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Courtship Song Analysis in Two Hybrid Zones between Sibling Species of the *Chorthippus albomarginatus* Group (Orthoptera, Gomphocerinae)

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Abstract—Two new hybrid zones between sibling species of the *Chorthippus albomarginatus* group were described on the basis of the courtship song analysis. Not only the sounds emitted but also the accompanying stridulatory movements of the hind legs were analyzed, which allowed the temporal parameters to be classified in a more reliable way. One hybrid zone between *Ch. albomarginatus* and *Ch. karelini* was found in Ulyanovsk and Samara Provinces of Russia. The other hybrid zone, presumably between *Ch. karelini* and *Ch. oschei*, was found in the protected Askania-Nova steppe area in Kherson Province of Ukraine. Based on comparison of the natural and laboratory hybrids, a hypothesis on the structure and dynamics of the hybrid zones is proposed.

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Hybrid zones are often regarded as natural laboratories in which the various processes associated with speciation can be studied (Barton and Hewitt, 1985, 1989; Harrison, 1986, 1990). Natural hybridization indicates the incompleteness of reproductive isolation between closely related, usually young species. It is noteworthy that species capable of hybridization often reveal partial precopulatory isolation but do not have any postcopulatory isolation mechanisms (see, e.g., Panov, 1989, 1993; Blinov and Kryukov, 1992; Jiggins et al., 1997; Kryukov and Gureev, 1997; Rolan-Alvarez et al., 1997; Bailey et al., 2004). Of special interest are species having similar biotopic and phenological preferences but strongly differing in their mating signals and sexual behavior. Such species are perfect objects for studying the role of sex selection in speciation, although the significance of this kind of selection for species divergence is still intensely debated.

In this communication, we describe two new hybrid zones between closely related grasshopper species of the *Chorthippus albomarginatus* group, which were differentiated based on analysis of the courtship songs. Three species of this group have relatively wide, almost non-overlapping ranges: *Ch. albomarginatus* occurs in North and Central Europe, extending as far eastwards as North Kazakhstan and West Siberia, *Ch. oschei* is distributed from the Balkans to Ukraine, and *Ch. karelini*, from Turkey and the southeast of European Russia to Irkutsk Province, also extending to Kazakhstan and Central Asia (Bey-Bienko and Mistshenko, 1951; Helversen, 1986; Vedenina and Helversen, 2009). These species are not only morphologically similar but also emit similar calling signals; however, their courtship signals are fairly different. Moreover, these species have a highly complicated courtship ritual: their acoustic signals consist of several elements with different amplitude-temporal patterns and are accompanied by display movements of the body, hind legs, and antennae (Helversen, 1986; Vedenina and Helversen, 2009). Earlier, we described a wide (about 200 km) hybrid zone between *Ch. albomarginatus* and *Ch. oschei* in the territories of Ukraine and Moldova (Vedenina and Helversen, 2003; Vedenina, 2011). Analysis of the isolation barriers revealed partial ethological isolation based on preference for conspecific courtship signals (Vedenina et al., 2007a).

It is remarkable that the courtship signals of the natural hybrids were found to contain new elements which were never recorded in the signals of the parent species. Genetic analysis of the courtship signals in the *Ch. albomarginatus* group revealed an unusual inheritance pattern of some of their parameters, suggesting the participation of duplicated loci in inheritance of homologous elements (Vedenina et al., 2007b). In this case, the hybrids emitting signals with new elements may gain an advantage over the parent species if such signals are favored by sex selection. At the same time, our analysis of clinal variation of quan-

The species studied, collection localities, and the number of individuals and signals recorded

No.	Species/song type	Collection locality	Collection date	Number of males (signals) recorded
1	*albomarginatus*	Russia, Penza Prov., env. of Nizhnii Lomov, near Lomovka River, 53°32′N, 43°43.2′E	15.VII.2012	7 (11)
2	hybrid	Russia, Ulyanovsk Prov., Nikolaevka, 53°07.4′N, 47°13.7′E	15.VII.2012	9 (15)
3	hybrid	Russia, Samara Prov., 14 km NW of Syzran, Zaborovka, 53°12.8′N, 48°16.6′E	15.VII.2012	5 (14)
4	*karelini*	Russia, Saratov Prov., 29 km SW of Krasnyi Kut, Dyakovka, 50°43.4′N, 46°46.2′E	15–16.VII.2010	3 (12)
5	*karelini*	Russia, Samara Prov., 25 km SE of Neftegorsk, Gerasimovka, 52°42.6′N, 51°30.6′E	12.VII.2012	1 (3)
6	*karelini*	Russia, 7 km E of Orenburg, near Ural River, 51°44.3′N, 55°20.8′E	13.VII.2012	1 (3)
7	*karelini*	Russia, Orenburg Prov., 53 km NE of Orenburg, Studentsy, 51°51.6′N, 55°51.3′E	14.VII.2012	3 (7)
8	hybrid	Ukraine, Kherson Prov., 28 km NE of Chaplinka, Askania-Nova, 46°27.68′N, 33°52.24′E	28.VII.2007	11 (22)
9	*karelini*	Ukraine, Kherson Prov., 30 km NE of Chaplinka, env. of Askania-Nova, 46°28.63′N, 33°52.88′E	28–29.VII.2007	9 (12)
10	hybrid	Ukraine, Kherson Prov., 24 km E of Chaplinka, env. of Khlebodarovka, 46°24′N, 33°50′E	14.VIII.2006	7 (16)

titative characters in the hybrid zone revealed a low level of introgression, which is more consistent with a stable equilibrium between selection and hybridization (Vedenina, 2011).

One of the hybrid zones described below, namely that between *Ch. albomarginatus* and *Ch. karelini*, was discovered by us in Ulyanovsk and Samara Provinces of Russia; the other hybrid zone, between *Ch. karelini* and probably *Ch. oschei*, was found in Kherson Province of Ukraine. Analysis of the courtship signals presented in this communication involves not only the sounds emitted but also the accompanying stridulatory leg movements, which allows the amplitude-temporal parameters of the signals to be measured and classified more reliably. We also compare the courtship signals of the natural and laboratory hybrids and on this basis try to determine the common traits and differences between the new hybrid zones and the one described earlier.

MATERIALS AND METHODS

The new material presented here was collected in 10 geographic localities in 2007–2012 (table; Fig. 1). In addition, reciprocal crossings of *Ch. oschei* from Greece (Macedonia, Grammos) and *Ch. karelini* from Volgograd Province of Russia (26 km E of Uryupinsk) were performed in the laboratory in 2003–2004. For this purpose, 10 virgin males of one species and 10 virgin females of the other species were kept in a cage (30 × 60 × 40 cm) for 3–4 weeks at the light regime 12 L :12 D and the daily temperatures varying from 20 to 25°C. Trays with humid sand placed in the cage served as the oviposition substrate. The collected egg pods were put in humid sand in Petri dishes and kept in the refrigerator at +4°C for at least 5 months. Then the pods were transferred into the room temperature; nymphs started to hatch 2–4 weeks later. The young nymphs were fed on *Poa annua*, the older nymphs and adults, on *Dactylis glomerata*.

The signals were recorded in the laboratory at temperatures 30–35°C. To record the courtship signals, the male was placed near the female. In most cases, we recorded not only the sounds but also the accompanying stridulatory movements of the legs. The latter were recorded with an opto-electronic device implementing the method developed by the German researchers

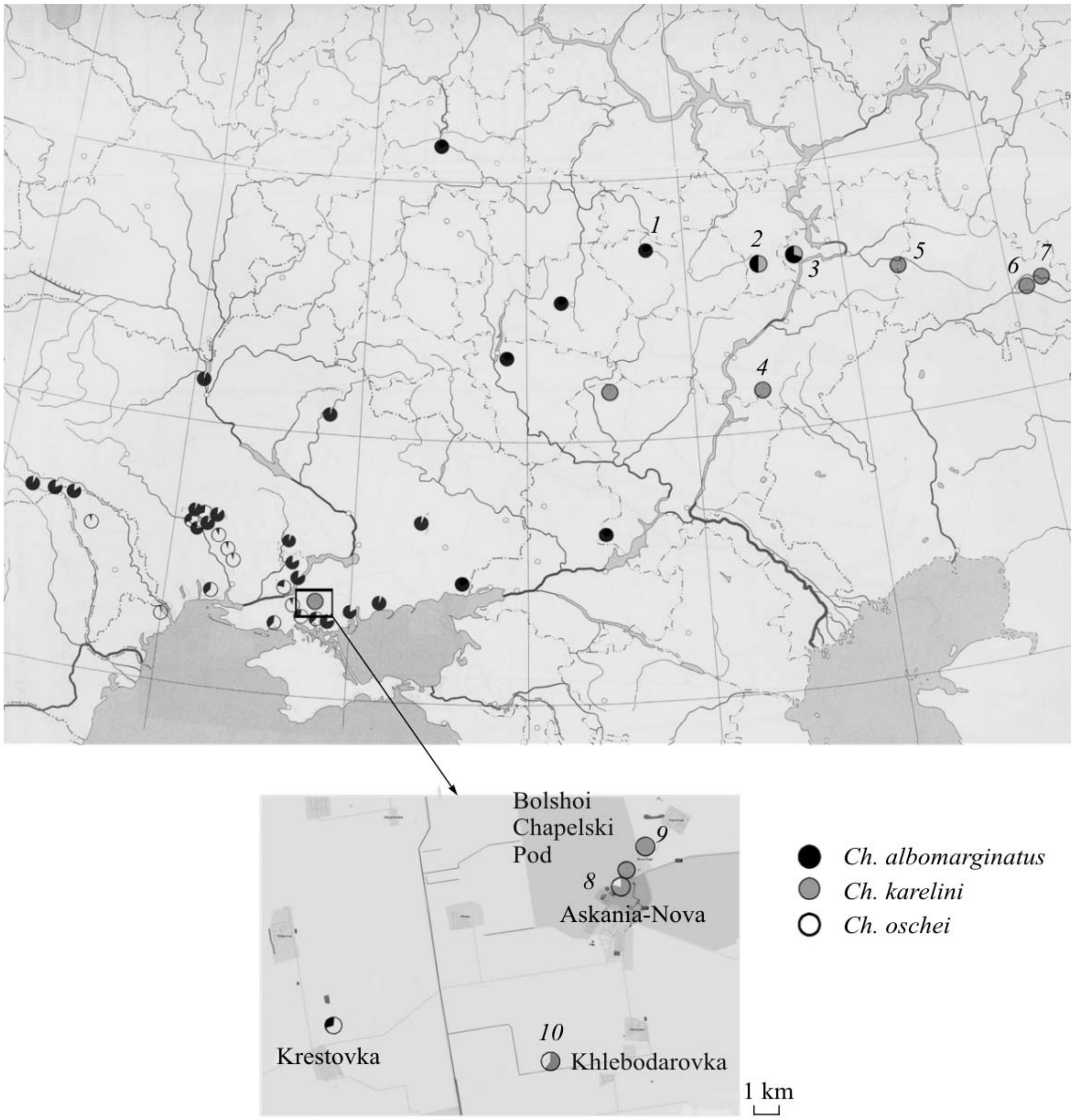

Fig. 1. Localities in Ukraine and European Russia where the courtship signals of *Chorthippus albomarginatus*, *Ch. karelini*, and *Ch. oschei* were studied. The new localities described in this communication are numbered. Circles with differently shaded sectors designate hybrid populations. The fragment below is the enlarged map of the Askania-Nova region.

(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 the 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 the frequency range 3 Hz–40 kHz, and fed to the computer via an amplifier and a self-made analog-digital converter. The sampling frequency was 100 kHz for acoustic signals and 2 kHz for leg movements. The amplitude-temporal parameters of the signals were measured using Turbolab 4.0 (Bressner Technology, Germany) and CoolEdit (Syntrillium, US) software; each parameter was measured 10 times in each signal where it was possible. The signals were analyzed using the Excel and Statistica software. For comparative analysis, we also used the signals described in our previous papers (Vedenina and Helversen, 2009; Vedenina, 2011).

RESULTS

The Courtship Signals of Three Species of the Ch. albomarginatus Group

Although the courtship signals of *Ch. albomarginatus*, *Ch. karelini*, and *Ch. oschei* were described in detail earlier (Helversen, 1986; Vedenina and Helversen, 2003, 2009), their brief descriptions are provided below since they are needed for comparative analysis. The courtship signal of *Ch. albomarginatus* consists of three elements forming a characteristic pattern (Figs. 2*a*, 2*b*): 4–8 alternating pairs of elements A and B are followed by element C. Elements A and B are produced by simultaneous movements of the two hind legs with different frequencies, so that the frequency of acoustic pulses in element A is higher than that in element B. The last element B preceding element C (designated as element B1) always has a characteristic pause in the middle. Element C is emitted by antiphase leg movements whose pattern is more complicated than that of elements A and B (Fig. 2*b*). Element C is followed by alternating A/B pairs, which are followed by one more element C, and so on. The courtship signal pattern of *Ch. oschei* is more complicated: 15–30 alternating A/B pairs are followed by a complex of three elements, B1-A1-C (Figs. 2*c*, 2*d*). Of them, B1 is the loudest and the longest element which is produced by synchronous high-amplitude leg movements with a gradually increasing frequency. The beginning of element C is marked by a very specific high-amplitude stroke of the hind legs and tibiae; then the legs return to the normal position and vibrate in antiphase. The leg movement pattern during element C is as complicated as that of *Ch. albomarginatus*. The relative amplitudes of elements A and B differ in the two species: in *Ch. albomarginatus* element A is louder than B, whereas in *Ch. oschei* element B is louder than A. The signal of *Ch. karelini* (Figs. 2*e*–2*g*) can also be differentiated into five elements. It begins with alternation of elements A and B, this phase being usually much longer than that in the signal of *Ch. oschei*. Elements A and B in the signal of *Ch. karelini* are similar in amplitude but differ in the duration and period of the pulses. Element A is produced by simple synchronous movements of the two legs, whereas element B results from a more complex pattern, in which the legs make double strokes with a phase shift. The frequency of leg strokes in element B is similar in the signals of all the three species; on the contrary, the frequency of strokes in element A of the signal of *Ch. karelini* differs strongly from the corresponding parameter of the two other species. The alternating A/B pairs are followed by a very long element B1 and short elements A1 and C. The leg movement patterns during elements B and B1 are almost identical. Element C in the signal of *Ch. karelini*, as well as in that of *Ch. oschei*, is accompanied by display movements in which, however, two phases can be distinguished: in the first phase only the femora make the stroke, whereas the second phase involves the abdomen, femora, and tibiae (Fig. 2*g*). The signal of *Ch. karelini* is characterized by the presence of two or three A1/C pairs, whereas in the signals of the two other species element C is emitted only once, after which the whole cycle is repeated.

For comparative analysis of the courtship signals, we selected eight amplitude-temporal parameters which were significantly different in at least two of the three species considered: the number of A/B pairs between the consecutive elements C, the ratio of the mean pulse amplitudes in elements A and B, the ratio of pulse frequencies in elements A and B, the ratio of the duration of elements A, B, B1, and A1 to that of element C, and the number of A1-C complexes emitted in succession (Fig. 3). Since most of the above parameters were ratios, we used log-transformed values for analysis.

The signals of males from locality 1 did not significantly differ from those of *Ch. albomarginatus* males from Moscow (Vedenina and Helversen, 2009) in seven parameters; differences were observed only in the number of A1-C complexes (Mann–Whitney test, $p = 0.03$). The signals of males from localities 4–7 did not significantly differ from those of the previously studied *Ch. karelini* males from Volgograd Province of Russia and the Askania steppe of Ukraine (Vedenina and Helversen, 2009) in six parameters; there were differences in the number of A/B pairs and the ratio of pulse frequencies in elements A and B ($p < 0.05$).

The Hybrid Zone between Ch. albomarginatus and Ch. karelini in Ulyanovsk and Samara Provinces of Russia

Hybrid courtship signals were recorded in males from two localities in Ulyanovsk and Samara Provinces (2 and 3 in the table), positioned about 70 km apart. These hybrid signals included almost all the elements (A, B, B1, A1, and C) transitional between *Ch. albomarginatus* and *Ch. karelini* (Figs. 4*a*, 4*b*; 5; 6). For example, element B characteristic of *Ch. karelini*, emitted by double strokes of two hind legs mov-

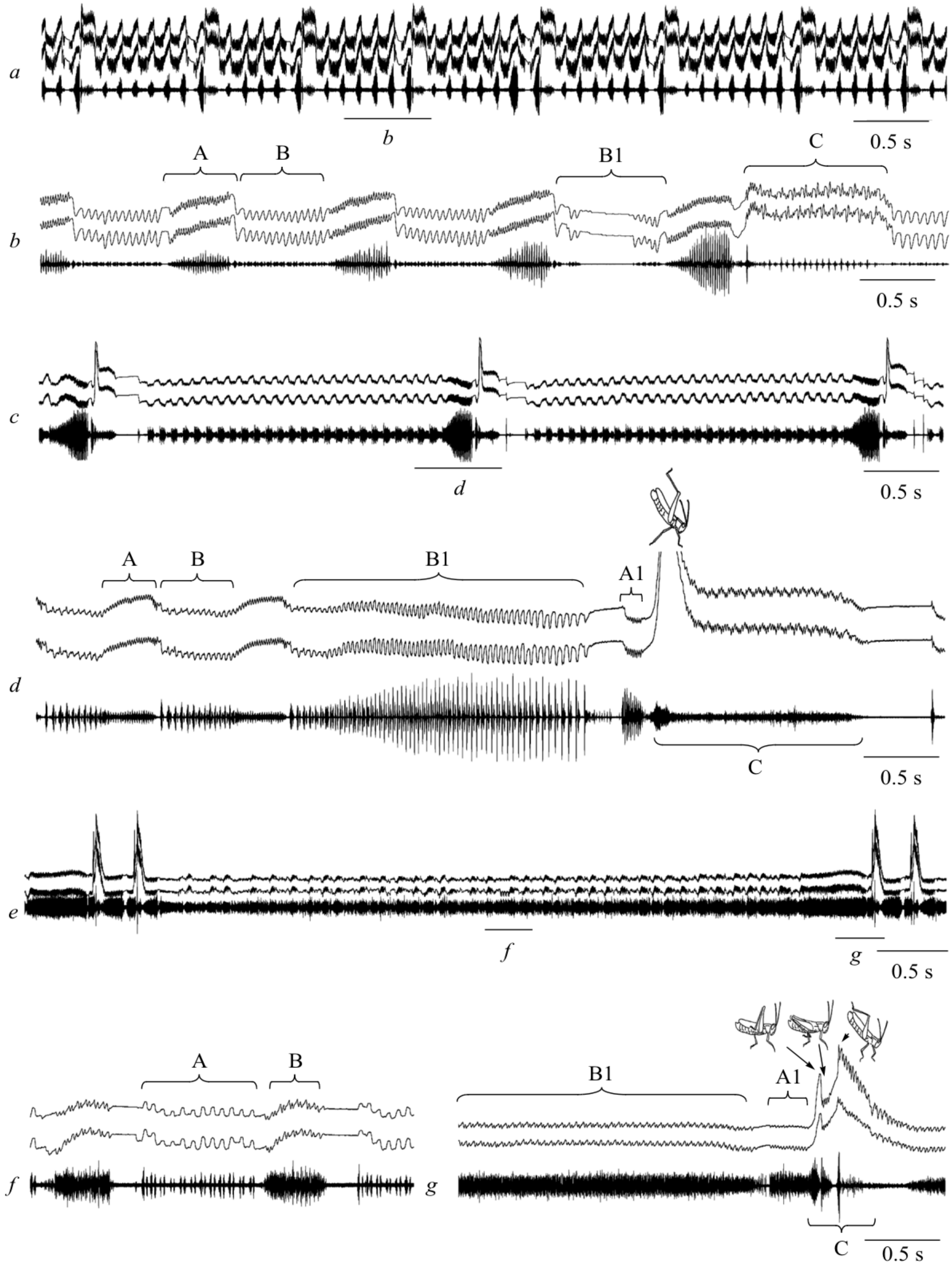

Fig. 2. Oscillograms of courtship signals of *Chorthippus albomarginatus* (*a*, *b*), *Ch. oschei* (*c*, *d*), and *Ch. karelini* (*e*–*g*). Fragments of oscillograms (*a*, *c*, *e*) are shown in (*b*, *d*, *f*, *g*) at a higher speed. Trajectories of movements of the hind legs are shown above each oscillogram on the same time scale. A, B, B1, A1, and C are elements of the signal. The drawings show the position of the hind legs and abdomen at different moments of generation of element C (*d*, *g*).

ing with a phase shift, sometimes alternated with element A of the *albomarginatus* type (Fig. 5*c*). Sometimes one signal included two variants of element A, corresponding to the *albomarginatus* and *karelini* types (Fig. 5*d*). Intermediate variants of the B1-A1-C complex could also be recorded in the hybrid signals (Fig. 6). For example, signals of the *albomarginatus* type almost completely lacked the pause in element B1, whereas element A1 did not differ from B1 in amplitude (Fig. 6*b*) as in the typical signal of *Ch. albomarginatus*. Sometimes the initial part of element C included a noticeable leg stroke characteristic of the *karelini* type, but the subsequent pattern of this element resembled that of the *albomarginatus* type (Fig. 6*c*). On the whole, the leg movement patterns of different individuals from the two hybrid localities (Fig. 6) demonstrated an almost smooth transition of the structure of element C from one species to the other.

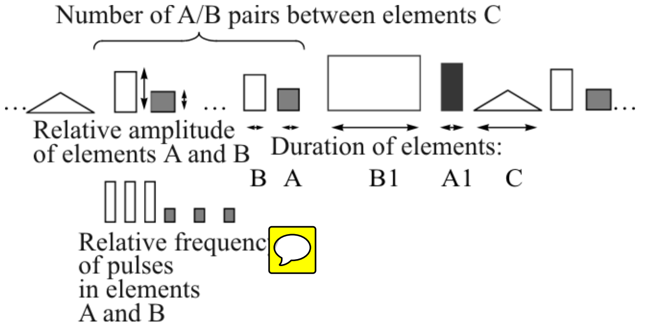

Fig. 3. A diagram of the courtship signal of *Chorthippus oschei* showing the parameters used for comparative analysis of signals in the *Chorthippus albomarginatus* group.

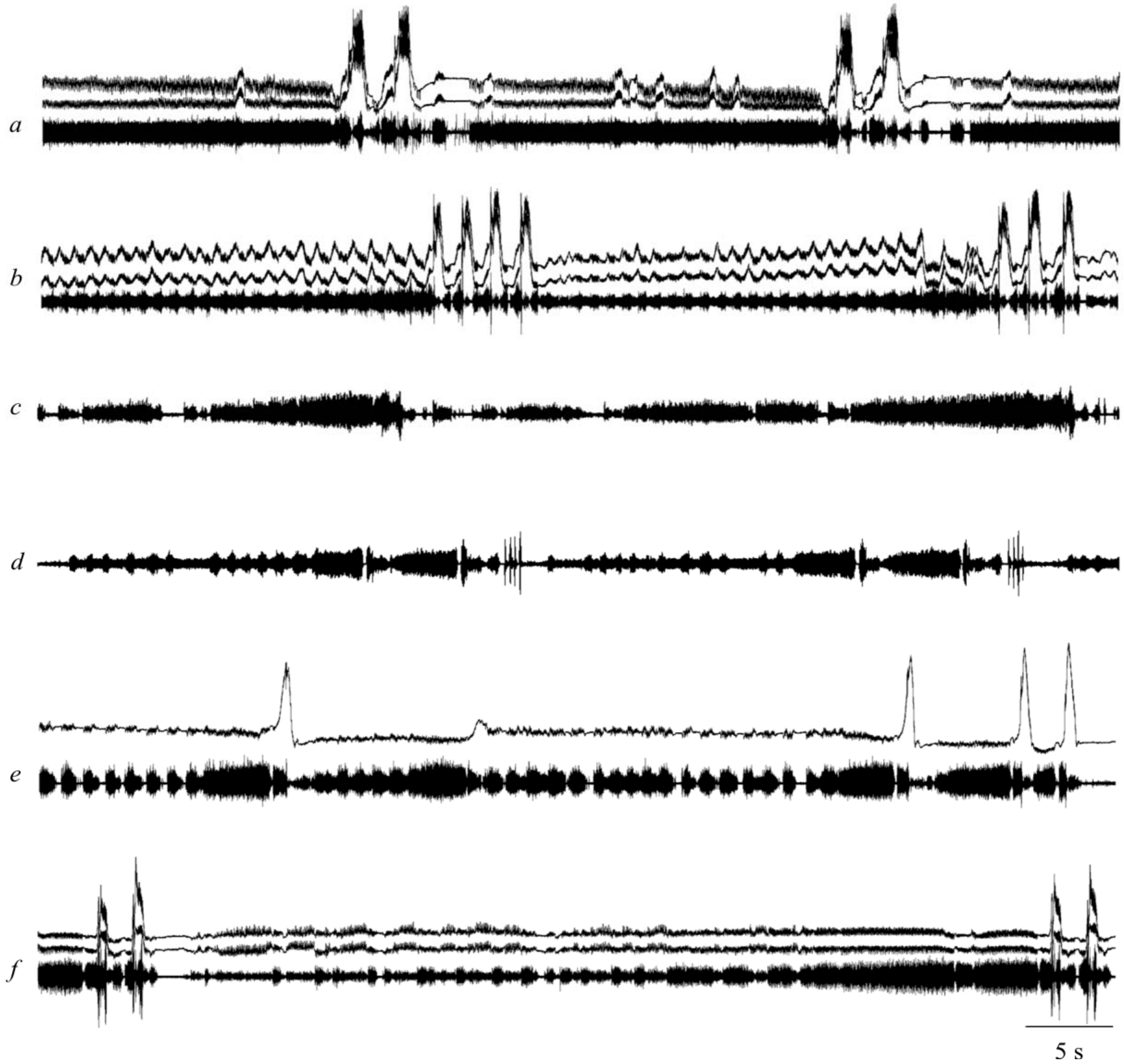

Fig. 4. Oscillograms of courtship signals of two males from localities 2 and 3 (*a*, *b*), two males from locality 10 (*c*, *d*), and two male F1 hybrids of *Chorthippus karelini* and *Ch. oschei* (*e*, *f*). Trajectories of movements of the hind legs are shown above the oscillograms (*a*, *b*, *e*, *f*) on the same time scale; the trajectory of only one leg is shown in (*e*).

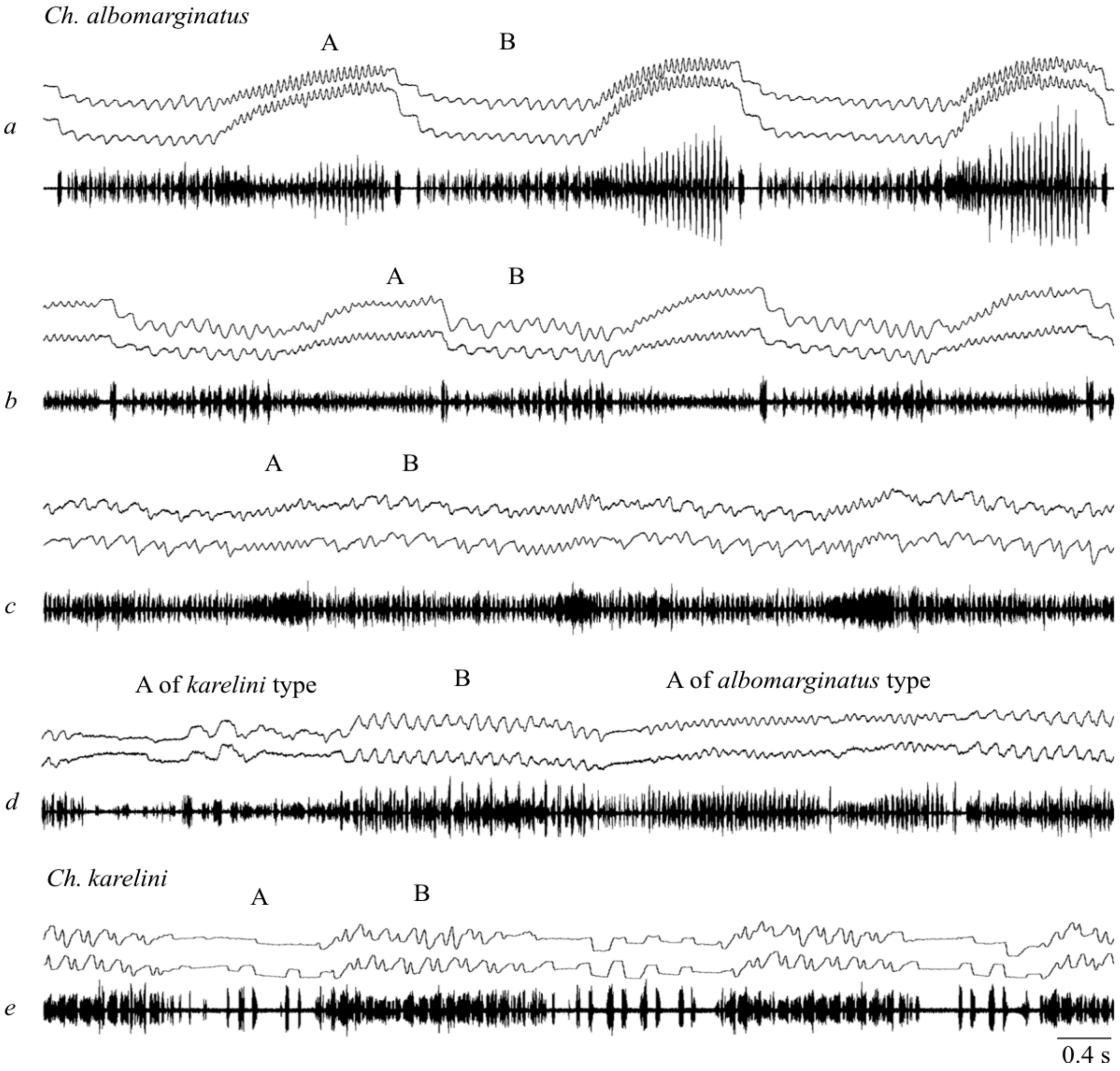

Fig. 5. Transformation of elements A and B in transition from the courtship signal of *Chorthippus albomarginatus* (*a*) to that of *Ch. karelini* (*e*), via the intermediate variants recorded in locality 2 within the hybridization zone (*b*–*d*). Trajectories of movements of the hind legs are shown above each oscillogram on the same time scale.

Comparison of the mean values of each parameter in the hybrid and allopatric populations showed that most parameters in the hybrid populations had intermediate values (Fig. 7). As shown by the Mann–Whitney test (confidence level 0.05), the population from locality 2 differed from the Moscow population of *Ch. albomarginatus* in six parameters, and from the allopatric populations of *Ch. karelini*, in four parameters. The variation of such signal parameters as the duration of elements B and B1 and the number of A1-C complexes was considerably higher in individuals from the hybrid populations 2 and 3 than in grasshoppers from the allopatric populations of *Ch. albomarginatus* and *Ch. karelini* (Figs. 4*a*, 4*b*; 7*e*, 7*f*, 7*h*). A higher level of variation of the hybrid signals was also revealed by the principal component analysis (PCA) (Fig. 8).

The Hybrid Zone in Kherson Province of Ukraine

Hybrid courtship signals were recorded in males from two localities in Ukraine: in Askania-Nova (locality 8) and near Khlebodarovka (locality 10) (Fig. 1, table). Locality 8 was positioned very close (less than 1 km) to Bolshoi Chapelski Pod, the biotope where a population of *Ch. karelini* had been found earlier (Vedenina and Helversen, 2009). However, only three out of 11 males in locality 8 emitted hybrid signals. By contrast, most of the males recorded in locality 10 (12 km from Askania-Nova) produced hybrid signals (Figs. 4*c*, 4*d*). In our opinion, hybridization between

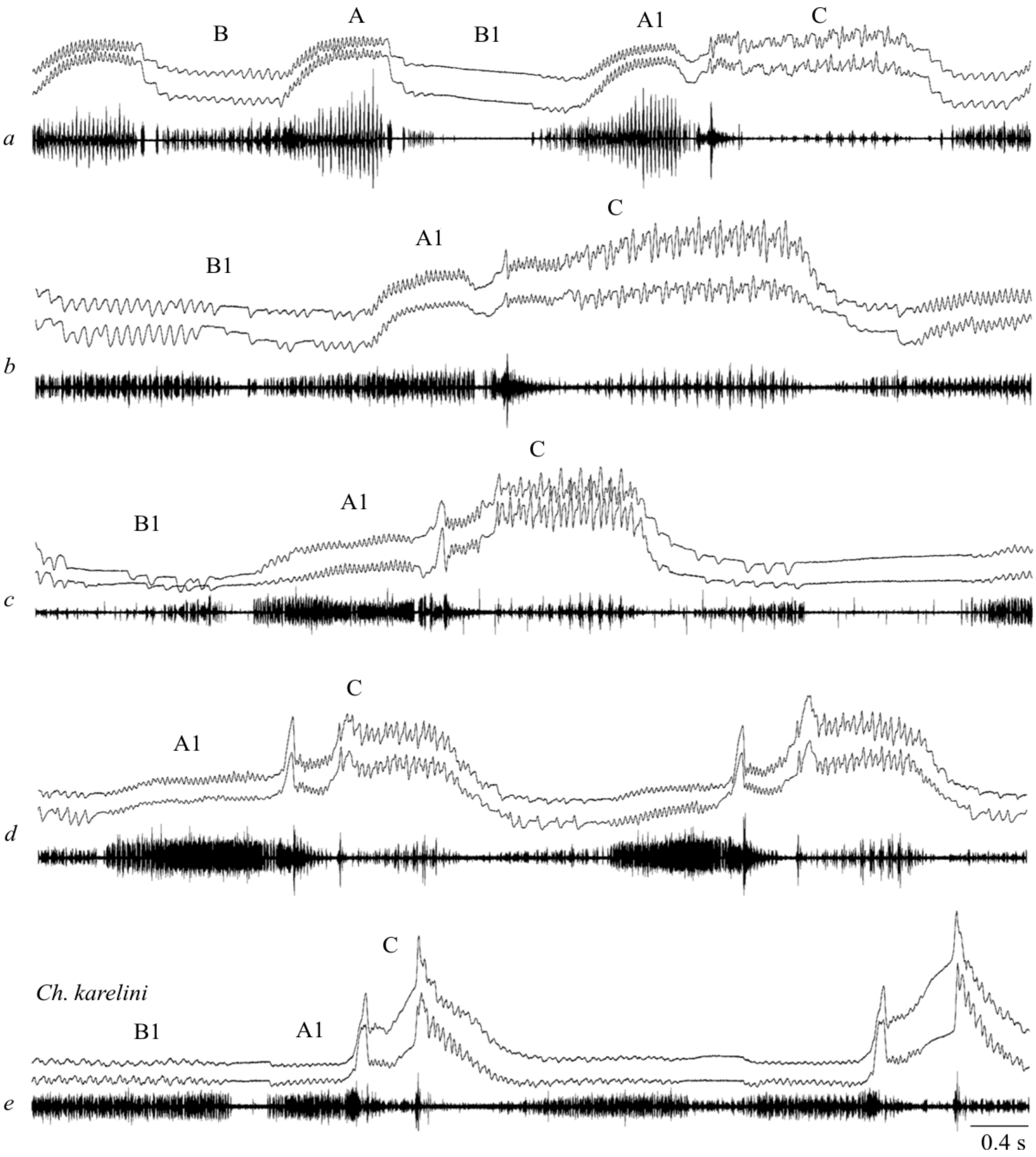

Fig. 6. Transformation of elements B1, A1, and C in transition from the courtship signal of *Chorthippus albomarginatus* (*a*) to that of *Ch. karelini* (*e*), via the intermediate variants recorded in the hybrid population in locality 2 (*b*–*d*). Trajectories of movements of the hind legs are shown above each oscillogram on the same time scale.

Ch. karelini and *Ch. oschei* was more likely than between other possible pairs of species; this problem is discussed in greater detail below. The intermediate signal variants shown in Fig. 9 clearly demonstrate the transition from *Ch. karelini* to *Ch. oschei*. In particular, changes in duration and amplitude modulation of element C are evident.

The values of all the eight parameters of signals recorded in locality 10 were in most cases intermediate between those of signals of *Ch. karelini* and *Ch. oschei* from allopatric populations (Fig. 7). Significant differences from *Ch. oschei* were observed in all the parameters, and from *Ch. karelini*, in six out of eight parameters (Mann–Whitney test, $p < 0.05$). It is remarkable that the values of one parameter, namely the number of A/B pairs, were lower than the corresponding values in both parent signals. Similar to the hybrid zone between *Ch. albomarginatus* and *Ch. karelini*, the variation of some signal parameters (the number of A/B pairs, the pulse frequency in elements

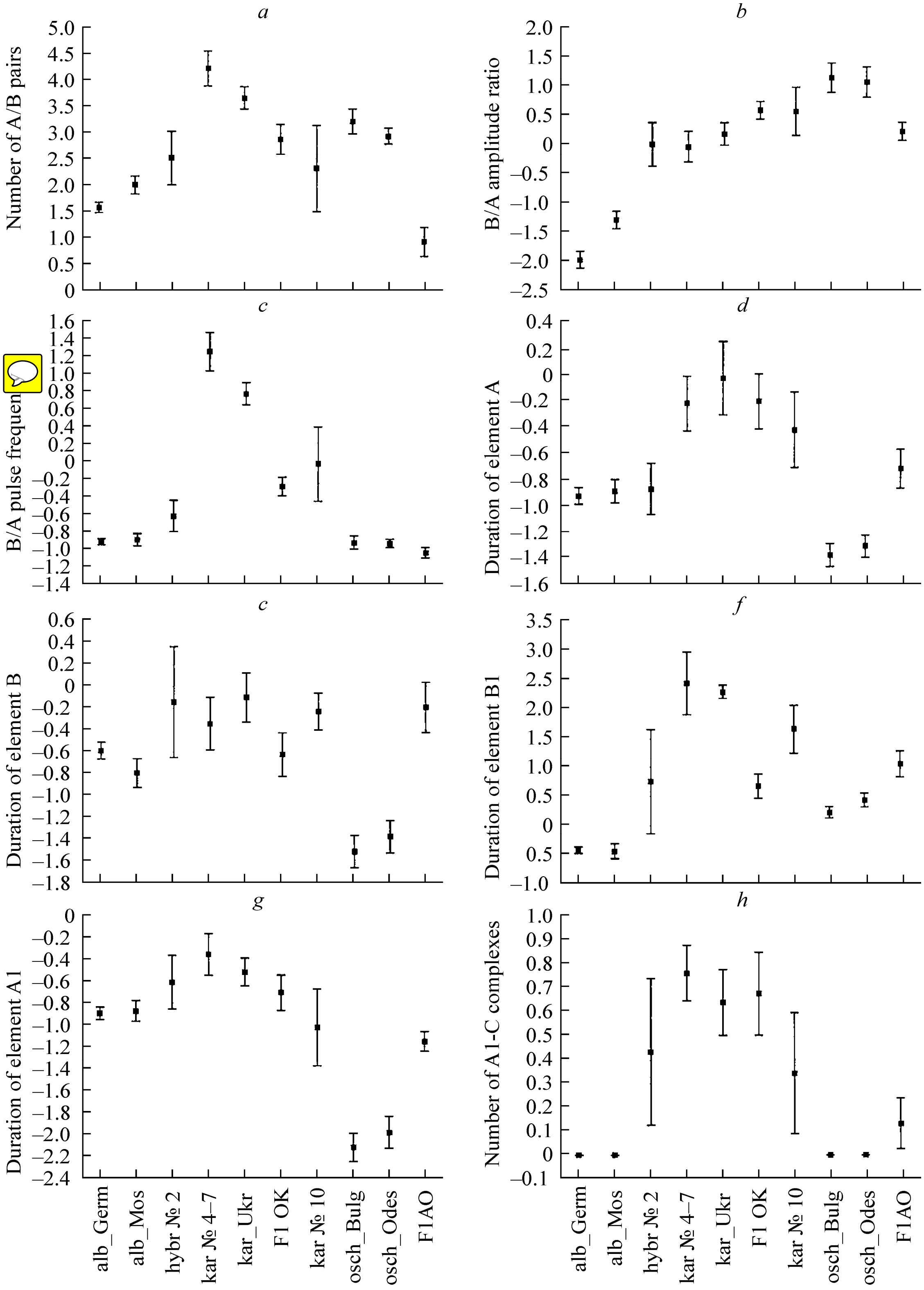

Fig. 7. The values (means and confidence intervals) of eight parameters of the courtship signals in different populations and F1 hybrids of the group *Chorthippus albomarginatus*.

A and B, the duration of element A1, and the number of A1-C complexes) in the hybrid Ukrainian populations exceeded that of the signals of *Ch. karelini* and *Ch. oschei* (Figs. 7, 10). According to the PCA results, the spread of signal parameters in locality 10 was considerably greater than in allopatric populations.

a

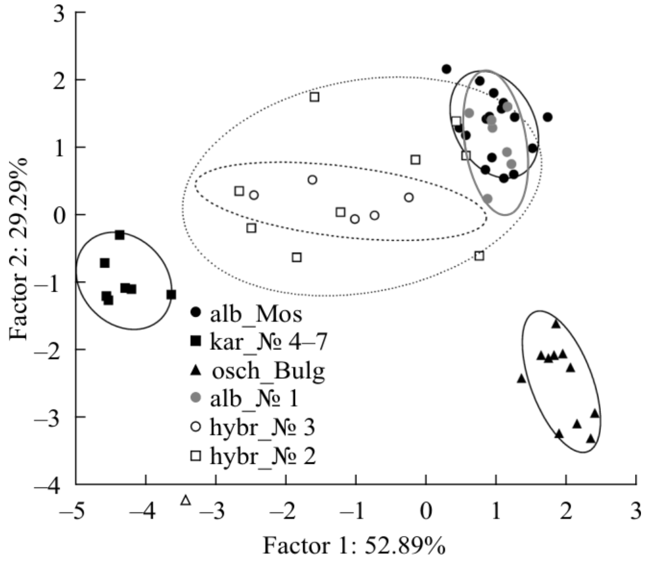

Fig. 8. The results of principal component analysis based on eight parameters of the courtship signal: (*a*) the values of the first two factors for allopatric populations of *Chorthippus albomarginatus*, *Ch. oschei*, and *Ch. karelini* and for hybrid populations from Ulyanovsk (locality 2) and Samara (locality 3) Provinces; (*b*) contribution of the parameters studied to factors 1 and 2.

b

Parameter	Factor 1	Factor 2
Number of A/B pairs	–0.57	–0.75
B/A amplitude ratio	–0.05	–0.89
B/A pulse frequency ratio	–0.89	–0.23
Duration of element A	–0.81	0.35
Duration of element B	–0.69	0.52
Duration of element B1	–0.81	–0.41
Duration of element A1	–0.73	0.62
Number of A1-C complexes	–0.89	–0.10

The Signals of the Laboratory Hybrids

Since the courtship signals of reciprocal F1 hybrids of *Ch. karelini* and *Ch. oschei* revealed no significant difference in any of the studied parameters, these signals were united in our analysis. Similar to the signals of the previously studied F1 hybrids of *Ch. albomarginatus* and *Ch. oschei* as well as natural hybrids, the signals of F1 hybrids of *Ch. karelini* and *Ch. oschei* demonstrated a high diversity of patterns. The signals of two hybrid males are shown in Figs. 4*e*, 4*f*. One signal was characterized by the highly variable number of A/B pairs between elements C and also the number of A1-C complexes; the other signal revealed high variability of the duration of elements B and B1.

It should be noted that the signals of F1 hybrids were very similar to those of males from the "hybrid" localities within the Askania-Nova region. Comparison of the PCA results (Figs. 10 and 11) revealed a significant overlap of the values of the first two factors for the signals of these groups. Significant differences between the signals of F1 hybrids and the signals recorded within the hybridization zone in locality 10 were observed in three parameters: the duration of elements B and B1 and the number of elements C (Mann–Whitney test, $p < 0.05$). It is interesting that on average, the signals emitted by F1 hybrid males were more similar to those of *Ch. karelini* than to those of *Ch. oschei* (Fig. 11). The same trend was observed in the natural hybrids in the environs of Askania-Nova (Fig. 10).

DISCUSSION

Earlier, we studied a wide hybrid zone between *Ch. albomarginatus* and *Ch. oschei* in the territories of Ukraine and Moldova (Vedenina and Helversen, 2003; Vedenina, 2011). The discovery of new hybrid zones described herein indicates that cases of hybridization in the *Ch. albomarginatus* group are not uncommon, and that three species of this group, in particular *Ch. albomarginatus*, *Ch. karelini*, and *Ch. oschei*, can hybridize in the nature. Analysis of the signals emitted by the natural and laboratory hybrids has shown that hybridization of these three species may occur in different combinations.

The recent reviews have shown that hybridization is a very common natural phenomenon that occurs in various forms and has a much greater influence on speciation than it was previously assumed. In some scenarios, hybridization may destroy the isolation barriers and completely eliminate the differentiation between populations (see, e.g., Gow et al., 2006). In other cases, hybridization may reinforce the isolation barriers and lead to the formation of "good" species (Wu, 2001; Via, 2009). In particular, the appearance of poorly adapted hybrids may result in reinforcement of ethological isolation between the hybridizing species (Servedio and Noor, 2003). The latter scenario

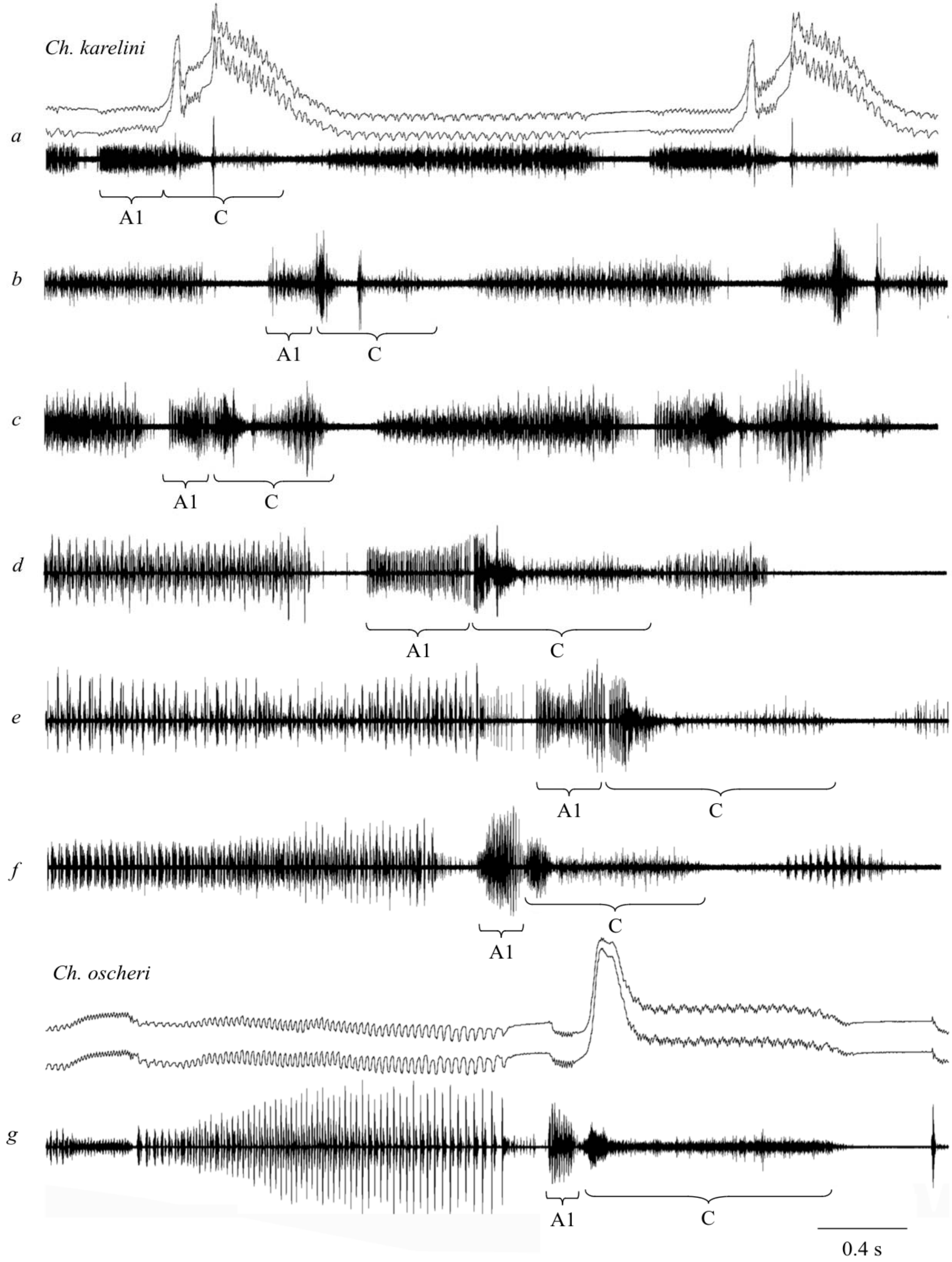

Fig. 9. Transformation of elements A1 and C in transition from the courtship signal of *Chorthippus karelini* (*a*) to that of *Ch. oschei* (*g*), via the intermediate variants recorded in the hybrid population in locality 10, Kherson Province of Ukraine (*b–f*).

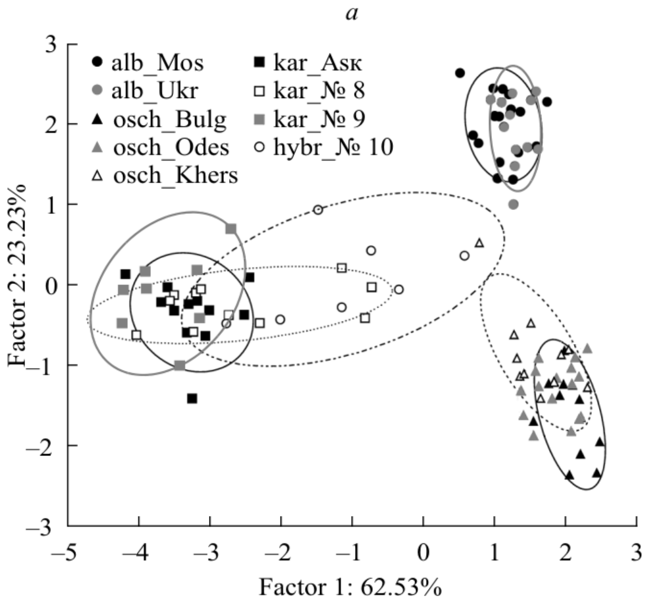

Fig. 10. The results of principal component analysis based on eight parameters of the courtship signal: (*a*) the values of the first two factors for allopatric populations of *Chorthippus albomarginatus*, *Ch. oschei*, and *Ch. karelini* and for hybrid populations from localities 8–10 in Kherson Province of Ukraine; (*b*) contribution of the parameters studied to factors 1 and 2.

b

Parameter	Factor 1	Factor 2
Number of A/B pairs	–0.59	–0.63
B/A amplitude ratio	0.11	–0.92
B/A pulse frequency ratio	–0.97	–0.12
Duration of element A	–0.89	0.15
Duration of element B	–0.84	0.33
Duration of element B1	–0.89	–0.37
Duration of element A1	–0.78	0.56
Number of A1-C complexes	–0.90	–0.14

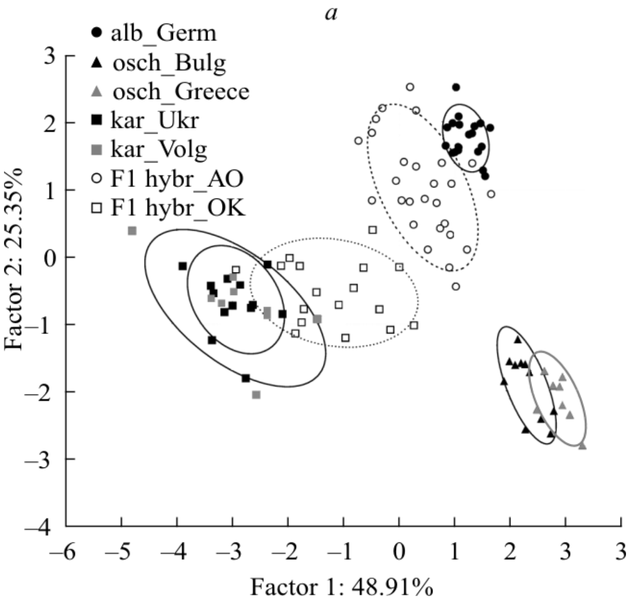

Fig. 11. The results of principal component analysis based on eight parameters of the courtship signal: (*a*) the values of the first two factors for allopatric populations of *Chorthippus albomarginatus*, *Ch. oschei*, and *Ch. karelini* and for F1 hybrids between *Chorthippus karelini* and *Ch. oschei* (OK) and between *Ch. albomarginatus* and *Ch. oschei* (AO); (*b*) contribution of the parameters studied to factors 1 and 2.

b

Parameter	Factor 1	Factor 2
Number of A/B pairs	–0.33	–0.80
B/A amplitude ratio	–0.06	–0.77
B/A pulse frequency ratio	–0.83	–0.33
Duration of element A	–0.84	0.15
Duration of element B	–0.65	0.63
Duration of element B1	–0.80	–0.11
Duration of element A1	–0.83	0.42
Number of A1-C complexes	–0.80	–0.28

has attracted the attention of many researchers since, despite the numerous convincing models, the empirical data supporting this hypothesis are still scarce. Besides, hybridization may facilitate adaptive divergence of populations and establishment of a new hybrid population isolated from the parent populations ("hybrid speciation"; see Lavrenchenko, 2013; Mallet, 2007; Abbott et al., 2010). Finally, selection and hybridization may exist in stable equilibrium with only partial introgression between the populations (Barton and Hewitt, 1985; Nosil et al., 2009). In such cases the isolation barriers are usually not reinforced, but the hybrid zone may become a source of speciation if the environment conditions change.

The relatively great distance between the two hybrid populations found in Ulyanovsk and Samara Provinces indicates the presence of a wide zone of hybridization of *Ch. albomarginatus* and *Ch. karelini*, which may be comparable to the hybrid zone between *Ch. albomarginatus* and *Ch. oschei* (Vedenina, 2011) both in its structure and in the processes occurring in it. Similar to the signals of the hybrids of *Ch. albomarginatus* and *Ch. oschei*, the signals of the hybrids of *Ch. albomarginatus* and *Ch. karelini* are more variable than those of the parent species and have some new characters. For example, some signals recorded in locality 2 contain two variants of element A, corresponding to the *albomarginatus* and *karelini* types (Fig. 5*d*); in other hybrid signals, the duration of element B exceeded that of both parent species (Fig. 1*e*). The hybrid courtship signals with novel traits may be preferred by females in mixed populations and may thus be supported by sex selection. Under the conditions of high population density which is often observed in grasshoppers, individuals of different sex may meet by chance, in which case the male may start courtship behavior without emitting the calling signal (Kriegbaum, 1989; Kriegbaum and Helversen, 1992). Field observations of grasshoppers of the *Ch. albomarginatus* group show that several males may simultaneously court one or several nearby females. This situation may be likened to the leks of birds in that it allows the female to compare the simultaneously courting males, which may considerably affect the female's choice. According to the theory of Kirckpatrick and Ryan (1991), females in leks often prefer the male that emits the most complex courtship signal.

Our previous analysis demonstrated an unusual pattern of inheritance of the courtship signals in *Ch. albomarginatus* and *Ch. oschei* (Vedenina et al., 2007b). It was shown that the signals of F1 hybrids of *Ch. albomarginatus* and *Ch. oschei* contained certain novel traits, well illustrated by the RCA results (Fig. 11). These traits are often manifested by the "off-scale" values of some parameters of the hybrid signals, which are significantly higher or lower than the corresponding values in the parent signals. We have proposed a hypothesis to explain the unusual inheritance of the signal structure in the group *Ch. albomarginatus* (Vedenina et al., 2007b), which presumes the participation of duplicated loci (type II of Templeton, 1981) in inheritance of the homologous signal elements. In the hybrids, both parental copies of such duplicated loci are expressed, which may result in unpredictable patterns of the neuronal network activity underlying the signal generation. The same inheritance pattern may manifest itself in the hybrids of *Ch. albomarginatus* and *Ch. karelini*, even though not all the signal elements are homologous in these species; the homology of elements B and C raises no doubts, but elements A seem to be non-homologous. We have not yet obtained mass material on the laboratory hybridization between *Ch. albomarginatus* and *Ch. karelini*; however, in pilot experiments we have obtained several F1 hybrid individuals and recorded their courtship signals.

Analysis of the hybrid signals and behavioral preferences in the hybrid zone between *Ch. albomarginatus* and *Ch. oschei* demonstrated significant asymmetry: the hybrid signals were significantly more similar to those of *Ch. albomarginatus* than to those of *Ch. oschei*, whereas females of *Ch. albomarginatus* were less selective than those of *Ch. oschei* (Vedenina et al., 2007a). The strong dominance of *Ch. albomarginatus* may be partly explained by a more ancient origin of this species and a simpler structure of its courtship signal. These factors undoubtedly affect the dynamics of the hybrid zone, which seems to be shifting towards *Ch. albomarginatus* (Vedenina, 2011). It may be assumed that females of *Ch. albomarginatus* will be also less selective than females of *Ch. karelini*, because the signal of *Ch. karelini* contains more elements and may be considered more complex than that of *Ch. albomarginatus*. In this case, the hybrid zone will also be shifting towards *Ch. albomarginatus*. In the previously studied zone of contact between *Ch. albomarginatus* and *Ch. oschei*, the populations of these species formed a mosaic pattern of spatial distribution determined by their different biotopic preferences. The existence of such differences between *Ch. albomarginatus* and *Ch. karelini* needs to be confirmed by further research.

The specificity of the second hybrid zone lies in the fact that *Ch. karelini* has a narrow local distribution in Ukraine, being present only in the Askania steppes, whereas *Ch. albomarginatus* and *Ch. oschei* occur in the remaining territory of Ukraine. Thus, the entire Ukrainian range of *Ch. karelini* is comparable in size to its hybrid; this case may be essentially different from the situations when the species range is considerably greater than the contact zone. In addition, *Ch. karelini* may hybridize with both *Ch. albomarginatus* and *Ch. oschei* in the Askania-Nova region. All these factors make the analysis of this hybrid zone a very complicated task.

The signals of hybrids from locality 10 and those of F1 hybrids of *Ch. karelini* and *Ch. oschei* did not differ significantly in five out of eight parameters. At the same time, comparison of the signals emitted by hybrid grasshoppers from locality 2 (where *Ch. karelini* and *Ch. albomarginatus* hybridize) and locality 10 revealed significant similarity in four out of eight parameters. Thus, hybridization between *Ch. karelini* and *Ch. oschei* is the most common in the Askania-Nova region, but the presence of some other combinations cannot be ruled out. Moreover, according to our previous results (Vedenina et al., 2007b; Vedenina, 2011), hybrid populations of the *Ch. albomarginatus* species group predominantly contain the backcross offspring of different combinations of species, whose signals may be very similar. For example, the signal of the hybrid (*albomarginatus* × *karelini*) × *karelini* may be identical to that of the hybrid (*oschei* × *karelini*) × *karelini*. Such hybrids can be differentiated only with the use of molecular markers.

The local distribution of *Ch. karelini* in the Askania-Nova region seems to be determined by the fact that in Ukraine the species is strictly associated with fescue-feathergrass steppes, which have been preserved only in the Askania-Nova Reserve. It may be assumed that 200–300 years ago this species was broadly distributed over the territory of Ukraine, a considerable part of which was then covered with steppes resembling the presently existing protected Askania steppe. With the spread of agriculture and active land tilling, *Ch. karelini* was replaced by the other two species, *Ch. albomarginatus* and *Ch. oschei*, which were expanding northwards from the Balkan refugia and probably happened to be better adapted to the new conditions (Vedenina and Helversen, 2009). So far, we have discovered two populations of *Ch. karelini* which we consider to be "pure:" the previously described population in Bolshoi Chapelski Pod (Vedenina and Helversen, 2009), and the population from locality 9, positioned several tens of meters from this protected territory (Fig. 1). If we assume that *Ch. karelini* mostly inhabits the territory of Bolshoi Chapelski Pod whose area is 24 km^2, the consequences of its hybridization there may be completely different from the consequences of hybridization of *Ch. karelini* in Ulyanovsk and Samara Provinces. In the latter case, the most probable scenario is that of stable equilibrium without the expansion of the hybrid zone, similar to the situation with the hybrid zone between *Ch. albomarginatus* and *Ch. oschei* in Ukraine (Vedenina, 2011). A different scenario, namely elimination of the isolation barriers and loss of differentiation, may be hypothesized in the case of *Ch. karelini* from the Askania steppe. Such a local population of *Ch. karelini* is much more vulnerable than the same species within its main range. Any environmental changes or anthropogenic impact may play the crucial role by triggering new mechanisms, which may lead to unpredictable results.

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