

Differential threshold effects of habitat fragmentation on gene flow in two widespread species of bush crickets

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Abstract

Effects of habitat fragmentation on genetic diversity vary among species. This may be attributed to the interacting effects of species traits and landscape structure. While widely distributed and abundant species are often considered less susceptible to fragmentation, this may be different if they are small sized and show limited dispersal. Under intensive land use, habitat fragmentation may reach thresholds at which gene flow among populations of small-sized and dispersal-limited species becomes disrupted. Here, we studied the genetic diversity of two abundant and widespread bush crickets along a gradient of habitat fragmentation in an agricultural landscape. We applied traditional (G_{ST} , θ) and recently developed (G'_{ST} , D) estimators of genetic differentiation on microsatellite data from each of twelve populations of the grassland species *Metrioptera roeselii* and the forest-edge species *Pholidoptera griseoptera* to identify thresholds of habitat fragmentation below which genetic population structure is affected. Whereas the grassland species exhibited a uniform genetic structuring ($G_{ST} = 0.020$ – 0.033 ; $D = 0.085$ – 0.149) along the whole fragmentation gradient, the forest-edge species' genetic differentiation increased significantly from $D < 0.063$ ($G_{ST} < 0.018$) to $D = 0.166$ ($G_{ST} = 0.074$), once the amount of suitable habitat dropped below a threshold of 20% and its proximity decreased substantially at the landscape scale. The influence of fragmentation on genetic differentiation was qualitatively unaffected by the choice of estimators of genetic differentiation but quantitatively underestimated by the traditional estimators. These results indicate that even for widespread species in modern agricultural landscapes fragmentation thresholds exist at which gene flow among suitable habitat patches becomes restricted.

Keywords: bush cricket, dispersal threshold, diversity, gene flow, landscape connectivity, population genetic structure

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Introduction

Biodiversity loss in agricultural landscapes over the last decades is largely attributed to structural changes associated with agricultural intensification (Fahrig 2003; Hendrickx *et al.* 2007; Billeter *et al.* 2008). Especially, habitat fragmentation, i.e. the loss and breaking apart of semi-natural habitats, such as extensive grasslands, hedgerows or forests, entails the loss of many species

from agricultural landscapes (Tscharrntke *et al.* 2002; Schweiger *et al.* 2005; Hoehn *et al.* 2007). Conversely, the remaining species are often assumed more tolerant to fragmentation, because they are often widespread (Swihart *et al.* 2003; Henle *et al.* 2004). However, it remains largely unknown to what extent fragmentation will also negatively affect these species by the reduced size and increased isolation of their habitats.

Reduced size and increased isolation of habitat patches have generally been found to lead to smaller effective population sizes and decreased inter-patch dispersal (Gaggiotti & Hanski 2004; Frankham *et al.* 2010).

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Decreased inter-patch dispersal and increased population bottlenecks because of fragmentation may lead to increased levels of inbreeding and loss of genetic variation within habitat patches. Increased genetic differentiation among patches (Keller *et al.* 2004), reduced fitness (e.g. Reed & Frankham 2003) and increased local extinctions (e.g. Nieminen *et al.* 2001) are the potential consequences and were found for various species (e.g. Madsen *et al.* 1996). However, few studies actually determine the threshold of habitat fragmentation at which these consequences become apparent (cf. Swift & Hannon 2010). Thus, determining the threshold at which habitat fragmentation affects abundant and widespread species is important to ensure species persistence and ecosystem functioning in modern agricultural landscapes and for better-informed decisions in nature conservation and landscape planning.

Individual dispersal, one of the major processes that impacts population sizes (Johannesen *et al.* 2003), persistence (Schtickzelle *et al.* 2006) or species interactions (Hunt & Bonsall 2009) is affected by habitat fragmentation (Fahrig 2007). However, determining the effects of habitat fragmentation on individual dispersal is often hindered by the difficulty of directly observing inter-patch dispersal or time lags in ecological responses. As a consequence, many studies now utilize indirect measures such as genetic population differentiation to estimate dispersal and deduce population persistence in response to different levels of habitat fragmentation (Keller *et al.* 2004; Hoehn *et al.* 2007). Lately, however, the suitability of widely used traditional measures of genetic population differentiation in determining the ecological effects of habitat fragmentation has been questioned (Hedrick 2005; Jost 2008, 2009; Heller & Siegismund 2009; Ryman & Leimar 2009).

Traditional methods for estimating genetic population differentiation as introduced by Wright (1951: F_{ST}) and in its extended versions by Nei (1977: G_{ST}) and Weir & Cockerham (1984: θ) relate the average within-subpopulation diversity to the overall genetic diversity. When gene diversity is high, however, the maximum level these measures can reach is reduced and even if the subpopulations are completely distinct, genetically they do not reach unity, leading to a downward bias of the estimates (Hedrick 2005; Jost 2008). To correct for this potential bias in the estimates of genetic differentiation for highly polymorphic markers, the differentiation measures G'_{ST} (Hedrick 2005) and D (Jost 2008) have recently been developed. In a meta-analysis, Heller & Siegismund (2009) found a considerable discrepancy between the traditional and newly developed measures with potentially severe implications in the fields of population and conservation genetics.

In this study, we used two traditional (G_{ST} and θ) and two newly developed (G'_{ST} and D) differentiation measures to investigate the genetic population structure of two bush-cricket species along a gradient of habitat fragmentation in an agricultural region in Germany. Both species, *Metrioptera roeselii* HAGENBACH 1822 and *Pholidoptera griseoptera* DE GEER 1773, are abundant and widespread throughout large parts of Europe. They differ in the level of disturbance of their preferred habitats and in their dispersal abilities, both of which are known factors that affect genetic diversity and genetic population structure (Marten *et al.* 2006; Bailey *et al.* 2007; Holzhauser *et al.* 2009; Vignieri 2010). Whereas *M. roeselii* occurs in grasslands, a land-use type that is frequently disturbed by mowing or grazing, *P. griseoptera* is found along the temporally more stable edges of hedges and woodlands in agricultural landscapes. In *M. roeselii*, approximately 1% of the population shows fully developed wings (e.g. Simmons & Thomas 2004). This might lead to a higher rate of dispersal than in *P. griseoptera*, for which macropterous individuals have never been observed (Ingrisch & Köhler 1998; Maas *et al.* 2002). Despite the presence of winged individuals in *M. roeselii*, both bush-cricket species are considered to represent only a low level of dispersal compared to highly mobile insects such as bees or butterflies (Thomas 2000; Reinhardt *et al.* 2005), and therefore may also be more susceptible to habitat fragmentation (Brouwers & Newton 2009).

Here, we were interested in how different levels of habitat fragmentation in agricultural landscapes affect genetic differentiation among populations of two species that represent different levels of dispersal ability. Specifically, we wanted to identify the thresholds of habitat fragmentation at which genetic differentiation significantly increases for these abundant and widespread bush-cricket species, if indeed these thresholds exist at all. We hypothesized (i) that both, *M. roeselii* and *P. griseoptera*, show increasing genetic population differentiation along a gradient from low to high habitat fragmentation, (ii) of these two species, *P. griseoptera* is affected more strongly by habitat fragmentation because of its lower dispersal ability, and (iii) at low levels of fragmentation, higher levels of habitat disturbance of frequently mown grasslands compared to forest edges are expected to cause higher differentiation in *M. roeselii* than in *P. griseoptera* owing to more frequent population bottlenecks accelerating genetic drift. By analysing genetic population differentiation using four different measures of genetic differentiation we also evaluated (iv) whether estimated fragmentation thresholds and thereby recommendations to nature conservation and landscape planning would differ depending on the differentiation measure applied.

Materials and methods

Study species

The bush-cricket species *Metrioptera roeselii* and *Pholidoptera griseoptera* are widespread and abundant throughout large parts of Europe. Both species belong to the same ensiferan clade and are omnivorous mesophilic generalists that show a great tolerance towards moisture and temperature of their habitats. They exhibit similar body sizes (not exceeding 20 mm) and fecundity (Reinhardt *et al.* 2005). The species differ in their preferred habitat type, dispersal capacity and generation time (Ingrisch & Köhler 1998; Maas *et al.* 2002). *M. roes-*

elii occurs in grasslands, including edges of roads, tracks and ditches. This grassland species shows about one per cent fully winged specimens (macropters) in the otherwise predominantly flightless (brachypters) population and has a generation time of 1 year. *P. griseoptera*, mainly found along forest edges and hedgerows, is completely brachypterous and has a generation time of 2 years (cf. Table 1).

Study sites and landscape evaluation

The study was conducted in two adjoining agricultural regions in central Germany that span a land-use gradient from highly intensive in the Wetterau to less inten-

Table 1 Ecological characteristics of the two bush cricket species *Metrioptera roeselii* and *Pholidoptera griseoptera* (Ingrisch & Köhler 1998; Maas *et al.* 2002; Simmons & Thomas 2004)

	<i>M. roeselii</i>	<i>P. griseoptera</i>
Habitat type	Grassland	Woodland edges and hedgerows
Mobility	~1% macropters per population	No macropters
Life cycle	Annual	Biennial
Longevity (month)	18	30
Density (Individuals per m ²)	0.07–1.1	0.08–0.72 (max. 2; Csencsics 2002)
Instars	(6–)7	7
Presence of imago in the year	End of June—Beginning of August	Mid of July—Beginning of August
Body size of imago (mm)	14–20	15–20
Temperature/moisture	Euryoecious	Euryoecious
Feeding	Polyphagous, mainly herbivorous + little insects and caterpillars	Polyphagous, omnivorous

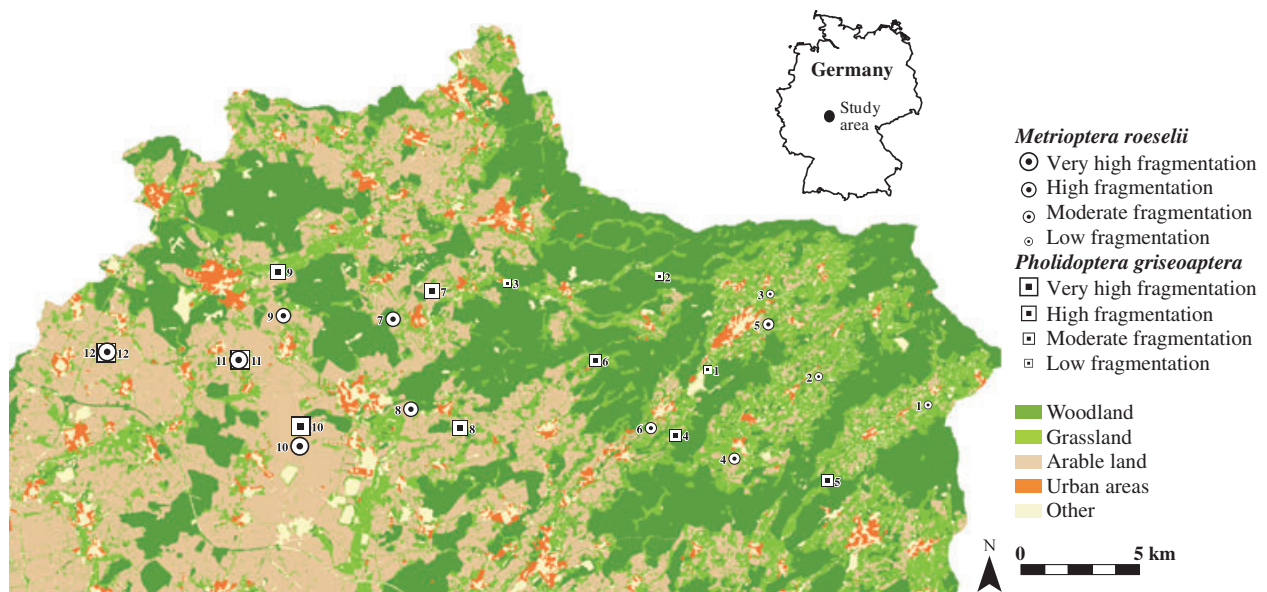


Fig. 1 Locations of *M. roeselii* (circles) and *P. griseoptera* (quadrats) populations sampled for genetic structure in landscapes of four different fragmentation classes (high to low) in Hesse, Germany, and land use within parts of the Wetterau region in the West and the Vogelsberg region in the East.

sive in the Vorderer Vogelsberg (Fig. 1). The region of high land-use intensity was characterized by large management units and large area proportions of arable land and only small area proportions of grassland and woodland. This relation of arable land to grassland and woodland changes in favour of the latter two land-use types, the less intensive agricultural management becomes along this gradient. A digital vector-based land-use map of this area (EFTAS Fernerkundung Technologietransfer GmbH 2007) was used to find all medium-sized (5000–10 000 m²) patches of suitable habitat for either species. Landscape structure at the local scale around each habitat patch was determined in circular sectors with a radius of 1 km. The choice of 1 km was based on several dispersal studies that suggest this distance to represent a suitable scale at which landscape structure is generally encountered in both species (Kindvall *et al.* 1998; Berggren *et al.* 2001; Holzhauer *et al.* 2006; Diekötter *et al.* 2007). Using the same but raster-based land-use map (grain size: 1 m²), the area proportion and proximity of suitable habitat, and the area proportion of arable land in the circular sectors was calculated. Suitable habitat was grassland for *M. roeselii*, which included the land-use types such as meadows, pastures, meadows with scattered fruit trees and grassy margins along fields, tracks and roads. For *P. griseoaptera*, woodland habitat comprised hedgerows, plane shrubs (>50% cover of woody growth) and mixed and deciduous forest. Proximity quantifies the spatial context of a habitat patch in relation to its neighbours and equals the sum of suitable area divided by the squared, nearest edge-to-edge distance between the focal patch and all patches of the same patch type. These proportions and proximity values at the local scale were used to distinguish four classes of habitat fragmentation: low, moderate, high and very high fragmentation (Fig. 2), which largely followed an east–west cline for both grasslands and woody vegetation (Fig. 1). For each of these four fragmentation classes, three focal habitat patches of similar size (median: *M. roeselii* = 8743 m², *P. griseoaptera* = 9092 m²) and inter-patch distances (average: *M. roeselii* = 5.6 km, *P. griseoaptera* = 6.7 km) were selected, totally 12 patches for each species. The maximum distance between patches among fragmentation classes was 35 km for *M. roeselii* and 31 km for *P. griseoaptera* (Fig. 1). Because inter-patch dispersal and therefore gene flow is affected by the landscape matrix of a fragmentation class, we also quantified the landscape structure as for the local scale but in an area including all three focal patches. We did so by finding the minimum circular sector including these three patches of each fragmentation class around their geographical centre ($r = 4000\text{--}6000\text{ m}$; Fig. 1) using a raster map with a grain size of 5 m² for the cal-

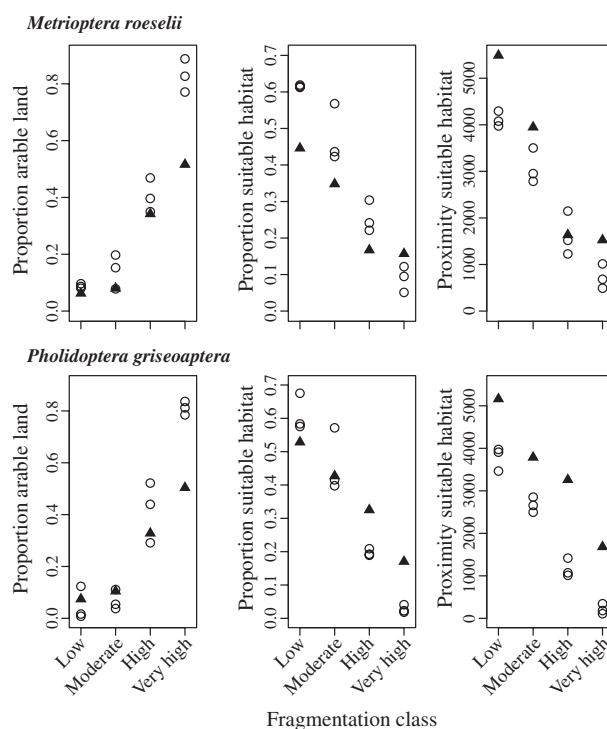


Fig. 2 Area proportion of the agricultural matrix and suitable habitat and proximity of suitable habitat quantified at the local scale (open circles) and at the landscape scale (filled triangles) for the different classes of habitat fragmentation. Suitable habitat included grassy land-use types for *M. roeselii* and woody land-use types for *P. griseoaptera*.

culations. Proximity indices of both local and landscape scale were standardized by extent and resolution. Values estimated at this landscape scale were smaller because of the inclusion of larger forest or urban areas, but in most cases ranked equally compared to the local scale (Fig. 2). Quantifications of landscape structure were performed using ArcGIS 9.2 and the LAMA extension (Aue & Ekschmitt 2010).

Sampling and microsatellite genotyping

For each bush-cricket species, tissue samples were collected by removing one hind leg of individuals in a total of 12 focal patches in September 2007 (*M. roeselii*) and between June and July in 2008 (*P. griseoaptera*). Within each focal patch, we attempted to sample 30 individuals (termed population hereafter), but at various sites, only a smaller number of individuals could be located, resulting in a mean sample size of 18 for *M. roeselii* and 30 for *P. griseoaptera* (Table 2). Individual legs were immediately put in 95% ethanol and stored until processing. Altogether, 220 individuals of *M. roeselii* were genotyped at six microsatellite loci: MR2-16, MR2-42, MR3-12, MR3-24 (redesigned reverse primer: 5'-CAAAGCAATAATC-

Table 2 Gene diversity at microsatellite loci within populations. Number of individuals (N), expected heterozygosity (H_E) and allelic richness (A_r)

Population ID	Fragmentation class	<i>Metriopectera roeselii</i>			<i>Pholidoptera griseoptera</i>		
		N	H_E	A_r	N	H_E	A_r
1	Low	33	0.723	6.1	31	0.654	5.6
2	Low	10	0.774	6.4	30	0.618	5.2
3	Low	11	0.711	6.4	35	0.650	5.8
	Average		0.736	6.3		0.641	5.5
4	Moderate	9	0.739	6.2	33	0.653	5.5
5	Moderate	31	0.754	7.1	29	0.656	5.5
6	Moderate	15	0.753	6.5	31	0.602	5.0
	Average		0.749	6.6		0.637	5.3
7	High	10	0.715	5.5	33	0.681	5.9
8	High	30	0.737	6.7	31	0.675	5.3
9	High	10	0.789	6.6	19	0.714	5.8
	Average		0.747	6.3		0.690	5.7
10	Very high	12	0.865	7.8	31	0.504	3.7
11	Very high	20	0.734	6.4	22	0.627	4.9
12	Very high	29	0.771	7.3	32	0.611	4.5
	Average		0.790	7.2		0.581	4.4

GTTGACCTTC-3'), MR3-34, MR3-44 (Holzhauer & Wolff 2005) and 357 individuals of *P. griseoptera* were genotyped at eight microsatellite loci: WPG1-28, WPG2-15, WPG2-16, WPG2-39, WPG7-11, WPG8-2, WPG9-1, WPG10-1 (Arens *et al.* 2005).

DNA was isolated from the femur muscle following a Chelex procedure (Walsh *et al.* 1991), involving a 10% H₂O-Chelex-solution, 4 μ L of Proteinase K (20 mg/L) and 1 μ L of RNase (80 μ g/mL). All loci were amplified in one multiplex polymerase chain reaction (PCR) for *M. roeselii*. For *P. griseoptera*, sets of two (set 1: WPG1-28, WPG8-2) and six (set 2) loci were independently co-amplified. Primers were labelled with the fluorescent dyes FAM, VIC, NED or PET, enabling unambiguous detection of similar sized amplicons from co-amplified loci. Amplification reactions were performed in 8 μ L volume following QIAGEN Multiplex PCR kit instructions (annealing temperatures: *M. roeselii*, 57 °C; *P. griseoptera*, 55 °C (set 1), 60 °C (set 2)). PCR products were genotyped on Applied Biosystems 3130xl Genetic Analyzer and fragment lengths estimated relative to the size standard LIZ 500. Microsatellite fragments were analysed and sized using GeneMapper® v3.7 software (Applied Biosystems).

Genotyping error, null alleles and neutrality of markers

To account for high proportions of null alleles in Orthoptera (Chapuis & Estoup 2007) and genotyping

errors, a blind and independent marker amplification was repeated for a random 10% of each species' samples (Pompanon *et al.* 2005). To allow for the detection of stochastic allelic dropouts and to enable standardization across genotyping plates, one negative and six positive controls were included in each run of 96 PCRs.

Mean error rate per locus was calculated based on the number of mismatches between a reference genotype and its replicates. Averages of 0.034 for *M. roeselii* and of 0.014 for *P. griseoptera* were found. Null alleles at a locus were assumed if repeated PCRs for a sample did not yield any product. Null allele frequencies were examined with Microchecker 2.2.3. High proportions of null alleles were detected in MR3-12, MR3-34 and MR3-44 (average frequency over all populations: 0.28, 0.25 and 0.37) and in WPG1-28, WPG8-2 and WPG2-39 (average: 0.07, 0.27 and 0.29) and were adjusted by introducing a new allele using the estimator of Van Oosterhout *et al.* (2004). As null alleles may affect allelic richness and heterozygosity, all calculations were repeated without null allele loci, which gave qualitatively similar results (results not shown). Given that the loci were corrected for null alleles assuming Hardy-Weinberg equilibrium, the calculation of observed heterozygosity and inbreeding coefficients is inappropriate (Van Oosterhout *et al.* 2004) and was not performed. Neutrality of microsatellite loci was tested by checking for outlying values of genetic differentiation using the method of Beaumont & Nichols (1996) in FDI22 (http://www.rubic.rdg.ac.uk/~mab/software.html). The empirical F_{ST} values of all loci of both species fell within the 95% confidence interval of expected F_{ST} as a function of H_E under a neutral model. Thus all loci can be expected to evolve neutrally.

Genetic data analysis

Genetic diversity per population was described by allelic richness and unbiased expected heterozygosity (H_E), (Nei & Chesser 1983) averaged over loci by using FSTAT 2.9.3 (Goudet 1995). H_E was not correlated to sample size for either species ($P \geq 0.409$). Allelic richness was calculated by rarefaction (El Mousadik & Petit 1996) for a minimum sample size of nine individuals for either species, representing the lowest sample size found in one population of *M. roeselii*. We compared within-population genetic diversity among fragmentation classes by generalized linear models with Quasi-poisson error distribution (allelic richness) or Gamma error distribution (H_E). If a significant main effect of habitat fragmentation on genetic diversity was apparent, the critical threshold of habitat fragmentation on genetic diversity was identified *a posteriori*. This was performed by contrasting the very high fragmentation

class with all the three remaining classes, the high fragmentation class with the two lower remaining classes and the moderate with the low fragmentation class. Potential impacts of microsatellite properties on the level of genetic diversity were checked by an analysis of covariance by relating species, repeat type (dinucleotide/trinucleotide), and the average number of repeats (allele length) per microsatellite to allelic richness and H_E (Goldstein & Schlötterer 1999; Marriage *et al.* 2009). There was a significant effect of average number of repeats on both allelic richness ($P < 0.001$) and heterozygosity ($P = 0.004$). Loci with a greater number of repeats showed greater values of genetic diversity (allelic richness: $r^2 = 0.738$, heterozygosity: $r^2 = 0.510$). There were no significant main effects or interactions of repeat type and species ($P \geq 0.157$).

Population genetic differentiation was first inferred by two traditional methods. We used G_{ST} , which is based on heterozygosities and defined as $(H_T - H_S)/H_T$ (Nei 1973), and θ which is based on variance components (Weir & Cockerham 1984). The latter was computed with the R-package HIERFSTAT (Goudet 2005). We then calculated two newly developed differentiation measures, standardized G_{ST} (G'_{ST}) following Hedrick (2005) and D (D_{est} , eqn. 12) as described in Jost (2008). For all calculations Nei & Chesser's (1983) unbiased estimates of H_T and H_S averaged across loci were used as computed in FSTAT 2.9.3 (Goudet 1995). For either bush-cricket species, we calculated G_{ST} , θ , G'_{ST} , D (i) among all 12 populations (overall differentiation) and (ii) among the three populations within each fragmentation class. Testing for significant differences was performed by permuting individual genotypes (i) among populations when testing overall genetic differentiation, and (ii) among populations within fragmentation classes when testing differentiation within fragmentation classes; 1000 permutations were performed in all tests.

We constructed approximately 95% confidence intervals (CI) for all four differentiation measures (G_{ST} , θ , G'_{ST} , D) using the range of the percentile values (2.5–97.5%) of 1000 differentiation estimates based on bootstrapping alleles within populations (Chao *et al.* 2008). When differentiation measures are either small or large (close to the boundaries of 0 or 1) CI are skewed and may not cover the observed differentiation value. In these cases, 95% CI were corrected according to the percentile method (Chao & Shen 2003). To do so, we performed a standard bootstrap procedure to obtain the 2.5% (a) and 97.5% (b) percentiles and the estimated average measure of differentiation (EMD). Then the distribution was shifted to vary around the observed measure of differentiation (OMD), so that we have a CI as $OMD - (EMD - a)$ and $OMD + (b - EMD)$. We tested whether genetic population differentiation increased

from low to high habitat fragmentation by repeatedly (1000 times) calculating Spearman's rho for the relationship between genetic differentiation and fragmentation class based on bootstrapped alleles. Genetic differentiation significantly increased with increasing habitat fragmentation, when rho values larger than 0 were found for more than 95% of the bootstrap trials ($P = 0.05$). Similar to Heller & Siegismund (2009), we investigated the relationship between the traditional and newly developed measures of genetic differentiation by regressing the observed class-specific differentiation measures of θ , G'_{ST} and D against G_{ST} .

An additional model-based Bayesian analysis of population structure was conducted with STRUCTURE 2.3.3 (Hubisz *et al.* 2009). Genetic clustering of either *M. roeselii* or *P. griseoptera* was tested with a Markov Chain Monte Carlo (MCMC) scheme for 1–12 clusters (K) applying the implemented admixture model. Because preliminary analyses showed that overall differentiation was low, we used sampling localities as prior information. Ten runs per K were carried out, each comprising a burn-in of 50 000 and a MCMC length of 100 000. We used the posterior probability of the data for a given K , called $\text{LnP}(D)$, to identify the most probable number of clusters using both ΔK values (Evanno *et al.* 2005) and guidelines of the STRUCTURE manual.

Isolation by distance was tested for by Pearson correlation and Mantel test by analysing the relation between pairwise genetic differentiation $\theta/(1-\theta)$ based on the θ estimator of Weir & Cockerham (1984) (Tables S1 and S2 Supporting information) and the logarithm of the pairwise geographical distance (Rousset 1997). Assuming also a great impact of habitat fragmentation on pairwise population differentiation, we first assessed the relationship of genetic distance and fragmentation by a classical Mantel test and second by a partial Mantel test to evaluate the relative importance of fragmentation and geographical distance. For both tests, in addition to the first matrix of pairwise genetic distances and the second matrix of log pairwise geographical distances, a third (fragmentation) matrix was constructed containing 1 if a population pair included at least one population from the very highly fragmented landscape, and 0 if not. Mantel tests were performed in FSTAT 2.9.3 (Goudet 1995). A total of 1000 randomizations were performed in classical and partial Mantel tests.

Because the sample sizes differed between the two species, we tested the consistency of our results by repeatedly (1000 times) reducing the sample size of *P. griseoptera* to that of *M. roeselii* in a random manner and recalculated the differentiation parameters. Because similar results were obtained, we present only the results of the full data set.

Unless noted otherwise, all the analyses were conducted using R version 2.8.1 (R Development Core Team 2008).

Results

Genetic diversity

For the grassland species *M. roeselii*, allelic richness averaged over loci ranged from 6.3 to 7.2 per fragmentation class. Mean expected heterozygosity (H_E) per fragmentation class ranged from 0.736 to 0.790 (Table 2). Among the four fragmentation classes neither allelic richness ($P = 0.232$) nor expected heterozygosity ($P = 0.456$) was significantly different. For the forest-edge species *P. griseoptera*, mean allelic richness averaged over loci ranged from 4.4 to 5.7 per fragmentation class. Habitat fragmentation had a significant negative effect on allelic richness ($P = 0.019$; Table 2), which was about one-fifth lower under very high fragmentation when compared to the three remaining classes (posterior contrast: $P = 0.004$). Mean expected heterozygosity (H_E) per fragmentation class showed a minimum of 0.581 and a maximum of 0.690 for this forest-edge species (Table 2). Similar to allelic richness, also expected heterozygosity tended to decrease with increasing habitat fragmentation ($P = 0.073$).

Fragmentation and population differentiation

Based on both traditional measures G_{ST} and θ , for the grassland species *M. roeselii*, a low overall population differentiation of 0.029 was observed (Table 3). Correcting for genetic variation within populations resulted

in an estimated differentiation of $G'_{ST} = 0.123$ and $D = 0.098$ that was 4.2 and 3.4 times higher than the traditional measures. *P. griseoptera* showed moderate overall population differentiation with $G_{ST} = 0.047$ and $\theta = 0.052$ (Table 3). Population differentiation estimated by $G'_{ST} = 0.139$ and $D = 0.096$ was about three and two times higher than G_{ST} and θ , respectively.

Differentiation within fragmentation classes for *M. roeselii* was significant but generally low when estimated by traditional measures with a maximum across fragmentation classes of 0.033 (G_{ST}) and 0.043 (θ) (Table 3). Substantially higher values of genetic differentiation were detected for both new measures with a maximum across fragmentation classes of 0.176 (G'_{ST}) and 0.149 (D). Genetic differentiation did not significantly increase with increasing habitat fragmentation for *M. roeselii* regardless of the measure used ($P \geq 0.296$). In contrast, genetic differentiation of *P. griseoptera* significantly increased with increasing habitat fragmentation as all simulated rho values were greater than zero (Spearman's rank test $P = 0.001$; Fig. 3). The species showed a moderate and significantly greater differentiation among populations within the very highly fragmented region than within the other three fragmentation classes, which were less differentiated and did not differ (Table 3, Fig. 3). Again, genetic differentiation within classes measured by G_{ST} and θ was numerically lower than evaluated by both newly developed measures, which showed a maximum of 0.226 (G'_{ST}) and 0.166 (D) in the very highly fragmented region; differentiation was significant in all classes (Table 3).

Bayesian analysis of population structure considering sampling locations revealed a single genetic cluster for *M. roeselii* and three clusters for *P. griseoptera* (Fig. S1

	G_{ST}	θ	G'_{ST}	D
<i>Metriopectera roeselii</i>				
Overall—				
Among all 12 populations	0.029*	0.029*	0.123*	0.098*
Among populations within the class of				
Low fragmentation	0.020**	0.034*	0.103**	0.085**
Moderate fragmentation	0.033*	0.043*	0.176*	0.149*
High fragmentation	0.023*	0.032*	0.127*	0.106**
Very high fragmentation	0.021*	0.028*	0.139*	0.121*
<i>Pholidoptera griseoptera</i>				
Overall—				
Among all 12 populations	0.047*	0.052*	0.139*	0.096*
Among populations within the class of				
Low fragmentation	0.008*	0.013*	0.028**	0.021*
Moderate fragmentation	0.010*	0.015*	0.034*	0.025**
High fragmentation	0.018*	0.026*	0.080*	0.063*
Very high fragmentation	0.074*	0.114*	0.226*	0.166*

Table 3 Genetic differentiation with traditional (G_{ST} , θ) and with newly developed differentiation measures (G'_{ST} , D)

Significance was obtained by permuting individual genotypes among populations; 1000 permutations were performed in all tests; * $P < 0.001$, ** $P < 0.01$.

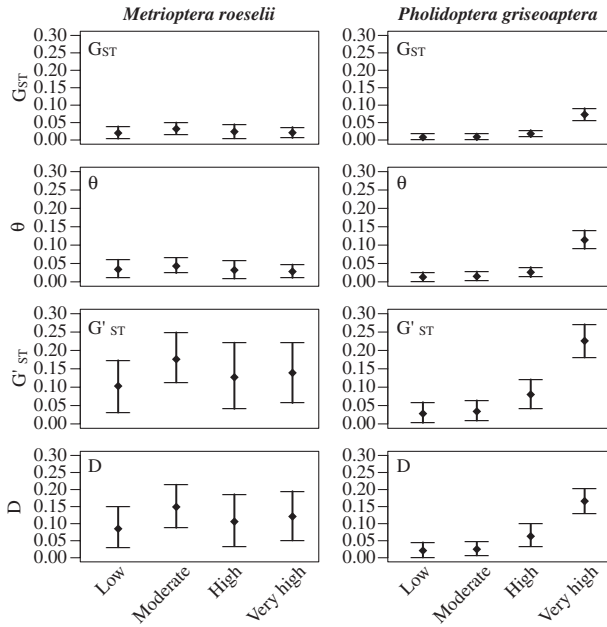


Fig. 3 Genetic differentiation within fragmentation classes measured by traditional measures G_{ST} and θ and both newly developed differentiation measures G'_{ST} and D . The 95% confidence intervals are the 2.5th and 97.5th percentile of 1000 simulation trials based on bootstrapping alleles within populations.

Supporting information). In *M. roeselii*, mean $\text{LnP}(D)$ decreased from $K = 1$ to $K = 2$ and at $K > 1$, there was considerable variation between individual STRUCTURE runs resulting in overall low ΔK values. Thus, all sampling sites were part of a single genetic population, irrespective of the level of fragmentation. In contrast, in *P. griseoptera*, two clusters corresponded to four populations in the high and very highly fragmented region, while all other less fragmented populations made up the third cluster.

Isolation by distance

For *M. roeselii*, no isolation by distance was apparent ($r^2 = 0.011$, $P = 0.418$; Fig. 4), whereas for *P. griseoptera* pairwise genetic distances between populations significantly increased with increasing geographical distances between populations ($r^2 = 0.106$, $P = 0.008$; Fig. 4). However, a Mantel test taking into account the affiliation of pairs to the class of very high fragmentation, indicated that habitat fragmentation was the major cause of pairwise population differentiation in *P. griseoptera* ($r^2 = 0.609$, $P = 0.001$). A partial Mantel test with both distance and very high fragmentation did not explain more variation of genetic distance than fragmentation alone. However, distance and fragmentation are intercorrelated, so their relative contribution cannot be disentangled ($r^2 = 0.125$, $P = 0.004$). A lack of isolation by distance was found when the three populations of the highly fragmented class were excluded, and only the nine remaining populations were analysed ($r^2 = 0.095$, $P = 0.073$). For *M. roeselii*, no effect of fragmentation or

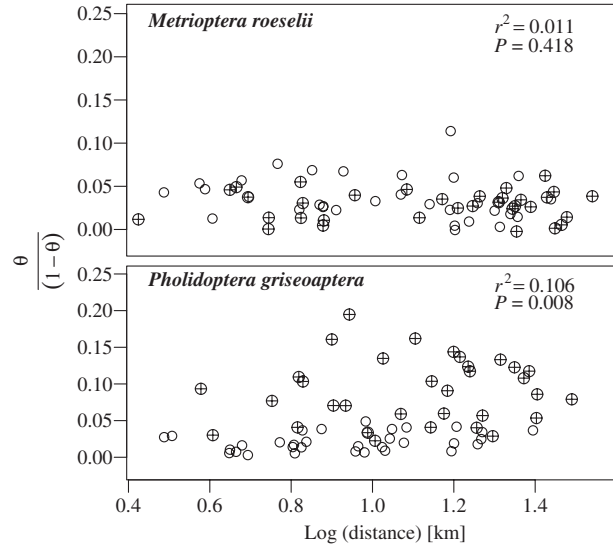


Fig. 4 Relationship between pairwise genetic differentiation $\theta/(1-\theta)$ and the logarithms of geographical distances for the grassland bush-cricket *M. roeselii* (above) and the forest-edge bush-cricket *P. griseoptera* (below). Circles with crosses represent pairwise distances including at least one population from the very highly fragmented landscape. Note that in *P. griseoptera* genetic distance was much better explained by fragmentation alone ($r^2 = 0.609$, $P = 0.001$) than by geographical distance.

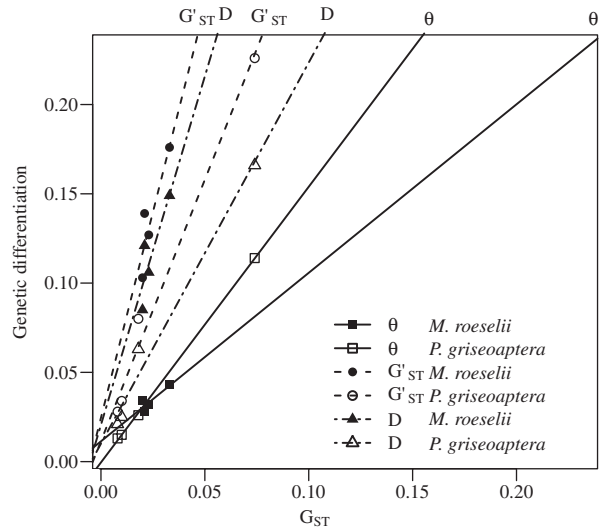


Fig. 5 Relationship between traditional differentiation measures G_{ST} and θ and both newly developed measures G'_{ST} and D . Genetic differentiation within the four fragmentation classes for each cricket species is shown.

geographical distance was obtained by the partial Mantel test.

Comparison of differentiation measures

Genetic differentiation as estimated by the two newly developed measures G'_{ST} and D was always higher than the differentiation estimated by the traditional measures (Table 3, Fig. 5). When differentiation measures among populations within fragmentation classes were correlated to traditional G_{ST} , the highest correlation for *M. roeselii* was found between G'_{ST} and G_{ST} ($r^2 = 0.903$, $P = 0.097$). Despite strong positive associations, neither test was significant for *M. roeselii* because of the small sample size of four (θ/G_{ST} : $r^2 = 0.887$, $P = 0.113$; D/G_{ST} : $r^2 = 0.865$, $P = 0.135$). However, all the correlations for *P. griseoptera* were significant, showing the highest correlation between θ and G_{ST} ($r^2 = 0.999$, $P < 0.001$); associations between D and G_{ST} ($r^2 = 0.989$, $P = 0.011$) or G'_{ST} and G_{ST} ($r^2 = 0.993$, $P = 0.007$) were slightly lower. Regression slopes of θ , D and G'_{ST} against G_{ST} differed considerably between the two species and were higher for the grassland species *M. roeselii* except for θ (θ : 0.94, D : 3.9, G'_{ST} : 4.6) than for *P. griseoptera* (θ : 1.5, D : 2.1, G'_{ST} : 2.9; Fig. 5). Genetic differentiation per fragmentation class remained in the same rank order among fragmentation classes for *P. griseoptera*, independent of the differentiation measure used. In contrast, for *M. roeselii*, the moderate fragmentation class was ranked highest by all differentiation measures, whereas the order for the remaining classes varied (Table 3).

Discussion

High genetic diversity is regarded as fundamental for evolutionary and ecological processes (Frankham 2003; Hughes *et al.* 2008). In modern agricultural landscapes, however, genetic diversity is—like other components of biodiversity—at risk because of the fragmentation of many habitat types. In fragmented landscapes, genetic diversity within populations is expected to decrease if populations become small and isolated (Madsen *et al.* 1996; Keller & Waller 2002; Gaggiotti 2003). Genetic differentiation, in contrast, is expected to increase with habitat fragmentation owing to disrupted gene flow among these isolated populations (Wright 1951). Effects of fragmentation, however, depend on landscape context (Holzhauer *et al.* 2006) and on species traits (e.g. Anton *et al.* 2007). Thus, empirical studies that compare selected species with differing traits are ideally suited to reveal the underlying mechanisms and potential thresholds of fragmentation.

Using two widespread and abundant bush crickets that differ in the level of disturbance of their preferred

habitats and in their dispersal abilities, we revealed differential genetic responses to habitat fragmentation. The grassland species *M. roeselii* did not follow the expectations by neither differing in genetic diversity nor genetic differentiation among the four classes of habitat fragmentation investigated. In contrast, the forest-edge species *P. griseoptera* showed reduced genetic diversity and increased genetic differentiation at very high levels of habitat fragmentation. These differential responses may have two reasons. First, *M. roeselii* has higher dispersal ability because of a small fraction of macropterous, i.e. long-winged morphs (~1%; Simmons & Thomas 2004). Macroptery has been shown to be induced in *M. roeselii* by stress associated with high densities (Poniatowski & Fartmann 2009) and also disturbance has been associated with macroptery (Zera & Denno 1997). Agricultural grasslands harbour high densities of *M. roeselii* (Ingrisch & Köhler 1998) and are frequently disturbed by mowing or grazing (Berggren 2004). Thus, a proportion of macropterous individuals and hence efficient dispersal and gene flow can be expected. Second, *M. roeselii* not only occurs on typical grasslands, but also on road edges and grassy margins along fields.

Road edges and grassy margins are common features in most agricultural landscapes (Berggren *et al.* 2001). These elements may act as corridors for dispersal (Berggren *et al.* 2002) and facilitate the exchange of individuals among more distant patches (Holzhauer *et al.* 2009). Grassy margins may also provide temporal retreats from which individuals could recolonise grassland patches after disturbances (Saarinen *et al.* 2005). Thus, even though grasslands in intensively used agricultural landscapes often seem highly isolated, our results suggest that the dense network of linear grassland structures assist to prevent the erosion of genetic diversity and local extinctions of *M. roeselii*. In addition, large population sizes and an annual generation time may keep genetic drift negligible and levels of genetic variation high enough for long-term persistence in disturbed habitats and fragmented landscapes (Gaggiotti 2003; Frankham *et al.* 2010).

The forest-edge species *P. griseoptera* showed lower levels of genetic differentiation than *M. roeselii* at the three lower fragmentation levels. In contrast to *M. roeselii*, however, a clear threshold of habitat fragmentation could be detected. In the most fragmented landscape, *P. griseoptera* showed significantly lower genetic diversity and higher genetic differentiation than in the three less fragmented landscapes. The STRUCTURE analysis considering sampling locations stresses the isolation of the populations in the most fragmented landscape. Together with the pattern of isolation by distance, which was driven by these most isolated populations, this

clearly indicates that gene flow among populations of *P. griseoptera* was not limited by geographical distance *per se*. Only when the amount of suitable habitat dropped below twenty per cent at the landscape scale did gene flow between populations become restricted. A similar threshold was also established for dispersal success, population persistence, species distribution and community composition in simulation approaches (e.g. Fahrig 1997; With & King 1999a,b) and empirical studies (Wiens *et al.* 1997; Tschardt *et al.* 2002; With *et al.* 2002; Schmidt & Roland 2006). This agreement of our results with those of simulations and studies in landscape of small to medium spatial extents indicates that indeed habitat fragmentation may be considered causal for the restricted gene flow at a larger landscape scale. The comparatively low fragmentation threshold (cf. Swift & Hannon 2010) and the lower differentiation of *P. griseoptera* than that of *M. roeselii* above this threshold and earlier findings (Diekötter *et al.* 2010) suggest that this flightless bush-cricket species has good dispersal abilities. Yet, revealing a threshold highlights that even widespread species may be threatened by habitat fragmentation in modern agricultural landscapes.

The threat of habitat fragmentation to species persistence, however, may not only depend on the amount and isolation of suitable habitat but also on habitat disturbance, matrix quality and landscape type and the interaction of these variables. Habitat disturbance, such as the mowing of grasslands, may lead to population bottlenecks accompanied by frequent founder effects. Thus, at low levels of fragmentation genetic drift owing to and following such population bottlenecks was expected to decrease genetic diversity within and to increase differentiation among populations of *M. roeselii* compared to *P. griseoptera* (Cleary *et al.* 2006; Vignieri 2010). Contrary to our expectations, the grassland species *M. roeselii* showed in all classes of habitat fragmentation greater allelic richness and heterozygosity than the forest-edge species *P. griseoptera*.

This higher genetic diversity in *M. roeselii* compared to *P. griseoptera* may be attributable to a more rapid accumulation of genetic variation because of the former species' shorter generation time. Despite the presence of macropters, levels of gene flow may not be high enough to equalize this variation among populations of *M. roeselii* leading to higher differentiation than in *P. griseoptera* (Hochkirch & Damerau 2009). Habitat disturbances may also force individuals to search for alternative habitat patches, thereby enhancing matrix effects on dispersal (cf. Ricketts 2001). Reduced matrix quality because of intensified or altered land use may be expected to result in increased fragmentation thresholds. Different levels of spatial or temporal heterogeneity within the matrix or the local habitat among

landscapes, in turn, may lead to different dispersal abilities and behaviour among populations of the same species from these different landscapes (Taylor & Merriam 1995; Hanski *et al.* 2004; R. Lange, S. Brand, S. U. Mollitor, V. Wolters, S. I. J. Holzhauser & T. Diekötter, unpublished data). Thus, fragmentation thresholds may not only differ among species but also within species in differently structured landscape contexts.

Identifying thresholds of habitat fragmentation at which the viability of species rapidly declines is essential in understanding the patterns of biodiversity in fragmented landscapes such as agricultural ones (Fahrig 1997; With & King 1999b). Genetic diversity represents one key aspect of biodiversity and crucially affects the viability of species (Niemininen *et al.* 2001; Reed & Frankham 2003). Thus, identifying fragmentation thresholds for inter-patch dispersal, genetic diversity and genetic differentiation is important to enable recommendations for sustainable landscape planning and conservation (Ezard & Travis 2006). The idea of using critical thresholds in habitat proportion has been criticized because threshold levels are likely to vary by species and landscape type, which will render general management recommendations difficult or impossible (cf. Swift & Hannon 2010). If sufficient empirical support exists, however, systematic variation or congruence in threshold levels among species, landscapes or response variables may provide valuable information to better understand the underlying ecological processes.

The usefulness of different estimators of population differentiation has been debated recently (Jost 2008; Heller & Siegismund 2009; Ryman & Leimar 2009). Here, we were able to show that the influence of landscape fragmentation on genetic differentiation was qualitatively unaffected by the choice of measure. All measures indicated reduced gene flow at very high fragmentation in *P. griseoptera*. Because the expected within-population diversity was higher than 0.5 for both species in our study, however, traditional measures necessarily, quantitatively underestimated genetic differentiation. This deviation between traditional and newly developed measures was larger in *M. roeselii* compared to *P. griseoptera* because of the former species' higher genetic diversity (cf. Jost 2008). Again, this difference in genetic diversity may be attributed to the shorter generation time of 1 year in *M. roeselii* when compared to 2 years in *P. griseoptera* (Ingrisch & Köhler 1998).

Conclusions

We conclude that even for widespread species, habitat fragmentation in modern agricultural landscapes may reach a critical threshold below which the long-term

survival of these species may become threatened. Our results show that these thresholds may differ substantially even for closely related species. Differences in the type and level of disturbance of the preferred habitat and potentially associated differences in their dispersal potential and behaviour result in a species-specific ability to cope with habitat fragmentation. Our results also show that the detected effect of habitat fragmentation on population genetic differentiation quantitatively differs depending on the measures of genetic differentiation applied. Therefore, to arrive at scientifically sound recommendations to landscape planning and conservation, we caution against generalizations even across closely related taxa and recommend the employment of newly developed measures of genetic differentiation that may better deal with levels of genetic diversity and enable valid comparisons among species.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Pairwise population $\theta/(1-\theta)$ matrix derived from the θ estimator of Weir & Cockerham (1984) for the grassland species *Metriopectera roeselii*; 1320 permutations, bold values represent significant pairwise genetic differentiation ($P = 0.05$). P -values were corrected for multiple testing. Calculations were done in FSTAT 2.9.3 (Goudet 1995)

Table S2 Pairwise population $\theta/(1-\theta)$ matrix derived from the θ estimator of Weir & Cockerham (1984) for the forest edge species *Pholidoptera griseoptera*; 1320 permutations, bold values represent significant pairwise genetic differentiation ($P = 0.05$). P -values were corrected for multiple testing. Calculations were done in FSTAT 2.9.3 (Goudet 1995)

Fig. S1 Results of STRUCTURE analyses for *Metriopectera roeselii* (A) and *Pholidoptera griseoptera* (B, C). A/B: Mean and individual posterior probabilities (LnP(D)) and ΔK values (Evanno *et al.* 2005) based on 10 runs (burnin 50 000 + 100 000 MCMC) for $K = 1-12$. C: Typical result of individual cluster membership and admixture for *P. griseoptera* at $K = 3$. Note dominance of a single cluster in most populations of low to high fragmentation (1–8) and strong differentiation of two additional clusters at the high and very high fragmentation level (9–12).

[Correction added after online publication 27 October 2010: the Supporting information file was corrected.]

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