



A narrow hybrid zone between the grasshoppers *Stenobothrus clavatus* and *Stenobothrus rubicundus* (Orthoptera: Gomphocerinae): female preferences for courtship songs

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Two grasshopper species, *Stenobothrus rubicundus* and *Stenobothrus clavatus*, were previously shown to hybridize in a narrow contact zone on Mount Tomaros in northern Greece. The species are characterized by complex and completely different courtship songs. In the present study, we investigated female preferences for the courtship songs of *S. rubicundus*, *S. clavatus* and hybrids in playback experiments. Playback of the courtship songs revealed assortative preferences in females of the parental species: they significantly more often preferred the songs of conspecific males. Hybrid females showed a lower selectivity than parental females, responding somewhat equally eager to playback of the songs of *S. clavatus*, *S. rubicundus*, and natural hybrid song, although less actively to the F₁ hybrid song. The results suggest that hybrid males may lose to males of parental species, whereas hybrid females would even have an advantage over parental females. Comparison of responses of females from allopatric populations and Mount Tomaros to different song types shows no evidence for reinforcement. Asymmetry found in female preferences may have implications for the structure of the hybrid zone. © 2013 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2013, 108, 834–843.

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INTRODUCTION

Hybrid zones provide model systems to examine partial barriers to gene flow, including the types of selection imposed by female preferences, when differentiated populations meet and produce hybrids (Barton & Hewitt, 1989). The maintenance and continued evolution of behavioural isolation is a crucial part of speciation. Behavioural contributions may not only be to premating isolation, but also to post-zygotic

isolation as a result of the reduced mating success of hybrid males. Hybrid males may suffer reduced fitness because their mating signals are intermediate between the parental signals and therefore unattractive to either of the parental females. Such hybrid unfitness that is also called 'behavioural hybrid dysfunction' (BHD) (Servedio & Noor, 2003; Bridle *et al.*, 2006) is usually environment dependent (i.e. it is most severe when hybrid females that may prefer intermediate mating signals are rare) (Coyne & Orr, 2004).

There are several examples demonstrating that intermediate signals may cause a reduced hybrid fitness. In the wolf spider species of the genus *Schizocosa*, male F₁ hybrids show intermediate courtship

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behaviour that is rejected by all females, including F_1 hybrids (Stratton & Uetz, 1986). Male advertisement call in F_1 hybrids between the green tree frogs *Hyla cinerea* and *Hyla gratiosa* differs from the parental calls and is unattractive to parental females (Höbel & Gerhardt, 2003). Finally, females of closely-related grasshopper species *Chorthippus brunneus* and *Chorthippus jacobsi*, as well as hybrid females, show reduced responsiveness to F_1 hybrid male songs (Bridle *et al.*, 2006). By contrast, females of hybrids between grasshopper species *Chorthippus albomarginatus* and *Chorthippus oschei* mate alike eagerly with hybrid males and males of one parental species, *Ch. albomarginatus* (Vedenina, Kulygina & Panyutin, 2007a). Such asymmetry in male mating success may be explained by the superiority of hybrid courtship songs that are not just intermediate but possess more features of one than of another species (Vedenina, Panyutin & von Helversen, 2007b; Vedenina, 2011).

Coyne & Orr (1989) suggested that BHD might be an important component of selection against hybrids in the context of reinforcement. Servedio & Noor (2003), however, consider that not only hybrid inviability or physiological sterility, but also behavioural hybrid unfitness makes an important contribution to reinforcement. One possible example may be *Heliconius* butterflies where sexual selection against intermediate colour patterns contributes to reproductive isolation and possibly to the evolution of greater divergence in sympatry (Jiggins *et al.*, 2001; Naisbit, Jiggins & Mallet, 2001).

Closely-related grasshopper species *Stenobothrus clavatus* and *Stenobothrus rubicundus* hybridize in a narrow contact zone on Mount Tomaros in northern Greece (Elsner, Klöpfel & Sradnick, 2009; Sradnick, 2010). They are remarkably different in several morphological characters, as well as in calling and courtship songs. The courtship songs of the two species are very complex, comprising several sound elements and accompanied by conspicuous visual display. *Stenobothrus clavatus* demonstrates characteristic movements with the club-shaped antennae when producing a particular phase of courtship, whereas *S. rubicundus* stridulates not only with the hindlegs, but also with the wings (Elsner & Wasser, 1995; Ostrowski *et al.*, 2009). Song analysis of natural and laboratory hybrids between *S. clavatus* and *S. rubicundus* has revealed that some hybrid songs have intermediate features between parental songs, whereas other hybrid songs comprise completely new elements. The *clavatus*-like song elements were found to dominate in hybrid songs (Vedenina *et al.*, 2012).

We found that the *S. clavatus* and *S. rubicundus* hybrid zone showed a mosaic distribution of song phenotypes. Mosaic hybrid zone models predict the persistence of parental populations throughout the

hybrid zone in ecologically divergent patches that are, to a great degree, relative to the mean dispersal of the organisms (Harrison, 1993). Therefore, it will be especially important to study song preferences in females of both parental species and hybrids to understand which processes are involved that allow the coexistence of differentiated genotypes in nature.

In the present study, we used playback experiments to test preferences of *S. clavatus*, *S. rubicundus* and hybrid females to courtship songs of *S. clavatus*, *S. rubicundus*, and hybrid males. First, we studied the premating isolation between the two species. Second, we compared the preferences of females from allopatric localities and from parental-like sites of the hybrid zone to test the reinforcement model. A comparison of the preferences in females of parental species and in hybrid females may also allow us to determine whether BHD contributes to reproductive isolation between these species and the maintenance of spatial structure in the hybrid zone.

MATERIAL AND METHODS

SPECIMENS

All specimens used for the behavioural experiments were collected in northern Greece during the summer seasons of 2008 and 2009. Females of allopatric *S. rubicundus* were collected on Mount Mitsikeli (20 females), Mount Valtou (eight females), Mount Pindos (four females), and Mount Vernon (three females). Females of allopatric *S. clavatus* were only collected on Mount Xerovouni (28 females) because it is the only known allopatric locality of *S. clavatus* (Ostrowski *et al.*, 2009; Vedenina *et al.*, 2012). Females collected on Mount Tomaros (where two species meet and hybridize) were divided into three groups. Females of one group were collected from areas where males produced *clavatus*-like courtship songs; females of the second group belonged to areas where males sang *rubicundus*-like songs; females of the third group were from areas where males demonstrated intermediate courtship (Vedenina *et al.*, 2012). We used 21 females from seven *clavatus*-like sites, 15 females from six *rubicundus*-like sites, and 14 females from five intermediate sites (Table 1). We also used ten laboratory-raised F_1 hybrid females obtained from the crosses of *clavatus* females with *rubicundus* males and ten females obtained from backcrosses of F_1 hybrids with *S. rubicundus*. Breeding details were previously described by Vedenina *et al.* (2012).

PLAYBACK EXPERIMENTS

For the playback experiments, we used four previously recorded courtship songs: the songs of allopatric

Table 1. List of sampling sites and the number of females from Mount Tomaros used in the behavioural experiments

Type of locality	Name of locality	Geographical coordinates	Height (m)	Number of females
<i>Clavatus</i> -like	ALM1	39°28'38,9"N; 20°47'17,8"E	1514	3
	NT1	39°32'35,2"N; 20°44'53,6"E	1776	6
	NTF	39°31'54,1"N; 20°45'28,7"E	1456	2
	ON1	39°28'55,7"N; 20°48'34,3"E	1313	1
	ON2	39°28'52,1"N; 20°48'35,1"E	1345	4
	1ST	39°28'27,7"N; 20°47'05,5"E	1368	1
	2ND	39°28'35,0"N; 20°47'09,4"E	1425	4
<i>Rubicundus</i> -like	LIC1	39°29'27,3"N; 20°46'37,3"E	1581	1
	LIN	39°29'30,3"N; 20°46'46,7"E	1555	7
	N2D	39°30'02,4"N; 20°47'37,2"E	1649	1
	N2E	39°30'06,1"N; 20°47'38,9"E	1618	1
	OH3	39°29'03,4"N; 20°48'26,2"E	1321	3
Intermediate	HYBGUT	39°29'09,2"N; 20°47'18,4"E	1833	2
	LIGIP	39°29'00,3"N; 20°46'56,4"E	1803	2
	N7	39°29'05,8"N; 20°47'01,5"E	1787	6
	ON5	39°28'50,1"N; 20°48'24,2"E	1455	3
	ON6	39°28'51,9"N; 20°48'19,5"E	1497	1

S. clavatus male, allopatric *S. rubicundus* male, a male from locality HYBGUT of Mount Tomaros ('natural hybrid'; Fig. 1), and a laboratory-raised F₁ hybrid male (Fig. 2). The ambient temperature near a singing male varied in the range 35–41 °C. We chose the most widespread types of the hybrid songs (i.e. the songs with some intermediate elements and other elements typical for *clavatus* song). The song of natural hybrid male had phase I of *clavatus* type (Fig. 1B), whereas phase I of the F₁ hybrid song contained pulses that were intermediate between pulses in corresponding phases of parental songs (Fig. 2B). The intermediate element also comprised alternation of simple pulses and complex series of short pulses, both produced by the leg movements (Figs 1C, 2C); this song part was suggested to be homologous to part II of *rubicundus* song. The F₁ hybrid songs contained few wing beats. However, the sound generated by the wing beats was relatively quiet compared to the wing-generating sound in *rubicundus* song (Fig. 2D, E). Both hybrid songs had phase III of *clavatus* type, comprising the sound generated by high-amplitude leg movements (Figs 1D, 2E). To study the importance of different phases of the courtship songs, we also played back these phases separately to females. We played back only the phases of pure courtship songs.

The four types of courtship songs, as well as different phases of pure songs, were played back to receptive virgin females (5–10 days after imaginal moult). The playback experiments were performed in an anechoic chamber. We were able to test four females

simultaneously because we used a custom-made gauze cage with four sections (each section 25 × 12 × 12 cm). Each female was placed into a separate section of this cage. The partitions between sections were made from nontransparent plastic; thus, the responding females could not see and influence each other. Females responded by rapid stridulatory movements of their hind legs, which were very soft and sometimes hard to hear. Therefore, the response of a female was visually documented by the experimenter. The cage was placed on a heating plate with a temperature varying in the range 37–38 °C. The loudspeaker (Soundcraft DT 25 P Conrad) was placed at a distance of 20 cm from the cage. The songs that were previously recorded on a computer via a data acquisition card (National Instruments) with the software LAB-VIEW7 (National Instruments) were transferred to wav-format with COOL EDIT (Syntrillium) and played back by AUDACITY (<http://audacity.sourceforge.net/>). An intensity of all the songs presented was comparable with the song intensity near a courting male. The songs were presented to each female alternately with 20-s pauses, no more than 12 times per day.

RESULTS

PREFERENCES OF FEMALES FROM ALLOPATRIC POPULATIONS

In experiments with playback of four song types, females from allopatric localities demonstrated

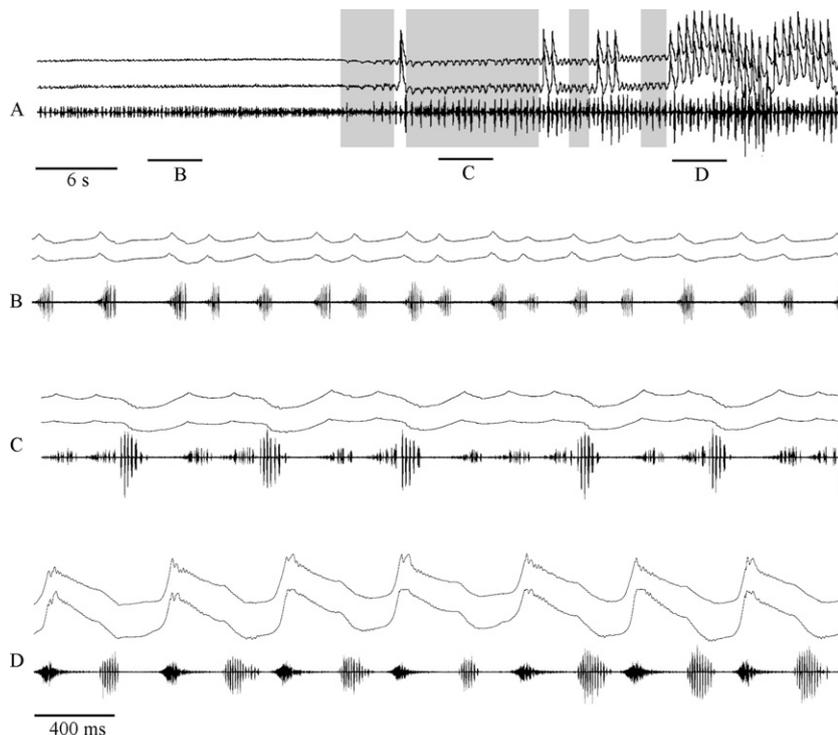


Figure 1. Oscillograms of the courtship song (the two upper lines are recordings of hind leg movements and the lower line is the sound recording) of a natural hybrid between *Stenobothrus clavatus* and *Stenobothrus rubicundus* from Mount Tomaros, locality HYBGUT. B, C, D, sections at an expanded time scale of the indicated parts of the song shown in (A). Shaded parts indicate intermediate elements.

distinct assortative preferences (Kruskal–Wallis test, $H = 47\text{--}56$, $P = 0.0001$; Fig. 3A, B). Females of *S. clavatus* from Mount Xerouvouni preferred to respond to a conspecific (= *clavatus*) song, whereas three other song types were less attractive (Mann–Whitney test, $U = 26.5\text{--}196.5$, $P < 0.001$). However, the percentage of responses to natural hybrid song was relatively high (40%). Females of *S. rubicundus* from allopatric localities also preferred to respond to a conspecific (= *rubicundus*) song. Three other song types were equally less attractive ($U = 111\text{--}682$, $P = 0.0001$).

In experiments with playback of different song phases, females of both species also showed the assortative preferences (Kruskal–Wallis test, $H = 55\text{--}92$, $P = 0.0001$; Fig. 4A, B). However, we found somewhat different results with respect to preferences between the females of two species. Females of *S. clavatus* responded with as much activity to phases I, III, and IV of conspecific song as they did to the complete courtship; however, they responded more rarely to phase II (Mann–Whitney test, $U = 97.5$, $P = 0.0009$). Responses of *S. clavatus* females to all phases of *rubicundus* song were very weak ($U = 0\text{--}4$, $P = 0.0001$; Fig. 4A). Females of *S. rubicundus* responded very actively to phase III, although much more weakly to phases I and II of conspecific court-

ship ($U = 2\text{--}12.5$; $P = 0.0001$). The attractiveness of *clavatus* song for *S. rubicundus* females varied depending on phases, although it was always lower than the attractiveness of phase III of conspecific song. Phases III and IV of *clavatus* song were more attractive than phases I and II (Fig. 4B).

PREFERENCES OF FEMALES FROM MOUNT TOMAROS

Females from *clavatus*-like localities of Mount Tomaros demonstrated assortative preferences (Kruskal–Wallis test, $H = 24$, $P = 0.0001$) similar to *S. clavatus* females from allopatric localities. In experiments with playback of four song types, they significantly more often responded to *clavatus* song, than to other song types (Mann–Whitney test, $U = 35.5\text{--}79$, $P < 0.009$; Fig. 3C). Females from *rubicundus*-like localities of Mount Tomaros also preferred to respond to conspecific song (Fig. 3D), similar to *S. rubicundus* females from allopatric sites. However, the differences between the response frequency to *rubicundus* song, *clavatus* song and natural hybrid song were not significant. A significant difference was only found between responses to *rubicundus* song and F_1 hybrid song ($U = 82.5$, $P = 0.03$). Females from intermediate localities demonstrated higher

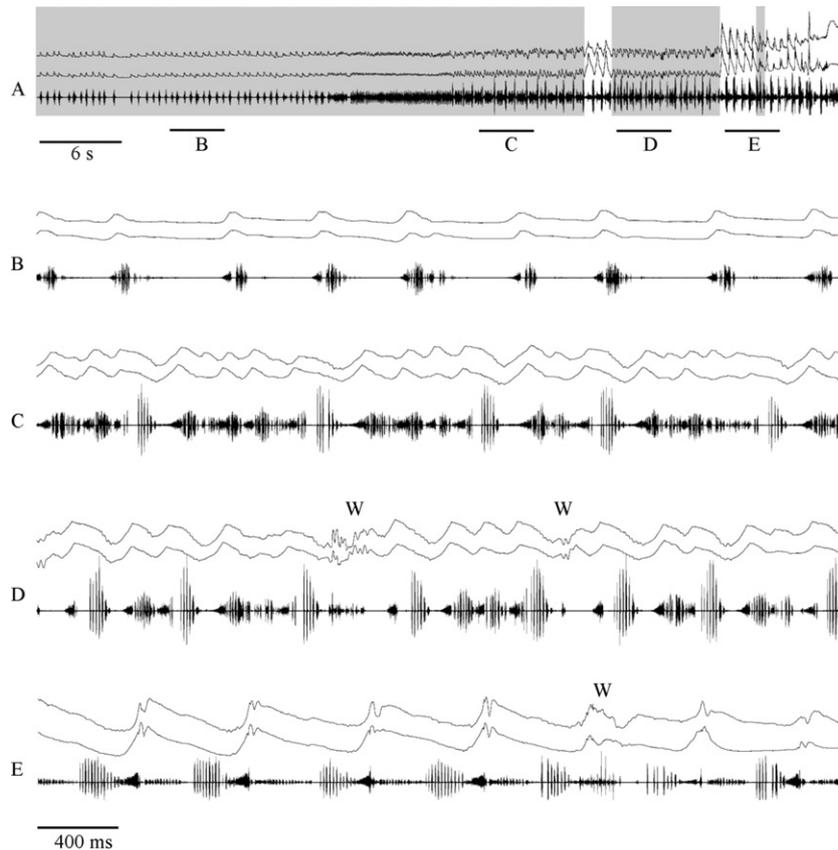


Figure 2. Oscillograms of the courtship song (the two upper lines are recordings of hind leg movements and the lower line is the sound recording) of a laboratory-raised F₁ hybrid between *Stenobothrus clavatus* and *Stenobothrus rubicundus*. B, C, D, E, sections at an expanded time scale of the indicated parts of the song shown in (A). Shaded parts indicate intermediate elements.

preferences for *clavatus* song than for the other three song types (Fig. 3E). They responded less often to *rubicundus* song (nonsignificant difference) and very weakly to both hybrid songs ($U = 30\text{--}32.5$, $P < 0.02$).

Analysis of responses of females from Mount Tomaros to different song phases showed different results depending on the localities. Females from *clavatus*-like localities demonstrated assortative preferences (Kruskal–Wallis test, $H = 54.6$, $P = 0.0001$), similar to *S. clavatus* females from allopatric localities: all phases except for phase II of *clavatus* courtship (Mann–Whitney test, $U = 53.5$, $P = 0.01$) were eagerly attractive (Fig. 4C). They responded much more weakly ($U = 8\text{--}14$, $P = 0.0001$) to all phases of *rubicundus* song. By contrast, females from *rubicundus*-like localities behaved differently from allopatric *S. rubicundus* females, responding rather actively not only to phase III of *rubicundus* song but also to phase III of *clavatus* song (Fig. 4D). All other song phases were either poorly attractive ($U = 49\text{--}53.5$, $P < 0.03$) or non-

attractive. Females from intermediate localities behaved similar to the females from *clavatus*-like sites: they actively responded to all phases of *clavatus* song except for phase II ($U = 12$, $P = 0.0003$), and showed a lower preference for the phases of *rubicundus* song ($U = 1\text{--}34.5$, $P < 0.02$).

PREFERENCES OF LABORATORY-RAISED HYBRID FEMALES

Females of F₁ hybrids did not show significant preferences for four song types (Kruskal–Wallis test, $H = 5.8$, $P = 0.12$; Fig. 3F). However, they tended to respond more actively to *clavatus* song and natural hybrid song. In experiments with playback of different song phases, they showed assortative preferences ($H = 35.9$, $P = 0.0001$) and behaved similarly to *S. clavatus* females or females from *clavatus*-like sites (Fig. 4F). All phases of *clavatus* song except for phase II were very attractive for F₁ hybrid females, whereas all phases of *rubicundus* song evoked a poor response (Mann–Whitney test, $U = 0\text{--}16.5$, $P < 0.02$).

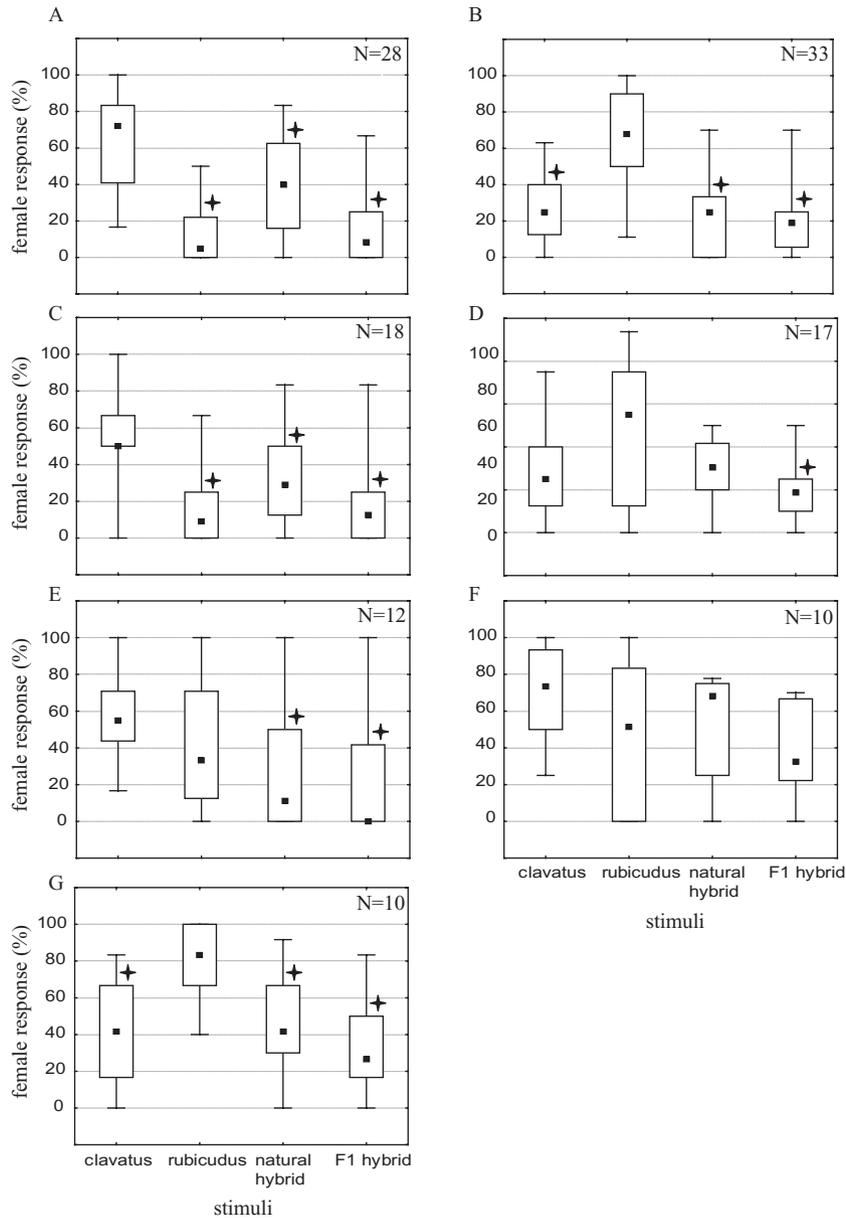


Figure 3. Responses of females (medians, quartiles and minimum/maximum) to playback of the courtship songs of *Stenobothrus clavatus*, *Stenobothrus rubicundus*, natural hybrid (from Mount Tomaros) and F₁ hybrid males. A, *S. clavatus* females from allopatric populations. B, *S. rubicundus* females from allopatric populations. C, females from *clavatus*-like sites of Mount Tomaros. D, females from *rubicundus*-like sites of Mount Tomaros. E, females from intermediate populations on Mount Tomaros. F, females of laboratory-raised F₁ hybrids between *S. clavatus* and *S. rubicundus*. G, females of backcrosses to *S. rubicundus*. Daggers indicate significant differences ($P < 0.05$) relative to the largest values. N , number of females tested.

Females of backcrosses with *S. rubicundus* showed assortative preferences (Kruskal–Wallis test, $H = 13.6$, $P = 0.004$), preferring to respond to *rubicundus* song (Fig. 3G). They responded significantly less often (Mann–Whitney test, $U = 7–16.5$, $P < 0.01$) to other song types.

DISCUSSION

PREMATING ISOLATION BETWEEN *S. CLAVATUS* AND *S. RUBICUNDUS*

In playback experiments, females from allopatric populations showed clear preferences for conspecific

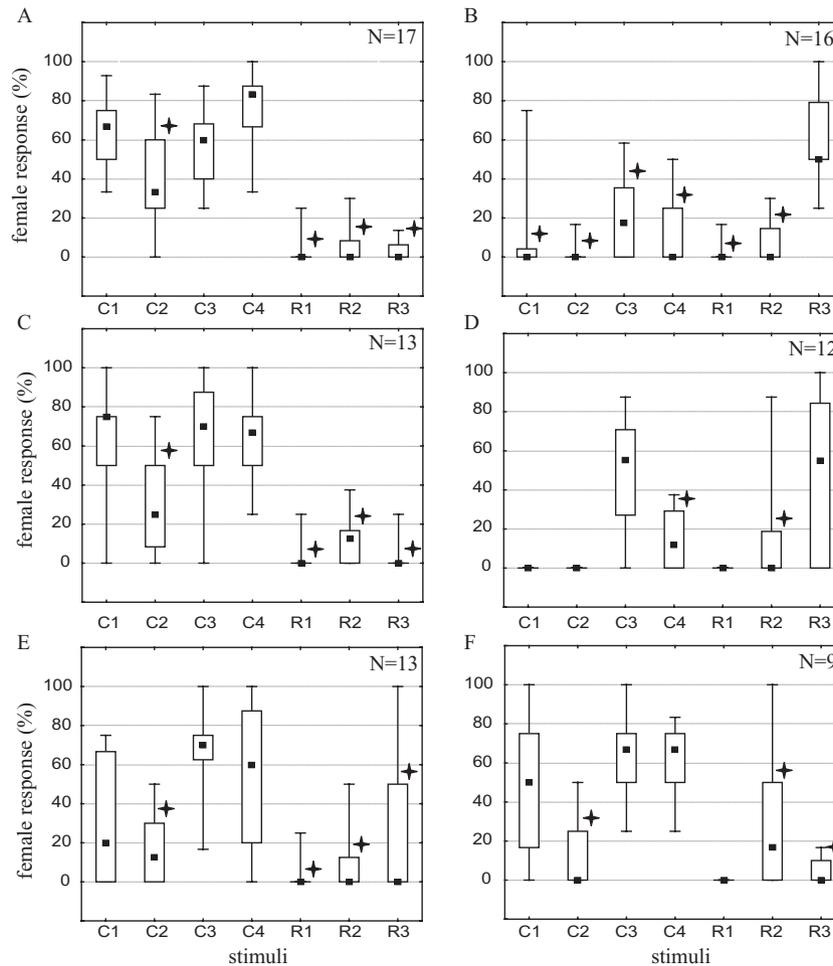


Figure 4. Responses of females (medians, quartiles and minimum/maximum) to playback of different phases of courtship songs of *Stenobothrus clavatus* (C1–C4) and *Stenobothrus rubicundus* (R1–R3). A, *S. clavatus* females from allopatric populations. B, *S. rubicundus* females from allopatric populations. C, females from *clavatus*-like sites of Mount Tomaros. D, females from *rubicundus*-like sites of Mount Tomaros. E, females from intermediate populations on Mount Tomaros. F, females of laboratory-raised F₁ hybrids between *S. clavatus* and *S. rubicundus*. Daggers indicate significant differences ($P < 0.05$) relative to the largest values for a corresponding female type shown in Fig. 3. N, number of females tested.

songs. Taking into account the quite different courtship songs produced by males of *S. clavatus* and *S. rubicundus*, this result is not unexpected. It is more surprising that females sometimes responded to heterospecific courtship. For example, *S. rubicundus* females responded to *clavatus* song in more than 20% of trials (Fig. 3B). The female response to heterospecific courtship may be explained by similarity of particular phases in the songs of two species. For example, the simple pulses alternated with complex series of short pulses during phase III of *clavatus* song and phase II of *rubicundus* song. This sound pattern is produced by different mechanisms, and probably, being nearby a male, a female would not respond to heterospecific courtship because of a completely different visual display accompanying each

song (Elsner & Wasser, 1995; Ostrowski *et al.*, 2009). However, if females are attracted by courting males at a distance, such similarity of the sound pattern in two species may confuse a female, and may promote more heterospecific contacts.

Analysis of the female responses to different courtship phases showed that the most attractive parts of courtship were phases I, III, and IV of *clavatus* song and phase III of *rubicundus* song. A high attractiveness of these parts of courtship may be explained by similarities of these parts to corresponding calling songs of the two species. The calling song of *S. clavatus* represents series of short pulses similar to phase IV (Ostrowski *et al.*, 2009). The calling song of *S. rubicundus* from Greece was not reported. According to the data obtained on *S. rubicundus* from alpine

populations (Ragge & Reynolds, 1998), the calling song may comprise all phases of the courtship song but of shorter sequences. According to our observations in Greece, solitary males more often used only wing beats generating the sound similar to part III of courtship. Wing clapping generates a very loud sound that can be perceived by females at a large distance. Females of many Gomphocerinae species are shown to respond acoustically to calling songs of conspecific males (von Helversen & von Helversen, 1975, 1983; Stumpner & von Helversen, 1992; Wirmer, Faustmann & Heinrich, 2010). Females may also respond acoustically to courtship songs; however, they may need not only acoustical, but also visual and chemical components of the courtship. Thus, we expected that, in our experiments with playback of the courtship songs, females would more eagerly respond to the calling component of the courtship. However, in the case of *S. clavatus* females, a relatively high response to other phases of conspecific courtship was found as well. We suggest that *S. clavatus* and *S. rubicundus* may have different strategies of courtship behaviour. For example, females in two subspecies of *Chorthippus parallelus*, *Chorthippus parallelus parallelus* and *Chorthippus parallelus erythropus*, rely on different channels of communication during courtship (Ritchie, 1990). The song plays a rather large role in producing assortment with *Ch. p. parallelus* females, whereas pheromones and/or tactile stimuli play a smaller role. By contrast, such a pheromonal/tactile stimulus is necessary in producing assortment with *Ch. p. erythropus* females. We suggest that females of *S. clavatus* and *S. rubicundus* may also rely on different communication channels during courtship. Females of *S. rubicundus* may appear to require visual cues (visual display represented by wing beats) in addition to the sound, whereas females of *S. clavatus* may have a reliance on sound only. However, because visual display of *S. clavatus* is also highly conspicuous, it is of interest to study the role of visual signals in the female assortment in both species.

FEMALE PREFERENCES FOR MALE SONGS SHOW NO EVIDENCE FOR REINFORCEMENT

The theory of reinforcement suggests that selection should favour divergence in mating behaviour and increase in the strength of assortative mating, as a result of the production of hybrids with reduced fitness (Liou & Price, 1994; Kelly & Noor, 1996; Noor, 1999; Jiggins *et al.*, 2001; Naisbit *et al.*, 2001). The most common test for reinforcement is to look for evidence that selection has altered female preferences in the area where the two divergent populations are in contact relative to allopatric populations (Butlin, 1998). A comparison of responses of females from

allopatric populations and from Mount Tomaros to the songs of *S. clavatus*, *S. rubicundus* and hybrids shows that the preferences for conspecific mates do not strengthen in the contact zone. Females collected from *clavatus*-like sites of Mount Tomaros preferred to respond to *clavatus* song and responded less significantly to all other song types, similar to *S. clavatus* females from allopatric populations. Females collected from *rubicundus*-like localities of Mount Tomaros showed even lower selectivity than *S. rubicundus* females from allopatric populations.

Despite many theoretical arguments for and against reinforcement, it is suggested that reinforcement can only complete the speciation process in the case of secondary contact between populations already showing very substantial barriers to gene exchange (Butlin, 1998). It is very likely that isolation barriers between *S. clavatus* and *S. rubicundus* are not sufficiently strong to be reinforced. According to the results of the present study, ethological isolation is not complete because the females respond to heterospecific songs. Post-zygotic isolation between these species appears to be rather weak: the crosses between *S. rubicundus* females and *S. clavatus* males resulted in a viable and fertile offspring, whereas the reciprocal crosses resulted in a smaller number of offspring, although these were still viable and fertile (Vedenina *et al.*, 2012). It is remarkable that, even in hybridizing species that show much stronger post-zygotic isolation, as in *Ch. p. parallelus* and *Ch. p. erythropus*, no evidence for reinforcement was found. Laboratory-raised F₁ hybrids between these subspecies were completely sterile but males collected from the hybrid zone were fertile as predicted if recombination reconstructs compatible ancestral genotypes (Butlin, 1998; Shuker *et al.*, 2005).

DO HYBRIDS BETWEEN *S. CLAVATUS* AND *S. RUBICUNDUS* SHOW BEHAVIOURAL HYBRID DYSFUNCTION?

Both parental and hybrid females showed significantly higher preferences for the songs of *S. clavatus* or *S. rubicundus* males than for the song of F₁ hybrid. By contrast, the natural hybrid song was as much attractive as the songs of the pure species for females from *rubicundus*-like sites of Mount Tomaros and F₁ hybrid females. The natural hybrid song possessed more *clavatus*-like elements and less intermediate elements than the song of the F₁ hybrid. Thus, hybrid males that produce intermediate song patterns demonstrate a reduced fitness because almost all female genotypes prefer the parental-like songs. Hybrid females, however, do not show the reduced fitness; they can even have an advantage over parental females because they do not distinguish between

the parental songs. The lower selectivity of hybrid females may lead to rapid introgression of alleles across the hybrid zone once hybridization begins. Similar results were shown by Vedenina *et al.* (2007a) on female preferences in the hybrid zone between *Ch. albomarginatus* and *Ch. oschei*. Hybrid females between these species were not selective at all, having an advantage over selective parental females. By contrast, hybrid males were likely to lose to parental males in mixed populations. Similar tendencies were also found by Bridle *et al.* (2006) in the hybrid zone between *Ch. brunneus* and *Ch. jacobsi*: selection against F₁ females appeared to be low, although selection against F₁ male song was apparently the major contributor to the reduction in hybrid fitness. Thus, in all examples, hybrid males tended to demonstrate BHD, whereas the hybrid females did not. Because the males of all Gomphocerinae species noted above have better flying capabilities than the females, we suggest that that BHD contributes to reproductive isolation between the hybridizing species to a substantial degree. At the same time, actual selection against hybrids within the hybrid zone is difficult to predict from data on behavioural hybrid fitness alone. The selection will depend on many factors, in particular, the abundances of female and male hybrids relative to parental genotypes, which may vary substantially between sites.

ASYMMETRY IN FEMALE PREFERENCES

The females obtained from backcrosses with *S. rubicundus* showed higher selectivity than females from *rubicundus*-like populations of Mount Tomaros. Backcross females significantly more often responded to *S. rubicundus* song, whereas females from *rubicundus*-like populations did not distinguish significantly between the songs of pure species and natural hybrid. This may be an indication that backcrosses to *S. rubicundus* do not comprise the major part of *rubicundus*-like populations on Mount Tomaros but, instead, occur there together with other genotypes. In addition, females from intermediate populations of Mount Tomaros favoured *S. clavatus* song over other song types. These results suggest a higher level of introgression of *rubicundus* alleles into *clavatus* genome on Mount Tomaros than vice versa. The F₁ hybrid females also preferred *S. clavatus* song over other song types, which was in concordance with an inheritance pattern of the courtship songs: *clavatus* elements were shown to dominate in the songs of laboratory hybrids (Vedenina *et al.*, 2012). We suggest that the song pattern of *S. clavatus* is more close to an ancestral pattern than the song of *S. rubicundus*. One of the main *clavatus*-like elements, the stepwise movements in the downstroke of the hind legs, is

suggested to be a plesiomorphic element, which is found in many Gomphocerinae species from different genera (Elsner, 1974; Helversen & Helversen, 1994; Elsner & Wasser, 1995; Vedenina & Mugue, 2011). Ancestral features are more likely to be dominant, whereas, at the first steps of speciation, mutations that cause the appearance of novel features are more likely to be recessive. The results of the present study on female preferences support the idea of ancestral origin of *S. clavatus* song.

Introgression of *rubicundus* alleles into *clavatus* genome on Mount Tomaros may have implications for the spatial structure of the hybrid zone. We suggest a displacement of the hybrid zone in favour of *S. clavatus*. However, because both species do not occur lower than 1300 m a.s.l., the hybrid zone can not move significantly, similar to a narrow hybrid zone between chromosomal races of the grasshopper *Podisma pedestris* in the Alpes Maritimes (Hewitt, 1975; Barton & Hewitt, 1981). It is very likely that other factors may influence differential introgression of the relevant alleles, such as habitat use or dispersal capabilities.

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