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ACOUSTIC BEHAVIOUR OF SOME BUTTERFLY SPECIES OF THE GENUS *EREBIA* (LEPIDOPTERA: SATYRIDAE)

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Abstract - During the summers of 1992 and 1993 we investigated the butterfly genus *Erebia* which, during preliminary research, had shown clear behavioural responses to non-specific acoustic stimuli. In this genus sound detection occurs through tympanal organs on the ventral base of the forewings. They react to sound stimuli at frequencies from 125 Hz to 16 kHz, with an escape behaviour comprised of a wing twitch, wing movement forward, partial or total wing opening and closing, fluttering around, and an escape flight. At a frequency of 1000 Hz, the startle response (a behavioural response to unexpected sound stimuli) had its lowest threshold at 49 dB SPL. The mean value for the suprathreshold sound intensity at the lowest frequency tested, 125 Hz, was 58 dB SPL and 68 dB SPL for the highest frequency. These sensitivities are much higher than those of other diurnal butterflies. Butterflies whose tympanal organs were covered with a wax and resin (colophonium) mixture or vaseline did not respond to sound stimuli.

Izvleček - AKUSTIČNO VEDENJE NEKAJ VRST METULJEV IZ RODU *EREBIA* (LEPIDOPTERA: SATYRIDAE)

V sezonah 1992 in 1993 smo raziskovali rod metuljev *Erebia,* ki je v preliminarnih poskusih pokazal jasen vedenjski odziv na nespecifične zvočne dražljaje. Za zaznavanje zvoka imajo ti metulji ventralno, na bazi sprednjega para kril timpanalni organ. V preizkušenem frekvenčnem območju od 125 Hz - 16 kHz so se na zvok odzivali s trzanjem s krili, premikom kril naprej, rahlim ali popolnim odpiranjem in zapiranjem kril, frfotanjem, ali pa so odskočili v zrak in pobegnili. Občutljivost metuljev na nepričakovane zvočne dražljaje je pri frekvenci 1000 Hz največja, saj se odzovejo že pri 49 dB SPL. Srednja vrednost nadpražne jakosti zvoka pri najnižji preizkušeni frekvenci 125 Hz znaša 58 dB SPL, pri najvišji frekvenci 16 kHz pa 68 dB SPL.

Dobljene vrednosti so precej nižje kot pri drugih dnevnikih. Metulji, katerih timpanalne organe smo premazali s kolofonijo ali vazelino, se na zvočne dražljaje niso odzivali.

Introduction

It has been known for some time that most species of moths have tympanal organs which warn the animals of the presence of echolocating bats (Roeder, 1967, cit. in FULLARD, 1984;). We also know that some of these species such as Arctiidae may emit ultrasonic clicks as anti-bat sounds to mislead insectivorous bats who are hunting (MILLER, 1991). In diurnal butterflies hearing and sound production are less well-known, although VOGEL (1912) described the position, morphology, and histology of a tympanal organ situated at the base of the forewings in the satyrid species *Epinephele jurtina* and *Coenonympha pamphilus*. Furthermore, SWIHART (1967) reported on the position of the tympanal organ in nymphalids: in *Heliconius erato* it laid at the base of the hindwings, while in *Ageronia feronia* it was at the base of the forewings. In the summer of 1992, accidental observations on the species *Erebia euryale* and *E. manto* demonstrated unusual motor reactions to air-borne sounds when stimulated with non-specific acoustic stimuli such as the clicks of a camera shutter, whistling and hand claps (M. GOGALA, D. RIBARIČ, 1992).

In the summers of 1992 and 1993 we tried to determine the frequency range and threshold needed to produce these specific motor reactions in *Erebia* butterflies. We also investigated whether they emit any sound signals themselves.

Materials and methods

Methods of sound stimulation

The sound stimuli utilised were pure sinusoidal sounds synthesized on an ATARI MEGA ST 4 computer using the AVALON II (Steinberg) software, and recorded on DAT (Digital Audio Tape). These sounds were than played back from the DAT via a SONY DTC - 57 ES recorder through a Bose Powered Acoustimass - 3-loudspeaker system. A bass module was used to obtain low frequencies. The motor reactions of the butterflies were video-recorded with either a Canon EX 1 Hi 8 or a JVC KY 25 super VHS camera. We used sound stimuli of 125, 250, and 500 Hz and 1, 2, 8, and 16 kHz. Each stimulus was of one second duration, with highest intensity between 70 and 170 ms. They were recorded three times, with a 10 second interval between each. The envelope of the sound stimuli was an ADRS Type (Attack - 110ms; Decay, to 60% of full volume - 90ms; Sustain - 620ms; Release - 180ms). Sound intensity was controlled with an EAL (Electro Acoustic Laboratory, M. Turk; Ljubljana) dB meter. The minimum distance between the loudspeakers and animals was about 1.4 meters. A dB meter with an inbuilt microphone was placed at the same height as the animals, and as near to them as possible, directed towards the loudspeakers. Such positioning allowed direct control of the intensity of the sound stimuli.

Morphological and histological methods

To determine the position and construction of the hearing organ in *Erebia manto* and *E. euryale,* classical morphological and histological techniques were used. Scales and bristles were carefully removed from the tympanal region in order to make morphological preparations for examination by SEM (Scanning Electron Microscopy). Histological preparations of the organ were made by the following procedures: animals were directly placed into Carnoy's fixative in the field. The tympanal organs were then isolated and put through serial alcohols and acetone, prior to being placed into durcopan medium. Sections were cut with a Reichert OM U2 ultramicrotome at 2.5 μm and then stained in an aqueous solution of fuchsin oxide, light green, acid orange, and acetic acid.

Results

Behavioural observations

Throughout the day butterflies usually flew around, making experiments impossible as they immediately flew away in response to any sound; evening appeared to be the best time of day for field experiments. In the evening, butterflies sat in small groups of 2 to 5 individuals on the grass staying there overnight. They responded to stimuli until darkness when the temperature fell to about 17°C. In single animals the response to repeated sounds decreased or almost vanished after 3-5 repetitions, while in a group such habituation was much slower and a response could last for ten minutes or longer. During the experiments, when the butterflies were sitting quietly on the grass, their wings were usually closed. Occasionally, a butterfly had its wings open or its forewings moved forward. Such butterflies responded to the next stimulus only with a wing twitch, or by reclosing their wings, although spontaneous wing closure could not be ruled out. We classified motor reactions into seven categories:

1. slight twitch of closed or open wings
2. forward movement of closed wings
3. partial wing opening and closing
4. total wing opening and closing
5. short-lived wing flinching
6. short fluttering around the spot
7. escape

These categories do not represent levels of response to increasing stimuli, and the distribution of these responses at a given frequency or intensity did not follow any rule or pattern. Environmental factors and the persistance of ongoing behavioural patterns do influence an animal's reactions. However, we can only say that the initial response to a given frequency and intensity was always greater than those to repeated stimuli of the same quality (habituation).

Any of the startle responses listed above were used to determine behavioural thresholds. All reactions were video-documented, allowing us to visually determine "response" or "no response". The behavioural startle response to pure tones for *Erebia manto* is shown in Figure 1.

The best response was at a frequency of 1000 Hz, where the lowest suprathreshold sound intensity was only 49 dB SPL. For the lowest frequency tested, 125 Hz, the mean value for the startle response was 58 dB SPL and for the highest frequency, 16 kHz, was 68 dB SPL. Sometimes the background noise was as loud as the stimulus but it was always in different frequency ranges than those with the best responses. Thus, butterflies only responded to our stimuli or to background stimuli in the same frequency range, such as the calls of cows or the sound of their bells.

We also tested the responses of butterflies with the tympanal organs covered with a wax and resin (colophonium) mixture or vaseline. Such animals did not show any behavioural response to acoustic stimuli.

Morphology and histology of the tympanal organ

In *Erebia* butterflies the tympanal organ lies at the base of the front pair of wings (Figure 2) and consists of the tympanum, the tracheal bubble, and the chordotonal organ.

The size of the tympanal membrane varies from species to species. In *E. euryale* it is about 700 μm long, and about 630 μm in *E. manto.* The width of the tympanum is about 400 μm in *E. euryale* and 320 μm in E. manto. Measurements were only made on two specimens of *E. manto* and one of *E. euryale.* The SEM pictures show the involution of the tympanum in the middle of its maximum width. This is the point of insertion of the chordotonal organ (Figure 3).

Histological sections through the cubital vein show the detailed construction of the organ. The tympanum is a smooth membrane on the underside of the wing, above which is the integumental chordotonal organ which consists of sense cells, envelope, and cover cells. The last-mentioned organ is surrounded by a tracheal bubble, and its base is attached to both the cuticle and the hypodermis (Figure 4).

Discussion

Startle responses similar to those in genus *Erebia* were described by SWIHART (1967) in *Heliconius erato adonis* (Heliconiidae) and by H. FRINGS and M. FRINGS (1956) in *Cercyonis pegala* (Satyridae), but these were measured with vibration transducers or by electrophysiological means.

It is notable that even electrophysiologically-determined hearing thresholds at different frequencies in *Heliconius erato* were about 10 dB higher than the startle response thresholds in our butterflies. This demonstrates that the sound sensitivity of *E. manto* is very high when compared with the above-mentioned species. *E. euryale* showed the same sound sensitivity throughout the day as *E. manto,* but because of different behaviour patterns in the evening it was impossible to make more precise experiments or to measure its behavioural startle response. This species never sat in groups on the grass in the evening, probably staying hidden between the spruce branches during the night.

The biological function of sound perception in the species investigated here is unknown at present, but it cannot be excluded that these *Erebia* species hear because of predation by birds; certainly the best responses lie in the frequency range of bird vocalizations. Although we never directly observed avian predation upon these butterflies, we did see some specimens with quite badly tattered wings, and this may have been the result of such predation. It may also be the case that our butterflies can perceive low frequencies with vibroreceptors in their legs. To examine this we will have to undertake experiments similar to those of Swihart, rather than observe the butterflies' reactions to sound. On the other hand, we have been unsuccessful in detecting any sound emission by *Erebia* butterflies, either in the audible or the ultrasonic ranges (in the latter case by the use of a bat detector). We also failed to find any structure thought to be associated with sound emission. From the references mentioned previously it is already known that some satyrids emit sounds, at least during the breeding season (S. KANE, 1982, MONGE-NAJERA, 1991) and this is also the case for some nymphalids (Scott, 1968 cit. in KANE, 1982). There is a report on a presumably stridulatory structure in some nymphalids of the genus *Maniola* (THOMSON, 1991). But the sound producing mechanism in *Hamadryas* butterflies is apparently not a stridulatory structure (MONGE-NAJERA, 1991). Further investigations are therefore needed to understand the acoustic behaviour of the *Erebia* butterflies.

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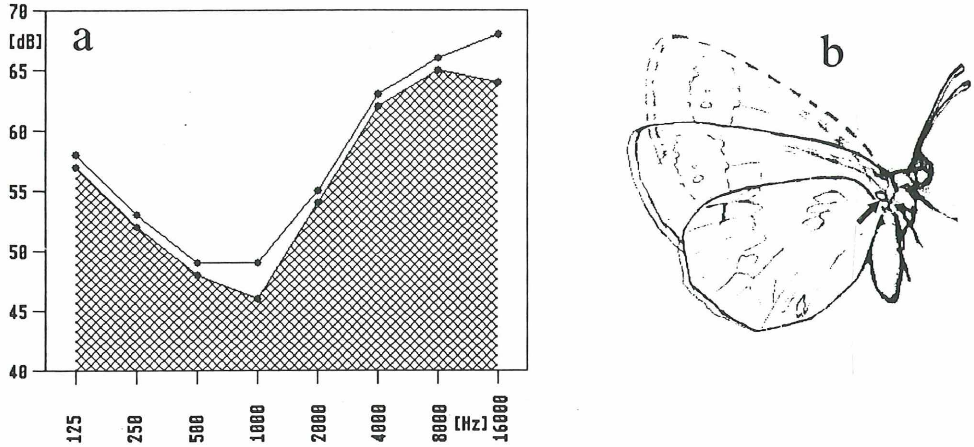

Fig. 1: a) Audiogram of startle responses in *E. manto.* The upper curve links the suprathreshold startle response values in the frequencies tested, the lower curve links the highest "no response" values. b) Startle response of the second category; the arrow shows the position of the tympanal organ.

Fig. 2: The position of the tympanal organ at the base of the front wing in *Erebia manto.*

Fig. 3: Scanning electron micrograph show the morphology of the tympanal organ in *E. manto.* The involution of the tympanum is the point of insertion of the chordotonal organ (marker: 100 μm).

100 µm

Fig. 4: Vertical section through the middle of the tympanal organ in *E. manto* (left = dorsal; right = ventral).

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