Title

Naturally-selected and sexually-selected wing structures synergistically enhance the attractiveness of katydid acoustic signals

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Abstract

Naturally-selected adaptations are often thought to impair the elaboration of conspicuous sexual signals. In katydids, males produce acoustic signals by rubbing together structures on their forewings; specialised forewing cells then radiate this sound. These dedicated sound-producing structures are found on a physically distinct basal area of the forewing, but in many species, the remainder of the wing has evolved enormous, exaggerated blade-like or leaf-like structures under natural selection. We tested whether removal of such adjacent, naturally-selected modifications enhances the attractiveness of male sexual signals by releasing bioacoustic constraints caused by their physical connection on the wing surface. Acoustic and resonance measurements of the leaf-mimic *Viadana brunneri* before and after removing the non-sound-producing, leaf-mimicking, portion of the forewing demonstrate that these regions decrease the frequency of male song and increase its amplitude (i.e. loudness). Using a playback experiment with calls from the intact versus wing-clipped males, we found that females prefer the lower frequency calls of males with the non-stridulatory portion of their forewings intact. Thus, the function and attractiveness of intraspecific signals can be enhanced by naturally-selected adaptations, contradicting the common assumption that adaptations for mate attraction and predator avoidance represent functional compromises between sexual and natural selection.

Keywords

Acoustic signalling, Laser Doppler Vibrometry, mate choice, Neotropics, stridulation, tegmina, Tettigoniidae.

Introduction

Animal acoustic signals can convey information about the identity and quality of prospective mates, and accordingly, these acoustic signals are expected to co-evolve with the sensory preferences of receivers [1-8]. Specialised sound generator morphology can underlie variation in the acoustic properties (e.g. pitch, bandwidth, and amplitude) of the signal produced [e.g. 9,10], but these sound generators do not function in isolation. Their performance might be influenced by physical associations with adjacent body parts which themselves can be under different selection pressures [11,12]. Such physical integration between traits could constrain the extent to which each function can be optimally adapted [13]. For acoustic signals, this is commonly predicted to result in reduced efficiency of transmission and reception [1,2,5]. In other words, acoustic sexual signals are 'checked' by traits under countervailing natural selection arising from physiological, structural, or ecological constraints [14-16]. These ideas are supported by theoretical models showing how sexual signals can displace naturally selected traits from their adaptive optima [17]. Despite extensive work demonstrating how predation imposes ecological constraints on signal efficacy [16,18], experimental tests of the reverse are far rarer: whether traits under natural selection can, in turn, impair acoustic signal function and fitness.

We studied this trade-off in a neotropical katydid (Orthoptera: Ensifera: Tettigoniidae), Katydids, like other acoustically-signalling species in the suborder Ensifera, use acoustic signals to attract mates [19]. Males produce sound by rubbing their forewings together, a process known as stridulation. In katydids, a scraper (or plectrum) on the anal margin of the right tegmen (sclerotized forewing) is moved against a stridulatory file on the ventral side of the left tegmen, which bears a series of precisely arranged cuticular teeth [20] (figure 1). This process creates vibrations that are radiated and enhanced by a specialised membranous cell on the right tegmen known as the mirror [21,22]. Most katydid species either lack a mirror on their left tegmen, or it is thought to be atrophied and/or highly damped [23-25]. These structures typically form a distinct area, hereafter referred to as the 'stridulum' (plural: 'stridula'), physically separated from the rest of the tegmen by the medial veins and is often held in a different orientation to the rest of the tegmen [26]. Comparative analyses of katydid stridulatory anatomy and mechanics across species have revealed that modifications in these structures are linked to shifts in the carrier frequency of male acoustic signals [27-29] and can be used to infer species identity [30]. These signal-morphology associations also occur within species [e.g. 31-33]. Subtle morphological variation can therefore measurably influence sexual signal output, but little is known about the influence of other components of the tegmina not directly involved in sound generation or radiation, such as areas not occupied by the stridulum. Physical coupling within these morphologically integrated units creates the potential for trade-offs whereby traits under different selection pressures cannot function optimally. Here we focus on a species of leaf-mimicking katydid, which provides an extreme yet experimentally tractable system for testing this hypothesis.

Many katydids possess large, non-stridulatory tegmina shaped by natural selection for flight and physical protection. In Neotropical leaf-mimicking species, these regions are often particularly enlarged, with the stridulum occupying only a small fraction of the total tegminal area. Predation pressure has shaped the size and shape of the non-stridulatory tegmina to mimic dead or living vegetation and thereby provide a specialised form of camouflage [34-36]. It has been proposed that the multicomponent nature of the tegmina in these species might prevent complete functional decoupling between naturally and sexually selected traits [13]. While much of the literature implies that such physical coupling should produce trade-offs, an alternative possibility is that selection has instead promoted functional synergy among these components. In one katydid species (*Typophyllum spurioculis*) for example, frequencies of maximal resonance in the non-stridulatory, leaf-mimicking tegminal regions closely match those at the mirror, though at lower amplitude, hinting that their role in signal transmission could be of biological relevance [37]. However, it is unclear whether, and in what ways, these exaggerated tegminal regions – shaped primarily by natural selection – directly contribute to the transmission and reception of acoustic sexual signals.

In this study, we experimentally removed the non-stridulatory tegmina of *Viadana brunneri* (Cadena-Castañeda, 2015; subfamily: Phaneropterinae), a small (0.38 ± 0.07 g; figure 1a) [38] katydid sampled on Barro Colorado Island, Panama. The parameters of the male pure tone call are well characterised at this locality and as with most Neotropical katydids, the stridula are located on the animal's dorsal surface with the non-stridulatory, leaf mimicking tegmen oriented perpendicularly on the lateral side [38] (figure 1). The tegmina of *V. brunneri* thus provide a prime exemplar of a morphological trait comprising sexual signalling structures adjacent to an exaggerated component shaped by natural selection (most conspicuously, leaf mimicry). This offers a valuable opportunity to experimentally manipulate structural components of sound generators, quantify their effects on male song, and test the effects on female preferences. Specifically, we tested whether these naturally selected, non-stridulatory wing regions alter the spectral, temporal, and radiative qualities of male courtship song. Finding that their removal increases song frequency, we then evaluated the functional compromise on male signals by testing whether females prefer acoustic signals from males with tegmina lacking these structures shaped by natural selection. However, we found the reverse. Females preferred the lower-frequency songs of intact males. Using laser Doppler vibrometry, we analysed how the biophysical properties of the stridulum and non-stridulatory portions of male tegmina interact to produce optimally attractive signals.

Methods

Animal collection and maintenance

Male and female individuals of *Viadana brunneri* were collected from research station lights on Barro Colorado Island (BCI), Panama (9.1647° N, 79.8367° W), during the 2025 dry season (January to April) between 04:00 and 06:00 and 20:00 and 01:00. Individuals were kept in single-sex communal mesh cages which were cleaned regularly and supplied with fresh green vegetation, apple, and water *ad libitum*. To ensure a natural acoustic background, cages were kept at ambient temperature (~25 °C) and humidity (~80%) in a screened building on the forest edge, as in ter Hofstede *et al.* [38]. The temperature and humidity of tropical rainforests, including BCI, are relatively stable, meaning that small fluctuations in these variables are unlikely to have affected katydid calling properties [39]. All behavioural experiments were conducted under these conditions, in Panama, after which any remaining females were released back into the wild. Any remaining live males were placed in transparent envelopes with some damp cotton wool and apple and exported live to the United Kingdom under export permit no. 461033 from the Ministerio de Desarrollo Agropecuario (MIDA). Upon arrival to the United Kingdom, individuals were kept in hermetic plastic boxes at a constant temperature of 27 °C and were regularly fed *ad libitum* bramble leaves, apple and water, until they were required for Laser Doppler vibrometry (LDV) scanning.

Male calling experiment

Acoustic recordings

To test whether non-sound-producing tegminal structures impact properties of male acoustic signals, male song was recorded before and after physical manipulation of the tegmen across two consecutive days (see Supplementary Methods for details). During recordings, live males were placed in custom-made mesh cages designed to hold individuals snugly without exerting physical pressure on the tegmina, which could have interfered with their natural calling behaviour. The cages also restricted movement sufficiently to ensure the anterior end of each male was always facing the microphone. Restrained males were placed inside the popup cage at a constant distance (5 cm) from the microphone, to allow for comparable relative amplitude measurements. Recordings were taken between 17:00 and 01:00 using Avisoft Recorder in sound-activated trigger mode (threshold: 1% energy from 10 – 30 kHz) and were saved as WAV files.

Tegmen manipulations

Approximately twelve hours after the first recording was taken for each individual, tegmina were randomly subject to one of three forms of manipulation. For a ‘removal’ experimental group, a single cut was made using microscissors along the medial vein (figure 1c). This detaches the rest of the tegminal regions involved in leaf masquerade from the stridulatory apparatus, leaving the latter completely intact and attached to the body. To control for the possibility that the tegmen cutting procedure itself affected acoustic signal differences, a separate group underwent a ‘sham’ manipulation. For these individuals, a cut of approximately the same length (~7.5 mm) as the one described above was made along the distal end of the anterior median vein, which did not remove any tegminal structures (figure 2). For a third group of ‘control’ individuals, no manipulation was performed. Males were recorded a second time, under the same conditions, approximately 12 hours after their tegmina had been manipulated.

Processing of recordings

A total of 1621 calls were recorded across 30 individuals ($N = 10$ per manipulation treatment group) with the number of calls per individual per day ranging from 2 to 212. From these, acoustic parameters were extracted using Avisoft SASLab Pro acoustic analysis software (version 5.3.2). In the absence of detailed information on tegmen movement, we follow the terminology of Morris *et al.* [40] for describing the temporal properties of males’ acoustic signals: we defined a call as “the most inclusive repetitive time-amplitude pattern in the insect’s sound emission” and a pulse as “a wave train, isolated in time by an amplitude modulation that declines to background noise level”. For each call, we used the automatic parameter estimation feature in Avisoft SASLab Pro to extract call duration (time from the start of the first pulse to end of the last pulse, in ms) and the number of pulses per call (automatically detected sub-elements with a 0.01 ms hold time) (FFT length 256, Hamming window, 98.43% overlap) with a temporal resolution of 0.013 ms.

Spectral parameters were also measured (FFT length 1024, Hamming window, 98.43% overlap) with a spectral resolution of 293 Hz. These included the carrier frequency (frequency with the most energy, in kHz), the bandwidth at -6 dB, and its upper and lower limits (hereafter referred to as the upper and lower frequencies), calculated with the “total” option selected. The quality factor Q is a dimensionless parameter that describes the sharpness of each call, where higher values of Q indicate low damping and sharp resonances [41-43]. Here, Q was calculated as the carrier frequency peak (f_o) divided by the bandwidth [44]. The mean value for each call parameter per individual per trial (i.e. before *vs.* after manipulation) was then calculated. Lastly, the maximum peak-to-peak (P-P) amplitude (the difference in maximum and minimum values in the waveform of maximum energy, in mV) of each call was measured, from which the relative amplitude (L) was calculated, using the sound pressure level of the calibration tone, with the formula:

$$L\ (dB) = Calibration\ tone\ SPL\ (dB) - 20 \times log_{10}(\frac{Calibration\ tone\ P\text{-}P\ (mV)}{Call\ P\text{-}P\ (mV)})$$

Consistent with previous studies, although there was some variation between individuals, the male call of *V. brunneri* was generally characterised by two pulses, with the second slightly greater in amplitude to the first, but both sharing the same spectral properties [38,45,46] (figure 1c; figure S1). Unmanipulated calls spanned a frequency range of approximately 14 – 18 kHz, had a carrier frequency of ~16 kHz, and a duration of ~5 ms (figure S1). Calls were visualised using the *seewave* package [47] (figure 1c) in R v.4.4.1 [48].

Statistical analysis

Visual inspection of the individual call data revealed that all continuous acoustic parameters were right-skewed, even after an attempted log-transformation. Therefore, to test for differences in acoustic properties before and after tegminal manipulation, data were analysed by constructing generalized linear mixed models (GLMMs) fitted with a gamma distribution and log link function using the *glmmTMB* package (function glmmTMB) [49]. Each acoustic parameter (call duration, relative amplitude, carrier/upper/lower frequency, and bandwidth) was regressed against Trial (two levels: before/after manipulation), Manipulation treatment (three levels: removal, sham, control), and their interaction as fixed effects. The ID of each male being measured was included as a random effect. For call duration, the number of pulses was included as an additional random effect. The overall model structure was as follows:

*(1) GLMM: Male call parameter ~ Trial*Manipulation treatment + (1 | Individual)*

"Number of pulses per call" was analysed as an ordinal factor by constructing a separate cumulative link mixed model within the *ordinal* package (function clmm) [50], using the same fixed and random effects. For all response variables, the significance of the Trial x Manipulation treatment interaction was determined by comparing models with and without the interaction using the anova() function. If a significant effect was found, the data were subsequently subset by Manipulation treatment for post-hoc analysis. After confirming its normal distribution, differences in the mean acoustic parameters (including call duration) between the two trials were analysed using a general linear model (GLM), with the null hypothesis being that the mean difference between the two trials is zero, as follows:

(2) LM: Mean difference in male call parameter (before vs. after) ~ Manipulation treatment

Female playback experiment

Playback song models

Based on the results of the male calling experiment, we next sought to test whether, and in what way, spectral differences in male calls - induced by the removal of non-stridulatory tegminal areas - affect conspecific female responses to those calls. Like nearly all studied phaneropterine katydids, *V. brunneri* form mating pairs through acoustic duets: females reply to the males by emitting a 'tick' at a particular interval after his call [51-53], a behaviour that likely facilitates mate searching. These female responses are clearly identifiable from spectrograms, allowing us to measure mate preference as the probability and/or latency of a female's response to a given male's call.

For each individual male, we selected a call whose carrier frequency was closest to that individual's mean, both before and after tegminal removal (i.e. two calls per male). The amplitude of each call was standardised to 80 dB peSPL (peak-to-peak equivalent SPL) in Avisoft SASLab Pro which is well above the acoustic neural thresholds studied in other Neotropical katydids [38,54]. To reduce background noise, a high pass filter of 1 kHz was applied to each clip. Clips were then exported as WAV files at a standardised length of 553 ms and imported into Avisoft Recorder.

Experimental trials

Acoustic clips were played from an Avisoft ScanSpeak ultrasonic dynamic speaker (frequency response: +/- 4.5 dB between 5 and 90 kHz) connected to a laptop running Avisoft Recorder via a digital-to-analog amplifier (UltraSoundGate Player 116H, model 70111, Avisoft). The speaker was placed 5 m away from a communal cage containing 15-25 females at any one time, under the same ambient rainforest conditions as the male calling experiment. Playback trials were repeated over 25 nights across the course of a month. The number of females within the cage remained relatively constant throughout, with many individuals present for the full duration of the experiment. Playback trials were attempted with individual females in pop-up cages. However, even after extensive testing (~10 females tested over the course of two weeks under different conditions), females never responded to male song playbacks when in isolation. The adaptive significance of this behaviour warrants targeted investigation, but it is likely an anti-predator strategy: aggregations of acoustically signalling katydids experience reduced predation risk from eavesdropping bat predators [55], and males of tropical species (including *V. brunneri*) are also known to increase calling when exposed to conspecific calls [46]. Although individual-level response data could not be collected, repeating the playback experiment on a single communal cage across 25 nights gave us a robust population-level measure of preference by repeatedly testing, and is analogous to playback studies on single wild populations [e.g. 56,57]. As there were multiple females within the cage potentially responding to each playback, and playbacks were looped several times during each trial (see below), this resulted in 1,250 discrete observations over the course of this lengthy, repeated-measures experiment. Our analytical approach to account for the statistical non-independence introduced by these repeated group-level measures is described below. Although this design sacrifices a degree of independent experimental replication, this was unavoidable due to the constraints on individual testing noted above. Moreover, it was offset by the extensive and granular data we were able to collect over nearly a month of test nights.

Clips were played in a random order at 18:00 each night, when *V. brunneri* is particularly active [58], at 1.6 s intervals - the average inter-call interval of all recorded males. Each trial consisted of 50 replicates per clip playback per night. Female responses were recorded using an AudioMoth 1.2.0 [59], positioned equidistant from the speaker and the communal cage to allow matching each female response ‘click’ to the specific male sound clip being played (see Supplementary Methods for further details).

Data processing

WAV AudioMoth response files were processed in Audacity v.3.5.1 where the total number of replies made by females from the communal cage were manually counted. For the first 10 replicate playbacks per night, the latency of each response was also obtained by measuring the time interval between the point of maximal energy in the male’s call and the point of maximal energy in the female’s response. For each trial, to eliminate the effects of potential female habituation, the initial response and latency of responses to the first set of playback replicates (per night) was also noted and treated as separate variables.

Statistical analysis

We confirmed that relative amplitude, call duration and bandwidth of the chosen playback calls did not differ before and after tegminal removal using the lmer() function in the *lme4* package [60], with Order (i.e. before or after tegminal manipulation) as a fixed effect and individual male as a random effect as follows:

(3) LMM: Playback male call parameter ~ Order + (1 | Individual)

For the total number of female responses, which consisted of a high proportion of zeros, zero-inflated generalized linear mixed models with a negative binomial distribution were constructed using the *glmmTMB* package [49]. Each relevant spectral variable (i.e. those that showed significant differences in the male calling experiment) was included as a fixed effect, alongside Order and their interaction. Three of the 20 playback calls consisted of a single pulse rather than two, which may have influenced female responses (these occurred in both manipulated and unmanipulated individuals). To account for this, the number of pulses was included as an additional fixed effect in these models. Individual male and night (as an ordinal factor) were treated as random effects. Additional models were also constructed to analyse the "before" and "after" Order data separately, using the same random effect structure. As in the male calling experiment, the significance of each relevant fixed effect was determined by comparing models with and without the variable in question using anova(). To summarise, the general model design was as follows:

*(4) GLMM: Female response parameter ~ Order*Male spectral variable + Number of male pulses + (1 | Individual male) + (1 | Night)*

The initial response to the first playback replicate per night (binary: yes/no) was modelled against a binomial distribution and a logit link function using the same fixed and random effects as above and was analysed under the same workflow. Visual inspection showed response latency to be normally distributed and so was analysed by constructing linear mixed models (lmer function in *lme4*) [60], again, using the same fixed, random effects, and workflow.

Laser Doppler vibrometry (LDV)

Tegmen resonance measurements

To explore how the removal of non-stridulatory tegminal areas impacts the resonance properties of the sound radiating parts of the stridulum, five males were studied using micro-scanning LDV (PSV-500, Polytec GmbH, Waldbronn, Germany) in a sound-attenuated booth at 21-23 °C. LDV scans measure displacement and velocity differences across the tegminal surface in response to a broad frequency stimulation, thereby identifying any frequencies at which the structures resonate within the stimuli range across all scan points along the tegmen surface (see Supplementary Methods for details). Separate scans of a) the entire tegmina and b) just the stridulatory apparatus were taken using 605-915 scan points, averaging 15 times for each point. A fast-Fourier transform (FFT) was performed at each point using a rectangular window and a sampling rate of 256,000 samples per second (sampling time: 64 ms, frequency resolution: 15.625 Hz). After scanning the intact tegmina, the non-stridulatory parts of the tegmina were physically removed using microscissors (see above for details). The remaining intact stridula were then repositioned and scanned in the same way.

Analysis

Two-tailed paired t tests were used to compare the resonant frequency of the mirror before and after the non-stridulatory parts were removed. Although our sample size was small, large effect sizes indicated by Cohen's *d* provides a measure of confidence in these comparisons (Cohen's *d* for right tegmina = 2.09, Cohen's *d* for left tegmina = 0.918) [61]. For scans of entire tegmina, resonance spectra were obtained from four broad non-overlapping areas similar to those studied in Baker *et al.* (1: precostal area, 2: anterior medial area, 3: posterior medial area, 4: stridulatory area) [26,62]. Doing so allowed us to describe resonance properties across the non-stridulatory regions of the tegminal surface. Magnitude-squared coherence (range: 0-1) values are shown in figure S2 (see Supplementary Methods).

Results

Non-stridulatory tegminal regions shape the properties of male courtship song

Removing the non-stridulatory areas of male tegmina increased the carrier frequency of their songs by a mean of 750.56 Hz (GLMM, $\chi^2_2 = 202.67$, $N = 30$, $p < 0.001$; removal: $z = 19.7$, $p < 0.001$; sham: $z = -0.9$, $p = 0.383$; control: $z = -1.5$, $p = 0.123$; figure 2a). The same pattern was observed for upper and lower frequency, but for the latter, 'sham'-manipulated individuals also called at a significantly higher pitch (see Table S1). Consequently, 'sham'-manipulated individuals produced sharper, narrowband calls, a finding that was also reflected in the analysis of Q (see Table S1).

Removing the non-stridulatory tegminal region made male songs quieter (GLMM, $\chi^2_2 = 219.84$, $N = 30$, $p < 0.001$; figure 2b). Individuals were significantly quieter following the 'removal' manipulation (mean difference: -4.60 dB, calculated by converting relative amplitude to a linear scale, finding the mean difference before and after manipulation, and then converting back to a logarithmic decibel scale), but those receiving the 'sham' treatment were significantly louder (mean difference: +1.12 dB, calculated as above) (GLMM, removal: $z = -20.5$, $p < 0.001$; sham: $z = 4.3$, $p < 0.001$; control: $z = 0.4$, $p = 0.673$). The trial*manipulation interaction was also significant for call duration (GLMM, $\chi^2_2 = 7.659$, $N = 30$, $p = 0.022$); however, post-hoc analyses revealed a significant effect only for the 'sham' treatment (see Table S1 for details). This was also true for the number of pulses per call (Table S1). All the above results were corroborated by equivalent analysis of the mean differences of each acoustic parameter between the two trials (Table S2). To summarise, non-sound-producing regions of the katydid tegmina contributed both to tuning the spectral properties of male calls and to improving the overall propagation of these sounds.

Females are more responsive to courtship songs from males with intact tegmina

Females responded more frequently and rapidly to lower frequency male calls (total number of responses: GLMM, $\chi^2_1 = 8.576$, $p = 0.003$; initial response: GLMM, $\chi^2_1 = 4.600$, $p = 0.032$; response time: LMM, $\chi^2_1 = 1.765$, $p = 0.184$; figure 3; Table S4). Similar, but not identical, results were found between female responsiveness and lower frequency, and when "before" and "after" Order data were analysed separately (see Table S4 for details). However, the interaction between tegmen manipulation and male carrier frequency had no discernible effect on the regularity of female responses or their response latencies (total number of responses: GLMM, $\chi^2_1 = 1.837$, $p = 0.175$; initial response: GLMM, $\chi^2_1 = 0.776$, $p = 0.378$; response time: LMM, $\chi^2_1 = 1.765$, $p = 0.184$). Therefore, females preferred calls from males with intact tegmina, and this appears to be completely explained by the lower carrier frequencies of these calls.

Removal of non-stridulatory regions alters stridulatory resonance properties

To provide biophysical insight to the acoustic shifts observed in the male calling experiment, we used microscanning LDV with broadband pulse stimulation to measure the resonance properties of the right mirror, the primary acoustic amplifier in katydids (Montealegre-Z & Mason, 2005; figure 1a,4), before and after removal of the non-stridulatory tegmina. For intact tegmina, the observed peak resonant frequencies of 12.6 – 15.4 kHz were within the range of carrier frequencies recorded from these males (14.9 – 16.1 kHz; figure 4). Removal of the non-stridulatory regions led to a significant increase in the peak resonant frequency (mean increase = 4.215 kHz, t = -4.671, d.f. = 4, p = 0.010; figure 4; Table S5), with displacement spectra indicating that manipulated samples showed resonances at considerably lower amplitudes, but with higher peak frequencies ranging from ca. 18 kHz to 22 kHz (the blue line in Fig 4B illustrates the mean of peak frequencies for the 5 samples). The damped left mirror of unmanipulated wings also vibrated within the range of recorded calling carrier frequencies, indicating that it functions as a resonator, albeit less efficiently than the right mirror. As with the right mirror, vibration amplitude decreased following manipulation; however, no significant differences in peak resonant frequency were observed ($t = -2.053$, d.f. = 4, $p = 0.109$). Figure 4 provides examples and resonance animations for exemplar individuals are provided in Movies S1 and S2.

Displacement spectra from scans of the entire tegminal surface also revealed components of resonance in non-stridulatory leaf-mimicking regions that align with those measured within the stridulatory areas (figure 5). This was true for both left and right tegmina. An exemplar resonance animation is provided in Movie S3. Indeed, in these scans, the peak displacement in the non-stridulatory regions was of greater amplitude than that measured at the stridulum. However, this effect may reflect differences in the orientation of these structures relative to the laser, and/or limited spatial resolution at the stridulum, given its much smaller area compared with the rest of the tegmen.

Discussion

Traits under sexual and natural selection are often considered to represent functional compromises, as these selective forces may favour different trait optima. This is particularly the case when such traits are physically connected to one another [13,14,63]. Historically, such outcomes have been tested by experimentally removing the sexual ornament and measuring the resulting effects on ecological viability [e.g. 18,64]. In this study, we took the opposite approach: we experimentally removed morphological structures presumed to be shaped by natural selection that are physically integrated with acoustic signalling components and measured the resulting effects on sexual signal production and reception. Our bioacoustics and behavioural data reveal a functionally synergistic relationship between structures shaped by different selective forces: the stridulatory apparatus, which is under sexual selection to produce attractive acoustic signals, and the non-stridulatory tegminal components, which are thought to be primarily shaped by natural selection for flight, physical protection, and – particularly in this species – leaf mimicry for camouflage. While subtle shifts in sound-producing morphology are known to have a profound influence on male courtship song [e.g. 28,65,66], and extended phenotypes can enhance their propagation [e.g. 67,68], our results show that physically contiguous, but non-sound producing, structures can also enhance the attractiveness of these signals, despite being shaped primarily by different selective pressures.

Removal of the non-sound-producing tegmina revealed significant shifts in both the carrier frequency and amplitude of male courtship song (figure 2). These are traits known to be important for acoustic communication in many taxa [69], including in Orthoptera [e.g. 70], and are thus under sexual selection. Confirming this, the calls of males with manipulated tegmina were less attractive to females in our playback experiment (figure 3). This result was explained by females preferring lower frequency song, which is known to provide an honest measure of male body size and/or age in amphibians [e.g. 71], birds [e.g. 72], mammals [e.g. 73], and other Orthoptera [e.g. 74,75]. For katydids, this is due to allometric scaling between mirror size and body size, both within and between species, where larger individuals, with larger mirrors, tend to produce lower-frequency sounds [28,33,70,76]. Larger body sizes – reflected by these lower frequency calls - might also indicate the size of male nuptial gifts, which are substantial in katydids and provide direct fitness benefits to females and their offspring [77]. Although body size was not measured in our calling experiment, we nevertheless expect this relationship to be reflected in the calls of both manipulated and unmanipulated males. It would be interesting to test whether natural variation in the non-stridulatory, elaborately shaped tegmina of *V. brunneri* provide an additional axis of acoustic information for female mate assessment.

In field crickets (*Gryllus spp.*), levels of frequency modulation are known to provide an honest indicator of stridulum asymmetry, which is an accurate predictor of mate quality [70,78,79]. We did not detect any obvious signs of frequency modulation in the short calls of *V. brunneri*, likely because in katydids, unlike in field crickets, sound is produced primarily by a single generator (the right mirror), so asymmetries between left and right tegmina would have negligible effects on call frequency modulation [80]. Similarly, although temporal parameters of male song were not affected by our experiments, the short, sporadic, disyllabic nature of *V. brunneri* calls might constrain the axes along which these parameters can vary, potentially limiting their usefulness to females during mate discrimination. We therefore hypothesise that carrier frequency and call amplitude are two of the key acoustic traits assessed during mate choice in this species, both of which were substantially impaired by our manipulations of non-stridulatory tegmina.

The tegmina of *V, brunneri* are a striking example of an exaggerated trait shaped by natural selection evolving despite strong and obvious physical connection to a sexually-selected structure located adjacent to it on the same wing surface. While our data suggest that functional synergy might optimise the multicomponent nature of these highly elaborated tegmina, little is known about how such relationships between traits under seemingly opposing selective forces initially evolve - particularly in the case of leaf mimicry, which has evolved multiple times independently in katydids [34,36] (figure 1a,b). Across katydid species, leaf mimicry is known to be under strong directional natural selection, increasing survival by causing predators to misclassify individuals as inedible or irrelevant objects [34-36]. While many non-leaf-mimicking katydids also exhibit exaggerated non-stridulatory tegminal regions, the specific geometric modifications required for convincing leaf mimicry may influence the acoustic properties of male song, potentially promoting arms races or positive coevolution between acoustic signal expression and receiver preferences. A comparative approach across species would be required to test this specific evolutionary hypothesis. Nevertheless, our experimental manipulations with a single leaf-mimicking species provide an example of how morphological multifunctionality can be maintained without compromising sexual signalling.

LDV acoustic resonance spectra from the stridula and non-stridulatory regions of live individuals provide further insight into the biomechanics underlying our results (figure 4,5). The increase in resonant frequency of the primary acoustic radiator, the right mirror [23], upon removal of the non-stridulatory tegmina can be explained by the inverse relationship between carrier frequency and the mass of the 'acoustic load' [67,81,82]. In other words, the additional resonating mass provided by the non-stridulatory regions, as indicated by our LDV scans of whole tegmina (figure 5), lowers the peak resonant frequency of the system by providing a resistive force (i.e. a high compliance). In addition, the reduced vibrational displacement of the right mirror following removal of the non-stridulatory structures might suggest that these regions help channel sound propagation in the anterior direction, minimising the dipole effect caused by destructive interference when sound is radiated from the upper and lower tegminal surfaces in opposite phases [25,41,43,83,84]. This may have caused the calls of males that underwent the 'removal' treatment to be more diffuse, potentially explaining the reduction in amplitude from the anterior direction. However, resonance spectra from LDV also suggest that non-stridulatory tegminal areas function as an additional resonating surface for call amplification (figure 5), as has already been shown in a distantly related leaf-mimicking katydid species [37]. In concordance with this, the higher call amplitude of individuals that underwent the "sham" treatment in the male calling experiment is likely explained by enhanced vibrational displacement of the non-stridulatory regions after they had been cut (figure 2b). Although not the primary objective of this study, these explanations offer valuable insight into the biophysics of sexually-selected signals in katydids – an area of growing interest given their unusually ultrasonic, brief and sporadic song characteristics that are broadcast in notoriously noisy tropical forest environments [38,46,85].

Resolving the fitness contributions of individual components within multifunctional morphological traits and understanding how evolution resolves the potential trade-offs inherent in their design, remains a key challenge in evolutionary biology. By integrating behavioural and biophysical assays of male bioacoustics with measures of female responsiveness, we identify exaggerated structures not traditionally considered to be involved in intraspecific communication - and very likely shaped by different, perhaps opposing, selection pressures - that have been functionally co-opted through their physical association with sound-producing structures to enhance male acoustic signals. In conclusion, synergistic interactions between trait components may facilitate multifunctionality, potentially accelerating the adaptive evolution of morphological traits and, in turn, the emergence of novel behaviours.

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87.

Ethics

This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility

All raw data, and analysis R code are available from Zenodo [86] (https://doi.org/10.5281/zenodo.18379928) alongside the .wav recordings from the male calling experiment, the chosen male playback audio files, GoPro female responses from the playback experiment, and all SVD laser Doppler vibrometry scans.

Declaration of AI use

We have not used AI-assisted technologies in creating this article.

Authors' contributions

JBW: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, visualization, writing – original draft, writing – review & editing; FAS-S: data curation, investigation, methodology, visualization, writing – review & editing; LBS: methodology, validation, writing – review & editing; FM-Z: funding acquisition, resources, software, supervision, validation, writing – review & editing; NWB: methodology, resources, supervision, validation, writing – review & editing.

Conflict of interest declaration

We declare we have no competing interests.

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Figure captions

Figure 1. Overview of *Viadana brunneri* bioacoustics. A) Photograph of a male *V. brunneri.* B) Photograph of the flattened tegmina of a male *V. brunneri* alongside a labelled, magnified image of the stridulatory apparatus, taken using an OMAX 18MP digital camera mounted on a Leica M60 dissecting microscope. C) Example oscillograms of an individual male *V. brunneri* call with and without its non-stridulatory tegmina intact (top left vs. top right, respectively), alongside spectrograms and power spectra (bottom) for each call, with the peak (carrier) frequency and quality factor (*Q*) labelled.

Figure 2. Male calling experiment results (*N* = 30 males) showing estimated means (points) and 95% confidence intervals (error bars) derived from fitted generalized linear mixed models that account for non-independence in the data (total n = 1621 calls; see Methods). The mean carrier frequency (A) and mean relative amplitude (B) of *Viadana brunneri* males with respect to all treatment groups, before and after tegmen manipulation. Significant differences are indicated by asterisks. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 3. Female playback experiment results (*N* = 20 male playback calls, each played 50 times on 25 different nights). (A) An illustration of male-female duetting behaviour in *Viadana brunneri* (above) accompanied by a labelled oscillogram of male playback calls and female response clicks (below). (B,C) Log_{10}-transformed mean total number of female responses within a given playback trial *(B)* and initial female response latency *(C)* as a function of the carrier frequency of male playback calls. Lines and 95% confidence interval shading are from fitted GLMM and LMMs respectively which had male carrier frequency as a single fixed effect; these models are for illustrative purposes only. Hollow circles indicate the mean number of female responses to each individual male playback call, colour coded by whether playback clips were recorded before or after manipulation.

Figure 4. Results from the stridulatory resonance experiment using laser Doppler vibrometry (LDV). Tegmen resonance plots for the left and right mirror of *Viadana brunneri* before (A) and after (B) removal of the non-stridulatory tegmina ($N = 5$). Bold lines indicate mean resonance with shading indicating +/- one standard deviation. Dashed blue vertical lines indicate the mean peak resonance frequency at the right mirror, the primary acoustic resonator. The inset of each panel shows an image of a male specimen before and after manipulation with the scanning mesh and density of scanning points overlayed (left), and alongside a vibrational 3D map at the peak resonant frequency for that individual (right).

Figure 5. Acoustic resonance across intact tegmina. A) A male *Viadana brunneri* specimen with its tegmina extended. Numbers and shading indicate the tegminal areas from which resonance measurements were taken. B) Same as A), with the scanning mesh and density of scanning points superimposed. C) 2D vibrational map showing the main areas of deflection at the peak resonance frequency at this individual's right mirror. D) 3D view of the vibrational map in C). E) Mean resonance spectra from the four tegminal areas (1-4; $N = 5$) illustrated in A), with shading indicating +/- one standard deviation. Dashed vertical brown and orange lines indicate the mean peak resonant frequency at the right mirror, and the mean call carrier frequency, respectively. Note that vibrations below 10 kHz are produced by the vibration of the entire tegmen and/or by minor resonances that occur naturally while live individuals are immobilised and are thus unlikely to represent adaptation for sound amplification in this species.