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SOUND PRODUCTION AND HEARING IN THE BLUE CRACKER BUTTERFLY *HAMADRYAS FERONIA* (LEPIDOPTERA, NYMPHALIDAE) FROM VENEZUELA

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Summary

Certain species of *Hamadryas* butterflies are known to use sounds during interactions with conspecifics. We have observed the behaviour associated with sound production and report on the acoustic characteristics of these sounds and on the anatomy and physiology of the hearing organ in one species, *Hamadryas feronia*, from Venezuela. Our observations confirm previous reports that males of this species will take flight from their tree perch when they detect a passing conspecific (male or female) and, during the chase, produce clicking sounds. Our analyses of both hand-held males and those flying in the field show that the sounds are short (approximately 0.5 s) trains of intense (approximately 80–100 dB SPL at 10 cm) and brief (2–3 ms) double-component clicks, exhibiting a broad frequency spectrum with a peak energy around 13–15 kHz. Our preliminary results on the mechanism of sound production showed that males can produce clicks using only one wing, thus contradicting a previous hypothesis that it is a percussive mechanism. The organ of hearing is believed to be Vogel's organ, which is located at the base of the forewing subcostal and cubital veins. Vogel's organ consists of a thinned region of exoskeleton (the tympanum) bordered by a rigid chitinous ring; associated with its inner surface are three chordotonal sensory organs and enlarged tracheae. The largest chordotonal organ attaches to a sclerite positioned near the center of the eardrum and possesses more than 110 scolopidial units. The two smaller organs attach to the perimeter of the membrane. Extracellular recordings from the nerve branch innervating the largest chordotonal organ confirm auditory sensitivity with a threshold of 68 dB SPL at the best frequency of 1.75 kHz. Hence, the clicks with peak energy around 14 kHz are acoustically mismatched to the best frequencies of the ear. However, the clicks are broad-banded and even at 1–2 kHz, far from the peak frequency, the energy is sufficient such that the butterflies can easily hear each other at the close distances at which they interact (less than 30 cm). In *H. feronia*, Vogel's organ meets the anatomical and functional criteria for being recognized as a typical insect tympanal ear.

Key words: butterfly, *Hamadryas feronia*, hearing, sound production, Vogel's organ, behaviour.

Introduction

The sounds of *Hamadryas* butterflies are well known to field biologists working in the neotropics (for a review, see Jenkins, 1983). Within the genus *Hamadryas*, male butterflies perch head downwards on tree trunks, with their wings outstretched, and typically take flight to interact with passing conspecific males and females: the resident male flies towards the newcomer and the two engage in an interaction, during which time the resident, or both the resident and newcomer, produce loud 'clacking' sounds (Swihart, 1967; Young, 1974; Muyshondt and Muyshondt 1975a–c; Otero, 1988; Monge-Nájera et al., 1998). Although sound production is observed predominantly during conspecific interactions, individuals have also been noted to fly towards a variety of other moving objects, including other insects, falling leaves, birds, thrown stones, vehicles, dogs and humans (Swihart, 1967; Young, 1974; Muyshondt and Muyshondt, 1975a; Jenkins, 1983; Otero, 1988; Monge-Nájera et al., 1998).

Many field observations of a variety of *Hamadryas* species have led to different ideas about both the mechanisms and the functional significance of sound production in this genus. Several hypotheses have been proposed concerning the mechanism of sound production and, although experimental

investigations have supported a percussive one involving the apical veins enclosing the discal cell of the forewings (Otero, 1990a; Monge-Nájera et al., 1998), the mechanism remains unknown. Darwin (1874) was the first to propose that the sounds play a role in courtship. Since then, most reports agree that the sounds are involved in conspecific communication, perhaps as a form of male–male territoriality, courtship or sexual recognition (Silberglied, 1977; Scott, 1986; DeVries, 1987; Otero, 1988; Monge-Nájera, 1992; Monge-Nágera et al., 1998). Others have suggested that the sounds serve to startle predators or potential competitors for food or 'guarded' aerial space (e.g. Young, 1974; Young and Borkin, 1985). There is still no single, satisfactory explanation for sound production, and it is possible that the adaptive function varies among different *Hamadryas* species or even among different populations of the same species.

Despite numerous descriptions of the sound-producing behaviours and attempts to explain the mechanisms associated with sound production, relatively little attention has been paid to the spectral characteristics of the sounds or the sensory mechanisms, if any, responsible for sound reception in *Hamadryas* butterflies. If sounds are indeed used for intraspecific communication, do sound-producing *Hamadryas* species possess ears that are capable of detecting conspecific sounds? There are few examples of *Hamadryas* spp. responding behaviourally to sounds: Collenette (1928) reported that *Ageronia* (=*Hamadryas*) *februa* of Central Brazil repeatedly responded to the clicking sounds of two fighting warblers by taking a brief flight while making its characteristic clicking sound. Others have noted that *Hamadryas* spp. may be disturbed by sounds, responding by spreading their wings (Muyshondt and Muyshondt, 1975a) or taking flight (Jenkins, 1983). The organs responsible for hearing were not investigated in these cases.

Vogel's organ, a pronounced membrane located at the base of the forewing cubital vein in certain Nymphalidae, has been commonly cited as a tympanal organ (e.g. Vogel, 1912; Bourgogne, 1951; Scott, 1986; Scoble, 1995; Hoy and Robert, 1996). Although this structure, first described histologically by Vogel (1912), resembles a typical insect tympanal ear, possessing a thinned membrane associated with a tracheal air sac and chordotonal sensory organ (Yack and Fullard, 1993; Hoy and Robert, 1996), only one study on satyrid butterflies has shown that it functions as a hearing organ (Ribaric and Gogala, 1996). The auditory sense in *Ageronia* (=*Hamadryas*) *feronia* was investigated physiologically by Swihart (1967), who recorded both from the main forewing nerve (IIN1c) and from single units of the thoracic ganglia. The author suggests, however, that the organ of sound reception was not Vogel's organ, but rather the membranous ampulla, a soft membrane immediately anterior to Vogel's organ. That the membranous ampulla functions as an ear is also suggested in a recent study by Monge-Nájera et al. (1998). Since an auditory sensory organ was not identified in either of these reports, the structure responsible for detecting sounds remains under debate.

In this paper, we provide a description of hearing and sound production in *H. feronia* (Fruhstorfer) of Venezuela (Fig. 1), which has been proposed to use sounds in male–male communications (Otero, 1988). We describe the typical behaviour associated with sound production in this population and report on the spectral characteristics of the sounds produced by males both in the laboratory and in the field. We extend the neuroanatomical description of Vogel's organ to *H. feronia* and describe physiological recordings from the wing nerve identified to innervate Vogel's organ in this species.

Fig. 1. The blue cracker *Hamadryas feronia*. Scale bar, 1 cm.

Materials and methods

Animals and behavioural observations

Hamadryas feronia (Fruhstorfer) were obtained from two different sources. Some animals were obtained after observation in the wild in a humid, lowland agricultural area close to El Vigia, Venezuela (9°N, 72°S), on 26 November 1995, 1 December 1995 or 30 September 1997. Observations of behaviour and sound recordings were made in the field for approximately 12 h on each of these dates. Following our observations, we captured several *H. feronia* with butterfly nets and traps baited with fermenting bananas (a modified Rydon-type butterfly trap; see Rydon, 1964). Animals were placed in glassine envelopes for transportation back to the laboratory at the Universidad de Los Andes, Mérida, Venezuela, where they were used for physiological tests (audiograms) and then preserved for later dissection. For additional morphological studies in our Canadian laboratory, some *H. feronia* were purchased as pupae from Costa Rica Entomological Supply (Alajuela, Costa Rica).

Sound production

Sounds were recorded in the field from free-flying males using three different recording systems. Some clicks were recorded using a bat detector (Pettersson D240) with a 1.7 s digital memory (sampling at 300 kHz) which was saved at 10 times reduced clock rate onto either a Sony Professional WM-D3 or a Sony DAT WM7 cassette tape recorder. The bat detector system has an effective bandwidth of 150 kHz, but could only record short 1.7 s sequences. Longer interaction sequences were recorded in the field using the Sony

Professional WM-D3 (in 1995) or the Sony DAT WM7 (in 1997) with a Sony audio microphone. The WM-D3 with audio microphone has a flat frequency response up to 15 kHz, which falls off by 25 dB between 15 and 20 kHz; the response of the DAT with audio microphone falls off at 20 kHz. Although these systems did not record high frequencies, they could be left running to record acoustic interactions over longer periods. These audio recordings were used only to analyze course time variables such as repetition rate and the duration of click trains. Recordings on the WM D3 were digitized with a sampling rate of 44.1 kHz and saved on a computer. The Sony DAT recorder digitized at 44.1 kHz, and the digital data were transferred directly from the tape to a computer hard disk using a ZA2 DAT-interface card (Zefiro Acoustics, Irvine, CA, USA). All acoustic data were analyzed using BatSound (Pettersson Elektronik AB, Sweden). Spectra were produced using a 1024-point rectangular Fast Fourier Transform (FFT) window. Since the clicks were played into the tape recorders at 10 times reduced speed, the effective sampling rate for the analysis was 441 kHz. Thus, the frequency resolution of the spectra was 431 Hz.

We recorded clicks from hand-held males both in the laboratory and in the field. The males were held 1 m from the microphone by a pair of forceps around the hindwings, such that they could flap their forewings freely (as described by Otero, 1990a). Here, we used only the digital high-frequency recording system. We also recorded from hand-held males in which the closing veins in the apical portion of the discal cell in one or both wings had been ablated between the insertion of veins R3+4+5 and M2 (see Otero, 1990a).

Morphology of Vogel's organ

The nomenclature used in the description of nerve roots, muscles and skeletal structures follows that of Nüesch (1953, 1957). For descriptions of the peripheral projections of nerve IIN1c, we followed the terminology of Vogel (1912). The term Vogel's organ is used to describe the entire tympanal structure, including the membrane and attached chordotonal organs. We have named the membrane found anterior to Vogel's organ the membranous ampulla, after Otero (1990b) ('costal cell membrane' of Monge-Nájera and Hernández 1991; Monge-Nájera et al., 1998).

For examinations of the peripheral nerve topography, the thoracic nervous system was dissected using a sagittal approach. After cooling to 5 °C for 30 min, the head, legs and abdomen of the butterfly were removed. The thorax was bisected sagittally, and the half containing the thoracic ganglia was pinned to a Petri dish lined with Sylgard (Dow Corning Corp., Midland, MI, USA) filled with saline [O'Shea and Adams (cited in Strausfeld et al., 1983)]. The pterothoracic ganglion (fused mesothoracic, metathoracic, abdominal I and II ganglia) and its peripheral nerves were stained with a 0.05 % solution of Janus Green B (Miller, 1979; Yack, 1993) and drawn with a Wild M7A dissection microscope and drawing tube attachment. Nerve branches were followed as close as possible to their peripheral target sites, with particular attention being paid to those branches approaching the region of Vogel's organ. When chordotonal organs were encountered, they were removed from the animal and placed in a drop of saline on a glass slide under a coverslip and viewed with an Olympus BH-2 compound microscope. Scolopale caps, stained with Janus Green B during the dissection, were counted and photographed.

For histological studies, live *H. feronia* were captured and returned to the Universidad de Los Andes laboratory, where the thoraces and wing bases were fixed by injection with a formalin-based fixative that renders the nervous tissue pliable for subsequent dissections (Chauthani and Callahan, 1966). Tissues were left in the fixative until we returned to our Canadian laboratories, whereupon the tissue was rinsed five times and stored in 70 % ethyl alcohol. Vogel's organs were dissected with surrounding wing base tissue, rinsed three times in 70 % ethanol, dehydrated to 95 % ethanol and embedded in glycol methacrylate (JB-4, Polysciences Ltd). The tissues were sectioned at 14–18 μm with a glass knife and stained with a variation (Yager and Hoy, 1987) of Masson's trichrome solution (Pantin, 1946).

Certain specimens were air-dried and, following the removal of overlying scales, the Vogel's organs were sputter-coated with gold/palladium and examined with a JSM-6400 scanning electron microscope.

Physiology of Vogel's organ

Animals were positioned with their dorsal surface up on a piece of modeling clay under a dissection microscope. The forewing and hindwing on one side were uncoupled, and the hindwing was placed over the top of the forewing so as not to cover the auditory organ during the audiogram procedure. The forewing was supported at an elevation of approximately 30 ° from the modeling clay surface, and a wide trench was dug into the modeling clay so as not to interfere with sound transmission to the auditory organ. All seven animals used for audiograms were males collected from the wild, and they were tested within 3 days of capture.

Nerve N.II was exposed by removing the tegula and the accompanying small amount of soft cuticle in the area. With the animal in the configuration described above, the nerve passes beneath the tegula and around the anterior margin of the wing, and it is easily visualized with the application of a small amount of Janus Green B solution. Once the nerve had been visualized (as the middle of the three nerves), it was isolated on a sharpened stainless-steel hook electrode. Because the recording could be accomplished from such a small opening, saline was often not needed. To maintain electrical isolation of the hook electrode from the hemolymph of the animal, and to prevent dessication of the nerve and surrounding tissue, petroleum jelly was injected under the hooked nerve and into the incision site using a blunted hypodermic needle and syringe. Activity in N.II was amplified with an a.c. preamplifier (Grass, model P15) and displayed on a portable digital oscilloscope (Tektronix, TekScope, model THS710A). To aid in monitoring auditory-evoked activity in the nerve, we

triggered the oscilloscope with the sound stimulus (below) and averaged sets of eight consecutive sweeps.

Stimulus presentation was controlled by a portable computer with custom-designed software interfaced to a digital function generator (Wavetek, model 23). The computer cycled through test frequencies between 0.5 and 30 kHz, which were generated by the digital function generator. Sine waves of a particular frequency were then passed through an envelope shaper (Coulbourn, model S84–04) to produce 5 ms shaped pulses with a 0.5 ms rise and fall time repeating at a period of 500 ms. The shaped pulses were then amplified (National Semiconductor, LM1875T) and broadcast at a distance of 1 m through either a Sony woofer (model XS-L6 MK2) for frequencies of 0.5–5 kHz or a Technics leaf tweeter (model EAS-10TH400B) for frequencies between 3 and 30 kHz. Thus, for 3, 4 and 5 kHz, we used both speakers. The Sony woofer was placed on several layers of foam and Styrofoam to avoid transmission of substratum vibrations to the animal. Sound pressure levels are given in dB SPL (rms re 20 μPa).

For each frequency presented to the animal, we determined the threshold by gradually increasing the broadcast intensity of the repeating stimulus pulses until a waveform (compound action potential) appeared on the oscilloscope, in time with the stimulus pulse, that had an amplitude of twice the background activity in the nerve. For three of the animals, we repeated the audiogram procedure and determined the threshold by monitoring the nerve activity with an audiomonitor and noted the threshold as that intensity when we clearly heard auditory-evoked activity (spikes synchronized with the stimulus pulses). The former method is more conservative at estimating auditory threshold since our ears consistently detected auditory-evoked activity in the nerve before it was at an amplitude twice the background noise displayed on the oscilloscope.

Sound intensities for both speakers were calibrated at all frequencies used in the audiogram procedure. A continuous sine wave generated by the digital function generator was broadcast from the speaker to a Brüel & Kjær microphone (model 4135, without protective grid, and with a Brüel & Kjær preamplifier, model 2633) placed at the same distance as the butterflies had been during the audiogram procedure. Sound intensities were read directly from the display of a Brüel & Kjær measuring amplifier (model 2610, set to slow averaging and using an external band-pass filter; Krohn-Hite, model 3500) with cut-off frequencies adjusted to exclude background room noise at low frequencies and broadcast intensities.

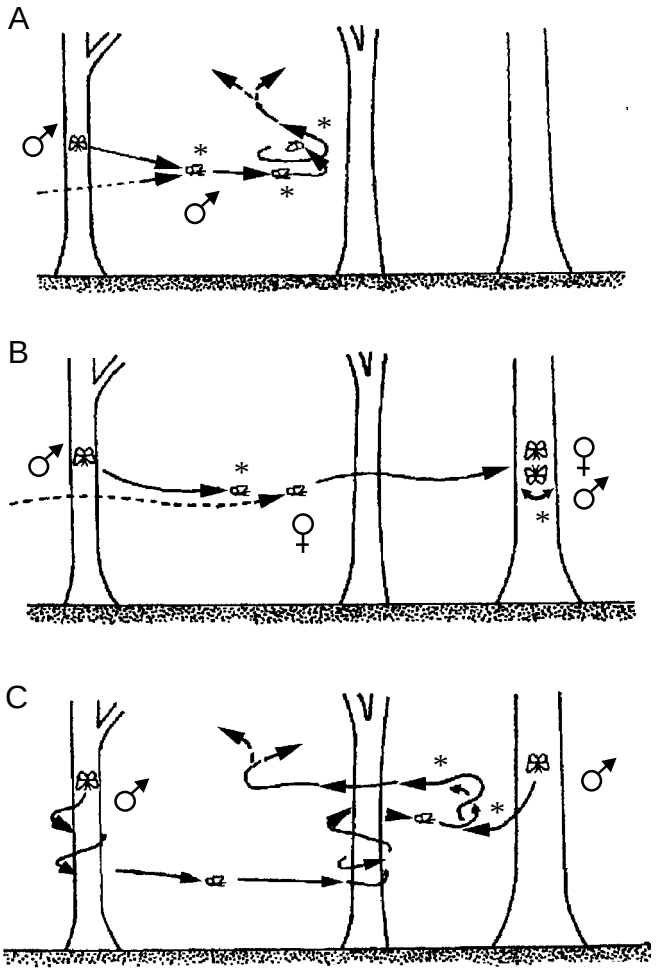

Fig. 2. Schematic diagram of some typical acoustic interactions between *Hamadryas feronia* butterflies. (A) In male–male interactions, an inverted perched male on the trunk of a tree takes flight as a second male flies past. The two males pursue each other until they fly off in separate directions. Sounds are produced (*) throughout the entire encounter, but especially when the two are in close proximity. (B) In male–female interactions, the male pursues the female until she alights on a perch; he then begins an on-the-wing display involving continuous clicking. (C) Males may leave their perched positions in an exploratory flight in which they circle tree trunks in search of other individuals. In this figure, the searching male is depicted as having encountered a conspecific male, resulting in a short interaction with continuous clicking followed by the two males flying their separate ways.

Results

Field behaviour

Our observations of the activity patterns of *H. feronia* were typical of those reported previously (Otero, 1988). Activity usually started in the morning and ended in the afternoon, with interactions beginning between 09:30 and 10:00 h and peaking before 12:00 h and with a decline in activity as the hottest time of the day was reached. A second bout of activity was often observed as afternoon temperatures cooled from approximately 14:00 h, and activity declined after approximately 16:00 h, after which the butterflies remained mostly perched until flying to their roosting sites just before sunset at approximately 18:00 h.

During our three observation days, we observed approximately 40 interactions between *H. feronia* males. Only the males were observed to produce sound, and they did so when interacting with other males and with females (Fig. 2). Interactions between butterflies were initiated when another individual flew past a perching male or when a male was

Table 1. *Temporal characteristics of acoustic emissions in free-flying* Hamadryas feronia *males*

	Duration of click components (ms)	Interval between clicks (ms)	Train duration (s)	Number of clicks per train
Mean	0.42	61.0	0.58	12.2
S.E.M.	0.02	5.4	0.08	1.7
S.D.	0.15	91.4	0.40	8.3
Minimum	0.2	0.1	0.14	3
Maximum	0.8	827	1.66	38
Number of clicks	55	267		
Number of trains		23	23	23

approached by another individual during flight. These interactions consisted of chases accompanied by sound production (sharp clicking sounds) by one or both males. Individuals interacting in flight did so at close distances to one another, often less than 10 cm and rarely over 30 cm apart.

Our observations support an earlier report (Otero, 1988) that males seem to rely on sounds as a means for sexual recognition. What happens immediately following an interaction depends on the sexes involved. If the interaction was between males (Fig. 2A,C), the two briefly pursued one another, with one or both producing sounds, and then abandoned their interaction. If the interaction was between a male and a female, the female might alight on a perch, in which case the male typically started an on-the-wing pendulous display involving continuous clicking (Fig. 2B). If the female is receptive, copulation will take place. If not, the male is likely to leave after displaying.

Sound production

Free-flying males

The temporal characteristics of click trains were determined from the long-term field recordings of flying males using audio-microphones. The trains of clicks occurred at highly unequal intervals ranging from less than 1 s up to several minutes. The duration of the click trains and hence the number of clicks in a train varied, but all trains were short, generally lasting less than 1 s with 10–15 clicks (Table 1). Most of the interactions we recorded were between two males, and it was impossible to assign the clicks to either of the two interacting individuals because they were flying close together. However, it appears from the recordings that in many cases both males were clicking (Fig. 3A). This complicated the determination of click intervals, but the most frequent interval of all 23 click trains analyzed was 50–80 ms (mean 61 ms; see Table 1), which was also the interval that dominated in recordings in which only a single male was clicking (Fig. 3B).

Recordings with the high-frequency system were used to determine the temporal fine structure and frequencies of the clicks. The clicks were 2–3 ms long, with a broad bandwidth and most energy between 10 and 15 kHz (mean peak frequency 14.4 kHz±2.3 kHz, mean ± S.D., Q_{10dB}=0.83, N=58 clicks, where Q_{10dB} is peak frequency divided by the bandwidth of the spectrum 10 dB below the peak), chosen at random from 10 different click trains (Figs 3, 4). There were many males in the area and we recorded at different sites, making it highly unlikely that all 10 click trains were produced by the same male. However, it is not unlikely that a couple of trains are from the same male. A closer inspection at high resolution

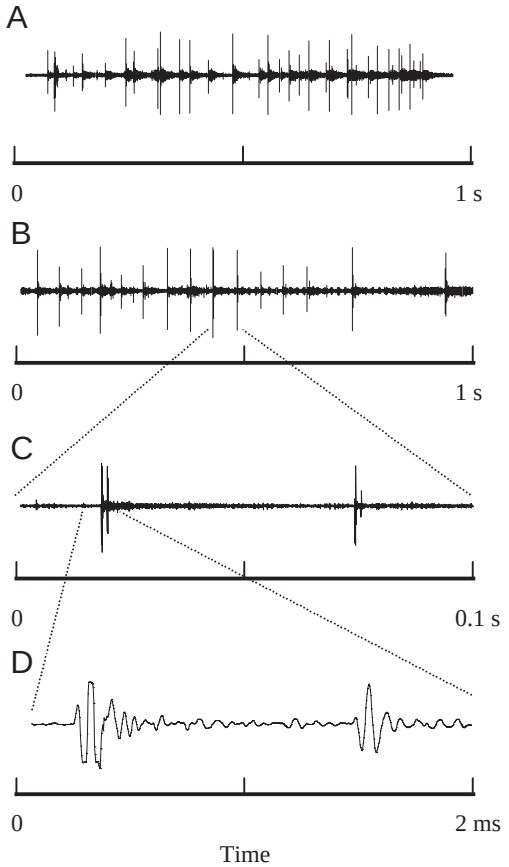

Fig. 3. Oscillograms of *Hamadryas feronia* sounds recorded from free-flying males. (A) Sounds recorded during a short interaction between two males. Click intervals vary and can be very short. (B) A click train in which only one male is clicking, showing the typical regular click pattern in which the only variation comes from skipping one or more clicks. (C) Two clicks from the train in B on an expanded time scale showing the typical double components of the clicks. (D) The first click in C on an expanded time scale. The duration of a single click component is typically approximately 0.4 ms.

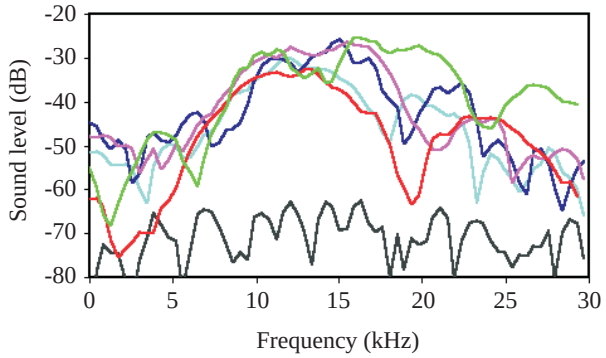

Fig. 4. Five examples of spectra of clicks from five different recordings. The bandwidth varies, but in all clicks most energy is between 8 and 18 kHz. The spectra of clicks and background noise are measured on the same relative dB scale. The level of the background noise (thick dark gray line) was between –80 and –70 dB and, thus, 40–50 dB below the peak of the click spectra.

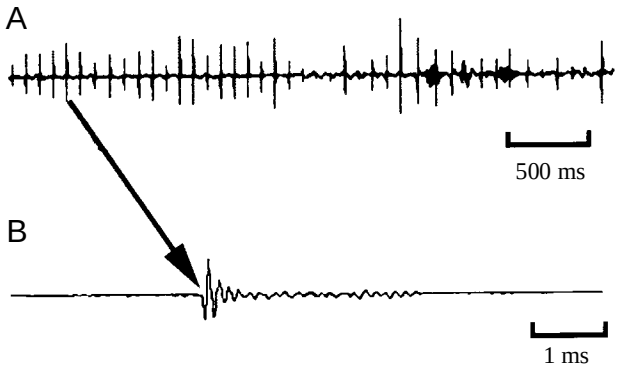

Fig. 5. (A) The click train from a male held in the hand. The click interval is approximately 80 ms in this case. (B) A single click on an expanded time scale showing that, in hand-held males, the clicks do not consist of double components (cf. Fig. 3).

revealed that almost all the clicks consisted of two components, each approximately 0.4 ms in duration (Fig. 3C,D).

We did not measure the distance to the free-flying clicking males and thus could not empirically measure the emitted intensity of the clicks. However, in spite of their short duration, the clicks were clearly audible to humans at distances in excess of 50 m in still air. A conservative sound pressure level at 10 cm can be estimated using the human threshold at 10 kHz of 20 dB SPL for one of the investigators (A.S.). The attenuation over the distance from 10 to 50 m by geometrical spreading loss and atmospheric attenuation (approximately 0.1 dB m^{-1} at 10 kHz) is approximately 60 dB. The click spectra show that the peak pressure at 13–15 kHz is approximately 2–10 dB above the value at 10 kHz. Assuming further that the threshold for short clicks (less than 1 ms) is at least 20 dB higher than for continuous tones, because integration time for humans is approximately 100–500 ms, we add 60–80 dB to the human threshold of 20 dB SPL to obtain a conservative estimate of the emitted click intensity of 80–100 dB SPL at 10 cm.

Hand-held males

The duration and spectra of the clicks from hand-held males were generally similar to those of clicks from flying males. In most cases, hand-held males clicked with quite regular intervals (Fig. 5), at an average of 77.6±18.2 ms (mean ± S.D., N=78 clicks, n=6 males). Usually, the hand-held males produced clicks consisting of only one component (Fig. 5). However, when both wings were intact, hand-held males occasionally produced two clicks per wingbeat cycle, either in the form of double clicks resembling those produced by free-flying males or with an extra click at irregular intervals (e.g. a click in the middle of an approximately 70 ms interval). We occasionally observed clicks being produced during short jerks of a single forewing, while the other wing was immobilized during the manipulation.

Ablation experiments on the hand-held males also indicated that each wing is capable of producing a click. If both wings were operated on, a very soft click could still be recorded, but the relative click amplitude was reduced by 20–30 dB. If only the vein in one wing was cut, many of the males still produced clicks with normal intensity.

Morphology of Vogel's organ

Peripheral projections of the mesothoracic wing nerve IIN1c

The mesothoracic nerve IIN1 leaves the anterior and lateral edge of the pterothoracic ganglion where the latter joins the pro-mesothoracic connectives (Fig. 6A). The first branch of IIN1, IIN1a, arises at the ganglion base of the nerve trunk and travels anteriorly to innervate the intersegmental muscle. The second branch, IIN1b, continues in a dorsal and posterior direction and supplies the dorsolongitudinal muscles and sensory structures of the posterior mesothorax. The main branch, IIN1c, contains the sensory neurons that innervate the base of the forewing and Vogel's organ.

IIN1c is a thick nerve that wraps around the anterior edge of the dorsoventral flight musculature and proceeds laterally to pass under the tegular arm (Fig. 6A), which is continuous with the pleural wing process (Fig. 6B). Immediately after passing under the tegular arm, a fine branch from IIN1c extends anteriorly to innervate the tegula. The main nerve then divides into three branches named, from anterior to posterior, N.I, N.II and N.III after Vogel (1911, 1912). N.I, the first of the three primary branches to leave the main nerve, projects anteriorly and divides into two branches before it enters the anterior wing veins. No chordotonal organs were detected in this branch. N.II is the largest of the three primary IIN1c branches and projects dorsolaterally towards the wingbase, where it supplies the large chordotonal organ COII (named after the nerve from which it originates) (Fig. 6B). Proximally, this branch is anchored to the internal side of a dorsal wingbase sclerite and descends to the ventral surface of the wing to COII. The chordotonal organ attaches *via* an attachment strand to a sclerite on the distal, or upper, half of the tympanal membrane (further description of

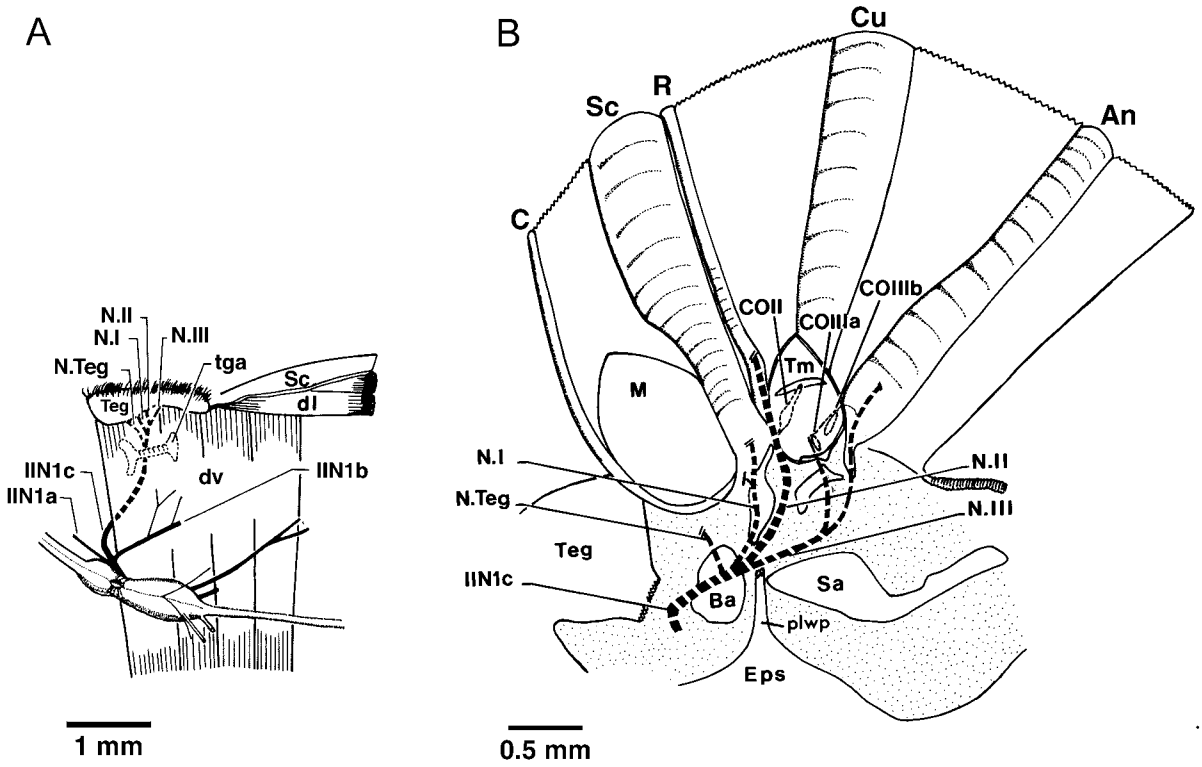

Fig. 6. Schematic diagrams of the peripheral projections of mesothoracic wing nerve IIN1c in *Hamadryas feronia*. (A) The right half of the mesothorax viewed from the midline, showing nerve root IIN1 and its branches a, b and c. The largest branch, IIN1c projects laterally, around the anterior edge of the dorsoventral flight musculature (dv), and passes under the tegular arm (tga). It then sends a fine branch (N.Teg) to the tegula (Teg) before dividing into three branches: N.I, the most anterior branch; N.II, the middle branch; and N.III, the most posterior branch. Dashed lines illustrate nerves and structures lying behind the dorsoventral flight musculature. Sc, Scutum; dl, dorsolongitudinal musculature. (B) Lateral view of the left mesothorax (anterior is to the left), with scales removed. The three primary wing nerve branches (lying beneath the integument) are shown: N.I innervates the anterior wing veins; N.II innervates chordotonal organ II (COII), which attaches to the inner surface of the tympanal membrane (Tm) of Vogel's organ and continues up the radial (R) vein; N.III innervates two smaller chordotonal organs, COIIIa and COIIIb, associated with the inner surface of the proximal edge of the Tm. An, anal vein; Ba, basilar sclerite; C, costal vein; Cu, cubital vein; Eps, episternum; M, membranous ampulla; plwp, pleural wing process; Sa, subalar plate; Sc, subcostal vein.

COII follows). The remainder of N.II continues up the wing, supplying the cubital and radial veins.

N.III, the smallest of the three branches, follows a dorsal and posterior course after leaving the main branch and immediately splits into two branches (Fig. 6B). One climbs dorsally and innervates a small chordotonal organ (COIIIa) estimated to have between 10 and 15 scolopale caps. This chordotonal organ appears to be anchored dorsally to a cuticular wing base

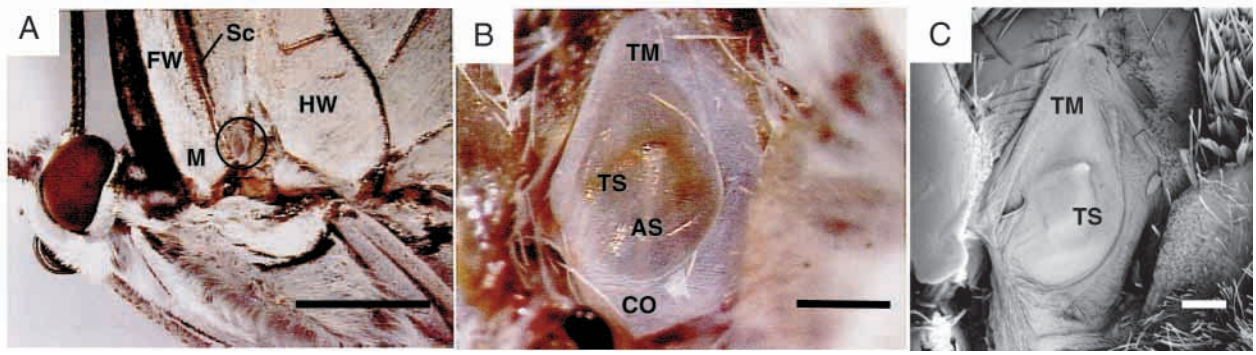

Fig. 7. The external morphology of Vogel's organ in *Hamadryas feronia*. (A) A view of the ventral surface of the forewing (FW), hindwing (HW), membranous ampulla (M) and subcostal vein (Sc). Vogel's organ (circled) lies at the base of the cubital vein (hidden by the hindwing). Scale bar, 3 mm. (B) Close-up of Vogel's organ. The crescent-shaped tympanal sclerite (TS) lies across the upper half of the opaque tympanic membrane (TM). Internally, the chordotonal organ (CO, hidden) projects its attachment strand (AS) to the center of the tympanal sclerite. Scale bar, 250 μm. (C) Scanning electron micrograph of Vogel's organ. Scale bar, 100 μm.

sclerite, and ventrally it is associated with tracheal tissue located against the inner proximal region of the tympanal membrane. The exact relationship between COIIIa and the tympanal membrane (i.e. whether it attaches directly or indirectly) was not determined, since the dissection necessitated the removal of tracheal tissue that was associated with the chordotonal organ, so that the chordotonal organ might have been detached from its attachment site during the dissection procedure. The second branch of N.III lies posterior to the first branch. It sends a very thin branch to a second chordotonal organ, COIIIb, estimated to have between 12 and 30 sensilla, and attaches to the soft membranous region of the inner proximal edge of the tympanal membrane. In two preparations, there appeared to be a connection between COIIIa and COIIIb. However, we cannot be certain if this is a supportive strand or nervous tissue. The remainder of N.III continues up the anal vein of the forewing.

External anatomy of the tympanal region and histology of COII

Since we recorded auditory responses from N.II only, we have limited our histological examinations to COII. The external features of Vogel's organ of *H. feronia* are illustrated in Fig. 7. The tympanic membrane is a large, tear-drop-shaped cuticular structure situated at the base of the forewing cubital vein facing obliquely forwards (Fig. 7A). In fresh specimens, the tympanal membrane is taut and dome-shaped with a tympanal sclerite 15–17 μm (*N*=1 butterfly) thick near to its center (Fig. 7B,C). An attachment strand runs from the tympanal sclerite for 233.4–442.8 μm (*N*=2) to the chordotonal organ, COII, which sits within an air sac at the base of the cubital vein. The attachment strand is 22.5–37.0 μm wide (28.6±6.2 μm, mean ± S.D., *N*=4) at its maximum diameter.

Fig. 8A shows a longitudinal section of the COII along with a portion of the attachment strand. The attachment strand attaches proximally to the center of COII where it meets 110–178 sensilla (139.8±35.0, mean ± S.D., *N*=4) (sensillar numbers were estimated from reconstructed sections by counting scolopale caps, there being one cap per neuron). The sensilla are organized in concentric rings around the attachment strand with layers of scolopale caps visible approximately 12 μm apart. The maximum diameter of the body of COII is 173.9–201.2 μm (194.4±13.7 μm, mean ± S.D., *N*=4), and its length (excluding the attachment strand) is 297.8 μm (*N*=1). COII is fastened dorsally to a wingbase sclerite where its neurons leave as the chordotonal nerve (N.II), which is 67.6 μm (*N*=1) thick at its exit point from the tympanic air sac. Fig. 8B shows a cross section of the attachment strand with a distal portion of COII. In this figure, the attachment strand is ringed by sensilla, each of which directs its scolopale rods and attachment caps in towards the attachment strand.

A second ventral membrane, the membranous ampulla (Figs 6B, 7A), is covered with scales and sits between the anterior edge of the forewing and the base of the subcostal vein. The membrane is soft and flexible, and it appears to be connected to Vogel's organ by tracheae, since the ampulla bulges outwards when the tympanal membrane in living or freshly killed specimens is pushed lightly inwards with forceps. No sensory organs associated with this membrane were revealed in our gross dissections of wing nerves or in histological examinations.

Physiology of Vogel's organ

Audiograms were determined from extracellular, whole-nerve recordings from seven male *H. feronia*. Thresholds, estimated when compound action potential amplitudes were twice the background activity of eight sweep-averaged traces, revealed a best frequency of 1.75 kHz at a threshold of 68 dB SPL, increasing to 90 dB SPL for frequencies above 6 kHz (Fig. 9A). Thresholds were between 5 and 15 dB SPL lower (approximately 58 dB SPL) for three additional audiograms determined by listening to nerve activity and recording threshold when auditory activity was 'just

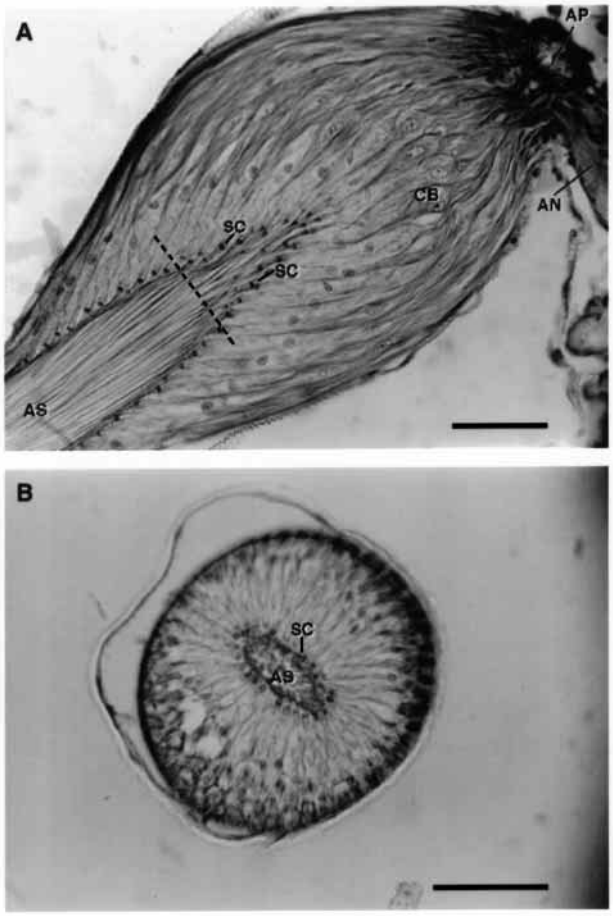

Fig. 8. (A) Longitudinal section of chordotonal organ II (COII) at its attachment point (AP) to the wingbase. The sensory cell bodies (CB) form the body of COII, projecting proximally into the auditory nerve (AN) of N.II and distally into the scolopidia (with their characteristic scolopale caps, SC), which terminate in the attachment strand (AS). Scale bar, 50 μm. (B) Cross section of COII at the approximate point of the dashed line in A. The attachment strand (most of which is not in the plane of the section) occupies a central position, surrounded by scolopidia. Scale bar, 50 μm.

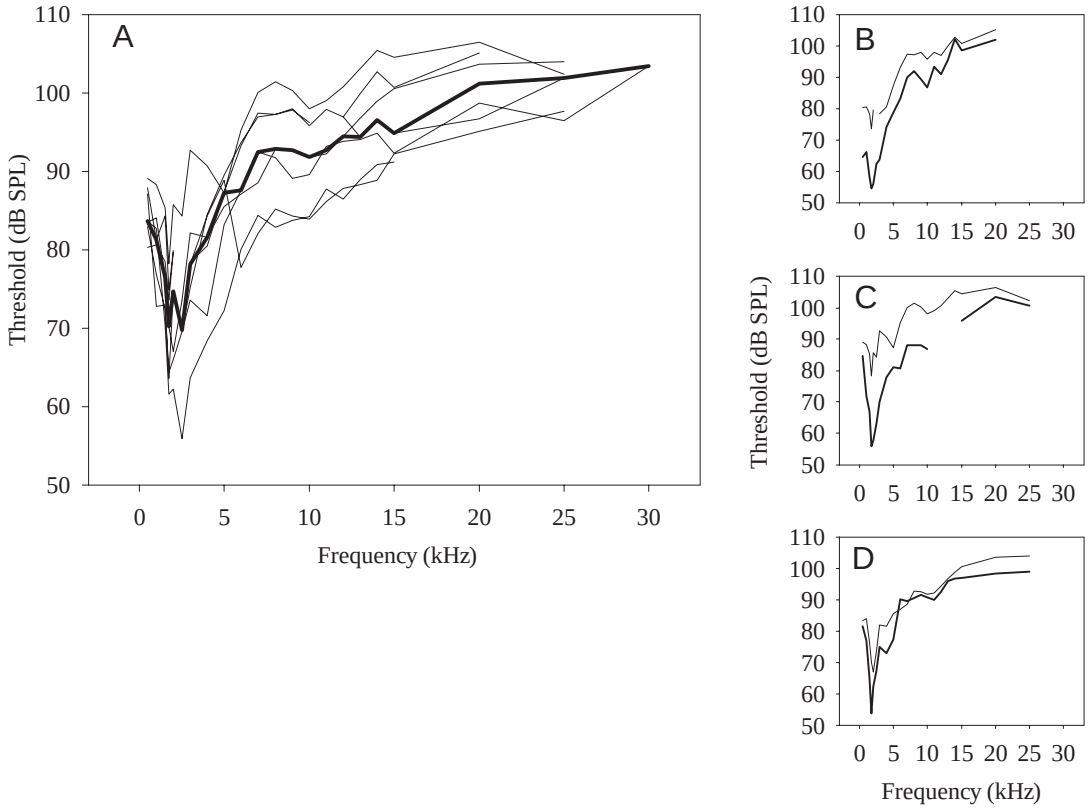

Fig. 9. Audiograms from seven *Hamadryas feronia* determined from extracellular whole-nerve recordings of N.II. (A) Median thresholds (bold line) and individual thresholds (thin lines) estimated from sweep-averaged oscilloscope traces when auditory-evoked compound action potentials were twice the amplitude of background activity. (B–D) Audiograms from three (of the original seven) *H. feronia* estimated by listening to the nerve activity and noting the threshold when activity synchronized with the stimulus pulses was just noticeable (bold lines). The thin lines are the respective individual audiograms from A.

noticeable' (Fig. 9B–D). Mean response latencies elicited by lower- (0.5–5 kHz) and higher- (5 kHz and above) frequency sounds were 6.8 ms (N=4) and 3.65 ms (N=5) respectively.

Discussion

Most butterflies (Rhopalocera, Lepidoptera) are known to possess keen visual and chemical senses that are used for intraspecific communication, for predator detection and for localizing food or oviposition sites (Boppré, 1984; Chew and Robbins, 1984; Silberglied, 1984). However, a sense of hearing, or the ability to produce sounds, appears to be an exception to the normal condition for butterflies. Of the few reported examples of sound production in butterflies, the conspicuous 'clacks' of *Hamadryas* spp. are the most commonly cited. Sound production has been noted primarily in males (and sometimes in females; e.g. Swinton, 1877b; Monge-Nágera et al., 1998) of several, but not all, species (Scott, 1986). Other reported cases of sound production in butterflies include that of the peacock butterfly *Inachis* (=*Nymphalis*=*Vanessa*) *io* (Nymphalidae, Nymphalinae) (Swinton, 1877a; Stainton, 1888; Wilks, 1889; Salisbury, 1940; Møhl and Miller, 1976), *Euvanessa antiopa*, *Aglais urticae* and *A. polycholoros* (Dumortier, 1963), *Pharneuptychia nr. pharnabazos* (Nymphalidae, Satyrinae) (Kane, 1982) and *Neptis hylas* (Nyphalidae) (Scott, 1968). In most of these cases, neither the functional significance nor the mechanism of sound production was investigated.

Reports of hearing in butterflies are fewer than those for sound production. This is partially due to the inherent difficulty in defining what constitutes a functional sense of hearing (see Yack and Fullard, 1993). In addition to the reports mentioned in the Introduction for *Hamadryas* species, behavioural responses to sounds in other butterflies include *Cercyonis pegala* and *C. alope* (Nymphalidae, Satyrinae) responding to intense low-frequency sounds (approximately 500 Hz to 10 kHz; >100 dB) by briefly flicking their antennae or wings, extending their proboscis or taking flight (Frings and Frings, 1956), and a similar type of response in *Heliconius erato* (Nymphalidae, Heliconiinae) (Swihart, 1967), *Erebia euryale* and *E. manto* (Nymphalidae, Satyrinae) (Ribaric and Gogala, 1996), but with lower response thresholds (approximately 75 dB SPL for *E. euryale* and 58 dB SPL for *E. manto*). To the best of our knowledge, there are no reports of sound emission for any of these species, and it is therefore unlikely that 'hearing' is involved in intraspecific communication. Ribaric and Gogala (1996) suggested that hearing functions in predator avoidance but, without experimental evidence, the significance

of these behavioural responses to sound is unknown. A single study (Swihart, 1967) has investigated neurophysiological responses to sound in two species of butterfly. In *H. erato*, the hindwing nerve IIIN1c responded to a best frequency of 1.2 kHz at a threshold of 61 dB, and it was argued that a small membranous sac located near the base of the hindwing was probably the site of sound reception. The forewing nerve IIN1c of *H. feronia* responded with an acoustic sensitivity similar to that of *H. erato*. For both nerve recordings, the frequency range investigated (Swihart, 1967) was limited to 0.4–5.0 kHz.

Although each of these cases of behavioural or physiological responses to sound in butterflies could indeed reflect an adaptive sense of hearing, each requires further study to demonstrate such. It is possible, for example, that those demonstrating motor responses to sounds at or exceeding 75 dB could be non-adaptive motor responses elicited by proprioceptors or vestigial hearing organs responding to cuticular vibrations (Yack and Fullard, 1993). For *H. feronia*, we believe that the present study provides sufficient evidence to argue for a functional sense of hearing, used for intraspecific communication, first by showing that sounds are used regularly in a social context, and second by showing that these butterflies possess typical tympanal hearing organs, the sensory cells of which are broadly sensitive to conspecific sounds. Although a mismatch exists between the best frequencies of Vogel's organ and the peak frequencies of the social sounds, the butterflies appear to be capable of hearing them at natural intensities.

Sound production

There is considerable speculation, based upon behavioural or anatomical observations, about the structures and mechanisms involved in sound production for *Hamadryas* butterflies (for reviews, see Otero 1990a; Monge-Nájera et al., 1998). Two studies have tested the problem experimentally, both concluding that sound production is associated with the thickened veins that close the discal cell of the forewing and that the mechanism is probably percussive, involving the two wings hitting one another during flight (Otero, 1990a; Monge-Nájera et al., 1998).

Although we cannot conclude from our study the exact mechanism of sound production, we can exclude the possibility that the sounds are produced by percussion because of our observation that sounds can be produced by a single forewing. Furthermore, our detailed analyses of individual clicks in free-flying males show that each click is composed of two components, suggesting that each perceived click is actually the clicking of both wings. Thus, we find it more likely that *H. feronia* produces clicks by buckling stiff mechanically bi-stable portions of the wings in a way similar to *Inachis io* (Møhl and Miller, 1976). It is interesting to note that photographic images of wing movements during sound production (see Fig. 10 in Monge-Nájera et al., 1998) show that the wings seem to 'flip-flop' from a straight to a concave form, which is also in accordance with the hypothesis of a wing-vein buckling mechanism.

Our observation that clicks of free flying males were produced in trains with inter-click intervals of approximately 61 ms suggests a click frequency of approximately 16 Hz, a value in line with the wingbeat frequencies reported for *H. feronia* and *H. amphimone* (Monge-Nágera et al., 1998). This suggests, in agreement with these authors, that the sounds are being produced during a particular phase of the wingbeat cycle. In our study, the mean click interval was slightly longer for hand-held males (77.6 ms) than for freely flying males (61 ms), perhaps because of the constraints on the forewing movements of holding the hindwings with forceps. The double component of the clicks in free-flying males, as mentioned above, may be explained by the two wings buckling almost simultaneously.

Only one other study has reported on the spectral characteristics of the sounds produced by *Hamadryas* spp. (Monge-Nágera et al., 1998). This report described the sounds as clicks 1.4 ms long with peak energies at 2 kHz and inter-click intervals of approximately 44 ms. There was no mention of click trains. The mean inter-click intervals are in reasonable agreement with our results (see Fig. 11c in Monge-Nájera et al., 1998), and differences may be accounted for by different sample sizes, species examined for sound analyses and whether free-flying or hand-held individuals were used for sound analyses (neither of the last two factors was specified in the study of Monge-Nágera et al., 1998). However, our results on the fine time structure and frequency spectra of the clicks do not agree with theirs. They report longer click durations (1.4 ms) with a much lower peak frequency (around 2.4 kHz) than we found. Monge-Nájera et al. (1998) digitized their recordings at a sample rate of 11 kHz. Hence, they could not analyze any frequency components above 5.5 kHz (Nyquist frequency). Even more important is the fact that, apparently, an anti-aliasing filter was not used. Hence, if there were any signal energy at frequencies above 5 kHz (as demonstrated by our analyses), those frequencies would alias ('fold down'), as a result of under-sampling, and obstruct the spectrum below 5 kHz and also obscure the fine temporal structure. Our high sampling rate (300 kHz) allowed us to detect frequency components up to 150 kHz and provided fine temporal resolution. We believe, therefore, that the short click durations, double click shape of most clicks and peak frequencies around 13–15 kHz that we report in the present paper represent a more accurate description of the clicks of *Hamadryas feronia*.

Anatomy

On the basis of our anatomical and physiological studies, we propose that the neurological site of sound reception in *H. feronia* is Vogel's organ, not the membranous ampulla as suggested previously (Swihart, 1967; Scott, 1986; Monge-Nájera et al., 1998), since no chordotonal organs were found to be associated with the membranous ampulla in *H. feronia* or in other species (Vogel, 1912). It is possible, however, that the membranous ampulla could serve as an accessory organ transmitting vibrational stimuli to Vogel's organ *via* tracheal interconnections.

The external anatomy and taxonomic distribution of Vogel's organ in nymphalid butterflies has been described by Vogel

(1912), Le Cerf (1926), Bourgogne (1951), Otero (1990b), Monge-Nájera and Hernández (1991), Ribaric and Gogala (1996) and Monge-Nájera et al. (1998), but only Vogel (1912) provides specific details concerning its innervation and internal anatomy. Vogel (1912) describes the peripheral projections of the forewing nerve in the satyrid *Epinephete jurtina* which, like *H. feronia*, possesses a Vogel's organ at the base of the cubital vein. The general distribution of IIIN1c (=V.N. of Vogel) nerve branches is similar in the two species. In *E. jurtina*, the most anterior nerve branch (N.I) supplies the costal and frontal wing veins, including a large group of sensory structures ('domes', which are probably campaniform sensilla) on the lower surface of the wing. As we observed in *H. feronia*, no chordotonal organs are associated with N.I. In *E. jurtina*, the middle nerve, N.II, is the largest branch and supplies two chordotonal organs ('Ch.O.A' and 'Ch.O.B'), each consisting of 12–40 sensilla. In *H. feronia*, N.II is also the largest of the three branches, but innervates just one chordotonal organ (COII) which is substantially larger (more than 110 sensilla) than either of those described for *E. jurtina*. Finally, in *E. jurtina*, the most posterior nerve branch (N.III) innervates a single chordotonal organ ('Ch.O.C') consisting of 12–40 sensilla, while in *H. feronia* this branch innervates two chordotonal organs (COIIIa and COIIIb), each with 10–30 sensilla. On the basis of anatomical similarities, we suggest that the three primary branches of forewing nerve IIIN1c in *H. feronia* are homologous to those in *E. jurtina*, since each branch innervates the same forewing veins in both species. Apparent differences between the two species include the numbers of chordotonal organs supplied by both N.II and N.III and the estimated number of cells observed in each chordotonal organ. Differences in the numbers of chordotonal organs probably reflect varying degrees of separation or fusion of chordotonal organ groups.

Physiology

Our audiograms, determined from recordings of the N.II branch of IIN1c, are in accord with recordings from the whole IIN1c branch in *H. feronia* made by Swihart (1967). Since N.II innervates COII, we are able to confirm that it is Vogel's organ, and not the membranous ampulla, that is the source of the auditory response in *H. feronia*. We can also exclude the possibility, on the basis of on our audiograms, that Vogel's organ is an ultrasound-sensitive ear used for bat detection, as proposed by Monge-Nájera et al. (1998).

We found a clear mismatch between the best frequency of the nerve recordings, at 1.75 kHz, and the mean peak frequency of the sounds produced (14.4 kHz), representing a reduction in sensitivity of approximately 20 dB during acoustic interactions. In most reported cases of intraspecific auditory communication in insects, hearing is obviously matched to species-specific sounds (eg. Cicadidae, Simmons et al., 1971; Huber et al., 1980, 1990; Popov, 1981; Orthoptera, Nocke, 1972, 1975; Field et al., 1980; Libersat et al., 1994; Meyer and Elsner, 1996; Lepidoptera, Surlykke and Gogala, 1986; Skals and Surlykke, 1999). In some cases, however, a mismatch has been reported between the frequency of the calling song and the best frequency of hearing determined from whole-nerve recordings (e.g. Popov, 1981; Popov et al., 1985; Bailey and Römer, 1991; Mason, 1991; Meyer and Elsner, 1996; Römer and Bailey, 1998; Fonseca et al., 2000).

Assuming that *H. feronia* is using sounds in a behavioural context, we offer the following possible explanations for the perceived mismatch. First, it could be argued, in support of the proposal of Mason et al. (1999), that the mismatch is due to the method used for determining our audiogram, which was generated from whole-nerve extracellular recordings. Whole-nerve multi-unit recordings are generally conservative in estimating auditory thresholds. The diameter of individual axons and their conduction velocities are two interacting factors that affect the amplitude of a summed action potential. It is possible that there are sensory cells that are either tuned to higher frequencies or broadly tuned to include both low and high frequencies, but that their responses are over-shadowed by a larger number of sensory cells responding only to low frequencies. This appears to explain the mismatch between the calling songs and auditory sensitivities of both *Cyphoderris monstrosa* (Orthoptera) (Mason, 1991; Mason et al., 1999) and *Tettigetta josei* (Fonseca et al., 2000). Second, Vogel's organ possesses three chordotonal organs, and it is possible that the different organs are sensitive to different sound frequencies, analogous to the multi-receptive fields of the locust Muller's organ (Michelsen, 1971a,b; Jacobs et al., 1999). We recorded from N.II, which receives projections only from the largest of the three organs, COII and, therefore, we may have been recording only part of the frequency range sensed by the tympanum. A third possible explanation for the auditory mismatch is that, during the audiogram procedure, the physiological conditions are different from those when the butterfly is flying and engaged in an interaction. It is possible that the tuning characteristics of the ear are determined, or affected by, pressures in the tracheae of the wings or the membranous ampulla or by the opened or closed state of the spiracles, all of which may be different during flight or may be under muscular control by the animal (see Römer and Bailey, 1998).

Regardless of the reasons for the auditory mismatch, the fact remains that there is sufficient energy in the sounds produced that Vogel's organ of *H. feronia* can still detect the sounds when individuals are in close proximity. A clearly documented feature of the behavioural interactions between *Hamadryas* spp. individuals is that they click while in pursuit and that the distances between individuals during pursuit is quite small, typically less than 10 cm. The sound intensity was estimated to be between 80 and 100 dB SPL at 10 cm. The spectrum shows that the energy at the best frequency of the ear, 1.75 kHz, is around 20 dB lower than the peak power frequency of the spectrum (approximately 14 kHz). Thus, at 1.75 kHz, the sound level is still well above the auditory threshold (68 dB SPL). Because of energy integration time (Tougaard, 1996), the thresholds for the 0.5 ms clicks may be up to 10 dB higher than for the 5 ms pulses we used for the audiograms. However, both

auditory thresholds and click intensity estimates were quite conservative.

A question of butterfly hearing

Our results suggest that male *H. feronia* are capable of hearing conspecific sounds and that they do so using Vogel's organ, which structurally resembles the tympanal ears of other insects. Since sounds are produced predominantly during social interactions, we further argue that, in this species of butterfly, sounds are probably involved in conspecific communication. We wish to caution, however, that our findings do not necessarily apply to other butterflies that possess such structures.

Current evidence suggests that Vogel's organ is found exclusively within the Nymphalidae. The structure is widespread throughout this large family, but its degree of development ranges from 'not well defined' (as in the Preponini of the Charaxinae, Amathusiini of the Morphinae) to very elaborate in the Eurytelinae (=Biblidini, Harvey, 1991; =Limenitinae, Ackery et al., 1998), where *Hamadryas* belongs, and in the Satyrinae (Otero, 1990b). According to recent arrangements (Harvey, 1991; Ackery et al., 1998), the presence of Vogel's organ would be interpreted as a homoplasic character appearing in different butterfly lineages. However, it seems that a consistent picture of relationships among nymphalid groups is still far from clear, and no definite assertion can be made with regard to the non-homologous status of Vogel's organ.

A superfamily of nocturnal moth-like butterflies, the Hedyloidea (Scoble, 1986), has been shown to possess a forewing tympanum that is ultrasound-sensitive and functions to elicit in-flight evasive behaviours similar to the bat-avoidance maneuvers of nocturnal moths (Yack and Fullard, 2000). Even though the hedylid ear differs from the nymphalid Vogel's organ in external appearance, there are several similarities in location and structure (with respect to wing venation and innervation) that suggest that the hedylid ear is homologous to Vogel's organ. Considering the current taxonomic placement of the Hedyloidea as a basal clade to the other butterfly superfamilies (Papilionoidea, Hesperoidea) (Kristensen and Skalski, 1998), it is possible that Vogel's organ represents a degenerate bat detector. Given this scenario, it may be that *Hamadryas* spp., and possibly other members of the Nymphalidae, have regained a sense of hearing at low frequencies and, concurrently or secondarily, evolved a means of sound production for communication purposes. The idea of a 'degenerate bat detector' is speculative, but it is corroborated by the changes in hearing found in diurnal moths. Two distantly related groups of moth, the Dioptinae (Notodontidae) (Fullard et al., 1997) and Archiearinae (Geometridae) (Surlykke et al., 1998), that presumably secondarily became diurnal, show the same basic change in their hearing, namely a loss of high-frequency hearing and tuning to lower frequencies. Comparative studies on the anatomical and physiological characteristics of Vogel's organ and its homologues will help resolve questions about its functional role and about the evolutionary history of hearing in butterflies.

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References

Ackery, P. R., de Jong, R. and Vane-Wright, R. I. (1998). The butterflies: Hedyloidea, Hesperoidea and Papilionoidea. In *Handbook of Zoology*, bol. 4. *Arthropoda: Insecta. Lepidoptera, Moths and Butterflies*, vol. 1, *Evolution, Systematics and Biogeography* (ed. N. P. Kristensen), pp. 263–300. Berlin: Walter de Gruyter.

Bailey, W. J. and Römer, H. (1991). Sexual differences in auditory sensitivity: mismatch of hearing threshold and call frequency in a tettigoniid (Orthoptera, Tettigoniidae: Zaprochilinae). *J. Comp. Physiol.* A **169**, 349–353.

Boppré, M. (1984). Chemically mediated interactions between butterflies. In *The Biology of Butterflies* (ed. R. I. Vane-wright and P. R. Ackery), pp. 259–275. Orlando, FL: Academic Press.

Bourgogne, J. (1951). Ordre des Lépidoptères. In *Traité de Zoologie*, *Insectes Supérieurs et Hémiptéroïdes*, vol. 10 (ed. P. P. Grassé), pp. 174–448. Paris: Masson Ed.

Chauthani, A. R. and Callahan, P. S. (1966). A dissection technique for studying internal anatomy of different stadia of Noctuidae. *Ann. Ent. Soc. Am.* **59**, 1017–1018.

Chew, F. S. and Robbins, R. K. (1984). Egg laying in butterflies. In *The Biology of Butterflies* (ed. R. I. Vane-wright and P. R. Ackery), pp. 65–79. Orlando, FL: Academic Press.

Collenette, C. C. (1928). An *Ageronia* responding to a noise made by birds. *Ent. Month. Mag.* **64**, 178–179.

Darwin, C. R. (1874). *The Descent of Man.* New York: Prometheus Books.

DeVries, P. J. (1987). *The Butterflies of Costa Rica and Their Natural History*, vol. I, *Papilionidae, Pieridae, Nymphalidae.* Princeton: Princeton University Press.

Dumortier, B. (1963). Morphology of sound emission apparatus in Arthropoda. In *Acoustic Behavior in Animals* (ed. R. G. Busnell), pp. 277–345. Amsterdam: Elsevier.

Field, L. H., Hill, K. G. and Ball, E. E. (1980). Physiological and biophysical properties of the auditory system of the New Zealand weta *Hemideina crassidens* (Blanchard 1851) (Ensifera: Stenopelmatidae). *J. Comp. Physiol.* **141**, 31–37.

Fonseca, P.J., Münch, D. and Hennig, R.M. (2000). How cicadas interpret acoustic signals. *Nature* **405**, 297–298.

Frings, H. and Frings, M. (1956). Reactions to sounds by the wood nymph butterfly, *Cercyonis pegala*. *Ann. Ent. Soc. Am.* **49**, 611–617.

Fullard, J. H., Dawson, J. W., Otero, L. D. and Surlykke, A. (1997). Bat-defenses in day-flying moths (Lepidoptera, Notodontidae, Dioptinae). *J. Comp. Physiol.* A **181**, 477–483.

Harvey, D. J. (1991). Higher classification of the Nymphalidae. In *The Development and Evolution of Butterfly Wing Patterns* (ed. H. F. Nijhout), pp. 255–273. Washington: Smithsonian Institution Press.

Hoy, R. R. and Robert, D. (1996). Tympanal hearing in insects. *Annu. Rev. Ent.* **41**, 433–450.

Huber, F., Moore, T. E. and Loher, W. (1990). *Cricket Behavior and Neurobiology*. Ithaca: Cornell University Press.

Huber, F., Wohlers, D. W. and Moore, T. E. (1980). Auditory interneurone responses to natural sounds in several species of cicadas. *Physiol. Ent.* **5**, 25–45.

Jacobs, K., Otte, B. and Lakes-Harlan, R. (1999). Tympanal receptor cells of *Schistocera gregaria*: Correlation of soma positions and dendrite attachment sites, central projections and physiologies. *J. Exp. Zool.* **283**, 270–285.

Jenkins, D. W. (1983). Neotropical Nymphalidae. I. Revision of *Hamadryas. Bull. Allyn Mus.* **81**, 1–146.

Kane, S. (1982). Notes on the acoustic signals of a neotropical satyrine butterfly. *J. Lepidopt. Soc.* **36**, 200–206.

Kristensen, N. P. and Skalski, A. W. (1998). Phylogeny and palaeontology. In *Handbook of Zoology*, bol. 4, *Arthropoda: Insecta. Lepidoptera, Moths and Butterflies*, vol. 1, *Evolution, Systematics and Biogeography* (ed. N. P. Kristensen), pp. 7–25. Berlin: Walter de Gruyter.

Le Cerf, F. (1926). Contribution à l'étude des organes sensoriels des Lépidoptères. *Encyclop. Ent.* **3**, 133–146.

Libersat, F., Murray, J. A. and Hoy, R. R. (1994). Frequency as a releaser in the courtship song of two crickets, *Gryllus bimaculatus* (de Geer) and *Teleogryllus oceanicus*: a neuroethological analysis. *J. Comp. Physiol.* A **174**, 485–494.

Mason, A. C. (1991). Hearing in a primitive ensiferan: The auditory system of *Cyphoderris monstrosa* (Orthoptera: Haglidae). *J. Comp. Physiol.* A **168**, 351–363.

Mason, A. C., Morris, G. K. and Hoy, R. R. (1999). Peripheral frequency mismatch in the primitive ensiferan *Cyphoderris monstrosa* (Orthoptera: Haglidae). *J. Comp. Physiol.* **184**, 543–551.

Meyer, J. and Elsner, N. (1996). How well are frequency sensitivities of grasshopper ears tuned to species-specific song spectra? *J. Exp. Biol.* **199**, 1631–1642.

Michelsen, A. (1971a). The physiology of the locust ear. I. Frequency sensitivity of single cells in the isolated ear. *Z. Vergl. Physiol.* **71**, 49–62.

Michelsen, A. (1971b). The physiology of the locust ear. II. Frequency discrimination based upon resonances in the tympanum. *Z. Vergl. Physiol.* **74**, 63–101.

Miller, T. A. (1979). *Insect Neurophysiological Techniques*. New York: Springer.

Møhl, B. and Miller, L. A. (1976). Ultrasonic clicks produced by the peacock butterfly: a possible bat-repellent mechanism. *J. Exp. Biol.* **64**, 639–644.

Monge-Nájera, J. (1992). Clicking butterflies, *Hamadryas*, of Panama: Their biology and identification (Lepidoptera: Nymphalidae). In *Insects of Panama and Mesoamerica* (ed. D. Quintero and A. Aiello), pp. 567–572. Oxford, New York, Tokyo: Oxford University Press.

Monge-Nájera, J. and Hernández, F. (1991). A morphological search for the sound mechanism of *Hamadryas* butterflies (Lepidoptera: Nymphalidae). *J. Res. Lepidopt.* **30**, 196–208.

Monge-Nájera, J., Hernández, F., González, M.I., Soley, J., Araya, J. and Zolla, S. (1998). Spatial distribution, territoriality and sound production by tropical cryptic butterflies (*Hamadryas*, Lepidoptera: Nymphalidae): Implications for the 'Industrial Melanism' debate. *Rev. Biol. Trop.* **48**, 297–329.

Muyshondt, A. and Muyshondt, A., Jr (1975a). Notes on the life cycle and natural history of butterflies of El Salvador. I B. – *Hamadryas februa* (Nymphalidae-Hamadryadinae). *N.Y. Ent. Soc.* **83**, 157–169.

Muyshondt, A. and Muyshondt, A., Jr (1975b). Notes on the life cycle and natural history of butterflies of El Salvador. II B. – *Hamadryas guatemalena* Bates (Nymphalidae- Hamadryadinae). *N.Y. Ent. Soc.* **83**, 170–179.

Muyshondt, A. and Muyshondt, A., Jr (1975c). Notes on the life cycle and natural history of butterflies of El Salvador. III B. – *Hamadryas amphinome* L. (Nymphalidae- Hamadryadinae). *N.Y. Ent. Soc.* **83**, 181–191.

Nocke, H. (1972). Physiological aspects of sound communication in crickets (*Gryllus campestris* L.). *J. Comp. Physiol.* **80**, 141–162.

Nocke, H. (1975). Physical and physiological properties of the tettigoniid (grasshopper) ear. *J. Comp. Physiol.* **100**, 25–57.

Nüesch, H. (1953). The morphology of the thorax of *Telea polyphemus* Cr. (Lepid.) I. Skeleton and muscles. *J. Morph.* **93**, 589–608.

Nüesch, H. (1957). Die Morphologie des Thorax von *Telea polyphemus* Cr. (Lepid.) II. Nervensystem. *Zool. Jb. Abt. Anat.* **75**, 615–642.

Otero, L. D. (1988). Contribución a la Historia Natural del género *Hamadryas*. Tesis doctoral, Universidad Central de Venezuela. Facultad de Agronomía, Instituto de Zoología Agrícola, Maracay. 108pp.

Otero, L. D. (1990a). The stridulatory organ in *Hamadryas* (Nymphalidae): Preliminary observations. *J. Lepidopt. Soc.* **44**, 285–288.

Otero, L. D. (1990b). Estudio de algunos caracteres para su uso en la clasificación de Eurytelinae (Lepidoptera: Nymphalidae). *Bol. Ent. Venez.* **5**, 123–138.

Pantin, C. F. A. (1946). *Notes on Microscopical Techniques for Zoologists.* Cambridge: Cambridge University Press.

Popov, A. V. (1981). Sound production and hearing in the cicada, *Cicadetta simuatipennis* Osh. (Homoptera, Cicadidae). *J. Comp. Physiol.* A **142**, 271–280.

Popov, A. V., Aronov, I. B. and Sergeeva, M. V. (1985). Calling songs and hearing in cicadas from Soviet Central Asia. *Zh. Evolyut. Biokhim. Fiziolog.* **21**, 451–562.

Ribaric, D. and Gogala, M. (1996). Acoustic behaviour of some butterfly species of the genus *Erebia* (Lepidoptera: Satyridae). *Acta Ent. Slov.* **4**, 5–12

Römer, H. and Bailey, W. (1998). Strategies for hearing in noise: peripheral control over auditory sensitivity in the bushcricket *Sciarasaga quadrata* (Austrosaginae: Tettigoniidae). *J. Exp. Biol.* **201**, 1023–1033.

Rydon, A. (1964). Notes on the use of butterfly traps in East Africa. *J. Lepidopt. Soc.* **18**, 51–58.

Salisbury, E. J. (1940). Stridulation in *Nymphalis io*. *Ent. Month. Mag.* **76**, 117.

Scoble, M. J. (1986). The structure and affinities of the Hedyloidea: a new concept of the butterflies. *Bull. Brit. Mus. Nat. Hist.* **53**, 251–286.

Scoble, M. J. (1995). *The Lepidoptera, Form, Function and Diversity*. New York: Oxford University Press.

Scott, F. W. (1968). Sounds produced by *Neptis hylas* (Nymphalidae). *J. Lepidopt. Soc.* **22**, 254.

Scott, J. A. (1986). *The Butterflies of North America.* Stanford: Stanford University Press.

Silberglied, R. E. (1977). Communication in the Lepidoptera. In *How Animals Communicate* (ed. T. A. Sebeok), pp. 362–402. Bloomington: Indiana University Press.

Silberglied, R. E. (1984). Visual communication and sexual selection among butterflies. In *The Biology of Butterflies* (ed. R. I. Vane-Wright and P. R. Ackery), pp. 207–223. Orlando, FL: Academic Press.

Simmons, J. A., Wever, E. G. and Pylka, J. M. (1971). Periodical cicada: Sound production and hearing. *Science* **171**, 212–213.

Skals, N. and Surlykke, A. (1999). Sound production by abdominal tymbal organs in two moth species: the green silver-line and the scarce silver-line (Noctuoidea: Nolidae: Chloephorinae). *J. Exp. Biol.* **202**, 2937–2949.

Stainton, M. T. (1888). The noise or sound produced by butterflies of the genus *Vanessa*. *Ent. Month. Mag.* **25**, 225.

Strausfeld, N. J., Seyan, H. S., Wohlers, D. and Bacon, J. P. (1983). Lucifer Yellow histology. In *Functional Neuroanatomy* (ed. N. J. Strausfeld), pp. 132–155. Berlin: Springer-Verlag.

Surlykke, A. and Gogola, M. (1986). Stridulation and hearing in the noctuid moth *Thecophora fovea* (Tr.). *J. Comp. Physiol.* A **159**, 267–273.

Surlykke, A., Skals, N., Rydell, J. and Svensson, M. (1998). Sonic hearing in a diurnal geometrid moth, *Archiearis parthenias*, temporally isolated from bats. *Naturwissenschaften* **85**, 36–37.

Swihart, S. L. (1967). Hearing in butterflies. *J. Insect Physiol.* **13**, 469–476.

Swinton, A. H. (1877a). On stridulation in the genus *Vanessa*. *Ent. Month. Mag.* **13**, 169–173.

Swinton, A. H. (1877b). On stridulation in the genus *Ageronia*. *Ent. Month. Mag.* **13**, 207–208.

Tougaard, J. (1996). Energy detection and temporal integration in the noctuid A1 auditory receptor. *J. Comp. Physiol.* A **178**, 669–677.

Vogel, R. (1911). Über die Innervierung der Schmetterlingsflügel und über den Bau die Verbreitung der Sinnesorgane auf Denselben. *Z. Wiss. Zool*. **98**, 68–134.

Vogel, R. (1912). Über die Chordotonalorgane in der Wurzel der Schmetterlingsflügel. *Z. Wiss. Zool.* **100**, 210–244.

Wilks, R. L. (1889). Stridulation in *Nymphalis io*. *Entomologist* **73**, 59.

Yack, J. E. (1993). Janus Green B as a rapid, vital stain for peripheral nerves and chordotonal organs in insects. *J. Neurosci. Meth.* **49**, 17–22.

Yack, J. E. and Fullard, J. H. (1993). What is an insect ear? *Ann. Ent. Soc. Am.* **86**, 677–682.

Yack, J. E. and Fullard, J. H. (2000). Ultrasonic hearing in nocturnal butterflies. *Nature* **403**, 265–266.

Yager, D. D. and Hoy, R. R. (1987). The midline metathoracic ear of the praying mantis, *Mantis religiosa. Cell Tissue Res.* **250**, 531–541.

Young, A. M. (1974). On the biology of *Hamadryas februa* (Lepidoptera: Nymphalidae) in Guanacaste, Costa Rica. *Z. Angew. Ent.* **76**, 380–393.

Young, A. M. and Borkin, S. S. (1985). Natural history notes for some *Hamadryas* butterflies (Nymphalidae: Nymphalinae; Aegeronini) in Northwestern Costa Rica during the tropical dry season. *J. Lepidopt. Soc.* **39**, 229–235.