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Directional and Frequency Characteristics of Auditory Receptors in Midges (Diptera, Chironomidae)

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Abstract—Individual characteristic frequencies and directional sensitivity of the Johnston's organ auditory receptors were measured in the midges *Chironomus plumosus* L. using the method of positive feedback stimulation: responses of receptors recorded with a glass microelectrode from their axons in the antennal nerve were amplified and fed to the stimulating speaker. With the amplitude and the stimulating signal phase properly adjusted, the whole feedback loop fell into auto-excitation with the frequency of oscillations close to the characteristic frequency of the receptor. Three separate groups of receptors were found with mean frequencies of 180, 221, and 264 Hz. These groups differ in their directional properties: the low-frequency receptors are mostly sensitive dorsoventrally, while the directional maxima of mid- and high-frequency ones are combined to provide equal sensitivity in the plane perpendicular to the flagellum. Our data suggest that in Chironomidae a single Johnston's organ together with the antenna can provide spatial localization of conspecific sounds and also perform the initial stages of frequency analysis.

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Males of non-biting midges (Chironomidae) make swarms in open areas, usually not far from the places of their development. The conspecific female flies into the swarm and mates there with the first male to approach it. Given strong competition between males in the swarm, a fast and successful response to a female would provide crucial competitive advantage. Therefore, the parameters of the sensory systems that determine the latency of detecting and locating the female must be subject to strong selection pressure favoring the speeding-up of all the consecutive stages of signal analysis or exclusion of those mechanisms which set unavoidable limits for the rates of information transfer and processing.

When seeking a female, the swarming males are guided by the sound of the female's flight. They perceive sound waves with their plumose antennae, at the base of which lie Johnston's organs, each with about 20 000 mechanosensory sensilla. Of them, sensilla of the most common type contain two receptors each (Zhantiev and Fedorova, 1999). The receptors transform the antennal oscillations into electric potentials whose pattern reflects, with some distortions, the pattern of the acoustic signal which has caused the oscillation. The potentials then spread along the axons of the antennal nerve, without transformations into spikes (Lapshin, 2010), and reach the zones of primary processing of auditory information (Ignell et al., 2005).

According to the latest experimental data, the receptors of Johnston's organs of non-biting midges are tuned to different frequencies within the range from 100 to 380 Hz (Lapshin, 2013). Thus, the auditory system of these insects can potentially perform spectral analysis based on information from several frequency channels.

To be able to move directly towards the female, the male should possess a mechanism of 3D localization of the source of sound waves. All the parts of this system would naturally be affected by sex selection favoring an increase in its response speed. The radial arrangement of mechanosensory sensilla in Johnston's organs (Zhantiev and Fedorova, 1999) suggests the possibility of single-stage assessment of the signal direction of arrival. Since the starting angular position of the female relative to the male may vary, the male's auditory system should be able to perform spectral analysis regardless of the plane of antennal oscillation. In other words, the groups of sensilla in each radial sector of Johnston's organs may be expected to include the sets of receptors sufficient for frequency analysis of conspecific signals. This communication reports the results of experimental testing of this hypothesis.

MATERIALS AND METHODS

Experiments were carried out on males of *Chironomus plumosus* L. captured from swarms on the Oka River bank (54°51′44″N, 38°21′28″E). Altogether, 46 experiments were performed. As a rule, during one experiment stereotyped series of measurements were made consecutively from two points of the antennal nerve.

The work consisted in estimating the relative threshold sensitivity of auditory receptors depending on the spatial orientation of the acoustic wave vector. The plot of such data in polar coordinates is commonly referred to as the polar pattern or the directional characteristic.

In earlier research, the polar pattern was assessed either by changing the spatial position of the acoustic emitter relative to the test object (Daley and Camhi, 1988; Lapshin and Rozhkova, 1997; Kämper and Vedenina, 1998), or by rotating the test object relative to the stationary emitters (Rozhkova, 1980). By contrast, a different principle of changing the direction of acoustic waves near the test insect was used in this study.

The main stimulating parts of the experimental setup were two Scandinavia 3C dynamic speakers (DLS, Sweden) positioned with their acoustic axes at the right angle (Fig. 1). The test insect was fixed at the intersection of these acoustic axes in such a way that the flagellum of the antenna associated with the studied Johnston's organ was normal to the plane defined by the axes of the two speakers.

The speakers were powered from an amplifier ($K_U = 4$) via a passive SC (Sin–Cos) transducer which produced two derived signals with amplitudes

$$A_1 = 0.25 \cdot U \cdot \cos\left(\frac{\pi}{180} \cdot (\varphi + 45)\right) \qquad (1)$$

$$A_2 = 0.25 \cdot U \cdot \sin\left(\frac{\pi}{180} \cdot (\varphi + 45)\right), \qquad (2)$$

where A_1 and A_2 are the amplitudes of the control signals for the first and the second speaker, respectively; U is the alternating voltage at the input of the SC transducer; φ is the angle between the vertical line passing through the insect fixation point and the vibration velocity vector of air particles. An increase in φ corresponded to counter-clockwise rotation of the velocity vector, with the insect's head viewed from the front (Fig. 1, inset).

The resulting direction of the air vibration velocity in the stimulating system was determined by the vector sum of the signals from both speakers. Changes in the sound wave direction relative to the test insect in increments of 15° were accomplished by coordinated switching of voltage dividers in the SC transducer. For those angles at which the values of sin( ) or cos( ) functions were negative, the signal polarity was inverted by switching the terminals of the speakers. This method of variation of the sound wave vectors did not require any construction elements to be moved inside the test zone, so that measurements could be made noticeably faster.

The movable parts of the speakers had a low resonant frequency (90 Hz). Due to the considerable response lag of the dome and its support, the emission phase delay increased with the signal frequency up to the point of inversion. To stabilize phase delay, a phase correction unit was added in the speaker control circuit.

Acoustic calibration of the stimulating device was performed with an NR-231-58-000 differential capacitor microphone (Knowles Electronics, US). This microphone was also used to control the angle of the acoustic vector near the test insects.

Before the experiments, the differential microphone and its amplifier were calibrated under free field conditions using a B&K 2235 sound-level meter and a B&K 4176 microphone (Brüel & Kjær, Denmark).

The measurements were brought to the logarithmic scale and expressed in decibels. The root-mean-square value of the sound particle velocity level (SPVL) of $4.85 \cdot 10^{-5}$ mm/s, corresponding to the standard sound pressure of $2 \cdot 10^{-5}$ Pa under free field conditions, was taken as 0 dB.

The directional and frequency characteristics of the midge auditory system were measured using the previously developed method of connecting the auditory receptors of the test insects in the positive feedback circuit (Lapshin, 2013; Lapshin and Vorontsov, 2013). The testing was based on using amplified potentials of the receptors as control signals for the speakers. To establish positive feedback, the air vibrations caused by the speakers had to induce the response receptor potential near the microelectrode tip in phase with the

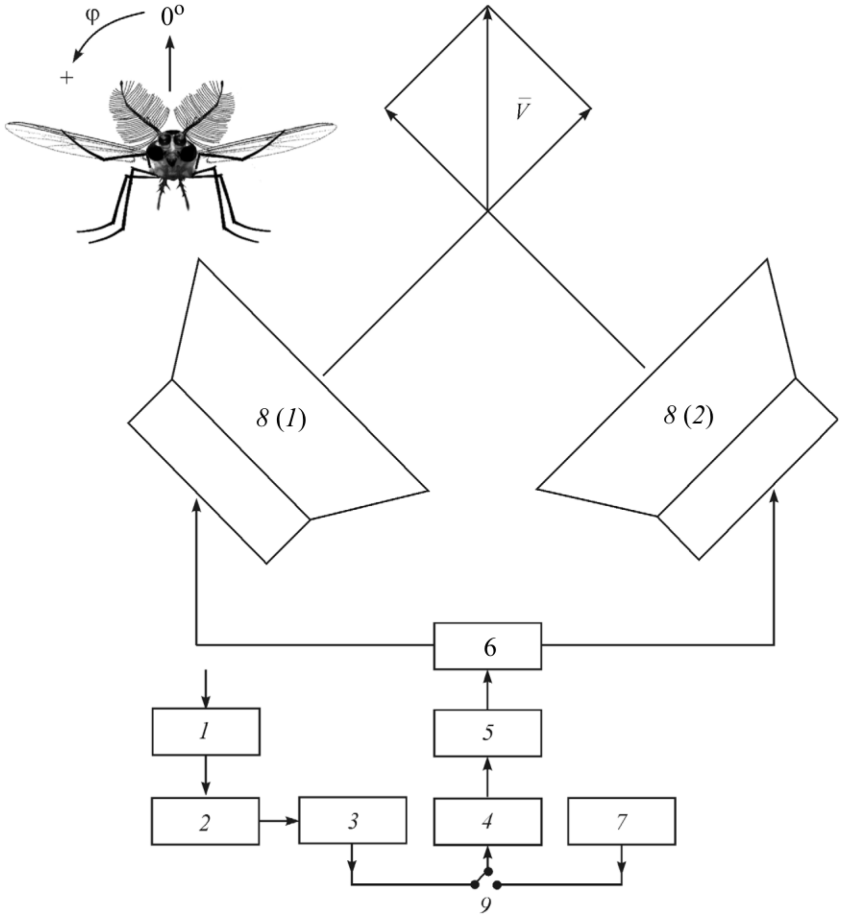

Fig. 1. The generator of acoustic stimuli: (*1*) microelectrode amplifier; (*2*) bioelectric potential amplifier; (*3*) phase correction unit; (*4*) attenuator; (*5*) amplifier; (*6*) SC transducer; (7) digital-analog converter; (*8*) speakers; (*9*) speaker switching unit. Arrow in the inset shows the increase of the acoustic vector rotation angle (φ).

control signal. When the conditions for autoexcitation were met, the system experienced periodic oscillations whose main frequency corresponded to the characteristic frequency of the receptor (Lapshin, 2013; Lapshin and Vorontsov, 2013).

Before testing, the insect was fixed on a metalized plastic stage with starch-based glue with addition of 0.9% NaCl solution. The thin layer of this glue applied on the conductive surface of the stage not only provided mechanical fixation of the midge but also acted as the silent electrode. The stage was fixed on a holder with two tiny ferrite magnets. This construction allowed the test insect to be positioned at any desired angle relative to the speakers. In most experiments the polar patterns were measured in midges fixed with the dorsal side up.

However, the stereotyped fixation of the test insects could result in selective recording of some particular groups of receptors. To avoid this bias, the position of the midge was changed periodically, either by turning it with the ventral side up, or by rotating it by 180° in the horizontal plane. The data of such tests were then corrected taking the position of the insect into account.

The receptor axon potentials in the antennal nerve were recorded extracellularly with microelectrodes made of 1B100F-4 borosilicate glass capillaries (WPI Inc.) and filled with 0.9% NaCl solution. The resistance at the microelectrode tip was 10–40 MOm. Extracellular recording of receptor activity was used because it was more stable than intracellular one over long time intervals.

The microelectrode signal was fed to a DC amplifier (Purves, 1983) with an input resistance of more than 10 GOm and $K_U = 10$, and then to an E14-440 analog-digital converter (ADC) (L-Card, Russia). The amplified signal from a differential microphone installed close to the test insect (control of the acoustic stimulus) was recorded simultaneously using a sepa-

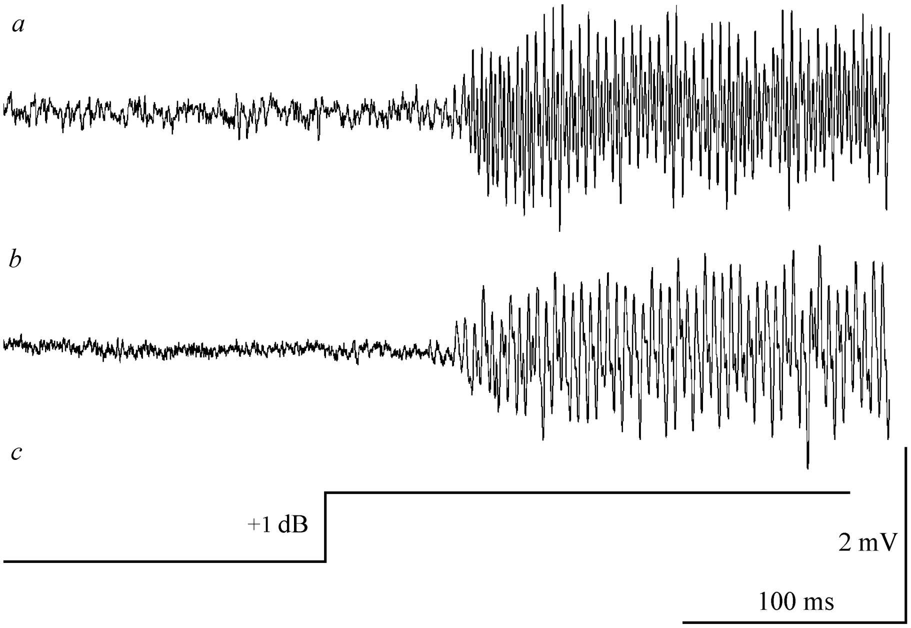

Fig. 2. An increase in the receptor electric activity in the positive feedback mode after crossing of the autoexcitation threshold: (*a*) receptor response; (*b*) acoustic feedback signal; (*c*) transfer ratio in the feedback circuit increasing by 1 dB (conventional representation). Voltage calibration is shown for oscillogram (*a*).

rate ADC channel. The digitized data were saved on a computer.

Apart from ADC, the signal from the output of the DC amplifier was additionally AC amplified ($K_U = 100$ within the frequency range of 5–5000 Hz) and fed to a digital attenuator system and an amplitude discriminator. The latter was used to measure the thresholds of receptor responses to external sinusoidal stimuli; the stimulus level could be adjusted in increments of 1 dB.

At the beginning of the test, as the electrode was shifted in the antennal nerve, the insect was continuously presented with a series of tonal pulses (sinusoidal frequency 240 Hz, amplitude 60 dB of SPVL, duration 80 ms, period 0.6 s). Since in the sinusoidal acoustic waves the air velocity is reversed during each period, one could expect activation of the receptors sensitive to the antenna tilting in two opposite directions. However, the receptors sensitive in the plane orthogonal to that of antennal oscillation could be overlooked during this procedure; therefore, the acoustic wave vector was periodically changed by 90°, by modifying the SC transducer settings.

After an abrupt increase in the amplitude of electrophysiological response, the stimulating system was set to the positive feedback mode, and the feedback level was adjusted to the point of autoexcitation (i.e., generation of autoexcitations). The threshold was defined as the level which required one more incremental step at the attenuator output (+1 dB, Fig. 2) for the system to enter the autoexcitation mode. One series of measurements yielded a set of attenuator settings which corresponded to the autoexcitation thresholds $Th_1 \ldots Th_i \ldots Th_{24}$ at different angular attitudes of the acoustic vector: 15°… $i \cdot 15°$… 360° (i being a whole number from 1 to 24). Since a series of measurements sometimes took quite a long time, extra measurements at certain attitudes (usually at 45° and 315°) were made periodically, not less than twice per series, to ascertain the stability of recording.

During the subsequent data processing, the maximum $Th_{\max}$ was determined among the values measured in one recording. Then a set of derived values describing the receptor sensitivity depending on the acoustic wave vector was obtained by the formula $A_i = Th_{\max} - Th_i$. In the polar patterns based on these data, the sectors of the highest sensitivity corresponded to the lowest recorded thresholds, and the central zero point corresponded to $Th_{\max}$. The acoustic vectors at which no excitation with the initial frequency was observed were given the value $A_i = 0$.

To estimate the absolute sensitivity of the receptors, in 24 experiments the feedback mode tests were followed by measuring the thresholds of responses to tonal (sinusoidal) pulses at different directions of acoustic waves. The stimuli were generated by an LA-DACn10m1 digital-analog converter (JSC "Rudnev-Shilyaev," Russia).

The technique for measuring the physiological thresholds of auditory receptors was described earlier (Lapshin, 2010; Lapshin and Vorontsov, 2013). The crossing of the receptor sensitivity threshold was indicated by an abrupt increase in the pulse frequency at the amplitude discriminator output, correlated with the beginning of an acoustic pulse.

To distinguish between the two above methods, I will use the term "polar patterns" for the results obtained by the positive feedback method, and "directional characteristics" for those obtained by tonal pulse stimulation.

In the directional characteristics based on the tonal pulse tests, the angular width of the sensitive zone was determined, with its boundaries drawn at the level of –6 dB of its maximum. The angular sensitivity range of the receptors was estimated based on directional characteristics rather than polar patterns, since the former were obtained under the conditions more closely resembling the natural activity of the Johnston's organ receptors.

The electrophysiological experiments were carried out under laboratory conditions at air temperature 19–21°C in August–September 2014, at Kropotovo Biological Station of the Institute of Developmental Biology of the Russian Academy of Sciences.

RESULTS

As the microelectrode was gradually shifted inside the antennal nerve, at a certain moment the amplitude of receptor response to tonal pulses exceeded 1 mV; this moment marked the beginning of the active phase of data collection. After that the microelectrode remained fixed while the stimulating circuit was set into the positive feedback mode; then the direction of the acoustic vector was changed stepwise, and the autoexcitation thresholds of the receptors were measured.

The diagram in Fig. 3*a* is an example of the polar pattern of an individual receptor, i.e., the receptor which was the only one responding to stimulation at a particular recording point. Since positive feedback was sensitive to the phase of the acoustic waves acting on the antenna, the polar pattern of an individual receptor looked as a lobe skewed relative to the origin of polar coordinates. The receptor also responded to the stimulating sound coming from the opposite direction but the phase of oscillations recorded from the antennal nerve was shifted by 180°. As a result, the stimulation system entered the mode which did not support autoexcitation of the receptor.

The directional characteristic of an individual receptor, resembling that of the classical differential receiver, is shown in Fig. 3*b*.

Altogether, nine individual receptors were tested. In the positive feedback mode, these cells produced autoexcitations at characteristic frequencies within the range of 182–224 Hz, the maxima of their polar patterns varying from 15 to 180°.

In most experiments, an electrode fixed in a certain position in the antennal nerve recorded the activity of two receptors which had different characteristic frequencies while their polar patterns were oppositely directed relative to the zero point (Fig. 3*c*). In other words, in any area of the nerve there was a high probability ($N = 56$, $p = 0.64$) of recording two receptors which responded in antiphase to the same shift of the antenna. The common axis of the polar patterns of these receptors could be differently oriented. After the electrode tip was shifted in the nerve, another pair of receptors started responding to stimulation. They had different characteristic frequencies, and their polar patterns were also oppositely directed, but not related in any way to the polar patterns of the preceding pair. Thus, the receptors responding in pairs possessed coordinated spatial characteristics. The orientation of the directional characteristic obtained by testing the same pair of receptors with sinusoidal stimuli largely coincided with the axis of their polar patterns (Fig. 3*d*). The slight mismatch between the angular positions of the polar patterns and directional characteristics of the same receptors may be explained by the influence of electric potentials of other axons passing near the recording point.

The width of the individual directional characteristic (limited by –6 dB of its maximum) was determined as the mean of two measurements of the angular width of its symmetrical parts (Fig. 3*d*). The mean width of the directional characteristic for all the receptors was 124° ($N = 24$, standard error $\sigma = 12°$). The sensitivity thresholds of receptors at the maxima of their characteristics varied between the tests within the range of 21–43 dB of SPVL (mean value 34 dB of SPVL, $\sigma = 6.1$ dB).

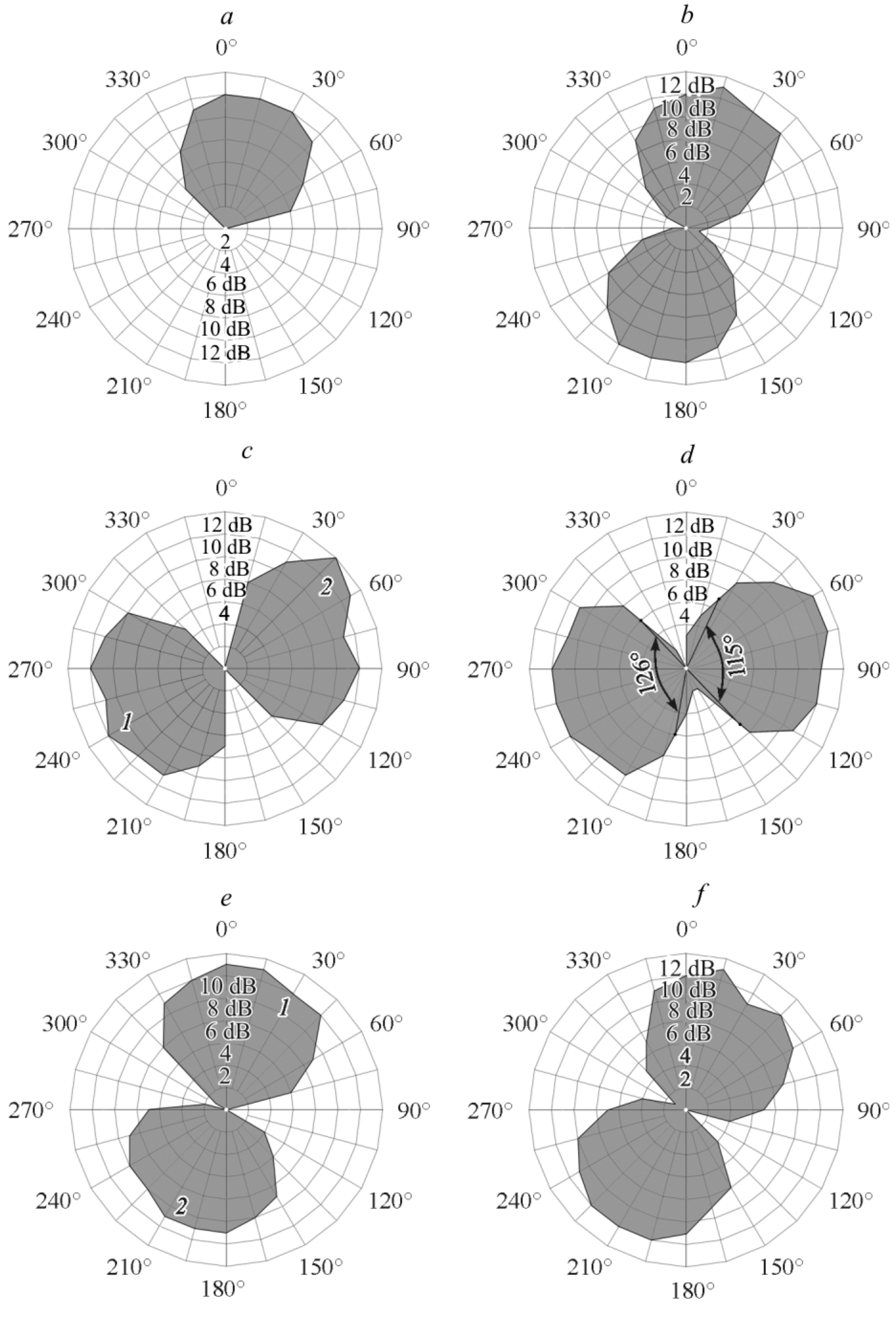

Fig. 3. Examples of polar patterns: (*a*) polar pattern of an individual receptor (characteristic frequency 204 Hz); (*b*) directional characteristic of the same receptor (threshold in the maximum sensitivity zone 33 dB of SPVL at 210 Hz); (*c*) patterns of two coordinated receptors responding to stimulation in antiphase (characteristic frequencies: 256 Hz for receptor with pattern "*1*" and 224 Hz for receptor with pattern "*2*"); (*d*) summarized directional characteristic of receptors *1c* and *2c* obtained by testing with sinusoidal stimuli (sinusoidal frequency 210 Hz, threshold in the maximum sensitivity zone 21 dB of SPVL); (*e*) polar patterns of a coordinated system of three receptors: (*1*) 234 Hz; (*2*) simultaneous autoexcitation at 186 and 284 Hz; (*f*) summarized directional characteristic of the system of receptors *1e* and *2e* (sinusoidal frequency 210 Hz, threshold in the maximum sensitivity zone 27 dB of SPVL). Numbers along the perimeter of the diagrams are angles between the acoustic stimulus vector and the vertical line passing through the insect fixation point (see inset in Fig. 1); numbers along the radii are relative sensitivity values, dB. The spatial characteristics of receptors shown in fragments (*a*, *b*), (*c*, *d*), and (*e*, *f*) were obtained in three different experiments, all with the fixed position of the electrode in the antennal nerve. Fragment (*d*) shows the method of estimating the width of the directional characteristic (limited by –6 dB of its maximum) as the mean of two measurements (126 and 115°; mean value 121°).

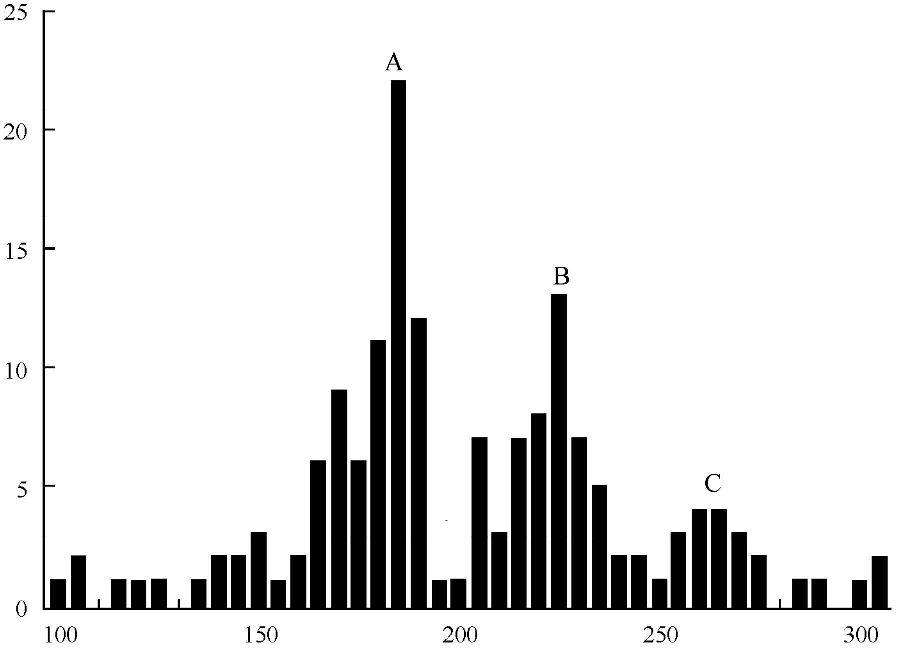

Fig. 4. Distribution of characteristic frequencies of the Johnston's organ receptors in males of *Chironomus plumosus* L. The main frequency groups: (A) 165–190 Hz, maximum at 185 Hz; (B) 205–245 Hz; (C) 255–275 Hz. Horizontal axis: frequency, Hz; vertical axis: number of recorded receptors (the frequency scale interval is 5 Hz).

In 14 experiments, autoexcitation within the limits of the polar pattern was observed simultaneously at two different frequencies, within the range of 170–190 Hz and 255–275 Hz, respectively. In other words, both sources responded in phase to the mechanical oscillations of the antenna. Comparison of such paired frequencies showed that their ratio in different tests was close to 1.5 ($M = 1.50$, $\sigma = 0.02$). At the same time, the characteristic frequencies changed differently and independently near the boundaries of the polar pattern, i.e., in the zones of unstable excitation. With the opposite angle, excitation was recorded only at one frequency which did not coincide with any of the two preceding frequencies. The shapes of the polar patterns (Fig. 3*e*) and directional characteristics of such triple systems (Fig. 3*f*) did not essentially differ from those of the paired receptors with oppositely directed polar patterns (Figs. 3*c*, 3*d*).

The distribution of characteristic frequencies of receptors based on 165 measurements is shown in Fig. 4. There are three peaks in the histogram: the first and the most distinct one (A) has the maximum at 185 Hz (mean frequency 180 Hz within the range of 165–190 Hz), while two other peaks lie within the ranges of 205–245 Hz (B, mean frequency 221 Hz) and 255–275 Hz (C, mean frequency 264 Hz), respectively. Most receptors of group C were included in the triple systems described above: 14 out of 16 receptors of this group were coordinated in both position and frequency with those of group A (with the frequency ratio close to 1.5).

The probability of random coincidence of the polar patterns of two receptors coordinated in frequency and recorded from the same point can be calculated by the Bernoulli formula for binominal distribution (Lakin, 1990). If two equiprobable positions of the polar patterns of paired receptors (either coinciding or oppositely directed) are taken as the null hypothesis, the probability of its realization in 14 out of 16 tests is very low: $H_0 < 2 \cdot 10^{-3}$. For three possible variants of mutual orientation of polar patterns (360°/124° ≈ 3) the probability of realization of the null hypothesis is already close to zero: $H_0 < 3 \cdot 10^{-6}$. Thus, simultaneous activity of two receptors with opposite polar patterns was not a mere coincidence, even if we do not consider the fairly stable frequency ratio in such pairs.

Of 66 studied receptors of group A, pairs with receptors of group B were recorded in 24 cases (frequency ratio in pairs 1.25, $\sigma = 0.09$). In five cases both receptors had similar characteristic frequencies, and in eight cases the second receptor was tuned to a lower frequency (ratio 0.82, $\sigma = 0.05$). These data suggest that the indistinct peak at 140–150 Hz (Fig. 4) was not random.

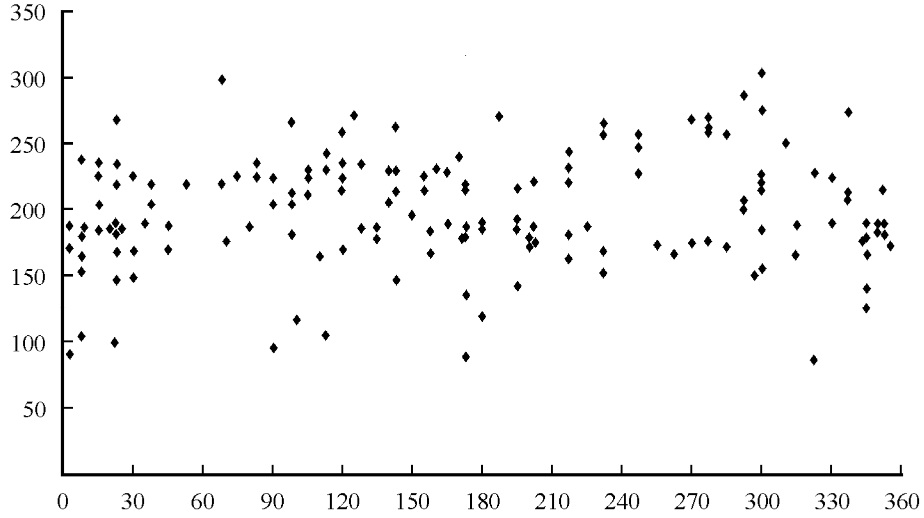

Fig. 5. Distribution of parameters of the Johnston's organ receptors in males of *Chironomus plumosus* L. Horizontal axis: angular position of the polar pattern maxima, degrees; vertical axis: characteristic frequency, Hz.

The total distribution of the testing results of the Johnston's organ receptors in the coordinates "angle of the polar pattern maximum—characteristic frequency" is shown in Fig. 5. As can be seen from the diagram, most of the angles were covered by the activity of at least two receptors with different characteristic frequencies. However, the density of dots along the horizontal axis seems to be lower in the areas of 60° and 240°, as compared with 0–30° or 150–180°, suggesting a certain relation between the characteristic frequencies of receptors and their spatial orientation.

It was found that the three groups of receptors, A, B, and C (Fig. 4) were indeed differently distributed by the orientation of their polar patterns (Fig. 6). The polar patterns of receptors of group A mostly lay within the ranges of 15–45° and 165–235°. The maxima of most receptors of group B concentrated within the range of 15–235°. Since the directional characteristics of the Johnston's organ receptors are symmetrical (Figs. 3*b*, 3*d*, 3*f*), receptors of group B ensured all-round coverage in the plane perpendicular to the antennal flagellum. The high-frequency receptors of group C did not reveal any distinct orientation trends, most probably due to the small sample size. However, as shown above, most receptors of this group were coordinated with those of group A, both in position and in frequency. The two cases not included in this group are shown in black in Fig. 6. Thus, group A was not uniform in its composition.

The histogram in Fig. 7 was obtained by removing from group A all the elements spatially associated with those of group C. It can be clearly seen that the polar patterns of the remaining receptors mostly lay within the ranges of 0–15° and 180–195°. This conclusion was also confirmed by statistical analysis: $H_0 < 0.02$ by the χ^2 test (Lakin, 1990).

DISCUSSION

The receptors tested in this research ($N = 165$) constituted only a small fraction of the total number of receptors in Johnston's organs. However, even such a small number seems to be sufficient for spectral analysis of acoustic signals coming from any direction (Fig. 5).

The mean width of the directional characteristic of receptors in *Chironomus plumosus* is 124°. Proceeding from this estimate, it may be concluded that four receptors with the same characteristic frequency would provide all-round coverage of all the sources of acoustic signals at this frequency. As can be seen in Fig. 6*c*, this condition is met even for the least representative group uniting the in-phase receptors of two frequency groups: 165–190 Hz (A) and 255–275 Hz (C).

At the same time, a few receptors may be not sufficient to determine the angular coordinates of the sound source with the necessary precision. According to the observations of Belton (1974), males of bloodsucking mosquitoes (Culicidae) were not attracted by acoustic signals from sources positioned far from the swarm, even if these signals contained frequencies typical of the flying conspecific females. The swarming males of *Ch. plumosus* fly in a weaving pattern and, similar to Culicidae, seem to be able to determine the distance to

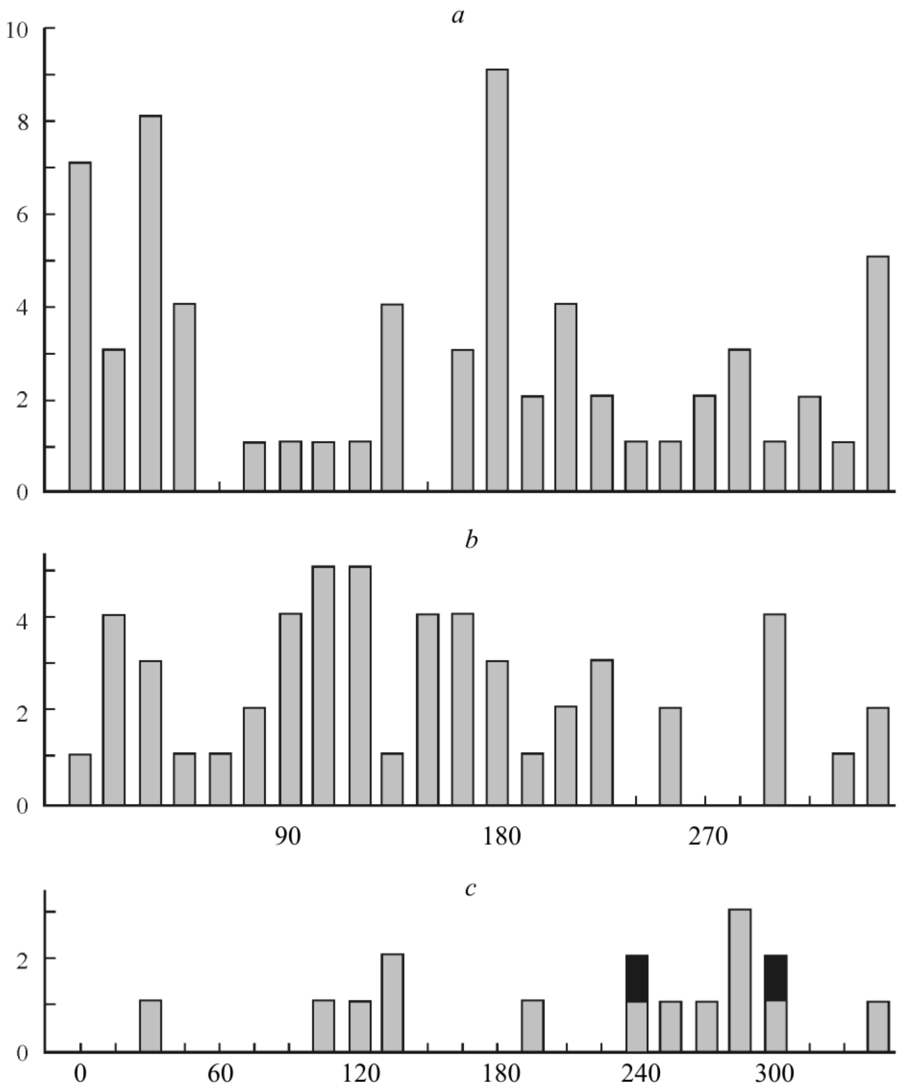

Fig. 6. Distribution of the angular positions of the polar pattern maxima of three main frequency groups of the Johnston's organ receptors in males of *Chironomus plumosus* L.: (*a*) 165–190 Hz; (*b*) 205–245 Hz; (*c*) 255–275 Hz. Horizontal axis: angle, degrees; vertical axis: number of receptors.

the sound sources by the degree of parallactic shift. In this case, their attention would be mostly restricted to the swarm zone while the sounds coming from greater distances would be ignored. The spatial selectivity of responses to acoustic stimuli increases the swarm stability and noise immunity of communication between males and females. However, the real-time triangulation tasks place stringent requirements on precision and speed of the system responsible for angular localization of signal sources. This may account for the seemingly excessive structural complexity of Johnston's organs in midges.

According to the data of Zhantiev and co-authors (2001), the wingbeat frequency of *Ch. plumosus* males exceeds that of females by 170 Hz. This value falls into the range of the most distinct peak (A) in Fig. 4. It may be supposed that receptors of group A ensure perception and subsequent analysis of the mixed harmonic produced by the difference between the wingbeat frequency of the male and the first harmonic of the sound of female's flight. The formation of mixed harmonics and their role in sound perception by flying insects were considered in detail in my earlier papers (Lapshin, 2010, 2012).

Most receptors of group A are oriented dorsoventrally with a certain shift (+15°) toward greater angles (Fig. 7). Zhantiev and co-authors (2001) noted that males catching up with the flying female approach it from above. Thus, the female acting as the sound source for the male is positioned in the lower sector

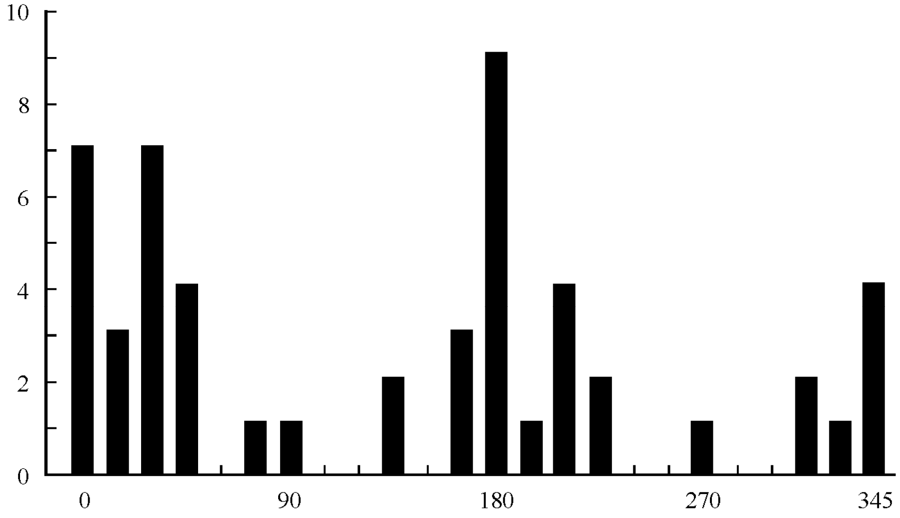

Fig. 7. Distribution of the angular positions of the polar pattern maxima of receptors of group A (165–190 Hz, see Fig. 4) which remained after removal of the receptors associated with those of group C. For designation of axes, see Fig. 6.

(180° in the coordinate system used herein), i.e., in the zone of the polar pattern maxima of receptors of group A.

This work has demonstrated the existence of numerous paired receptors with different characteristic frequencies and oppositely directed polar patterns (Fig. 3*c*). This means that in response to the same mechanical shift of the antenna, the two receptors produce antiphase electric signals in their axons. It is also known that the main structural elements of Johnston's organs in non-biting midges are sensilla containing two bipolar receptors each (Zhantiev and Fedorova, 1999). The total number of such sensilla was estimated by the cited authors at 20–21 thousand. It may be supposed that the axons of the receptors of one such sensillum may extend within a common sheath along the antennal nerve. If this is true, the high probability of recording antiphase paired receptors can be explained by the electrode tip getting under the common sheath and recording the activity of both axons simultaneously. At the same time, the positive feedback method allowed the individual parameters of the two receptors to be studied separately.

The pairwise grouping of antiphase signals in the midge receptor system is in some way analogous to the opponent color-coding mechanism in the eye's retina (Svaetichin and MacNichol, 1958; Daw, 1973). The opponence of auditory receptors with different characteristic frequencies may facilitate inhibition at the subsequent stages of auditory information processing in those cases when the signal has a continuous (i.e., noise-like) spectrum, rather than a linear one typical of the signal of a flying female.

The presence of a harmonic close to the differential frequency is not by itself sufficient for the signal to be recognized as conspecific. The electric responses of receptors also contain frequency components multiple to the main tone of female's flight (Lapshin, 2010). Correct interpretation of an acoustic signal requires not only receptors tuned to the differential frequency but also receptors with different characteristic frequencies. Very suitable for this purpose would be spatially associated in-phase elements with a 1.5-fold difference in frequency. Since auto-excitation appeared simultaneously at both frequencies in the same angular sector, it would be safe to suppose that the sources of these signals were morphologically associated, both within Johnston's organ itself and within the antennal nerve. In addition, the consistent ratio of the characteristic frequencies of paired receptors (about 1.5) indicates that their parameters are somehow physiologically determined.

In an earlier study (Lapshin, 2013), the method of connecting the test insect in the positive feedback circuit was used to obtain the statistical distribution of characteristic frequencies of the Johnston's organ receptors in the males of *Ch. plumosus*. Comparison of this distribution with the histogram in Fig. 4 reveals two pairs of similar peaks: 140–190 Hz and 165–190 Hz (group A); 240–290 Hz and 255–275 Hz (group C). The zone of maximum acoustic sensitivity in the tuning curve of *Ch. plumosus* males (Lapshin, 2010) coincides with peak C.

At the same time, comparison of the two distributions also reveals some differences: the previously obtained distribution lacked the peak corresponding to peak B in Fig. 4, but had a high-frequency group at 320–370 Hz. Such discrepancies may be explained by the fact that in the previous work stimulation was presented only dorsoventrally, whereas in the present research testing was performed with all the possible angles. Since many receptors of group B are oriented laterally (Fig. 6*b*), the number of active receptors should decrease during dorsoventral stimulation within this frequency range. The same reasoning may be valid for the high-frequency peak (320–370 Hz): receptors with such characteristic frequencies were more likely to respond to dorsoventral stimulation.

Based on the experimental data, it may be concluded that a single Johnston's organ together with the antenna can accomplish perception of a conspecific signal, spatial localization of its source, and the initial stages of its frequency analysis. This conclusion is confirmed by the long-known fact that a mosquito with only one remaining antenna can still orient itself correctly with respect to a sound source (Roth, 1948).

Thus, the multichannel organization of the midge auditory system (in terms of both direction and frequency) combined with short electrotonic pathways of the antennal nerve can ensure much faster processing of auditory information without reducing the quality of analysis.

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REFERENCES

1. Belton, P., "Trapping Mosquitoes with Sound," Proc. Calif. Mosq. Control Assoc. **35**, 98 (1967).
2. Daley, D.L. and Camhi, J.M., "Connectivity Pattern of the Cercal-to-Giant Interneuron System of the American Cockroach," J. Neurophysiol. **60** (4), 1350–1367 (1988).
3. Daw, N.W., "Neurophysiology of Color Vision," Physiol. Rev. Publ. **53** (3), 571–611 (1973).
4. Ignell, R., Dekker, T., Ghaninia, M., and Hansson, B.S., "Neuronal Architecture of the Mosquito Deutocerebrum," J. Comp. Neurol. **493**, 207–240 (2005).
5. Kämper, G. and Vedenina, V.Yu., "Frequency-Intensity Characteristics of Cricket Cercal Interneurons: Units with High-Pass Functions," J. Comp. Physiol. **182**, 715–724 (1998).
6. Lakin, G.F., *Biometrics* (Vysshaya Shkola, Moscow, 1990) [in Russian].
7. Lapshin, D.N., "Perception of Acoustic Signals by Male Midges (Diptera, Chironomidae) during Flight Simulation," Sensornye Sistemy **24** (2), 129–138 (2010).
8. Lapshin, D.N., "Mosquito Bioacoustics: Auditory Processing in *Culex pipiens pipiens* L. Males (Diptera, Culicidae) during Flight Simulation," Entomol. Obozr. **91** (1), 36–57 (2012) [Entomol. Review **92** (6), 605–621 (2012)].
9. Lapshin, D.N., "Frequency Parameters of the Auditory Receptors of Non-Biting Midges (Diptera, Chironomidae)," Sensornye Sistemy **27** (1), 35–46 (2013).
10. Lapshin, D.N. and Rozhkova, G.I., "A Device for Studying Wind-Sensitive Systems of Insects," Sensornye Sistemy **11** (2), 168–174 (1997).
11. Lapshin, D.N. and Vorontsov, D.D., "Frequency Tuning of Individual Auditory Receptors in Female Mosquitoes (Diptera, Culicidae)," J. Ins. Physiol. **59** (8), 828–839 (2013).
12. Purves, R.D., *Microelectrode Methods for Intracellular Recording and Ionophoresis* (Academic Press, 1981; Mir, Moscow, 1983) [in Russian].
13. Roth, L.M., "A Study of Mosquito Behavior: an Experimental Laboratory Study of the Sexual Behavior of *Aedes aegypti* (Linnaeus)," Amer. Midl. Nat. **40**, 265–352 (1948).
14. Rozhkova, G.I., "Directional Sensitivity of Neurons of the Cercal System of a Cricket Differently Positioned in the Gravitational Field," Neirofiziologiya **12** (6), 604–611 (1980).
15. Svaetichin, G. and MacNichol, E.F., "Retinal Mechanisms for Chromatic and Achromatic Vision," Ann. New York Acad. Sci. **74**, 385–404 (1958).
16. Zhantiev, R.D. and Fedorova, M.V., "Fine Structure of Male Johnston's Organ in *Chironomus plumosus* L. (Diptera, Chironomidae)," Entomol. Obozr. **78** (2), 287–295 (1999) [Entomol. Rev. **79** (2), 148–155 (1999)].
17. Zhantiev, R.D., Fedorova, M.V., Chukanov, V.S., and Esipovich, N.G., "Morphofunctional Organization of the Antennae and Johnston's Organs in Non-Biting Midges (Diptera, Chironomidae)," Sensornye Sistemy **15** (1), 73–84 (2001).