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Ultrasonic Emission by Noctuid Moths (Lepidoptera, Noctuidae): Main Characteristics of Signals and Possible Mechanisms of Their Generation

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Abstract—Morphological and acoustic studies of the sound-producing mechanism in noctuid moths were carried out on *Amphipyra perflua.* Two laterally symmetrical pairs of complementary areas adjacent to each other are responsible for generation of ultrasonic bursts. One pair is located on the ventral surface of the fore-wing base, and the other, on the metascutum. The surface of these areas is covered with inclined cone-like structures 18-25 μm high. The density of cones is as high as 3 per 100 μm^2. The aculeae of both the complementary areas are oriented toward each other and can serve to couple the wings to scutum. When uncoupling, these structures produce an ultrasonic pulse. Moths with removed wing coupling areas cannot emit clicks. Noctuid moths produce ultrasonic clicks during flight. The peak amplitude of these clicks varies between 79 and 84 dB SPL at a distance of 5 cm from a moth, with their mean duration being 67 μs. The clicks have a broad-band (> 100 kHz) spectrum with the main peaks, at 57 and 78 kHz. Artificially generated clicks have a similar spectrum. The regular pattern of emission with one click per second or every third wing-beat was often observed in the nature and in conditions of tethered flight. The adaptive importance of the acoustic behavior of moths is discussed.

Noctuid moths are known to emit ultrasonic bursts in flight. This phenomenon was first discovered by Roeder and Treat (1957). According to Kay (1969), signals of the noctuid moth *Heliothis zea* have the form of rapidly damped paired clicks with filling frequency of 50 kHz and duration of 180 us each. The pulses in a pair are separated by 190 μs. The sound intensity is 98.6 dB SPL at a distance of 1.5 cm from the source. Kay assumed that the acoustic pulse generator is of the tymbal type and, by analogy with arctiid moths (Arctiidae), is located on the metepisterna. However, this assumption has not been verified experimentally so far.

Relying on stroboscope observations of flying noctuid moths, Agee (1971) concluded that acoustic pulses may either arise from the wing flapping at the upper point or be an experimental error resulting from the contact of wings with the mounting holder. In 1993, Agee's conclusions were revised in view of new data. Using the technique of moth photographing at the moments of click recording, Zhantiev *et al.* (1993) demonstrated that, in noctuid moths, the position of wings in emission is close to horizontal. This fact is contradictory to the model of the generation mechanism, proposed by Agee. It should be mentioned that short ultrasonic pulses do appear at the upper point in mutual contact of the hind wings of a flying moth (Waters and Jones, 1994), but their amplitude, as a rule, does not exceed 65 dB SPL, which is at least 10 dB (20 dB on the average) less than the amplitude of clicks recorded by Kay and Agee. In the paper by Zhantiev *et al.* (1993), the basic types of noctuid signals and examples of their spectra were given. The free-flying moths were demonstrated to produce ultrasound too, i.e. the acoustic emission of noctuids cannot be accounted for by insect fixation in experimental conditions. Parameters of the recorded signals were independent of species, sex, and age of moths.

Several noctuid moth species are known, hypothetically using the acoustic signals for intraspecific communication: *Thecophora fovea* employs the stridulation mechanism of emission (Surlykke and Gogala, 1986); *Amyna natalis,* the tymbal mechanism on the wings (Heller and Achmann, 1993); and *Pseudoips fagana,* the tymbal mechanism on the abdominal segment (Skals *et at.,* 1996). However, only males emit sounds in all the examples. In all three cases, the intensity of the acoustic signals is 1-2 orders of magnitude higher than the values recorded by Kay and by Zhantiev *et al.* Up to now, the search for a more general mechanism of the sound emission for noctuid moths has not been successful.

In many nocturnal moths, including noctuid moths, the fore wing in the resting position is coupled with the body by means of complementary areas covered with cone-shaped processes—microtrichia (Kuijten, 1974). The first area is located on the posterior margin of the hind wing, and the second one lies on the metascutum surface. Both males and females possess such structures. When these areas are uncoupled artificially (manually), a loud click is distinctly heard. Presumably, the moths generate ultrasound by this mechanism.

Roeder and Treat (1957) assumed that noctuids survey the surrounding space by emitting clicks. The possibility of echolocation in moths was discussed in different aspects by all those who studied the emission of clicks. In the recent decade, data confirming this hypothesis have been obtained (Lapshin *et al.*, 1993; Lapshin, 1995; Lapshin and Vorontsov, 1998). Thus, the biological function of the signals under study became more definite. In this connection, the problem of identification of the mechanism of the ultrasonic burst emission has assumed special importance.

It can be seen from the oscillogram of a series of bursts, presented in the paper by Roeder and Treat (1957), that the experimental moth produced clicks at every wing-beat. Similar examples were also given in other reports (Agee, 1971; Zhantiev *et al.*, 1993). However, the latest investigations showed that in some cases the emission rate of "one click per each wing-beat" is not obligatory and even not the basic (Lapshin, 1996). It was found that, when presented with visual stimuli, the noctuid moth *Amphipyra perflua* tends to emit with an interval $3T_b$ between the clicks (T_b the wing-beat period, approximately 30 ms).

From the standpoint of the echolocation system efficiency, the interpulse time of the probing signals is one of the basic parameters determining the rate at which the information is updated. In this connection, it was necessary to investigate in more detail the dynamics of the spontaneous acoustic emission by noctuids in addition to the studies in which the dependence of the click emission frequency on visual or echolocation information was revealed (Lapshin, 1996; Lapshin and Vorontsov, 1998).

The purposes of this study were as follows: (1) to determine the characteristics of the acoustic emission by noctuids—amplitude, duration, spectrum and dynamics of the click sequence; (2) investigate the possibility of generation of ultrasonic signals by the areas of the wing-thorax coupling and by the cuticular structures on the body of noctuids (including metepisterna); and (3) carry out a comparative analysis of the acoustic parameters of both natural and artificial clicks.

MATERIALS AND METHODS

For the most part, experiments was carried out with *Amphipyra perflua* F. These noctuid moths of both sexes were trapped using a food attractant (fermented mixture of beer and honey) in Moscow and environs in July-August 1996-1999. A total of 50 specimens of this species were used in the experiments.

In addition, 12 specimens of the noctuid moth *Hydraecia micacea* Esp. captured at light of a fluorescent lamp were tested for capability of acoustic emission.

The experiments were performed in the evening and at night in laboratory at a temperature of 18-20°C.

A Study of Spectral Characteristics of Moths' Clicks

An insect was pasted with warm wax to a thin wire, so that the wings did not touch the bracing in flapping. To record acoustic signals, a RFT 301 (1/4") microphone was placed at a distance of 5 cm from the moth, in front of it and above its longitudinal axis. The electric signal from the microphone output was amplified (microphone amplifier RFT 00023), passed through high-pass filtering (cutoff frequency 1 kHz), and fed into the input of a Hewlett Packard HP54601B digital storage oscilloscope. The sampling frequency of the built-in analog-to-digital converter of the oscilloscope is 1.25 MHz, which corresponds to a quantization interval of 0.8 μs. From the oscilloscope output the digital data were delivered to a computer by means of the RS-232 interface.

The subsequent processing of the results included normalization of click oscillograms to the maximum amplitude, alignment of the base line slope, measuring the duration of signals, and calculation of their spectra, The duration of a separate impulse was conditionally taken equal to the total length of an integer number of component half-waves with amplitude of no less than 0.2 of the maximum value. The spectra were calculated by the method of sequential analysis of an inscribed piecewise linear function in the frequency range 1-100 kHz with a step of 1 kHz (D'yakonov, 1987). Original specially designed computer programs were used to solve particular problems of this study.

Measurement of Click Amplitude

In the first set of experiments, clicks were recorded from moths in tethered flight. Twenty tests with *A. perflua* were made. The peak amplitude of each click from a continuous sequence was recorded using a specially designed analog-to-digital converter. A digital code was delivered from the output of the analog-to-digital converter to the 16-bit input-output port of the computer. The results of measurements were used to build histograms of the amplitude distribution of 20 impulses or more (up to 100 impulses), depending on the stability of the ultrasound emission and research task. Such measurements were performed in the range 60-90 dB SPL. The sound pressure level of 20 μPa was taken as 0 dB SPL.

Identification of the Acoustic Emission Mechanism

In the second set of experiments, 20 acoustic impulses were recorded from each moth. A total of 400 oscillograms from 20 moths were obtained.

The position of wings of a flying insect at the moments of acoustic emission was observed visually with switched-off external lighting, using a stroboscope operating in the waiting mode. As a source of short (3 ms) light pulses in the stroboscope was used a red light-emitting diode matrix placed in the focus of a projection lens.

Flying noctuids were photographed at the moments when their clicks were recorded, using a previously developed technique (Zhantiev *et al.,* 1993). The camera was placed below the insect.

After recording acoustic impulses from intact moths, we removed with microscissors the forewing coupling areas from 10 specimens with the most stable emission (amplitude of the loudest impulses was 70 dB SPL and more at a distance of 5 cm from the insect, with a repetition rate was no less than 4 s^{-1}). Thereafter the same program of acoustic measurements was repeated.[1]

Another part of insects of the second set (10 specimens) was used in experiments in which the coupling areas were studied as sound generating structures. A moth narcotized with chloroform was placed under the microphone, and acoustic impulses were produced by moving manually the distal part of the fore wing. With certain experience gained, it was possible to obtain a sequence of clicks similar in shape to the natural signals, by varying the angle of inclination of the fore wing with respect to the body and the extent of coupling. A total of 20 oscillograms of artificial bursts was registered from every specimen.

In the third set of experiments, the possibility of generating the acoustic impulses in deformation of thoracic sclerites was studied in 20 specimens. For this purpose, a thin (0.3 mm in diameter) pin connected to an electromechanical vibrator was brought orthogonally under visual control to the surface of the sclerite studied. The amplitude of the rhythmic translation of the pin did not exceed 0.2 mm. The control signal (1- or 10-Hz frequency meander) was applied to the vibrator from the output of an ESL-2 stimulator via a filter smoothing the sharp fronts of the control current. In the course of the test, we found the points on the thorax surface, in which the cuticle deformation was accompanied by generation of clicks.

Recording of the Acoustic Activity of Noctuids in Nature

To record clicks, noctuid moths were attracted by a food attractant applied to the bark of a birch at a height of 1 m. The tree was situated at a spruce forest edge near the laboratory. We mounted the sound-receiving device with a parabolic reflector on the same tree at a height of nearly 2m. The directional lobe of the receiver was oriented downwards along the trunk, so that moths flying up to the attractant were in the coverage area. This experimental pattern allowed us to decrease the probability of recording of sounds unrelated to moth activity, e.g. cries of bats or inviting signals of grasshoppers. On transforming the acoustic signals to square electric impulses with duration of 1 ms, the information was delivered to the laboratory and tape-recorded.

Analysis of Intervals between the Clicks

The processing of tape records included the conversion of analog data into a digital code and construction of histograms of interpulse intervals. In this part of the study, the following data were analyzed:

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[1] During preparation to the experiments, it was found that it is necessary to keep intact the adjacent structures of wings when removing the areas. Otherwise, a dramatic increase in the flight noise to 70 dB SPL and more is possible. In this case, every flapping acquires the typical additional crisp sound, and the damaged insect becomes unsuitable for further use.

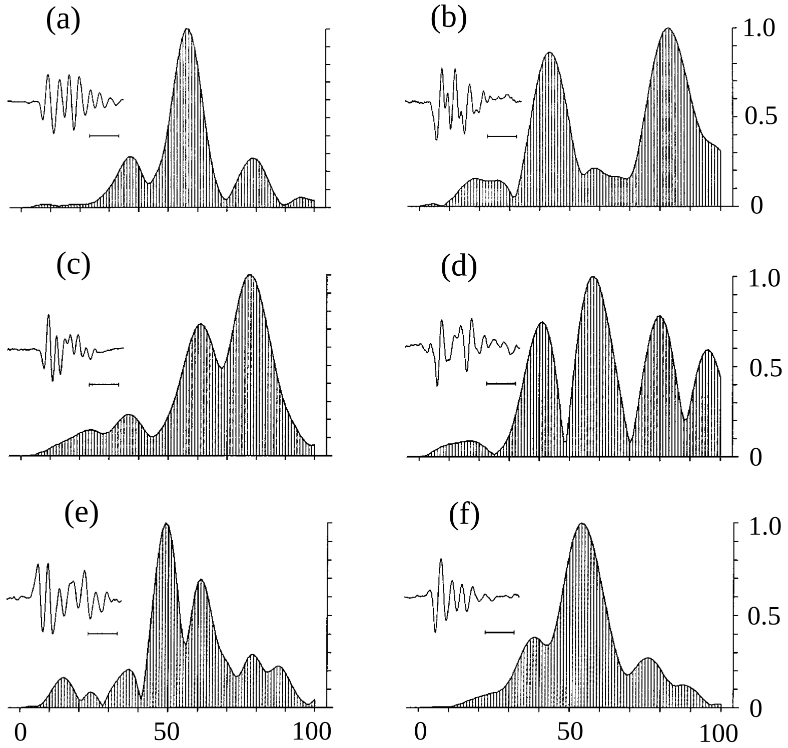

Fig. 1. Oscillograms and spectra of acoustic signals of *Amphipyra perflua;* (a-e) in tethered flight; (f) the acoustic impulse appearing in uncoupling of the coupling areas of the moth wing and body. Oscillograms: time scale - 50 μs: spectra: abscissa, frequency in kHz: ordinate, amplitude of harmonic components in linear scale, relative units. In order to distinguish the spectral components, signals with duration longer than 100 μs were used.

(1) Data obtained previously in studying the capability of *A. perflua* for echolocation in a "carousel" device (10 tests, Lapshin *et al.,* 1993) and in recording the flying paths of moths (12 tests, Lapshin, 1995). These data were processed so as to avoid artifacts related to contacts of wings with model barriers in the final array.

(2) Records of the acoustic activity of *A. perflua* in tethered flight with simultaneous recording of the motion activity (8 specimens) by means of the technique described earlier (Lapshin and Vorontsov, 1998).

(3) Records of the acoustic activity of noctuids in natural conditions.

Morphological Investigations of the Coupling Areas

The basal pan of the fore wings, sclerites of the metathorax, and coxae of *A. perflua* were fixed in 70° alcohol, dehydrated in acetone, and dried in air flow in paper containers. Being dried, the material was mounted on stubs, in such a way as to orient the ventral side of wing fragments upwards, and coated with platinum-palladium mixture. Microphotographs were taken with a Hitachi S-405A scanning electron microscope.

Macrophotographs of *A. perflua* were taken with a Zeiss Stemi 2000-C binocular microscope equipped with an AVT HORN AVT-BCI TV camera. The resolution of the CCD matrix of the camera is 500 x 575 dots. Video information was fed into the computer using a special interface. The obtained digital images were subjected to software contrast enhancement with separation of the cuticle surface relief.

RESULTS

Spectral Characteristics of Clicks

The acoustic clicks of noctuids had sharp fronts of amplitude rise. Their duration was in the range from 20 to 140 μs (average 67 μs, standard error 2.6 μs, sample size 400 oscillograms). The click envelope shape could vary significantly during a single test. Accordingly, the click spectra were variable as well (Figs, 1; 2a). A comparative analysis distinguished at least 4 narrow-band components (Figs. 1a-1d) appearing in the spectra in different amplitude ratios. The individual variability of the positions of the most significant peaks (i.e., those having the amplitude higher than 0.3 of the maximum value) along the frequency axis did not exceed ±5 kHz. Occasionally, we observed a "fine structure in the spectra," i.e. separation of the spectral peaks into even narrower components (Fig. 1e).

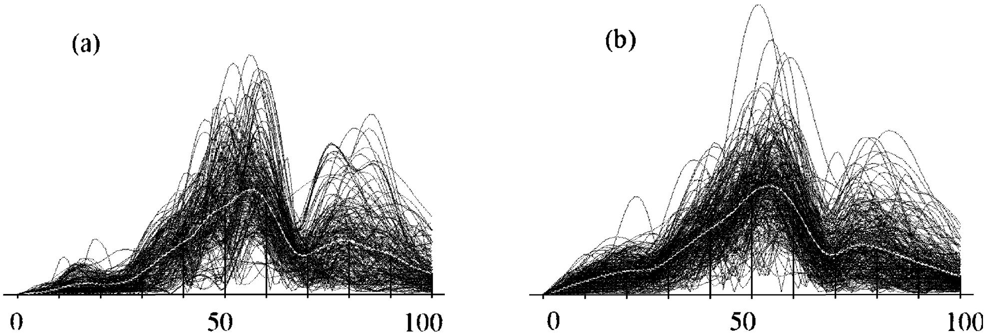

Fig. 2. Variability of spectral characteristics of (a) natural and (b) artificial acoustic impulses of *Amphipyra perflua,* normalized to the peak amplitude. Each figure is plotted by sequential superposition of 200 spectra of clicks. Abscissa, frequency in kHz; ordinate, relative amplitude of harmonic components. White lines against the background of the spectra represent the mean values ("the averaged spectra").

Position of Wings at the Moments of Emission

In order to determine the position of wings at the moments of click emission, a moth in tethered flight was illuminated with a stroboscope whose flashes were synchronized with the moments of recording of the inherent signals of insects. It was found that the noctuids can produce sounds in different phases of the flapping, including the position of wings at the upper point, but more often, in the horizontal position of wings.

A phenomenon was revealed in one of the experiments, which is likely to account for the contradiction with the results presented by Agee (see Introduction). One of the moths clicked when its wings were in the horizontal position; however, it was enough to direct the light beam of the stroboscope at its head to change abruptly the phase of fluttering at the moments of emission—the moth began to click when its wings were in the upper position. Simultaneously, the amplitude of signals increased by 6-10 dB. Once the light spot was moved from the head to the periphery of wings, the mode of emission returned to the preceding state. Several repetitions of this operation produced the same effect. For two other moths, the stroboscope flashes synchronous with their clicks caused distinct changes in the dynamics of the ultrasound emission: an increase in the click rate and variability of the wing position at the moments of the click emission. However, in these cases the changes did not disappear after the stroboscopic illumination was terminated. The stimulation of moths by light flashes unsynchronized with their own clicks did not cause any response. (The flicker rate was 30 Hz, which approximately corresponded to the wing-beat frequency of the investigated moths.) From these experiments follows that the light flashes produced soon after the emission of clicks can influence the acoustic behavior of moths. These observations indicate that the findings by Agee (1971) are erroneous owing to the use of pulsed illumination synchronized with the acoustic emission. To reduce the influence of light flashes on the results, the use of stroboscope in our further investigations was limited, especially in recording the oscillograms of clicks and measuring their amplitude.

Dynamics of Changes in the Peak Amplitude of Clicks

Zhantiev *et al.* (1993) demonstrated that the amplitude of clicks may vary essentially. According to our observations, the most comprehensive picture of signal level variation in the course of an experiment can be divided into 3 stages.

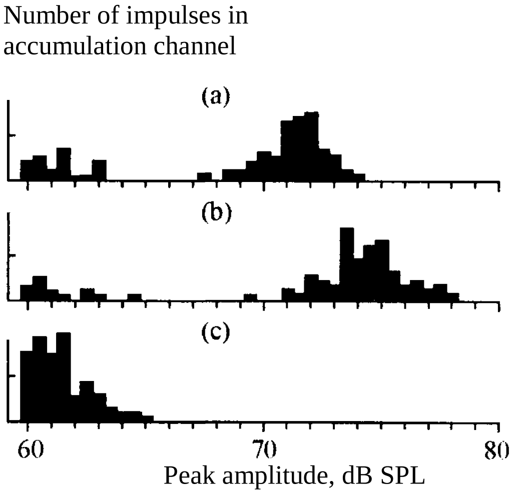

Fig. 3. Histograms of amplitude distribution of acoustic clicks of *Amphipyra perflua,* constructed using the results of the same experiment: (a, b), intact insect, successive accumulations in the same test; (c), the same moth with removed coupling areas of wings. Each histogram is plotted using the results obtained in measuring the amplitude of 100 clicks. Accumulation channel width 0.5 dB.

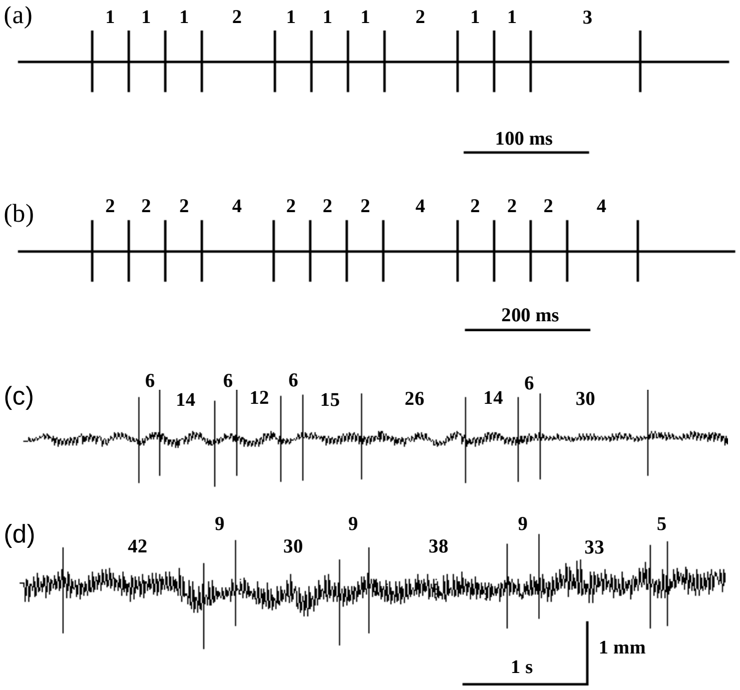

Fig. 4. Examples of click series by *Amphipyra perflua*: (a) in partially restricted flight in "carousel" device; (b-d) in tethered flight. Vertical lines represent the clicks, with interpulse interval multiplicities of the wing-beat period given at the lines. Continuous lines in oscillograms (c, d) reflect the horizontal movement of a moth, fast oscillations correspond to separate wing-beats.

The first stage: during the first minutes of flight, the distribution of the peak amplitudes of the clicks has no clear peaks in the range from 60 to 74 dB SPL. The stroboscopic observations of several specimens of moths evidence that the generation of signals with amplitude less than 64 dB SPL is confined to the moments of contact of hind wings of the flying insect. According to the data of Waters and Jones (1994), these are the flight noises. For 3 moths of 20, these noises were lower than the signal threshold, i.e. less than 60 dB SPL.

The second stage is the stabilization of the amplitude of clicks. The distribution breaks up into two clearly distinguished groups (Fig. 3a): the position of the first is still stable at the level of 60 dB SPL, while the second group lies in the range 70-74 dB SPL at the beginning of the stage (Fig. 3a). The last group drifts gradually to higher amplitudes (Fig. 3b), occasionally reaching 84 dB SPL (usually 76-80 dB SPL). The purely random fluctuations of the amplitude were imposed upon this process. Some experiments were suspended at this stage, and the coupling areas on wings of the insects were removed (see below).

The third stage of emission from intact insects is characterized by reverse drift of the amplitude distribution to lower values, with subsequent disintegration of the main peak into several smaller groups. After that either the emission of clicks was terminated or the flight stopped, presumably as a result of moth tiredness.

Measurements of the diagram of the acoustic emission of noctuids revealed a maximum on the dorsal side and minima in the rostral and caudal directions. These results coincide with the data of Kay (1969), obtained for another species of noctuids.

Temporal Dynamics of the Acoustic Emission of Moths in Partially Restricted and Tethered Flight

An analysis of the data obtained from experiments with the "carousel" device (Lapshin *et al.*, 1993; Lapshin, 1995) showed the predominance of the interpulse intervals equal to the wing-beat period (T_b), (Figs. 4a; 5a). In general, moths emitted short series consisting of 2-5 clicks separated by longer (> $10T_b$) intervals. The wing-beat period of different

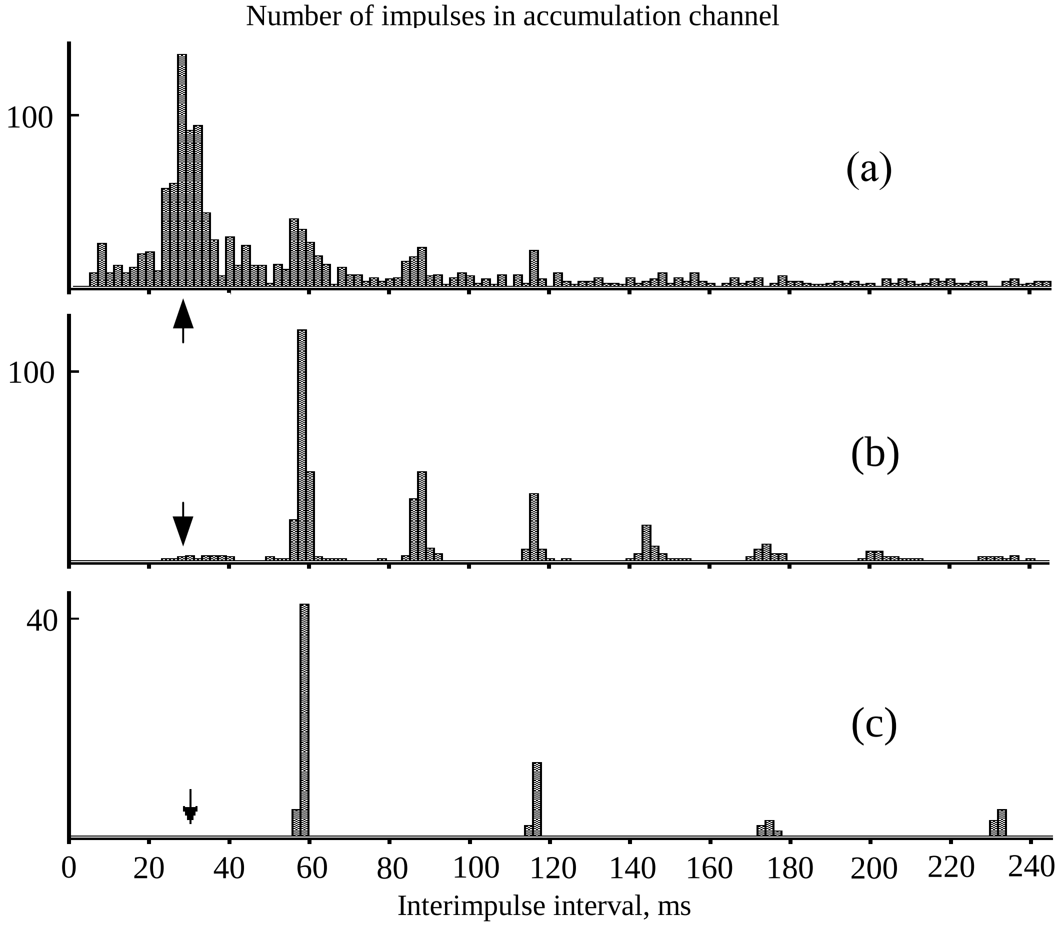

Fig. 5. Histograms of intervals between the acoustic clicks of *Amphipyra perflua*: (a), moths in partially restricted flight (10 tests, a total of 1800 intervals); (b, c), spontaneous activity of 2 specimens in tethered flight. Arrows mark the points on the time axis corresponding to a single wing-beat period (T_b nearly 29 ms). Histogram (b) is plotted upon accumulation of a sequence of 500 clicks, histogram (c) the same for 100 clicks. Discretization step 2 ms.

specimens of *A. perflua* varied within 27-33 ms, the mean value for 18 specimens was 29 ms.

The dynamics of the acoustic activity of moths in tethered flight showed a distinct deficit of short intervals ($1T_b$); Figs. 4b-4d, 5b, 5c; recording made at room light). One of the moths in our tests clicked with intervals that were multiples of $2T_b$, rather than of T_b as usual (Figs. 4b, 5c). Another typical feature of the acoustic behavior of moths is the tendency to produce clicks in equal-interval pairs divided by outwardly arbitrary time intervals (Fig. 4c, 4d).

The fact that the emission intervals with repeated rhythmic pattern, shown in Figs. 4a and 4b, were revealed suggests that the signals of noctuids may carry a species-specific encoded information. To verify this hypothesis, a special program recognizing repeated fragments in a special program recognizing repeated fragments in a sequence of clicks was used. However, we could not find any statistically significant regularity in the alternation of intervals of one or another duration.

A study of the acoustic behavior of moths brought up a question as to whether there are any species-specific differences in the patterns of the click emission and, as an extreme case, whether there exist non-clicking species. The results of our many years' observations give an affirmative answer to the second question. The most complete observations were carried out on the noctuid moth *Hydraecia micacea* (12 specimens). We failed to record in experiments a spontaneous acoustic emission in the form of a stable sequence of impulses. Only when stimulating the abdomen or antennae of these moths, we occasionally got single

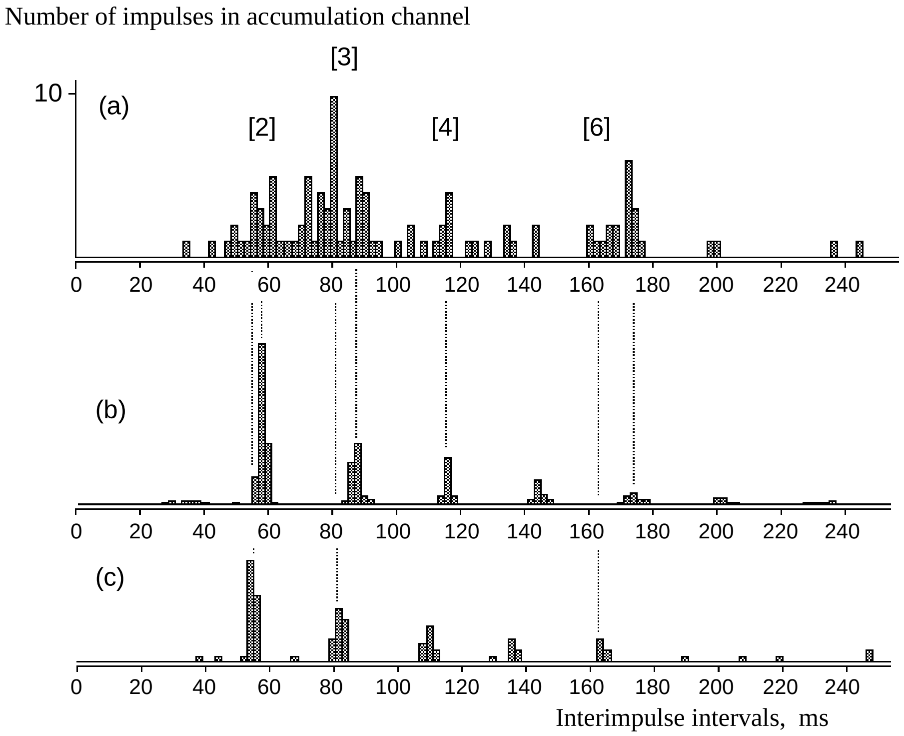

Fig. 6. Histograms of interpulse intervals: (a), acoustic activity of noctuids in free flight in natural conditions (a total of 194 clicks); (b. c), analogous histograms of noctuid activity in tethered flight, presented for comparison: (b), *Amphipyra perflua* and (c), *Crino satura.* In parentheses, coefficients of the interpulse interval multiplicity to wing-beat period are given. Discretization step 2 ms. Abscissa: interpulse interval, ms; ordinate: number of impulses in accumulation channel.

clicks not differing in parameters from the ordinary signals of moths. These observations are confirmed by the earlier results of experiments on 8 moths of the same species, which did not click, either, and were used as a control in tests in the "carousel" device (Lapshin *et al.,* 1993).

Acoustic Activity of Noctuid Moths in Natural Conditions

Moth clicks were recorded in nature in the late August 1998 at air temperature of 17-19°C. The period of the maximum activity was at 23-24 hours by the local astronomic time. Before and after this period, no clicks were observed. During 4 evenings, 28 series were recorded. Each series consisted, on the average, of 7 clicks.

The subsequent statistical analysis of the interpulse intervals was complicated by the presence of moths of at least 2 species in the coverage area of the recording device (*Amphipyra perflua, Crino satura* Schiff., and, possibly, *Meganephria oxyacanthae* L.). No reliable identification of species in flight at low illumination of the experimental site was possible.

A comparison of the cumulative histogram with the histograms obtained for the stationary flight revealed and, in some cases, made it possible to distinguish groups related to the activity of different species (Fig. 6). The sum total of the analysis is close to the results obtained previously in a study with visual stimulation (imitation of an approaching stem of a tree) of moths (Lapshin, 1996). In both cases, the majority of recorded intervals were multiples of the wing-beat period: $2T_b$ or $3T_b$ (Fig. 6).

Morphology of the Coupling Areas

The coupling of wings with the moth body is accomplished by paired formations in the form of a system of two complementary areas: the first located on the lower surface of the fore wing (Fig. 7a: *Az* area, dorsal view), and the second, on the surface of the metascutum (Fig. 7a: *Sz* area).

A narrow area *(Az)* devoid of scales and hairs is located on the lower surface of fore wing near its base along the posterior margin. The length of this area is nearly 1750 μm, and its maximal width, 250 μm. The area gradually narrows to wing base and, following wing relief, is somewhat convex in shape. Along the wing margin, the area partially extends onto the upper surface. This entire area has microstructured surface densely covered with cone-like structures adjoining

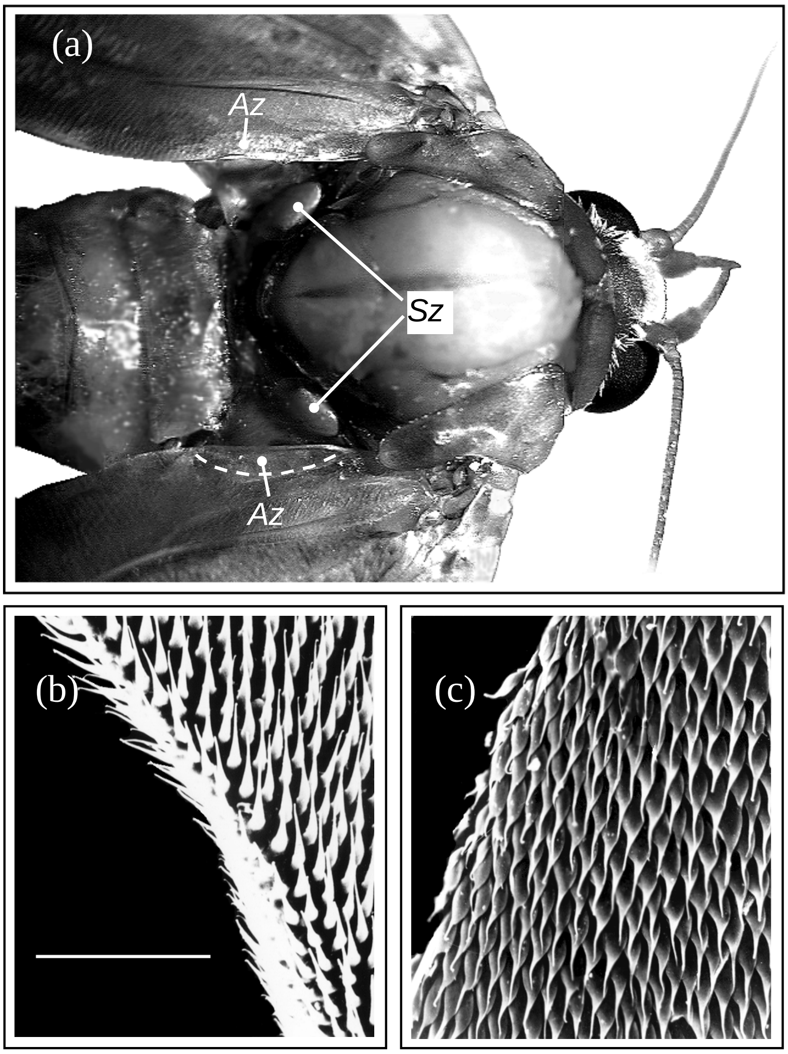

Fig. 7. Mechanism of coupling of fore wings with the body in *Amphipyra perflua*: (a) location of active coupling areas on wings (*Az* the border is marked with dotted line) and on scutum (*Sz*); (b) surface of *Az* area, wing margin (ventral view); (c) surface of *Sz* area. The moth wings are moved apart so that the coupling areas on metascutum are entirely visible. Scale bar 50 μm.

the wing cuticle (Fig. 7b). The apices of the cones are generally oriented toward the wing apex throughout the area. The density of the cones reaches 3 per 100 μm^2, with their diameter at base equal to 4 μm. The height of the cones varies from 18 to 25 μm, depending on their position on the area.

A similarly structured area (*Sz*) is present on the metascutum *(Sct₃)* (Fig. 7c). It is located on the dorsal surface of a convex carina 1.7 mm long at the posteriolateral edge of the mesothoracic scutellum (Fig. 7a) and is normally covered from above with overhanging long hairs. Its maximal width in the first one third is 420 μm. The carina of the scutum is narrowed in the caudal direction to 140 μm. The height of cones is 12 μm near the posterior margin of the area, and up to 40 μm near the anterior margin. In this direction, the longer cones gradually replace the shorter ones, along with a change in their orientation. Close to the posterior margin, the cones are oriented along the longitudinal axis of the carina. In going to the anterior margin, the direction of their orientation changes by 90°.

The cones of both complementary areas are normally oriented toward each other. When the posterior margin of the fore wing is moved over the carina of the scutum, the cones can couple and fix the fore wing in rest with respect to the insect's body.

Experiments on Acoustic Identification of the Mechanism of Acoustic Emission

After the removal of the coupling areas from fore wings *(Az)* the moths in tethered flight emit no high-amplitude clicks, whereas the flight noises either remain at the same level (Fig. 3c) or increase somewhat (by 1-2 dB). Incomplete removal or partial damage of wing parts bearing the coupling areas did not result in the loss of the acoustic emission capability. Moreover, after such an operation, the amplitude of the recorded signals occasionally increased by 6 dB, on the average, compared with the former value, and clicks started to accompany every wing-beat of an insect. A repeated operation for complete removal of the specialized wing areas resulted in the loss of ability to generate ultrasonic signals with amplitude higher than 66 dB SPL.

The coupling is possible in the case of incomplete overlapping of the coupling areas, and even upon a light contact of the wing edge with the area on scutum. The acoustic impulses appearing upon artificial uncoupling of the areas, varied in shape similarly to the natural impulses. The signals closely similar to the natural ones were recorded repeatedly (Fig. 1f). The illustrations in Fig. 2 are constructed by superposition of the spectra of impulses emitted by moths in flight (Fig. 2a) and artificial clicks (Fig. 2b). The averaged spectral characteristics plotted in Fig. 2 by white lines against the backgrounds of the spectra illustrate the similarity of the frequency parameters of the natural and artificial signal. Thus, an acoustic impulse similar to the natural signals in spectral characteristics can appear at the moment of uncoupling of the body and wing.

The generation of click at the moment of uncoupling of the wing and body is possible at a certain orientation of wings relative to the thorax, allowing a mechanical contact of the areas *Az* and *Sz*. To check this condition, we photographed the moths using a flash lamp synchronized with their own clicks. It was found that the position of moth wings at the moments of generation allows emission of clicks upon uncoupling of the coupling areas.

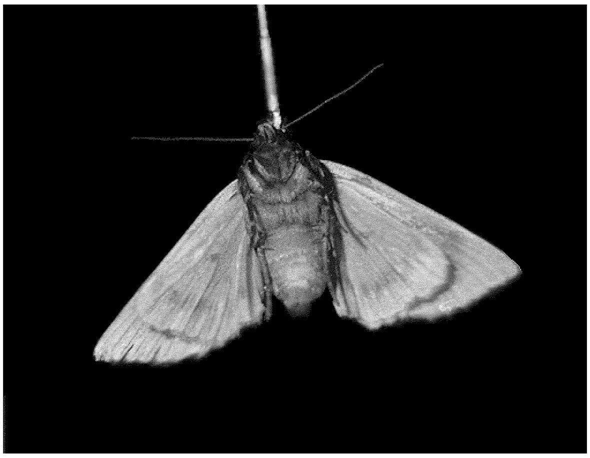

Fig. 8. Photograph of *Amphipyra perflua* at the moment of click recording, ventral view.

Search for Other Mechanisms of Click Generation

Among the numerous sclerites forming the exoskeleton of a moth, the closest attention was given to the metepisternum *(Eps_3)* as a potential place of click generation. Microtymbals are present on the surface of this sclerite in some moths belonging to Arctiidae, the family being taxonomically close to Noctuidae. Moths can emit clicks, using these structures (Blest *et al.,* 1963; Fullard and Heller, 1990).

The metepisternal cuticle of moths (Fig. 9, *Eps_3)* is very tough. With gradually increasing mechanical loading with a vibrator pin, the sclerite surface yielded with a click. However, the form of click and its spectrum did not conform to the parameters of the own signals of noctuids, in the first place because of the excessive level of spectral components at frequencies higher than 80 kHz.

Mechanical vibrations of the surface of the hind coxa (Fig. 9, Cx_3) can cause the generation of clicks very similar in physical parameters to the natural signals. Occasionally, the clicks appeared twice during the stimulation cycle, on pressing on the cuticle and upon unloading. However, measurements of the directional characteristics of the acoustic emission appearing upon deformation of this sclerite demonstrated that the axis of the maximum is oriented downwards, at an angle of about 30° to the horizontal plane, rather than in the dorsal direction typical of the natural emission of noctuids (Kay, 1969).

A slight pressing to some points of the metameron (Fig. 9, *Mer_3)* also resulted in a spasmodic deflection of its cuticle accompanied by a click. Generally, the spectra of these clicks had a peak strongly shifted to the low-frequency part of the range (30-40 kHz) as compared with the normal sounds of noctuids (50-60 kHz).

We also examined the possibility of click generation by any structures on the wing surface, e.g., by bistable membranes. A mechanism based on this principle has been described for the butterfly *Inachis io* L.

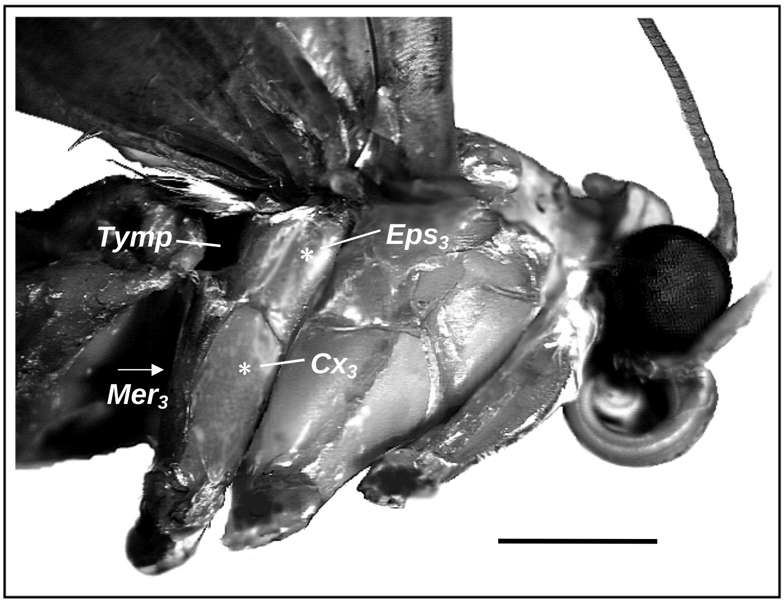

Fig. 9. Photograph of *Amphipyra perflua,* lateral view. Asterisks and arrow point to zones of the thorax, where mechanical deformation was accompanied by emission of ultrasonic impulses. *Eps*$_3$, metepisternum; *Cx*$_3$, hind coxa; arrow, direction of the force deforming the metameron (*Mer*$_3$). Scale bar 2 mm.

(Nymphalidae) (MØhl and Miller, 1976). However, no functionally similar structures were found in noctuid moths.

In conclusion, it should be mentioned that the disruption of wing integrity, e.g., partial fracture of veins or minor ruptures of membranes can cause formation of zones of acoustic impulse generation. The sounds generated by these zones mainly coincide with a certain phase of the flapping motion and exhibit spectral characteristics very similar to the natural ones. On the one hand, the last circumstance suggests that it is the wings that emits clicks and defines their spectral features. On the other hand, these artifacts markedly complicate the acoustic identification of the signal generator in moths.

DISCUSSION

By convention, the acoustic emission of a click is divisible into two main stages: excitation of a vibration impulse (generation) and its transformation into sound signal (emission). The processes associated with these stages may be spatially separated and occur on different physical structures.

As the results of our study testify, the characteristics of clicks appearing at the moment of uncoupling of the corresponding areas of the wing and body ("generation"—coupling areas, "emission"—wings) show the closest conformity to the parameters of the acoustic emission by noctuids. Other possible mechanisms of generation and emission considered in our study do not fit in one or another parameter the system of the acoustic emission characteristics of noctuid moths.

As the most strained parts of the coupling areas of the fore wing *(Az)* and metascutum *(Sz)* are uncoupling, the boundary of the maximum force will move in discrete steps over the surface of the interaction zone. As this take place, the cascade repeat of the coupling-uncoupling cycles is possible at intervals several times as long as the acoustic impulse duration. This creates the conditions for the generation of time-separated paired clicks, as originally described by Kay (1969).

The high variability of the physical parameters of the emitted impulses is a characteristic feature of the emission in noctuids. Signals with complicated and changeable spectrum are most likely to be formed by the proximal areas of the fore wings with a complicated pattern of compartments (membranes), each potentially capable of resonating at several frequencies (modes). The mechanical impulse appearing at the moment of uncoupling can serve as an excitation source for these structures.

The fore-wing length of a moth (20-30 mm) is several times the wave-length of the significant components of the signal spectrum, (e.g., 8.5 mm for 40 kHz). At such linear dimensions one would expect narrowing of the directional diagram of emission and appearance of additional directional lobes.

These effects must become more pronounced with decreasing wave-length. If the emitting surface of the forewing oscillated along the normal to its plane, the principal axis of the direction characteristic of emission would be oriented perpendicular to the plane of the emission zone. However, the major lobes of the emission diagram will be oriented at different angles to the wing surface at different frequencies because of the finite speed of the vibration wave propagation along the wing. Inasmuch as the position of moth wings varies from time to time at the moments of emission, some spectral components at a point of an acoustic impulse recording will be intensified or, conversely, sharply suppressed in comparison with the mean level, depending on the instantaneous orientation of the directional diagram relative to the microphone. The fallout or increase of certain peaks in the spectra of clicks are attributable to this mentioned effect (Fig. 1a-1c). The gradual change in the amplitude of the recorded signals (Figs. 3a, 3b) seems to be associated with a shift of the time of acoustic impulse generation with respect to the flapping cycle, with the result that the emitting segments of wings change their orientation diagram relative to the microphone. Thus, the basic features of the acoustic emission of moths can be accounted for in terms of the described mechanism of generation and emission of ultrasonic clicks.

The complex spectral composition of the ultrasonic impulses emitted by moths may have adaptive value in ultrasonic orientation. When a probing signal with broad spectrum is reflected from a surface with irregularities of size more than 1/4 of the acoustic wave length (e.g., tree bark), the interference effects suppress some frequency components but not the entire signal, i.e. the broadband probing signals increase the noise immunity of the echolocation system as a whole.

A. perflua moths are characterized by stable emission of clicks at intervals multiple to the wing-beat period (Fig. 4b; Figs. 5b, 5c; Fig. 6). The multiple division is likely to occur under illumination, since this effect was weakly pronounced (Fig. 5a) in experiments with a "carousel" device performed in the dark (Lapshin *et al.*, 1993; Lapshin, 1995). We assume that the observations of the multiplicity of interpulse intervals to 2 wing-beat periods ($2T_b$,) (Figs. 4b, 5c), along with the capability of moths to generate click pairs with stable intervals (Figs. 4c, 4d), are of particular importance. The possibility of occurrence of such regimes suggests the presence of a system of two-level control over the emission rhythm in the CNS.

A decrease in the average repetition frequency of the probing clicks impairs the echolocator efficiency, and therefore, the effect of exact division must have a certain biological sense. It is well known that the strongest behavioral responses in bats have been recorded on presenting the bait insects with wing-beat periods of 40-60 s^{-1} (Schnitzler *et al.*, 1983). Another observation testifies that small moths run the risk of bat attack to a greater extent (Fullard, 1977). In turn, the noctuids of lesser size flap more frequently on the average. Infrequent but rhythmic clicks can give to a bat an impression of the presence in the air of a large and, thus, a strong insect, which is why it is quite probable that the low frequency of the probing clicks rate in noctuids (multiple division of the wing-beat frequency) is a direct consequence of the predatory pressure of bats.

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