomys*). Grasshopper mice are predatory rodents of the desert that produce both loud, long-distance advertisement calls and USVs in close-distance mating contexts. Using live-animal recording in normal air and heliox, laryngeal and vocal tract morphological investigations, and biomechanical modelling, we found that grasshopper mice employ two distinct vocal production mechanisms. In heliox, changes in higher-harmonic amplitudes of long-distance calls indicate an airflow-induced tissue vibration mechanism, whereas changes in fundamental frequency of USVs support a whistle mechanism. Vocal membranes and a thin lamina propria aid in the production of long-distance calls by increasing glottal efficiency and permitting high frequencies, respectively. In addition, tuning of fundamental frequency to the second resonance of a bell-shaped vocal tract increases call amplitude. Our findings indicate that grasshopper mice can dynamically adjust motor control to suit the social context and have novel morphological adaptations that facilitate long-distance communication.

1. Introduction

Acoustic signals are shaped by social selection [1,2] and constrained by the signalling environment [3–6] or ecological adaptation [7,8] in a broad range of taxa. Mechanistically, acoustic divergence is determined by several variables in senders: morphological adaptations of the sound source (larynx) and/or vocal tract filter (pharynx, nasal and oral cavities), changes in motor control of the source and filter, and selection on driving force (lung pressure). Identifying the relative contributions of each component to acoustic variation is critical to understanding the evolution of vocal communication systems.

Most terrestrial mammals produce sounds by airflow-induced vibrations of vocal folds [9]. By contrast, rodents produce ultrasonic vocalizations (USVs; greater than 20 kHz) in close-distance mating contexts via an aerodynamic whistle mechanism whereby tightly constricted larynges serve as an orifice to pass expired air [10–12]. However, our understanding of vocal functional morphology in rodents is limited to laboratory mice (*Mus musculus*) and rats (*Rattus domesticus*; [10–15]). The great diversity of rodent mating and social systems make the clade an excellent system to examine production mechanisms underlying signal diversification [16].

Cricetid rodents in the subfamily Neotominae [17] commonly produce audible (less than 20 kHz) vocalizations [18,19]. In particular, grasshopper mice (*Onychomys*) are predatory rodents of western North America known for their

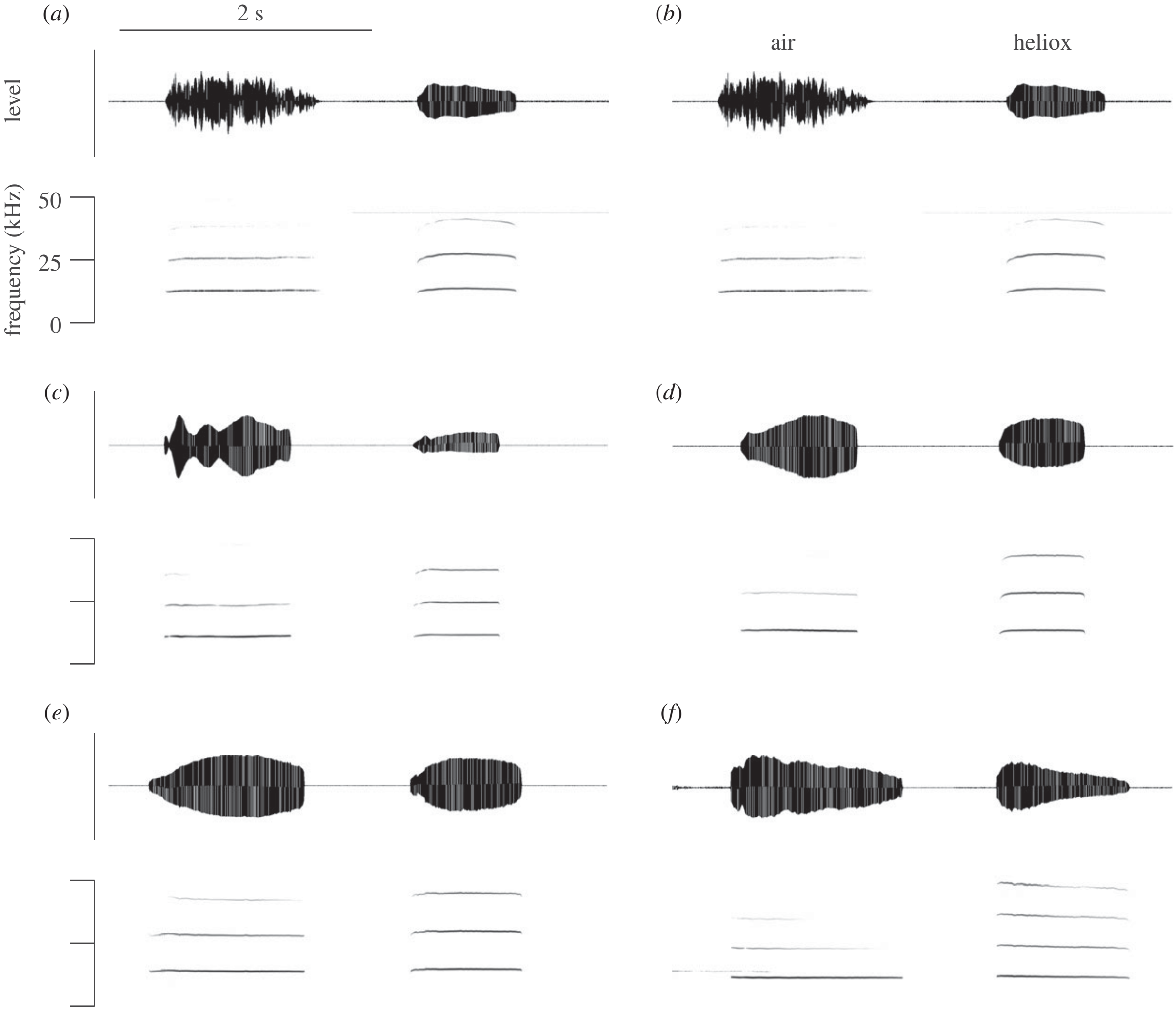

Figure 1. Long-distance calls of *Onychomys* in normal air and heliox. Each panel (*a–f*) shows two calls from each of six mice in air (*a*,*c*,*e*) and heliox (*b*,*d*,*f*). Sound is depicted as a waveform (*a–d*) and spectrogram (*e*,*f*). The first call in (*f*) is preceded by a long-distance call of another animal in the same room.

'wolf's howl in miniature' [20]. Both males and females produce long-distance advertisement vocalizations used in mate attraction and territorial advertisement ([21,22]; figure 1). Animals often assume an upright posture and open their mouths widely to generate a remarkably loud call (electronic supplementary material, videos S1 and S2; [22]). Grasshopper mice also produce USVs in close-distance mating bouts ([18]; herein), allowing exploration into how sound production mechanisms vary in distinct social contexts.

In this study, we used heliox experiments, laryngeal and vocal tract morphological investigations, and biomechanical modelling to investigate how grasshopper mice produce spectacular long-distance calls. If vocalizations are produced via a whistle mechanism, fundamental frequency (F_0) is predicted to increase under heliox atmosphere [23]. Conversely, if sound is produced via airflow-induced tissue vibration, then F_0 is predicted to remain constant, but amplitudes of higher harmonics will change relative to F_0 [24]. The morphological study identified adaptations that could facilitate long-distance call production. However, since vocal production is based on complex coupled mechanisms between airflow, vocal fold tissue properties and supraglottal structures, computational simulations are required [25]. Thus, our morphological findings were used to develop computational models of vocal fold movements to inform the physical mechanisms underlying call production.

2. Material and methods

(a) Animals

Grasshopper mice are predatory rodents that inhabit deserts, grasslands and prairies of the western United States and northern Mexico. We captured northern (*Onychomys leucogaster*), southern (*Onychomys torridus*) and Chihuahuan grasshopper mouse (*Onychomys arenicola*) in an area of sympatry near Tank Mountain (31°48′46.90″ N 108°48′49.90″ W) in the Animas Valley, New Mexico, USA [26,27]. Mice were transferred to animal facilities at Northern Arizona University, Flagstaff, AZ, USA, maintained on a 14 L : 10 D cycle (21 ± 2°C) and provided rodent chow and water ad libitum. Animals used in the experiments were F_1 or F_2 from same-species pairs or opposite-species pairs (hybrids). A subset of animals was transferred to Midwestern University, Glendale, AZ, USA, for heliox experiments and morphological investigations.

(b) Acoustic recording

We recorded long-distance calls and close-distance mating vocalizations in normal air and heliox atmosphere. Individual male mice (for spontaneous long-distance vocalizations) or a male–female pair (for close-distance USVs) were placed inside a custom-made cage consisting of 10 mm mesh inside a larger standard rat cage. The cage was equipped with bedding, a running wheel, food and water gel. Heliox gas (80% He, 20% O_2) was injected into the rat cage at flow rates between 20 and 40 l min^{-1} through a 12 mm wide tube placed on the cage floor. Small holes along the tube facilitated uniform distribution of gas. In light gas, F_0 of a whistle increases in proportion to the amount of gas present. Predicted effects of light gas concentrations were estimated with a small whistle placed at the floor of the cage and connected externally by a silastic tube. The whistle was blown and recorded at regular intervals in order to monitor the heliox concentration. The ratio of the frequency of the whistle in air and in heliox allowed an estimation of the expected effect for any given heliox concentration. We also recorded long-distance calls in air within individual male home cages to control for the introduction of animals into a novel environment.

Long-distance calls from seven males produced in normal air and in heliox atmosphere were analysed for total duration, F_0 at mid-call and sound pressure level (decibel). Short-distance mating USVs from eight males were analysed for mean F_0. All measurements were performed using PRAAT sound analysis software (v. 5.3.80 for Windows; www.praat.org). Reported means of maximum sound pressure level values are not relative to a common standard and were only compared within individuals between treatments.

(c) Histology

Four male mice (two *O. torridus* and two *O arenicola*) were euthanized with isoflurane and then transcardially perfused. Larynges were dissected and fixed in 10% buffered formalin phosphate (SF100-4; Fisher Scientific) for one week. Vocal folds were investigated for the presence of collagen, elastin and hyaluronan, all of which play an important role in vocal fold biomechanics [28–30]. Mid-membraneous coronal sections (5 mm thick) were stained with haematoxylin–eosin for a general overview, Masson's Trichrome for collagen fibre stain, Elastica–Van Gieson for elastic fibre stain and alcian blue (AB) stain (pH 2.5) for mucopolysaccharides and glycosaminoglycans. We also performed a digestion procedure with bovine testicular hyaluronidase (2 h 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, AB stain fully degrades. Sections were scanned with an Aperio CS 2 slide scanner and processed with IMAGESCOPE software (v. 8.2.5.1263; Aperio Tech.).

(d) Computational simulation of the sound production mechanism

We expanded our previous biomechanical model on voice production [31] to investigate how morphological features affect the sound production mechanism and why long-distance calls reach high sound amplitude. The larynx model is based on a two-mass model of the vocal folds [32,33]. Acoustic characteristics of a flared vocal tract were estimated by using the transmission line model [34]. Details of the simulations are summarized in the electronic supplementary material.

3. Results

(a) Acoustic recordings

Long-distance calls were flat, stable vocalizations lasting between 0.8 and 1.4 s in air. Figure 1 shows examples of one call recorded in normal air and one in heliox for six animals. Long-distance calls recorded in heliox were slightly higher in F_0 (515 ± 85 Hz; paired *t*-test, $t_6 = -6.03$, $p = 0.001$) but much lower (−6.52 ± 0.6 kHz; paired *t*-test, $t_6 = 10.72$, $p < 0.0001$) than predicted in the presence of He, indicating an airflow-induced tissue vibration mechanism (figure 2*a*). Interestingly, calls produced in heliox were slightly shorter in duration (−0.27 ± 0.03 s; paired *t*-test, $t_6 = 8.82$, $p < 0.0001$; figure 2*b*), potentially indicating auditory-feedback-mediated vocal control [35].

Next, we tested how different filter properties of the oropharyngeal space affected sound characteristics in normal air and in heliox. Visual inspection of spectrograms suggested that upper harmonics in heliox were more prominent relative to F_0 (figure 1). We measured the amplitude of $2F_0$ and $3F_0$ in normal air and in heliox relative to F_0 at the center of the long-distance call. The data are summarized in table 1 and in figure 2*c*,*d*. In all animals, amplitude of $2F_0$ and $3F_0$ relative to F_0 were significantly higher in heliox than in normal air ($2F_0$: paired *t*-test, $t_6 = 8.9$, $p < 0.0001$; $3F_0$: paired *t*-test, $t_6 = 9.9$, $p < 0.0001$; figure 2*c*). The mean relative amplitude of $2F_0$ ranged from −18.7 to −30.7 dB in normal air compared with −2.8 to −16.7 dB in heliox (figure 2*c*; table 1). The mean relative amplitude of $3F_0$ ranged from −42.5 to −53.7 dB in normal air compared with −14.7 to −30 dB in heliox (figure 2*d*; table 1).

Mating vocalizations consisted of frequency downsweeps, trills, chevrons and a low-amplitude version of the long-distance call (figure 3). A non-receptive female also produced agonistic barks or 'chits' [21]. The F_0 of downsweeps produced during the mating bouts increased to 69.2 ± 7.0 kHz in heliox from 48.3 ± 2.6 kHz in air (paired *t*-test, $t_7 = 8.9$, $p < 0.0001$; figure 3). The F_0 of trills and chevrons, which were produced less often, also increased in heliox compared with air (trills, air: 49.9 ± 4.6 kHz, heliox: 70.8 ± 11.7 kHz; paired *t*-test, $t_5 = 4.9$, $p = 0.004$; chevron, air: 46.7 ± 4.5 kHz, heliox: 64.2 ± 7.0 kHz; paired *t*-test, $t_4 = 8.9$, $p > 0.001$; figure 3). The results suggest that USVs are produced by an aerodynamic whistle mechanism, whereby airflow passes through the glottis, enters the supraglottal space, and impinges on a downstream structure [10,12,13]. By contrast, the low-amplitude version of the long-distance call and agonistic barks did not shift in frequency (figure 3), suggesting an airflow-induced tissue vibration mechanism [9]. In summary, animals appear capable of switching between two production mechanisms within the same social interaction.

(b) Laryngeal histology and vocal tract geometry

In order to better understand the vocal production mechanism in grasshopper mice, we investigated the anatomy of the laryngeal sound source and geometry of the vocal tract filter. Analysis of coronal sections from four grasshopper mice larynges revealed that vocal folds are composed of thyroarytenoid muscle, lamina propria and epithelium (figure 4). In grasshopper mice, the lamina propria is composed of a 19.7 µm (s.d. = 1.5 µm) thin layer of protein fibres, which are shaped into vocal membranes at the vibrating edge (figure 4*a*–*e*). Vocal membranes were symmetrical on the left and right vocal fold. Their length was measured by adding 5 µm coronal sections. The mean dorsoventral length of vocal membranes is 570 µm (s.d. = 90 µm) (*O. arenicola*: 660 and 610 µm; *O. torridus*: 480 and 510 µm). Histological preparations can cause some shrinkage of soft tissue (approx. 20% in laryngeal tissue according to Kimura *et al.* [36]), making a mean vocal membrane length of approximately 600 µm plausible.

The lamina propria consists of collagen fibres (figure 4*f*), a thin layer of elastin fibres below the epithelium (figure 4*g*),

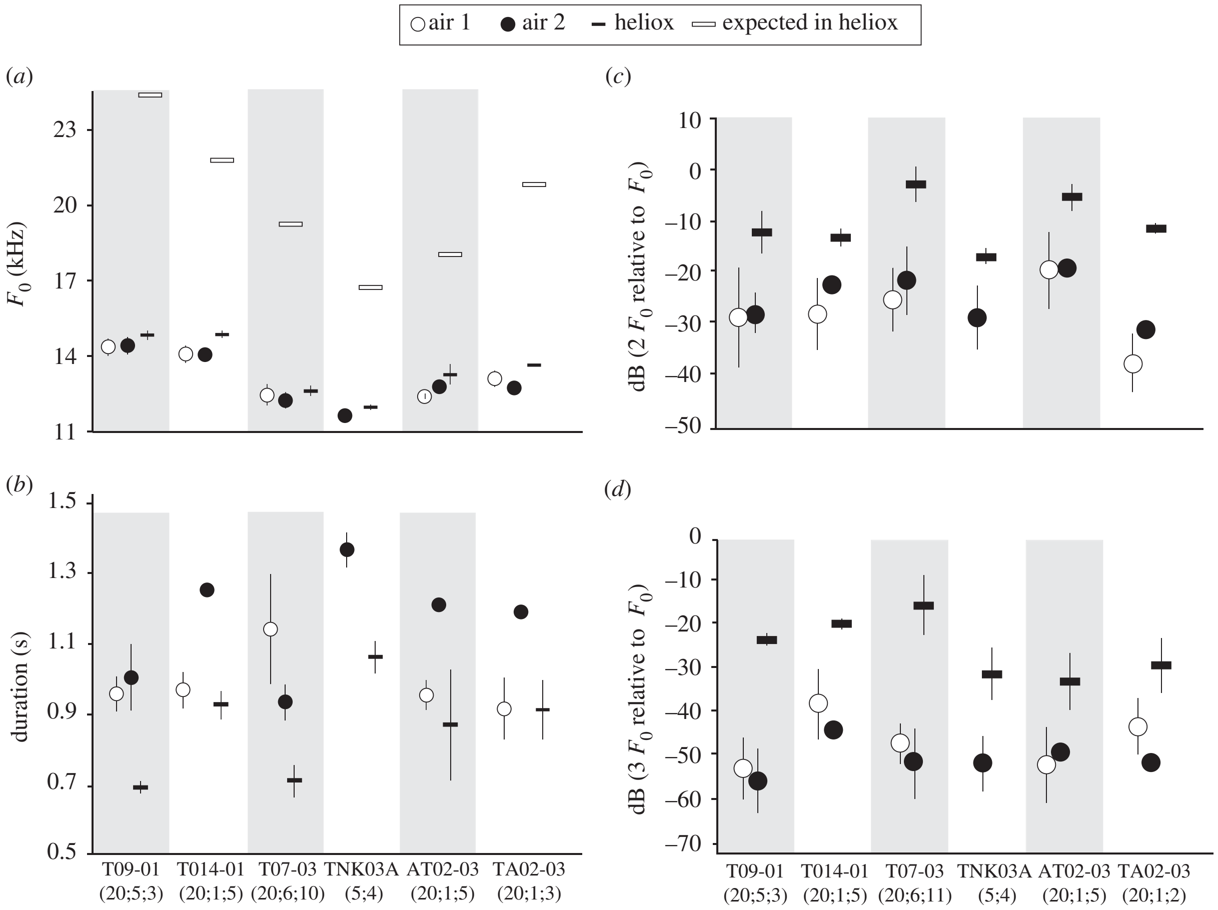

Figure 2. Acoustic properties of *Onychomys* long-distance calls produced in normal air ('air 1', in home cage; 'air 2' in experimental cage) and heliox. (*a*) Centroid fundamental frequency (F_0) of long-distance calls in air and heliox. Fundamental frequency was slightly higher in heliox but remained far below the predicted frequency estimated from the frequency response of a whistle ('expected in heliox'; see Material and methods). (*b*) Duration of long-distance calls produced in air and heliox. Relative level (dB) of the second (*c*) and third (*d*) harmonics of long-distance calls in air and heliox. Error bars indicate mean ± s.d. for individual mice. Home cage recordings were not available for TNK03A.

Table 1. Fundamental frequency (F_0) and dB levels of the second ($2F_0$) and third ($3F_0$) harmonic relative to F_0 in normal air and in heliox.

mouse ID	body mass (g)	F_0 (kHz) of long-distance call in air	F_0 (kHz) of long-distance call in heliox	$2F_0$ (dB) in air	$2F_0$ (dB) in heliox	$3F_0$ (dB) in air	$3F_0$ (dB) in heliox
T09-01	23.3	14.4 ± 0.3	14.8 ± 0.2	−27.6 ± 3.8	−11.9 ± 4.1	−53.7 ± 7.3	−22.2 ± 1.5
T014-01	27.3	14.1	14.8 ± 0.1	−21.8	−13.0 ± 1.7	−42.5	−18.8 ± 1.3
T07-03	36.1	12.2 ± 0.3	12.6 ± 0.2	−21.4 ± 6.6	−2.8 ± 3.5	−50.0 ± 7.8	−14.7 ± 6.6
TANK03-A	47.9	11.6 ± 0.1	11.9 ± 0.1	−28.5 ± 6.2	−16.7 ± 1.4	−49.9 ± 6.3	−30.0 ± 5.8
AT02-03	28.9	12.8	13.3 ± 0.4	−18.7	−5.2 ± 2.6	−47.6	−31.5 ± 6.4
TA02-03	29.8	12.7	13.6 ± 0.05	−30.7	−11.3 ± 1.0	−49.9	−27.9 ± 6.2
L012-01	32.4	12.5 ± 0.2	12.8 ± 0.1	−29.7 ± 7.1	−7.8 ± 1.3	−50.6 ± 4	−24.1 ± 0.8

and hyaluronan (figure 4*h*,*i*). The removal of hyaluronan by hyaluronidase digestion with subsequent AB staining indicated that much of the positive stain in the vocal membrane was attributed to hyaluronan (figure 4*h*,*i*).

We studied the geometry of oral and pharyngeal cavity in additional male specimens of *O. leucogaster* ($n = 2$) and *O. arenicola* ($n = 1$) by dissecting the thorax and injecting dental cast into the trachea and upper respiratory tract. Neck, head and mouth were positioned to resemble live animals with erect posture and wide mouth gape during long-distance vocalization (figure 5). Once the cast was solidified, specimens were deep-frozen and sectioned in the sagittal plane (figure 5). The total length of the oral and pharyngeal cavity measured between vocal folds and upper incisive was 20.3 ± 1.1 mm (mean ± s.d.; $n = 3$). The first segment of the vocal tract (hereafter 'segment 1') was narrow and tube-like (1 mm diameter) and measured 7.0 ± 1.0 mm (mean ± s.d.) in length. The second segment (segment 2) resembled a flared tube, with a diameter of 1 mm at the base and a 5 mm diameter at its opening, and a length of 13 mm.

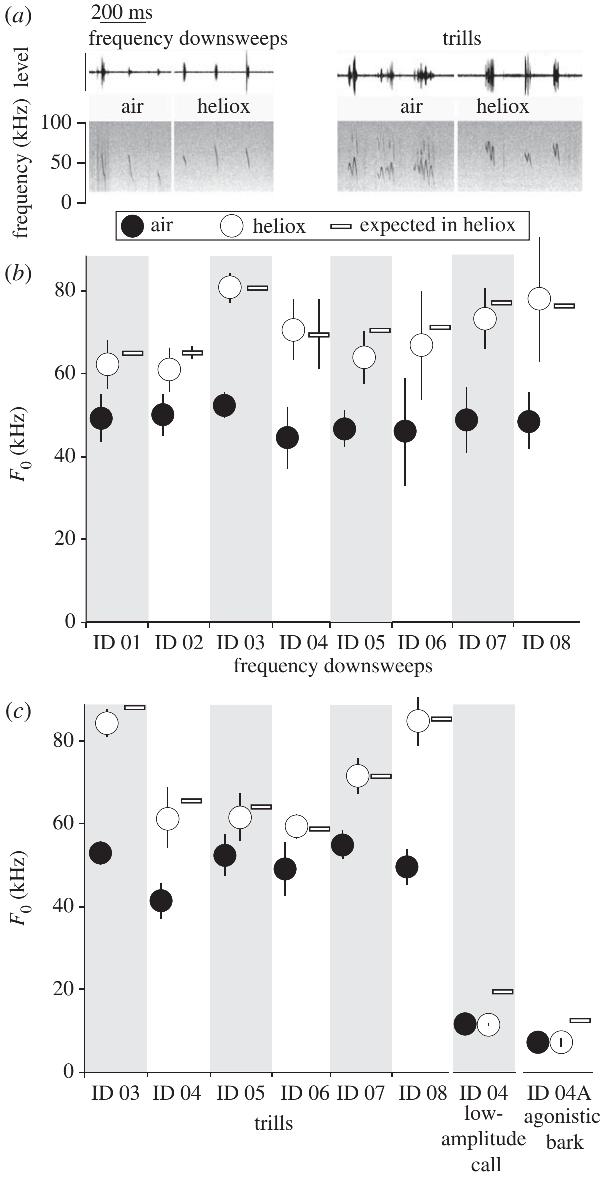

Figure 3. Ultrasonic vocalizations emitted during mating bouts are produced via an aerodynamic whistle mechanism. (*a*) Call types include frequency downsweeps and trills. Sound is depicted as a waveform (top panel; relative dB level) and a spectrogram (bottom panel). (*b*) Mean fundamental frequency (F_0) of frequency downsweeps in air and heliox. (*c*) F_0 of trills, low-amplitude calls, and agonistic barks (female) in air and heliox. Error bars indicate standard deviation. 'Expected in heliox' refers to the predicted frequency estimated from the frequency response of a whistle (see Material and methods).

(c) Computational simulation of the sound production mechanism

(i) The sound source

The two-mass model of vocal fold vibration (figure 6*a*) was simulated for grasshopper mice and house mice (*Mus*; see the electronic supplementary material for details) to determine the phonation threshold pressure (PTP) and F_0 with varying Young's moduli. The lamina propria of a house mouse vocal fold is 80–100 µm thick mediolaterally and does not possess vocal membranes [37]. F_0 at PTP is illustrated in a two-dimensional graph, wherein vocal production is realized only above the threshold line (figure 6*b*). In house mice, PTPs between 0.4 and 1.0 kPa are required to produce 1–5 kHz vocalizations. Interestingly, thinner lamina propria allows grasshopper mice to produce much higher frequency (10–17 kHz) calls with similar PTPs (0.5–1.2 kPa). The presence of vocal membranes increases glottal efficiency by reducing the PTP required to initiate phonation (figure 6*b*).

(ii) The vocal tract filter

A 20 mm long vocal tract (segment 1 + segment 2) shaped like a uniform tube with a 1 mm inner diameter generates resonances at 4.3 and approximately 13 kHz (red line in figure 6*c*). If segment 2 is modelled as a flared tube with a 1 mm diameter at the transition between segment 1 and 2, and an increasing diameter towards the open end, the first resonance increases to 8 kHz and the second remains close to 13 kHz (figure 6*c*). The simulation also suggests that the flared mouth opening radiates approximately 30 dB more sound power at the second formant than a uniform tube (figure 6*c*).

4. Discussion

Conspicuous acoustic displays are often matched by striking morphological innovations underlying vocal production [1]. As a consequence of their wide-ranging predatory lifestyle, grasshopper mice have evolved long-distance calls to advertise their presence over large distances. We found that long-distance call production is aided by laryngeal and supralaryngeal adaptations, including the presence of vocal membranes. In addition, we found that grasshopper mice alternate between an airflow-induced tissue vibration mechanism and a whistle mechanism to produce audible and USVs, respectively. We discuss our findings in relation to dynamic changes in motor control of vocal production necessary to produce distinct call types. We then consider functional adaptations of the vocal apparatus that promote energetic efficiency and social factors driving the evolution of long-distance signalling.

(a) Vocal motor control of distinct production mechanisms

Although production of audible vocalizations in rodents is well-documented in aversive contexts (*Rattus*; [38]; pine voles, *Microtus pinetorum*: [39]; Mongolian gerbil, *Meriones unguiculatus*: [40]; house mice: [41]) and more recently described in prosocial contexts ([18,19]; herein), the underlying production mechanisms are unknown. Our heliox experiments indicate that loud long-distance calls, softer pure tones and agonistic barks are all produced by an airflow-induced tissue vibration mechanism, whereas USVs are produced by a whistle mechanism. Alternation between two production mechanisms, in some cases within the same mating bout, probably necessitates recruitment of distinct motor programmes. For example, frequency patterns of rat USVs precisely match laryngeal muscle activation, requiring call-type specific motor programmes [14]. Furthermore, USVs are generated with lower subglottal pressures (2 kPa) than audible vocalizations produced by airflow-induced vibration of the vocal folds (5 kPa) in rats [11]. At the very least, grasshopper mice appear to dynamically adjust motor control of subglottal pressure to produce discrete call types. Further experimentation is needed to assess the boundaries and degrees to which laryngeal motor patterns are conserved or recombined to generate acoustic diversity [42,43].

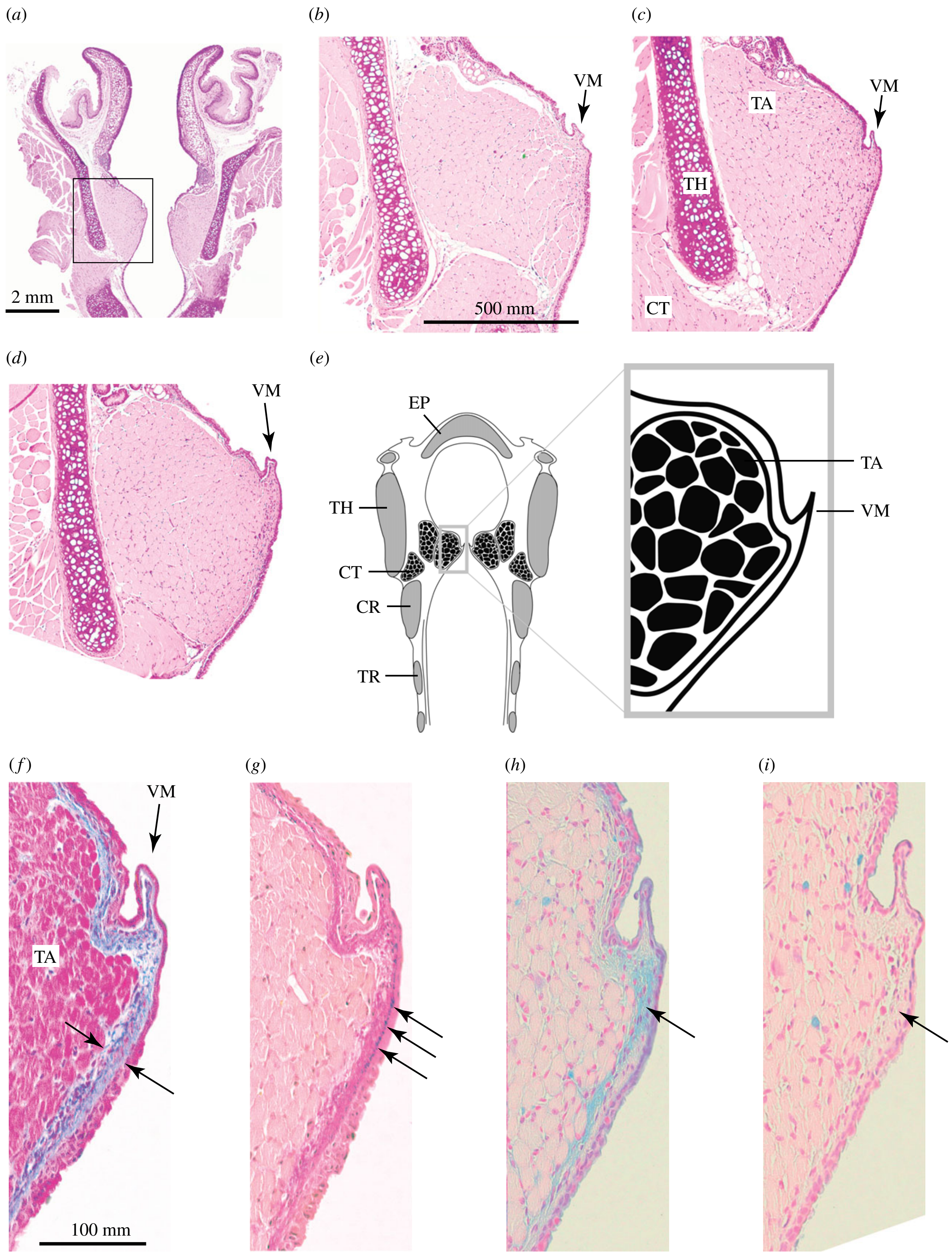

Figure 4. Grasshopper mouse larynx reveals vocal membranes. (*a*) Coronal cross section of a larynx. (*b–d*) Higher magnification images (from the square in (*a*) of three male mice vocal fold cross sections (H&E stain). On the medial edge of both vocal folds, all mice had vocal membranes consisting of a double layer of epithelium with a small amount of connective tissue between the epithelial layers. (*e*) Schematic of a grasshopper mouse larynx. (*f*,*g*) Lamina propria of vocal membranes contains collagen (blue stain in *f*), elastin (black stain in *g*) and hyaluronan (blue stain in *h*). (*i*) Removal of hyaluronan by hyaluronidase digestion with subsequent AB staining. CR, cricoid cartilage; CT, cricothyroid muscle; EP, epiglottis; TA, thyroarytenoid muscle; TH, thyroid cartilage; TR, tracheal rings; VM, vocal membrane. (Online version in colour.)

(b) Adaptations for high vocal intensity facilitating long-distance communication

In general, lung pressure and morphological features within and above the larynx mediate the intensity of laryngeal vocal signals. Although we do not have empirical measures of lung pressure in grasshopper mice, we found two intralaryngeal adaptations that probably promote loud, high-frequency calls. First, grasshopper mouse vocal folds are composed of thin lamina propria (approx. 20 µm) compared

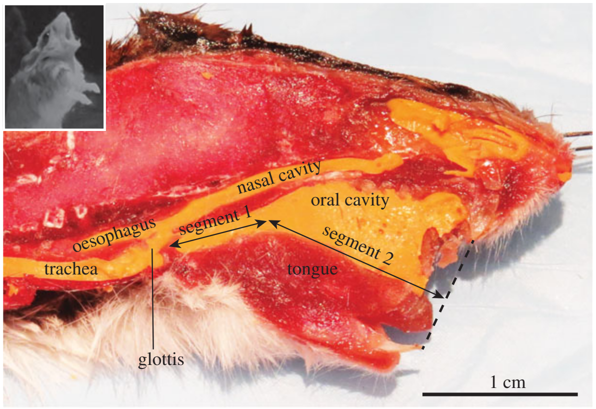

Figure 5. Adult males produce a long-distance call in an upright posture with wide-open mouths (inset). Midsagittal section of a grasshopper mouse specimen. The upper respiratory tract (trachea, part of oesophagus, nasal and oral cavity) are filled with yellow dental cast. Note that the vocal tract is narrow and uniform between the glottis and a point in the oral cavity about 7 mm upstream (segment 1). The remainder of the vocal tract (segment 2) widens like the bell of a horn from 1 mm to approximately 5 mm in diameter. (Online version in colour.)

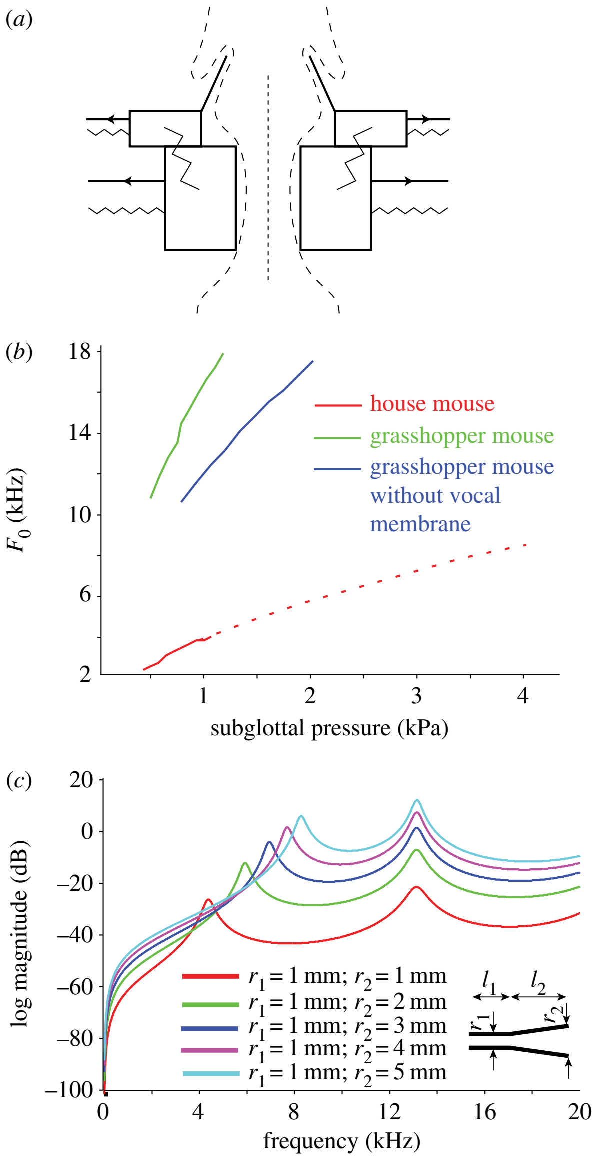

Figure 6. Sound production mechanism models in grasshopper mice (*Onychomys*). (*a*) Schematic of vocal membrane model. (*b*) Phonation threshold pressures (PTP) for house mouse and grasshopper mouse phonation. Note that the smaller lamina propria facilitates higher fundamental frequencies in grasshopper mice (blue line) than in house mice (red line). The dotted red line extrapolates the PTPs theoretically needed for house mice to produce high-frequency calls typical of *Onychomys*. The vocal membranes (green line) increase glottal efficiency by reducing the lung pressure required to initiate phonation. (*c*) Vocal tract models ranging between a uniform tube and a tube with bell-shaped mouth opening. The latter simulates a mouse producing a long-distance call with a wide-open mouth (figure 5). Relative radiated power level as a function of frequency, for a constant input volume flow at the larynx, with a vocal tract shape ranging from a uniform tube to vocal tract with flared bell-shaped opening. r_1, radius of the vocal tract; l, length of segments 1 and 2 of the vocal tract; $l_1 = 7$ mm and $l_2 = 13$ mm; $r_1 = 1$ mm for all experiments; r_2 is flared and was altered from 1 to 5 mm. (Online version in colour.)

with similarly sized (20–40 g) house mice (80–100 µm; [37]). Thin lamina propria enables faster tissue vibration, thus permitting higher F_0. Indeed, the highest F_0 for spontaneously produced audible calls in *Mus* (6 kHz; [44]) is much lower than grasshopper mice (11–14 kHz; table 1). Assuming identical vocal fold tissue density and viscoelasticity between *Mus* and *Onychomys*, lamina propria thickness probably accounts for the observed differences in F_0. The degree to which lamina propria size contributes to species differences in call F_0 within *Onychomys* awaits further investigation.

Second, grasshopper mice possess vocal membranes, or thin narrow upward extensions on the medial surface of the vocal fold. Vocal membranes have evolved independently in at least four mammalian orders (bats: [45]; non-human primates: [46]; cats: [47]; rodents: this study) and are associated with production of high F_0 at high intensities. Similar to Mergell *et al.* [48], our model simulations confirm that vocal membranes increase glottal efficiency by lowering phonation threshold pressures, particularly at high frequencies [49,50]. Airborne acoustic signals are notorious for being energetically inefficient owing to heat loss and weak coupling between the sound source and the environment [51]. Thus, vocal membranes appear to be a common solution to producing high-frequency sounds with less effort, adding to other laryngeal innovations that facilitate specific acoustic objectives (e.g. fibrous mass in frogs: [52,53]; vocal fold composition in songbirds: [54]).

Finally, grasshopper mice possess supralaryngeal adaptations that facilitate loud calls. Grasshopper mice assume a conspicuous posture whereby the head is raised and the mouth is widely opened to produce a call that can purportedly travel in excess of 100 m [55]. Empirical and modelling results suggest that F_0 of long-distance calls is tuned to the second resonance of the vocal tract (formant tuning), and that the bell-shaped mouth opening increases vocal intensity. The contribution of the mouth opening theoretically amplifies the signal by close to 30 dB, in accordance with empirical measures of upright calls (∼95 dB sound pressure level re: 20 µPa at 33 cm) and softer mating calls produced while prone with little mouth opening (∼52 dB; B. Pasch 2017, unpublished data). Such formant tuning is a strategy to adjust one of the vocal tract's lower resonances to match one of the lower harmonics. Songbirds, for example, tune the first vocal tract resonance to F_0 during their songs by elaborate movements of the oropharyngeal–oesophageal cavity [8,14]. Similarly, human singers adjust the second or third resonance to the second or third harmonic while singing high notes [56,57]. Anatomical specializations of the head and neck associated with a predatory, carnivorous lifestyle may confer grasshopper mice with greater flexibility in mouth opening [58,59].

Simulation of the mouse's vocal tract transfer function also suggests increased radiation efficiency for frequencies between 8 and 15 kHz caused by a flare-shaped mouth. The amplifying effect of a bell-shaped mouth opening is based on improved coupling between the sound source and air, a phenomenon well-documented in horn acoustics, loudspeaker design [60], and mouth opening and lip configuration of singing humans [61]. The flared resonance chamber transfers higher frequencies better than lower frequencies [60]. A similar effect occurs in crickets [62], frogs [63] and bats [64] that exploit bell-shaped structures in the environment to improve sound propagation. Finer scale recordings coupled with detailed modelling efforts are needed to better quantify radiation efficiency.

In summary, our findings suggest that optimization of vocal fold morphology is associated with adjustments to vocal tract resonance to facilitate long-distance signalling. Comparative analyses across a wider range of vocal rodents of different body sizes and vocal tract lengths will clarify the relative importance of peripheral mechanisms in the evolution of acoustic displays. We predict that specializations for long-distance vocalizations are associated with morphological adaptations in the vocal apparatus to increase intensity, while USVs used in mating favour complexity arising from central control of breathing and laryngeal movements [65].

(c) Evolution of production mechanisms for long-distance communication in muroid rodents

Although USV production in close-distance social contexts is ubiquitous among muroid rodents [66–69], lower frequency audible vocalizations are common in at least one subfamily (Neotominae; [18,70]). What processes catalysed the evolution of such vocalizations? One hypothesis posits that USV production confers immunity from acoustically orienting predators [71,72], and evolutionary release from predators permitted production of lower frequencies. However, many mammalian predators are sensitive to frequencies of more than 50 kHz [73,74], and reptilian and avian predators can hear up to 5 and 10 kHz, respectively [75]. Thus, while ultrasonic signalling probably confers senders a relatively private channel owing to the high directionality, scattering and attenuation [13,76,77], factors other than predation probably contribute to the origin of long-distance audible vocalizations [78].

Advertisement vocalizations often function to mediate both intra- and intersexual interactions, implicating social structure and spatial organization as critical factors in the evolution of long-distance calls [1]. In non-human primates, the evolution of loud calls is associated with resource and/or mate defence and mate attraction [79]. Moreover, call F_0 and the active space (carrying distance) of vocalizations are strongly associated with home range size [79,80]. Indeed, home ranges of grasshopper mice are five to nine times larger than predicted by body mass [81,82]. Wide spacing among individuals, either owing to resource availability and/or strong territoriality, necessitates a mechanism to facilitate detection of mates and/or competitors over large distances. Under this scenario, selection acted on small vocal organs to produce high intensity signals that propagate far distances. Indeed, biomechanical models suggest that sound pressure level increases with F_0 at rates up to 10 dB per octave provided that lung pressure is raised proportionally [61,83]. Comparative analyses that explore the rich diversity of rodent social and spatial organization in relation to sound production mechanisms will help clarify the adaptations enabling this extraordinary mode of communication.

Ethics. Founder animals were captured with a permit from the New Mexico Department of Game and Fish (no. 3562) and all procedures were approved by the Institutional Animal Care and Use Committees at Northern Arizona University (no. 16-001) and Midwestern University, Glendale, AZ, USA (no. 2478).

Data accessibility. Data are available from Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.8m70g [84].

Authors' contributions. B.P., I.T.T. and T.R. conceived and designed the study. B.P. and T.R. acquired the acoustic and morphological data. I.T.T. developed the biomechanical model. B.P., I.T.T. and T.R. analysed and interpreted the data. B.P., I.T.T. and T.R. wrote the manuscript.

Competing interests. We declare we have no competing interests.

Funding. Funding was provided by Northern Arizona University and Midwestern University, Glendale, AZ, USA.

Acknowledgements. We thank Haley Szczublewski and Mandi Johnson for assistance with animal care, Aubrey Funke for initial exploratory histological preparations, and Polly Campbell and two anonymous reviewers for constructive feedback on the manuscript.

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