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Complex courtship in a bimodal grasshopper hybrid zone

Abstract Grasshoppers of the *Chorthippus albomarginatus*-group, which is outstanding with respect to its complex courtship song, were studied at fifteen localities in the Ukraine and Moldova. The analysis of the courtship songs revealed two species: *C. albomarginatus* in northeastern Ukraine and *Chorthippus oschei* in the southwestern Ukraine and Moldova. In a belt about 200 km wide, not only were one or the other pure species found, but also males with intermediate song characters. *C. albomarginatus* and *C. oschei* were hybridised in the laboratory, and F1 hybrid males as well as F2 hybrid males produced intermediate song patterns, quite similar to those recorded in the field. We defined a "hybrid song score" for intermediate songs. The score showed a bimodal distribution with most songs resembling one or other parental type, but with only a few intermediates. At several localities, where hybrids with songs similar to one of the parental species dominated, some individual males sang more similarly to the other species. In one locality, two hybrid populations only 3 km apart had different parental types. Hybrid songs can contain novel elements, even more complex than the parental ones, which may offer a new starting point for sexual selection. We suggest that genetic introgression occurs between the two sibling species *C. albomarginatus* and *C. oschei* within a wide hybrid zone stretching over a distance of several hundred kilometres, but with a patchy spatial distribution.

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Introduction

Hybrid zones, where genetically distinct groups of organisms interact leaving at least some offspring of mixed ancestry, are found in all major groups of higher organisms (e.g. Harrison 1986, 1993; Barton and Hewitt 1989; Jiggins and Mallet 2000). Hybrid zones are supposed to be natural laboratories that offer experimental material for the study of the characters and processes involved in divergence and speciation. Hybridising taxa may be at a crucial stage of speciation, when reproductive isolation between them is incomplete. Many hybrid zones appear to be long-term rather than transient phenomena and some authors would consider speciation to be complete at this stage (Hall and Selander 1972; Jackson 1972). Most of the literature and theory concentrates on the examples of so-called unimodal hybrid zones, in which intermediate forms are found to be numerous or even dominant (Hewitt et al. 1987; Butlin 1998; McMillan et al. 1999). Hybrid zones that consist largely of parental forms with few intermediates (so-called bimodal hybrid zones) seem to be rarer and are less well investigated (Harrison 1986; Jiggins and Mallet 2000). According to Jiggins and Mallet (2000), bimodal hybrid zones are typically coupled with prezygotic isolation, often leading to assortative mating within the hybrid populations. Assortative mating may be a by-product of changes in habitat use or temporal isolation. More intriguing processes occur when assortative mating evolves as a result of sexual selection when competition

over mates takes place, either by male contest or by mate choice (West-Eberhard 1983; Andersson 1994).

In gomphocerine grasshoppers, the song is supposed to be the most important component of reproductive isolation. Postmating isolating mechanisms seem to be of less importance, as some sibling grasshopper species, even those with sympatric distributions (e.g., *Chorthippus biguttulus*-group, *Chorthippus dorsatus*-group), can be easily hybridised under laboratory conditions (Perdeck 1957; von Helversen and von Helversen 1975; Stumpner and von Helversen 1994), and offspring are fertile. However, these hybrids are only rarely found in nature. Some hybrid forms in the *C. biguttulus*-group have been found in local mountain populations, e. g. in the Alps (Ragge 1981, 1984; Ingrisch 1995). A well-documented hybrid zone between *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus* was found in a rather narrow region in the Pyrenees (Butlin and Hewitt 1985; Butlin 1998). A recently discovered hybrid zone between *Chorthippus brunneus* and *Chorthippus jacobsi* in northern Spain is of special interest as it has a patchy spatial distribution with unimodal as well as bimodal local hybrid populations (Bridle and Butlin 2002).

We investigated two sibling species of gomphocerine grasshoppers of the *Chorthippus albomarginatus*-group. Four sibling species of this group, *C. albomarginatus* (De Geer 1773), *Chorthippus oschei* von Helversen 1986, *Chorthippus karelini* (Uvarov 1910) and *Chorthippus lacustris* La Greca and Messina 1975, are very similar in morphology but have very different courtship songs. They are outstanding among all European grasshopper species since they have an extremely complex and elaborate courtship behaviour (von Helversen 1986).

The calling song and courtship song of *C. albomarginatus* have already been described in detail by Faber (1953) and Harz (1957) as well as by Ragge and Reynolds (1998). All species of this group seem to use a similar mating strategy. Males court females for a long time, up to half an hour or more, during which the song pattern can change depending on the phase of courtship and degree of excitation. The calling songs of all four species are rather simple and similar, but the courtship songs are quite different, consisting of many sound elements with different and complex temporal patterns. Moreover, three species (*C. oschei, C. karelini* and *C. lacustris*) demonstrate spectacular movements of the legs, palps and antennae during the courtship (von Helversen 1986). The species are geographically separated over most of their ranges. *C. albomarginatus* inhabits northern and central Europe, reaching western Siberia in the east; *C. karelini* occurs in Asia Minor, the south-eastern part of European Russia, Kazakhstan and Middle Asia; *C. oschei* is found in the Balkans and *C. lacustris* is endemic to a very small area around Lake Joannina in northern Greece (Bei-Bienko and Mishchenko 1951; La Greca and Messina 1975; von Helversen 1986). One more species of this group, *Chorthippus bruttius* Fontana and La Greca 1999, has recently been described from southern Italy (Fontana and La Greca 1999). However, the morphology and
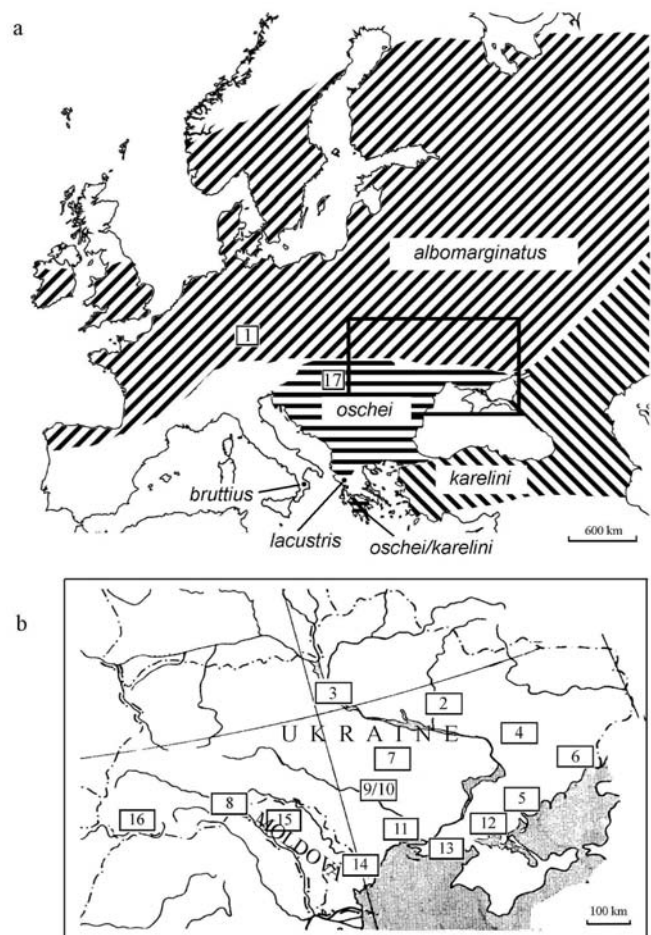
Fig. 1 a Map of the distribution of several species of the *Chorthippus albomarginatus*-group in Europe. The *large rectangle* indicates the area of the collections shown in **b** in more detail, *small numbered rectangles* give the position of collecting sites. Localities 1 and 17 are situated in Germany and Hungary, respectively. **b** shows the localities of the 15 populations studied in the Ukraine and Moldova

courtship song of this species are both very similar to those of *C. karelini* (Vedenina and von Helversen unpublished data).

To date, it is not known whether these species, which are very similar morphologically, have parapatric distributions or whether hybridisation occurs at the borders of their distributional ranges (Fig. 1a).

We studied *C. albomarginatus* and *C. oschei* in the Ukraine and Moldova, where a contact zone between these species might be expected. Since the species of this group can be easily distinguished by their courtship song, we recorded and analysed the songs and also the underlying stridulatory movements of the hind legs of numerous male specimens from different populations. As we found animals singing intermediate songs, we hybridised the two species in the laboratory and analysed the songs and leg movements of the hybrids.

Table 1 Localities where grasshoppers were collected

Locality	Geogra-phical coordi-nates	Date of collection	Number of males recorded
1. Germany: Bavaria, 13 km N of Erlangen, Effeltrich	49°40′ N 11°15′ E	20 July 1999	6
		29 August 2001	20
2. Ukraine: Poltava region, 24 km NE of Mirgorod, Velikie Sorochintzy	50°01′ N 33°57′ E	12–14 August 1997	10
3. Ukraine: Kiev	50°25′ N 30°30′ E	29 August 1998	5
4. Ukraine: Donetzk region, 25 km W of Krasnoarmeisk, near the Byk river, Kamenka	48°21′ N 36°50′ E	22 July 2000	9
5. Ukraine: Zaporozh'je region, 40 km E Melitopol, surroundings of Vladimirovka	46°43′ N 35°50' E	20 July 2000	10
6. Ukraine: Donetzk region, 40 km E Mariupol, Gusel'shikovo	47°10′ N 38°04′ E	21 July 2000	9
7. Ukraine: border between Nikolaev and Kirovograd regions, 35 km NE of Pervomaisk	48°07′ N 31°18′ E	5 August 1997	6
		30 June 1999	11
8. Moldova: surroundings of Of Lipkan'	48°15′ N 26°49′ E	3 August 1997	8
9. Ukraine: Nikolaev region, 7 km E of Pervomaisk, Migija, near the Juzhnyi Bug river. Meadow no. 1	48°01′ N 30°57′ E	30 June 1999	15
		15 July 2000	9
10. Ukraine: Nikolaev region, 4 km E of Pervomaisk, Migija, near the Juzhnyi Bug river. Meadow no. 2	48°01′ N 30°54′ E	27 August 1998	12
		15 July 2000	9
11. Ukraine: Nikolaev region, beach of Tiligul'skii liman, Koblevo	46°40′ N 31°11′ E	8 July 1999	10
12. Ukraine: Kherson region, 7 km NW of Novotroitzkoe	46°23′ N 34°17′ E	19 July 2000	5
13. Ukraine: Kherson region, 15 km E of Skadovsk, surroundings of Tarasovka	46°08′ N 33°06′ E	18 July 2000	10
14. Ukraine: Odessa region, 20 km N of Belgorod-Dnestrovskii, beach of Dnestrovskii liman, surroundings of Semjonovka	46°17′ N 30°08′ E	2 July 1999	7
		8 July 2001	12
15. Moldova: Beltz	47°46′ N 27°54′ E	4 August 1997	8
16. Ukraine: Zakarpatskaja region, 4 km NW of Tjachev	48°02′ N 23°31′ E	29 July 1997	6
17. Hungary: province Békés, 7 km NW of Battonya, Kistompapuszta	46°16′ N 20°39′ E	5 September 1999	3
		16 July 2002	10

Methods

Male and female grasshoppers of the *C. albomarginatus*-group were collected in various localities in the Ukraine and Moldova during the summer seasons of 1997–2002 and their songs recorded (Table 1, Fig. 1b). Some sampling sites were investigated twice in different years. There were no significant differences in the data obtained in the different years (Mann-Whitney U-test: locality 7, $n=6$ and 11, $U=15$, $P=0.063$; locality 9, $n=15$ and 9, $P=0.052$; locality 10, $n=12$ and 9, $U=38$, $P=0.25$, locality 14, $n=7$ and 12, $U=38$, $P=0.703$). Therefore, for the calculation of a hybrid index score , we used all song recordings from the different years. On average, the courtship songs of ten males were recorded from each locality. In addition, songs of *C. albomarginatus* from Germany were recorded during the same period (locality 1). The latter locality was chosen since it was relatively close to the type locality of the species. Songs of *C. oschei* were also recorded from Hungary (locality 17).

To record the courtship song, a male was placed nearby a female collected at the same locality.

Field recordings of the songs were made with an Elektronika-302-1 cassette recorder and a Sony F-V610 microphone; the upper frequency limit was 14 kHz. For song analysis, a PC with an A/D board and the Turbolab 4.0 program (Germany, Bressner Technology) was used.

In the laboratory, not only the sound but also the movements of the hind legs during stridulation were recorded with an opto-electronic device (von Helversen and Elsner 1978). A piece of reflecting foil was glued to the distal part of each hind leg femur and two opto-electronic cameras were focused on the illuminated reflecting dots. Each camera was equipped with a position-sensitive photodiode that converted the upward and downward movements of the hind legs into voltage signals. These signals, together with the microphone (1/2″ Brüel and Kjaer) recordings of the sounds, were A/D-converted with a custom-built DSP card and stored as .wav files. The recordings were analysed with the Turbolab program. The sampling rate for recording the stridulatory move-

ments was 2 kHz and for the sound recordings 250 kHz, so that the complete spectrum of the songs up to about 40 kHz (limited by the microphone) could be recorded and analysed. Courtship behaviour was also recorded with a digital video camera (Sony DCR-TRV 900 E); the video signals were transferred to a PC for analysis.

The courtship songs were recorded for 210 males, in 88 of which the stridulatory movements were recorded simultaneously. In the laboratory, songs and stridulatory movements were recorded at 30–31°C, the temperature of field recordings ranged between 25 and 36°C. The broad temperature range of the field recordings was not crucial for the song analysis since we did not compare absolute time parameters, only relative ones.

Specimens of *C. albomarginatus* and *C. oschei* collected from different populations were hybridised in the laboratory. For these experiments, *albomarginatus* specimens from the border of the Nikolaev and Kirovograd regions (locality 7) and *oschei* specimens from the Odessa region (locality 14) were used. From eight to ten females of one species and the same number of males of the other species were put together in cages measuring 30×60×40 cm. The females, supplied with moist sand for oviposition, were allowed to mate over 2 weeks. Egg pods were collected and kept in a refrigerator at 4°C for 5 months. Larval offspring were bred at the ambient temperature of 25–30°C and were fed with fresh grass, *Poa annua* and *Dactylis glomerata*. Adults obtained from each cross were kept together and allowed to mate only with the animals from the same cross combination. F2 generation larvae were obtained and bred in the same way. The courtship songs of several hybrid males from both generations were recorded and analysed.

Results

Songs of the pure species

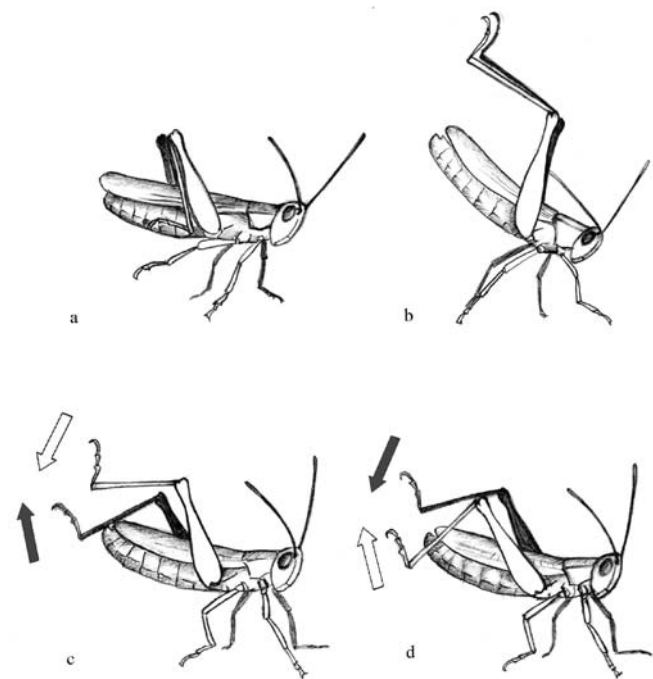The courtship song of *C. albomarginatus* starts with an alternation of two elements, the A and B syllables (von
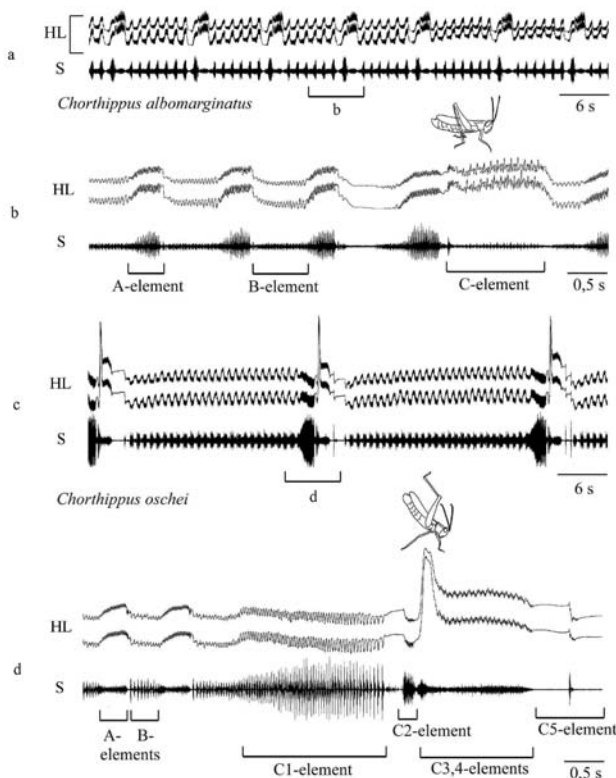
Fig. 2 Oscillograms of the courtship song (hind leg movements *HL* and sound *S*) in **a, b** *Chorthippus albomarginatus* from the Poltava region (locality 2) and in **c, d** *C. oschei* from Hungary (locality 17); **b** and **d** sections at an expanded time scale of the indicated parts of the songs shown in **a** and **c**, respectively. *Drawings* show the highest positions of hind legs at the corresponding moments of the song

Fig. 3 Highest positions of the hind legs during the courtship song of **a** *Chorthippus albomarginatus*, **b** *C. oschei* and **c, d** a hybrid during the C element of the courtship. *C. albomarginatus* does not show any stroke with its hind tibiae, *C. oschei* almost straightens the hind legs during the stroke, showing the "headstand" posture; a kind of hybrid male that we found several times, incompletely stroked out the hind legs and then made conspicuous up and down movements with the hind tibiae (indicated by *arrows*), not known from either parental form

Helversen 1986). The A syllables are produced with legs vibrating rapidly (about 40–65/s) in a high position, the B syllables with legs held in a lower position and vibrating much more slowly (about 20–25/s) (Fig. 2a, b). Typically, about 15–30 s after the beginning of the courtship, a third element, the C syllable, appears. It is produced by a complex movement pattern of small up and down strokes of the hind legs while the legs are in an unusually high and steep position vibrating at a rate of about 30–40/s. After the end of the C element, A and B elements alternate again, always starting with a B element. In a fully developed courtship song, each C element follows after 3–7 pairs of A/B elements (Fig. 2). Therefore, the song can be expressed schematically as ...-A-B-A-B-A-C-B-A-..-A-B-A-C-B-A... . During A and B elements, the legs are vibrated synchronously (syndromally), during the C element the legs are vibrated alternately (antidromally). In the fully developed courtship, A syllables are always louder than B syllables. The courtship song can continue for 3–10 min and even longer before the first attempted copulation occurs. Copulation attempts always directly follow the C element.

The courtship song of *C. oschei* also starts with an alternation of two elements, A and B syllables. The leg position and the rate of leg vibration are similar to those in *albomarginatus*. However, in the *oschei*-song, B syllables always have a higher sound intensity than A syllables (Fig. 2c, d). After a series of alternating A and B elements, the C element starts. The number of A/B pairs between two C elements is higher than in the *albomarginatus*-song (15–30 pairs). The C element of the *oschei*-song can be subdivided into five parts. First, a loud and relatively long C1 syllable is produced by synchronously vibrating the legs, which move with a high amplitude. Then the legs vibrate more rapidly with a lower amplitude at a rather low position (C2 element). The C3 syllable follows immediately, when the legs move into an extra-high and steep position and make a very spectacular flicking out movement with the tibiae—the so-called headstand posture (Figs. 2d, 3b). This posture is completely absent in the courtship of *C. albomarginatus* (compare Figs. 2b, d; 3a, b; videoclip S1, videoclip S2). Then the tibiae are again pressed against the femur and the legs come down to a lower position and vibrate alternately producing a hissing sound of low intensity (C4 element). After the C4 element, a pause of up to 2 s follows and then, in a fully developed courtship, from one to five loud ticks are produced (C5 element). The number of ticks increases in the course of courting. The scheme of the complete song can be written as ...-A-B-A-B-C1-C2-
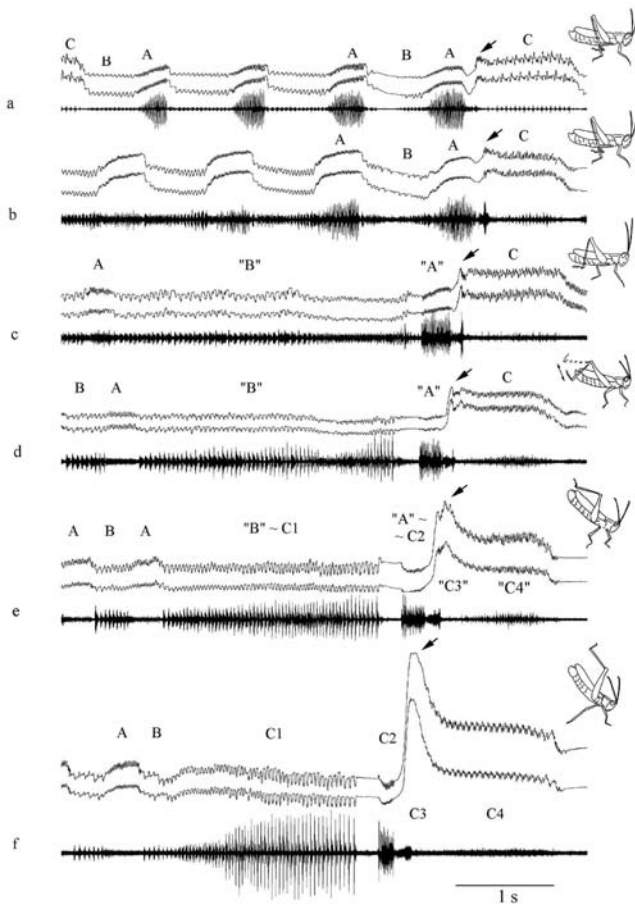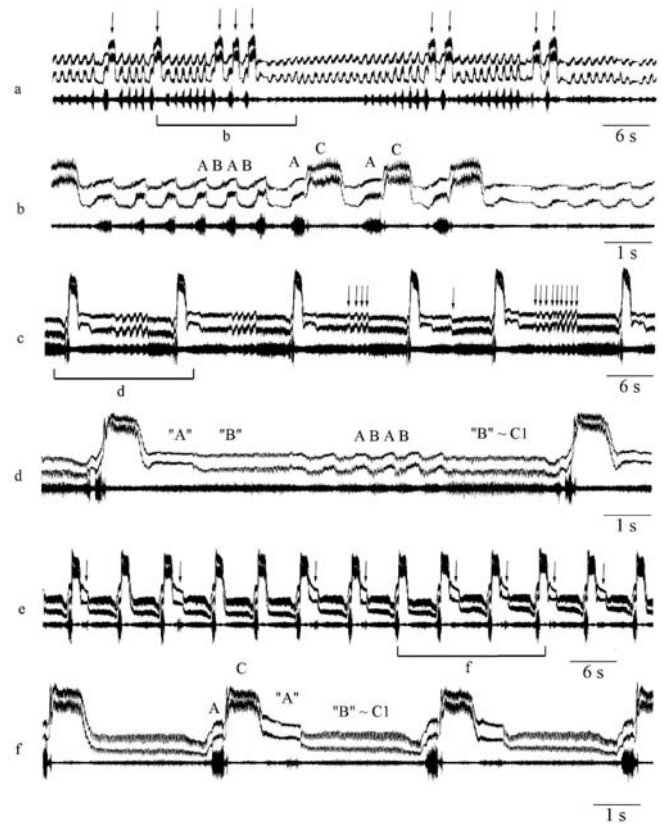
Fig. 4 Transitions from **a** the pure *albomarginatus* courtship song to **f** the pure *oschei* song through **b–e** intermediate variants. Hind leg movements and song patterns were recorded from different individuals: **a** locality 1, **b** locality 7, **c** and **e** locality 10, **d** locality 11 and **f** locality 14. *A*, *B* and *C* are different song elements. Drawings of the highest positions of the hind legs in different males are shown on the *right*; the moments when they occur are marked with *arrows*

Fig. 5 The courtship songs (hind leg movements and sound) of three natural hybrids in the *Chorthippus albomarginatus*-group from localities 9 (**a**, **b**, **e**, **f**) and 11 (**c**, **d**). *Arrows* indicate the occurrence of A/C pairs, A/B pairs and unusual "A" syllables in song **a**, **c** and **e**, respectively. *A*, *B* and *C* are different elements of the songs; **b**, **d** and **f** are sections at an expanded time scale of the indicated parts of the songs shown in **a**, **c** and **e**, respectively

C3-C4-pause-B-A-...-A-B-C1-C2-C3-C4- pause- C5-B-... . Such a song pattern is typical for males from Yugoslavia and Hungary (Vedenina and von Helversen, unpublished data) and differs slightly from that described for specimens from Greece (von Helversen 1986).

In our study, all males from Erlangen (Germany, locality 1, Fig. 1a) and most specimens from five localities in the north-eastern Ukraine (localities 2–6, Fig. 1b) sang the songs typical for *C. albomarginatus*. On the other hand, most males in one locality of the south-eastern Ukraine, one locality in Moldova and in the Hungarian locality (localities 15–17, Fig. 1b) sang more or less identically to the *oschei*-song (Fig. 2).

Intermediate songs

In several localities in the Ukraine and in Moldova (localities 7–13), we found individuals whose songs were intermediate between the *albomarginatus* and *oschei* type with respect to the temporal parameters of the song and leg-movement patterns (compare Fig. 4a, f with Fig. 4b–e). In these songs, not only was the number of A/B pairs between the C elements intermediate, but so were other parameters that differ between *albomarginatus* and *oschei*. In some males, A/B elements were of the *oschei* type, whereas the C element was of the *albomarginatus* type, without any tibial stroke of the hind legs. In the example presented in Fig. 4c, A and B elements were equal with respect to loudness and the B element preceding each A/C pair was unusually long. In another example shown in Fig. 4d, the pulse amplitude and the pulse rate of the prolonged B element were not stable and varied over a wide range; this male produced incomplete tibial strokes with the hind legs. In some individuals, the stroke with the hind legs was only weak (Fig. 4c), in others it was stronger (Fig. 4d), but nevertheless lower in amplitude than in *C. oschei*. Figure 4e gives an example of a hybrid song in which the stroke was comparable to the C3 element of *oschei* (Figs. 3b, 4f).

The C element could sometimes be repeated twice or three times (Fig. 5a). The duration of the A and B

Table 2 Song parameters chosen for the calculation of a hybrid index score

Parameters	*albomarginatus*-type (=0)	Intermediate type (=0.5)	*oschei*-type (=1)
1. Number of A/B pairs	The number of A/B pairs between C elements 3–9	The number of A/B pairs between C elements 10–14, highly variable (Fig. 5a, c), or 1–2 (Fig. 5e)	The number of A/B pairs between C elements 15 or more
2. Relative loudness of A and B elements	A syllables louder than B syllables (Fig. 4a)	A and B syllables approximately of equal sound intensity or variable (Fig. 4b, c)	A syllables have lower intensity than B syllables (Fig. 4d–f)
3. C1 element	C1 syllable is absent (Fig. 4a, b)	"B" syllable preceding each A/C pair longer than other B elements and consisting of irregularly repeated pulses of variable amplitude (Fig. 4c, d)	C1 syllable consisting of regularly repeated pulses of monotonically increasing amplitude (Fig. 4e, f)
4. Relative loudness of A and C elements	A element preceding each C element of larger amplitude than the previous B element (Fig. 4a-c)	Prolonged "B" syllable (a supposed C1 syllable) preceding each A/C pair is of about equal loudness to the following A syllable (a supposed C2 syllable) (Fig. 4d, e)	C1 element louder than C2 element (Fig. 4f)
5. C4 element	C element consisting of low intensity but distinct and regularly repeated pulses (Fig. 4a, b)	C element consisting of distinct but irregularly repeated pulses (Fig. 4c-e)	C4 element present, as a hissing sound without distinct pulses (Fig. 4f)
6. Pause after C element	Pause and ticks after each C element missing and alternating A/B pairs following immediately (Fig. 2b)	A pause after each C element sometimes absent, sometimes present	C5 element (pause and ticks after each C4 element) present (Fig. 2d)

elements (in terms of the numbers of up and down movements) also varied greatly. In the pattern shown in Fig. 5d, there were both unusually long and unusually short A and B syllables between two C elements. The C element could be immediately followed by a B element with an A element sometimes intervening (Fig. 5e, f).

In those localities where intermediate song patterns were recorded, a high degree of inter- as well as intra-individual variability was found compared to the rather narrow range of variability known from the pure, allopatric populations (compare Figs. 2 and 5).

Novel elements and novel combinations
in the hybrid songs

Some males produced very conspicuous up and down movements with the hind tibiae in the course of the C element, when they demonstrated an incomplete head-stand posture with the hind femur elevated to a median position (Figs. 3c, d; 4d; videoclip S3). These movements can be regarded as a new element of a complex courtship behaviour that is not known from either of the pure species.

Another feature not known from the parental species was the repetition of C elements. Several hybrids (for example see Fig. 5a, b) sometimes produced a series of C elements instead of only one C framed by a series of A/B pairs, as is usual in the parental species.

Figure 5e, f shows the song pattern of a male without the alternating A/B pairs between C elements, with only one pair of modified A and B; the order of this male's song pattern was ...A-C-A-B-A-C-A-B-.... or ...A-C-B-A-C-B-... . In another respect, the song pattern shown in Fig. 5c and d was more elaborate than is known from either of the pure song patterns: two types of A elements

(long and short) and two types of B elements (also long and short ones) followed each other in this song in a stereotyped manner. Such songs could be stereotyped, complex, and could have entirely new patterns unknown from the parental species.

A "hybrid song score"

At least six temporal parameters in the intermediate songs differed between the two pure song patterns. These parameters were chosen to calculate a "hybrid index" (Table 2).

We calculated the hybrid index score for male courtship song from different localities in the following way: "0" was given to the song parameter if it was typical for the *albomarginatus* song, "1" if it was typical for the *oschei* song, and "0,5" if it had an intermediate expression. These values were summed for each individual. The minimum score, 0+0+0+0+0+0=0, corresponded to a "pure" *C. albomarginatus*, the maximum score, 1+1+1+1+1+1=6, to a "pure" *C. oschei*. An example of a hybrid song could be: 0+1+0,5+0,5+0+1=3.

Geographical distribution of pure species and hybrids

In Fig. 6, histograms of the distribution of hybrid song scores for the 17 populations studied are presented. At all locations in the northern parts of Ukraine, the song patterns recorded were of the *albomarginatus* type (localities 2, 3, 4, see Figs. 1b and 6). Going farther south, the number of A/B elements in the songs increased and became more variable (localities 5, 6). More to the south-east, a broad belt of hybrid populations was found (localities 7–14), and only the western Moldavian and

Fig. 6 The distribution of pure and intermediate courtship songs in the hybrid belt. Seventeen localities in Germany (*1*), Ukraine (*2–7, 9–14, 16*), Moldova (*8, 15*) and Hungary (*17*) were investigated. Index scores are counts of six different song parameters in each individual. The minimum score (0) corresponds to a "pure" *C. albomarginatus*, the maximum score (6) to a "pure" *C. oschei* (for the definition of the hybrid index score see the text and Table 2). The score data summed for all pure and hybrid localities are shown in the three *lowest histogram*s
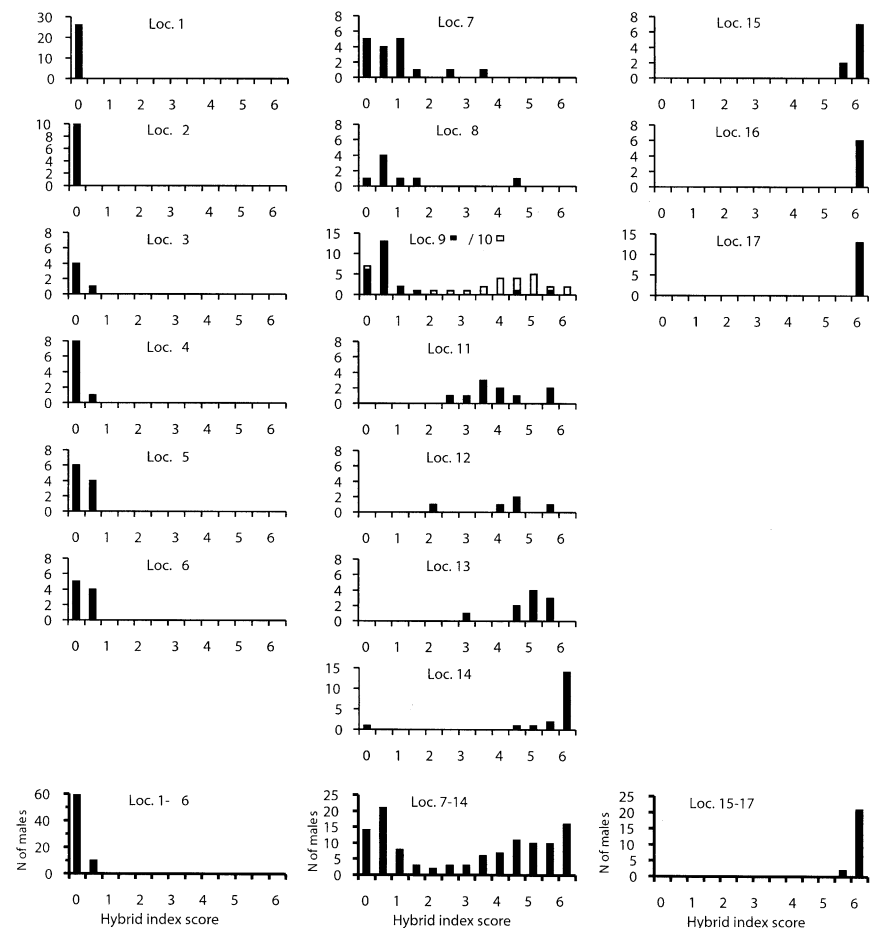
Ukrainian populations (localities 14 and 15), as well as the Hungarian (locality 17) were more or less pure *oschei*.

In most hybrid populations, males singing a strictly intermediate song pattern were in the minority, and most of the males sang more similarly to one of the parental types. In some populations, both parental song types (or intermediates similar to one of them) were recorded; however, one type dominated, whereas the other type was quite rare (Fig. 6, localities 8–10, 14).

Localities 9 and 10 were only 3 km apart (Table 1, Fig. 1b), but at locality 9 *albomarginatus* dominated and at locality 10 *oschei* (and intermediates nearer to *oschei*) prevailed. The meadow at locality 9 was a moist pasture with an abundance of *Agropyron repens* just near the Juzhnyi Bug river, whereas the meadow at locality 10 was much drier, with *Triticum*, *Agrostis* and *Agropyron* grasses, along a small agricultural field at a higher beach of the river.

When the score data for all hybrid localities (7–14) were added together, the song pattern distribution was bimodal: most of specimens sang similar songs to *albomarginatus* or *oschei*, and less were of an intermediate type (Fig. 6).

Correlations between song parameters

When all localities were included, the parameters selected for the hybrid index (Table 2) except for parameter 1 (number of A/B pairs) were highly significantly correlated with each other. Correlation coefficients between parameters 2–6 were between 0.62 and 0.85 (Spearman rank correlation, all $P<0.001$). Only the number of A/B pairs between C elements seemed to follow different rules. In general (Fig. 7a), this parameter also showed an intermediate character in the populations from localities 7–14, but the numbers reached unusually high as well as unusually low values. This number can vary even within one song, e.g., from 5 to 20 (Fig. 5a) or from 1 to 10 (Fig. 5c). Figure 7b–d shows the degree of variability in this parameter for two pure populations and one hybrid population. Populations from locality 10 and 11, despite of more *oschei*-like song types, showed very low numbers of A/B pairs (Fig. 7a, d). Such low numbers of A/B elements were typical for some hybrids (e.g. Fig. 5e, f) and do not seem to prove a closer relation to *albomarginatus*.
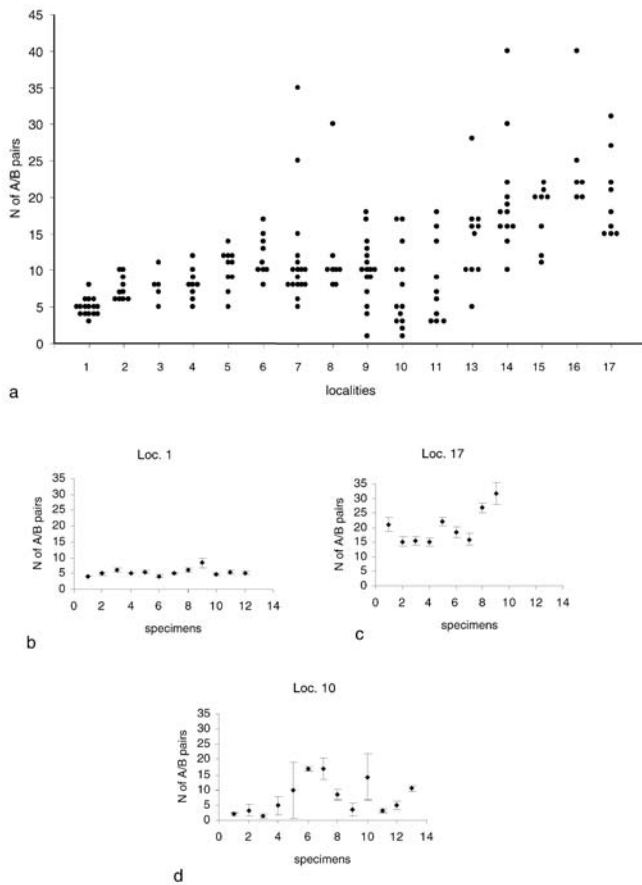
Fig. 7 a Mean values of the number of A/B pairs framed by two C elements for each individual male in the *Chorthippus albomarginatus*-group recorded at localities 1–17. For three localities, mean values and standard deviations of A/B pair number for each specimen is shown (**b–d**)
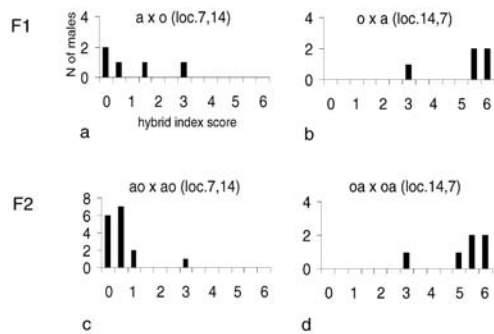
Fig. 8 Distribution of courtship song types in F1 (**a**, **b**) and F2 (**c**, **d**) laboratory hybrids between *C. albomarginatus* and *C. oschei*. **a** F1 generation obtained from a cross between a*lbomarginatus* females from locality 7 and *oschei* males from locality 14; **b** F1 generation obtained from a reciprocal cross, between *oschei* females from locality 14 and *albomarginatus* males from locality 7; **c** F2 generation: offspring of F1 hybrids obtained from a cross between a*lbomarginatus* females from locality 7 and *oschei* males from locality 14; **d** F2 generation: offspring of F1 hybrids obtained from the reciprocal cross
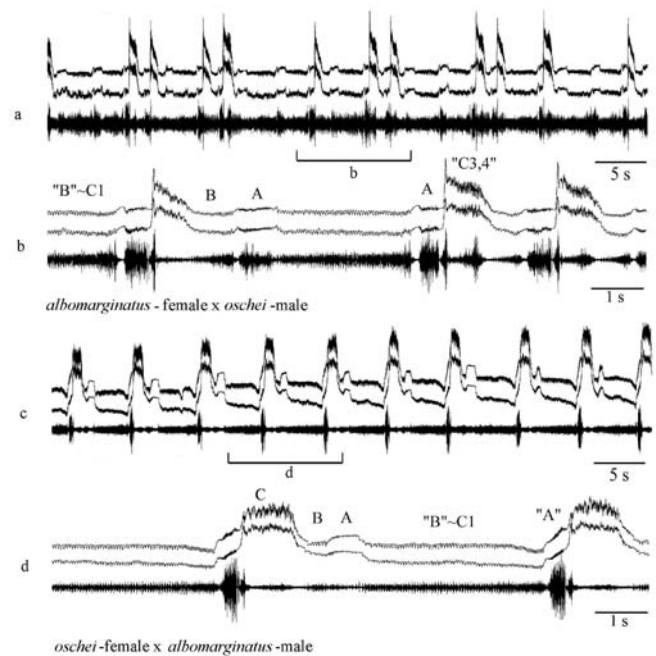
Fig. 9 The courtship songs of two laboratory F1 hybrids between *C. albomarginatus* and *C. oschei*. **a**, **b** Song of a hybrid male that was obtained from a cross between an *albomarginatus* female from locality 7 and an *oschei* male from locality 14; **c**, **d** song of a hybrid male obtained from a reciprocal cross between an *oschei* female and an *albomarginatus* male. The songs of laboratory hybrids were similar in pattern and variation to natural hybrid songs (compare with Fig. 5). *A*, *B* and *C* are different elements of the songs; **b** and **d**: sections at an expanded time scale of the indicated parts of the songs shown in **a** and **c**, respectively

Songs of laboratory hybrids

F1 and F2 hybrids between *C. albomarginatus* and *C. oschei* were raised in the laboratory and did not show a visible reduction of viability. Some hybrid males sang rather similarly to the pure species, whereas others sang intermediate songs. F1 hybrids from both reciprocal crosses had a clear tendency to resemble the maternal type (Fig. 8a, b). Even the hybrid males of the F2 generation tended to sing more similarly to their grandmother's species (Fig. 8c, d).

All intermediate song patterns recorded in the laboratory hybrids were similar to the "natural" intermediate songs found in the field, in the Ukraine and Moldova. This included features like missing alternating A/B pairs between the C elements (compare Figs. 5e and 9c), or a high variability in the number, amplitude and duration of A and B elements. During the incomplete headstand, laboratory hybrids also produced the new type of up and down movement mainly with the hind tibiae, with the femur half elevated, similar to the type which we observed in some natural hybrid males (Fig. 3c, d). In some intermediate songs, the C elements were repeated twice (Fig. 9a).

Discussion

Premating and postmating barriers between *C. albomarginatus* and *C. oschei*

Our observations demonstrate a hybrid belt between the two sibling grasshopper species *C. albomarginatus* and *C. oschei*. The hybrid zone is supposed to be relatively wide, since the hybrids are found over an area extending for 200 km. In almost all localities studied, males singing more or less similarly to the parental types predominated, whereas males that produced an intermediate song pattern were comparatively rare. Bimodal hybrid zones have been shown in many cases to be coupled with strong assortative mating (Jiggins and Mallet 2000). In the *albomarginatus-oschei* hybrid zone, assortative mating also seems to predominate. This is probably based on the courtship signal. The courtship songs of the two species are extremely different in temporal structure, and behavioural experiments with other gomphocerine grasshoppers have demonstrated that the song is the most important component of reproductive isolation for many species (von Helversen and von Helversen 1983, 1994; Vedenina and Zhantiev 1990; Stumpner and von Helversen 1994). Preliminary behavioural experiments with *C. albomarginatus* and *C. oschei* (not detailed here) showed that females from both species preferred to mate with conspecific males.

From the crossing experiments, we obtained both F1 and F2 hybrid generations. However, we cannot be completely sure that no postzygotic incompatibilities exist between the two species since we could not control hatch rate and larval mortality in comparison to the pure species. An analysis of F1 and F2 hybrid songs (Fig. 8) revealed a strong matrocline effect, probably caused by sex linked inheritance of the song pattern and/or by maternal influences. Similar effects were described in different Orthopteran species (von Helversen and von Helversen 1975; Ritchie and Phillips 1998). In the F2 generation, the distribution of the song patterns was similar to the distribution in the first generation, i.e. grandsons sang similarly to their grandmother's species. We cannot explain this result.

Should *C. albomarginatus* and *C. oschei* be regarded as distinct species?

Despite incomplete intrinsic barriers to gene flow, *C. albomarginatus* and *C. oschei* should probably be considered as different species. The two forms differ not only in their courtship songs. In spite of a high similarity in most morphological characters, the two species differ in the structure of the stridulatory file and in number of stridulatory pegs (Vedenina unpublished data), as well as in the white colour of the hind tarsae of *oschei* that are flicked out during the "headstand" (von Helversen 1986).

The two species probably also have slightly different ecological niches. The occurrence of *albomarginatus*-like and *oschei*-like populations in close vicinity but in different types of meadows (localities 9 and 10) might result from different habitat preferences between the two species. A mosaic structure (spatial deviations from smooth clines) of some hybrid zones is due to habitat heterogeneity and strong associations between habitats and genotypes (Harrison and Rand 1989). In particular, this was demonstrated for two hybridising *Gryllus* species in North America, where two parental types were associated with different soils (Harrison 1986), and for two species of *Bombina* toads hybridising in Croatia (MacCallum et al. 1998). It was not found in the mosaic hybrid zone between *C. jacobsi* and *C. brunneus* in northern Spain (Bridle et al. 2002).

Homologous elements in the *albomarginatus* and *oschei* songs

A comparison of the leg-movement patterns in hybrid grasshoppers allowed some hypotheses about the homology of sound elements in the *albomarginatus* and the *oschei* songs. Although the sound patterns of the A and B elements are different in the two species, the respective leg-movement patterns appear to be similar (Figs. 2, 4) and are connected by a continuous row of transitions in the hybrids. A similarity of the C elements is not so evident in the two pure species. However, the study of hybrid songs and the underlying leg movements shows a transition between the C element of the *albomarginatus* song and C3 and C4 elements of the *oschei* song, as well as between the last A syllable preceding the C element in the *albomarginatus* song and the C2 syllable of the *oschei* song (Fig. 4). The prolonged "B" element preceding the A/C pair in the hybrid pattern (Fig. 4c, d) is supposed to be transient between the B element of the *albomarginatus* song and the C1 element of the *oschei* song.

Thus, by observing the hybrid song patterns, it was possible to follow the transition from one pure song type to the other pure pattern through intermediate variants, suggesting homologies between certain elements. This transition probably reflects how the complex pattern of the headstand in *oschei* evolved by integration and specialisation of the neighbouring elements of the "headstand" to form the extraordinarily complex C element.

Elaborate courtship as a result of sexual selection?

How did such elaborate grasshopper courtship songs evolve? The evolution of male trait and female preference in the *C. albomarginatus*-group may be faster than in other grasshopper species groups due to a different mating strategy: during courtship, several competing males often sing around a female, and courtship songs are very long and frequent. When a female does not accept the first attempt at copulation, but does not escape, a male can

continue to sing for two or even more hours. Females can hear and compare different courting males for many hours in the field. This may facilitate female choice and favour competition among males similar to a "lek"-situation. Kirkpatrick and Ryan (1991) called it the "paradox of the lek", that females prefer elaborate male mating displays just in those species in which they receive little more from males than sperm.

The evolution of female preferences driving males to extreme ornamentation or—like in these grasshopper species—to extremely elaborate behaviour patterns, has been discussed by von Helversen and von Helversen (1994). Following the idea of Fisher's "runaway process" (Fisher 1958; Searcy and Andersson 1986), females may have preferred males with a more extravagant courtship, e.g., those males that demonstrate a headstand and a stroke with the hind legs in addition to the song pattern, as well as more complex sound patterns around this stroke. The sons of such females are likely to inherit the attractive features and may find more females than other males.

The preference of elaborately courting males by females may also directly facilitate finding males with "good genes" (Hamilton and Zuk 1982; Zahavi 1987; Maynard Smith 1991), if the ability of a grasshopper male to produce "supernormal" signals like a headstand is indicative of superior genetic quality. A more elaborate signal can also be a supernormal one. The main differences between the courtship songs of *C. albomarginatus* and *C. oschei* lie in the degree of complexity of the C element, and just the C element seems to be the most important component of the courtship, as copulation attempts by the male always follow it. A female sometimes responds acoustically to the courtship song; in this case the female response also follows the C element (Vedenina et al. 2001). In *C. oschei*, the C element consists of a greater number of subelements, and the longest subelement, C1, is extremely loud. Subelements C3 and C4 are additionally accompanied by the visual display. Production of a loud sound and of the very fast stroke of the hind legs probably require much energy by the singing male and thus may be used by the female as an indicator of vigour that cannot be imitated by mere bluff (Gerhardt 1991; von Helversen and von Helversen 1994).

Novel hybrid song elements—an offer for sexual selection by female choice?

Hybrid songs having new elements as compared to both parental songs may offer "material" for sexual selection. For example, during the headstand posture, some hybrids produced quite conspicuous movements with their hind tibiae that are not known from the parental forms (Fig. 3c, d). If females favour males that produce particularly important courtship elements more loudly, more elaborately or more frequently than normal males, they may be seduced to prefer these types of hybrid songs over the pure songs.

Some hybrids between *C. albomarginatus* and *C. oschei* repeated the C element more often than their parental species (Figs. 5, 9). Interestingly, one of the related species, *C. karelini,* always repeats the C element of the courtship twice (von Helversen 1986), and another closely related species, *C. lacustris,* even repeats this element four times: males of the latter species demonstrate an especially elaborate headstand posture during the C phase of the courtship, not only making the strokes with the hind legs but also turning the body around the vertical axis (von Helversen 1986). It seems possible that in the evolution of the extremely complex courtship behaviour within this group of species, former hybridisations favoured by glacial shifts already played a role.

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