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Acoustic Signals in Insects: a Reproductive Barrier and a Taxonomic Character

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Abstract—In singing insects, the song is an important component of the specific mate recognition system (SMRS). In communities of sympatric singing species, there is a partitioning of communication channels, the so-called "acoustic niches." Within one community, the songs of different species always differ in temporal or frequency characters, i.e. occupy different acoustic niches. However, conspecific songs do not always act as an interspecific reproductive barrier, despite always being a SMRS component. The species that do not communicate acoustically due to allopatry, different timing of vocalization, inhabiting different biotopes, or unmatched food specializations can produce similar songs while forming reproductively isolated communities. Individuals of different sexes need not only to recognize a conspecific mate but also to evaluate its "quality." The close-range signal (courtship song) provides more opportunities for choosing the "best" male than does the distant signal (calling song). In many species of Orthoptera, courtship includes not only acoustic but also vibrational, visual, chemical, and mechanical signals. An analysis of cricket songs showed the courtship songs to be on average more elaborate and variable than the calling songs. At the same time, due to the difference in mating behavior between the two groups, the acoustic component of courtship is used for mate quality evaluation to a greater extent in grasshoppers than in crickets. The courtship songs of grasshoppers are generally more elaborate in temporal structure than cricket songs; moreover, they may be accompanied by visual displays such as movements of various body parts. Thus, song evolution in grasshoppers is more strongly driven by sexual selection than that in crickets. According to the reinforcement hypothesis, the premating barrier between hybridizing species becomes stronger in response to reduced hybrid fitness. However, our behavioral experiments with two groups of hybridizing grasshopper species did not confirm the reinforcement hypothesis. We explain this, firstly, by a low level of genetic incompatibility between the hybridizing species and secondly, by high hybrid fitness when attracting a mate. A high competitive capability of hybrids may be accounted for by attractiveness of new elements in hybrid courtship songs. When we divide similar forms based on their songs, we in fact distinguish biological species using the criterion of their reproductive isolation. Acoustic differences between species are usually greater than morphological ones. Therefore, song analysis allows one to determine the real status of doubtful species-rank taxa, to distinguish species in a medley of sibling forms, and to reveal cryptic species in the cases when morphological studies fail to provide a univocal result. At the same time, songs are subject to intraspecific variation the range of which is different in different groups. Therefore, it is necessary to study which degree of difference corresponds to the species level before interpreting the status of some forms based on song comparisons. Besides, song similarities cannot indicate conspecificity of acoustically isolated forms; on the other hand, song differences between these forms prove that they are full-rank species.

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In the biological species concept originally formulated by Dobzhansky (1937) and Mayr (1947, 1971, etc.), the species is interpreted as an integral reproductive system, with the emphasis placed on the mechanisms of isolation between different reproductive communities. The isolation mechanisms are commonly divided into two categories: pre-copulatory and post-copulatory ones. Pre-copulatory isolation is the result of selective mating, which may be either a side effect of seasonal, geographic or ecological differences, or the consequence of ethological isolation. In the opinion of Mayr (1974), the ethological factors constitute the largest and the most important class of isolation mechanisms. By contrast, the recognition species concept proposed in the middle of the 1980s by Paterson (1985) attaches the greatest importance to the pre-copulatory mechanisms facilitating reproduction within the limits of the species. In the general case, such mechanisms are referred to as the specific mate recognition system (SMRS). In different taxa, SMRS is represented by a complex of acoustic, olfactory, visual or other stimuli (sound signals, pheromones, nuptial attire, specific postures, etc.) which help the conspecific partners to recognize one another.

Although this concept was originally proposed as an alternative to the "isolation-based" approach of Mayr, it soon became evident that the differences between the isolation and recognition concepts were not as great as some advocates of the latter concept often choose to believe (Borkin et al., 2004). Indeed, SMRS is essentially a "friend or foe" identification system, both words representing the key aspects in the biological context: for an individual ready for reproduction it is equally important to find a "friend," i.e., a conspecific partner without which the act of reproduction would be impossible, and to avoid a "foe," i.e., a heterospecific partner since mating with it would be a waste of resources. In view of this, many authors now consider an extended variant of the biological species concept, according to which species are groups of organisms that reproduce sexually and have a unified SMRS (Claridge, 2009a, 2009b). Correspondingly, reproductive isolation is insured by interspecific differences in the structure of the SMRS. It follows that it is the unique SMRS that constitutes the primary criterion of a species, all the other interspecific differences being of secondary nature.

Indeed, in many cases the emergence of differences in the SMRS structure between populations (or groups of populations) is the first stage of formation of the pre-copulatory reproductive barrier, which may ultimately lead to the splitting of the ancestral form into independent species. This is why a comparative study of SMRS often allows the researcher to distinguish cryptic species or to make unambiguous conclusions about the taxonomic status of some dubious species-rank forms. On the other hand, sometimes, for example, in case of geographic or ecological isolation, speciation may take place without any differences emerging in the SMRS structure. Therefore, the definition of a species as a group of individuals or populations with the same SMRS is far from being universal, since two forms that are reproductively isolated and even clearly different in morphology may still produce similar signals to attract the mate.

Besides, it is now customary to distinguish between the prezygotic and postzygotic mechanisms of post-copulatory isolation (Coyne and Orr, 2004). Prezygotic isolation works at the level of zygote formation. The eggs are selectively fertilized with the sperm of conspecific males, whereas the spermatozoa of heterospecific males die in the female's genital tract before they can reach the eggs. It has been shown in a number of cases that such a cryptic variant of mate selection is fairly common in the nature. Moreover, such isolation may be established very quickly. An example of this kind is provided by the crickets *Allonemobius fasciatus* (De Geer, 1773) and *A. socius* (Scudder, 1877) which hybridize in the parapatric zone. The females of these species respond positively to both conspecific and heterospecific signals, despite their differences. At the same time, the sperm of a conspecific male is significantly preferred to that of a heterospecific male (Doherty and Howard, 1996; Howard et al., 1998). Still, in insects that possess intraspecific sound or vibrational communication, acoustic signals often constitute the main component of SMRS.

In the Russian literature there are only two summarizing monographs on the bioacoustics of insects, both published early in the 1980s (Zhantiev, 1981; Popov, 1985). Since that time, new directions of research have appeared in this field, in particular the study of acoustic communication in communities of sympatric species and the study of the role of courtship signals and their evolution under the influence of sexual selection. The range of the studied taxa has been considerably enlarged. Besides the traditional objects of insect bioacoustics, such as Orthoptera and Cicadidae, research has embraced a number of other taxa, including the insects which produce not sound but vibrational signals: small representatives of Homoptera (Auchenorrhyncha, Psyllinea, and Aleyrodinea), Heteroptera, Neuroptera, some families of Coleoptera and Diptera, etc. The number of taxonomic publications prepared with the use of the bioacoustic characters has increased manifold, and acoustic analysis has become a commonly accepted method in the taxonomy of some groups. The goal of this paper is to review the data on the above topics, namely, the bioacoustics of insect communities and the evolution of signals under the influence of sexual selection, and also to consider the possibility of using acoustic characters in taxonomic research.

MATERIALS AND METHODS

This communication is mainly based on our original data on crickets and grasshoppers (Orthoptera: Gryllidae and Acrididae), which emit sound signals propagating in air, and also on planthoppers and psyllids (Homoptera: Auchenorrhyncha and Psyllinea), which use solid substrates as the acoustic medium for their vibrational signals.

Orthopterans and psyllids produce their signals by means of frictional sound organs consisting of movably coupled sclerites, one of which bears a row of denticles or pegs (pars stridens), and the other, a sharp edge or vein (plectrum). The sound-producing organ of planthoppers is the complex paired tymbal apparatus (Zhantiev, 1981). In crickets sound is generated by friction of one tegmen against the other, in grasshoppers, by friction of the hind femora against the veins on the tegmina, and in psyllids, by friction of the anal margins of the wings against the serrated ridges on the meso- and metanotum (Tishechkin, 2006b).

The following terms are used herein for description of the signals. The signals of Orthoptera consist of *pulses*, each pulse being generated by a single action of the frictional organ, i.e., a single shift of its movable elements in one direction. The sound apparatus of the small Auchenorrhyncha is concealed under the wings, and there is currently no technical possibility to observe the work of their minute tymbals. Correspondingly, in the bioacoustic of these insects the pulse is usually defined as a short discrete fragment of the signal (or as a sequence of oscillations) characterized by a rapid increase and a subsequent decrease in the amplitude, i.e., a fragment separated by amplitude minima from other similar fragments. Both in Orthoptera and in Homoptera, the periodically repeated groups of pulses are referred to as *syllables*; these syllables, in turn, may be united into *phrases*. The *repetition period* of the rhythmical signal elements (pulses, syllables, sometimes phrases) is the time interval from the beginning of one of the similar elements to the beginning of the next element.

The signals of insects were recorded both under the field and the laboratory conditions. The vibrational signals of homopterans were recorded by means of a GZP-311 piezoelectric cartridge (a pickup head) from a vinyl record player, from which the signal was directed to the recording device via a matching amplifier. Sound signals in the field were captured using MD-383, Spirit IM-01 or Audio-Technika ATR 25 condenser microphones and recorded with Sony Walkman MZ-NH900, MZ-RH910 or Sharp MD-MT 190H minidisk recorders or with a customized Elektronika-302-1 cassette tape recorder. The upper frequency limit of the equipment was 14 kHz or higher. To avoid amplitude distortions, the recording level was adjusted manually in all the cases.

In grasshoppers under laboratory conditions, we recorded not only the sounds but also the stridulatory movements of the hind legs. The latter were recorded with a custom-made opto-electronic device implementing the method developed by the German researchers (Helversen and Eisner, 1977; Hedwig, 2000). Pieces of reflecting foil were glued onto the outer distal lobes of the male's hind femora. Two cameras were focused on the illuminated foil pieces in such a way that the reflected light was directed by a set of mirrors onto position-sensitive photocells. The light beam falling on the photocells was converted into the electronic signal and recorded simultaneously with the sounds. The sound signals were recorded using a Brüel & Kjær 4191 microphone with a frequency range of 3 Hz–40 kHz, and fed to the computer via an amplifier and a custom-made analog/digital converter. The sampling frequency was 100 kHz for sound signals and 2 kHz for leg movements.

The sound signals of crickets were recorded in the laboratory using the same equipment. The insects for experiments were obtained from the laboratory cultures of McGill University in Montreal and the Moscow Zoo.

The calling sound signals were recorded from a solitary male positioned 10–15 cm from the microphone; to record the courtship signals the female was placed near the male. Data on the material collection localities are given in the text below or in the cited publications.

RESULTS AND DISCUSSION

Segregation of Acoustic Communication Channels, or Acoustic Niches in Insect Communities: Do Species Always Differ in Their Signals?

Each species producing acoustic communication signals occupies a certain communication channel, or the so-called acoustic niche. This term usually refers to the complex of physical (amplitude-temporal and frequency) parameters of the signal, in particular, the presence or absence of periodicity of elements of different levels (pulses, syllables, and phrases), the specific temporal pattern of these elements and, finally, the ranges of their duration and repetition period. A certain combination of these parameters determines the "position" of each species' signal in the soundscape of the biotope, i.e., the acoustic niche, which is a component of the ecological niche occupied by a given species (Zhantiev, 1981). Alternatively, the acoustic niche may be defined as a range of acoustic parameters of the environment within which successful communication of a given species is possible (Bukhvalova, 2006). It should be borne in mind that the acoustic parameters of the environment of a particular species include, among other components, the biogenic noise, i.e., signals of sympatric species.

Segregation of acoustic niches is always observed in communities of species engaged in acoustic contact. This is true not only of closely related species from one genus or family, but also of supraspecific taxa. For example, during the study of singing insects and other acoustically active animals in the Bornean tropical rain forest it was found out that sounds produced by representatives of the same group of animals shared a number of features, so that different higher taxa were characterized by different "acoustic images" (Riede, 1996). Due to this phenomenon, it is nearly always possible to tell whether the singing animal hidden deep in the forest is a mammal, a bird, an amphibian, or a member of a certain taxon of insects. Among the latter, the groups easily identifiable by their sounds are crickets (they emit prolonged signals with a narrow-band spectrum within the range of 4–9 kHz), katydids (their songs are similar to those of crickets but have broader frequency spectra extending into the ultrasonic range), and singing cicadas (their signals contain many high-frequency harmonics).

Within each low-rank taxon of insects (genus, subfamily, or family), segregation of acoustic niches is usually based on differences in the amplitude-temporal patterns of signals. These differences are usually of qualitative nature; sometimes they are so great that the signals of closely related and almost morphologically identical species may have nothing in common. In many cases, analysis of signals is the only way to prove that the forms in question are indeed different species.

A classic example of this kind is given by four common representatives of the *Chorthippus biguttulus* species group: *Ch. biguttulus* (Linnaeus, 1758), *Ch. brunneus* (Thunberg, 1815), *Ch. mollis* (Charpentier, 1825), and *Ch. maritimus* Mistshenko, 1951 (Orthoptera, Acrididae). Their identification by morphological characters is very difficult; they are also very similar ecologically and can often be found in the same biotope. At the same time, their sound signals can be easily distinguished even with the unaided ear (Fig. 1, *1–9*).

Among planthoppers there are also many closely related species that differ almost exclusively in the temporal patterns of their calling signals. For example, two close species of *Macropsis* living on the Russian olive *Elaeagnus angustifolia* (Elaeagnaceae), namely *M. elaeagni* Emelyanov, 1964 and *M. elaeagnicola* Dubovsky, 1966 (Homoptera, Cicadellidae), are totally identical in external morphology (Fig. 1, *10*) and differ only in the fine details of the apodemes of abdominal segment II and the male genitalia (Fig. 1, *11–12* and *15–16*). The two species are strictly sympatric in the plains and low mountains of Central Asia, and both can often be collected from the same tree. However, there is almost no similarity between any elements of their signals (Fig. 1, *13–14* and *17–18*).

In cases when the general signal structure is more or less similar in different species, the differences are of a quantitative nature. This is observed, in particular, in grasshoppers of the subfamily Gomphocerinae (Orthoptera, Acrididae) (Bukhvalova, 2006; Tishechkin and Bukhvalova, 2010). Their calling signals may be classified into several types depending on the structure of their phrases (short and regularly repeated, or single and prolonged ones) and syllables (composed of discrete pulses, merged pulses, etc.). In turn, the main species-specific character within each type is the syllable repetition period. It is interesting that other quantitative parameters, such as the number of pulses in the syllable or the duration of phrases and intervals between them, do not seem to be important for acoustic niche segregation: they are either highly variable or strongly overlapping in different species. In each community of grasshoppers, the signals of different species belonging to the same type always differ in the syllable repetition period. No cases of co-occurrence of two species emitting signals of the same type with a similar syllable period have been recorded so far.

As an example, let us consider our data on the grasshopper community in the environs of Obluch'e, the west of Khabarovsk Territory (Tishechkin and Bukhvalova, 2010). This community included five species emitting signals of the same type, with short and regularly repeated phrases and with syllables consisting of merged pulses (Fig. 2, *1–12*). The signals of all the species were clearly different in the syllable repetition period, so that each signal occupied a certain range only insignificantly overlapping with the neighboring ranges (Fig. 2, *13*). The other signal parameters were either highly variable, as the phrase repetition period in *Chorthippus intermedius* (Bey-Bienko, 1926) (Fig. 2, *1*), or practically identical, as the phrase duration in *Ch. intermedius* and *Chrysochraon dispar major* Uvarov, 1925 (Fig. 2, *8* and *10*). Even the presence of a facultative additional component in the signal of *Ch. intermedius* (Fig. 2, *1*, *7*) did not allow this signal to get outside the acoustic niche determined by the syllable repetition period in the main part of the song: the signal still occupied the free range on the temporal scale, not overlapping with the other species in this character (Fig. 2, *13*).

The calling signals of the small Auchenorrhyncha are more complex and diverse in their amplitude-temporal pattern; the oscillograms of signals of species of the genus *Handianus* (Homoptera, Cicadellidae, Deltocephalinae) are given as an example in Fig. 3 (Tishechkin, 2000). However, in this group there are also some rare cases of segregation of acoustic niches by quantitative parameters, such as the duration of some elements of the signal (in the genus *Doratura*, Cicadellidae; Tishechkin, 2011c) or their repetition period (in spittlebugs of the genus *Aphrophora*, Aphrophoridae; Tishechkin, 2011a). Two of the studied species of the genus *Doratura*, namely *D. stylata* (Boheman, 1847) and *D. homophyla* (Flor, 1861), are distributed over the whole territory of Russia. In the south of Moscow Province they occur in the same biotopes as *D. impudica* Horvath, 1897; in the North Caucasus they are sympatric with *D. exilis* Horvath, 1903, and in the steppes of Tyva (South Siberia), with *D. gravis* Emeljanov, 1966 and *D. lukjanovitshi* Kusnezov, 1929. Comparison of the oscillograms shows clear similarity in the temporal signal pattern of all the species (Fig. 4, *1–6*), but the duration of signal elements, and sometimes their number (in *D. impudica*: Fig. 4, *2*), are species-specific.

Finally, insects demonstrate some examples of acoustic niche segregation by frequency. It was shown that in sympatric species of Cicadellidae, the signals similar in their amplitude-temporal structure may differ in the main frequency or the frequency modulation pattern (Tishechkin and Burlak, 2013). In the orthopteran communities, signals of some species differ in their frequency parameters as clearly as in the amplitude-temporal ones; for this reason, they stand out against the sounds of other insects in sonograms (Benediktov, 2015).

However, singing insects may avoid competition not only due to differences in the physical parameters of their signals. Sometimes clearly different species produce similar or even identical calling signals, i.e., occupy the same acoustic niche. This is possible if the species do not engage in acoustic contact with one another, so that the similarity of their signals does not hinder their intraspecific communication. This phenomenon can be observed not only in allopatric species, which are acoustically isolated for obvious reasons. Sympatric species may also be acoustically isolated due to the differences in the time of acoustic activity, biotopic associations, trophic specialization, etc. For example, most grasshoppers of the tribe Bryodemini (Orthoptera, Acrididae, Oedipodinae) in the South Siberian steppes perform display flights accompanied by loud sound signals during the whole day. However, *Bryodemella tuberculatum* (Fabricius, 1775) has only one or two brief surges of crepuscular activity, in the evening after sunset and sometimes in the morning before sunrise; some individuals may be active in the daytime but only in cloudy weather when the other species of Bryodemini do not fly (Tishechkin, 2010). This pattern may be related to the fact that the signals of *B. tuberculatum* and one of the diurnal species, *Angaracris barabensis* (Pallas, 1773), sound quite similar even though technically they differ in their temporal parameters. Since Bryodemini often reach very high abundance, their signals during mass display flights may be mutually jammed despite the clear interspecific differences (Tishechkin, 2010).

Even in the so-called “dusk chorus,” i.e., a brief surge of acoustic activity before nightfall typical of the tropics, different insect species can be clearly separated by the time of singing, even though the period of activity of each species may be only half an hour long. For example, the typical dusk chorus in the forests of Borneo Island starts with the singing of cicadas, and at 18.30 sharp the cicadas cease to sing, to be replaced by the chorus of mole crickets (Riede, 1996). Of the singing cicadas (Homoptera, Cicadidae) forming the dusk chorus in Panama, one species starts singing at the beginning of sunset, another, on average 11 min after sunset, and one more species, approximately 19 min later, this gap being gradually reduced to

11 min by the end of the reproduction period (Wolda, 1993). Such exact timing of the singing activity is reflected in the names of insects: for example, some species of the Asian genus *Pomponia* (Homoptera, Cicadidae) are locally known as “six o’clock cicadas” (Boulard, 2006).

Besides its significance for reproductive isolation, separation of communication channels also allows the insects to avoid acoustic noise. This is indicated by the segregation of acoustic niches of the higher taxa (for example, different families of insects) based on differences in the physical parameters of the signals and/or the time of vocal activity (Riede, 1996). It is evident that crossbreeding between singing cicadas and mole crickets would be impossible even without special isolation mechanisms; but mass singing of one species would jam the signals of the other species, leading to segregation by the time of their activity in the dusk chorus.

A similar phenomenon is sometimes also observed in species of one taxon. An example of this kind is the two species of Bryodemini (Orthoptera, Acrididae, Oedipodinae) mentioned above, one of which sings in the daytime and the other, in the twilight. Their signals can be quite easily differentiated in oscillograms; however, under the field conditions, with the sounds of tens and hundreds of individuals forming a continuous background noise, it would be very difficult to identify these two species by their signals if we did not know beforehand that one of them is diurnal and the other is crepuscular.

It is also known that the response of small homopterans (planthoppers and psyllids) to signals of sympatric species does not differ from their response to abiotic noise produced by fluctuations and contacts of leaves and stems: under the natural conditions, each individual tries to “put in” its signal during the brief intervals between the songs of other species and/or the gusts of wind (Tishechkin, 2012).

In actual practice of studying intraspecific acoustic interactions, one of the main problems is to determine whether the species in question are indeed acoustically isolated, or they do perceive each other’s signals and therefore compete for acoustic niches within the same community.

When we are dealing with insects emitting sound signals, the problem is relatively simple. Sound oscillations propagate uniformly in all directions from their source, even though they can be weakened by physical obstacles, first of all dense vegetation. Still, if species co-occur in the same biotope and have overlapping periods of seasonal and daily activity, they will inevitably compete for communication channels. If species occupy the same acoustic niche, they are always spatially isolated due to allopatry or different ecological preferences.

An example of allopatric species with similar signals is two species of grasshoppers: *Chorthippus macrocerus* (Fischer-Waldheim, 1846) and *Ch. hammarstroemi* (Miram, 1907) (Orthoptera, Acrididae, Gomphocerinae). The first species occurs in the steppes of European Russia and West Kazakhstan, from the foothills to the subalpine zone in the Caucasus, and in Transcaucasia. The range of the second species extends from Altai to the Pacific, reaching Yakutia in the north and the northern regions of Mongolia and China in the south. Such a broad distribution indicates high ecological plasticity of these two species; however, they do not contact one another in any place. This may be related to the fact that their signals belong to the same type (Fig. 4, *7–10*): they are formed by single phrases with a highly variable duration (on average from 4–5 to 15–17 s in *Ch. macrocerus* and from 5–6 to 20–30 s in *Ch. hammarstroemi*), while the structure and repetition period of the syllables are similar (170–340 ms in *Ch. macrocerus* and 180–270 ms in *Ch. hammarstroemi*) (Tishechkin and Bukhvalova, 2010).

Cases of biotopic vicariance are also known in this subfamily. For example, *Ch. macrocerus* and *Ch. vagans* (Eversmann, 1848) occupy the same acoustic niche; however, in Rostov Province where our research was conducted, the former occurs in steppes, agrocenoses, and roadsides, and the latter, only in glades within forest plantations (Tishechkin and Bukhvalova, 2010).

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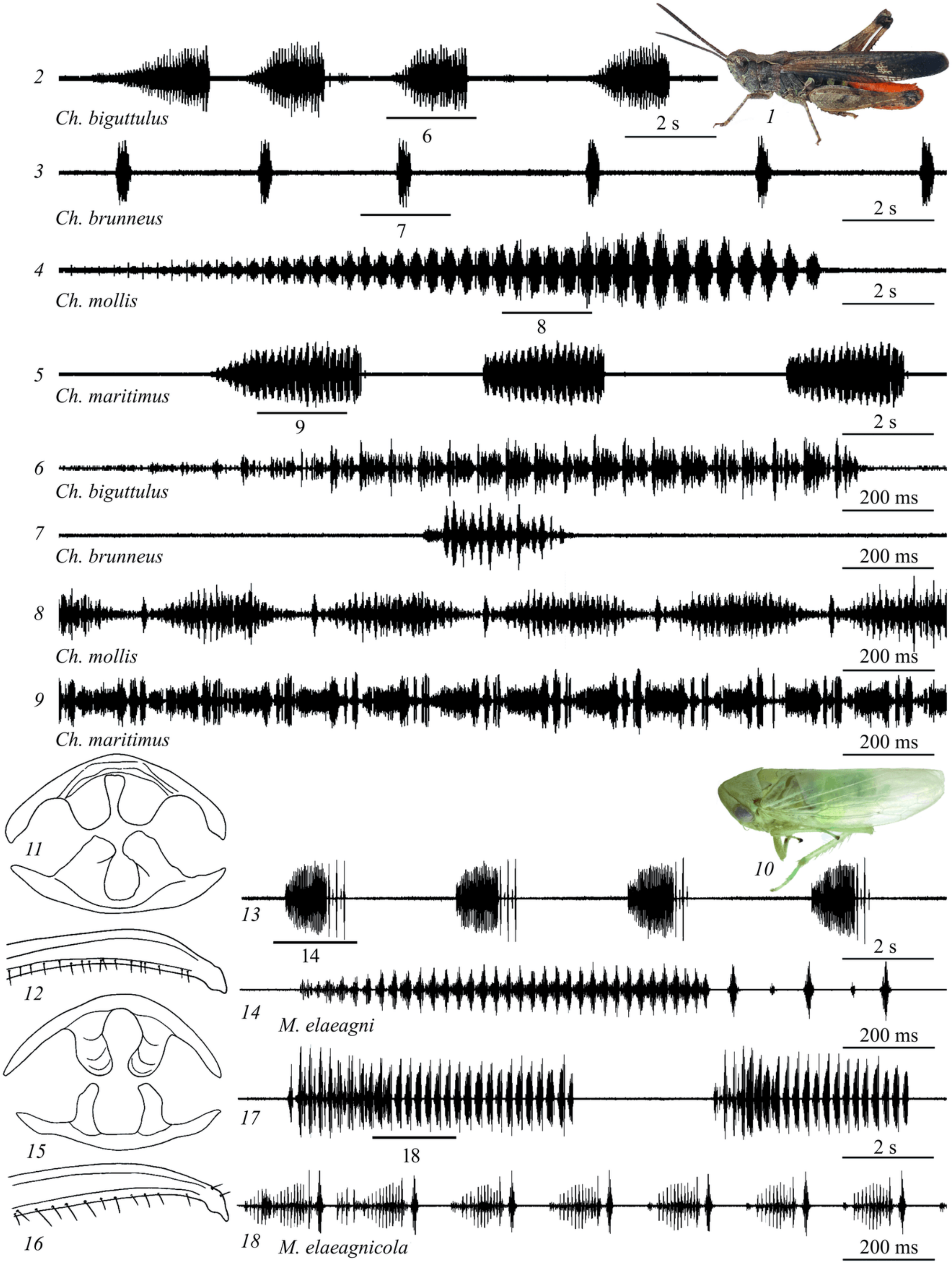
Fig. 1. Differences in the structure of acoustic signals between morphologically close species: (*1–9*) grasshoppers of the *Chorthippus biguttulus* species group: (*1*) habitus; (*2–9*) oscillograms of calling signals; all the records made in the environs of Ul'yanino, Khvalynsk District, Saratov Province); (*10–18*) leafhoppers of the genus *Macropsis* living on *Elaeagnus angustifolia*: (*10–14*) *M. elaeagni*; (*15–18*) *M. elaeagnicola*: (*10*) habitus; (*11*, *15*) apodemes of abdominal segment II of males; (*12*, *16*) tip of the stylus; (*13–14*, *17–18*) oscillograms of calling signals; all the records made in the foothills of West Tien Shan. Fragments of signals designated by numbers 6–9, 14, and 18 are shown at higher speed in oscillograms under the same numbers.


1
2
Ch. biguttulus
6
2 s
3
Ch. brunneus
7
2 s
4
Ch. mollis
8
2 s
5
Ch. maritimus
9
2 s
6
Ch. biguttulus
200 ms
7
Ch. brunneus
200 ms
8
Ch. mollis
200 ms
9
Ch. maritimus
200 ms
10
11
12
13
14
2 s
14
M. elaeagni
200 ms
15
16
17
18
2 s
18
M. elaeagnicola
200 ms

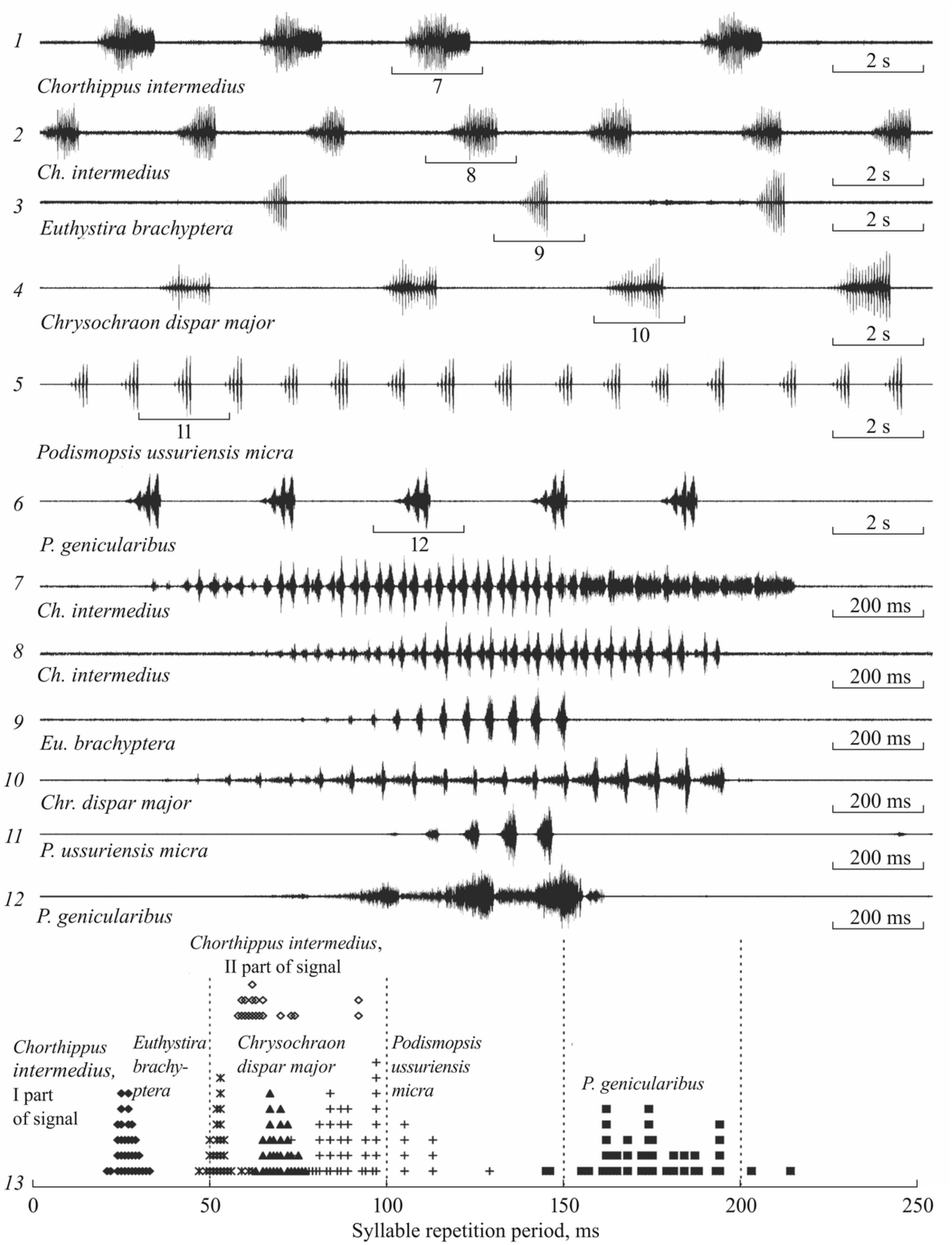


Fig. 2. Segregation of acoustic niches in the community of grasshoppers of the subfamily Gomphocerinae in the environs of Obluch'e, Khabarovsk Territory. Only the species emitting regularly repeated phrases of syllables not divided into pulses are presented: (*1–12*) oscillograms of signals; (*13*) distribution of syllable repetition periods in different species. Oscillograms of a complete signal consisting of two parts (*1*, *7*) and a reduced signal (*2*, *8*) are given for *Chorthippus intermedius*. Fragments of signals designated by numbers 7–12 are shown at higher speed in oscillograms under the same numbers.

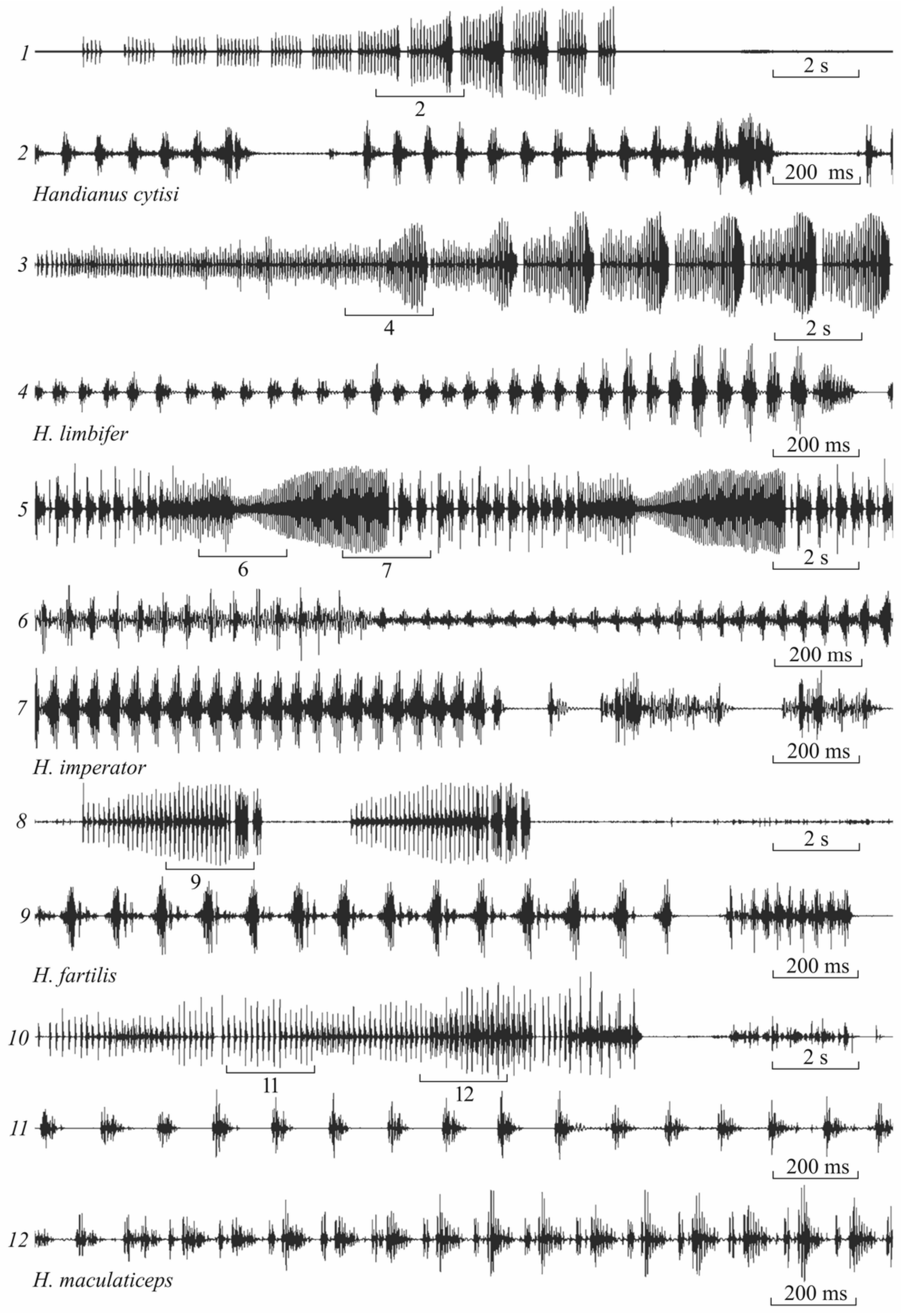


Fig. 3. Oscillograms of calling signals of leafhoppers of the genus *Handianus*. Fragments of signals designated by numbers 2, 4, 6, 7, 9, 11, and 12 are shown at higher speed in oscillograms under the same numbers.

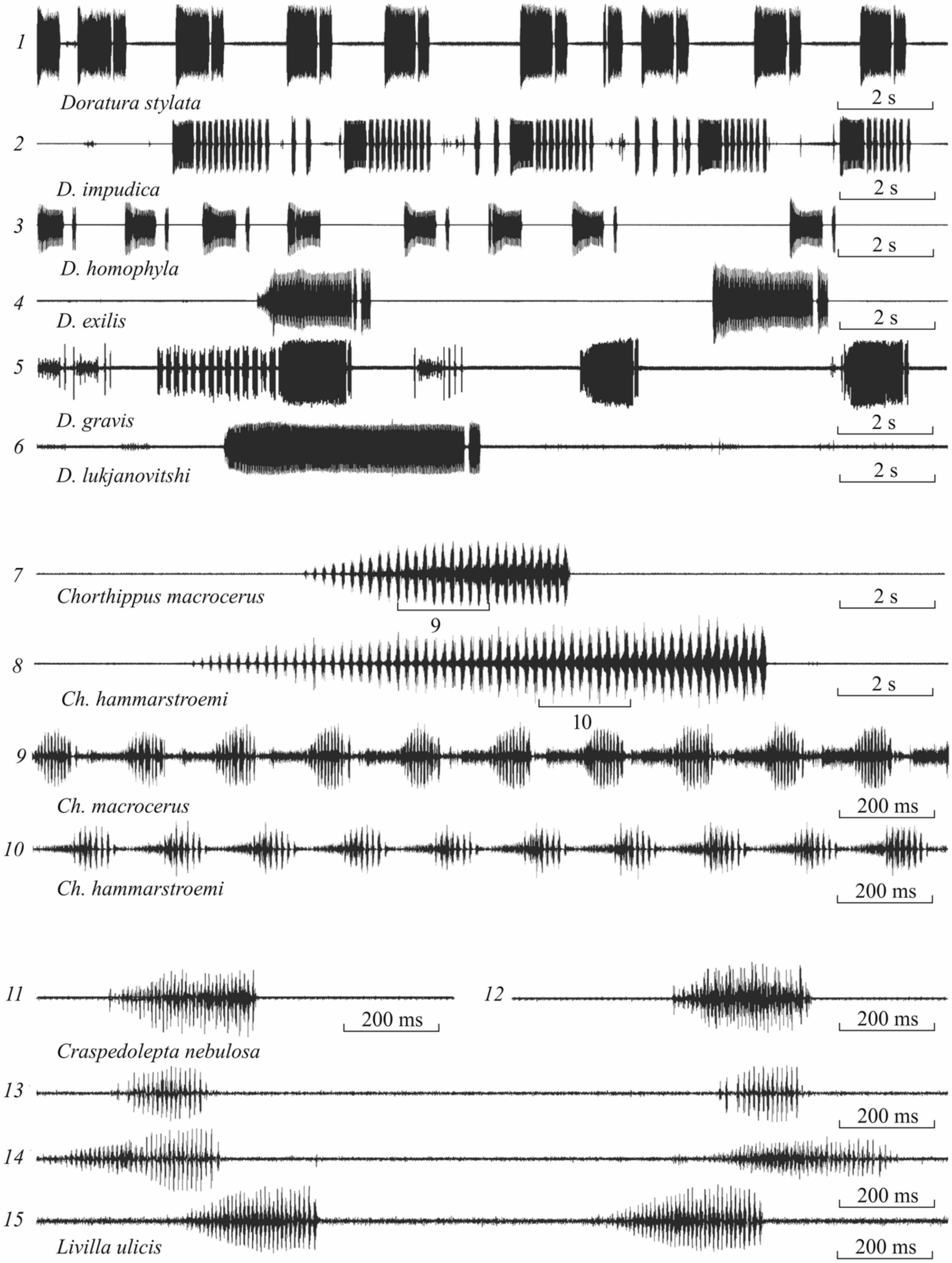


Fig. 4. Oscillograms of calling signals: (*1–6*) leafhoppers of the genus *Doratura*; (*7–10*) two vicariant species of grasshoppers of the genus *Chorthippus*; (*11–15*) two psyllid species that are formally sympatric but occupy different plant communities. Fragments of signals designated by numbers 9 and 10 are shown at higher speed in oscillograms under the same numbers.

Unlike sounds, vibrational signals spread only in the solid substrate, mostly along the stems and leaves of plants on which the singing individuals are positioned. Experiments with recording such signals under the natural conditions showed that the range of communication was much greater than could be assumed, even in small representatives of Homoptera (Auchenorrhyncha and Psyllinea) with the body length not exceeding 3–4 mm. Vibrational signals not only propagated along all the branches of one stem, but they could be also transferred from stem to stem via the touching overground parts, via the common root system, and even via the contacting roots of different plant species. This was indicated, first, by the possibility of recording signals from an insect positioned on one stem, with the vibrational sensor attached to another stem; second, by the responses of the males to each other's signals. In most insects the singing of one individual stimulates the acoustic activity of others. This was exactly what we observed in the nature: as soon as one male emitted the calling signal, the individuals positioned on the neighboring stems growing from one rhizome or touching with their leaves responded immediately. It follows from the above that among the insects using vibrational communication, even strict monophages do not have their own "dedicated" communication channels. In the dense herbage they inevitably perceive the signals of all the other species that occupy other plants in the same community (Tishechkin, 2011b).

At the same time, even in a small area there may be several communities of plants that have practically no contact with each other, so that transmission of vibrational signals between them is impossible. The same is true of the different life forms of plants: the vibrational signals of insects living on trees may be only occasionally heard by the species living in the herbaceous layer under the canopy of the same forest. It should be noted that technically such species are strictly sympatric because they may be collected in one biotope or during net-sweeping along a transect only several tens of meters long.

For example, two species of psyllids (Homoptera, Psyllinea), *Craspedolepta nebulosa* (Zetterstedt, 1828) (Aphalaridae) and *Livilla ulicis* Curtis 1836 (Psyllidae), can often be found practically in the same locality in the south of Moscow Province. However, the former species is a monophage of the fireweed *Chamerion angustifolium*, forming almost pure grass stands on forest edges and in clearings, whereas the latter occurs on the greenweed *Genista tinctoria*, growing in dry glades. The signals of these species are nearly identical, except for the fact that the phrases of *L. ulicis* are usually emitted with shorter and more regular intervals (Fig. 4, *11–15*) (Tishechkin, 2006a).

Thus, although the signals used for attracting conspecific mates always represent a component of SMRS in the meaning of Paterson, they do not always constitute a reproductive barrier in the meaning of Mayr. In forms that do not engage in acoustic contacts due to allopatry or ecological differences, speciation may proceed without divergence by the characters pertaining to the signal structure, even though these characters remain fully functional in terms of SMRS. One of the best known examples of this kind is treehoppers of the *Enchenopa binotata* (Say, 1824) complex (Homoptera, Membracidae). The evolution of this group, including the role of vibrational signals in speciation, was studied in detail by the American researchers; the review of the aspects related to acoustic communication was published by Cocroft and co-authors (2008).

In all likelihood, speciation in this group started with transition of the ancestral form onto several species of trees and shrubs from different families. Treehoppers, similar to most Auchenorrhyncha, lay eggs in incisions made by the ovipositor on the branches of the host plants. The development of eggs and hatching of larvae in spring is triggered by the beginning of sap flow in the stem. Since different species of plants differ in their phenology, the development cycles of the trophic races of treehoppers associated with them became desynchronized. Correspondingly, the gene flow between them was drastically reduced, and each race got specialized to feeding on its respective host plant and gradually became a distinct species. At present, this group is a complex of cryptic monophagous species. In the absence of reliable morphological differences between them, most of these species have not yet been formally described. They are also practically indistinguishable by the temporal patterns of their signals, but this does not prevent their existence as biological species because differences in their trophic specialization, and consequently in phenology, ensure reliable reproductive isolation even under the conditions of sympatry.

The signals of some representatives of *E. binotata* group differ in their carrier frequency, this parameter being the main character used for recognition of the conspecific song. The dependence of the signal frequency on the physical properties of the host plant was studied in two species of this group, living on *Cercis canadensis* (Fabaceae) and on *Ptelea trifoliata* (Rutaceae). A study of the frequency response of thin branches, leaf stalks, and laminae of these plants showed it to be different both in the two species and in different organs of the same plant species. The singing males of treehoppers living on *C. canadensis* mostly concentrated on the twigs, and those of the species associated with *P. trifoliata* preferred the leaf stalks. The carrier frequency of the signals was about 140 Hz in the first species and about 340 Hz in the second. These values coincided quite well with the frequency maxima of the plant parts inhabited by the insects: lower frequencies were more efficiently transmitted by the thicker twigs, and higher frequencies, by the thinner leaf stalks. It may therefore be assumed that the differences in the carrier frequency of the signals originally appeared as the result of "tuning" to the frequency parameters of the specific substrate, and only secondarily became a factor of reproductive isolation.

Similarity of signals in different species may be also observed in cases of allopatric speciation. For example, *Macropsis flavida* Vilbaste, 1980 and *M. remota* Tishetshkin, 1998 (Homoptera, Cicadellidae, Macropsinae) emit almost identical signals (Fig. 5, *1–4*) but differ in size (the latter being noticeably larger), in small details of the apodemes of abdominal segment II in males, and partly in trophic specialization: the former species feeds on many willows while the latter is a monophage of *Salix schwerinii* (Salicaceae). The two forms do not engage in acoustic contact because the former is distributed in the continental part of Eurasia from West Siberia to the Pacific coast, and the latter is reliably known only from Sakhalin Island. In our opinion, the two forms have presently reached the stage of divergence at which they may be regarded as distinct species, although it is obvious that originally they were the continental and insular populations of the same species, separated due to geographic isolation.

An even more significant example is that of *Macropsidius sahlbergi* (Flor, 1861) and *M. chazarianus* Logvinenko, 1981 from the same subfamily (Tishechkin, 2014). The great majority of species of the genus *Macropsidius* are externally identical, their diagnostics being based exclusively on the male genital morphology (Tishechkin, 2006a, 2014). Identification of most species is quite easy since the morphological differences between them are obvious (Fig. 5, *5–6*, *9–10*, *13–14* and *17–18*). They can also be easily differentiated by their signals (Fig. 5, *11–12*, *15–16* and *19–20*), except for the above pair of species which have an almost identical temporal pattern of phrases (Fig. 5, *7–8* and *11–12*). Both species feed on wormwoods of the subgenus *Dracunculus* (Artemisia, Asteraceae), but the former is broadly distributed in the forest and steppe zones of Eastern Europe and the steppes of Kazakhstan, and is also known from several localities of Western Tien Shan, while the latter is an endemic of the sandy deserts of the North Caspian Sea basin, from Makhachkala in the western coast to Mangyshlak in the eastern one. The structure of their signals has not been affected by directional selection, probably due to allopatry, whereas their divergence by morphological characters has advanced considerably (Fig. 5, *5–6* and *9–10*).

As a rule, however, the signals do evolve together with the morphological characters in case of allopatric speciation. Moreover, divergence by the signals may proceed at a greater-than-average rate, which may lead to the emergence of externally similar forms differing in their signals. Therefore, similar signals are rarely found even in geographically isolated species; it should be noted that these particular forms do not fit the definition of species as groups of individuals or populations differing from other such groups in the structure of SMRS. At the same time, such groups preserve their reproductive isolation, which means that they totally correspond to Mayr's traditional definition of species.

Evolution of Signals under the Influence of Sexual Selection: Examples from Two Groups of Orthoptera

Individuals of different sexes face the task of not only recognizing and locating the conspecific mate but also assessing its "quality" (Andersson, 1994). The problem of selecting the best mate is more urgent for females because the number of males ready for copulation at a given moment is usually greater than the number of receptive females (e.g., Kriegbaum, 1989; Helversen and Helversen, 1994). In Orthoptera, the relative stability and low intraspecific variation of the calling signals (the signals produced by a solitary male) provide little opportunity for selecting the "best" male. The female can estimate the quality of the male at a distance only by preferring, other conditions being equal, the males with stable and loud songs and thus ignoring the old or diseased males (Zhantiev, 1981).

A different situation emerges when the male gets sufficiently close to the female and starts emitting a different type of signal, namely the courtship song. In different species of Orthoptera courtship may include not only sound but also vibrational, visual, olfactory, and mechanical signals (Balakrishnan and Pollack, 1997; Vedenina, 2005; Kortet and Hedrick, 2005; Heller, 2006; Finck et al., 2016). Some parameters of such a polymodal signal may be more stable and serve for recognition of a conspecific individual, while other, more variable parameters may be used to estimate the mate quality (Vedenina, 2005; Vedenina and Shestakov, 2013). The variable signal provides a greater possibility of choice for females and of contest for males, especially in case of certain strategies of sexual behavior, such as "lekking" (Kirkpatrick and Ryan, 1991). The contest may lead to differential reproductive success if the signals of some males happen to be more attractive for most females than those of other males (Andersson, 1994).

According to one of the theories of sexual selection, the signaling theory or the "good genes" theory (Zahavi, 1987; Hamilton and Zuk, 1982), the conspicuous characters of the male are preferred by females because they indicate its quality or better fitness, which would be inherited by its offspring. For example, the sound signal in which a certain element has a greater duration or intensity as compared with the norm is more attractive to females. A possible explanation of this phenomenon is that such a signal requires more energy to be spent by the male; therefore it manifests its high quality (Stumpner and Helversen, 1994; Gerhardt and Huber, 2002). The sound signal accompanied by display movements of different body parts, for example, by swinging of the antennae or legs, is also more "expensive" than the signal without movements, and may therefore serve as an indicator of a strong and healthy mate (Vedenina and Helversen, 2003, 2009). Another, equally common theory of sexual selection was proposed by Fisher (1930, 1958). According to it, the first stage is the emergence in the population of a mutant allele affecting either a certain character of the male or the preferences of the females. This allele may be initially neutral and spreading by genetic drift. At the subsequent stages, however, the males possessing this character obtain an advantage over the males lacking it: females start to gain from selecting males with the new character because their male offspring will inherit the character, attract more females, and produce more offspring of their own. It should be noted that, even though both theories are commonly accepted, there have been few empirical tests proving the existence of any of these mechanisms.

Below we will show to which extent the acoustic signals may evolve under the influence of sexual selection, by the example of two groups of Orthoptera: crickets (Gryllidae) and grasshoppers (Acrididae).

Crickets have a relatively large range of communication: up to 10 m (Zhantiev, 1981; Popov, 1985). Crickets of many species have shelters or burrows; the male producing the calling signal is often positioned near the entrance to its burrow, in a small pit which enhances the signal acting as a sound reflector or resonator. The pulses of the cricket signals have a sine carrier; therefore the frequency spectra of their calling signals lie in narrow frequency ranges, unlike the broadband frequency spectra of the signals of most katydids and grasshoppers. Thus, the sound signals of different cricket species may differ not only in the temporal but also in the frequency characteristics (Eisner and Popov, 1978; Zhantiev, 1981). As for the temporal parameters, the signals of closely related species may differ in the duration and period of pulses, the number of pulses in a syllable, and also by the grouping of pulses into syllables, as, for example, the signals of different species of the genus *Gryllus* (Fig. 6, *1–3*).

Female crickets often fly towards the singing males, but they mostly cover the last meters walking over the substrate. During courtship the males produce acoustic signals which considerably differ from the calling song in not only the temporal but also the frequency parameters. The courtship signal has lower ampitude than the calling signal while its frequency spectrum includes more high-frequency components. As a rule, two elements can be distinguished in the courtship signals of different species of the genus *Gryllus*: high-amplitude pulses with higher frequencies (referred to as *clicks* for convenience of description) and low-amplitude pulses with low frequencies, alternating in a specific pattern (Fig. 6, *4–9*) (Zhantiev and Chukanov, 1972; Nocke, 1972; Zhantiev and Dubrovin, 1974; Fitzpatrick and Gray, 2001; Vedenina and Pollack, 2012). Thus, the temporal structure of the courtship signal is more complex than that of the calling signal which only comprises one element (Fig. 6, *1–3*). The male emits its courtship signal while moving backwards toward the female, which has to mount the male for successful copulation. It was shown that the conspecific courtship signal was needed for triggering

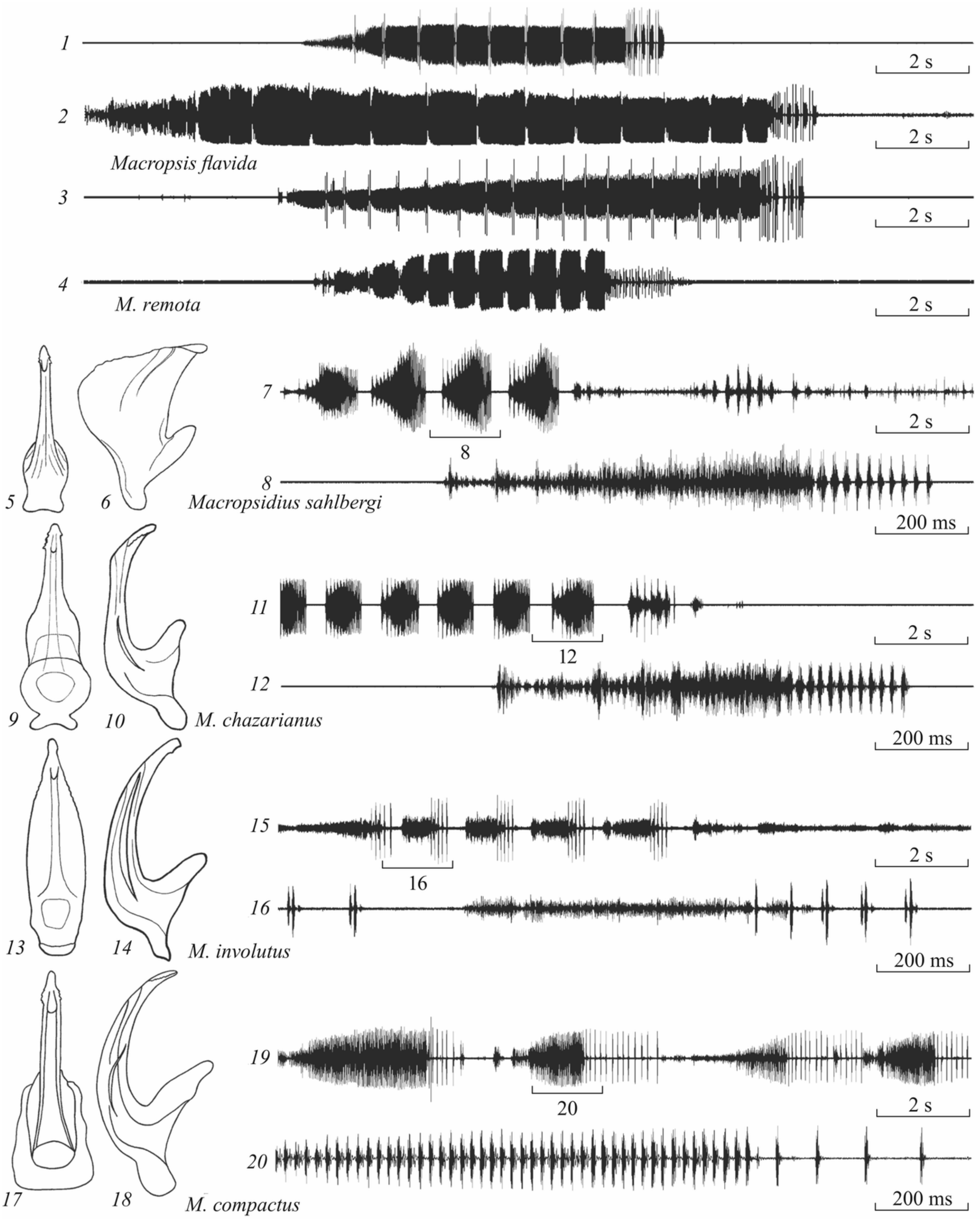


Fig. 5. Similarity of acoustic signals in allopatric species: (*1–4*) oscillograms of signals of close species of leafhoppers of the genus *Macropsis*: (*1*) male from Amur Province; (*2*) male from South Primorye; (*3*, *4*) males from the south of Sakhalin Island; (*5–20*) leafhoppers of the genus *Macropsidius*: (*5*, *9*, *13*, *17*) penis in posterior view; (*6*, *10*, *14*, *18*) penis in lateral view; (*7*, *8*, *11*, *12*, *15*, *16*, *19*, *20*) oscillograms of calling signals. The four species differ equally well in morphology but the first two species have an almost identical signal structure (*7*, *8* and *11*, *12*). Fragments of signals designated by numbers 8, 12, 16, and 20 are shown at higher speed in oscillograms under the same numbers.

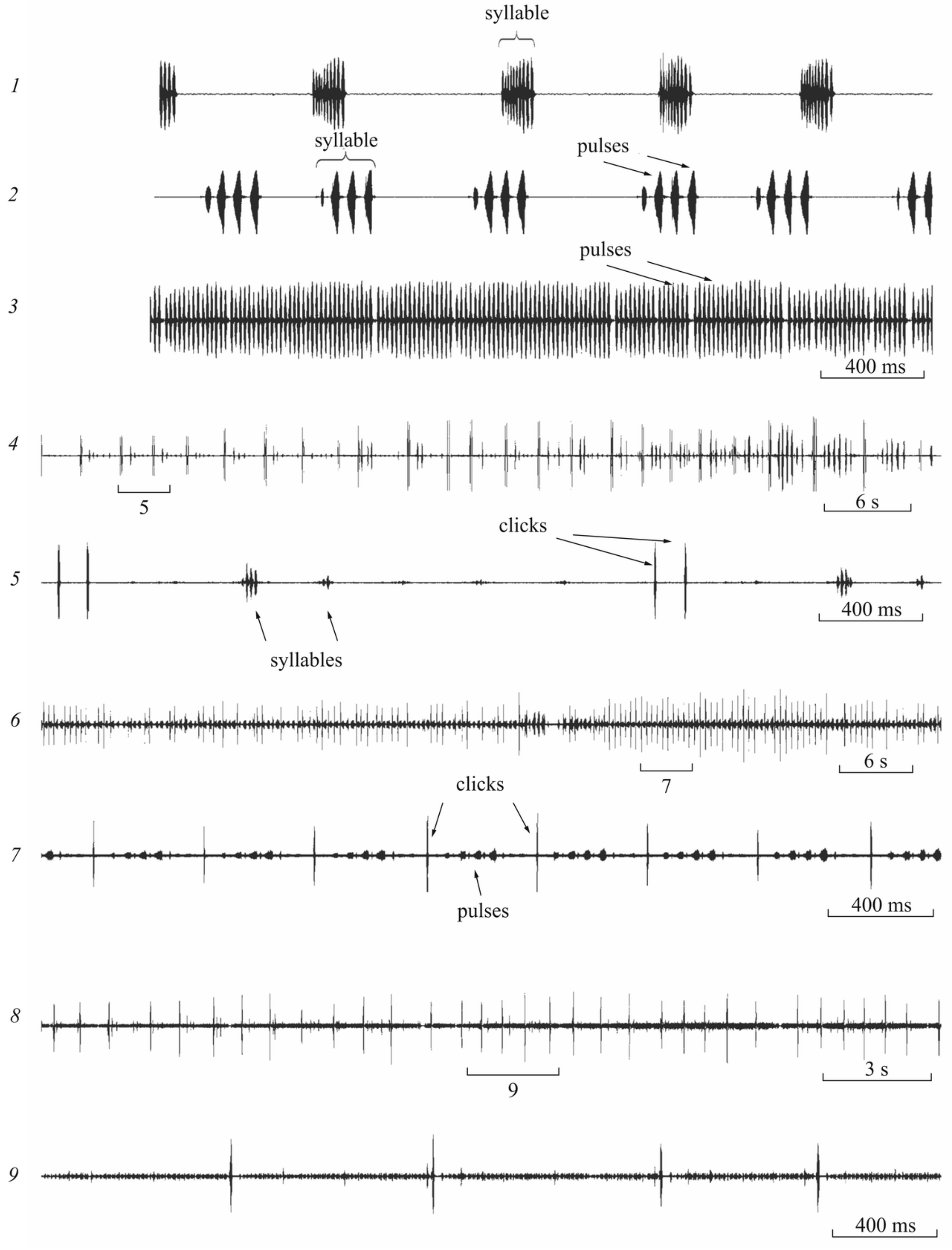


Fig. 6. Calling (*1–3*) and courtship signals (*4–9*) of three American species of crickets: (*1*, *4*, *5*) *Gryllus assimilis*; (*2*, *6*, *7*) *G. firmus*; (*3*, *8*, *9*) *G. rubens*. Fragments of signals designated by numbers 5, 7, and 9 are shown at higher speed in oscillograms under the same numbers.

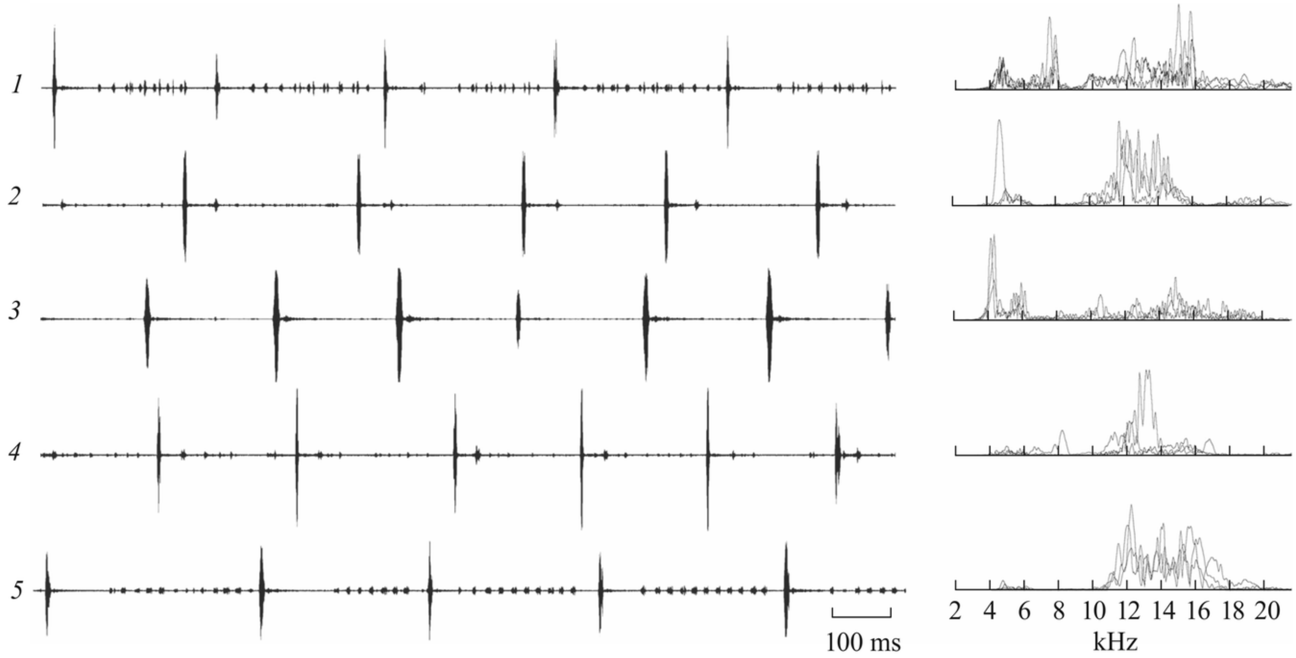


Fig. 7. Courtship signals and frequency spectra of clicks of five males of *Gryllus bimaculatus* (*1–5*). Each diagram on the right contains spectra of three different clicks from the same signal.

the stereotyped behavior pattern of the female leading to successful spermatophore transfer; at the same time, the percentage of successful copulations decreased significantly when the temporal structure of the model signal was changed (Balakrishnan and Pollack, 1996; Vedenina and Pollack, 2012; Shestakov and Vedenina, 2015).

At the same time, analysis of variability of the different parameters of the courtship signals of *G. bimaculatus* (De Geer, 1773) (Shestakov and Vedenina, 2015) and *G. assimilis* (Fabricius, 1775) (Vedenina and Pollack, 2012) showed that some parameters were relatively stable (coefficient of variation 6–17%) while others were highly variable (CV 20–93%). The most variable parameter in both species was the amplitude ratio of the clicks and the pulses, and the most stable parameters were the carrier frequencies of the pulses (*G. bimaculatus*) and the clicks (*G. assimilis*). The variation of the relative amplitude of clicks and pulses and the frequency spectrum of clicks in different males of *G. bimaculatus* is shown in Fig. 7. Experiments with synthesized model signals presented to females of *G. bimaculatus* showed that the different parameters of the signal were not equally important. The stimuli containing only clicks and no pulses (st7, st8 in Fig. 8, *3*, *4*) were even more efficient than the stimuli containing both clicks and pulses (st4, st6 in Fig. 8, *1*, *2*). The synthesized songs with different carrier frequencies of the clicks were equally attractive (Fig. 8, *1*, *2*). By contrast, changes in the click duration (Fig. 8, *3*, *4*) reduced the females' response down to the level of the negative control (courtship of "mute" males with no signal being played). Thus, changes in the variable parameter of the signal did not affect its efficiency or even increased it, whereas changes in the stable parameter decreased the efficiency of the signal. The same tendency was observed earlier for the long-range signals of anurans (Gerhardt, 1991; Gerhardt and Huber, 2002) and some orthopterans (Stumpner and Helversen, 1992; Shaw and Herlihy, 2000). Our data show that this rule holds true for short-range communication as well.

It should be noted that assessment of the mate quality in crickets may be based not only on acoustic signals. For example, in tree crickets (Oecanthinae) the female mounting the male during copulation consumes the secretion of its metanotal glands (Brown, 1999). The females of true crickets eat the spermatophore which, despite the absence of the nutritional spermatophylax typical of many katydids (Boldyrev, 1915), still serves as a factor of attraction during courtship. A characteristic behavioral feature of many species of crickets is defense of the individual territory, and also of the burrow or shelter. The owner of

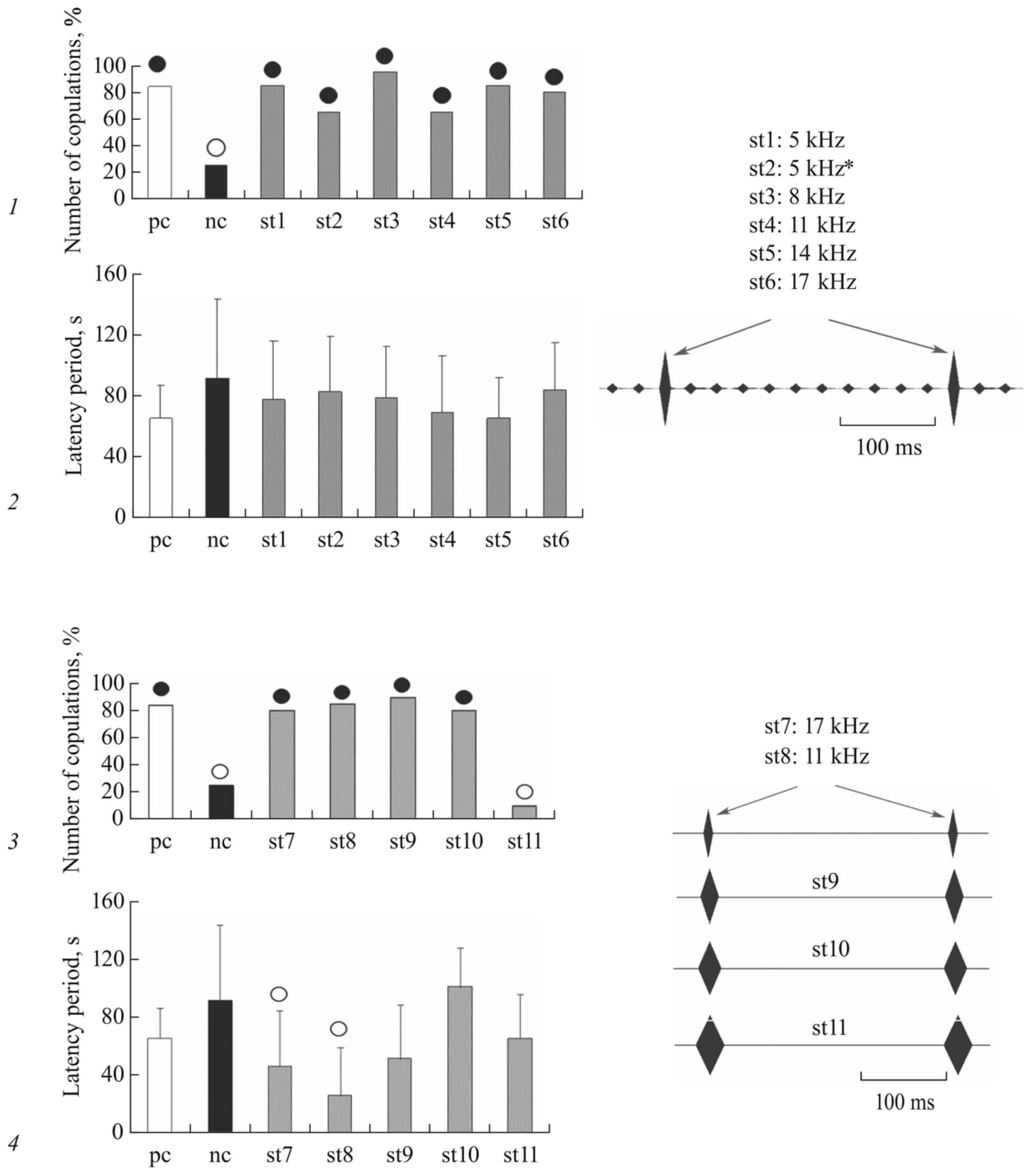


Fig. 8. Results of presentation of synthetic models of the courtship signal (shown on the right) to females of *Gryllus bimaculatus:* (*1*, *2*) model signals (stimuli, st) with different carrier frequencies of clicks; (*3*, *4*) model signals with different durations of clicks (duration of clicks with carrier frequency 11 kHz varied from 12 to 36 ms in st8–st11). All the tests except st2 were performed with females 3–8 days after imaginal molt; test st2 was performed with females 14–17 days old (asterisk). On the left: (*1*, *3*) fraction of copulating females; (*2*, *4*) latency period from the beginning of courtship signal to copulation. White bars: results of presentation of natural signal during courtship of a mute male (positive control, pc); black bars: results of courtship of a mute male without any signal (negative control, nc); gray bars: results of presentation of different model signals during courtship of a mute male; white circles: significant difference from positive control; black circles: significant difference from negative control ($p < 0.05$). The means and standard deviations are shown for the latency period ($N = 19–20$).

the territory usually wins the fight against the invader, though in general the outcome depends on the size and aggressiveness of the fighting males (Simmons, 1986). The female mated with the winner, one of the reasons for this choice being the fact that the loser did not display courtship behavior in the presence of the winner (Burk, 1983). On the other hand, if the female was presented with one courting male at a time, it more frequently mated with the dominant male, apparently orienting by its scent (Simmons, 1986; Kortet and Hedrick, 2005; Thomas and Simmons, 2009). Besides, females often prefer males possessing a burrow or a shelter because they use burrows for oviposition (Alexander, 1961).

Among acridid grasshoppers, representatives of the family Gomphocerinae use sound communication most actively. They have the shortest range of acoustic communication: 1–3 m (Haskell, 1958; Lang, 2000). These grasshoppers stridulate while resting on the ground or on herbaceous plants, mostly grasses. In this case, reflection and absorption of sound waves by the vegetation and soil constitute major obstacles to sound propagation. Unlike the crickets facing the same problem, grasshoppers do not use any external constructions to direct and enhance their signals. Besides, the calling signals of Gomphocerinae have lower intensity than those of crickets, and occupy a broad frequency spectrum (Vedenina and Zhantiev, 1990; Meyer and Eisner, 1996). Some representatives of Gomphocerinae not only produce sound signals by femoro-tegminal stridulation but may also use their wings. For example, some veins of the hind wing of *Stenobothrus rubicundus* (Germar, 1817) are heavily sclerotized, so that the males can produce loud sounds by striking one wing against the other, both in flight and while resting on the substrate (Eisner and Wasser, 1995).

The signals of Gomphocerinae may be very elaborate in their amplitude-temporal parameters (Bukhvalova and Vedenina, 1998; Vedenina and Bukhvalova, 2001; Ragge and Reynolds, 1998; Bukhvalova, 2003; Savitsky, 2005). This is partly accounted for by the specific features of femoro-tegminal stridulation. In most species studied, the hind legs move with a certain phase shift which may change in the process of stridulation; besides, the very pattern of movement may be different in the right and left leg (Eisner, 1974; Helversen and Helversen, 1994; Vedenina, 2005; Vedenina and Helversen, 2009; Vedenina and Shestakov, 2013). For example, if the legs move in phase opposition and the sound pulse is generated by the leg movement in one direction only, the resulting pulse frequency will be doubled. If the legs work in phase at the beginning of the phrase and then start moving in phase opposition, the distinct pulses within the syllables may disappear and the boundaries between the syllables become blurred. Different stridulatory pegs may be used for sound generation depending on the position of the hind femur; in this case, the non-uniform density and arrangement of pegs along the row may also affect the signal structure (Vedenina, 2005). Thus, the temporal pattern of the signal may become extremely complex due to the use of the paired sound-producing apparatus.

The receptive females of most species of Gomphocerinae respond acoustically to the male's calling signal. The male and female move toward one another while emitting sound signals in turns. Having approached the female, the male starts emitting the courtship signal. In most species of the tribes Chrysochraontini, Dociostaurini, and Arcypterini the male courtship signals are almost identical to the calling signals, differing only in slightly greater duration. The courtship signals of many species of the tribe Gomphocerini, in particular the genus *Euchorthippus*, *Chorthippus parallelus* (Zetterstedt, 1821), *Ch. montanus* (Charpentier, 1825), *Ch. macrocerus* (Fischer-Waldheim, 1846), and most species of the *Ch. biguttulus* group, also strongly resemble the calling signals (Ragge and Reynolds, 1998; Savitsky, 2000; Savitsky and Lekarev, 2007; Vedenina and Mugue, 2011).

By contrast, the courtship signals of nearly all the species of the tribe Stenobothrini are considerably longer and more complex than the calling ones. As a rule, they comprise more elements and are often accompanied by display movements. In many species of the genus *Omocestus* the beginning of the courtship signal is almost identical to the calling signal that is generated by simple up-and-down movements of the legs; this is followed by additional elements which are typical only of the courtship signal. For example, in the courtship signal of *O. minutus* (Brullé, 1832) the first part (element A) resembles the calling signal while the second part (element B) contains syllables with lower amplitude (Fig. 9). It is followed by abrupt upward strokes of the right and left legs in turn, the legs assuming a nearly vertical position at the end of the stroke (element C). The abrupt downward movement of the leg produces a single pulse. At the end of the signal, before the attempt at copulation, the male uses both its legs to generate nonspecific pulses that are similar in different species. In most species of the genus *Stenobothrus* the complexity of the courtship signal is also determined by specific alternation of the calling signal and other elements that are typical only of courtship. For example, the courtship signal of *S. nigromaculatus* contains two additional elements, and that of *S. fischeri* contains three additional ele-

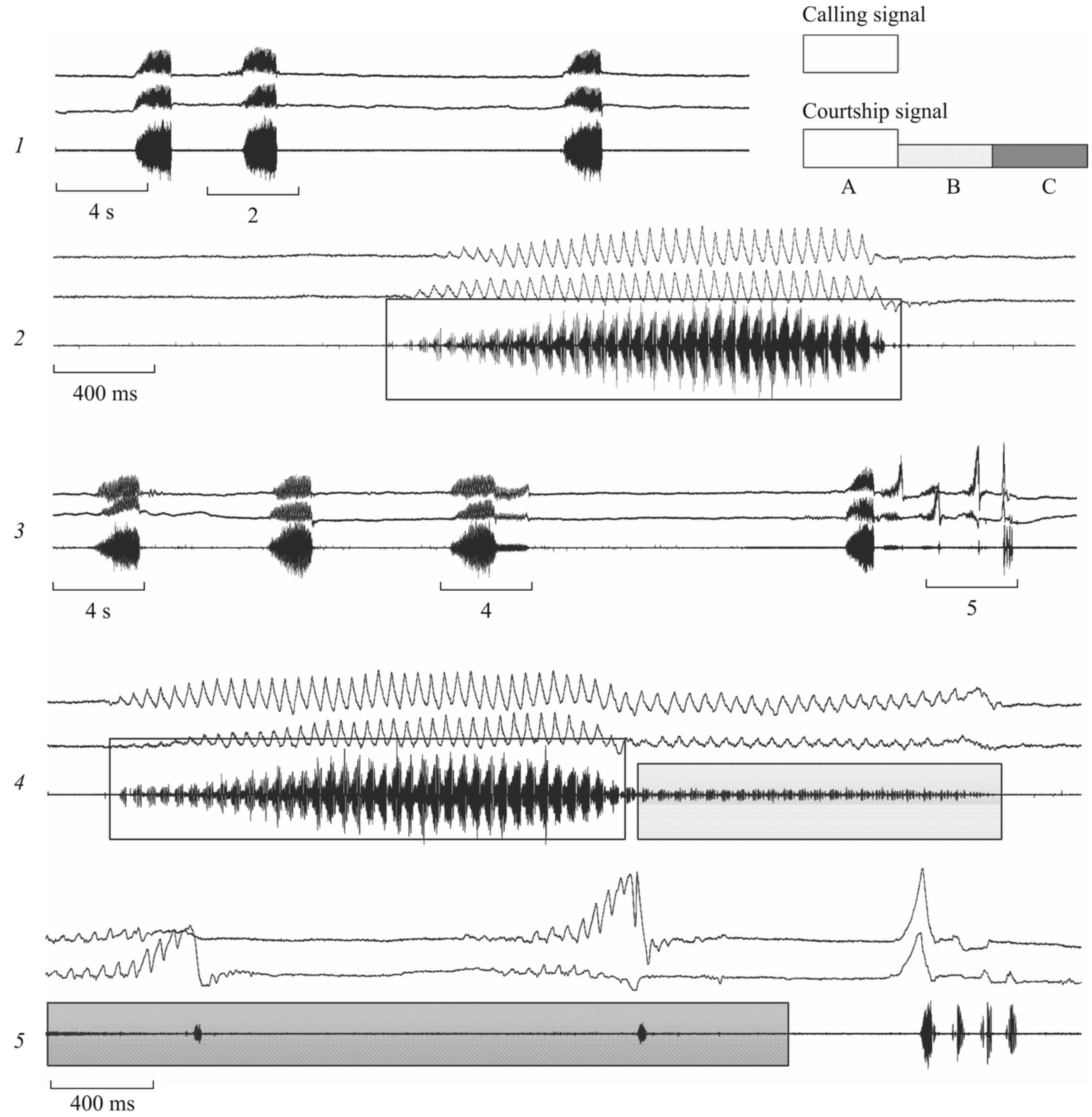


Fig. 9. Oscillograms of signals of *Omocestus minutus* from Saratov Province: (*1*, *2*) calling signal; (*3–5*) courtship signal. Trajectories of the hind legs are shown above each oscillogram on the same time scale. Schemes of the signal composition are shown in the upper right part. Fragments of signals designated by numbers 2, 4, and 5 are shown at higher speed in oscillograms under the same numbers.

ments alternating with the element of the calling signal (Vedenina and Shestakov, 2013). Very typical of species of the genus *Stenobothrus* are visual demonstrations (swiping movements of the antennae and tibiae, movements of the abdomen), which accompany the process of courtship but do not generate any sounds by themselves.

Males of closely related species of the *Ch. albomarginatus* (De Geer, 1773) group produce a complex courtship signal, in which none of the elements is even similar to the calling signal. Four out of five European species perform characteristic display movements at specific moments of courtship. The signal of one species of this group, *Ch. karelini* (Uvarov, 1910)

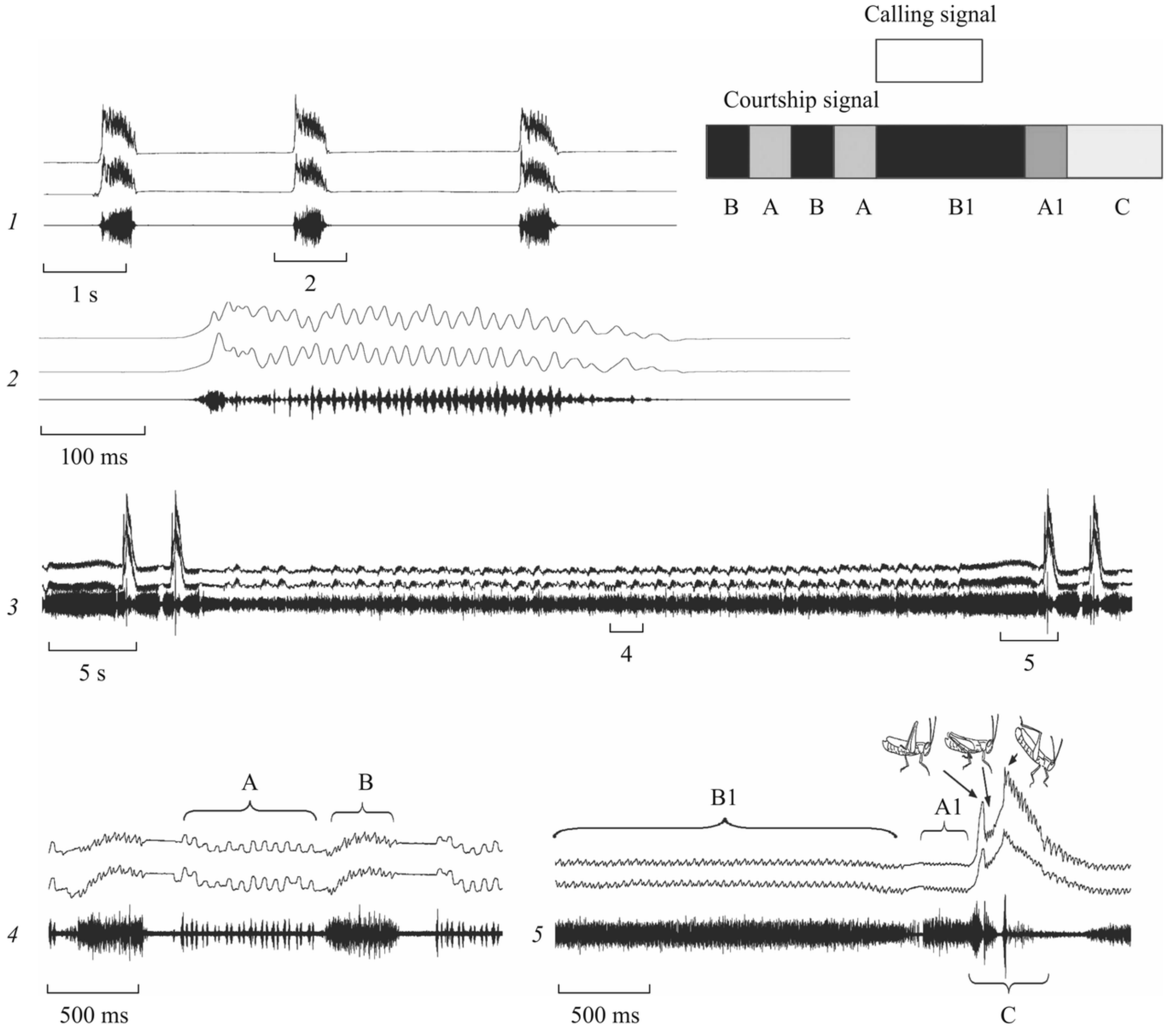


Fig. 10. Oscillograms of signals of *Chorthippus karelini karelini* from Askania-Nova, Ukraine: (*1*, *2*) calling signal; (*3*–*5*) courtship signal. Trajectories of the hind legs are shown above each oscillogram on the same time scale. Drawings show the position of the hind legs and abdomen at certain moments of courtship. Schemes of the signal composition are shown in the upper right part. Fragments of signals designated by numbers 2, 4, and 5 are shown at higher speed in oscillograms under the same numbers.

comprises five elements (Fig. 10). Courtship starts with alternation of elements A and B which are similar in amplitude but differ in the duration and repetition period of pulses. Element A is produced by simple synchronous movements of the two legs, while element B is generated by a more complex movement pattern that includes double strokes of each leg with a phase shift. The alternation of A/B pairs is followed by a prolonged element B1 and two or three short elements A1 and C. The leg movements during generation of elements B and B1 are almost identical. Each element C is accompanied by display movements and includes two distinguishable phases: the swiping movement involves first only the hind femora, then the abdomen, femora, and tibiae. After this, the whole cycle is repeated.

Comparison of the degree of variation of the amplitude-temporal parameters of courtship signals in different species of the subfamily Gomphocerinae showed that the most stable parameters in all the studied species was the repetition period of syllables or pulses (coefficient of variation less than 20%) (Fig. 11). In this feature the courtship signal is similar

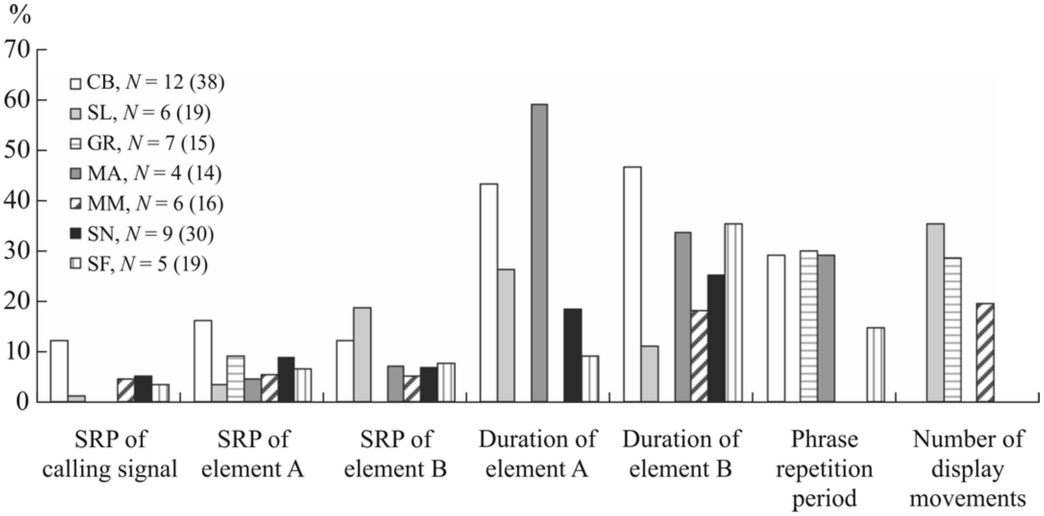


Fig. 11. Coefficient of variation of the syllable repetition period (SRP) of calling signal and six parameters of courtship signal (syllable repetition periods of elements A and B, duration of these elements, phrase repetition period, and number of display movements) in seven species of grasshoppers: *Chorthippus biguttulus* (CB), *Stenobothrus lineatus* (SL), *Gomphocerippus rufus* (GR), *Myrmeleotettix antennatus* (MA), *M. maculatus* (MM), *S. nigromaculatus* (SN), and *S. fischeri* (SF). Element A in the courtship signal is identical to the calling signal. *N* is the number of recorded males (signals).

to the calling signal, since in the latter the syllable repetition period is also one of the most stable parameters, regarded as the species-specific character (Bukhvalova, 2006; Tishechkin and Bukhvalova, 2010). By contrast, such parameters as the number of syllables in a phrase, the duration and period of phrases, and the number and duration of different elements are more variable (CV 20–60%). The repetition period of visual stimuli (display movements) also varies, though the very pattern of these movements is fairly stereotyped. The most variable are those elements of the sound signal which are accompanied by the display movements of the legs, antennae, head, etc. For example, element B is present in the signals of some males of *Ch. biguttulus* but absent in the signals of other males (Vedenina and Shestakov, 2013). This element is accompanied by an upward movement of the hind legs, which may provide an additional visual stimulus for the female. The number and period of the high-amplitude leg movements during the generation of short pulses in *Gomphocerippus rufus* (Linnaeus, 1758) vary strongly even within the signal of one male. In *Stenobothrus flscheri* (Eversmann, 1848) the duration of element C, whose generation is accompanied by swiping movements of the hind tibiae and sideward turns of the whole body, is also highly variable, while the element itself is repeated irregularly both within the signal of one male and between the signals of different males. Considering the variability of these parameters, we suppose that they may be used for assessment of the individual quality of the male.

It is worth noting that the syllable and pulse repetition periods are stable not only in the element of the courtship signal resembling the calling signal, but also in its other elements that are typical only of courtship. This indicates the possibility of using courtship signals in taxonomic practice. Analysis of the courtship signals is not only possible but necessary in some species groups having similar calling signals, for example, *Stenobothrus rubicundus* (Germar, 1817), *S. eurasius* (Zubovsky, 1898), and *Ch. albomarginatus* (Eisner and Wasser, 1995; Berger, 2008; Vedenina and Helversen, 2009). Besides, comparison of phylogenetic reconstructions and acoustic signals of grasshoppers (Vedenina and Mugue, 2011) showed that in more ancient species the calling signal was usually almost identical to the courtship signal, whereas courtship comprising not only a complex acoustic signal but also display movements was mostly found among the young species. The pattern of the calling signal may be similar and relatively simple in some closely related species while their courtship signals may be complex and very different; this fact suggests that courtship signals may evolve faster than calling ones. Therefore we assume that the courtship signal may be more strongly affected by sexual selection than the calling signal (Vedenina, 2005).

It is assumed that prolonged and complex courtship may be important at high population densities that are frequently observed in grasshoppers, in which case individuals of different sexes may often meet by chance, using no calling signals (Kriegbaum, 1989; Kriegbaum and Helversen, 1992). Having approached the female, the male touches it with its antennae and, if the female happens to be conspecific, proceeds to courtship without emitting the calling signal. Males of some grasshopper species may court the same female for a long time (1–2 h), with periodical attempts at copulation. For this purpose, the male mounts the female; if the female is not ready for copulation, it raises the tip of its abdomen to prevent coupling or pushes the male away with its hind legs. However, the female does not necessarily retreat but may remain in its place, so that the male can continue its courtship. It was shown that a prolonged courtship signal may affect the hormonal status of the female, facilitating the onset of the receptive phase (Bull, 1979; Riede, 1983). It is also known that the acoustic signal inhibits the female's locomotion. If the female is in the so-called semi-receptive phase and is not yet ready to copulate, it may rapidly reach the receptive state in the process of courtship.

What, then, are the parameters by which the female may assess the male's quality? In crickets the female's choice and successful copulation depend on many short-range signals, which include not only the courtship signal but also the outcome of the fight between the males, the presence of a territory or shelter, and "nuptial gifts" (nutritious metanotal gland secretion consumed by females of tree crickets of the genus *Oecanthus*, and spermatophores consumed by females of true crickets). The mate quality may be estimated based on a complex of characters. The polymodal courtship signal may also serve this purpose. However, comparative analysis of the acoustic courtship signals of different cricket species reveals low diversity and complexity of their temporal structure. On the other hand, courtship of true crickets always starts with antennal touching (Adamo and Hoy, 1994), during which not only the species and sex of an individual are ascertained but also its social status, determined by the males' fights, is assessed (Simmons, 1986). It is still unknown whether the social status of a male is reflected by its acoustic signals.

By contrast with crickets, fertilization in grasshoppers does not involve external transfer of a spermatophore, so that the female obtains no nutrients during copulation. Male grasshoppers reveal no territorial behavior; unlike crickets, they do not engage in fights and have no shelters or burrows. On the contrary, grasshoppers typically form aggregations of individuals of both sexes, in which several males may court females simultaneously; such aggregations may be regarded as some kind of leks. Under such conditions, comparison and assessment of the mate quality may be based on the polymodal courtship signal including the acoustic, visual, and possibly chemical components. Some closely related species of grasshoppers are known to differ in the composition of their cuticular carbohydrates (Tregenza et al., 2000); such contact pheromones may provide an additional isolation barrier between these species. It was experimentally shown that species-specific carbohydrates triggered courtship in sympatric species of the *Ch. biguttulus* group (Finck et al., 2016). Still, the high complexity of the acoustic and visual components of courtship in grasshoppers is most probably the result of sexual selection. Thus, the evolution of the acoustic signals in grasshoppers may be more driven by sexual selection than that in crickets.

Acoustic Signals in the Zones of Hybridization of Closely Related Species: an Example of Two Groups of Grasshoppers

Natural hybridization, defined as crossing between representatives of genetically different populations (Barton and Hewitt, 1985), occurs at nearly all the stages of speciation. An exception is the case of allopatric speciation, when hybridization is impossible due to geographic barriers between the populations. The recent reviews concerning the role of hybridization in speciation (e.g., Nosil et al., 2009; Sobel et al., 2010; Abbott et al., 2013) show that hybridization is very common in the nature, existing in a great variety of forms and facilitating speciation to a much greater extent than it was assumed previously. On the one hand, hybridization may slow down the divergence of populations by enhancing gene flow and recombination. On the other hand, it may accelerate speciation as the result of adaptive introgression and even lead to instantaneous speciation by polyploidy.

In the speciation context, hybridization may have several consequences, which attract the attention of researchers to a varying extent. For example, hybridization may lead to reinforcement of isolation barriers and formation of "good" species (Wu, 2001; Via, 2009). According to the hypothesis of reinforcement of ethological isolation between hybridizing species, the degree of mating assortativity is increased in the zones of contact as compared with the allopatric areas if the hybrids are nonviable or markedly less fit than the parental species (Dobzhansky, 1937, 1940). So far, however, there has been little empirical evidence in support of this hypothesis, and all these data have been obtained in insects. One of such studies was carried out on butterflies of the genus *Agrodiaetus* (Lepidoptera, Lycaenidae) (Lukhtanov, 2010; Lukhtanov et al., 2005). Using the comparative molecular phylogenetic methods, the cited authors showed that interspecific differences in wing coloration mostly emerged in the zones of secondary contact, rather than in those of allopatry. Moreover, it was shown that the wing coloration was differentiated by the ethological scenario (i.e., due to improvement of behavioral mechanisms) and not by the ecological one. The latter was rejected based on the absence of significant correlations between the ecological parameters (microclimatic conditions and altitude of the habitats) and the wing coloration.

Another way of testing this hypothesis is comparative analysis of sexual preferences of females from allopatric and sympatric populations. If selectivity is found to be higher in sympatric populations than in allopatric ones, this difference would support the hypothesis. For example, butterflies of the genus *Heliconius* (Lepidoptera, Nymphalidae) living under the conditions of sympatry engage in interspecific courtship and copulation less frequently than the same species from allopatric populations (Jiggins et al., 2001; Naisbit et al., 2001). Similar results were also obtained for the phasmid *Timema cristinae* (Phasmatodea, Timematidae) (Nosil et al., 2003), beetles of the genus *Ochthebius* (Coleoptera, Hydraenidae) (Urbanelli and Porretta, 2008), and different species of fruit flies (Noor, 1995; Higgie et al., 2000).

Many cases of natural hybridization between closely related grasshopper species of the subfamily Gomphocerinae were described in the literature (e.g., Ragge, 1976, 1984; Butlin, 1998; Bridle and Butlin, 2002; Vedenina and Helversen, 2003; Willemse et al., 2009; Vedenina et al., 2012; Vedenina, 2015), but the processes of reinforcement of ethological isolation were studied only in two hybrid zones. One of such zones is the extensive (about 200 km wide) zone of hybridization between the sibling species *Chorthippus albomarginatus* and *Ch. oschei* in Ukraine and Moldova (Vedenina and Helversen, 2003; Vedenina, 2011). These species are not only morphologically similar but they also emit identical calling signals; at the same time, they differ well in their courtship signals. The courtship signal of *Ch. albomarginatus* comprises three elements with different temporal patterns while the signal of *Ch. oschei* comprises five elements, one of them being accompanied by display movements of the abdomen and the hind tibia (Vedenina and Helversen, 2003, 2009; Vedenina, 2015). The other hybrid zone, between *Stenobothrus rubicundus* and *S. clavatus*, is a narrow zone of contact on Mount Tomaros in the north of Greece, the area of distribution of the two species on this mountain being only 16 km^2 (Vedenina et al., 2012). Unlike the representatives of the *Ch. albomarginatus* group, *S. rubicundus* and *S. clavatus* are not sibling species but can be easily differentiated by several morphological characters. The antennae are club-shaped in *S. clavatus* and filiform in *S. rubicundus*. The tegmina and wings of the two species differ well in size, venation, and coloration (Eisner and Wasser, 1995; Ostrowski et al., 2009). The calling and courtship signals of *S. rubicundus* and *S. clavatus* also reveal considerable differences. Their courtship signals are described below in detail, as an example.

The courtship signal of *S. clavatus* (Fig. 12, *1–4*) starts with low-amplitude strokes of the hind legs, with low-intensity syllables being generated only by the downward movements (part I). This part may last for up to 15 min and is followed by a relatively short part II (10–20 s), which comprises the same element repeated with a higher frequency. Then the male starts high-amplitude strokes with its hind legs and simultaneously, with its club-shaped antennae. The upward leg movement and the initial phase of their downward movement produce a noise-like pulse, and a more high-amplitude series of pulses is generated at the end of the downward stroke (part III). This is followed by part IV, which is essentially the calling signal: the leg strokes generate series of pulses similar to those of part III only during the stepwise downward movement.

A characteristic feature of the acoustic communication of *S. rubicundus* is the combination of femoro-tegminal stridulation, typical of Gomphocerinae, and loud fluttering of the wings; the calling signal and a certain part of the courtship signal are emitted simultaneously with such fluttering. Similar to that of *S. clavatus*, the courtship signal of *S. rubicundus* (Fig. 12, *5–8*) starts with low-amplitude leg strokes

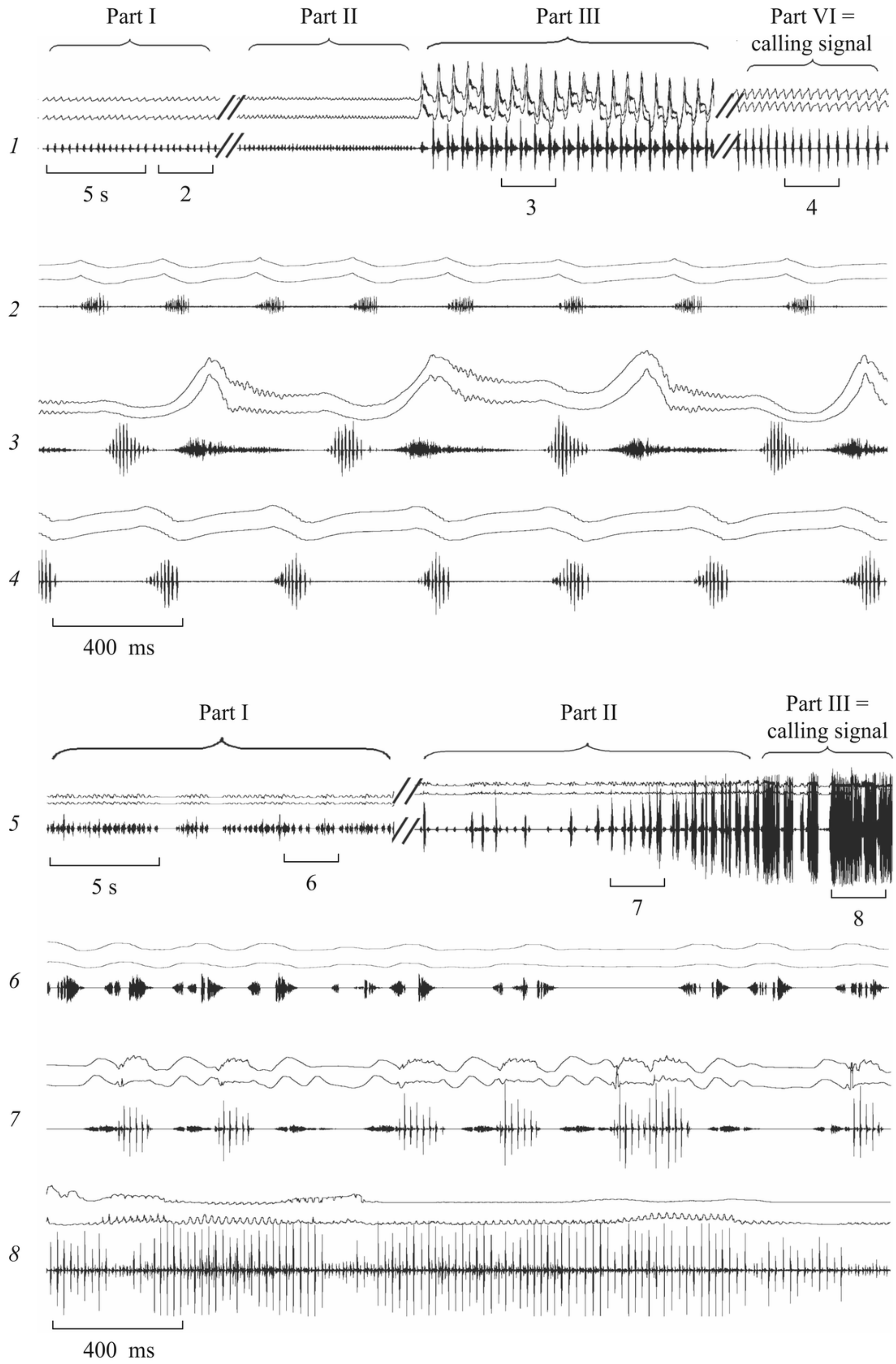


Fig. 12. Oscillograms of courtship signals: (*1–4*) *Stenobothrus clavatus*; (*5–8*) *S. rubicundus*. Trajectories of the hind legs are shown above each oscillogram on the same time scale. Fragments of signals designated by numbers 2–4 and 6–8 are shown at higher speed in oscillograms under the same numbers. The amplitude of wing movements in *S. rubicundus* was so great that pieces of reflecting foil glued onto the hind tibiae were regularly obscured by the wings; thus, regular oscillations visible in the leg trajectories (*7*, *8*) actually reflect the frequency of movements of the wings, rather than the legs.

which, however, produce sounds during both the upward and the downward movements (part I). This part may also last for a considerable time, after which the leg strokes start to alternate with the wing-generated signals (part II). As a result, the low-amplitude syllables with two pulses each are emitted in turn with louder syllables comprising 5–8 short pulses. The wing-generated signals are rare at the beginning, but during the progress of courtship they become more frequent, so that at the end of part II one or two syllables formed by simple pulses generated by leg movements alternate with a complex syllable emitted by the wings. According to the data of Eisner and Wasser (1995), the sound is produced by striking of the two maximally raised wings. Part III of the courtship signal is a trill of pulses generated only by the wings.

Preferences in two groups of hybridizing species were studied by different methods. In experiments with *Ch. albomarginatus* and *Ch. oschei*, females of one species were placed in a cage containing the conspecific and heterospecific males in equal numbers; the females' response was estimated by the number of copulations (Vedenina et al., 2007a). We used individuals from three allopatric and four hybrid populations. The specific feature of all the hybrid populations from the contact zone between *Ch. albomarginatus* and *Ch. oschei* was the prevalence of one of the parental acoustic phenotypes (Vedenina, 2011). Given free choice among the equal numbers of conspecific and heterospecific males, females from both allopatric and hybrid populations mated with males of the same species or of the same acoustic phenotype in 80–90% of the cases (binomial test, $p < 0.005$) (Fig. 13, *1–4*). No difference was found between the degree of selectivity of females from allopatric and hybrid populations. When females of the parental species were allowed to choose between conspecific males and males of F1 hybrids obtained in the laboratory, selectivity was different in females of the two species (Fig. 13, *5*, *6*). Hybrid males had no reproductive success with females of *Ch. oschei* (copulation recorded only in 10% of cases; $p < 0.001$), whereas their success with females of *Ch. albomarginatus* was comparable to that of the conspecific males ($p = 0.172$). We also studied the selectivity of females of the laboratory hybrids (Fig. 13, *7*, *8*). The number of copulations of F1 hybrid females with hybrid males was nearly the same as that with males of *Ch. albomarginatus*, while the number of copulations with males of *Ch. oschei* was significantly smaller ($p = 0.013$). The number of copulations of F2 hybrid females with all the types of males was nearly the same, all the differences being non-significant.

In order to study preferences in the *S. rubicundus* group, recorded courtship signals were played to the females via a speaker, and the presence or absence of their acoustic response was noted (Vedenina et al., 2013). The presented signals included those of males from allopatric populations, the signal of a male from the center of the hybrid zone, and the signal of an F1 hybrid male. In contrast with the hybrid zone between *Ch. albomarginatus* and *Ch. oschei*, the populations from the hybrid zone between *S. rubicundus* and *S. clavatus* could be subdivided into three groups, marked with the prevalence of the signal of *S. rubicundus*, *S. clavatus*, and intermediate acoustic phenotypes, respectively. The females of *S. rubicundus* and *S. clavatus* from allopatric populations showed clear selectivity with respect to the four types of courtship signal (Kruskal-Wallis test, $p = 0.0000$): in particular, they preferred the conspecific signal to all the others (Mann-Whitney test, $p < 0.001$) (Fig. 14, *1*, *2*). However, it should be noted that the signal of the natural hybrid male proved to be quite attractive to the females of *S. clavatus* (40%). Females from the hybrid populations of the *clavatus* type behaved similarly to those from the allopatric population (Fig. 14, *3*). By contrast, females from populations of the *rubicundus* type were less selective: although they preferred the *rubicundus* signal, they did not differentiate significantly between the signals of *S. rubicundus*, *S. clavatus*, and the natural hybrid (Fig. 14, *4*). Females from the center of the hybrid zone preferred the *clavatus* and *rubicundus* signals to the hybrid ones (Fig. 14, *5*), whereas F1 hybrid females did not show significant preference for any of the four signal types (Kruskal-Wallis test, $p = 0.12$) (Fig. 14, *6*).

Thus, females from allopatric populations and those from the sympatry zones demonstrated similar levels of selectivity in our behavioral experiments with both groups of hybridizing species. Moreover, females from hybrid localities of the *rubicundus* type were even less selective than those from allopatric populations of *S. rubicundus*. Therefore, our results do not confirm the theory of reinforcement of ethological isolation.

It is assumed that reinforcement of ethological isolation is more likely to occur at the last stages of speciation, i.e., in the presence of a relatively high level of postzygotic isolation between the hybridizing

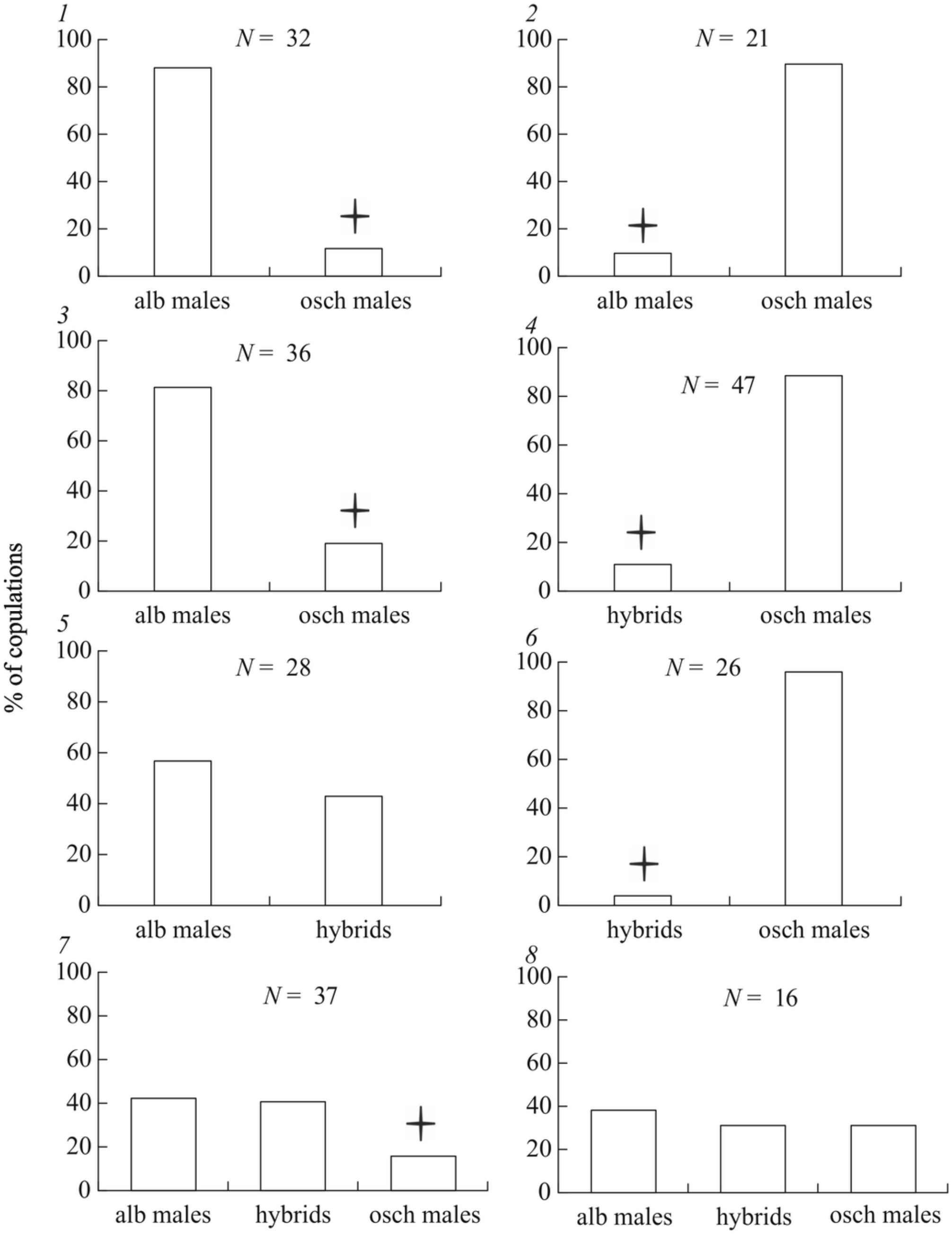


Fig. 13. Results of behavioral experiments (percentage of copulations under free-choice conditions) with females of the *Chorthippus albomarginatus* group: (*1*, *5*) *Ch. albomarginatus* females from allopatric populations; (*2*, *6*) *Ch. oschei* females from allopatric populations; (*3*) females from hybrid biotopes of type *albomarginatus*; (*4*) females from hybrid biotopes of type *oschei*; (*7*) F1 hybrid females; (*8*) F2 hybrid females. *N* is the number of females used. Significant differences are marked with crosses.

species (Butlin, 1998). However, the species studied by us do not follow this scenario. First, the level of genetic incompatibility both between *Ch. albomarginatus* and *Ch. oschei* and between *S. clavatus* and *S. rubicundus* is still not sufficiently high for such a mechanism to be enabled. Laboratory experiments have shown the hybrids between these species to be viable and fertile (Vedenina et al., 2007, 2012). Besides, in both hybrid zones the hybrids may successfully compete for mates with the parental species, due to the lower selectivity of the hybrid females. Judging by the results of our behavioral experiments, the hybrid females in mixed populations have a better chance of finding males and producing offspring than the females of the parental species. In addition, since the hybrid signals are attractive to the females of both

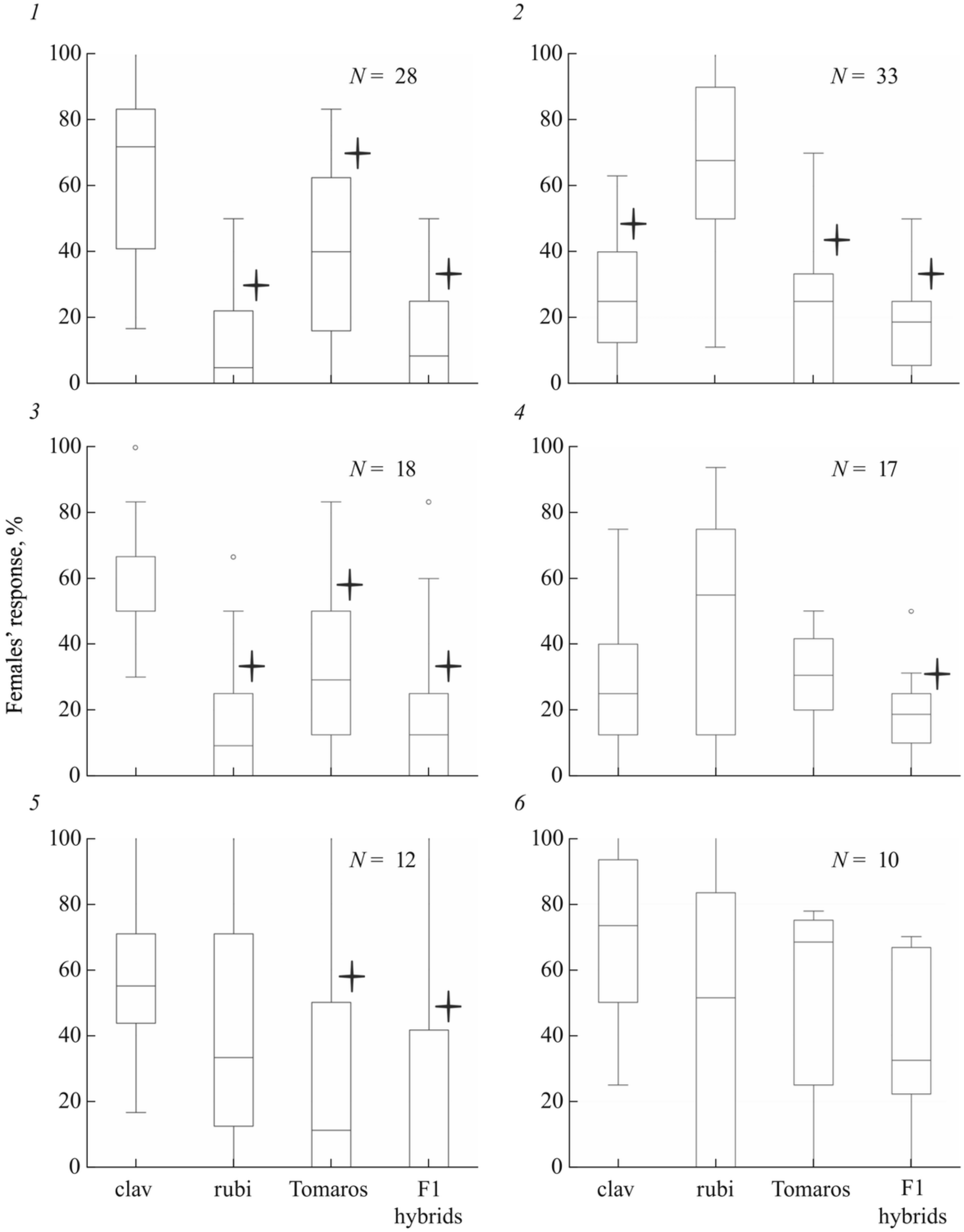


Fig. 14. Responses of females of *Stenobothrus rubicundus* group to presentation of four types of courtship signal (medians, quartiles, and max–min): (*1*) *S. clavatus* females from allopatric populations; (*2*) *S. rubicundus* females from allopatric populations; (*3*) females from hybrid biotopes of type *clavatus*; (*4*) females from hybrid biotopes of type *rubicundus*; (*5*) females from hybrid biotopes of intermediate type; (*6*) F1 hybrid females. *N* is the number of females used. Significant differences are marked with crosses.

Ch. albomarginatus and *S. clavatus*, it may be assumed that the hybrid males can also compete with males of the parental species.

How can one explain the high attractiveness of hybrid signals to females of the parental species and low selectivity of hybrid females choosing their mates?

In our opinion, when interpreting the above results one should take into account the polyfunctional nature of the courtship signal and polymodality of the courtship ritual. It was noted in the previous chapter that the courtship signals of Gomphocerinae may evolve faster than the calling signals, due to the fact that the courtship signals may be used both for species recognition and for mate quality assessment. The courtship signals of the studied hybridizing species have a very elaborate temporal structure, and it is highly probable that not all the elements of these signals constitute the key characters for recognition of conspecific forms. Since the hybrid signals in both groups studied include some elements of the signals of parental species and some new elements, it may be assumed that it is the new elements that attract the females. In this case, the courtship signals may evolve under the influence of sexual selection. As for the polymodality aspect, the courtship of Gomphocerinae includes the acoustic, visual, and chemical components, whereas in our experiments with *S. clavatus* and *S. rubicundus* the females were presented only with the acoustic component. Therefore, the females' responses may have been distorted in the absence of the visual and chemical signals, i.e., the degree of their selectivity may have been underestimated in the experiment.

Why are the empirical examples of enhancement of ethological barriers so rare, particularly in vertebrates? As a rule, this mechanism is studied in the hybridizing species which are still genetically close, so that the fitness of their hybrids is relatively high. By contrast, at the stages when this mechanism does work, the postzygotic barriers are already so strong that cases of natural hybridization can hardly be recorded.

The Use of Acoustic Characters in Taxonomy: Possibilities and Limitations

For many decades, taxonomy has been limited by morphological characters. Correspondingly, generations of museum experts have restricted their work to the study of collection material within the framework of the typological species concept dating back to Linnaeus' time. In the middle of the past century, this concept was replaced by the biological species concept (Dobzhansky, 1937; Mayr, 1947, 1971) which quickly established itself as the dominant one. It has become evident that "morphological" taxonomy does not quite correspond to the new criteria, since it cannot always allow one to differentiate biological species or to determine the taxonomic status of dubious forms. Researchers had to consider other types of characters, such as molecular and ethological, in particular bioacoustic ones. The use of bioacoustic characters is certainly limited since not all animals use acoustic communication, let alone the fact that signals can only be studied in the living individuals. At the same time, bioacoustic characters have one considerable advantage: according to the data of ethological experiments, in many groups it is the differences in the calling signals emitted by males to attract conspecific females that constitute the principal precopulatory reproductive barrier. Therefore, when differentiating close forms by their signals, we in fact differentiate the biological species by the criterion of their reproductive isolation. By contrast, molecular taxonomy mainly operates within the framework of the same typological concept, in which the taxonomic status is determined by the degree of similarity/difference in certain characters (not only the traditional morphological ones but also characters of any other kind; see Borkin et al., 2004). However, a certain level of difference in the nucleotide composition of, for instance, the COI gene between two forms does not necessarily imply their inability to mate and produce fertile offspring in the nature. On the other hand, some closely related species may reveal no difference at all in a particular gene, this situation being indicative of a recent divergence (Vedenina and Mugue, 2011).

Besides, experiments with retranslated signals and comparison of signals in the complexes of sympatric species allow the researchers to determine the parameters playing the key role in signal recognition, which, therefore, constitute the most reliable diagnostic characters. For example, the study of responses of grasshoppers of the subfamily Gomphocerinae to natural signals and artificial stimuli showed that the key parameters included the repetition period and the internal structure of the syllables (Vedenina and Zhantiev, 1990; Stumpner and Helversen, 1992, 1994; Eiriksson, 1993; Dagley et al., 1994; Helversen and Helversen, 1994). Similar results were obtained somewhat later by comparison of signals in the grasshopper communities: it was found out that songs of sympatric species belonging to the same type (prolonged single or short and regularly repeated ones) were always different in at least one of these characters (Bukhvalova, 2006; Tishechkin and Bukhvalova, 2010).

The potentials of bioacoustic analysis in taxonomy became evident already in the second half of the past century. It was shown that in some cases, simple comparison of oscillograms allowed the researcher to recognize distinct species in groups where taxonomic chaos had prevailed for decades. One of the best examples among grasshoppers is *Chorthippus* gr. *biguttulus*, the group already considered above, which includes common and numerous insects inhabiting both natural and anthropogenic landscapes from Western Europe to the Far East and from Scandinavia to the south of Central Asia (Fig. 1, *1–9*). Before the beginning of bioacoustic studies, many forms of uncertain status were described among them, and the distribution boundaries of even the accepted species remained obscure. By now, a considerable part of taxonomic problems of this group has been solved; in particular, it has been shown that only five species occur in the territory of Russia and adjacent countries, and that *Ch. biguttulus*, previously assumed to be trans-Palaearctic, does not extend eastwards beyond West Siberia (Bukhvalova, 1993, 1998; Tishechkin and Bukhvalova, 2009a).

The taxonomy of some genera of European singing cicadas (Homoptera, Cicadidae) was recently reassessed using the acoustic characters (Puissant and Sueur, 2010, etc.). In particular, it was found that the New Forest cicada *Cicadetta montana* Scopoli, 1772, extending farther to the north than other cicadas (as far as Karelia within European Russia) and earlier believed to be trans-Palaearctic, was in fact a complex of cryptic species (Sueur and Puissant, 2007; Gogala et al., 2008).

The sound communication of insects has been studied for many decades, and at present the signals of orthopterans and singing cicadas have been almost completely inventoried in many countries. Nearly all the taxonomic works and even some faunistic publications on these groups (Puissant and Sueur, 2011) now include oscillograms and sonograms of signals; material lacking such data is considered unreliable by most experts.

The current situation in the field of insect vibroacoustics is somewhat different. It was only recently realized that species emitting sound signals represented exceptions rather than the rule among insects. Vibrational communication has been described, besides Auchenorrhyncha and Psyllinea mentioned above, also in Plecoptera, Heteroptera, Aleyrodinea (Homoptera), Neuroptera, some Coleoptera, Trichoptera, Diptera, Hymenoptera, and a number of other taxa (Drosopoulos and Claridge, 2006). Thus, the great majority of insects, especially small ones, communicate by vibrational signals transmitted through solid substrates.

Although the study of vibrational signals of insects is just beginning, analysis of these signals has already been accepted as a method of practical taxonomy of some groups. The best example of this kind among leafhoppers is the large genus *Macropsis* (Homoptera, Cicadellidae, Macropsinae) counting at least 100 species in the Palaearctic. The uniform genital morphology combined with extensive coloration polymorphism, sometimes manifested by parallel forms in different species, has resulted in description of a great number of uncertain forms within this group. In the largest and most reputable reviews, this genus has for a long time remained the only one lacking complete identification keys (Emeljanov, 1964; Anufriev and Emeljanov, 1988). This taxonomic confusion was ended only after the study of the male calling signals. It was found that the signals of most species of *Macropsis* had virtually nothing in common, although morphologically these species differed only in insignificant details of male genitalia and apodemes of abdominal segment II (Fig. 15). Without the bioacoustic data, these morphological differences seemed to be too subtle to justify the species status of different forms; besides, in some cases they could not be discerned in samples containing a mixture of species. At the same time, some of the previously described forms proved to be conspecific, so that our analysis of representatives of this genus in the fauna of Russia and adjacent countries yielded even more new synonyms than newly described species (Tishechkin, 1999, 2002, 2015a).

A comparative study of signals in the genus *Gargara* (Homoptera, Membracidae) not only confirmed the species status of the Asian *G. mongolica* Dlabola, 1965 but also revealed a new, previously undetected species *G. stepposa* Tishechkin, 2005 in the steppes of European Russia and the South Urals (Tishechkin, 2005). This finding is noteworthy since before it, only three species of Membracidae were known in Europe, of which two were originally described by Linnaeus and Fabricius, and the third was introduced much later from North America.

Other examples of the use of acoustic characters in the taxonomy of Homoptera were considered in our recent review (Tishechkin, 2013). Analysis of vibrational signals was also successfully applied to the taxonomy of lacewings (Neuroptera, Chrysididae) (see

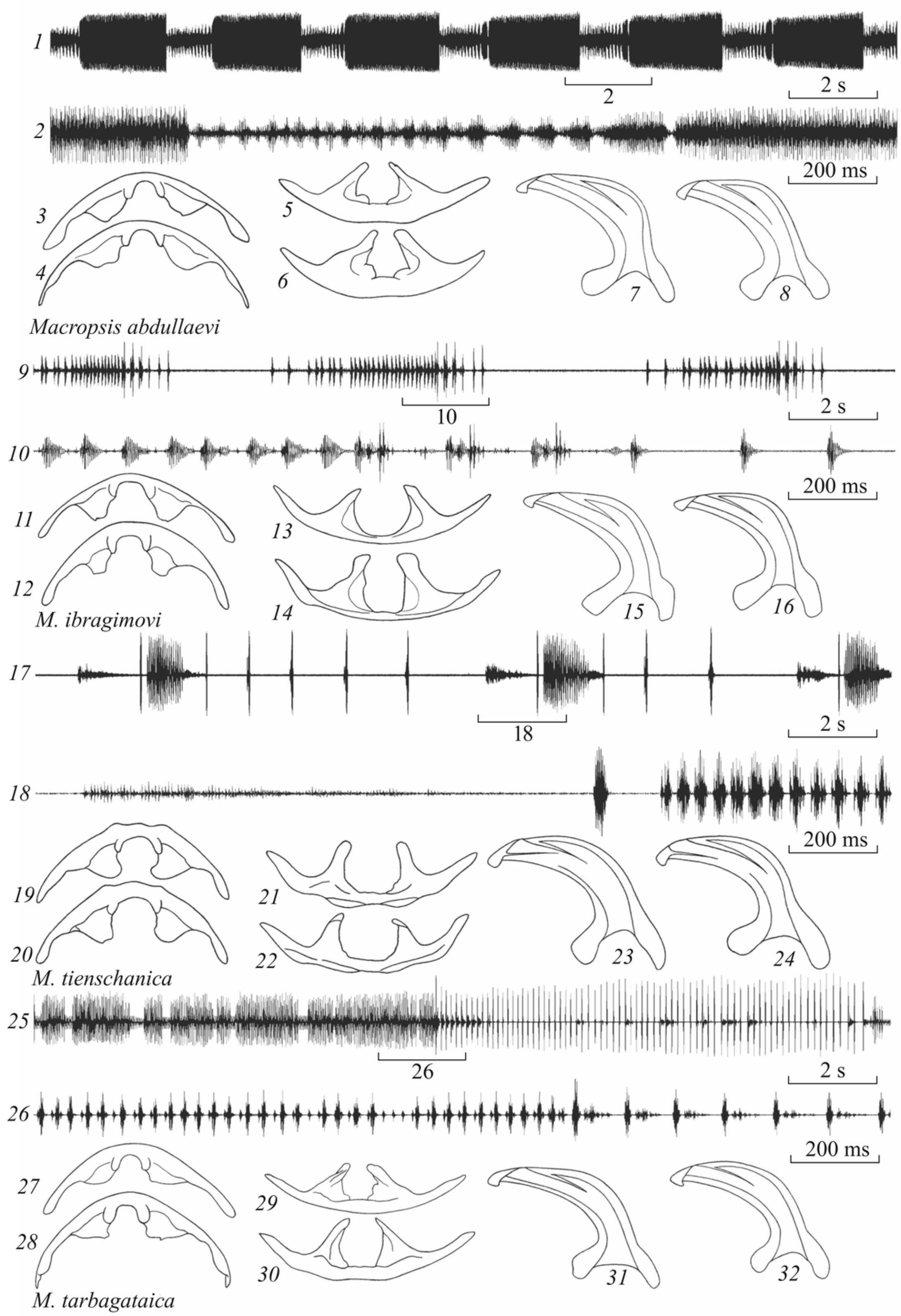


Fig. 15. Externally identical Central Asian species of leafhoppers of the genus *Macropsis*: (*1*, *2*, *9*, *10*, *17*, *18*, *25*, *26*) oscillograms of calling signals; (*3*, *4*, *11*, *12*, *19*, *20*, *27*, *28*) tergal apodemes of abdominal segment II of males; (*5*, *6*, *13*, *14*, *21*, *22*, *29*, *30*) sternal apodemes of abdominal segment II of males; (*7*, *8*, *15*, *16*, *23*, *24*, *31*, *32*) penis in lateral view. Fragments of signals designated by numbers 2, 10, 18, and 26 are shown at higher speed in oscillograms under the same numbers.

review in Henry, 2006). For the other taxa mentioned above, there have been almost no taxonomic publications making use of acoustic analysis, even though the differences between the signals of different species of these groups are usually no less distinct.

The studies of Homoptera and Orthoptera clearly demonstrate that acoustic characters have a considerably greater "resolving power" as compared with morphological ones, and that the use of acoustic characters may solve a number of problems.

Firstly, many taxa include long-known and easily distinguishable forms in which, however, the level of morphological differences is not sufficient for these forms to be regarded as "good" species. Acoustic analysis allows one to determine their true status, i.e., to decide if these forms are species or subspecies, species or color morphs, etc.

Secondly, an equally common situation in taxonomy is a medley of apparently blending forms. In this case, the study of signals would allow the researcher to differentiate between the biological species and to reveal the characters suitable for their diagnostics in the collection material. For example, following the preceding researchers, we tried to identify species of the genus *Hephathus* (Homoptera, Cicadellidae, Macropsinae) by their genital morphology, only to realize that none of the morphological characters worked when applied to series of specimens. However, analysis of signals allowed us to "sort out" the material by species and to reveal interspecific differences in coloration; in turn, these findings opened the possibility of processing extensive collection material and eventually led to establishment of some new synonyms and to reconsideration of all the data on the distribution of these insects (Tishechkin, 2015b).

Thirdly, comparison of signals allows one to distinguish cryptic species, which are difficult or impossible to identify by morphological characters.

Yet any taxonomist, when facing the need to use a "non-morphological" method for the first time, naturally questions whether the costs of introducing the new method would be justified. This is perfectly true of the acoustic techniques as well. Indeed, only a short time ago a study of insect signals was impossible without a whole set of specialized expensive equipment, which considerably limited the possibility of using acoustic characters together with morphological ones. As a result, most taxonomists restricted themselves to morphology while the studies of sound and especially vibrational signals were left to the narrow circle of experts in bioacoustics. However, the situation changed drastically with the appearance of digital recording techniques. The modern magneto-optical, minidisk, and flash recorders can record sounds in the format readable by any PC; the sound files can be copied from them onto the computer in the same way as images are transferred from a digital camera. The processing of records has also been simplified to the limit because the whole complex of analyzing equipment can be now replaced by a couple of computer programs. Besides, the modern recorders are highly portable, so that even the multipart devices for recording vibrational signals can be installed in a field base within a nature reserve, in a tent, and even directly in the nature if necessary. Some difficulties with recording under the natural conditions may emerge during the studies of the signals of katydids (Zhantiev, 1980; Korsunovskaya, 2008) and the broadband signals of grasshoppers, which extend considerably into the ultrasonic part of the sound spectrum (Vedenina and Zhantiev, 1990; Meyer and Eisner, 1996) and thus fall outside the working range of the common portable recorders. This limitation may affect not only the frequency but also the amplitude-temporal parameters of the recorded signal. For example, if different elements of the courtship signal of a grasshopper have maxima in different frequency ranges (Vedenina et al., 2007b; Ostrowski et al., 2009), the amplitude ratio of these elements may be distorted in the oscillogram. There are some complex techniques for recording the signals simultaneously with the activity of the sound-producing apparatus (the movements of the sound-producing structures or the muscle contractions), which cannot be used under the field conditions, either. However, such techniques are seldom required for solving taxonomic problems; they are needed, in particular, during analysis of the elaborate courtship signals (Vedenina and Helversen, 2009; Vedenina and Shestakov, 2013).

As a rule, interpretation of the results does not require any special training, since in most cases it amounts to qualitative comparison of oscillograms. The humorous assertion that a bioacoustics expert differs from a traditional taxonomist only in studying the morphology of oscillograms instead of insect genitalia, is not far from the truth; in fact, "morphological differences" between oscillograms are usually more pronounced than those between morphological structures (e.g., compare the oscillograms and drawings of the genitalia in Fig. 1, *11–18*).

However, the use of acoustic characters in insect taxonomy has its limitations. Similar to any other character, the signals evolve gradually, so that in the nature one may find some forms which have not yet completely diverged not only in their morphology but also in their signals. Small but stable differences in the song structure are sometimes observed between the subspecies of grasshoppers (Vedenina and Bukhvalova, 2001; Tishechkin and Bukhvalova, 2009). The morphological differences between them are not great enough to consider these forms as separate species, but comparison of their calling signals does not lead to a univocal conclusion about their status, either. For example, in *Chorthippus albomarginatus* (De Geer, 1773) species group, only a detailed study of the courtship signals and the associated display movements allowed us to prove that the forms which used to be regarded as subspecies were in fact separate species (Vedenina and Helversen, 2009).

Besides, signals are subject to intraspecific variation, which is manifested in considerably different ways in different groups (Tishechkin, 2013). In the simplest case, the quantitative parameters of the signal, such as the durations and repetition periods of its rhythmic elements, may vary around a certain mean value; this phenomenon is observed in all the singing insects. Some parameters may also change regularly: for example, the syllable repetition period may increase or decrease toward the end of the signal, i.e., the male may start singing “slower” or “faster.” In the extreme case, the gaps between some elements may increase to the point where the phrase becomes split into separate syllables emitted with irregular intervals. The signals of some species may include additional or facultative fragments. Such a case was described above for *Ch. intermedius*, in which the second part of the phrase may be absent. Usually the male emits only complete or only reduced signals (Fig. 2, *1*, *7* and 2, *8*), but sometimes one type is replaced by the other during one song.

The range of variation is different in different taxa, so that the level of differences which in one group is observed between distinct species, in some other group may be recorded even between different signals of the same individual. Therefore, before assessing the status of some form based on comparison of the signals, one should study their variation and determine what degree of difference corresponds to the species level in the given taxon.

Finally, in some situations the use of acoustic characters for differentiating biological species is impossible in principle. It should be reminded here that the calling signal, which is the main component of SMRS, does not necessarily perform an additional isolation function. If two species cannot hear each other’s songs in the nature due to allopatry, different ecological preferences, different daily or seasonal timing of acoustic activity, and other factors, they may produce identical signals and still exist as reproductively isolated entities. Correspondingly, the similarity of signals in acoustically isolated forms, for example, living on different islands or mountain ranges, associated with different host plants or inhabiting different biotopes, gives no ground for synonymizing these forms. On the other hand, if the signals of such forms are clearly different, this is a convincing proof of their status as separate species.

In conclusion, let us consider the effect of using acoustic characters on the stability of the system. It is no secret that the cautious attitude of some taxonomists towards new methods is largely caused by the fact that the schemes inferred from different types of characters often contradict one another. In particular, this is true of the molecular data, the use of which often yield results totally inconsistent with the traditional concepts.

The acoustic method has already proved its validity in this respect. As the result of a total inventory of the sounds emitted by grasshoppers in Western Europe, the status of some forms was reconsidered but the system as a whole did not undergo any substantial change (Ragge and Reynolds, 1998). In the taxonomy of the European singing cicadas, the greatest part of taxonomic changes occurred in the problematic genus *Cicadetta* and the related obscure groups. Our study of the vibrational signals of over 500 species of the small Auchenorrhyncha of Russia and adjacent countries, carried out in the latest decades, allowed us to solve a number of problems but all of them involved the taxonomically difficult groups, which had been known to contain many uncertain forms long before the emergence of bioacoustics as a separate discipline. We have not yet encountered a single case when a morphologically and ecologically homogenous species would have to be split based on the results of the signal analysis.

Thus, within the framework of the biological species concept in its broad interpretation (i.e., including

Paterson's recognition concept), the key characters used for differentiating cryptic species and for elucidating the status of dubious forms usually constitute the main components of SMRS. However, in order to avoid taxonomic errors, it should be borne in mind that the character serving for attraction of the conspecific mate does not necessarily act as an interspecific reproductive barrier.

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