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The unusual inheritance pattern of the courtship songs in closely related grasshopper species of the *Chorthippus albomarginatus*-group (Orthoptera: Gomphocerinae)

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Abstract

Sibling species of the *Chorthippus albomarginatus*-group are exceptional among all European grasshopper species because they have an extremely elaborate courtship behaviour. Here, we present a genetic analysis of the courtship song differences between two closely related grasshopper species *Ch. albomarginatus* and *Ch. oschei*. Measurements of seven courtship song characters and one parameter of courtship visual display were compared among parentals, F_1 and F_2 hybrids, and backcrosses. Variation in one morphological character, the number of stridulatory pegs, was studied as well. The distributions of song phenotypes were more consistent with a type-III genetic architecture, which involves complementary or duplicate pairs of loci. We suggest that this type of architecture may provide the novel elements of the hybrid songs, even more complex than the parental ones, which may offer a new starting point for sexual selection. One parameter of the visual display appeared to be controlled by a single locus, but differences in other characters of visual display could be explained by a more complex inheritance pattern. Inheritance of the peg number differences was consistent with a simple polygenic additive model. Sex linkage and/or maternal effects were not detected for any trait. We found a disproportionate influence of the *Ch. albomarginatus* parent on most hybrid song characteristics and the visual display, and this may support a previously suggested idea that *Ch. albomarginatus* is an ancestral form and *Ch. oschei* is a derived species.

Introduction

Within different groups of animals, there are many sister species that differ only in mating signals. They are similar morphologically and show little or no genetic differentiation based on various genetic markers like allozymes and mitochondrial genes. These species can often be hybridized under no-choice conditions and the offspring can be viable and fertile. Nevertheless, they do not hybridize in nature, or the hybrids are only rarely found in contact zones. Thus, only ethological isolating mechanisms evolved between the species. These species have become the focus of studies on the role of sexual selection in rapid speciation, although the importance and explicit function of sexual selection in their formation has been the subject of debate (Maynard Smith, 1991; Andersson, 1994; Panhuis *et al.*, 2001; Mead & Arnold, 2004).

The genetic analysis of mating signals is of considerable interest in evolutionary biology. Knowledge of genetic architecture of these traits is essential for studying various models of speciation. Polygenic control may suggest that new forms have evolved gradually, whereas single genes may imply that they can appear more suddenly (Templeton, 1981; Coyne, 1992; Civetta & Singh, 1998; Henry *et al.*, 2002). Data supporting

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polygenic inheritance of sexually selected traits are known in different animals. The most detailed studies available concern *Drosophila* (Ehrman, 1961; Hoikkala & Lumme, 1984; Kyriacou & Hall, 1986; Tomaru & Oguma, 1994; Pugh & Ritchie, 1996; Nuzhdin & Reiwitch, 2000; Gleason & Ritchie, 2004), but there are studies on other insect groups, in particular, Orthoptera (Butlin & Hewitt, 1988; Shaw, 1996; Ritchie & Phillips, 1998; Ritchie, 2000; Shaw & Parsons, 2002). When mating signals are encoded by single genes, allelic substitutions will cause major, arbitrary changes to these signals, and are therefore more likely to produce new species that differ only in their choice of mates. Ritchie & Phillips (1998) suggested that the likelihood of major genes varies with the mode of signalling. Thus, pheromonal systems showed many examples of major gene effects (Coyne *et al.*, 1994; Takahashi *et al.*, 2001; Dopman *et al.*, 2004; Gleason *et al.*, 2005). In contrast, acoustic systems were suggested to have a polygenic mode of inheritance. This is not surprising, as acoustic behaviour involves the coordinated action of multiple morphological traits, muscles and neurons.

In the past decade, however, a substantial amount of evidence suggesting a simple genetic architecture (few major genes) for traits previously thought to be polygenic accumulated. Estimates using biometrical methods suggested a low number of genes affecting differences in vibration signals between host races of the planthopper *Nilaparvata* (Butlin, 1996), and variation in the substrate-borne songs between two sibling species of lacewings *Chrysoperla* (Henry *et al.*, 2002). A quantitative trait loci (QTL) mapping approach provided further examples of major genes for acoustic traits in *Drosophila*. A low number of genes were shown to control the differences in courtship songs of *Drosophila virilis* and *D. littoralis* (Hoikkala *et al.*, 2000), *D. pseudoobscura* and *D. persimilis* (Williams *et al.*, 2001), and between different lines of *D. melanogaster* (Gleason *et al.*, 2002). Moreover, the data accumulated through QTL analysis on *Drosophila* show that the trait differences between species are more likely to be polygenic, but the same trait differences within species are more often controlled by major genes. It is suggested that the large-effect QTL of the within-species study reflect recent mutations at genes that have not yet been fixed, whereas between-species differences arise over a much longer timescale and are therefore more likely to reflect numerous QTL of minor effect (Orr, 1998; Gleason & Ritchie, 2004).

The studies on inheritance of acoustic signals among Orthoptera are not as numerous as the *Drosophila* studies, although Orthoptera are an excellent model for the study of acoustic communication (Ritchie & Phillips, 1998). Just recently, a detailed study on the inheritance of the calling song differences was conducted on the closely related grasshopper species *Chorthippus brunneus* and *Ch. jacobsi* (Saldamando *et al.*, 2005). Two song parameters were found to be controlled by few genes, whereas the third parameter appeared to be controlled by many loci. The authors suggested that divergence in two parameters might be driven by sexual selection, and this was consistent with the results on female song preference. However, in another study available on grasshoppers of the same group, *Chorthippus biguttulus* and *Ch. mollis*, a much more elaborate pattern of inheritance was observed (von Helversen & von Helversen, 1975). The authors did not go beyond the F1 generation, but the results were intriguing. Hybrids between *Ch. biguttulus* and *Ch. mollis* produced songs that were either intermediate or parental-like in structure, however, the females could prefer the song of one parental species but sing like another parental species. Sex linkage and/or maternal effects were shown to influence the expression of the song pattern. In contrast, no sex linkage was detected in the expression of the song characters in *Ch. brunneus* and *Ch. jacobsi*. The different results obtained in these two studies are currently difficult to explain. We still need many more empirical studies on the grasshoppers to suggest any reliable generalizations.

In this paper, we perform a genetic analysis of the courtship song differences between two closely related grasshopper species of the *Chorthippus albomarginatus*-group. Four sibling European species of this group are quite similar in morphology and have similar and rather simple calling songs. However, they have different and extremely elaborate courtship behaviour (von Helversen, 1986). Their courtship songs consist of many sound elements with different and complex temporal patterns, and these elements alternate in a very characteristic order. During the courtship, three species of this group, *Ch. oschei*, *Ch. karelini* and *Ch. lacustris*, periodically demonstrate a very characteristic visual display – lifting of the abdomen and a stroke with the hind tibiae.

A bimodal mosaic hybrid zone between two species of this group, *Ch. albomarginatus* and *Ch. oschei*, has been documented recently by Vedenina & Helversen (2006). *Chorthippus albomarginatus* inhabits northern and central Europe, *Ch. oschei* inhabits the Balkans, and they meet in a contact zone, about 200 km wide, in the territory of Ukraine and Moldova where they have been found to hybridize. In all hybrid localities studied, males singing intermediate courtship songs were in a minority, whereas males singing the song of one or another parental type dominated. Crosses in the laboratory showed that the hybrids were viable and fertile, which indicated little or no intrinsic post-zygotic isolation (Vedenina & Helversen, 2006). The behavioural experiments revealed relatively strong assortative mating based on the courtship song recognition (Vedenina *et al.*, 2006). One can suppose that *Ch. albomarginatus* and *Ch. oschei* diverged recently, probably through the last glaciations, and elaborate courtship songs rapidly evolved as a result of sexual selection.

In the present analysis of the courtship songs of *Ch. albomarginatus* and *Ch. oschei*, more comprehensive measurements were conducted than in the previous paper (Vedenina & Helversen, 2006), when only the temporal song parameters were used and relatively roughly scored. In the following, we measured the temporal parameters in absolute values. The frequency characteristics of the song as well as a specific visual display accompanying the courtship song were also analysed. Analysis of the hybrid songs has supported our previously suggested hypotheses about the homology of the sound elements in the two species. We also studied the structure of the stridulatory file that was not previously investigated in the members of the *Ch. albomarginatus*-group, although this trait was found to differ markedly between other *Chorthippus* species (Ragge & Reynolds, 1988; Bukhvalova, 1993; Bridle *et al.*, 2001). The number and density of stridulatory pegs appeared to be one of the few morphological characters that allowed us to distinguish between *Ch. albomarginatus* and *Ch. oschei* when the song recordings were not available. We tried to reveal the pattern of inheritance of the complex courtship behaviour and the stridulatory peg number by using biometrical methods. We present estimates of the additive and dominance effects on courtship song characters and the peg number (Mather & Jinks, 1982; Lynch & Walsh, 1998).

The patterns of inheritance of most song parameters did not fit the standard genetic models. Therefore, we presented estimates of the minimum number of loci contributing to divergence of only two song characters and the peg number (Lande, 1981; Zeng, 1992). On the basis of genetic analysis, the most likely scenario for the evolution of the *Ch. albomarginatus*-group is discussed.

Materials and methods

Grasshoppers and crosses

Specimens of *Ch. albomarginatus* and *Ch. oschei* collected from four populations were used for the crosses. Nymphs of the last instar of *Ch. albomarginatus* were collected in Moscow, Russia in 2001, and in the surroundings of Erlangen, Bavaria, Germany in 2002; larvae of *Ch. oschei* were collected in Odessa region, Ukraine in July, 2001, and at the beach of the Batak lake, Bulgaria in 2002. All populations at localities except the Ukrainian population were allopatric; the Ukrainian locality was at the southern border of the hybrid zone (Vedenina & Helversen, 2006). An F_1 generation was obtained from the reciprocal crosses between (1) Russian *albomarginatus* and Ukrainian *oschei* (RU crosses) and (2) German *albomarginatus* and Bulgarian *oschei* (GB crosses). To obtain the F_1 generation, 10 virgin males of one parental species were placed with 10 virgin females of another parental species in cages (30 × 60 × 40 cm) and were kept together for 3–4 weeks. The females were supplied with moist sand for oviposition. Egg pods were collected and kept in a refrigerator at 4 °C for 5 months. Nymphs were reared at an ambient temperature of 25–30 °C and fed with fresh grass, *Poa annua* and *Dactylis glomerata*. F_1 hybrids obtained from each cross, i.e. albR × oschU, oschU × albR, albG × oschB, oschB × albG (female × male), were kept separately and allowed to mate only with the animals from the same cross combinations. The F_2 generation was reared in the same way as the F_1 generation. In the case of the hybrids originating from the German and Bulgarian populations, backcrosses of the hybrid males to the females of pure species, alb × (alb × osch) and osch × (osch × alb), were generated as well.

Recording and analysis of songs

Parents, F_1 and F_2 hybrids of the grasshoppers were reared and recorded at different times and in different laboratories: in the Zoological Institute of Erlangen, Germany in 2001–2002 and in the Institute for Information Transmission Problems, Moscow in 2003–2004. However, the environmental conditions during breeding and song recordings were similar. During recording of each courtship song, a male was sitting near a female; the ambient temperature near the courting male was 30–32 °C. During stridulation of most males studied not only the sound but also the movements of the hind legs were recorded with a custom-built opto-electronic device (von Helversen & Elsner, 1977; Hedwig, 2000). A piece of reflecting foil was glued to the distal part of each hind leg femur of a male 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 PC card. The sampling rate was 2 kHz for recording the stridulatory movements and 100 kHz for sound recordings. The recordings were analysed with the Turbolab 4.0 program (Bressner Technology, Groebenzell, Germany). The courtship songs were recorded from 176 males, in 115 of which the stridulatory movements were recorded simultaneously with the sound. Courtship behaviour was also recorded with a Sony DCR-TRV 355E digital video camera (Sony, Tokyo, Japan); the video signals were transferred to a PC for analysis of visual display.

During the song analysis, the spectral features of different song elements as well as frequency of the leg movements producing each song element were measured. Of all measured frequency and temporal song parameters, only temporal features were chosen for analysis of crosses as they differed significantly between the parental songs. Besides temporal song characteristics, the visual display accompanying the courtship song was evaluated. The strength of the stroke with the hind tibiae was measured as the maximal angle between the femur and tibia (Figs 1 and

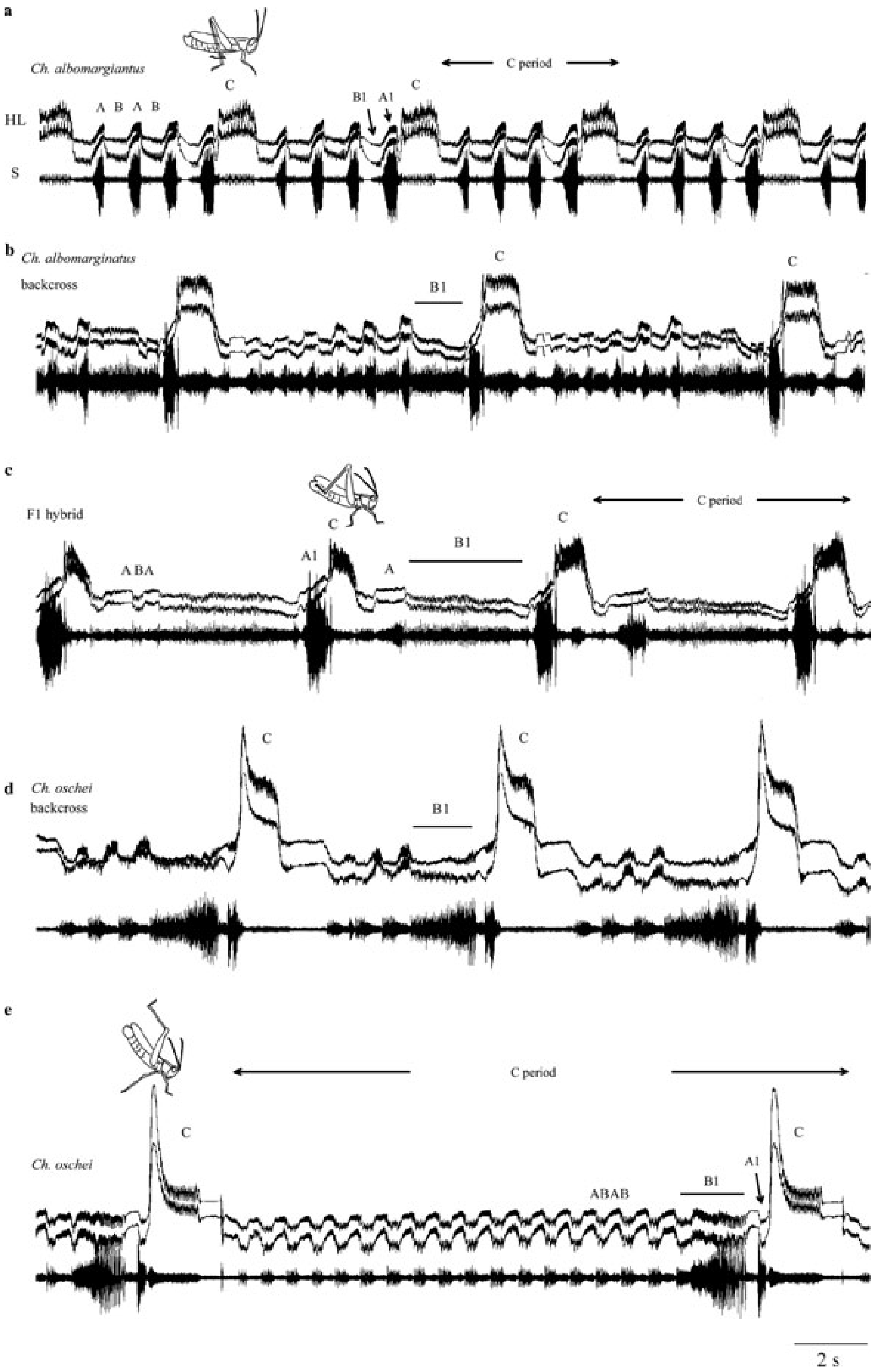


Fig. 1 Oscillograms of the courtship songs (hind leg movements HL and sound S) of (a) *Chorthippus albomarginatus*, (e) *Ch. oschei*, and hybrid progeny: (b) backcross to *Ch. albomarginatus*, (c) F_1 hybrid, and (d) backcross to *Ch. oschei.* A, A1, B, B1 and C are different song elements. Drawings show the highest positions of hind legs at the corresponding moments of the song.

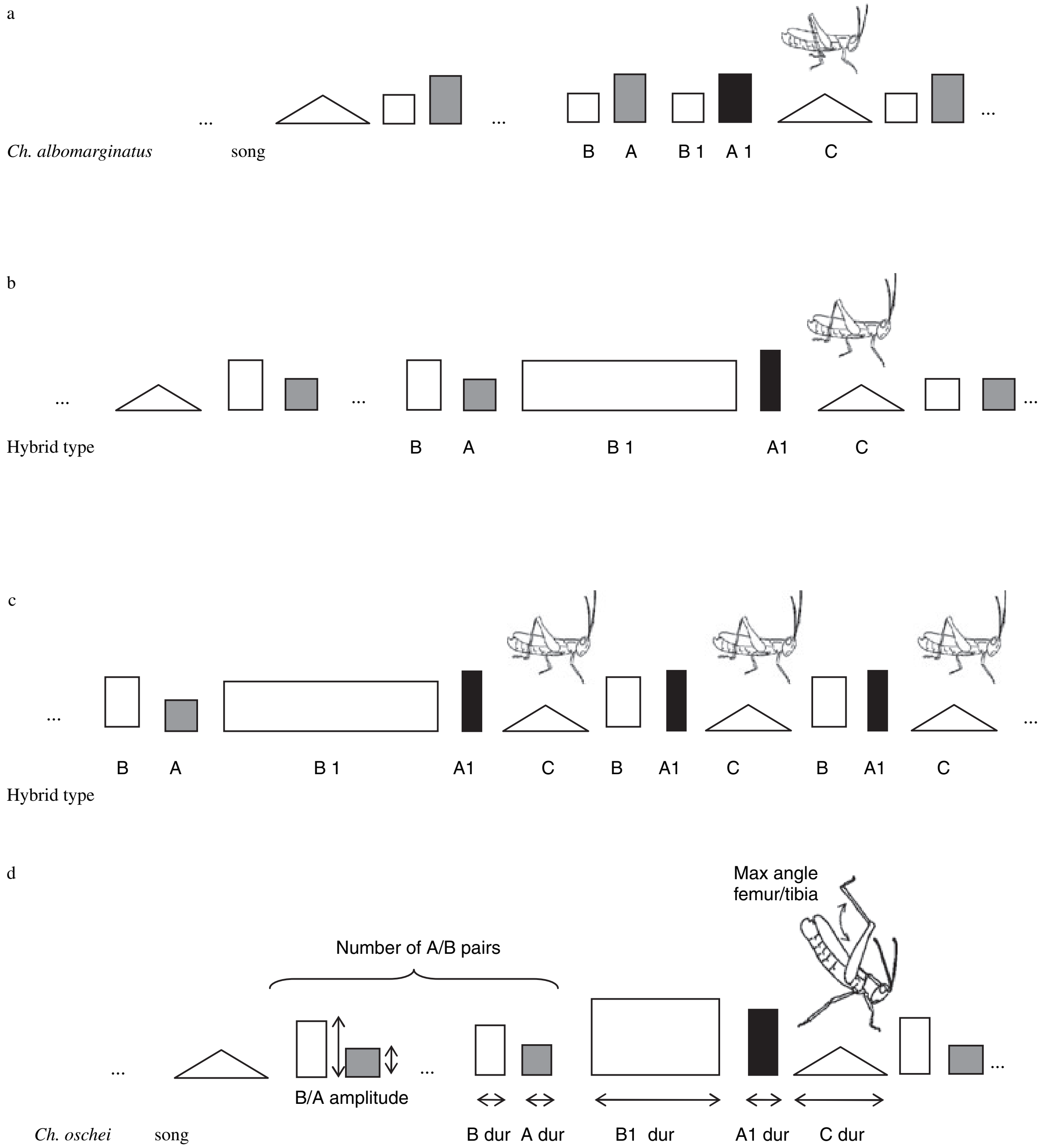


Fig. 2 Schemes of the courtship songs of (a) *Chorthippus albomarginatus*, (d) *Ch. oschei* and (b, c) two types of hybrid songs. A, B, B1, A1 and C are different song elements. Drawings show the highest positions of hind legs at the corresponding moments of the song. The seven temporal parameters (number of A/B pairs, B/A amplitude ratio, duration of B, A, B1, A1 and C elements) and one character of visual display (maximal angle between femur and tibia) used for genetic analysis are shown in (d).

2). We determined the mean value of each song feature for each individual male from at least five cycles of the courtship song; one cycle corresponded to a period of the song C element (Fig. 1a,c,e). Coefficients of variation (CV) were calculated for each individual (within-individual variation) and for each species or cross (among-individual variation). To further visualize and clarify differences between parental and hybrid songs, a principal component analysis (PCA) was applied to temporal song features. All statistical analyses were performed using STATISTICA 6/Win v (StatSoft, Tulsa, OK, USA).

Morphological analysis

Numbers of stridulatory pegs on the inner side of hind femora were counted using a MBS-9 light microscope at 56× magnification. Morphological analysis was conducted for all males that were used for the song analysis.

Biometrical genetic methods

We estimated the effects of different genetic factors (additive, dominance and epistatic) with the joint scaling test using the weighted least squares method (Mather & Jinks, 1982; Lynch & Walsh, 1998). The observed generation means were first tested for fit to a simple additive-dominance model and a model with epistatic interactions. We also estimated the number of effective factors influencing the difference in the song characters and the peg number by the Castle–Wright method (Lande, 1981). To estimate the segregation variance, we used formulae 4b and 8b from Lande (1981). However, this estimator is unbiased if the following assumptions are true: (1) all alleles increasing the value of the character are fixed in one population and all alleles decreasing the value of the character are fixed in the other population; (2) allelic effect differences are equal at all loci; (3) all loci are unlinked and (4) all alleles interact additively within and between loci. In reality, all four true assumptions rarely occur and violations of the assumptions can seriously bias the estimator. Most of the bias comes from unequal allelic effect differences and linkage (Zeng, 1992). We applied Zeng's correction of the Castle–Wright method, estimating the recombination frequency between loci as $r = 0.468$ (the haploid chromosome number $M = 9$) and the function of variation of allelic effect differences as $z = 1.57$ (when individual allelic effects are normally distributed) and $z = 2$ (leptokurtic distribution of allelic effects).

Results

Parental song phenotype and homologous elements

The courtship song of *Ch. albomarginatus* consisted of three alternating elements, A, B and C. After alternation of A and B elements (three to seven pairs), one C element followed, and afterwards the cycle repeated without a pause (Fig. 1a). The last B and A elements that preceded C element were indicated as B1 and A1 respectively (Figs 1–3). The only difference between the last A/B pair and other A/B pairs was a short break produced during generating the B1 element (Figs 1a and 3). All three elements differed in the leg-movement pattern and sound characteristics. Frequency spectra of the leg vibration showed peaks at about 60 and 22 Hz for the elements A and B respectively (Fig. 3). The pattern of the leg movements producing the C element was much more complicated, and the spectrum of the leg vibration showed several peaks corresponding to the main frequency of about 12 Hz and several harmonics. During A and B elements, the two legs vibrated synchronously, whereas during the C element the legs vibrated alternately. Sound syllables A, B and C differed in temporal parameters (pulse rate and pulse amplitude) as well as in frequency characteristics. The spectrum of syllable B had a band of dominant frequencies between 15 and 20 kHz, whereas syllables A and C had maximal energy in the high-frequency band – in a range of 30–40 kHz.

The courtship song of *Ch. oschei* was more complicated and consisted of at least five different elements (Figs 1e and 4). Similarly to *Ch. albomarginatus*, the song of *Ch. oschei* started with alternation of two elements, A and B. Frequency spectra of the leg vibration showed that the legs vibrated at a rate of about 58 and 17 Hz during A and B elements respectively (Fig. 4). After 15–30 alternations of A/B pairs, a complex of B1–A1–C elements followed. In the course of the B1 element, the vibration rate of the legs decreased, therefore the frequency spectrum had a broad band between 18 and 25 Hz. The element A1 was produced by a rapid leg vibration at a rate of about 75 Hz. The complex element C started with lifting of the abdomen, a fast movement of the legs into an extra-high position and a very characteristic stroke with the tibiae (Fig. 1e). Then the tibiae came again to their normal position and the legs vibrated in a complex pattern. The spectrum of the leg vibration producing the C element had several peaks, the first one at about 13 Hz and the following ones corresponding to the harmonics. The two legs were moved synchronously when producing all elements except for the element C; after the stroke with the tibiae, the legs were vibrated alternately. The sound elements also differed in frequency and temporal characteristics. In the frequency spectra, a low-frequency band (15–20 kHz) was typical for the B syllable and a high-frequency band (30–35 kHz) was characteristic for the A syllable. The syllable B1 was a more broad-band sound (15–35 kHz), whereas the subsequent elements A1 and C had narrow bands in a high-frequency range, at about 35 and 25 kHz respectively (Fig. 4).

Although the temporal characteristics of the A, B and C syllables were different in the songs of the two species, there were many similarities between them. The positions of the femora when producing different elements were similar in both species: the lowest position during B element, the higher position during A element, and the highest position during C element. Elements A in the songs of both species were produced with the legs vibrating at a similar high rate, and the frequency of the sound was relatively high. The elements B were produced with the legs vibrating at a similar low rate, and the sound frequency was low. The leg pattern producing C element was complex in the songs of both species: the leg movement spectra had several harmonics, the sound was of a relatively high frequency, the legs were moved

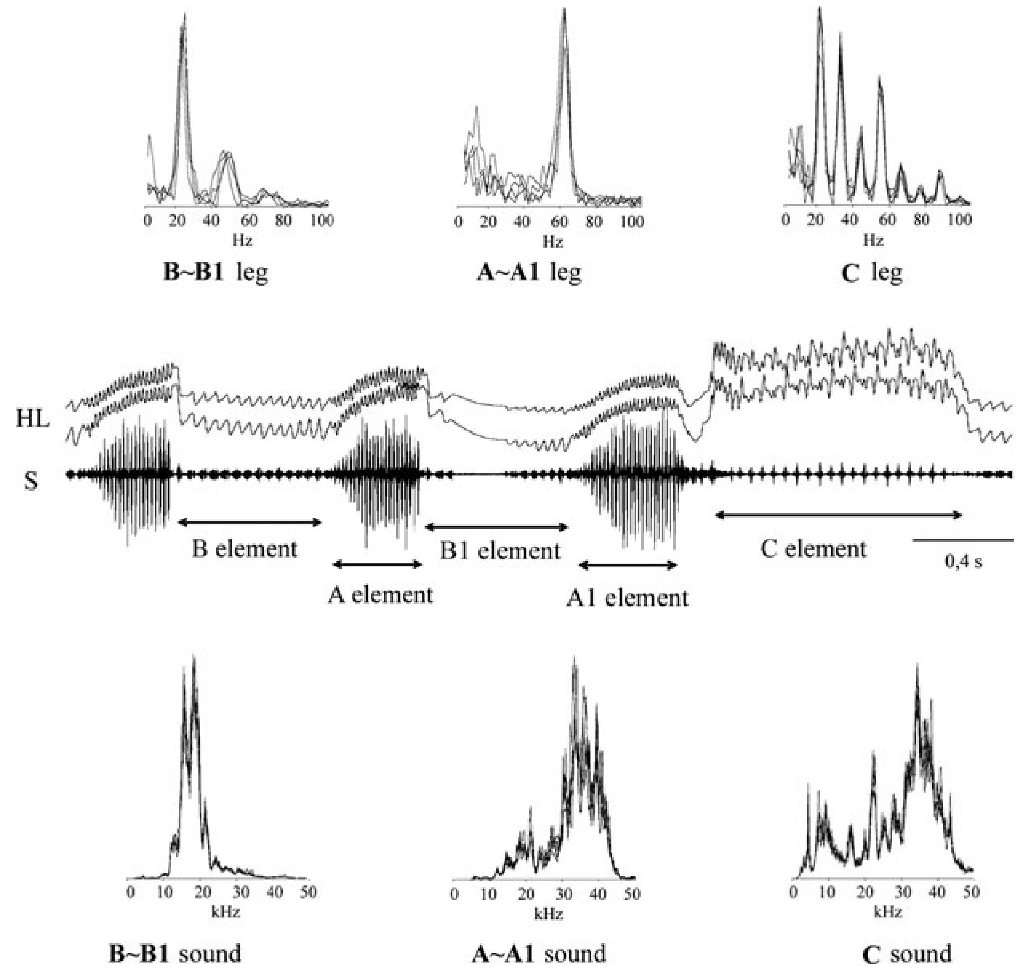


Fig. 3 Courtship song (hind leg movements HL and sound S) of *Chorthippus albomarginatus* and frequency spectra of A, B, B1, A1 and C song elements. Upper panel shows spectra of movements of one leg and lower panel shows sound spectra of the corresponding song parts (their length marked). Vertical scales of spectra are linear. Each spectrum was obtained from five to six elements.

synchronously in the very beginning of the element and then started to move alternately. We therefore suggested the elements A, B and C in the *albomarginatus*- and *oschei*-songs to be homologous. Analysis of the laboratory hybrid songs revealed that B1 and A1 elements may be homologous to the last B/A pair preceding the C element in the *albomarginatus*-song (see below). Based on the estimated homology of the song elements, schematics of the parental and hybrid songs were drawn to illustrate the parameters used for genetic analysis (Fig. 2). The temporal song traits used were the following: the number of A/B pairs between C elements, the ratio of B and A syllable amplitudes, the duration of B, A, B1, A1 and C elements.

Song differences between the two parental *Ch. albomarginatus* populations and the two *Ch. oschei* populations were minor (Tables 1 and 2). Significant differences between the two *Ch. albomarginatus* populations were found in the means and/or variances of three song parameters (the number of A/B pairs, B/A amplitude and duration of B element), whereas the difference between the two *Ch. oschei* populations was found in only one parameter, the number of A/B pairs (Mann–Whitney test, $P < 0.05$). Two parameters, the number of A/B pairs and B/A amplitude, exhibited relatively high among-individual variation, but other parameters, especially the duration of A1 and C elements, were less variable (Table 2).

Songs of F_1 hybrids

Among F_1 hybrid songs, we found significant heterogeneity and several different song phenotypes. One of the most common song types contained two forms of element B consisting of short and long syllables (Figs 1c and 2b–c). They were identical in the leg-movement pattern and sound amplitude but differed in the syllable duration. The long B1 syllable always preceded the pair of A1 and C elements. In some hybrid songs, however,

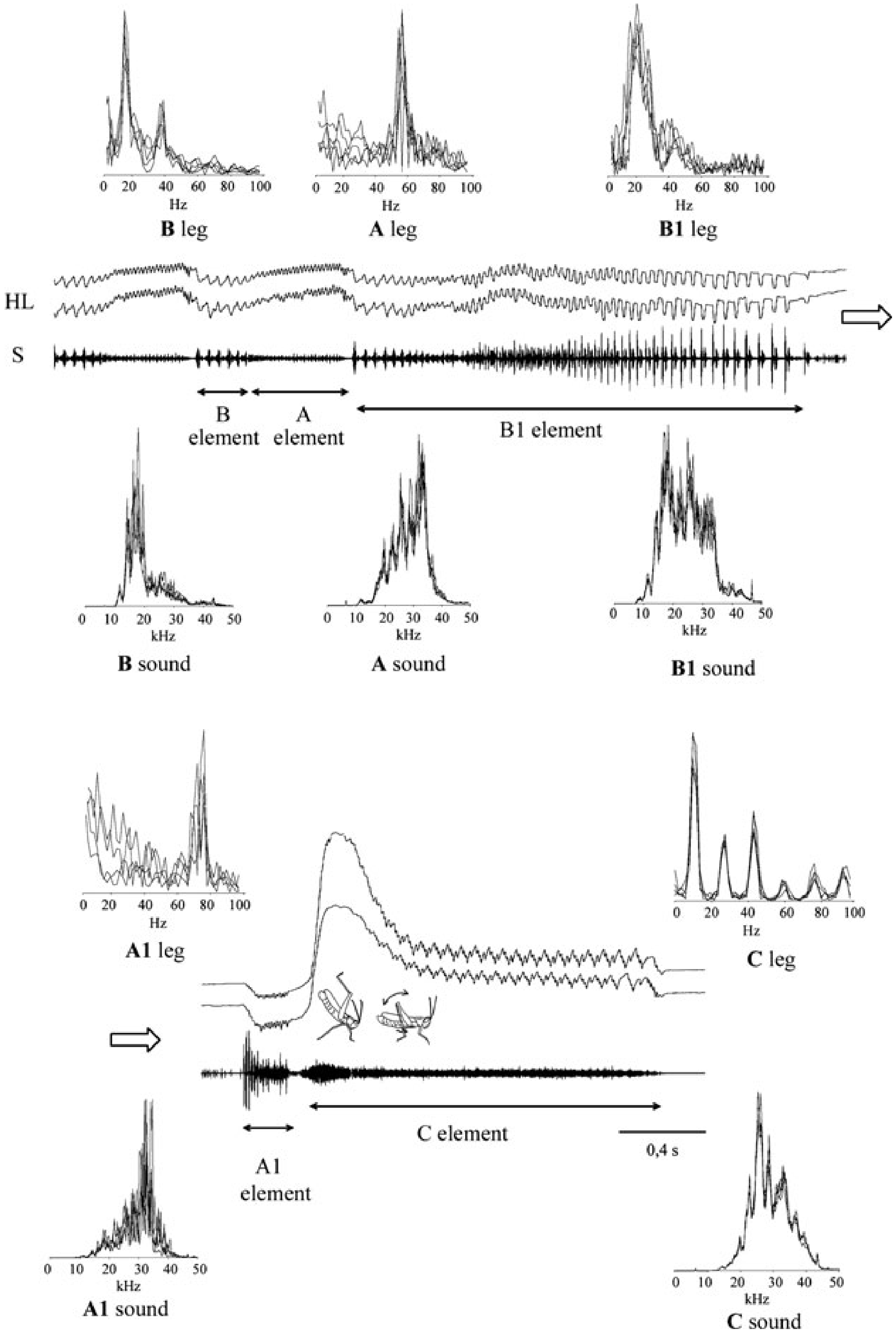


Fig. 4 Courtship song (hind leg movements HL and sound S) of *Chorthippus oschei* and frequency spectra of A, B, B1, A1 and C song elements. Upper panel shows spectra of movements of one leg and lower panel shows sound spectra of the corresponding song parts (their length marked). Vertical scales of spectra are linear. Each spectrum was obtained from five to six elements. Drawings show that tibial stroke is produced at the very beginning of the C element, and the complex leg-movement pattern of C element is produced after the tibiae come to their normal position.

Table 1 Mean and standard deviations (in brackets) of seven courtship song characters and number of stridulatory pegs in *Chorthippus albomarginatus* from Germany (alb G) and Russia (alb R), *Ch. oschei* from Bulgaria (osch B) and Ukraine (osch U), F_1 and F_2 hybrids (female × male), and backcross (BC) hybrid generations.

Species/cross	*n*	Number of A/B pairs	B/A amplitude	Duration of element A, s	Duration of element B, s	Duration of element B1, s	Duration of element A1, s	Duration of element C, s	Peg number
alb G	20	5 (1)	0.14 (0.05)	0.47 (0.08)	0.66 (0.11)	0.77 (0.09)	0.48 (0.07)	1.18 (0.13)	107.1 (6.3)
osch B	11	26.4 (9.4)	3.30 (1.47)	0.36 (0.04)	0.32 (0.04)	1.80 (0.31)	0.17 (0.03)	1.45 (0.31)	186.3 (13.6)
F_1 (a × o) GB	13	2.5 (1.9)	1.23 (0.47)	0.54 (0.31)	1.17 (0.74)	4.10 (1.79)	0.40 (0.08)	1.17 (0.13)	142.1 (14.9)
F_1 (o × a) GB	14	3.6 (2.8)	1.38 (0.60)	0.66 (0.17)	1.08 (0.52)	3.57 (1.90)	0.36 (0.07)	1.18 (0.15)	147.2 (10.8)
F_2 (a × o) GB	16	3.1 (1.9)	0.94 (0.44)	0.53 (0.24)	1.05 (0.97)	2.71 (1.45)	0.33 (0.08)	1.04 (0.22)	143.1 (16.5)
F_2 (o × a) GB	4	2.0 (0.8)	1.19 (0.66)	0.85 (0.19)	1.40 (0.58)	2.45 (0.66)	0.41 (0.09)	0.95 (0.06)	139.0 (8.1)
BC alb GB	7	6.1 (4.1)	0.41 (0.26)	0.41 (0.09)	0.77 (0.16)	1.32 (1.20)	0.35 (0.04)	1.19 (0.22)	119.2 (6.3)
BC osch GB	9	9.4 (5.4)	3.0 (1.2)	0.37 (0.08)	0.35 (0.12)	1.81 (0.31)	0.23 (0.04)	1.18 (0.19)	166.8 (11.3)
alb R	13	8.2 (3.4)	0.28 (0.08)	0.46 (0.08)	0.53 (0.09)	0.75 (0.15)	0.54 (0.09)	1.21 (0.20)	110.5 (9.6)
osch U	16	19.7 (6.9)	3.28 (1.68)	0.34 (0.07)	0.33 (0.10)	2.00 (0.52)	0.18 (0.04)	1.27 (0.17)	171.9 (12.5)
F_1 (a × o) RU	19	3.2 (2)	0.83 (0.88)	0.54 (0.15)	0.90 (0.58)	2.30 (1.00)	0.45 (0.10)	1.20 (0.18)	138.4 (14.6)
F_1 (o × a) RU	18	4.8 (4.2)	0.98 (0.69)	0.61 (0.27)	0.87 (0.31)	2.42 (0.99)	0.45 (0.12)	1.13 (0.17)	148.0 (15.9)
F_2 (a × o) RU	10	4.2 (2.4)	1.32 (0.89)	0.56 (0.15)	0.84 (0.39)	1.64 (0.66)	0.45 (0.10)	1.26 (0.21)	140.6 (18.0)
F_2 (o × a) RU	6	7.5 (8.8)	2.01 (0.94)	0.77 (0.29)	0.83 (0.54)	2.32 (1.25)	0.42 (0.11)	1.27 (0.23)	131.2 (9.0)

n, the number of males studied.

Table 2 Average coefficient of variation (CV) calculated for seven courtship song characters and number of stridulatory pegs in *Chorthippus albomarginatus* from Germany (alb G) and Russia (alb R), *Ch. oschei* from Bulgaria (osch B) and Ukraine (osch U), and F_1, F_2 and backcross (BC) hybrid generations.

		Number of A/B pairs					Duration of element B1				
Species/cross	*n*	Among	Within	B/A amplitude ratio	Duration of element A	Duration of element B	Among	Within	Duration of element A1	Duration of element C	Peg number
alb G	20	20.6	7.2	35.7	17.0	16.7	11.7	3.9	14.6	11.0	5.9
osch B	11	35.4	9.0	44.5	11.1	18.8	17.2	6.1	17.6	21.4	7.3
F_1 GB	27	79.8	53.8	41.4	41.1	56.6	48.3	21.6	19.8	12.0	8.9
F_2 GB	20	62.6	58.3	49.7	38.9	80.9	50.0	24.8	23.7	19.4	10.7
BC alb GB	7	67.4	20.9	63.4	22.0	20.8	90.9	6.4	11.4	18.5	5.3
BC osch GB	9	56.8	22.9	40.3	21.6	34.3	17.1	4.9	17.4	16.1	6.8
alb R	13	41.2	12.5	30.0	17.4	17.0	20.0	4.5	17.4	16.5	8.7
osch U	16	34.9	6.9	51.4	21.4	30.1	25.9	5.9	25.1	13.1	7.3
F_1 RU	37	81.6	37.8	88.0	37.8	52.6	42.2	16.0	25.0	15.1	10.6
F_2 RU	16	104.8	65.7	57.6	33.9	53.9	48.8	21.0	23.6	17.5	11.2

For two parameters, both among- and within-individual variations are shown, for all others only among-individual variation is presented. *n*, refers to the number of males studied.

the A1/C pairs were repeated two-three times one after another, and only short B syllables followed in between (Fig. 2c). Sound intensity of the A elements was highly variable in most hybrid songs. Each cycle started with relatively quiet A syllables but the sound intensity increased by the end of the cycle. In most cases, the transition between quiet and loud A elements was very abrupt. Thus, all A syllables were quiet and only the last A (=A1) syllable preceding the C element was loud (Fig. 2c). The last A syllable differed in the frequency of the leg movements; it was about 70 Hz (Fig. 5), whereas other A elements showed the peak at 60 Hz. Thus, the B1 and A1 syllables in the hybrid song resembled the B1/A1 pair in the *oschei*-song.

Among GB hybrid songs, there were no parental-like phenotypes. Among RU hybrids, however, five males (two specimens from alb × osch crosses and three specimens from the reciprocal crosses) sang the song identical to the *Ch. albomarginatus* parent.

Some F_1 hybrid songs had features not found in the parental songs. One such feature has already been mentioned: the repeat of the B-A-C complex, several times, without alternation of A/B pairs in between. Another feature was a specific leg-movement pattern producing the C element (Fig. 5). This pattern was relatively simple and the spectrum of the leg vibration showed no harmonics in contrast to the parental species, both of which demonstrated a very complex leg-movement

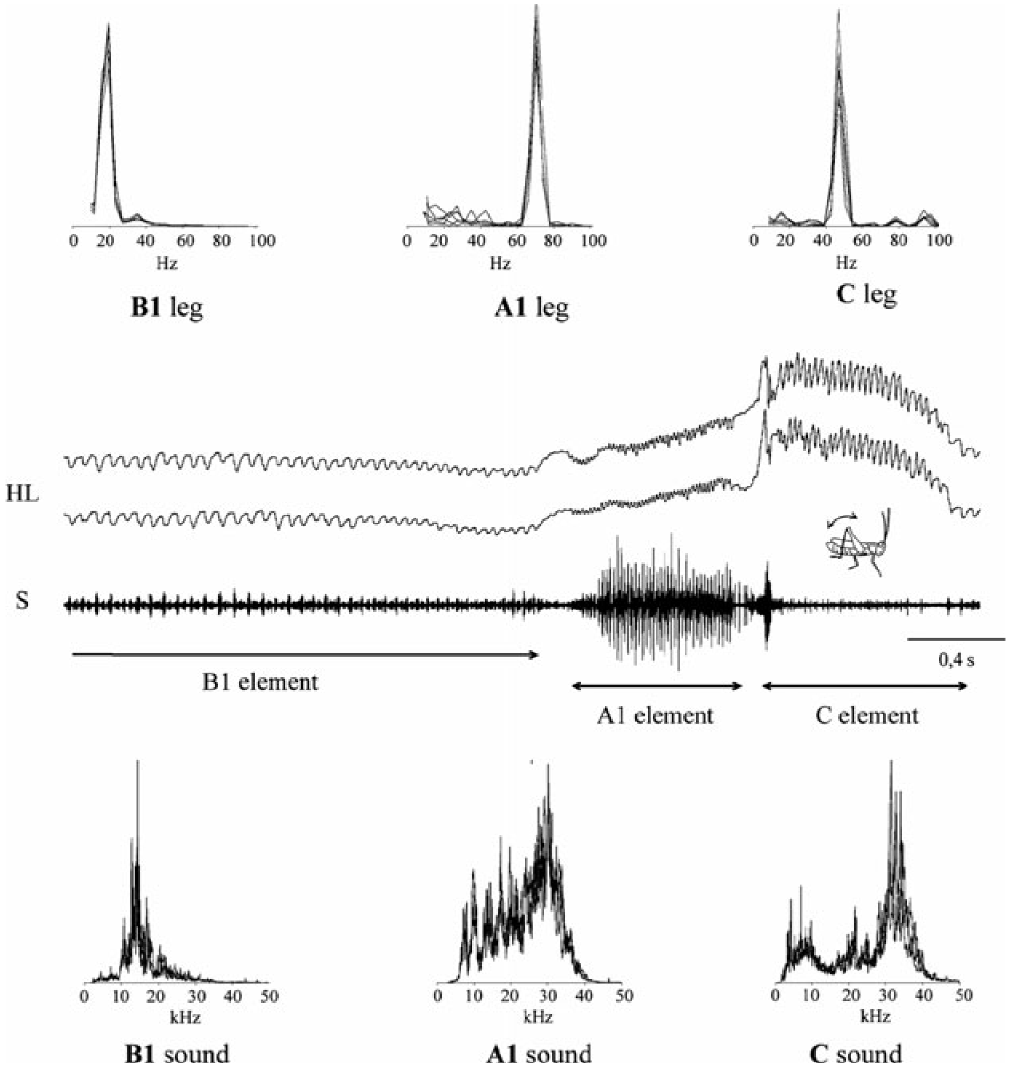


Fig. 5 Courtship song (hind leg movements HL and sound S) of F_1 hybrid between *Chorthippus albomarginatus* and *Ch. oschei* and frequency spectra of B1, A1 and C song elements. Upper panel shows spectra of movements of one leg and lower panel shows sound spectra of the corresponding song parts (their length marked). Vertical scales of spectra are linear. Each spectrum was obtained from five to six elements. Drawings show that during C element, the hind legs are moved with incompletely straightened tibiae, and tibiae come to their normal position only at the end of C element.

pattern of the C element (Figs 3 and 4). Some novelty in the hybrid songs was also evident from the analysis of the different temporal song parameters. Only two parameters, the relative amplitude of B/A elements and the duration of A1 element, had intermediate values between parental characteristics (Fig. 6, Table 1). Other parameters had extreme values that exceeded one or another parental song feature. The number of A/B pairs between C elements was significantly lower in the hybrids than in the *Ch. albomarginatus* parent, the parent with a lower value of this parameter (Figs 1 and 6). The duration of element B1 was longer in the hybrids (in GB hybrids – significantly longer) than in the *Ch. oschei* parent, the parent with a larger value of this parameter (Figs 5 and 6).

In the F_1 hybrid songs, both within- and among-individual variation in the four characters (the number of A/B pairs, the durations of A, B and B1 elements) was much higher than the variation in the parental songs (Fig. 6). The number of A/B pairs showed three to seven times greater within-individual variability and two to three times greater among-individual variation in F_1 progeny than in parentals. The duration of B1 element showed three and two to three times greater within- and among-individual variation, respectively, in F_1 hybrids when compared with parentals (Table 2). The results of a

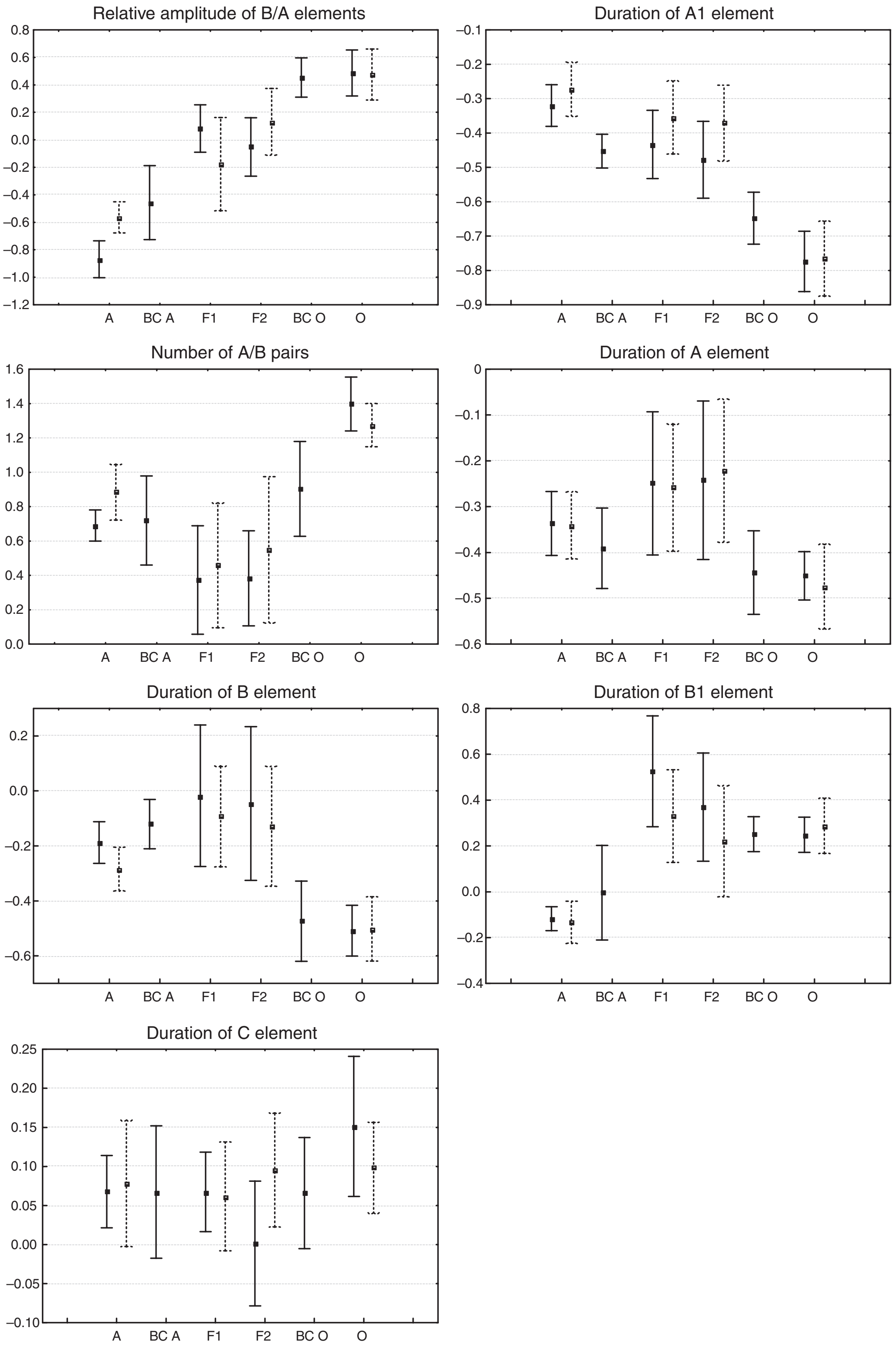


Fig. 6 Mean and standard deviations (all measurements log 10 transformed) of seven courtship song parameters in *Chorthippus albomarginatus* (A), *Ch. oschei* (O), F_1 and F_2 hybrids, and backcrosses (BCA, BCO). Crosses obtained from German and Bulgarian parental populations are shown by solid lines, those from Russian and Ukrainian parental populations are shown by dashed lines.

PCA using all seven song features evidence that the F_1 hybrids had higher variability than the parental species (Fig. 7).

The differences in all song parameters between reciprocal F_1 hybrids were insignificant (Mann–Whitney test, $P = 0.18–0.88$). Therefore, the data on reciprocal crosses were taken together in the further analysis.

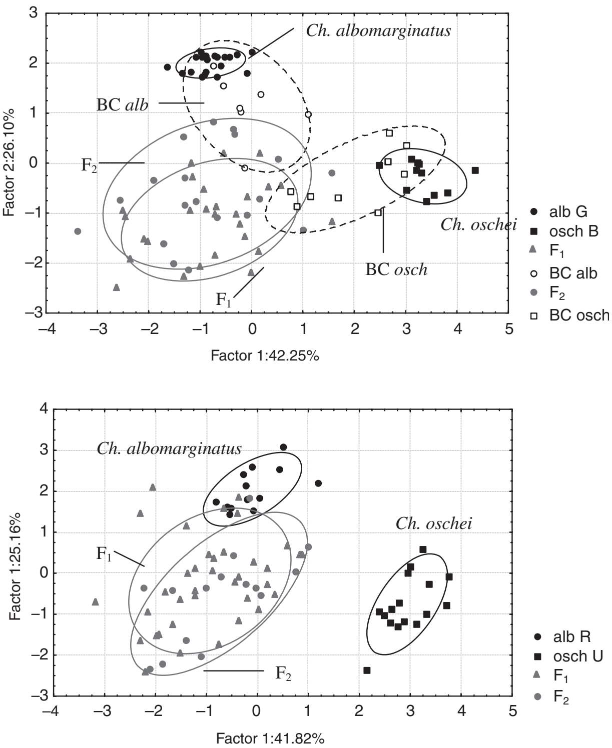
Songs of F_2 hybrids

Most of F_2 hybrids produced courtship songs resembling those of F_1 hybrids, both in the population means and in the degree of variability (Tables 1 and 2, Fig. 6). Among F_2 progeny of GB hybrids, we found a wider range of the song phenotypes as compared to F_1 hybrids. Several F_2 hybrids sang more similarly to *Ch. albomarginatus*. There were significant differences between F_1 and F_2 progenies of GB hybrids in three song parameters, B/A amplitude, B1 duration and C period (*t*-test, $P < 0,05$). For RU hybrids, only one parameter, B/A amplitude, was found to considerably differ in F_1 and F_2 generations. Among-individual variation of most parameters was only slightly higher in F_2 than in F_1 progeny of GB hybrids; for the number of A/B pairs and the A element duration, the CV were even lower in F_2 than in F_1. In RU hybrids, the CV for the B/A amplitude and the durations of A and A1 elements were lower in F_2 than in F_1 (Table 2).

In Fig. 7, the Factor 1 scores of the F_1 and F_2 hybrids overlapped with the *Ch. albomarginatus* scores, whereas the Factor 2 scores of the hybrids overlapped with the *Ch. oschei* scores. For the parameters measured, the contribution of different song parameters to the first principal component was about twice as large as to the second principal component. The high value of Factor 1 indicated a dominant influence of the *Ch. albomarginatus* parent on the hybrid song characteristics.

Songs of backcrosses

The majority of song features of the backcross hybrids resembled those of the backcross parent (Fig. 1b,d). In the *Ch. albomarginatus* backcross progeny, only two song parameters, B/A amplitude and A1 duration, differed significantly from the *Ch. albomarginatus* parent (Mann–Whitney, $P < 0.05$). In the *Ch. oschei* backcross progeny, three song parameters (number of A/B pairs, C period and A1 duration) significantly differed from the *Ch. oschei* parent. The parameters that were found to be different had intermediate values of means between hybrid and backcross parents. Variability in the number of A/B pairs, and in the duration of A and B elements among backcrosses was also intermediate between hybrid and backcross parental variations. However, the CV for the B/A amplitude and B1 duration were very high (63.4 % and 90.9 % correspondingly) in the *Ch. albomarginatus* backcrosses (Table 2).



Song parameter	GB Factor1	GB Factor2	RU Factor1	RU Factor2
Number of A/B pairs	0.82	0.35	0.83	0.30
B/A amplitude ratio	0.57	–0.74	–0.51	–0.71
Duration of A element	–0.66	–0.31	–0.79	–0.30
Duration of B element	–0.82	–0.24	–0.79	–0.08
Duration of B1 element	–0.06	–0.93	–0.17	–0.89
Duration of A1 element	–0.81	–0.36	–0.83	0.37
Duration of C element	0.42	–0.06	0.11	–0.38

Fig. 7 The values of the first and second factors of a principal component analysis (PCA) using seven song parameters in (a) German *Chorthippus albomarginatus*, Bulgarian *Ch. oschei*, and their hybrids, F_1, F_2 and backcross (BC) hybrid generations, and in (b) Russian *Ch. albomarginatus* and Ukrainian *Ch. oschei* and their F_1 and F_2 hybrids, and (c) loadings of different parameters in the two PCA factors.

Courtship visual display in hybrids

All males of *Ch. oschei* demonstrated a characteristic stroke with their hind tibiae in the beginning of C element; the average of the estimated angles between femur and tibia was 112° (Table 3). In contrast, no F_1 hybrid demonstrated any stroke with their hind tibiae, consistent with the *Ch. albomarginatus* parent. The same results were obtained for the *Ch. albomarginatus* backcrosses. Among F_2 hybrids, one fourth of all males

Table 3 Courtship visual display in *Chorthippus albomarginatus* from Germany (alb G), *Ch. oschei* from Bulgaria (osch B), and F1, F2, and backcross (BC) hybrid generations.

Species/cross	*n*	No. (%) of individuals demonstrating tibiae stroke	Maximal angle between femur and tibia, mean ± SD (grad)
alb G	20	0 (0)	–
osch B	11	11 (100)	112 ± 9
F1	27	0 (0)	–
F2	20	5 (25)	11 ± 6
BC alb	7	0 (0)	–
BC osch	9	5 (55)	45 ± 20

recorded demonstrated the stroke. The stroke was, however, weak: the mean maximal angle between femur and tibia was 11°. In the *Ch. oschei* backcrosses, about half of progeny demonstrated the stroke, and the mean maximal angle between femora and tibia was 45° (Table 3).

There was a characteristic feature of the hybrid visual display not found in the *Ch. oschei* parents. In *Ch. oschei*, the tibial stroke was produced in the beginning of C element, then the tibiae came quickly to their normal position, and the femora with the pressed tibiae vibrated alternately (Fig. 4). When a hybrid male produced the tibial stroke, the tibiae came to their normal position only in the very end of C element. Thus, the legs with the slightly opened tibiae were moved alternately from the beginning to the end of C element (Fig. 5). Such conspicuous movements were found in natural hybrids as well (Vedenina & Helversen, 2006).

Morphology of the stridulatory file

The parental species were found to differ significantly in the number of the stridulatory pegs situated on the inner side of the hind femora (Fig. 8). In *Ch. albomargintus* from both parental populations the peg number varied in a range of 100–120, in *Ch. oschei* from two parental populations this number varied from 160 to 200 (Table 1). The *albomarginatus*- populations did not differ significantly from each other, whereas the difference between the *oschei*- populations was significant ($t = 2.32$, $P < 0.05$). In F_1 hybrids, the peg number was intermediate between the parentals. The difference in peg numbers between reciprocal F_1 hybrids was not significant (Mann–Whitney, $P = 0.07–0.16$), and F_1 hybrids did not differ from F_2 hybrids. In backcross hybrids, the mean peg number was intermediate between hybrid and backcross parents (Fig. 8b, Table 1).

We found that in *Ch. oschei*, the peg density on the proximal and middle thirds of the stridulatory file was much higher than in *Ch. albomarginatus* (Fig. 8a), whereas on the distal third, the peg density was similar in the two species. We suggest that the very proximal part of the keel will be used during generation of the C element, when the femora come into an extra-high position, the middle part of the keel will be used during generation of the A

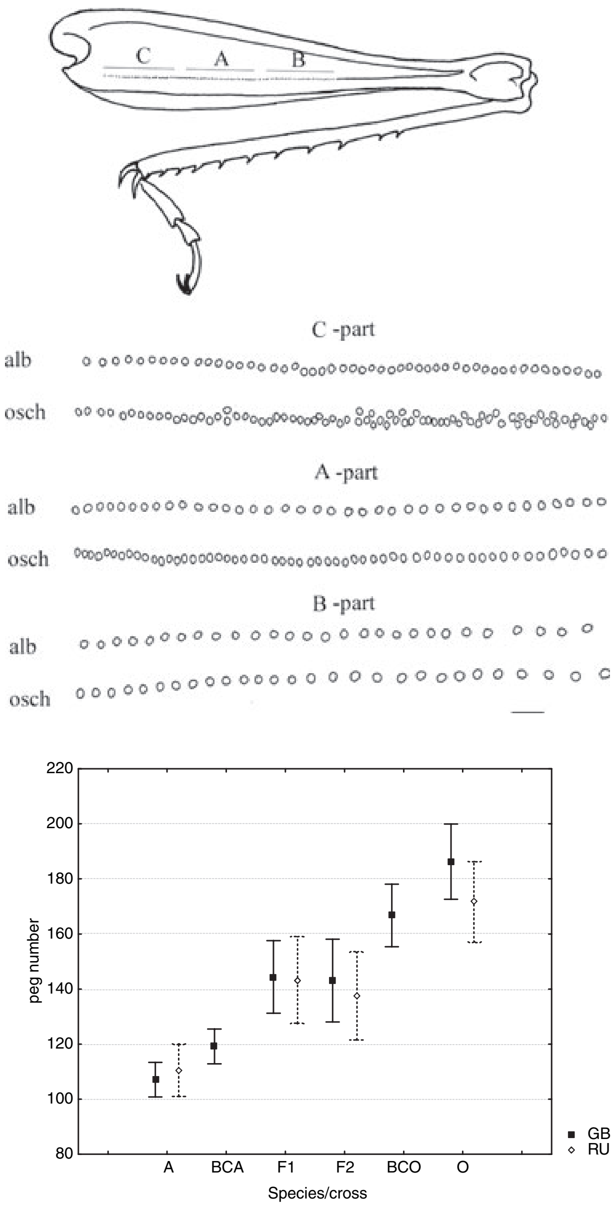


Fig. 8 (a) Stridulatory pegs situated on the inner side of hind femur in *Chorthippus albomarginatus* (alb) and *Ch. oschei* (osch). A proximal third of stridulatory file (C-part) is supposed to be used in generation of the C-element of the courtship song, a middle third (A-part) – in generation of the A element, a distal third (B-part) – in generation of the B element. Scale 100 μm. (b) Mean and standard deviations of stridulatory peg number in *Ch. albomarginatus* (A), *Ch. oschei* (O), F_1 and F_2 hybrids, and backcrosses (BCA, BCO). Crosses obtained from German and Bulgarian parental populations are shown by solid lines, those from Russian and Ukrainian parental populations are shown by dashed lines.

Table 4 Joint scaling test estimates for mean, additive and dominant interaction components (standard error) of the generation means for six courtship song characters and peg number in *Chorthippus albomarginatus* and *Ch. oschei*.

	Character						
	Number of A/B pairs	B/A amplitude ratio	Duration of element A	Duration of element B	Duration of element B1	Duration of element A1	Peg number
Midparent	0.71 (0.16)	−0.05 (0.1)	−0.32 (0.08)	−0.18 (0.13)	0.30 (0.12)	−0.49 (0.06)	145.67 (7.02)
Additive	0.35 (0.09)*	0.68 (0.11)*	−0.06 (0.04)	−0.16 (0.06)*	0.18 (0.05)*	−0.23 (0.05)*	39.57 (6.52)*
Dominant	−0.34 (0.16)*	0.14 (0.068)*	0.07 (0.075)	0.17 (0.125)	0.23 (0.119)*	0.06 (0.042)	−0.97 (5.29)
Scaling tests							
A test	0.375 (0.615)	−0.128 (0.581)	−0.195 (0.245)	−0.036 (0.323)	−0.416 (0.481)	−0.153 (0.152)	−13.5 (17.3)
B test	0.036 (0.654)	0.337 (0.374)	−0.188 (0.246)	−0.421 (0.40)	−0.271 (0.296)	−0.089 (0.20)	2.7 (27.1)
C test	−1.219 (1.26)	−0.001 (0.96)	0.265 (0.745)	0.537 (1.26)	0.295 (1.06)	0.048 (0.50)	−13.49 (64.9)

All characters except peg number were $\log_{10}$ transformed before analysis.
*Significant sources of variance, $P < 0.05$.
A, B and C tests are presented for the adequacy of the additive-dominant model.

element, and the distal part – during generation of the B element (Figs 1 and 3). The difference in peg density of the two species could influence some features of the C and A elements. The analysis conducted in the current study did not allow us to distinguish if the differences in temporal structure of the C syllables resulted from the leg-movement pattern or from the stridulatory peg morphology. In contrast, the differences in the relative amplitude of the A and B elements may result from the differences in the peg morphology in the middle part of the keel.

Genetic analysis

We applied the biometrical analyses only to the GB crosses, as both parental populations used for these crosses were allopatric. Table 4 presents estimates of the additive and dominance components and tests of an additive-dominance model (after Mather & Jinks, 1982), which fitted the data well. For almost all song parameters and peg number, the additive component was found to be significant, whereas dominant effect was significant for the three song parameters, the number of A/B pairs, B/A amplitude and duration of B1 element.

For this biometrical analysis, we did not use one song parameter, the duration of the C element, because of the small difference between the parental means. The test for the adequacy of the model implying nonallelic interactions did not reveal these interactions for any trait. All epistatic interactions tested (homozygote–homozygote, homozygote–heterozygote, heterozygote–heterozygote) were not significant and we do not present the data.

Nonadequacy of additive + dominant + epistatic model for the song parameters is a surprising result. For those parameters that had extreme values in F_1 hybrids (Fig. 6) one can suggest strong nonallelic interactions. For these parameters, the Castle–Wright estimates of the number of effective factors are not meaningful. The results obtained from the Castle–Wright and Zeng estimators are only presented for the two song parameters and the peg number, the characters that showed intermediate F_1 values between the parentals (Table 5). The results suggest relatively high number of effective factors for both song parameters, B/A amplitude (Ne = 10–19) and duration of the A1 element (Ne = 5–9), and for peg number (Ne = 8–14).

Table 5 Effective number of factors (±SE) underlying the difference in two courtship song characters and peg number between *Ch. albomarginatus* and *Ch. oschei*.

Character	Castle-Wright Ne ± SE	Zeng, $z = 1.57$ Ne ± SE	Zeng, $z = 2$ Ne ± SE
B/A amplitude	10.78 ± 8.31	14.97 ± 2.61	19.18 ± 2.62
Duration of A1 element	5.44 ± 4.98	7.27 ± 2.82	9.18 ± 2.87
Peg number	8.22 ± 6.32	11.28 ± 3.07	14.89 ± 3.09

All characters except peg number were $\log_{10}$ transformed before analysis.
z, function of variation of allelic effect differences.

Discussion

Pattern of inheritance of the courtship song

Our data demonstrated an unusual pattern of inheritance of the courtship song differences in *Ch. albomarginatus* and *Ch. oschei*. Of seven song parameters, four traits had exaggerated values in F_1 hybrids compared with the parentals, i.e. the magnitude of the hybrid trait was significantly larger than that in the parent with the largest value, or significantly smaller than that in the parent with the smaller value. The extreme mean values for the same parameters were also found in F_2 hybrids. As a whole, F_1 hybrid songs demonstrated significantly higher among- and within-individual variability than the parental songs. The variability of most song parameters in the F_2 hybrids did not exceed significantly the variability in the F_1 hybrid progeny.

According to Templeton (1981), three types of genetic architecture of traits involved in reproductive isolation can be distinguished. Type I involves many genes of small effect, type II – one or a few major genes with epistatic modifiers, type III – complementary or duplicate pairs of loci. Based on the different biometrical methods used, different authors suggested either type-I (Shaw, 1996; Ritchie, 2000) or type-II (Butlin, 1996; Henry *et al.*, 2002) of genetic architecture. The genetic analysis conducted in the current study gave some contradictory results. The joint-scaling test revealed a simple additive-dominance model for all song parameters. However, the distribution of the four song characters shown on the graphs (Fig. 6) is difficult to explain by simple additive and dominance effects. Such distribution may be only explained by more elaborate interactions between non-allelic loci. Nonsignificance of elaborate interactions could result from the high variances of the hybrid song parameters.

High song variability in the F_1 generation allows us to suggest that the inheritance of the song parameters in *Ch. albomarginatus* and *Ch. oschei* may be consistent with the model III genetic architecture. We know from several studies that the acridid grasshoppers possess extremely large genomes (i.e. Rees *et al.*, 1978; Westerman *et al.*, 1987). It is supposed that the duplications or/and invasion of transposable elements happen in different organisms with similar rate, but in comparison with the species with relatively small genomes, like *Drosophila*, nonessential DNA is removed much more slowly from genomes of studied representatives of Orthoptera (Hartl, 2000; Petrov *et al.*, 2000). In the grasshoppers of the *Ch. biguttulus*-group, the sequence polymorphism shown for almost all genes studied so far may be explained by the presence of multiple slightly divergent loci (J. Ustinova, personal communication). From the current study, we speculate that the homologous elements in the songs of *Ch. albomarginatus* and *Ch. oschei* could be controlled by the different copies of the duplicate loci. In the hybrids, both parental copies may be expressed and this could result in the development of a highly variable pattern.

In the study of von Helversen & von Helversen (1975), the hybrid song patterns exhibited an increased degree of variability and complexity compared with the songs of parental species *Ch. biguttulus* and *Ch. mollis* because of superposition of independent parental song elements. Based on revealed nonhomology of the parental elements, two pattern-generating neuronal networks were suggested to be formed in the CNS of the hybrids. The outputs of the two networks converge in a common final pathway, probably at the level of the motoneurons, and may lead to the superimposed pattern of the hybrid song. Although the song elements in *Ch. albomarginatus* and *Ch. oschei* were found to be homologous, we suggest similar processes in the hybrid neuronal network as was suggested for the *biguttulus/mollis*-hybrids. The homologous song elements may be generated by slightly different neuronal networks; this difference may be, for example, determined by the various synaptic connections between the same neurons. The hybrid network may be organized so that more synaptic connections than in both parental networks are formed. As a result, an extremely unbalanced neuronal programme of stridulation and, therefore, a high variability of some song parameters appears in hybrids.

We suggest that the song parameters that appeared highly variable in hybrids could be driven by sexual selection. According to our hypothesis, these parameters of high variability, like the number of A/B pairs and duration of the A, B and B1 elements, may be controlled by more divergent loci in the parental species thus indicating stronger selection. According to our estimates, two song parameters, the relative amplitude of B/A elements and A1 duration, seemed to be controlled by many genes and the divergence in these parameters might occur without selection. Comparing our results with the study on song inheritance of *Ch. brunneus* and *Ch. jacobsi* (Saldamando *et al.*, 2005), we see some similarities in the inheritance pattern of the song differences between the two pairs of species. Some traits in the songs of *Ch. brunneus* and *Ch. jacobsi* were suggested to diverge rapidly due to sexual selection, whereas one song parameter might have evolved without selection, i.e. by drift. However, contributions of epistatic effects between *Ch. brunneus* and *Ch. jacobsi* were minor, and genetic architecture underlying the song differences was supposed to be simple. It is possible that the divergence between these species is smaller than it is between *Ch. albomarginatus* and *Ch. oschei*, although in both cases two naturally hybridizing species only differ in incomplete premating barriers to gene exchange. One explanation for the greater divergence between *Ch. albomarginatus* and *Ch. oschei* could be the different strength of selection causing the calling and courtship song evolution. The evolution of male trait and female preference in the *Ch. albomarginatus*-group may be faster due to a different mating strategy, when several courting males sing around a female for two or even more hours. This may facilitate female choice and favour competition among males (Vedenina & Helversen, 2006).

Inheritance of visual display

The F_1 hybrids behaved like their *albomarginatus*-parent: they did not demonstrate any stroke with the hind tibiae. The stroke was registered in 25% of the F_2 hybrid males and in 50% of the *Ch. oschei* backcross generation. These data indicate that possibly a single-locus genetic architecture underlies the absence or presence of the tibiae stroke in the two species, with the full dominance of *Ch. albomarginatus*. On the other hand, the strength of the stroke measured as the maximal angle between femur and tibia is suggested to have a more complex pattern of inheritance. In the current study, we did not precisely measure the duration of the stroke, or the time from its beginning to the moment when the tibiae were pressed to the femora. As this time was much longer in the hybrids than in the *Ch. oschei* parents whereas in the *Ch. albomarginatus* parent the stroke was completely absent, such character fell outside the parental range. Similarly to several song characters analysed above, this character of visual display cannot be interpreted by simple genetic models.

Inheritance of the stridulatory peg number

Our genetic analysis suggested a simple additive polygenic inheritance of the stridulatory peg number in *Ch. albomarginatus* and *Ch. oschei*. Therefore, one may consider this morphological parameter not to be under sexual selection. It is interesting that a much lower number of factors underlying the differences between *Ch. brunneus* and *Ch. jacobsi* in peg number was revealed (Saldamando *et al.*, 2005). The authors, however, expected to find polygenic inheritance of this parameter, as this feature was not related to the song characteristics and assumed to be a neutral character. We argue against a complete neutrality of this parameter. First, one cannot exclude the influence of peg morphology on the song characters in the *Ch. albomarginatus*-group, especially on such parameter as B/A amplitude, but this requires more investigations. Secondly, a proprioceptive function of the stridulatory pegs (Hustert *et al.*, 1999) may also promote the divergence between species. Thus, a selection pressure stemming from the high leg position during the stroke could work on the peg density in the proximal part of the stridulatory keel.

What can genetic analysis of the hybrid songs tell us about the evolution of the *Ch. albomarginatus*-group?

Our genetic analysis indicated a disproportionate influence of the *Ch. albomarginatus* parent on most hybrid song characteristics and visual display. The dominance of *Ch. albomarginatus* was found to be even stronger in RU hybrids than in GB hybrids (Fig. 7). The Ukrainian locality of *Ch. oschei* is on the border of the hybrid zone. We may expect the presence of *albomarginatus*-genes in the genomes of the Ukrainian *Ch. oschei*, and therefore, some F_1 hybrids could possess more *albomarginatus*- than *oschei*-genes. For this reason we did not use the data on RU crosses for the genetic analysis. In our previous paper describing the natural hybrid zone between *Ch. albomargiatus* and *Ch. oschei*, we discussed the preliminary results of the hybridization experiments (Vedenina & Helversen, 2006). However, both parental populations used were taken from the localities adjacent to the hybrid zone and, therefore, we cannot compare the crosses obtained with our present results.

In the case of the GB hybrids, both parental populations were allopatric and situated far away from the hybrid zone. A dominance of *Ch. albomarginatus* that was clearly expressed in the songs of GB hybrids could suggest a more ancient origin of this species. Ancestral features are more likely to be dominant whereas at the first steps of speciation, mutations that cause appearance of novel features are more likely to be recessive. The suggestion about a more ancient origin of *Ch. albomarginatus* is supported by the fact that the courtship song of *Ch. oschei* is regarded to be more elaborate than the *Ch. albomarginatus* song, as the *Ch. oschei* song possesses more different temporal elements and the C element is accompanied by a conspicuous tibial stroke. Evolution of a more complex *oschei* song from a simpler *albomarginatus* song (or from some ancestor similar to *Ch. albomarginatus*) was very likely a result of sexual selection, taking into account the mating strategy in this grasshopper group, when courting continues for hours in a group of males and females similar to a ‘lek’. The evolution of female preferences driving males to extremely elaborate behavioural patterns has been discussed by von Helversen & von Helversen (1994). According to the idea of ‘runaway process’ (Fisher, 1958; Andersson, 1994), females may prefer males with a more extravagant courtship, e.g. those males that demonstrate a stroke with the hind legs in addition to the song pattern, or a more complex sound pattern accompanying the stroke. The sons of such females are likely to inherit the attractive features and may find more females than other males. According to the ‘good genes’ hypothesis (Zahavi, 1987; Maynard Smith, 1991), the females testing ‘the quality’ of a male must prefer the most costly features of the signal. In *Ch. oschei*, the B1 element is extremely loud. Production of a loud sound and a stroke with the hind legs probably requires much energy from a singing male and thus may be used by a female as an indicator of vigour.

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