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Climatic effects on population declines of a rare wetland species and the role of spatial and temporal isolation as barriers to hybridization

Katja Rohde*, Yvonne Hau, Nicole Kranz, Jasmin Weinberger, Ortwin Elle and Axel Hochkirch

Department of Biogeography, Trier University, D-54286 Trier, Germany

Summary

1. Climate change and climatic extremes may affect species directly or indirectly. While direct climatic effects have been intensively studied, indirect effects, such as increasing hybridization risk, are poorly understood.

2. The goal of our study was to analyse the impact of climate on population dynamics of a rare habitat specialist, *Chorthippus montanus*, as well as the fine-scale spatial overlap with a sympatric habitat generalist, *Chorthippus parallelus* and the dispersion of hybrids. We were particularly interested in the role of spatiotemporal overlap on heterospecific encounter frequencies.

3. We conducted high-precision mark-recapture studies on two sites over 7 years and genotyped 702 individuals of two *C. montanus* generations to detect hybrids. We tested the performance of three programs (STRUCTURE, NEWHYBRIDS and ADEGENET) and accepted only hybrids detected by the two best performing programs. We then tested for correlations between yearly population trends and climatic variables. Furthermore, we analysed the spatial dispersion of both taxa and the hybrids to calculate variation in spatial and temporal overlap and infer heterospecific encounter probabilities.

4. Our results revealed that droughts during the egg phase and rainy weather during nymphal development were strongly correlated with population declines in the habitat specialist. The highest hybridization rate (19·6%) was found in the population with lowest population size. The combined effects of spatial and temporal niche overlap decreased heterospecific encounter probabilities to 4·2–7·6% compared to 20–28% and 11–19% calculated alone from phenology or spatial overlap respectively. Hybrids were detected in areas of higher heterospecific encounter probability, mainly at the edge of the specialists' occupied habitat in areas with intermediate soil moisture conditions compared to the parental species.

5. This illustrates that the combination of spatial and temporal segregation provides an effective barrier to hybridization. However, the high hybridization rate in one of the populations suggests that this function may decrease with decreasing population size. This supports the hypothesis that climatic extremes threaten rare species directly by reducing reproductive success and may indirectly increase hybridization risk.

Key-words: climate change, extreme climatic events, genetic displacement, grasshopper, microsatellite, Orthoptera, reproductive barriers, sympatric species

Introduction

It is widely agreed that climate change can have dramatic consequences for ecosystems, biodiversity and species' distributions. Habitat modifications, range shifts of species as well as altered species interactions are considered the most important consequences (Visser & Both 2005; Parmesan 2006; Chunco 2014). The extent and rate of responses to climate change vary strongly depending on the species involved and their physiological tolerances, dispersal ability and life-history strategies (Parmesan 2006; Chunco 2014; Sánchez-Guillén *et al.* 2014). Short-lived species

*Correspondence author. E-mail: rohdek@uni-trier.de

generally seem to respond faster than long-lived species, however, even closely related species could differ in their response as a result of their differing ecological specialization (Tingley *et al.* 2009; Sánchez-Guillén *et al.* 2014). Habitat generalists are predicted to be less sensitive to climate change than specialists due to their wider range of tolerances to environmental changes (Gilchrist 2000). Moreover, the effects of climate change are unlikely to proceed in a constant pattern. It is more likely that climatic extremes, such as droughts and strong rainfall, will lead to sudden changes in population dynamics (Hochkirch & Damerau 2009).

The effects of climate change on reproductive interactions between closely related species are poorly understood (Hochkirch & Lemke 2011; Chunco 2014). If the species' responses to climatic changes are asynchronous, equilibria of interactions are likely to become disrupted. This has the potential to alter the function of reproductive barriers or the equilibrium of species interactions leading to an increased potential for hybridization (Engler *et al.* 2013). Range shifts between two species could lead to secondary contact between formerly allopatric species, resulting in novel sympatric populations, which may result in new interspecific interactions and ultimately hybridization (Gröning & Hochkirch 2008; Chunco 2014; Sánchez-Guillén *et al.* 2014). Furthermore, such an asynchrony may alter the dynamics of sympatric populations by increasing their degree of sympatry (Sánchez-Guillén *et al.* 2014). Although sympatric species may have evolved reproductive barriers as a result of negative selection to hybridization (Rundle & Schluter 1998; Hochkirch & Lemke 2011), environmental change could lead to a collapse of reproductive barriers (Seehausen 2006; Crispo *et al.* 2011; Hasselman *et al.* 2014). Besides the effects of shifting ranges, climate change may also influence the phenology of species and thus the temporal reproductive isolation of closely related species (Cleland *et al.* 2007; Parmesan 2007). Additionally, hybrid fitness itself could be affected by climate change (Chunco 2014) and hybrids may even have a better adaptation to the novel environmental conditions than the parental species (Rieseberg 1997; Arnold, Sapir & Martin 2008).

Climatic effects on hybridization interactions are particularly interesting, as they could have severe evolutionary consequences, such as on speciation (Abbott *et al.* 2013), but are also of conservation concern because of genetic displacement processes and outbreeding depression (Rhymer & Simberloff 1996). The consequences of hybridization can vary considerably. Hybridization could lead to new adaptations (Mallet 2005) or may enhance genetic diversity and prevent small populations from inbreeding depression (Mallet 2005; Arnold, Sapir & Martin 2008; Schulte, Veith & Hochkirch 2012). These positive effects of hybridization were generally described for natural hybridization processes, whereas anthropogenic hybridization is more often considered a threat, particularly to rare species and small populations (Rhymer & Simberloff 1996; Seehausen *et al.* 2008). Anthropogenic hybridization is mainly discussed in the context of biological invasions of non-native or domesticated species (Huxel 1999; Allendorf *et al.* 2001; Witzenberger & Hochkirch 2014), whereas indirect facilitation of hybridization via habitat loss or climate change has received only little attention (Seehausen *et al.* 2008; Chunco 2014). Anthropogenic hybridization may lead to a population collapse due to genetic displacement which may result in local extinction of populations or even the complete extinction of a species (Rhymer & Simberloff 1996; Seehausen *et al.* 2008).

The two grasshopper species *Chorthippus montanus* (Charpentier, 1825) and *Chorthippus parallelus* (Zetterstedt, 1821) represent an interesting study system concerning the potential effects of climate on population decline and hybridization risk. Both species occur sympatrically across large parts of Eurasia (Fig. S1, Supporting Information). *Chorthippus montanus* is a habitat specialist adapted to permanently moist habitats, which are naturally fragmented, whereas *C. parallelus* is a widely distributed habitat generalist occurring in a variety of non-arid grasslands (Detzel 1998). Both species are closely related and morphologically very similar, but have distinctive songs (Reynolds 1980). Hybridization between both species in the field has been first assumed by Reynolds (1980) and confirmed in a recent study (Rohde *et al.* 2015). Laboratory experiments have shown that hybrids are fertile at least until the third generation (Bauer & von Helversen 1987; Köhler 2013). Hybrids are morphologically either intermediate or similar to *C. parallelus* and perform intermediate songs (Hochkirch & Lemke 2011; Köhler 2013). Even though *C. montanus* females strongly prefer conspecific males as mates, mate choice is frequency dependent and increasing heterospecific frequency increases hybridization probability (Rohde *et al.* 2015).

During the last two decades, *C. montanus* has disappeared from sites below 400 m in the Hunsrueck Mountains (Rhineland-Palatinate, Germany; Weyer, Weinberger & Hochkirch 2012), possibly as an effect of climatic extremes, like the summer heatwave in 2003 or spring drought in 2007 (Figs S2–S4). As single intermediate morphotypes were found at localities with former occurrence of *C. montanus*, we hypothesized that hybridization with *C. parallelus* poses an additional threat to small populations. To test the hypothesis that climatic fluctuations influence the population trend of *C. montanus*, we studied the dynamics of two populations from 2010 to 2016 using a mark-recapture study. We tested for correlations with climatic parameters, including climatic extremes, such as the drought in April 2011. We further hypothesized that the low spatiotemporal overlap between both species is the major pre-mating barrier and more effective than the spatial or temporal overlap alone. Therefore, we conducted a mark-recapture study also for the population of *C. parallelus* on one of the sites from 2011 to 2013 and used a GIS analysis to calculate the spatiotemporal overlap. To test the hypothesis that hybridization rate increases with

decreasing population size and that hybrids are mainly located in areas with high heterospecific encounter probabilities at the edge of the *C. montanus* distribution, we genotyped all *C. montanus* specimens captured in 2012 and 2013 and used two assignment programs (ADEGENET and NEWHYBRIDS) to detect hybrids. Finally, we hypothesized that the species strongly differ in their soil moisture preferences and that hybrids occupy zones of intermediate moisture. We therefore tested for differences in the average soil moisture values of both species and the hybrids.

Materials and methods

STUDY SPECIES

Chorthippus montanus is a univoltine grasshopper species, which occurs in moist habitat types due to the high water requirements of the eggs (Ingrisch 1983; Kleukers *et al.* 1997). Ongoing habitat deterioration caused by land use change represents the main threats for *C. montanus* populations in the study region (Weyer, Weinberger & Hochkirch 2012). The species is classified as Near Threatened in Germany (Maas, Detzel & Staudt 2002) and Vulnerable in Switzerland, the Netherlands and Luxembourg (Proess & Meyer 2003; Monnerat *et al.* 2007; Bakker *et al.* 2015). The species is flightless and its mobility is low (Weyer, Weinberger & Hochkirch 2012). Nymphs hatch in May and adults are found from July to November. *Chorthippus parallelus* is a common univoltine grasshopper species (Kleukers *et al.* 1997). The phenology of both species is slightly shifted, with the nymphs of *C. parallelus* hatching earlier than those of *C. montanus* and becoming adult c. 1 month earlier (Ingrisch & Köhler 1998; Hochkirch & Lemke 2011).

STUDY SITE

Our previous study on the hybridization rate between both species has shown that the populations Reinsfeld1 (R1: N49°40′26·674″; E6°52′59·516″) and Reinsfeld2 (R2: N49°41′11·504″; E6°53′58·412″; Fig. S1) had the highest hybridization rate (R1: 6%; R2: 8·9%; Rohde *et al.* 2015). Although the study sites are located in close vicinity (linear distance 1·7 km) and at similar altitudes (R1: 480 m; R2: 520 m a.s.l.), both *C. montanus* populations showed a striking difference in phenology in 2010 (population maxima R1: 10 August; R2: 1 September). Based on these phenological differences and the potential consequence for phenological overlap with *C. parallelus*, these sites were chosen for our studies of the spatiotemporal population dynamics and occurrence of hybrids. Both study sites represent moist meadows. The *C. montanus* populations are strongly isolated with no gene flow to other populations in the vicinity (J. Weyer, K. Rohde & A. Hochkirch, unpubl. data). The habitat size of R1 is slightly smaller (9353 m^2) than of R2 (11 747 m^2). Both *C. montanus* populations were surrounded by large (and continuous) *C. parallelus* populations.

DATA COLLECTION

In 2010, we conducted a first sampling of c. 40 individuals of each species on both study sites for a preliminary genetic study (Rohde *et al.* 2015). To study the population dynamics of *C. montanus*, a mark-recapture study was performed during 7 years (2010–2016) for both populations. From 2011 to 2013, the *C. parallelus* population on R2 was also studied by mark-recapture to investigate the spatial and temporal population overlap of both species. During each visit, the study site was completely combed and each individual was caught with a net. Each specimen was individually marked with a non-toxic permanent paint marker (Edding 780) using a modified 1-2-4-7 method (Weyer, Weinberger & Hochkirch 2012) and was subsequently released at the same position. Additionally, the following parameters were recorded: number, date, sex and geographical coordinates of the capture position. To analyse the spatial distribution of both species and hybrids, the geographical coordinates were determined with the Trimble®GeoXT™ GPS device (GeoExplorer®2008 series) and a Trimble®GeoBeacon™, (Hesse, Germany) a receiver for high quality real-time differential corrections (accuracy <30 cm). To improve the accuracy, each geographical coordinate was measured 100 times (1 per s) and the mean value was stored. In 2012 and 2013, a hind tarsus of each individual was sampled for genetic analyses. The mark-recapture study was performed from end of July to end of October. The sites were visited every 2–5 days depending on weather conditions. The study was stopped when the number of individuals dropped below three per site in one season, i.e., at the end of October.

MARK-RECAPTURE ANALYSIS

The program MARK 4.3 (White & Burnham 1999) was used to estimate population sizes of both species and sites for each year to analyse population trends. Furthermore, daily population sizes were calculated for each species to test for temporal overlap. The module POPAN was used to perform Jolly–Seber calculations allowing the calculation of population sizes of open populations with differing death and recruitment rates over lifetime. Three different parameters are estimated: the daily survival probability Phi_i including death and emigration, the daily recapture probability P_i and the daily immigration probability $pent_i$ regarding immigration and birth. With these parameters and the recapture data, the total daily population size N_i and the total population size N are estimated (Fric *et al.* 2009). To test the quality of our data, we first ran the full model $(Phi(g \times t)P(g \times t)pent(g \times t)N(g)$; g = sex, t = time) and performed a goodness-of-fit test subsequently. Afterwards, predefined and simplified models were calculated to reduce the number of parameters included. For each of the three parameters, we modified the explanatory variable and combined these in all possible combinations. We tested the interaction term of sex and time ($g \times t$), the addition of sex and time ($g + t$) as well as sex (g) and time (t) independently or as constant parameters (.). For Phi_i, we also tested the addition of sex and a linear trend for temporal effects ($g + T$), which often applies to grasshopper populations as the survival probability decreases with time over the capture season. The best model for population size was chosen using the Akaike Information Criterion with correction for finite sample sizes (AICc).

WEATHER DATA

To investigate the impact of weather on population dynamics of *C. montanus*, we analysed the daily weather data for the years 2010–2014 from the weather station 'Trier Petrisberg' of the German Meteorological Service (DWD). As the sites were strongly affected by anthropogenic measures in 2015, the years 2015–2016 were excluded from the analysis. The weather data were divided into three different phases (phase1 = reproductive phase of the previous year, August–October; phase2 = egg phase, November–April; phase3 = nymphal development, May–July). We tested the impact of the weather variables on the yearly population change using hierarchical partitioning (package hier.part in R), to detect the average independent contribution of each weather variable (Chevan & Sutherland 1991). The following weather variables were selected as they are likely to be important considering the ecology of *C. montanus*: days without rainfall, longest arid period (consecutive days without precipitation), precipitation, air temperature (average, maximum, minimum at ground level), cloud cover,

wind velocity, snow (only in the egg phase) and duration of sunlight on population change. Afterwards, the correlation of population changes with the three strongest explanatory variables predicted by hierarchical partitioning was tested in linear regression models. To illustrate the multidimensional correlations between weather variables during the three different phases and their correlation with population changes, we performed principal component analyses (PCA) in R using the vegan 2.0–10 package (Oksanen *et al.* 2013). Each factor was scaled by its proportional eigenvalue due to the strong variability in the scales of our data. We used environmental fitting (env.fit) with 1000 permutations to test for correlations of the population changes on each site with the PCA functions. This method tests if the true correlation is greater than estimated correlations after random permutation and produces an R^2 measure and significance values. All tests were carried out in R 3.0.2 (R Development Core Team 2014).

GENETIC ANALYSES

To detect hybrids and study the genetic consequences of population size changes, we used microsatellites. DNA was extracted using the DNeasy Blood & Tissue Kit (Quiagen Multiplex, Hilden, Northrhine-Westphelia, Germany). All individuals (N_{total}: 702) were genotyped at 10 polymorphic microsatellite loci. Four microsatellite markers were designed for *C. montanus* prior to this study (Table 1) and six were developed for *C. parallelus* (BF1, BD5, BH5, BD7, BF9 and CD6; Abercrombie *et al.* 2009).

The Quiagen Multiplex was used in multiplexed PCR protocols for a combination of two to four loci with the following annealing temperatures (BF1, BH5, CD6 and CM37: 54 °C; BD5 and CM5: 48 °C; CM33 and CM19: 51 °C; BD7 and BF9: 58 °C). We filled PCR tubes with 10 μL reaction mixes (5·5 μL MultiplexMasterMix, 2 μL water, 1·4 μL genomic DNA (2–10 ng), 1·1 μL primer mix (1 μM per primer)). Amplification was carried out in a Multigene Gradient Thermal Cycler (Labnet) with the following PCR conditions: Initialization: 94 °C per 10 min; Denaturation: 94 °C per 45 s; Annealing: primer specific per 45 s; Extension: 72 °C per 45 s; Final Extension: 72 °C per 30 min; 37 cycles. Each forward primer was labelled with a fluorescent dye (FAM, HEX or TAMRA) at the 5′-end. Fragment lengths of PCR products were determined on a MEGABACE1000 automated sequencer (GE Healthcare, Munich, Bavaria, Germany) and scored with Fragment Profiler 1.2 (Amersham Biosciences, Freiburg im Breisgau, Germany).

NULL ALLELES AND SCORING ERRORS

Potential null alleles and scoring errors due to stuttering or large allele drop-out were investigated using MICROCHECKER v.2.2.3 (Van Oosterhout *et al.* 2004). As Dabrowski *et al.* (2014) found high inconsistencies between and within different methods of null allele detection we also tested for null alleles using FreeNA (Chapuis & Estoup 2007). FreeNA was also used for estimating pairwise F_{ST} values of Weir & Cockerham (1996) between populations and years both without using and using the ENA correction as described by Chapuis & Estoup (2007). To compare corrected and uncorrected F_{ST} values a pairwise t-test was performed in R (R Development Core Team).

SIMULATING AND DETECTING HYBRIDS

To detect hybrids in the dataset, we first simulated hybrids in HYBRIDLAB 1.1 (Nielsen, Bach & Kotlicki 2006). This simulation was based upon a subset of 40 purebred individuals of each parental species and each study site identified during a previous study (Rohde *et al.* 2015). Four classes of hybrids (F1, F2 and backcrosses with both species) with 50 individuals of each class were simulated. We then tested the performance of hybrid detection in this simulated dataset with three programs, STRUCTURE 2.3.4, NEWHYBRIDS (which represent Bayesian approaches) and the R package ADEGENET 1.4–1 (which uses a discriminant analysis of principal components; Pritchard, Stephens & Donnelly 2000; Anderson & Thompson 2002; Jombart 2008). The program NEWHYBRIDS assigns individuals to different hybrid classes (i.e. F1, F2 and backcrosses; Anderson & Thompson 2002). For our analysis, we just distinguished between the two purebred parental species and a category 'hybrid' (including F1, F2 and backcrosses). Posterior distributions were evaluated after 10^5 iterations of the MCMC and a burn-in period of 10^4 iterations. ADEGENET is using a discriminant analysis of principal components (DAPC) and a *post hoc* classification of individuals to one of three classes (both purebred species and 'hybrid'). For STRUCTURE, we first determined the optimal threshold (q-value) to minimize the number of misassignments based upon a larger simulated dataset of 200 individuals per hybrid class as well as 119 purebred *C. montanus* and 118 purebred *C. parallelus* from a preceding study (Rohde *et al.* 2015). The STRUCTURE analysis was run with the admixture model, a burn-in of 10^4 simulations followed by 10^5 Markov chain Monte Carlo (MCMC) simulations and a K of 2 with 10 iterations. The optimal threshold was detected at $q = 0{\cdot}91$, i.e. hybrid assignment between 0·09 and 0·91. However, even with this optimal q-value the percentage of misassignments in STRUCTURE was substantially higher (15·9%) than in ADEGENET (11·0%) and NEWHYBRIDS (12·0%; Fig. S5; Table S1). Therefore, we only used the latter two programs for hybrid assignment. As both programs, overestimated the number of hybrids (25–35% of purebred individuals were assigned as backcrosses by a single program), we only defined those individuals as hybrids, which were detected by both programs. This increased the correct assignment rate to 96·1% with no F1 or F2 hybrid being assigned to a wrong class (Table S1). The hybridization rate refers to the number of hybrids in relation to the total number of genetically sampled *C. montanus* individuals.

Table 1. Characterization of four polymorphic microsatellite primers for *Chorthippus montanus* with: locus name; repeat motif; primer sequence of forward (for) and reverse (rev) primer; allele size range (bp) and fluorescence dye name (Tag)

Locus	Repeat motif	Primer sequence 5′-3′	Allele size range (bp)	Tag
CM 5	(ATC)21	F: TGTACCCATGAGCTACTGTCA R: TGGCAAACTGGCGAGCTTCT	306–432	HEX
CM 19	(TCTG)4(TCCG)3	F: CGATCGCCTTTTGACAGCTC R: CCATATTCTCGCGTGGCTTG	410–450	FAM
CM 33	(GAT)11	F: ACAAACTGTCTCGAATACTTGC R: GGTAGTAGCTATTCTTGAGTTG	301–349	TAMRA
CM 37	(TCA)6	F: GTTTCCGTGATCCTGAGCG R: AGGTACTTGGATTCGGTGAG	219–339	TAMRA

GENETIC DIVERSITY

To analyse differences in genetic diversity between populations and years, we calculated the expected and observed heterozygosities (H_E and H_O) using GenAlEx 6.5 (Peakall & Smouse 2006, 2012) as well as the allelic richness (Ar) using F_{stat} 2.9.3.2 (Goudet 2001). To analyse the influence of hybrids on the genetic diversity, we performed these analyses including and excluding hybrids.

SPATIAL ANALYSES

Density distribution and encounter probabilities

To calculate spatial, temporal and spatiotemporal encounter probabilities as a proxy of hybridization risk, we used the GPS data from the mark-recapture studies in 2011–2013 for further analysis in ArcGIS 10.1 (ESRI 2011). For *C. montanus*, we analysed the spatial density distribution at both sites and years to calculate its density in 1 m^2 grid cells. For *C. parallelus*, we calculated the same for the area covering the complete *C. montanus* distribution to test the overlap of both species in this area. The calculation was done (1) for the complete study period (i.e. pure spatial overlap) and (2) for seven periods of 2 weeks each from end of July to end of October (i.e. spatiotemporal overlap) using the Spatial Analyst tool *Kernel Density* (settings: cell size = 1; search radius = 1; unit = m^2). To receive values per m^2, we created a 'distribution' grid shapefile (1 × 1 m^2) across the corresponding site and transformed the density grid into a point shapefile using the conversion tool *Grid to Point*. Afterwards, we merged both grids using the function *Join*. Using these data, we calculated the abundance of both species per m^2 for each time period and defined the relative frequency of *C. parallelus* in cells of *C. montanus* as heterospecific encounter probability in that grid cell ($EP_i = N_{para(i)}/(N_{mont(i)} + N_{para(i)})$, with EP = heterospecific encounter probability, N = abundance, i = grid cell). We then weighted each grid cell with the relative number of *C. montanus* individuals occurring within it to obtain the overall heterospecific encounter probability for the complete grid in that time period ($EP_t = (\Sigma(EP_i \times N_{mont(i)}))/N_{mont(t)}$, t = total). To compare the heterospecific encounter probabilities in areas of the next generation hybrids with those in areas of the next generation purebred *C. montanus*, we calculated the mean heterospecific encounter probabilities of the preceding year within a radius of 5 m of each individual using the Geoprocessing Tools *Buffer* and *Intersect*. A two-way ANOVA was used to test for significant differences in the encounter probabilities between purebred *C. montanus* and hybrids as well as among years. The response variable was Box-Cox-transformed using the MASS library for R to infer the optimal lambda to achieve an optimal data distribution for ANOVAs (Venables 2002).

For creating GIS maps, the locality of each recaptured individual was averaged using the Data Management Tool *Multi Convex Polygon* with the geometry Convex_Hull (no angle over 180°) to avoid pseudoreplication. Afterwards, the Spatial Analyst Tool *Kernel Density* with the settings: cell size = 1; search radius = 5 and unit = m^2, was used to create a density map of *C. montanus* for each site and year. A search radius of 5 m^2 was chosen, because the species has generally a very low mobility (Weyer, Weinberger & Hochkirch 2012).

Soil moisture

To analyse the soil moisture preferences of both species and hybrids, the soil moisture of each capture position was recorded using a UMS Infield7 tensiometer with a Theta probe (type ML2x) during the mark-recapture study. A two-way ANOVA was used to test for significant differences in the soil moisture of capture position between species (*C. montanus*, *C. parallelus* or hybrid), site and year. To visualize the soil moisture variation on each site and to identify possible ecological boundaries, we created a soil moisture map in ArcGIS10.1 (ESRI 2011). For this purpose, a 5 × 5 m grid was established on both sites marked with bamboo sticks in 2012 and 2013. We measured the soil moisture at each grid node three times and calculated a mean. The GPS coordinates were recorded using the Trimble®GeoXT™ (GeoExplorer®2008 series) and the Trimble®GeoBeacon™. Soil moisture fluctuates strongly depending on the current waterlogging. However, our intention was to obtain data on relative soil moisture variation across the study site. The mean soil moisture across study years was finally calculated for each grid node to infer a soil moisture map using the Spatial Analyst Tool *Interpolation* (Kriging) with the settings Krigingmethod = spherical and search radius = 12 points.

Results

POPULATION SIZE AND THE CORRELATION WITH WEATHER DATA

Both *C. montanus* populations strongly declined over the years. From 2010 to 2016, the R1 population of *C. montanus* decreased by 99·3% and the R2 population by 74·7% (Fig. 1), with the strongest decline from 2011 to 2012 with 72% for R1 and 35% for R2 and from 2015 to 2016 with 91·5% for R1 and 52·5% for R2. The *C. parallelus* population decreased from 2011 to 2013 by 21·3% (Table 2).

Hierarchical partitioning revealed that the weather parameters explaining population changes varied among the three phases. During the reproductive phase of the preceding year, the length of the arid period had the highest impact on population change and showed a significant negative correlation ($R^2 = 0{\cdot}5$, $F_{1,6} = 6{\cdot}1$, $P < 0{\cdot}05$). Wind velocity and precipitation were the next important variables identified, but they were not significantly correlated with population change. During the egg phase, days without rain and length of the arid period had the highest impact. The regression analysis showed a significant negative correlation for days without rain ($R^2 = 0{\cdot}5$, $F_{1,6} = 7{\cdot}3$, $P < 0{\cdot}05$) and a similar trend for length of the arid period ($R^2 = 0{\cdot}48$, $F_{1,6} = 5{\cdot}4$, $P = 0{\cdot}058$). During the nymphal phase, population change was significantly negatively correlated with cloud cover ($R^2 = 0{\cdot}5$, $F_{1,6} = 6{\cdot}4$, $P < 0{\cdot}05$) and showed a trend for a positive correlation with days without rain ($R^2 = 0{\cdot}43$, $F_{1,6} = 4{\cdot}6$, $P = 0{\cdot}076$).

The PCAs for the weather data during the three different phases were rather different. The two-first principal components explained 88% (reproductive phase, PC1: 68·4, PC2: 19·9%), 83% (egg phase, PC1: 52·5, PC2: 30·4%) and 88% (nymphal phase, PC1: 58·3, PC2: 31%) of the variance (Figs 2A–C). During the reproductive phase, the first function was mainly explained by days without rain (0·74) and cloud cover (−0·70), while the second function was explained by minimum air temperature (0·71). During the egg phase, the first function was mainly

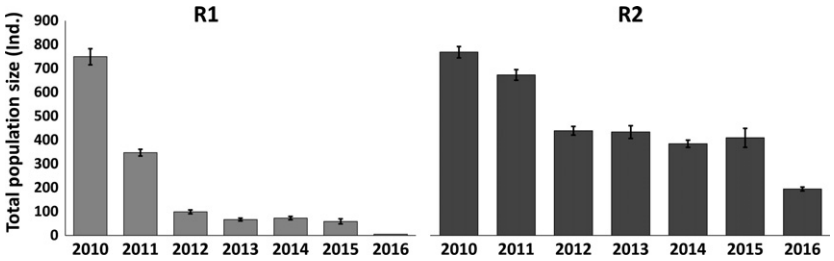

Fig. 1. Estimated total population size of *Chorthippus montanus* on the two sites Reinsfeld1 and Reinsfeld2 using the program MARK. Error bars are standard errors.

Table 2. Estimation of the total population size of *Chorthippus montanus* and *Chorthippus parallelus* on Reinsfeld1 (R1) and Reinsfeld2 (R2), and the estimated population size of *C. parallelus* for the overlapping area (OA) of both species (R2) using the best models calculated with the program MARK

Species and site	Year	Best model (MARK)	Total population size	Population change (%)	Date of max. daily population size
C. montanus R1	2010	$Phi(g + T)P(g)pent(g \times t)N(.)$	749 ± 34		10 Aug
	2011	$Phi(g + T)P(g + t)pent(t)N(g)$	347 ± 14	−53·8	26 Aug
	2012	$Phi(.)p(g)pent(t)N(.)$	99 ± 8	−71·5	27 Aug
	2013	$Phi(g + T)p(g)pent(g + t)N(g)$	67 ± 6	−32·3	22 Sep
	2014	$Phi(g + T)p(g)pent(g + t)N(g)$	73 ± 7	9	28 Jul
	2015	$Phi(g + T)p(g)pent(t)N(g)$	59 ± 11	−9·2	13 Aug
	2016	No model possible (two captures)	<5	−91·5	–
C. montanus R2	2010	$Phi(g + T)p(g + t)pent(t)N(g)$	755 ± 23		01 Sep
	2011	$Phi(g + T)p(g + t)pent(t)N(g)$	661 ± 22	−12·5	01 Sep
	2012	$Phi(g + T)p(g + t)pent(t)N(.)$	431 ± 18	−34·8	31 Aug
	2013	$Phi(g + T)p(g)pent(g \times t)N(g)$	426 ± 26	−1·2	13 Sep
	2014	$Phi(g + T)p(t)pent(g \times t)N(g)$	378 ± 15	−11·3	01 Sep
	2015	$Phi(g + T)p(g + t)pent(g \times t)N(g)$	402 ± 39	6·4	24 Aug
	2016	$Phi(g + T)p(g + t)pent(g + t)N(g)$	191 ± 8	−52·5	12 Sep
C. parallelus R2	2011	$Phi(g + T)p(t)pent(g + t)N(g)$	1517 ± 69		04 Jul
	2012	$Phi(g + T)p(g + t)pent(g + t)N(g)$	1435 ± 167	−5·4	31 Jul
	2013	$Phi(g + T)p(t)pent(g \times t)N(g)$	1194 ± 91	−16·8	31 Jul
C. parallelus OA	2011	$Phi(g + T)p(t)pent(g + t)N(g)$	416 ± 39		26 Jun
	2012	$Phi(g + T)p(g + t)pent(g + t)N(g)$	384 ± 123	−7·7	26 Jul
	2013	$Phi(g + T)p(t)pent(g \times t)N(g)$	289 ± 45	−24·7	29 Aug

Population change is the percentage change in population size from 1 year to the next. *Phi*: daily survival probability; *P*: daily recapture probability; pent: daily immigration probability; *N*: population size. The parameters *g*: sex and *t*: time may be constant (.), independently, in interaction ($g \times t$) or in addition ($g + t$). *T* represents a linear trend for temporal effects.

explained by mean air temperature (0·74), while the second axis was explained by days without rain (0·68) and length of the arid period (0·65). During the nymphal phase, the first function was mainly explained by duration of sunlight (−0·72) and days without rain (−0·72), while the second axis was explained by cloud cover (−0·61) and length of the arid period (0·61). Only during the nymphal phase, population change in the R1 population was significantly correlated with the principle components (environmental fitting: $P = 0{\cdot}03$, PC1 = 0·72, PC2 = −0·69), whereas no significant correlation was found for R2.

NULL ALLELES AND SCORING ERRORS

We found no evidence for stuttering or large allele dropout, but null alleles were suggested for nearly all loci in several populations by all three programs. However, only locus CD6 had null allele frequencies >0·2 in four (out of six) populations (Table S2). Generally, null alleles were much rarer in *C. parallelus* populations (Table S2). Pairwise F_{ST} values calculated in FreeNA using ENA correction differed only slightly from uncorrected F_{ST} values showing consistently lower F_{ST} values (mean difference: 0·0039). In order to test the effect of the most critical microsatellite locus (CD6) on the performance of hybrid assignment, we performed the simulation study with and without this locus. The results showed a slight decrease in correct assignment rate (from 96 to 95%), and thus, we decided to retain this locus. Discarding loci deviating from HWE could generally weaken the interpretation of biological phenomena (Dharmarajan, Beatty & Rhodes 2013) as HWE cannot be assumed for hybridizing populations.

HYBRIDIZATION RATE

The hybridization rate of the *C. montanus* population with *C. parallelus* on R1 was 15·6% (10/64) in 2012 and 19·6%

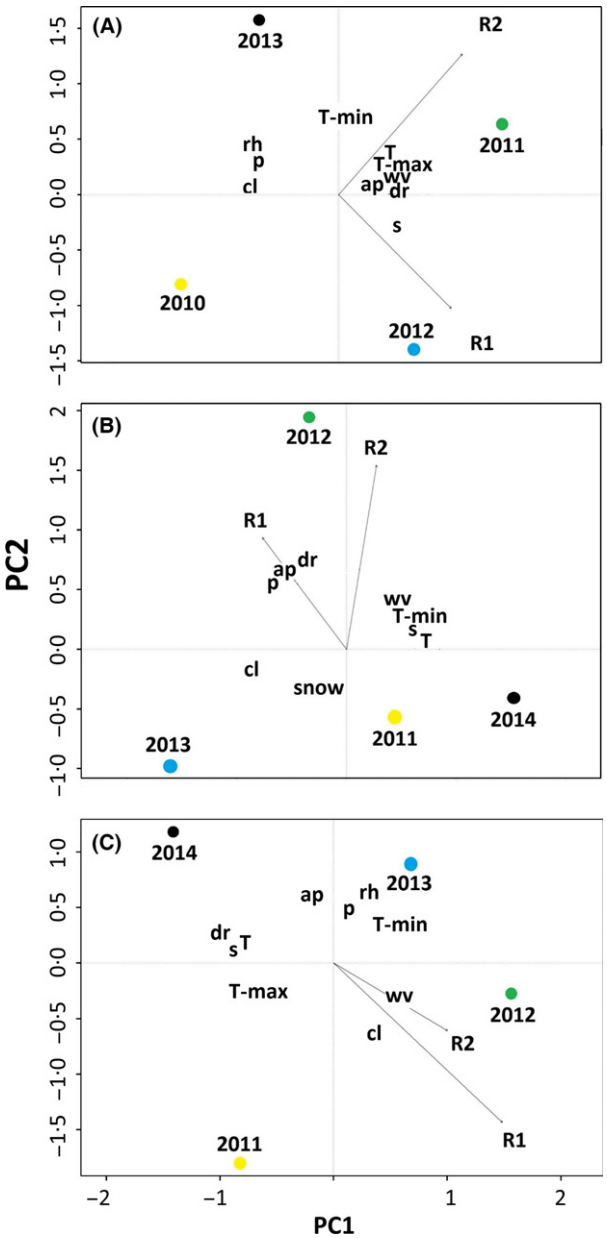

Fig. 2. Plot of the first two functions of the principal component analyses (PCA) on the variables averaged air temperature (*T*), minimal air temperature (*T*-min), maximal air temperature (*T*-max), days without rainfall (dr), duration of sunlight (s), wind velocity (wv), cloud cover (cl), length of the arid period (ap), precipitation (p) and relative air humidity (rh) for the reproductive phase in the previous year (A), the egg phase (B) and the nymphal phase (C), explaining 88% (A: PC1: 68·4%, PC2: 19·9%), 83% (B: PC1: 52·5%, PC2: 30·1%) and 89% (C: PC1: 58·3%, PC2: 31%) of the total variance. Each point represents 1 year (yellow: 2010–2011; green: 2011–2012; blue: 2012–2013; black: 2013–2014). Arrows show the correlation of population shift of R1 and R2 using environmental fitting.

(11/56) in 2013 (Fig. 3, Table S3). On R2, the hybridization rate was 7·4% (23/298) in 2012 and 6·0% (17/284) in 2013 (Table S3).

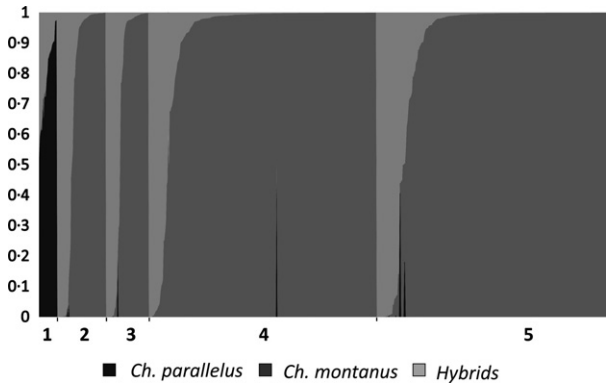

Fig. 3. NEWHYBRIDS assignment estimates generated from microsatellite data for hybrid detection. (1) *Chorthippus parallelus* individuals as reference population, (2) R1 2012, (3) R1 2013, (4) R2 2012, (5) R2 2013. An individual was assigned to one of the three classes (*C. parallelus*, *Chorthippus montanus*, and Hybrids) if $q > 0{\cdot}5$.

GENETIC DIVERSITY

For the allelic richness as well as for the observed and expected heterozygosities, there were only slightly differences between the populations R1 and R2 in both study years (Table S4). Including hybrids in the dataset, the allelic richness increased slightly in both populations. There was no difference in observed and expected heterozygosities (Ho) between the datasets including and excluding hybrids (Table S4).

SPECIES DISTRIBUTION AND OVERLAP

In all study years, *C. parallelus* reached its estimated maximum daily population size between the beginning and end of July (Table 2). The date of maximum daily population size of both *C. montanus* populations varied between the sites. While on R2, the maximum daily population size was rather consistently at the end of August or beginning of September, it fluctuated strongly between the years on R1 between end of July (2014) and end of September (2013; Table 2, Fig. 4).

The GIS analyses showed that the occupied area of *C. montanus* on R1 had a size of 2986 m² in 2011 which decreased during the following years (2012: 581 m^2, 2013: 560 m^2). On R2, *C. montanus* had an occupied area of 1186 m^2 in 2011 (2012: 713 m^2, 2013: 720 m^2), of which 585 m^2 (49·3%; 2012: 327 m^2, 45·9%; 2013: 326 m^2, 45·3%) were also occupied by *C. parallelus*.

The ratio of the daily population sizes of both species within the overlapping zone of *C. montanus* and *C. parallelus* (R2) changed end of June (2011), mid of August (2012) or end of July (2013). At the beginning of the season, *C. parallelus* was generally more frequent than *C. montanus* in the overlapping zone; afterwards, *C. montanus* became more frequent (Fig. S6). The heterospecific encounter probability calculated from phenology alone was 19·9% (2011), 19·6% (2012) and 28·0% (2013), while

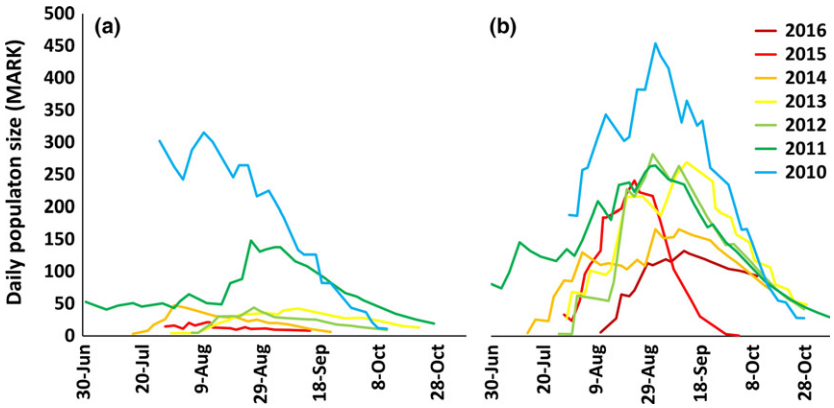

Fig. 4. Estimated daily population size of *Chorthippus montanus* on the Reinsfeld1 (a) and Reinsfeld2 (b) using the program MARK. There are no data on daily population size on Reinsfeld 1 in 2016 as only two individuals were captured.

Table 3. Mean probability per *Chorthippus montanus* individual to encounter *Chorthippus parallelus* (in %) on R2 during seven periods á 2 weeks from 11 July to 22 October. The (–) marks periods without *C. montanus* individuals

Year	Period 1 30·6–25·7	Period 2 26·7–09·8	Period 3 10·8–24·8	Period 4 25·8–09·9	Period 5 10·9–24·9	Period 6 25·9–08·10	Period 7 08·10–22·10	Mean encounter probability
2011	8·2	4·0	4·5	4·3	3·0	2·5	2·6	4·2
2012	–	3·0	11·6	4·0	3·2	3·1	1·5	4·4
2013	–	17·9	3·9	7·0	4·8	7·0	4·8	7·6

calculated from spatial overlap alone it amounted to 11·4% (2011), 12·3% (2012) and 19·0% (2013).

SPATIOTEMPORAL ENCOUNTER PROBABILITY

The mean probability per *C. montanus* individual to encounter *C. parallelus* (across all grid cells) on R2 differed between the 2-week periods and years (Table 3). The highest heterospecific encounter probability was found during the first 4 weeks of the adult season of *C. montanus* with a maximum during the second period in 2013 (17·9%). Afterwards, the probability decreased with ongoing season. In 2011, 24% of the overlapping area had a mean encounter probability greater 10% (across all periods), whereas in 2012 and 2013, this area increased to 27 and 35% respectively. Overall, the spatiotemporal overlap increased from 4·2% (2011), 4·4% (2012) to 7·6% (2013). Hybrids were mainly localized at the edge of the previous year's main distribution of *C. montanus* (Figs 5 and 6). The previous year's mean heterospecific encounter probability was significantly higher within a 5-m radius of the hybrids' position than

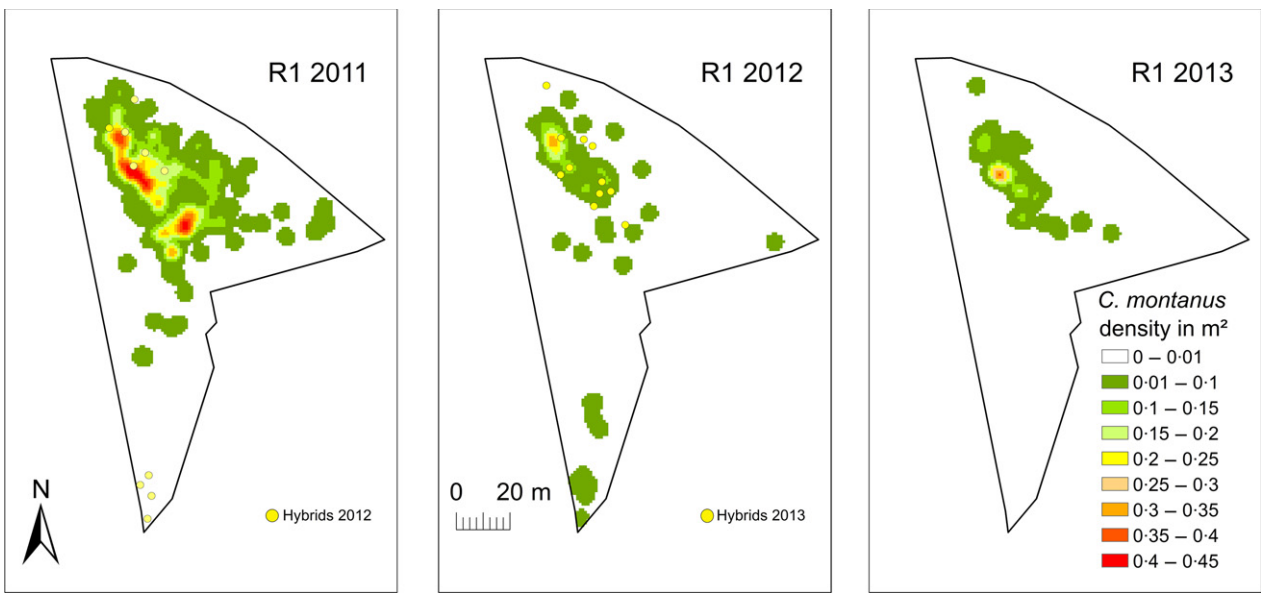

Fig. 5. Density distribution of *Chorthippus montanus* in 2011–2013 on Reinsfeld1 including the averaged capture point of hybrids from the following year. There are no data on hybrids in 2013 as no individuals were genotyped in 2014.

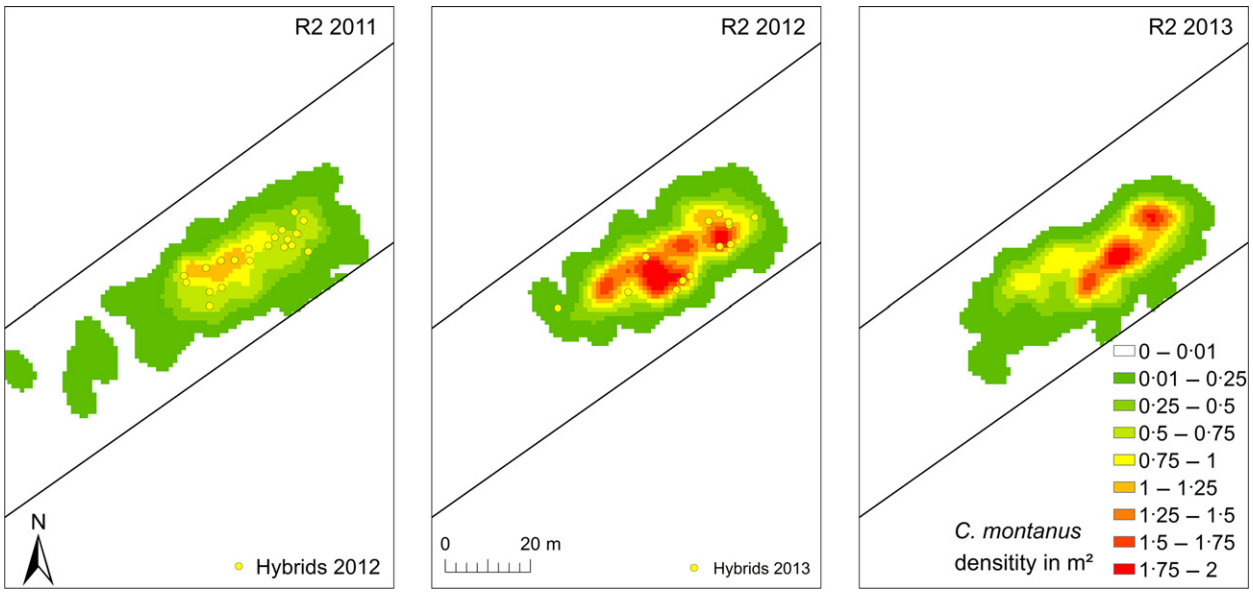

Fig. 6. Density distribution of *Chorthippus montanus* in 2011 to 2013 on Reinsfeld2 including the averaged capture position of hybrids in the following year. There are no data on hybrids in 2013 as no individuals were genotyped in 2014.

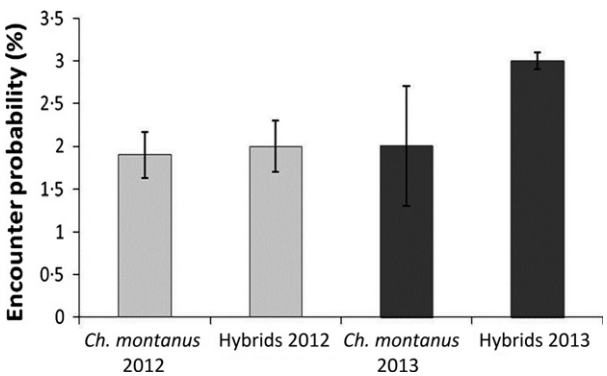

Fig. 7. Averaged encounter probability of the previous years (2011 and 2012) at the capture position within a radius of 5 m for hybrids and the remaining *Chorthippus montanus* population of the next generation (2012 and 2013).

in the areas surrounding purebred *C. montanus* (ANOVA, $\lambda = 0{\cdot}22$, $F_{1,469} = 11{\cdot}6$, $P < 0{\cdot}001$; Fig. 7). We also detected a significant difference in the encounter probabilities between years (ANOVA, $\lambda = 0{\cdot}22$, $F_{1,469} = 30{\cdot}9$, $P < 0{\cdot}001$; Fig. 7), but no significant interaction between year and hybrid status (ANOVA, $\lambda = 0{\cdot}22$, $F_{1,469} = 3{\cdot}8$, $P = 0{\cdot}053$; Fig. 7).

MEAN SOIL MOISTURE AT THE CAPTURE LOCATION

The mean soil moisture at the capture locations of *C. montanus* (R1: 40–43%, Fig. S7a and b; R2: 39–40%; Fig. S8a and b) was significantly higher than at the capture locations of *C. parallelus* (R2: 27–28%; ANOVA, $\lambda = 0{\cdot}44$, $F_{1,61239} = 238{\cdot}2$, $P < 0{\cdot}001$). The mean soil moisture at the capture positions of the hybrids varied between 29 and 38% (R1: 2012: 29%; 2013: 36%; R2: 2012: 38%; 2013: 33%; Figs S7 and S8) and was significantly higher than for *C. parallelus* (ANOVA, $\lambda = 0{\cdot}38$, $F_{1,6724} = 24{\cdot}4$, $P < 0{\cdot}001$), but not significantly different from *C. montanus* (ANOVA, $\lambda = 0{\cdot}56$, $F_{1,471} = 1{\cdot}99$, $P = 0{\cdot}16$).

Discussion

Our results show that both populations of *C. montanus* declined strongly and in parallel during a period of 7 years. The strongest yearly population declines occurred after severe droughts and therefore population dynamics correlated strongly with climatic parameters, supporting the hypothesis that climatic fluctuations are a major driver of the population decline of this species. Generally, we found significant correlations of droughts during the previous adult season and during the egg stage as well as cloudy or rainy conditions during the nymphal period with population decline. We also found changes in the spatiotemporal overlap of *C. montanus* with its congener *C. parallelus*. Spatiotemporal overlap was generally lower than spatial or temporal overlap alone, confirming the hypothesis that spatiotemporal segregation is a major pre-mating barrier between both species. It remained quite constant (although slightly increasing during the last year), but hybrids were mainly detected in areas with increased heterospecific encounter probabilities. This can be explained by the finding that mate choice is strongly influenced by encounter probability in this species pair (Rohde *et al.* 2015). An increase in hybridization rate was also only noted in the smaller population of *C. montanus* (R1), which showed the strongest population decline of *C. montanus*. Hybridization rate was also substantially higher on R1, supporting the hypothesis that the hybridization rate is mainly driven by the encounter probability. Large populations appear to be

better buffered against hybridization than smaller populations. Our results also confirm that hybrids occupy intermediate soil moisture conditions, which could either be a consequence of or the reason for their locations at the edge of the *C. montanus* distribution.

We assume that ongoing climate change and more frequent extreme weather events may further decrease population sizes of *C. montanus* and may subsequently change the spatiotemporal overlap on sites with increasing risk of hybridization. It has been shown for other taxa that hybridization might ultimately lead to genetic displacement (Schulte, Veith & Hochkirch 2012). It remains uncertain, whether *C. montanus* might have a similar fate in the future, becoming displaced by the habitat generalist *C. parallelus*, or whether hybridization might increase the adaptive potential of the specialist. However, recent continuation of the mark-recapture experiment has shown that *C. montanus* is meanwhile close to extinction on R1 (with only two male individuals found in 2016). It will thus be interesting to study the genetics of *C. parallelus* at this site in the future to test if some genetic information of *C. montanus* survived in the heterospecific population.

DIRECT EFFECTS OF CLIMATE CHANGE AND EXTREME WEATHER EVENTS

Climate-mediated range shifts to higher elevations have been reported for many species (Warren *et al.* 2001; Parmesan 2006). For *C. montanus*, altitudinal shifts are virtually impossible, as the species already occurs at the highest elevations of the Hunsrueck Mountains, but has lost nearly all populations <400 m a.s.l. Furthermore, it has a very low dispersal ability and its habitats are strongly fragmented (Weyer, Weinberger & Hochkirch 2012). The main reason for the strong habitat specialization of *C. montanus* is the low drought tolerance of the eggs, which depend upon permanently high soil moisture (Ingrisch 1983). Extreme climatic events such as the three long arid periods observed in April and November 2011, as well as in March 2012 (Barbosa *et al.* 2012) are likely to have had direct negative impacts on the *C. montanus* populations. However, even other extreme climatic events like extreme precipitation during the nymphal phase may lead to population declines. Such extreme weather events are likely to increase in Europe during the next decades (Lehner *et al.* 2006; Prudhomme *et al.* 2014). Thus, the time available to compensate population declines between weather extremes is likely to decrease and might drive the populations to extinction. This decline is further promoted by ongoing habitat degradation due to the lack of habitat management. Both sites have not been mown since we started our project (except for R2 in 2015) and the accumulation of tangled matted grass is known to negatively affect *C. montanus* as well (Weyer, Weinberger & Hochkirch 2012). However, the extreme population declines in 2011 and 2012 are more likely to be an effect of the extreme climatic conditions as we would expect a gradual decline by the slow process of habitat degradation. The site R2 has been mown in summer 2015, but the population size of *C. montanus* still decreased here. In this case, we assume that the use of heavy machines for mowing during the reproductive phase of the grasshopper has led to a high mortality and is responsible for the population decline in 2016. The overall population decline will increase the effects of other threats, such as hybridization, a phenomenon known as the extinction vortex (Gilpin & Soulé 1986).

INDIRECT CLIMATIC EFFECTS ON HYBRIDIZATION

Indirect effects of climate change on hybridization are little understood (Chunco 2014). An asynchronic response to climate change may affect the relative frequencies of species and alter their coexistence (Heard, Riskin & Flight 2012; Sánchez-Guillén *et al.* 2014). The two grasshopper species studied differed strongly in their population response to climatic extremes. While *C. montanus* strongly declined after arid periods, *C. parallelus* populations remained nearly stable. Habitat specialists like *C. montanus* are more sensitive to changing environmental conditions than habitat generalists as they have lower tolerances, which have the potential to change the hybridization dynamics among both species. The remaining *C. montanus* population becomes increasingly restricted to the wettest areas, whereas the *C. parallelus* population can expand and immigrate into the *C. montanus* population. The changes in the relative frequencies of both species as well as the changing spatial overlap increases the probability for *C. montanus* to encounter *C. parallelus* and thus for hybridization (Rohde *et al.* 2015). In our study, hybrids were mainly found at the edge of the *C. montanus* populations, in areas with high heterospecific encounter probabilities of the previous year. This result is surprising given the ability of the grasshoppers to move throughout their nymphal and adult stage. However, it is known that adults of this species do not move large distances (Weyer, Weinberger & Hochkirch 2012) and this is also likely for nymphs, which spend more time in feeding than in locomotion.

THE ROLE OF PRE-MATING BARRIERS

The two species have large overlapping ranges, suggesting that hybridization interactions may occur in many populations, similar to a mosaic hybrid zone (Köhler 2013). However, hybridization seems to be minimized by the low spatiotemporal overlap of both species. The combination of spatial and temporal niche divergence reduces hybridization risk dramatically. Spatial population overlap alone would suggest an 11–19% heterospecific encounter probability and temporal overlap a 20–28% probability. However, both dimensions together reduce the mean heterospecific encounter probabilities on R2 to 4·2%

(2011), 4·4% (2012) and 7·6% (2013), which is much closer to our observed hybridization rates of 7·4% (2012) and 6·0% (2013). In the smaller population (R1), hybridization has already reached frequencies between 15·6 and 19·6% (compared to 6·1–10·8% in 2010; Rohde *et al*. 2015). Hence, the population decline of *C. montanus*, which was initially triggered by climatic factors and habitat deterioration, may now increasingly cause an additional threat from hybridization. Even under favourable conditions for *C. montanus*, the populations of both species are likely to fluctuate permanently and thus hybridization equilibria are probably also under permanent fluctuation.

Due to the earlier adult season of *C. parallelus*, heterospecific encounter probabilities were highest during the first month of the adult season of *C. montanus*. Even though freshly molted individuals might not be sexually receptive immediately (Kriegbaum 1988), hybridization risk probably peaks as soon as they are receptive. Interestingly, *C. parallelus* varied strongly in phenology in the overlapping area of both populations. In 2011, the highest daily population size of *C. parallelus* was 2 months earlier than for *C. montanus*. In 2012, the difference was only 1 month and in 2013 only 2 weeks. This phenology shift was much less pronounced when considering the complete spatial distribution of the *C. parallelus* population, suggesting that this species might react much more sensitive to climatic fluctuations under ecological unfavourable conditions. A possible explanation might be a delayed egg development under wetter and colder conditions. This shows that although phenology may play an important role as reproductive barrier, it can fluctuate dramatically in response to environmental changes (Parmesan & Yohe 2003; Parmesan 2006).

The differing water requirements of the eggs of both species (Ingrisch 1983) are thought to be a major reason for their spatial segregation (Köhler 2013) as our study corroborates. *C. parallelus* preferred areas with lower soil moisture than *C. montanus*, but it had a broader ecological amplitude and also entered areas of higher soil moisture. This suggests that the ecological barrier alone would not be sufficient to prevent hybridization. However, decreasing soil moisture after long arid periods would probably increase *C. parallelus* densities in the centre of the habitat of *C. montanus*. Interestingly, hybrids were mainly found in areas of intermediate soil moisture. It remains unknown, whether this is a consequence of intermediate water requirements, or if it is a secondary result caused by the higher hybridization probability in zones of highest spatial overlap, which are likely to have an intermediate soil moisture.

As hybrid fitness appears to be quite high and backcrossing of hybrids has been documented in the lab (Köhler 2013), it is unlikely that reinforcement processes, i.e. selection against hybrids, affect the populations. The intermediate songs and intermediate spatial distribution of hybrids is likely to decrease pre-mating isolation compared to the parental species.

Conclusion

Our results show that extreme climatic events present a major threat for habitat specialists such as *C. montanus*. The heterospecific encounter probabilities with the habitat generalist *C. parallelus* were strongly reduced by the combined effect of spatial and temporal isolation, but hybrids were mainly found in areas with increased heterospecific encounter probabilities of the preceding year. If the population decline of *C. montanus* alters the spatiotemporal distribution, it may thus face an increased hybridization risk. The increasing probability of extreme climatic events as predicted by climate models represents a major threat to small and fragmented populations of many wetland specialists. It remains to be studied, whether hybridization risk further increases with decreasing population sizes. Other studies have shown before that anthropogenic disturbances can affect existing reproductive barriers between syntopic populations and promote hybridization (Seehausen *et al*. 2008). Reproductive barriers remain effective as long as syntopic populations fluctuate around an equilibrium, but we hypothesize that as soon as population trends lead to a directional change in the relative frequencies, reproductive barriers may break down and the smaller population may be 'genetically swamped'.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Y.H. (2011), N.K. (2012), J.W. (2011–2014) and K.R. (2012–2016) performed the mark-recapture study and analysed the respective population size with MARK. K.R., J.W. and N.K. collected samples for the genetic analysis. K.R. genotyped the collected samples and performed the genetic analyses. O.E. and K.R. performed the GIS analysis. K.R. and A.H. performed the statistical analyses and discussed the interpretation of the data. K.R. wrote the manuscript with input by A.H.

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Data accessibility

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.364b3 (Rohde *et al*. 2016).

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Supporting Information

Details of electronic Supporting Information are provided below.

Fig. S1. *Top left*: Distribution of *Chorthippus montanus* (red), *Chorthippus parallelus* (yellow) and the overlapping distribution of both species(orange) (modified after Kleukers *et al.* 1997).
Fig. S2. Overview of the precipitation rate in selected month (a. March, b. April, c. November; egg phase *Chorthippus montanus*) from 1948 to 2014.
Fig. S3. Overview of the longest arid period during the egg phase of *Chorthippus montanus*) from 1948 to 2014.
Fig. S4. Temperature profile of the month August from 1948 to 2014.
Fig. S5. NEWHYBRIDS assignment estimates for a performance test using a simulated dataset.
Fig. S6. Total daily population size of *Chorthippus montanus* and *Chorthippus parallelus* within the overlapping habitat of Reinsfeld2 (a = 2011; b = 2012; c = 2013).
Fig. S7. Soil moisture distribution on R1 with the maximal distribution boundary of *Chorthippus montanus* for the year 2012 (a) and 2013 (b) and the corresponding hybrids.
Fig. S8. Soil moisture distribution on R2 with the maximal distribution boundary of *Chorthippus montanus* for the year 2012 (a) and 2013 (b) and the corresponding hybrids.
Table S1. Performance test. Comparison of all three programs.
Table S2. (a) Microchecker – Test for Null alleles. (b) Mircochecker. (c) FreeNA: Estimating null allele frequency using the EM algorithm (Dempster, Laird & Rubin 1977). (d–e) FreeNA: Estimating global F_{st} of Weir & Cockerham (1996) both using and without using the ENA correction described in Chapuis & Estoup (2007).
Table S3. Hybrid detection with two different programs (NEWHYBRIDS, ADEGENET) in both populations of *C. montanus* in the years 2012 and 2013.
Table S4. Genetic parameters of both *C. montanus* population with hybrids (+) and excluding hybrids detected with the conservative approach (−); *N*: sample size; Ar: allelic richness; H*o* and H*e*, observed and expected heterozygosities; numbers in parentheses are standard errors.