Sympatry with the devil: reproductive interference could hamper species coexistence

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Summary

1. As species are often considered discrete natural units, interspecific sexual interactions are often disregarded as potential factors determining community composition. Nevertheless reproductive interference, ranging from signal jamming to hybridization, can have significant costs for species sharing similar signal channels.

2. We combined laboratory and field experiments to test whether the coexistence of two congeneric ground-hopper species with overlapping ranges might be influenced by sexual interactions.

3. In the laboratory experiment the number of conspecific copulations of *Tetrix ceperoi* decreased substantially in the presence of *Tetrix subulata*. Males of *T. ceperoi* performed more mating attempts with heterospecific females, whereas females of *T. subulata* rejected these heterospecific approaches more often than those of conspecifics. Although no heterospecific matings occurred in the laboratory, the reproductive success of *T. ceperoi* was reduced substantially in field experiments. Negative effects on *T. subulata* were found only at high densities.

4. Our results suggest that reproductive interference could have similar consequences as competition, such as demographic displacement of one species ('sexual exclusion'). As reproductive interference should be selected against, it may also drive the evolution of signals (reproductive character displacement) or promote habitat, spatial or temporal segregation.

Key-words: mate choice, mistaken identity, outbreeding, pre-mating isolation, specific mate recognition systems.

Introduction

Unravelling the mechanisms of species coexistence is fundamental for understanding the factors determining biodiversity (Chesson 2000). Competition has been discussed as the main type of interaction affecting the coexistence of closely related species (Gause 1934; Schoener 1974; Connell 1983), although its significance remains a matter of controversy (Strong, Lawton &

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Southwood 1984; Denno, McClure & Ott 1995; Friggens & Brown 2005). A fundamental feature of competition is a shared limited resource, which species compete for either through exploitation or interference (Begon, Mortimer & Thompson 1996). However, the identification of such a limited resource is often difficult, particularly in herbivorous insects (Strong *et al*. 1984).

An alternative explanation for the missing coexistence of some species might be found in interspecific sexual interactions (reproductive interference). Reproductive interference is defined as any kind of interaction between species associated with their mating system, which is caused by incomplete species recognition systems and adversely affects the fitness of at least one of the species involved. Such sexual interactions have received much attention in the evolutionary literature, as they might drive speciation due to reinforcement of pre-mating barriers and reproductive character

displacement (reviewed in Spencer, McArdle & Lambert 1986; Butlin 1989; Servedio & Noor 2003; Seehausen 2004). However, the ecological significance of reproductive interference is still often undervalued, except for hybridization, which has been discussed in the context of biological invasions (reviewed in Rhymer & Simberloff 1996; Mallet 2005). Reproductive interference is principally possible at any stage of mate acquisition, ranging from signal jamming during mate attraction to hybridization and can be associated with high fitness costs independently of the fertilization of eggs. It may involve wastage of energy, time and gametes and can negatively affect reproductive success or even survival of a species (Ribeiro & Spielman 1986; Kuno 1992).

It has been argued that the fitness loss associated with reproductive interference can influence species coexistence and lead to demographic displacement of one species (Kuno 1992; Söderbäck 1994; Takafuji, Kuno & Fujimoto 1997; Westman, Savolainen & Julkunen 2002). Information on such ecological effects of reproductive interference is still sparse and missing even in modern ecology textbooks (e.g. Begon *et al.* 2005). This might be affected by the frequent view of species as discrete reproductively isolated units (Mallet 2005). Sexual interactions between species with a strong overlap in distribution are thought to be unlikely as their mate recognition systems should have evolved in response to such interactions (Paterson 1985). However, species with overlapping ranges ('sympatric' in its original evolutionary sense) do not necessarily co-occur on a local scale, a pattern termed 'microallopatry' (Coyne & Orr 1989) or 'allotopy' if they utilize different habitats (Schaefer 2003). Such mosaic types of sympatry are of high ecological interest, particularly if the species involved have overlapping niches. The question arises, whether such a distribution pattern can be caused by reproductive interference.

The two ground-hoppers *Tetrix ceperoi* (Bolívar, 1887) and *Tetrix subulata* (Linnaeus 1758) broadly overlap in distribution and habitat preferences, but rarely co-occur at the same site (Kleukers *et al.* 1997; Gröning, Kochmann & Hochkirch 2005). Here, we test whether sexual interactions occur between these species, which might explain their missing coexistence. We conducted two experiments in order to examine the occurrence, extent and reproductive consequences of interspecific sexual interactions. In a substitutive laboratory experiment we tested whether the behaviour pattern and particularly the number of copulations is influenced by the presence of a related species. In a substitutive field experiment we analysed the reproductive consequences of co-occurrence.

Methods

NATURAL HISTORY OF THE STUDY OBJECTS

Ground-hoppers (Tetrigidae) are an ancient group of Orthoptera, inhabiting mainly damp, open habitats (Paranjape, Bhalerao & Naidu 1987). *T. ceperoi* is distributed in the Mediterranean and western Europe, whereas *T. subulata* has a holarctic distribution. The area of overlap includes northern Spain, France, southern England, central Europe, Italy and the Balkans (Kleukers *et al.* 1997). Both species are terricolous and confined to damp, warm habitats, but *T. subulata* is generally more widespread and also occurs in wet grasslands (Gröning *et al.* 2005). *T. ceperoi* has been found in similar habitat types in areas, where *T. subulata* is rare or missing, such as the German Wadden Sea islands (Gröning, Krause & Hochkirch 2006). Tetrigidae feed on a variety of algae, mosses, small plants and detritus (Hochkirch *et al.* 2000).

Adults of both species reproduce in May and June (Kleukers *et al.* 1997) and utilize visual cues for mate recognition. Although both species show remarkable differences in their courtship displays (Hochkirch, Deppermann & Gröning 2006), heterospecific matings have been observed in the laboratory (Gröning & Finger, unpublished data). In all Tetrigidae sexual size dimorphism is distinct: females are substantially larger than males as they pass through one additional nymphal instar (Ingrisch & Köhler 1998). Both sexes of *T. ceperoi* are smaller than the corresponding sex in *T. subulata* (Kleukers *et al.* 1997). Females of both species oviposit in moist soil. The egg clutches are slightly larger in *T. subulata* (16·1 eggs ± 1·2, *n* = 26) than in *T. ceperoi* (14·1 ± 0·8 eggs, *n* = 27). Nymphs hatch after 20–30 days, depending on temperature (Forsman 2001). In the red data book for Lower Saxony and Bremen *T. subulata* is listed as vulnerable and *T. ceperoi* as endangered (Grein 2005). It has been suggested that reproductive interference with *T. subulata* might represent a threat for *T. ceperoi* (Gröning *et al.* 2005).

THE BEHAVIOURAL EXPERIMENT

Experimental design

To analyse the effect of species co-occurrence on mating frequencies, we conducted a substitutive behavioural experiment with five treatments (two conspecific, two heterospecific and one mixed treatment). In each treatment two males and two females were transferred into a $15 \times 26 \times 19$ cm plastic enclosure with a sand-covered floor and fresh food, which was placed under an Osram HQIT 250-W bulb (white daylight). In conspecific treatments, two males and two females of one species were placed (control samples for each species). In heterospecific treatments, two males of one species were confronted with two heterospecific females (no-choice). In mixed treatments one male and one female of each species were kept together. The animals were allowed to habituate for 5 min and then observed for 30 min. We noted the behaviour of each specimen every 30 s, including also the direction of courtship displays, mating attempts and defensive

behaviour for later analysis of mate preferences. The treatments were replicated 30 times in a reshuffled order to avoid effects of diurnal activity patterns. In order to minimize the impact on the natural populations of the threatened species, we did not collect the large number of insects required to run completely independent replicates. Instead, individuals were randomly drawn from a pool of 160 specimens (40 individuals per species and sex), which were sampled from three sites near Osnabrück (Germany), where both species co-occurred at different relative abundance (see Gröning *et al.* 2005). To maximize the probability that virgin females were used, specimens were collected as early in the year as possible (14–15 April 2003). The sexes were kept separately in order to increase the mating motivation. After each copulation, females were kept in isolation for at least 3 days until they were receptive again to avoid confounding effects from previous matings. Recent evidence suggests that *Tetrix* females are highly polyandrous and readily remate within a period of 48 h (Caesar, Ahnesjö & Forsman 2007). The experiment was performed in a greenhouse of the University of Osnabrück at a temperature of 23–26 °C and a relative humidity of 50–55% between 23 April and 5 June 2003.

Data analysis

For each replicate, we calculated the relative frequencies of behaviour types for each sex. To test for general changes in the behaviour patterns, we performed three-way ANOVAs for each behaviour type, using species, sex and treatment as explanatory variables. We simplified our models, by removing stepwise nonsignificant three-way or two-way interactions (Crawley 2005). Pairwise *t*-tests with Bonferroni correction were used to identify the differing treatments. As ANOVA assumes normally distributed residuals and homogeneous variances, it was necessary to transform our data. We applied Box–Cox transformation using Venables and Ripley's MASS library for R (Venables & Ripley 2002), which reveals the optimal power transformation (λ) to fit the data to meet the model assumptions. As our main interest was the number of copulations (and not the time invested in copulations), we compared also the number of intraspecific copulations between the conspecific and mixed treatments using Pearson's χ^2 test (Crawley 2005). To analyse the mate preferences in the mixed treatment, we calculated the relative frequencies of approaches towards the conspecific and heterospecific female for each replicate, including directed locomotion, courtship and mating attempts. Data were included only if approaches occurred ($N_{ceperoi} = 18$ males, $N_{subulata} = 19$ males). These data were analysed with Kruskal–Wallis rank sum tests. The females' reaction was examined by calculating the proportions of rejected approaches of heterospecific and conspecific males for each female that was approached by a male ($N_{subulata} = 25$ females, $N_{ceperoi} = 18$ females). This rejection

frequency was analysed with Kruskal–Wallis rank sum tests. All statistical analyses were carried out with 'R 2·3·0' (R Development Core Team 2005).

THE REPRODUCTIVE FIELD EXPERIMENTS

Experimental design

Even if the presence of heterospecifics in the laboratory might influence the number of conspecific matings, this does not necessarily affect reproduction, as in females of many insect species a single conspecific copulation is sufficient to maximize the reproductive success. In order to analyse the effects of co-occurrence on the reproductive success, we conducted two substitutive field experiments in a former clay pit near Osnabrück (Germany), where both species occurred. Within each experiment the sex ratio was balanced and the total abundance was kept constant (experiment 8: eight specimens, and experiment 16: 16 specimens), while the species ratio was varied. Each experiment consisted of five treatments with differing species combinations (*T. subulata* unispecific: 8 : 0, 16 : 0; *T. subulata* dominance: 6 : 2, 12 : 4; mixed: 4 : 4, 8 : 8; *T. ceperoi* dominance: 2 : 6, 4 : 12, *T. ceperoi* unispecific: 0 : 8, 0 : 16), each of which was replicated four times. We chose a randomized block design, accounting for a slight environmental gradient in soil moisture.

The experiments were performed in 40 cages (35 × 35 × 35 cm), which had an aluminium structure covered with close meshed (0·6 × 0·6 mm) white synthetic fibre (Econet L, Svensson), allowing light and rain penetration. Corner posts (20 cm) were used to anchor the cages in the ground. A thermally sterilized (80 °C) soil mixture of sand and compost was filled in the cages to a height of 3 cm. Each cage was supplied with moss (*Rhytidiadelphus squarrosus*) and soil algae as food for the ground-hoppers. The moss was collected in Osnabrück, dried, hackled and heated in a microwave to destroy spider eggs. Algae were cultured from soil samples containing algal mats, which were stirred up with water. The cages were prepared in February 2005 by removing the original soil and vegetation and filling in the sterile soil, hackled mosses and algae suspension. During the following weeks, the cages were homogenized by removing excessive vegetation until the experiments were started on 26 April 2005.

We collected a total of 480 specimens from populations with sufficiently high abundance in north-western Germany. To maximize the probability that virgin females were used, specimens were collected as early in the year as possible (5–21 April 2005) and both sexes were kept isolated until the experiment was started. During the experiment the condition of the cages was monitored regularly. Potential predators, such as spiders and Carabidae were removed, and the cages were watered to account for the high water demand of the hygrophilous species. Data on reproductive success were achieved by counting and removing nymphs with

an exhaustor during the period of emergence (27 June, 18 July, 24 and 25 August). As the first instars of Tetrigidae are difficult to identify, we reared them in plastic enclosures in a greenhouse until their identification was possible (from the fourth instar onwards). Specimens, which were not exactly identifiable, were excluded from the analysis. The mortality of nymphs from the unispecific treatments did not differ between the species (ANOVA, $F_{1,8} = 2.44$, $P = 0.16$). The enclosures were supplied with moist soil, algae and mosses as food.

Data analysis

Combined effects of blocks, species and treatment were tested with ANCOVA. Data were Box–Cox transformed using the MASS library for R (Venables & Ripley 2002). We fitted four different nonlinear and linear regression models to our data using the nlsList function for grouped data in R (Crawley 2005). Model simplification techniques were applied in order to fit the minimal adequate models to our data by minimizing residual deviance (Crawley 2005). We started with a three-parameter Michaelis–Menten function ($c + ax/(1 + bx)$), with c = intercept, a = initial slope (rate of increase) and $c + a/b$ = asymptote (maximum reproduction). We reduced one variable subsequently (usually first the intercept c, as a density of zero should produce no offspring).

Results

BEHAVIOURAL EXPERIMENT

Behaviour patterns

The average time spent in copulations differed significantly between the treatments (ANOVA, $\lambda = -0.87$, $F_{2,354} = 24.35$, $P < 0.001$), with more time invested in copulations in the conspecific than in the mixed treatments (pairwise *t*-tests with Bonferroni correction, $P < 0.001$). No copulations were observed in the heterospecific treatments. There was a significant interaction between the factors 'species' and 'treatment' (ANOVA, $\lambda = -0.87$, $F_{2,354} = 5.53$, $P = 0.004$): While *T. subulata* spent a similar proportion of time in copulations in mixed and conspecific treatments, no copulation of *T. ceperoi* was found in the mixed treatment. A similar pattern was found when the number of copulations per specimen was considered instead of the invested time. The average number of copulations per observed individual decreased significantly in *T. ceperoi* from 0·37 to 0·0, while it remained almost constant (0·17 vs. 0·1) in *T. subulata* (Fig. 1, χ^2 two-sample test, d.f. = 1, $\chi^2 = 4.90$, $P = 0.027$).

Males were more often engaged in mating attempts than females, mainly due to homosexual mounts (ANOVA, $\lambda = -0.29$, $F_{1,353} = 65.11$, $P < 0.001$). There was a significant interaction between 'species' and 'treatment'
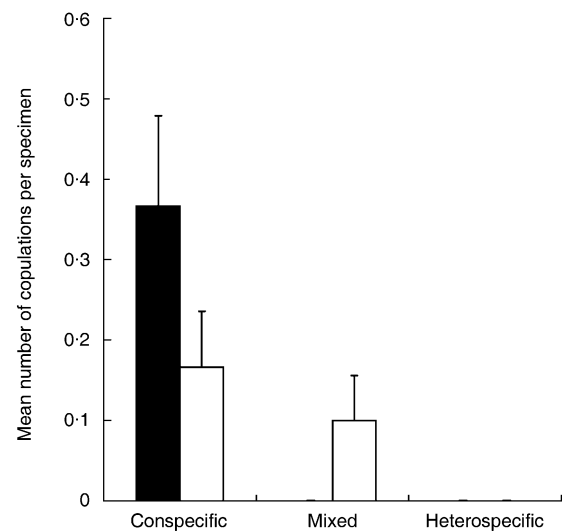
Fig. 1. Average number of successful copulations per individual for *Tetrix ceperoi* (black columns) and *Tetrix subulata* (white columns) in the conspecific, mixed and heterospecific treatment. No heterospecific matings were observed. The number of copulations decreased significantly in *T. ceperoi* in the mixed treatment, while it did not differ significantly in *T. subulata* (χ^2 two-sample test, d.f. = 1, $\chi^2 = 4.90$, $P = 0.027$).

for this type of behaviour (ANOVA, $\lambda = -0.29$, $F_{2,353} = 5.17$, $P = 0.006$). While *T. subulata* spent significantly more time in mating attempts in the mixed treatments, the frequency remained almost constant in *T. ceperoi*. Courtship was exclusively performed by males (ANOVA, $\lambda = -0.47$, $F_{1,348} = 108.29$, $P < 0.001$). In general, *T. subulata* invested significantly more time in courtship than *T. ceperoi* (ANOVA, $\lambda = -0.47$, $F_{1,348} = 6.03$, $P = 0.001$). We also found an effect of the treatment on this type of behaviour (ANOVA, $\lambda = -0.47$, $F_{2,348} = 5.11$, $P = 0.006$). Courtship was more common in conspecific treatments than in mixed or heterospecific treatments (pairwise *t*-test with Bonferroni correction, $P = 0.046$), while the latter two treatments did not differ significantly. This effect was only found for *T. subulata*, resulting in a significant interaction between 'species' and 'treatment' (ANOVA, $\lambda = -0.47$, $F_{2,348} = 4.44$, $P = 0.012$).

Defensive behaviour in Tetrigidae includes leg movements and body shaking. This type of behaviour is usually related to male mating attempts (Uvarov 1977). Hence, defensive behaviour was observed significantly more often in females than in males (ANOVA, $\lambda = -0.07$, $F_{1,354} = 13.08$, $P < 0.001$). We also found a significant effect of the treatment on this behaviour type (ANOVA, $\lambda = -0.07$, $F_{2,354} = 4.59$, $P = 0.01$). Defensive behaviour was more common in the mixed treatment than in the conspecific treatment (pairwise *t*-test with Bonferroni correction, $P = 0.019$). However, this pattern was only produced by defensive behaviour of males, which was nearly exclusively found in the mixed treatment (ANOVA, $\lambda = -0.07$, $F_{2,354} = 3.77$, $P = 0.02$).
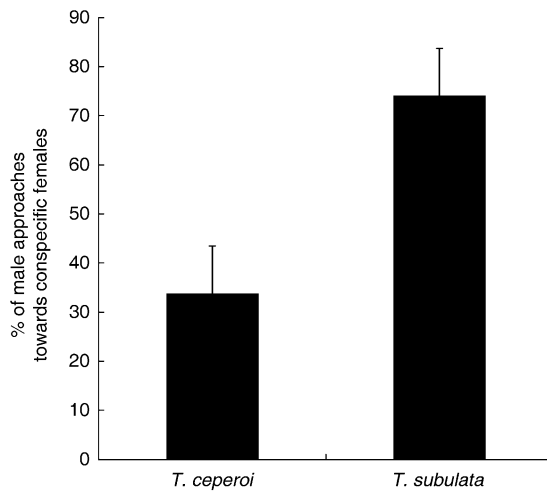
Fig. 2. Average proportion of male approaches to conspecific females in the mixed treatment. The proportion differed significantly between both species (Kruskal–Wallis test, d.f. = 1, χ^2 = 6·39, P = 0·011). *T. subulata* males were stronger attracted to females of their own species, *T. ceperoi* to heterospecific females. Error bars are standard errors ($N_{ceperoi}$ = 18 males, $N_{subulata}$ = 19 males).

Fig. 3. Average percentage of female defensive reactions towards male approaches in the mixed treatment. *T. ceperoi* females rejected approaches of males of both species to a similar proportion, whereas females of *T. subulata* repelled heterospecific males (white columns) significantly more often than conspecific ones (black columns). Error bars are standard errors (*T. ceperoi*: 10 replicates with conspecific and eight with heterospecific male approaches; *T. subulata*: 13 replicates with conspecific and 12 with heterospecific male approaches).

Directed movements and female defence

The direction of male approaches in the mixed treatment differed significantly between the two species (Fig. 2, Kruskal–Wallis test, d.f. = 1, χ^2 = 6·39, P = 0·011). *T. subulata* males preferably attempted to copulate with females of their own species (on average 73·9%), whereas *T. ceperoi* were more attracted by heterospecific females (on average 66·3%). While *T. ceperoi* females repelled approaches of males of both species to a similar proportion (Kruskal–Wallis test, d.f. = 1, χ^2 = 0·034, P = 0·85), females of *T. subulata* rejected heterospecific males (72·1%) significantly more often than conspecific ones (Fig. 3 and 31·1%, Kruskal–Wallis test, d.f. = 1, χ^2 = 6·83, P = 0·009).

REPRODUCTIVE FIELD EXPERIMENTS

In both experiments, the reproductive success of both species was significantly affected by the intraspecific density (ANCOVA, experiment 8: λ = 0·205, $F_{1,34}$ = 69·50, P < 0·001; experiment 16: λ = 0·24, $F_{1,34}$ = 67·91, P < 0·001). However, a cross-comparison of both experiments using treatments with equal intraspecific abundance (i.e. 4 and 8 specimens) revealed a significantly reduced reproductive success in the presence of heterospecifics (ANCOVA, λ = 0·46, $F_{3,27}$ = 3·81, P = 0·021). In the mixed treatments of experiment 8, the reproductive success of *T. ceperoi* was lower than expected based on a linear relationship, while in *T. subulata* it was higher (Fig. 4a). The maximum total reproduction of the latter species had already been reached in the mixed treatment (4 : 4). The opposite was true for *T. ceperoi*, which had on average 40 and 43% lower individual reproduction rates in the mixed treatment (4 : 4)
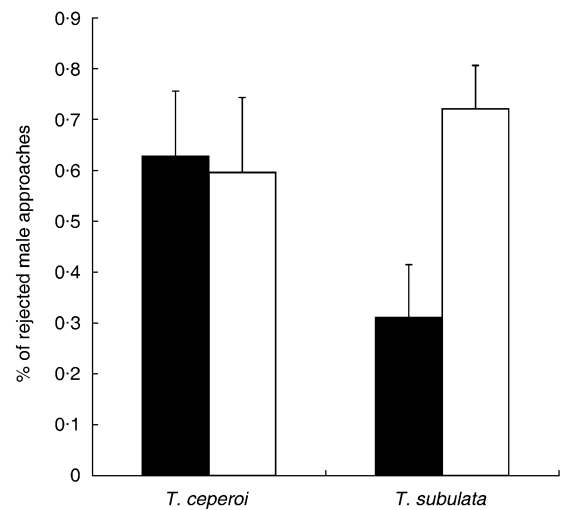
and in the *T. subulata* dominance treatment (6 : 2), respectively.

In the mixed treatments of experiment 16 the reproductive success of both species was lower than expected based upon a linear relationship (Fig. 4b). Both species had reduced individual reproduction rates in the mixed and dominance treatments compared with a linear response. Compared with the unispecific treatments (8 : 0, 0 : 8), the mean reproduction rate in the mixed treatments of similar intraspecific density (8 : 8) was reduced in both species, but to a different degree (*T. ceperoi*: –45%, *T. subulata*: –26%). In both experiments, *T. subulata* had a significantly higher reproduction than *T. ceperoi* (ANCOVA, experiment 8: λ = 0·205, $F_{1,34}$ = 4·26, P = 0·046; experiment 16: λ = 0·24, $F_{1,34}$ = 3·88, P = 0·057) and no block effect was found.

To explain the relationship between intraspecific abundance and reproductive success, we fitted linear and nonlinear models with or without intercept to our response variable (reproduction of each species). All models explained more of the variation among the treatments than would be expected by chance. The minimal adequate models for both experiments were two-parameter Michaelis–Menten functions passing through the origin. These models had a minimal residual deviance and were less complex than three-parameter models. Linear models provided a worse fit (higher residual deviance, Tables 1 and 2).

Discussion

Our results from the laboratory experiments revealed that reproductive interference occurs between *T. ceperoi*
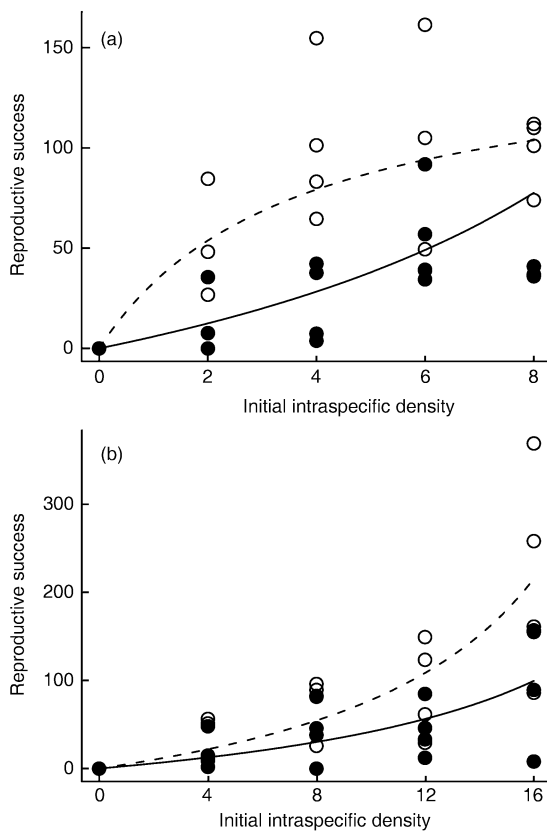
Fig. 4. Results of the substitutive field experiment, measured as the reproductive success (number of nymphs) of *Tetrix ceperoi* (●) and *Tetrix subulata* (○) in response to the intra-specific density. Heterospecifics were added to gain an overall initial density of (a) 8 and (b) 16, respectively. The lines represent the minimum adequate models to explain the response for *Tetrix ceperoi* (solid line) and *Tetrix subulata* (broken line). The asymptotic response of *Tetrix subulata* (a) indicates a higher reproduction in mixed treatments than expected from the unispecific treatments. The exponential responses (all other lines) indicate a reduced reproduction in the presence of heterospecifics.

and *T. subulata*. Although heterospecific matings were not observed during these experiments, *T. ceperoi* failed to achieve conspecific matings in the mixed treatment. Moreover, in the field experiments the reproductive success of *T. ceperoi* decreased substantially in the presence of *T. subulata*. Although these experiments do not represent a direct test, they support the hypothesis that reproductive interference could hamper the co-existence of species with incomplete mate recognition systems. Our results also suggest that the outcome of this relationship is density-dependent, as has been previously shown in a hybrid zone of *Chrysochus* beetles (Peterson *et al.* 2005). While *T. ceperoi* was negatively affected in the presence of *T. subulata*, the reproduction of *T. subulata* was only reduced at high total densities. It is reasonable to suggest that demographic displacement of the species facing the higher costs might be a con-sequence of reproductive interference in a longer term. This process has been compared with competitive exclusion (Kuno 1992), although it would be more appropriate to call it 'sexual exclusion'. Reproductive interference differs from competition in the lack of a common resource. The most apparent and most frequently discussed type of reproductive interference is hybridization (Rhymer & Simberloff 1996; Mallet 2005), which is more related to negative heterosis than to competition (Spencer *et al.* 1986). In the case of *T. ceperoi* and *T. subulata*, hybridization is unlikely, as the two species are genetically comparatively distantly related with a p-distance of 10·4% in the mitochondrial ND1 gene. Moreover, no evidence for introgression was found in phylogenetic analyses using sequences of four gene fragments (Gröning & Hochkirch, un-published data).

The reduced mating success of *T. ceperoi* in the mixed treatments seems to be a consequence of asymmetric mate preferences. *T. ceperoi* males performed fewer

Table 1. Comparison of models to explain the variation in reproduction rates across the four treatments with an overall density of eight specimens. The minimum adequate model is marked with an asterisk at the residual deviance. Errors following parameter estimates are standard errors

Model	Species	Parameter estimates	Residual deviance	Residual d.f.	Equation
Three-parameter asymptotic	*T. subulata*	$a = 43\cdot84 \pm 34\cdot04$ $b = 0\cdot29 \pm 0\cdot32$ $c = -1\cdot97 \pm 17\cdot75$	35·64	34	$y = c + (ax/1 + bx)$
	T. ceperoi	$a = 6\cdot07 \pm 6\cdot13$ $b = -0\cdot05 \pm 0\cdot07$ $c = -1\cdot88 \pm 15\cdot85$			
Two-parameter asymptotic (passing through the origin)	*T. subulata*	$a = 41\cdot88 \pm 25\cdot69$ $b = 0\cdot28 \pm 0\cdot28$	34·64*	36	$y = ax/1 + bx$
	T. ceperoi	$a = 5\cdot57 \pm 3\cdot72$ $b = -0\cdot05 \pm 0\cdot06$			
Linear	*T. subulata*	$a = 12\cdot37 \pm 2\cdot88$ $c = 16\cdot30 \pm 14\cdot11$	36·43	36	$y = c + ax$
	T. ceperoi	$a = 9\cdot82 \pm 2\cdot88$ $c = -6\cdot29 \pm 14\cdot11$			
Linear model, passing through the origin	*T. subulata*	$a = 15\cdot08 \pm 1\cdot65$	36·21	38	$y = ax$
	T. ceperoi	$a = 8\cdot77 \pm 1\cdot65$			

Table 2. Comparison of models to explain the variation in reproduction rates across the four treatments with an overall density of 16 specimens. The minimum adequate model is marked with an asterisk at the residual deviance. Errors following parameter estimates are standard errors

Model	Species	Parameter estimates	Residual deviance	Residual d.f.	Equation
Three-parameter asymptotic	*T. subulata*	$a = 3 \cdot 61 \pm 2 \cdot 15$	50·03	34	$y = c + (ax/1 + bx)$
		$b = -0 \cdot 05 \pm 0 \cdot 01$			
		$c = 10 \cdot 07 \pm 20 \cdot 11$			
	T. ceperoi	$a = 1 \cdot 96 \pm 2 \cdot 47$			
		$b = -0 \cdot 04 \pm 0 \cdot 02$			
		$c = 6 \cdot 39 \pm 20 \cdot 54$			
Two-parameter asymptotic (passing through the origin)	*T. subulata*	$a = 4 \cdot 55 \pm 1 \cdot 62$	48·84*	36	$y = ax/1 + bx$
		$b = -0 \cdot 04 \pm 0 \cdot 01$			
	T. ceperoi	$a = 2 \cdot 70 \pm 2 \cdot 01$			
		$b = -0 \cdot 04 \pm 0 \cdot 02$			
Linear	*T. subulata*	$a = 12 \cdot 41 \pm 2 \cdot 06$	52·14	36	$y = a + bx$
		$c = -16 \cdot 50 \pm 20 \cdot 20$			
	T. ceperoi	$a = 5 \cdot 74 \pm 2 \cdot 06$			
		$c = -4 \cdot 70 \pm 20 \cdot 20$			
Linear model, passing through the origin	*T. subulata*	$a = 11 \cdot 03 \pm 1 \cdot 17$	51·26	38	$y = ax$
	T. ceperoi	$a = 5 \cdot 35 \pm 1 \cdot 17$			

mating attempts with conspecifics than with *T. subulata* females, which possibly offer superstimuli due to their larger body size (Hochkirch *et al*. 2006). A preference for larger females has been shown in a number of other insect species (Bonduriansky 2001; Thornhill & Alcock 2001) and might represent an ancestral sensory bias (Ryan 1998). On the other hand, female mate recognition seems to be more reliable in *T. subulata* than in *T. ceperoi* as females of the former species rejected a significantly higher proportion of *T. ceperoi* mounts than those of conspecifics. The reason might either be found in the different courtship displays of both species (Hochkirch *et al*. 2006) or in the smaller body size of *T. ceperoi* males. Pheromones are unlikely to play a role in communication of Tetrigidae as males ignore motionless females even if they are sitting in close proximity to them. Moreover, males sometimes even climb across females accidentally, while they readily mount moving objects of appropriate size, such as flies and male Tetrigidae (Hochkirch *et al*. 2006). Recent studies suggest that vibrational communication might also play a role in the communication of Tetrigidae (Benediktov 2005).

It has often been doubted that results from laboratory behavioural experiments are comparable with field situations, as heterospecific sexual interactions have been detected more often in the laboratory (Coyne & Orr 1989; Verrel 1990; Ficetola & De Bernardi 2005). In the field, the frequency of heterospecific encounters can be substantially lower than in small arenas with unnaturally high abundance. Species might utilize different microhabitats, occur in different abundance or intraspecific aggregations, leading to small-scale segregation and a reduced frequency of encounters in nature (Verrel 1994). Our field experiments support the laboratory data with decreased reproductive success in the mixed treatments, but it has to be considered that

we used cages wherein the species cannot avoid each other. Although experiments can be useful to investigate mechanisms of interspecific interactions (Dame & Petren 2006), field observations are necessary to understand the significance of reproductive interference in nature (Deering & Scriber 2002). Field data from a recently restored floodplain, where both species co-occur at high densities, suggest that mating attempts of males are strongly correlated with encounter frequencies (Gröning, Lücke, Finger & Hochkirch, unpublished data).

CHARACTERISTICS OF REPRODUCTIVE INTERFERENCE

Our data show that reproductive interference should more often be considered as a potential force shaping animal communities. Reproductive interference differs from resource competition in the lack of a shared limited resource. It is caused by incomplete mate recognition systems (Reitz & Trumble 2002; Dame & Petren 2006) and can be associated with high fitness costs. The behavioural stage at which reproductive interference acts can be rather variable, ranging from signalling during mate attraction to hybridization. The latter is just the most obvious mechanism of reproductive interference, a phenomenon that is frequently discussed in the context of *ex-situ* conservation (outbreeding) and biological invasions (Rhymer & Simberloff 1996; Bell & Travis 2005; Mallet 2005). Hybridization is often suggested to be the most costly form of reproductive interference, involving gamete wastage and hybrid offspring, which might lead to gene pool swamping (Barton & Hewitt 1985; Rhymer & Simberloff 1996). However, heterospecific matings without hybridization might involve even higher fitness costs, as no genes are transferred to the next generation (Liou & Price 1994).

Female insects are often not receptive for some days after mating or might even be physically blocked (Andrews, Petney & Bull 1982), reducing the conspecific mating success. In extreme cases, females are damaged during heterospecific copulations and produce less offspring afterwards ('Ripper', Ribeiro & Spielman 1986).

Many authors concluded that the costs of indirect types of reproductive interference, such as signal jamming or heterospecific mating attempts, are relatively low (Singer 1990; Collins & Margolies 1991; Doherty & Howard 1996; Gregory, Remmenga & Howard 1998). These costs, however, have rarely been measured in terms of reproductive success or survival. Moreover, fitness loss is not necessarily dependent on the type of interaction and can be high even in sexually incompatible species (Verrel 1994). Our results support this hypothesis, as the reproductive success of *T. ceperoi* was significantly reduced in the mixed treatments. It has already been argued that interspecific mating attempts can reduce fitness and lead to decreased conspecific matings in the presence of heterospecific individuals (McLain & Shure 1987; Singer 1990; Verrel 1994). This is particularly true if the heterospecific is more attractive than the conspecific female (Deering & Scriber 2002). In some cases even heterospecific males can be more attractive than conspecific females (Verrel 1994).

Similar to competition, the significance and outcome of reproductive interference is density-dependent (Collins & Margolies 1991; Westman *et al.* 2002). As assortative mating is influenced by the social environment, sexual isolation may be disrupted by a high abundance of heterospecifics (Hettyey & Pearman 2003; Peterson *et al.* 2005). If both species are equally affected by reproductive interference, the initial density should determine the reproductive success and survival (similar to negative heterosis, Foster *et al.* 1972). Nevertheless, asymmetric types of reproductive interference are probably more common in nature, as it is rather unlikely that two species have completely similar reproductive properties. In the present study, *T. subulata* had higher reproductive rates even in unispecific treatments and was less affected in mixed treatments and at lower densities. In some cases the degree of asymmetry can be even stronger and only one species might suffer from reproductive interference. This is particularly true, if only one species utilizes the signal for mating and the other one for a different behaviour type, such as rivalry (*Chorthippus dorsatus* and *Chorthippus parallelus*, Keithahn & Hochkirch, unpublished data).

ECOLOGICAL AND EVOLUTIONARY CONSEQUENCES

As the outcome of reproductive interference (decreased reproduction) is similar to competition (Ficetola & De Bernardi 2005), the consequences of both types of interaction are comparable. Sexual exclusion is a reasonable effect of reproductive interference (Kuno 1992; Söderbäck 1994; Reitz & Trumble 2002; Westman *et al.* 2002) and might explain the missing coexistence of several closely related species. It might therefore represent a potential threat to endangered species. In the regional red list for Lower Saxony *T. ceperoi* is listed as endangered and *T. subulata* as vulnerable (Grein 2005). It remains speculation, whether the rarity of *T. ceperoi* is a consequence of displacement due to reproductive interference with *T. subulata*. The latter species also inhabits a broader range of habitats. Interestingly, *T. ceperoi* seems to have a broader niche where *T. subulata* is missing or rare, such as on the German Wadden Sea islands (Gröning *et al.* 2005).

Although sexual exclusion seems to be a logical consequence of reproductive interference, several ecological and evolutionary mechanisms are thinkable, which allow species to coexist. These mechanisms include spatial, temporal or habitat segregation (Singer 1990; Kuno 1992; Fujimoto, Hiramatsu & Takafuji 1996; Hettyey & Pearman 2003), dilution effects from intraspecific aggregations (Ficetola & De Bernardi 2005) or local abundance (Söderbäck 1994; Takafuji *et al.* 1997; Westman *et al.* 2002; Hettyey & Pearman 2003), different speeds of colonization or population dynamics (Westman *et al.* 2002) or reproductive character displacement (Brown & Wilson 1956). The latter phenomenon has been frequently discussed in the speciation literature (Dobzhansky 1937; Butlin 1989; Servedio & Noor 2003; Coyne & Orr 2004). If two species co-occur that utilize overlapping signal channels during mate recognition, those individuals that are able to recognize conspecifics correctly will have a higher reproductive success. This will lead to a selective pressure on mate recognition systems and consequently new communicative traits may arise (Dobzhansky 1937). This process will subsequently lead to reproductive isolation of populations with such new signals and other populations, which utilize ancestral signals. Hence, it would be interesting to compare sympatric and allopatric populations of the two ground-hoppers.

Conclusions

Reproductive interference is a phenomenon, which is still widely ignored in the ecological literature. Our data indicate that the consequences of reproductive interference could be dramatic. In many cases, where closely related taxa do not co-occur, reproductive interference may be a more likely cause than resource competition. Hence, its importance in shaping natural communities should receive more attention (Hettyey & Pearman 2003). Until now, studies on reproductive interference have focused on classical reinforcement situations in narrow hybrid zones (e.g. Butlin & Ritchie 1991) or on invasive species (Rhymer & Simberloff 1996). The presence of heterospecifics utilizing similar signals should be addressed more often also in species with broadly overlapping distributions.

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