



Reproductive interference in two ground-hopper species: testing hypotheses of coexistence in the field

Julia Gröning, Niklas Lücke, Alexander Finger and Axel Hochkirch

J. Gröning (julia.groening@biologie.uni-osnabrueck.de), N. Lücke, A. Finger and A. Hochkirch, Dept of Biology/Chemistry, Div. of Ecology, Univ. of Osnabrück, Barbarastr. 13, DE-49076 Osnabrück, Germany.

Similar to resource competition, reproductive interference may hamper the coexistence of closely related species. Species that utilize similar signal channels during mate finding may face substantial fitness costs when they come into contact and demographic displacement of the inferior species (sexual exclusion) is a likely outcome of such interactions. The two ground-hopper species *Tetrix ceperoi* and *Tetrix subulata* broadly overlap in their ranges and general habitat requirements, but rarely co-occur on a local scale. Results from laboratory and field experiments suggest that this mosaic pattern of sympatry might be influenced by reproductive interference. Here, we examine the significance of sexual interactions for these species in the field and test hypotheses on mechanisms of coexistence. Our results show that heterospecific sexual interactions also occur under field conditions, but in contrast to the experiments *T. ceperoi* was not the inferior species. The number of male mating attempts of both species was strongly correlated with encounter frequencies. Males discriminated between the sexes but not between the species, suggesting an incomplete mate recognition system in both species. The analysis of microhabitat preferences and spatial distribution revealed that habitat partitioning is not a suitable mechanism of coexistence in this system. Instead, the costs of reproductive interference are substantially mitigated by different niche breadths leading to different degrees of aggregation. Despite a considerable niche overlap *T. ceperoi* displayed a stronger preference for bare ground and occurred more aggregated than *T. subulata*, which had a broader niche. These differences may reduce the frequencies of heterospecific encounters and interactions in the field. Our results demonstrate that coexistence in the presence of reproductive interference is comparable to resource competition, being strongly influenced by ecological traits of the involved species, such as niche breadth and dispersion pattern.

Interspecific interactions are believed to represent powerful mechanisms influencing species coexistence. Apart from competition, predation and mutualism, sexual interactions between species (reproductive interference) can have important ecological and evolutionary consequences (Ribeiro and Spielman 1986, Kuno 1992). 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 (Hochkirch et al. in press). Interspecific sexual interactions produce costs in terms of time, energy and gametes wasted at the expense of conspecific mating success (Singer 1990). Similar to resource competition,

reproductive interference is density-dependent (Hettyey and Pearman 2003) and in most cases asymmetric (Fujimoto et al. 1996). It can affect population dynamics, abundance, habitat choice and distribution of species (Kuno 1992, Ficetola and De Bernardi 2005). It has been suggested that demographic displacement of one species caused by reproductive interference (sexual exclusion) is even more likely than competitive exclusion (Ribeiro and Spielman 1986, Kuno 1992, Hochkirch et al. in press). Nevertheless, a variety of ecological and evolutionary mechanisms might enable sexually interacting species to coexist. These mechanisms include temporal, spatial or habitat segregation (Singer 1990), dilution effects from intraspecific aggregations (Ficetola and De Bernardi 2005) or local

abundance (Söderbäck 1994), different speeds of colonization or population dynamics (Westman et al. 2002) or reproductive character displacement (Brown and Wilson 1956). While the ecological mechanisms facilitate coexistence by decreasing the frequencies of heterospecific encounters, the latter represents an evolutionary adaptation to reduce the costs of reproductive interference.

Species with overlapping ranges, which do not co-occur on a local scale are among the most promising systems to study reproductive interference. Two species, which show such a mosaic type of sympatry are the two ground-hopper species *Tetrix ceperoi* and *Tetrix subulata* (Orthoptera, Tetrigidae). These species broadly overlap in their ranges and general habitat requirements, but are rarely found at the same site (Gröning et al. 2005). Evidence from laboratory and field experiments suggest that reproductive interference could hamper their coexistence: The mating frequencies and reproductive success of *T. ceperoi* decreased substantially in the presence of *T. subulata*, while the latter species was only affected at high densities. Males of *T. ceperoi* preferably courted heterospecific females, but were not accepted as mates, whereas *T. subulata* males preferably attempted to mate with conspecifics (Hochkirch et al. in press). However, many studies revealed a poor concordance between results from laboratory experiments and field observations (Andrews et al. 1982 vs Bull and Burzacott 1994, Hettyey and Pearman 2003 vs Ficetola and De Bernardi 2005). Hence, it is of crucial importance to validate experimental findings in wild populations.

Here, we use data from field observations and experiments to evaluate the importance of reproductive interference between both *Tetrix* species in their natural environment. We test two hypotheses (microhabitat partitioning and aggregation) to explain how these species might mitigate the costs of reproductive interference. (1) We studied the behaviour of undisturbed free-ranging ground-hoppers, focussing on the magnitude and direction of heterospecific sexual interactions in the field. (2) We examined whether the species utilize different microhabitats (0.28 m²), which may lead to a segregation on a micro-scale. (3) We mapped the abundance and spatial distribution of both species on a meso-scale (650 m²) to test for intraspecific aggregations and interspecific segregation and examined correlations with the vegetation structure. (4) To analyze whether microhabitat partitioning can produce spatial segregation, we experimentally manipulated parts of the habitat according to the detected differences in microhabitat preferences. We then tested, if the distribution of the ground-hoppers is influenced by these changes.

Methods

Study objects

Tetrigidae are small, terricolous Orthoptera, which feed on algae, mosses, small plants and detritus (Hochkirch et al. 2000). *Tetrix subulata* and *T. ceperoi* are diurnal species, which reproduce in May and June and hibernate during a late nymphal instar or as adults (Kleukers et al. 1997). Hence, a temporal segregation can be excluded as a mechanism of coexistence. Ground-hopper males exhibit visual courtship displays for mate acquisition (Hochkirch et al. 2006). Males of *T. ceperoi* perform a fast movement of high amplitude of the hind legs and the pronotum (“pronotal bobbing”), whereas *T. subulata* males show only minor movements of the body (“lateral swinging”, “frontal swinging”). Males of both species attempt to mate with moving objects of similar size, such as other Tetrigidae (including males) or even flies. Sexual size-dimorphism is distinct, as females pass through one additional nymphal instar (Hochkirch et al. 2007). They are, therefore, larger than the males (Ingrisch and Köhler 1998). *Tetrix ceperoi* and *T. subulata* are genetically comparatively distantly related with a p-distance of 10.4% in the mitochondrial ND1 gene. Hybridization is thought to be unlikely (Hochkirch et al. in press) as no evidence for introgression was found in phylogenetic analyses using sequences of four gene fragments (Gröning and Hochkirch unpubl.).

Cepero’s ground-hopper, *T. ceperoi*, is mainly distributed in the Mediterranean and reaches the northern edge of its range in central Europe, whereas the slender ground-hopper, *T. subulata*, has a holarctic distribution (Kleukers et al. 1997). Their ranges overlap substantially in central Europe, France, Italy, the Balkans, northern Spain and southern England. Both species are highly mobile pioneers that are adapted to dynamic habitats, such as floodplains, moist dune slacks, sand pits, drainage ditches or ponds. Although both species are hygrophilous and prefer damp, open habitats, they rarely co-occur at the same site (Kleukers et al. 1997, Gröning et al. 2005). *T. subulata* is generally more widespread and also inhabits wet grassland, while *T. ceperoi* is believed to be more thermophilous. In areas, where *T. subulata* is rare or missing (e.g. East Frisian Islands, Germany), *T. ceperoi* has been found in similar habitat types (Gröning et al. 2006). In the regional red list for Lower Saxony and Bremen (Germany) *T. ceperoi* is listed as endangered and *T. subulata* as vulnerable (Grein 2005). Although habitat loss is generally considered to be the major threat, reproductive interference might also influence the coexistence of both species (Gröning et al. 2005, Hochkirch et al. in press).

Study area

The data were obtained at a restored floodplain of the river Hase near the town Haselünne (Emsland, Germany). The restoration measures were carried out in winter 2001/2002 resulting in a mosaic of ephemeral ponds, moist swales and inland sand dune complexes. The area (37 ha) is characterized by high dynamics due to winter flooding and is managed by extensive cattle grazing. *T. ceperoi* and *T. subulata* have been recorded from the site since 2004 (Gröning et al. 2005). They co-occur in high numbers in moist hollows, ditches and along the open shore of ephemeral ponds. These habitats are dominated by bulrush (*Juncus effusus*, *J. articulatus*, *Eleocharis palustris*), young sprouts of willows and alder (*Salix* spp., *Alnus glutinosa*), diverse forbs (e.g. *Lythrum salicaria*, *Mentha aquatica*, *Lycopus europaeus*, *Trifolium repens*) and grasses (e.g. *Agrostis stolonifera*). They are flooded during the winter and run dry in the course of the summer months depending on rainfall. Since *T. subulata* and *T. ceperoi* have rarely been found on the same site in northwestern Germany (Gröning et al. 2005), this floodplain offers an ideal opportunity to examine the magnitude of reproductive interference in the field and to study potential mechanisms of coexistence.

Data collection

Reproductive interference

To analyse the frequency and direction of heterospecific interactions, we observed the behaviour of free-ranging, undisturbed adult *T. ceperoi* and *T. subulata* during their reproductive period (3 May to 24 June 2006). We obtained data during their daily time of activity (between 10:30 and 17:00) and only when the weather was warm and sunny. All observations were made along the shore of an ephemeral pond in the centre of the floodplain, where both species occurred in high numbers. Behavioural records were obtained from 116 individuals (*T. ceperoi*: 29 males, 31 females, *T. subulata*: 30 males, 26 females). Observed specimens were chosen in a sequential order to avoid confounding effects of daily activity patterns or weather conditions. To allow a comparison with previous laboratory experiments (Hochkirch et al. in press), we applied a similar method: each insect was observed for 30 min and its behaviour was noted every 15 s, including any interaction with other Tetrigidae. To analyse mate preferences we recorded the direction of male courtship displays and mating attempts as well as defensive behaviour of females. In addition, time, date, species, sex and the substrate on which the focal insect perched were noted. The body temperature (pronotum) was measured every 10 minutes using a digital infrared

thermometer to control for temperature-dependent activity. In order to analyse correlations between the frequency of encounters and mating attempts, we also noted species, sex and behaviour of each specimen in a maximum distance of 5 cm from the focal individual (= encounters). After 30 min the insect was marked with a non-toxic paint-marker (edding 780) to avoid pseudoreplication and released at the location of capture.

Microhabitat preferences

To compare the microhabitat preferences of both species, we recorded microclimate and vegetation parameters at the exact location of randomly chosen individuals and compared these data with non-occupied sites (control samples). For each ground-hopper, we noted species, sex, behaviour, time and date, as well as the substrate, on which the insects perched (including the categories bare ground, litter, grasses, forbs, mosses and shrubs). We measured the body temperature (pronotum) and the temperature of the substrate with a digital infrared thermometer. Radiation was measured using a luxmeter. In a circle of 30 cm diameter surrounding the focal insect, we recorded the maximum vegetation height and estimated the vegetation cover, including the relative frequencies of bare ground, litter, grasses, forbs, mosses and shrubs. Simultaneously to each individual, a corresponding control sample was taken in one metre distance in a random direction (Gröning et al. 2006). The microhabitat records were made at the same sites as the behavioural observations during favourable weather conditions (3 May to 28 June). In total 460 individual records were obtained (115 for each species and sex).

Grid mapping and experimental habitat manipulation

To map the abundance and spatial distribution of both species, a 650 m² grid containing 62 grid cells (3 × 3 m) was established in an ephemeral pond in the western part of the floodplain. The area was marked out by placing numbered wooden pegs at 3 m intervals throughout. The pond was completely flooded during winter and gradually fell dry in spring. The spatial distribution of *Tetrix* individuals was mapped at three visits (15 May, 8 June, 21 June 2006). For this purpose, three persons counted synchronously the number of specimens in each grid cell with each person observing a transect of one metre. The ground-hoppers were caught with a small goldfish net or by hand, identified (species, sex), and marked with a paint-marker (edding 780). Afterwards the specimens were released at the location of capture.

To test whether the species segregate spatially in response to the distribution of their preferred habitat structure, we experimentally manipulated the vegetation in the grid on 16 May 2006. We mowed the vegetation in every second grid cell to a height of 5–10 cm using a trimmer. Litter was removed by hand to enhance the availability of bare ground in the treated cells. The vegetation cover (%) in each grid cell was estimated on 9 June 2006, including the relative frequencies of the following categories: shrubs (young willows and alder), bulrush, forbs, grasses, mosses, litter and bare ground. Moreover, soil moisture (%) was measured at three spots of each grid cell (low, medium, high elevation) using time domain reflectometry.

Statistical analyses

We used χ^2 cross table tests to analyse whether the relative frequencies of conspecific and heterospecific encounters differ between *T. ceperoi* and *T. subulata* males. The same method was applied to test if the relative frequencies of male mating attempts with con- and heterospecific individuals correspond to the relative frequencies of encounters. Fisher's exact test was used to examine females' defensive reactions towards mating attempts of con- and heterospecific males. To analyse the microhabitat preferences of *T. ceperoi* and *T. subulata*, we performed two-way ANOVAs for metric data (body and substrate temperature, vegetation cover, vegetation height). Since we obtained individual data sets rather than abundance data, we used "sex" and "species" as explanatory variables and the measured parameters as response variables. If necessary, data were Box–Cox-transformed using Venables and Ripley's MASS library for R (Venables and Ripley 2002), which reveals the optimal power transformation (λ) to fit the data to meet the model assumptions. We used χ^2 cross table tests to analyze nominal data (substrate) and applied Fisher's exact test when the expected frequencies were < 5 (Crawley 2005). To compare the locations of the insects with the corresponding control samples, we carried out paired t tests for all metric abiotic and biotic variables. The variances associated with the means of the insect's location and the control sample were analysed with Fisher's F-test (Crawley 2005).

We performed a standardized principal component analysis (PCA) in order to identify correlations between the environmental factors. Due to the variable scales of our data set (lux, °C, cm, % cover) we used the function "rda" of the community ecology package vegan 1.6–10 for R (Oksanen et al. 2005) to scale the factors by their proportional eigenvalue. The factors were standardized to unit variance using correlation coefficients to achieve a more balanced ordination. The locations of *T. ceperoi* and *T. subulata* were fitted as vectors onto

the ordination using the function "envfit" for R. This method also generates an R^2 measure and significance values based on the probability that random permutations would yield a higher degree of fit than the true data (Oksanen et al. 2005).

We chose the Czechanowski index to quantify niche overlap between *T. ceperoi* and *T. subulata* for the following microhabitat parameters: radiation, substrate temperature, substrate type, vegetation height and the different aspects of the vegetation cover. The Czechanowski index ranges from 0 (no shared resource states) to 1 (identical resource utilization). To test whether the observed niche overlap differed from a random pattern we carried out null model analyses with EcoSim 7.0 (Gotelli and Entsminger 2001). EcoSim simulates patterns of niche overlap and compares these randomized results with the observed data matrix. We used the algorithm RA3 (Winemiller and Pianka 1990) to test for non-random niche overlap. In this procedure, the observed niche breadth of each species is retained, but the utilization of any resource state is allowed, including categories that were available but not used by the species. We defined the relative availability of each resource state using the data of the corresponding control samples. For each data set 10 000 replicates were created in the simulation.

To examine the spatial distribution of both species in the grid, we first tested whether the data are negatively binomial distributed (Hanski 1981). Since this was not true for all species and/or census dates, we calculated Morisita's index of dispersion, which is independent of population density (Krebs 1999). An index of $I_d > 1$ indicates an aggregated dispersion, whereas the dispersion is uniform if the index is $I_d < 1$. The null hypothesis of randomness was tested with a χ^2 test. To evaluate the spatial niche overlap of *T. ceperoi* and *T. subulata* in the grid, we used again the Czechanowski index. Observed and expected indices were tested for statistical significance with EcoSim 7.0 as described above, but with equiprobable resource states, suggesting that the chance for the insects to occur is equally high in each grid cell (Gotelli and Entsminger 2001). To assess the effect of the habitat manipulation, we conducted a MANOVA and one-way-ANOVAs using "treatment" (manipulated vs non-manipulated) as explanatory variable and the number of specimens per grid cell as response variable. Species and census dates were tested separately.

We applied a non-metric multidimensional scaling (NMDS) to test for correlations between the vegetation parameters (vegetation cover, maximum vegetation height) and soil moisture in the grid using the function "metaMDS", which is implemented in the community ecology package vegan 1.6–10 for R (Oksanen et al. 2005). This function first transforms the data by Wisconsin double standardization and applies Bray–Curtis dissimilarities as a measure of ecological distance.

To evaluate the most important variables for the species, the locations of *T. ceperoi* and *T. subulata* were fitted as vectors onto the NMDS plot, using the function “envfit” with 1000 random permutations as outlined above (Oksanen et al. 2005). All statistical analyses were carried out with “R 2.4.0” (Anonymous 2006). All error measures provided refer to standard errors.

Results

Reproductive interference

The frequencies of encounters with con- and hetero-specific individuals differed significantly between the species (Fig. 1, χ^2 cross table test: DF = 3, $\chi^2 = 32.02$, $p < 0.01$). The proportion of total conspecific encounters was higher for *T. ceperoi* males (76.1%) than for *T. subulata* males (42.8%). In both species, the number of encounters with heterospecifics and conspecifics correlated with the frequencies of male mating attempts (Fig. 1). Males of both species directed more mating attempts towards females than towards males compared to the relative frequencies of encounters, but did not discriminate against heterospecific females (Fig. 1, χ^2 cross table test, DF = 3, *T. ceperoi*: $\chi^2 = 11.08$, $p < 0.05$, *T. subulata*: $\chi^2 = 15.70$, $p < 0.01$). Females of *T. ceperoi* rejected significantly more heterospecific mating attempts (100%, $n = 6$) than conspecific approaches (36%, $n = 11$, Fisher’s exact test, $p = 0.035$). Defensive reactions of *T. subulata* females were rarely observed (3 reactions per 19 approaches) and no significant difference was found in the number of defensive reactions towards con- and heterospecific males.

Microhabitat preferences

Tetrix ceperoi and *T. subulata* differed significantly in microhabitat utilization with respect to vegetation

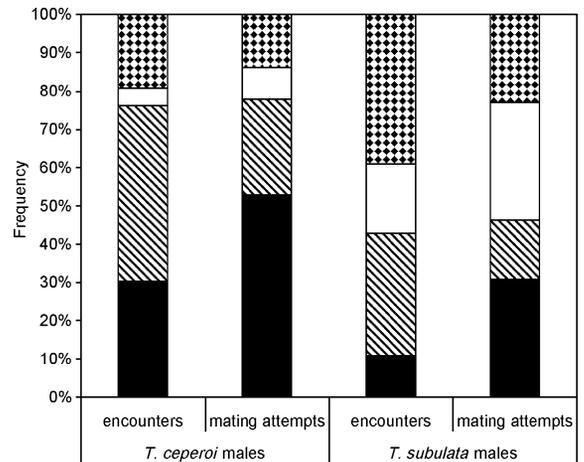


Fig. 1. Relative frequencies of encounters and mating attempts with con- and heterospecific individuals for males of *T. ceperoi* and *T. subulata*. Black segments: conspecific females, hatched segments: conspecific males, white segments: heterospecific females, chequered segments: heterospecific males. *T. ceperoi* males: encounters $n = 109$, mating attempts $n = 36$; *T. subulata* males: encounters $n = 138$, mating attempts $n = 26$. Note, that males of both species were able to correctly identify the sexes, but not the species.

height and the cover of bare ground, grasses and litter (Table 1, Fig. 2). The vegetation height was higher at the locations of *T. subulata* ($23.0 \text{ cm} \pm 1.16$) than at the locations of *T. ceperoi* ($18.6 \text{ cm} \pm 1.02$). A similar pattern was found for the cover of grasses and litter, which were more abundant at the locations of *T. subulata*, whereas the cover of bare ground was lower than in the surrounding of *T. ceperoi* (Fig. 2). Other vegetation parameters, as well as the temperatures of the insects’ body and the substrate did not differ significantly between the species. The radiation was significantly higher at the male than at the female locations (males: $74\,942 \text{ Lux} \pm 1,895$, females: $67\,923 \text{ Lux} \pm 1,977$). No significant statistical interactions

Table 1. Results of the two-way ANOVAs, using species (*T. ceperoi* vs *T. subulata*) and sex as explanatory variables (DF = 1,456). The transformation (λ) refers to the optimal power transformation to fit the data to meet the model assumptions. Note that the sexes only differed significantly with respect to radiation. No significant interaction occurred. The factors mosses, shrubs and forbs were tested with Kruskal–Wallis rank sum test, but were not significant for species or sex.

Factor	Transformation λ	Species		Sex		Species : sex	
		F	p	F	p	F	p
Radiation	–	3.30	0.070	6.59	0.011*	0.12	0.729
Substrate temperature	–	1.51	0.219	0.96	0.328	1.84	0.175
Body temperature	–	1.12	0.292	1.04	0.309	0.88	0.350
Vegetation height	0.23	10.37	0.001*	0.42	0.518	0.68	0.411
Vegetation cover							
Bare ground	–	9.72	0.002*	0.40	0.528	2.79	0.096
Grasses	0.56	4.30	0.039*	0.65	0.420	1.67	0.197
Litter	–	4.84	0.028*	0.73	0.392	1.37	0.242

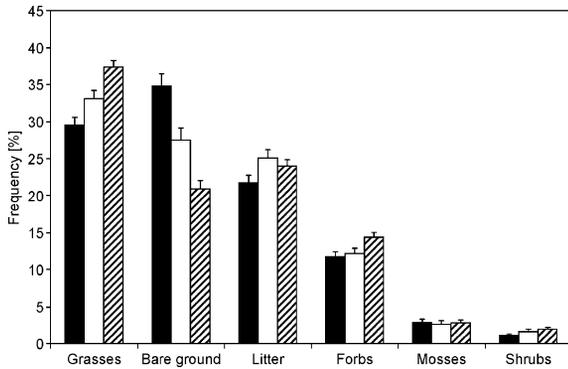


Fig. 2. Average cover of vegetation parameters in a circle of 30 cm surrounding the location of *T. ceperoi* (black segments, n = 230), *T. subulata* (white segments, n = 230) and the control (hatched segments, n = 460). The control samples of both species were combined since they were not significantly different. Error bars are standard errors. Note, that the differences to the controls were generally higher in *T. ceperoi* than in *T. subulata*.

occurred (Table 1). The relative frequencies of substrate types on which the insects perched differed significantly between the species (χ^2 cross table test, DF = 4, $\chi^2 = 27.62$, $p < 0.001$), but not between the sexes. While *T. ceperoi* was found more often on bare ground and mosses, *T. subulata* used to perch more frequently on litter.

We compared the variances of each parameter between the species to estimate differences in niche breadths (Gröning et al. 2006). The variances of radiation, vegetation height and cover of shrubs were smaller at the locations of *T. ceperoi* than at the locations of *T. subulata* (Fisher's F-test, DF = 229, radiation: $F = 0.75$, $p = 0.033$; vegetation height: $F = 0.76$, $p = 0.042$; shrubs: $F = 0.43$, $p < 0.001$). The observed niche overlap of the species was significantly greater than expected by chance for the habitat descriptors radiation, vegetation height, cover of grasses and forbs. In these four parameters the values

were rather high, ranging from 84.2% (radiation) to 96.3% (vegetation height, Table 2). For all other microhabitat features (substrate, substrate temperature, cover of bare ground, mosses, shrubs and litter), the niche overlap between *T. ceperoi* and *T. subulata* did not differ from a random pattern.

The analyses also revealed significant differences between the locations of the insects and the associated control samples in one metre distance, suggesting a non-random distribution of the ground-hoppers (Table 3, Fig. 2). While temperature, radiation and cover of bare ground were significantly higher at the locations of *T. ceperoi* and *T. subulata*, vegetation height and cover of grasses and forbs were higher at the controls. The parameter "cover of shrubs" was only significant for *T. ceperoi*, with higher values at the location of the control. No significant differences were found for "cover of mosses" and "cover of litter".

The frequencies of utilized substrates also differed between the species and the controls (*T. ceperoi*: χ^2 cross table test, DF = 4, $\chi^2 = 78.05$, $p < 0.001$, *T. subulata*: Fisher's exact test, DF = 4, $p < 0.001$). The insects were found more often on bare ground, but less frequently on grasses. *T. subulata* perched more often on litter than would be expected by the availability of this substrate. The variances of nearly all environmental parameters were significantly smaller at the location of *T. ceperoi* than at the control, except for the factors "substrate temperature" and "cover of bare ground". For *T. subulata* only the variances of vegetation height, cover of forbs and shrubs differed significantly, being smaller at the location of the insect than at the control (Table 4).

A plot of the first two principal components (explaining 44.4% of the total variance) is given in Fig. 3, including the vectors of the insects' locations and the control samples. In this multidimensional framework there was a positive correlation between *T. ceperoi* locations and the factors "bare ground", "temperature" and "radiation", whereas the locations were negatively correlated with dense and high vegetation. This

Table 2. Observed and expected mean niche overlap of *T. ceperoi* and *T. subulata*, calculated with EcoSim 7.0. For each data set 10.000 replicates were created in the simulation. *denotes significant p-values.

	Observed mean niche overlap	Expected mean niche overlap	p observed > expected
Radiation	0.842	0.747	0.009*
Substrate temperature	0.332	0.337	0.491
Substrate	0.734	0.615	0.125
Vegetation height	0.963	0.189	0.003*
Vegetation cover			
Bare ground	0.775	0.707	0.061
Mosses	0.729	0.653	0.150
Grasses	0.895	0.646	<0.001*
Forbs	0.952	0.600	<0.001*
Shrubs	0.560	0.594	0.545
Litter	0.162	0.214	0.744

Table 3. Results of the paired t tests between the habitat parameters at the location of both ground-hopper species and the corresponding control sample (DF =229, except for vegetation height: *T. ceperoi* DF =228, *T. subulata* DF =227). Positive t values indicate a higher value at the location of the insects than at the control and vice versa. *denotes significant p-values.

Factor	<i>T. ceperoi</i>		<i>T. subulata</i>	
	t	p	t	p
Radiation	4.66	<0.001*	3.56	<0.001*
Substrate temperature	5.06	<0.001*	7.14	<0.001*
Vegetation height	-5.67	<0.001*	-3.08	0.002*
Vegetation cover				
Bare ground	8.87	<0.001*	3.99	<0.001*
Mosses	-0.54	0.591	0.07	0.943
Grasses	-5.49	<0.001*	-2.47	0.014*
Forbs	-2.75	0.006*	-2.15	0.033*
Shrubs	-2.66	0.008*	-0.55	0.580
Litter	-1.33	0.184	0.30	0.765

correlation was slightly stronger in *T. ceperoi* males ($R^2 = 0.028$, $p < 0.001$) than in females ($R^2 = 0.011$, $p = 0.009$). The locations of *T. subulata* were not significantly correlated with the environmental parameters, whereas the pooled control samples for both species were correlated with the vegetation matrix ($R^2 = 0.058$, $p < 0.001$).

Grid mapping and habitat manipulation

In total 360 individuals of *T. ceperoi* and 577 individuals of *T. subulata* were marked on the three census dates. While during the first count (15 May) *T. subulata* was much more common than *T. ceperoi* (360/128), its abundance decreased during the season until *T. ceperoi* was more abundant at the last census date (21 June: 108/146). Morisita's index of dispersion (Table 5) revealed a clumped dispersion of *T. ceperoi* at each date (mean $I_d = 2.50$), whereas the distribution of *T. subulata* was less aggregated (mean $I_d = 1.44$). The degree of interspecific aggregations (both species

combined) was lower compared to the intraspecific aggregations of *T. ceperoi*, but greater than the intraspecific aggregations of *T. subulata*. On each census date, the spatial niche overlap of *T. ceperoi* and *T. subulata* was greater than expected by chance (mean 54.6%, Table 5). There was no substantial change in niche overlap and dispersion indices after the habitat manipulation.

The abundance of *T. ceperoi* and *T. subulata* differed significantly between the mowed grid cells and the controls (MANOVA, DF = 1, $p < 0.001$, $F = 6.90$). This effect was stronger for *T. ceperoi* than for *T. subulata*. More individuals of *T. ceperoi* were found in the mowed grid cells than in the controls on both dates after the manipulation (8 June: ANOVA, $\lambda = 0.20$, $F_{1,60} = 20.97$, $p < 0.001$; 21 June: ANOVA, $\lambda = 0.17$, $F_{1,60} = 13.73$, $p < 0.001$). For *T. subulata* a higher number of specimens in the mowed grid cells was only found at the first date after the habitat manipulation (8 June: ANOVA, $\lambda = 0.33$, $F_{1,60} = 4.37$, $p = 0.041$).

Table 4. Results of Fisher's F-tests between the location of both ground-hopper species and the corresponding control sample (*T. ceperoi* DF =228, *T. subulata* DF =227). Note that in all significant cases V_{TC} (variance at the location of *T. ceperoi*) and V_{TS} (variance at the location of *T. subulata*) are smaller than V_{CC} and V_{CS} (variance at the corresponding control samples), respectively. The values for radiation (Lux) were divided by 1 000 000. *denotes significant p-values.

Factor	<i>T. ceperoi</i>				<i>T. subulata</i>			
	V_{TC}	V_{CC}	F	p	V_{TS}	V_{CS}	F	p
Radiation	748.05	1023.71	0.73	0.018*	982.88	1181.13	0.83	0.167
Substrate temperature	15.66	16.25	0.96	0.780	16.91	16.75	1.01	0.941
Vegetation height	237.94	473.40	0.50	<0.001*	309.71	412.51	0.75	0.031*
Vegetation cover								
Bare ground	650.88	588.76	1.11	0.450	616.83	544.96	1.13	0.351
Mosses	46.51	65.15	0.71	0.011*	56.71	60.24	0.94	0.650
Grasses	231.99	408.79	0.57	<0.001*	294.96	358.13	0.82	0.145
Forbs	111.27	181.91	0.61	<0.001*	120.22	211.03	0.57	<0.001*
Shrubs	10.43	27.57	0.38	<0.001*	22.66	27.21	0.83	<0.001*
Litter	240.61	319.97	0.75	0.032*	292.62	325.50	0.90	0.423

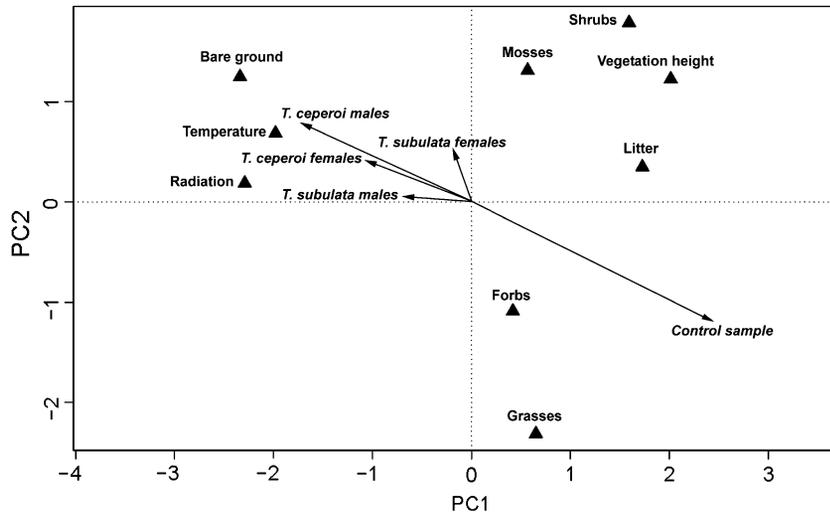


Fig. 3. Plot of the first two principal components of a standardized PCA of the environmental parameters at the exact location of the insects (explaining 44.4% of the total variance). The factors were standardized to unit variance using correlation coefficients to achieve a more balanced ordination. The locations of *T. ceperoi* and *T. subulata* were fitted as vectors onto the ordination. There was no significant correlation for *T. subulata* males and females with the environmental parameters. The strong correlation of *T. ceperoi* locations with the factors “bare ground”, “temperature” and “radiation” is apparent.

Fig. 4 illustrates the correlation of the species' distributions with the vegetation parameters and soil moisture in the grid after the habitat manipulation. The distribution of *T. ceperoi* was correlated with the vegetation matrix on both dates (8 June 2006: $R^2 = 0.245$, $p = 0.002$; 21 June 2006: $R^2 = 0.260$, $p = 0.001$). Its distribution pattern was mainly explained by NMDS 2, which was positively correlated with bare ground and negatively with vegetation height and cover of shrubs. A significant correlation between the distribution of *T. subulata* and the vegetation parameters was only found on 8 June 2006 ($R^2 = 0.204$, $p = 0.006$). It was positively correlated with cover of litter, mosses and bare ground. Both *Tetrix* species were concentrated in the manipulated grid cells, which are well separated from the control cells by the second axis of the NMDS. The controls had a higher variability than the treated cells and were positively correlated with “cover of shrubs” and “vegetation height”. The

manipulated grid cells were less variable and characterized by a higher proportion of bare ground.

Discussion

Our results show that reproductive interference between *T. ceperoi* and *T. subulata* does also occur among free-ranging individuals in the field. Thus, sexual interactions between these species do not simply represent an experimental artefact. However, there are some important differences between the laboratory results and the field observations, stressing the need of field validations as suggested by many authors (Verrel 1994, Zhang et al. 2004, Ficetola and De Bernardi 2005). Based on the experimental data, *T. ceperoi* seemed to be the inferior species in this system (Hochkirch et al. in press). The strong asymmetric mate preference of *T. ceperoi* males for *T. subulata*

Table 5. Dispersion of *T. ceperoi*, *T. subulata*, as well as both species combined (X_i = mean number of specimens per grid cell, I_d = Morisita's index of dispersion, χ^2 = test for random distribution, $DF = 61$, all $p < 0.05$) for the three census dates. Observed (C_O) and expected (C_E) mean niche overlaps were tested with EcoSim 7.0. The p-value indicates the probability for the observed niche overlap to be greater than the expected. *denotes significant p-values.

Census date	<i>T. ceperoi</i>			<i>T. subulata</i>			Both species			Spatial niche overlap		
	X_i	I_d	χ^2	X_i	I_d	χ^2	X_i	I_d	χ^2	C_O	C_E	p
15 May 2006	2.07	2.72	279.84	5.81	1.29	163.90	7.87	1.42	266.42	0.57	0.41	<0.001*
8 June 2006	2.60	2.56	310.74	3.39	1.58	182.08	5.98	1.80	356.79	0.58	0.36	<0.001*
21 June 2006	2.36	2.20	235.34	1.74	1.45	109.00	4.10	1.57	204.90	0.49	0.37	0.008*

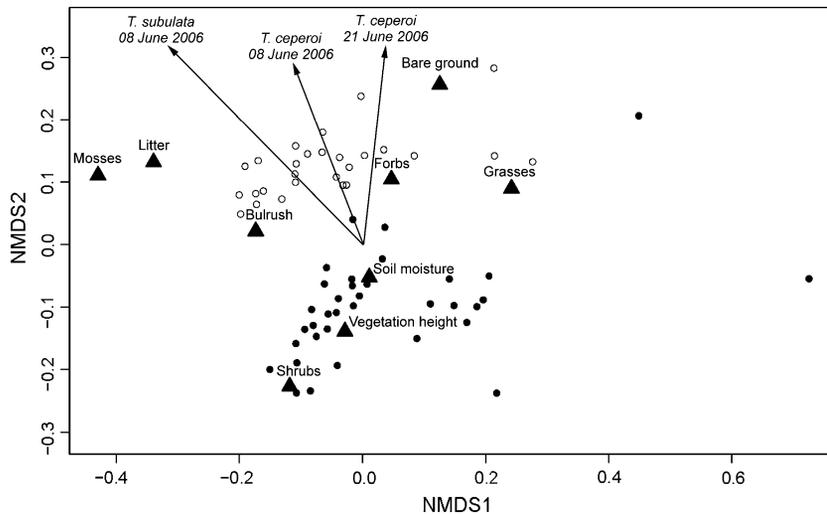


Fig. 4. Plot of the first two axes of a nonmetric multidimensional scaling (NMDS), illustrating the correlation of the species distribution (fitted as vectors onto the plot) with the vegetation matrix in the grid (open circles: manipulated grid cells, black dots: controls). Manipulated grid cells were characterized by a higher amount of bare ground, whereas cover of shrubs and vegetation height were higher in the controls. The distribution of *T. ceperoi* was strongly correlated with bare ground, whereas *T. subulata* had a higher preference for litter and was only correlated to the vegetation matrix at the first census date.

females was not confirmed by our field data. Instead, males of both species misdirected their mating behaviour in the field and *T. subulata* was even more affected. The sexual interactions depended on the encounter frequencies and the sex of the encountered individuals. Since *T. subulata* males came across heterospecifics more often than *T. ceperoi* males did, the number of heterospecific mating attempts was also higher in the former species. While males of both species failed to recognize their own species, they had a better ability to distinguish between the sexes, as they performed more mating attempts with females than expected by chance. Thus, the mate recognition system seems to be incomplete in males of both species and even mating attempts with males sometimes occurred. Indiscriminate mate choice has been reported in males of many taxa (Andersson 1994) and seems to be a suitable strategy, if the encounter rates with receptive females are low and the courtship is of short duration (Dukas et al. 2006). However, in laboratory experiments the number of heterospecific interactions may be increased due to artificially high densities or small arena sizes (Ficetola and De Bernardi 2005). Indeed, the encounter frequencies were much higher in the mixed treatments of the laboratory experiment (10% of the observation time of both species and sexes) compared to the field situation (ca 1%) where the population density was lower. These results show that reproductive interference is density-dependent, as has been proposed by several authors (Kuno 1992, Westman et al. 2002, Hettley and Pearman 2003).

Certainly, the outcome of reproductive interference is also influenced by female mate recognition, since females are generally considered to be the “choosy sex” (Andersson 1994). Males of both *Tetrix* species differ substantially in their visual courtship displays, which might help females to identify mates correctly (Hochkirch et al. 2006). In laboratory experiments *T. subulata* females rejected *T. ceperoi* males to a higher proportion than mounts of conspecific males. In the field *T. subulata* females were less choosy (only three rejections were observed), which might be related to the small number of conspecific contacts compared to the laboratory. In contrast, females of *T. ceperoi* were more selective in the field than in the laboratory, where they did not discriminate against heterospecific males. Hence, it remains difficult to assess in which species the female mate recognition system is more reliable. Erroneous female choice has also been reported in other taxa, such as *Drosophila* flies (Tomaru et al. 2000), crickets (Gray 2005) and fishes (Ryan and Wagner 1987) and sometimes lead to heterospecific matings (Tomaru et al. 2000).

It has been shown that even in the absence of heterospecific matings, reproductive interference can substantially reduce the reproductive success (Hochkirch et al. in press). The consequences of such interactions can be rather dramatic and might lead to demographic displacement of the inferior species (Ribeiro and Spielman 1986, Söderbäck 1994, Westman et al. 2002). However, similar to resource competition the costs of reproductive interference can be mitigated by several ecological and evolutionary

mechanisms, allowing sexually interacting species to coexist. The ecological mechanisms enhance the fitness by decreasing the relative frequencies of heterospecific encounters (Verrel 1994). These mechanisms can be divided into segregative mechanisms (spatial, temporal or habitat segregation), dilution effects (intraspecific aggregations, higher abundance of the inferior species) and life history effects (dispersal and colonization ability, population dynamics).

Initially, we aimed at examining two major hypotheses of coexistence, resource partitioning (Schoener 1974) and aggregations (Hanski 1981, Atkinson and Shorrocks 1984), which are frequently discussed in the context of resource competition. Our data support the idea that coexistence can be promoted by intraspecific aggregations alone even in the absence of resource partitioning (Wertheim et al. 2000). In the case of *T. ceperoi* and *T. subulata* the niche overlap was too strong to cause spatial separation. Nevertheless, the spatial overlap of the species was reduced due to their differential dispersion patterns, which seem to be strongly determined by the niche breadth of the species. Niche breadth should, therefore, be more often considered in aggregation models of coexistence. *T. ceperoi* was much more specialized than *T. subulata* as illustrated by the variances of the microhabitat parameters. Although both ground-hoppers preferred bare patches with a warm microclimate, the preference for bare ground was more distinct in *T. ceperoi*, leading to stronger aggregations compared to *T. subulata*, which also occurred at more densely vegetated locations and generally had a broader niche. This is supported by the results of the habitat manipulation, which did not increase the spatial segregation. The aggregation model proposes that coexistence is facilitated if intraspecific aggregations are stronger than interspecific aggregations (Kuno 1988). Indeed, this pattern was found in *T. ceperoi*, which was the inferior species in the laboratory, but not in *T. subulata*. Aggregation models of coexistence assume an enhanced intraspecific competition within the conspecific aggregations (Ives 1988). In the case of reproductive interference, conspecific aggregations should increase the fitness of a species, since the probability of conspecific mating is higher. Thus, in contrast to resource competition the costs of interspecific interactions are substantially higher than the costs of intraspecific encounters. Corresponding to its broader ecological niche, *T. subulata* was more evenly distributed on the site and faces higher costs within the aggregations of *T. ceperoi*. However, it may compensate these costs due to its broader niche that enables the species to occur at places where *T. ceperoi* is missing.

Despite the potential of different dispersion patterns to facilitate coexistence, it is striking that these two species rarely co-occur in northwestern Germany

(Gröning et al. 2005). Kuno (1992) hypothesized that the stable coexistence of interfering insect species depends primarily upon the almost complete avoidance of mating interference. However, this hypothesis does not take into account that the outcome of species interactions may also be altered by other forces, such as predation or life history parameters (Gherardi and Cioni 2004): For instance, the reproductive capacity seems to be higher in *T. subulata* (Hochkirch et al. in press), which may affect the costs of reproductive interference (Westman et al. 2002). Moreover, different colonization speeds may influence the coexistence of sexually interfering species (Hastings 1980). The colonization of a site which is already occupied might be difficult for the second species ("preemption competition" Calcagno et al. 2006), as the immigrants will have problems to find conspecific mates. Since the study site has been colonized only recently, the coexistence of *T. ceperoi* and *T. subulata* might still be dynamic (Boyer and Rivault 2006). It remains to be examined whether one species will be excluded in a longer term. Changes in habitat quality might be of higher importance for the survival of *T. ceperoi* than reproductive interference. Since the species depends on the availability of bare patches (Gröning et al. 2006), the extinction of *T. ceperoi* might be driven by natural succession before the costs of reproductive interference will have any effect on population dynamics. The damp areas of the study site presently undergo a fast succession by young willows and bulrush, which might render the floodplain habitats unsuitable, particularly for *T. ceperoi*.

In conclusion, our results demonstrate that the significance of reproductive interference in the field is strongly influenced by the ecological specialization of the involved species. Based on the principle of competitive exclusion (Gause 1934), we expected that reproductive interference is altered by resource partitioning (Schoener 1974). However, our results show that coexistence is possible despite a strong niche overlap as long as the two species involved differ in niche breadth. These differences strongly affect the dispersion patterns and, thus, mitigate the costs of reproductive interference. Intraspecific aggregations are widespread in animals (Parish and Edelstein-Keshet 1999) and generally believed to reduce the costs of interspecific interactions, such as competition (Hanski 1981, Ives 1988) or predation (Ruxton and Sherratt 2006). The significance of aggregations has rarely been addressed in the context of reproductive interference (but see Ficetola and De Bernardi 2005), whereas habitat partitioning has often been considered (Samways 1977, Kuno 1992, Fujimoto et al. 1996, Jackson and Tinsley 1998, McLain and Pratt 1999). Our results illustrate that aggregations are strongly influenced by

niche breadths, which should be addressed more often when analysing mechanisms of species coexistence.

Acknowledgements – We wish to thank Sebastian Dittrich, Andreas Flohre, Sascha Krause, Andreas Michalik, Claudia Normann, Monika Riepl, Felix Schaefer, Stefan Thiele and Axel Tschuschke for their excellent assistance in the field. We are grateful to the Ecology group of the Univ. of Osnabrück, especially Kathrin Witzemberger and Anselm Kratochwil, for helpful discussions and valuable comments on the manuscript. Financial support was provided by research grants to JG (GradFöG, Reinhold-Tüxen-Gesellschaft e.V., Forschungspool of the Univ. of Osnabrück).

References

- Andersson, M. 1994. Sexual selection. – Princeton Univ. Press.
- Andrews, R. H. et al. 1982. Reproductive interference between three parapatric species of reptile tick. – *Oecologia* 52: 281–286.
- Anonymous 2006. R: a language and environment for statistical computing. – R Development Core Team 2006. <http://www.R-project.org>
- Atkinson, W. D. and Shorrocks, B. 1984. Aggregation of larval Diptera over discrete and ephemeral breeding sites: the implications for coexistence. – *Am. Nat.* 124: 336–351.
- Boyer, S. and Rivault, C. 2006. Habitat selection and coexistence of invasive cockroach species (Dictyoptera) in sugar-cane field on Réunion island. – *Acta Oecol.* 29: 16–26.
- Brown, W. L. and Wilson, E. O. 1956. Character displacement. – *Syst. Zool.* 7: 49–64.
- Bull, C. M. and Burzacott, D. 1994. Reproductive interactions between two Australian reptile tick species. – *Exp. Appl. Acarol.* 18: 555–565.
- Calcagno, V. et al. 2006. Coexistence in a metacommunity: the competition–colonization tradeoff is not dead. – *Ecol. Lett.* 9: 897–907.
- Crawley, M. J. 2005. Statistical computing: an introduction to data analysis using S-Plus. – Wiley.
- Dukas, R. et al. 2006. Courtship of male insects: when is learning advantageous? – *Anim. Behav.* 72: 1395–1404.
- Ficetola, G. F. and De Bernardi, F. 2005. Interspecific social interactions and breeding success of the frog *Rana latastei*: a field study. – *Ethology* 111: 764–774.
- Fujimoto, H. et al. 1996. Reproductive interference between *Panonychus mori* Yokoyama and *P. citri* (McGregor) (Acari: Tetranychidae) in peach orchards. – *Appl. Entomol. Zool.* 31: 59–65.
- Gause, G. F. 1934. The struggle for existence. – Williams and Wilkins.
- Gherardi, F. and Cioni, A. 2004. Agonism and interference competition in freshwater decapods. – *Behaviour* 141: 1297–1324.
- Gotelli, N. J. and Entsminger, G. L. 2001. EcoSim: Null models software for ecology (ver. 7.0). – Acquired Intelligence Inc. and Kesey-Bear. <http://homepages.together.net/~gentsmin/ecosim.htm>.
- Gray, D. A. 2005. Does courtship behavior contribute to species-level reproductive isolation in field crickets? – *Behav. Ecol.* 16: 202–206.
- Grein, G. 2005. Rote Liste der in Niedersachsen und Bremen gefährdeten Heuschrecken, 3. Fassung. – *Inf.dienst. Nat.schutz. Niedersachs.* 25: 1–20.
- Gröning, J. et al. 2005. Dornschröcken (Orthoptera, Tetrigidae) auf den Ostfriesischen Inseln – Verbreitung, Koexistenz und Ökologie. – *Entomol. Heute* 17: 47–63.
- Gröning, J. et al. 2006. Habitat preferences of an endangered insect species, Cepero's ground-hopper (*Tetrix ceperoi*). – *Ecol. Res.* doi: 10.1007/s11284-006-0315-2.
- Hanski, I. 1981. Coexistence of competitors in patchy environments with and without predation. – *Oikos* 37: 306–312.
- Hastings, A. 1980. Disturbance, coexistence, history, and competition for space. – *Theor. Popul. Biol.* 18: 363–373.
- Hetttyey, A. and Pearman, B. 2003. Social environment and reproductive interference affect reproductive success in the frog *Rana latastei*. – *Behav. Ecol.* 14: 294–300.
- Hochkirch, A. et al. 2000. Specialized diet and feeding habits as key factors for the habitat requirements of the grasshopper species *Tetrix subulata* (Orthoptera: Tetrigidae). – *Entomol. Gen.* 25: 39–51.
- Hochkirch, A. et al. 2006. Visual communication behavior of three pygmy grasshoppers (Orthoptera, Tetrigidae). – *J. Insect. Behav.* 19: 559–571.
- Hochkirch, A. et al. 2007. Intersexual niche segregation in Cepero's ground-hopper, *Tetrix ceperoi*. – *Evol. Ecol.* doi: 10.1007/s10682-006-9147-3.
- Hochkirch, A. et al. in press. Sympatry with the devil – reproductive interference could hamper species coexistence. – *J. Anim. Ecol.* doi: 10.1111/j.1365-2656.2007.01241.x.
- Ingrisch, S. and Köhler, G. 1998. Die Heuschrecken Mitteleuropas. – Westarp Wissenschaften.
- Ives, A. R. 1988. Aggregation and the coexistence of competitors. – *Ann. Zool. Fenn.* 25: 75–88.
- Jackson, J. A. and Tinsley, R. C. 1998. Reproductive interference in concurrent infections of two *Protopolystoma species* (Monogenea: Polystomatidae). – *Int. J. Parasitol.* 28: 1201–1204.
- Kleukers, R. et al. 1997. De Sprinkhanen en Krekels van Nederland (Orthoptera). *Nederlandse Fauna I.* – KNNV Uitgeverij and EIS-Nederland.
- Krebs, C. J. 1999. Ecological methodology. – Addison Wesley Longman.
- Kuno, E. 1988. Aggregation pattern of individuals and the outcomes of competition within and between species: differential equation models. – *Res. Popul. Ecol.* 30: 69–82.
- Kuno, E. 1992. Competitive exclusion through reproductive interference. – *Res. Popul. Ecol.* 34: 275–284.
- McLain, D. K. and Pratt, A. E. 1999. The cost of sexual coercion and heterospecific sexual harassment on the fecundity of a host-specific, seed-eating insect (*Neacoryphus bicrucis*). – *Behav. Ecol. Sociobiol.* 46: 164–170.
- Oksanen, J. et al. 2005. The vegan Package. <http://cc.oulu.fi/~jarioksa/>.

- Parish, J. K. and Edelman-Keshet, L. 1999. Complexity, pattern, and evolutionary tradeoffs in animal aggregation. – *Science* 284: 99–101.
- Ribeiro, J. M. C. and Spielman, A. 1986. The Satyr effect: a model predicting parapatry and species extinction. – *Am. Nat.* 128: 513–528.
- Ruxton, G. D. and Sherratt, T. N. 2006. Aggregation, defence and warning signals: the evolutionary relationship. – *Proc. R. Soc. Lond. B.* 273: 2417–2424.
- Ryan, M. J. and Wagner, W. E. 1987. Asymmetries in mating preferences between species: female swordtail fish prefer heterospecific males. – *Science* 236: 595–597.
- Samways, M. J. 1977. Effect of farming on population movements and acoustic behaviour of two bush crickets (Orthoptera: Tettigoniidae). – *Bull. Entomol. Res.* 67: 471–481.
- Schoener, T. W. 1974. Resource partitioning in ecological communities. – *Science* 185: 27–39.
- Singer, F. 1990. Reproductive costs arising from incomplete habitat segregation among three species of *Leucorrhinia* dragonflies. – *Behaviour* 115: 188–202.
- Söderbäck, B. 1994. Reproductive interference between two co-occurring crayfish species, *Astacus astacus* and *Pacifastacus leniusculus*. – *Nordic J. Freshwater Res.* 69: 137–143.
- Tomaru, M. et al. 2000. Courtship song recognition in the *Drosophila melanogaster* complex: heterospecific songs make female receptive in *D. melanogaster* but not in *D. sechellia*. – *Evolution* 54: 1286–1294.
- Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S. – Springer.
- Verrel, P. A. 1994. Is decreased frequency of mating among conspecifics a cost of sympatry in salamanders? – *Evolution* 48: 921–925.
- Wertheim, B. et al. 2000. Species diversity in a mycophagous insect community: the case of spatial aggregation vs resource partitioning. – *J. Anim. Ecol.* 69: 335–351.
- Westman, K. et al. 2002. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a small, enclosed Finnish lake: a 30-year study. – *Ecography* 25: 53–73.
- Winemiller, K. O. and Pianka, E. R. 1990. Organization in natural assemblages of desert lizards and tropical fishes. – *Ecol. Monogr.* 60: 27–56.
- Zhang, J. et al. 2004. Influence of increasing number of mating partners on mating behaviour and reproduction in Brandt's voles. – *Folia Zool.* 53: 357–365.