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Effects of habitat fragmentation on the genetic structure of the monophagous butterfly *Polyommatus coridon* along its northern range margin

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Abstract

Population genetic patterns of species at their range margin have important implications for species conservation. We performed allozyme electrophoresis of 19 loci to investigate patterns of the genetic structure of 17 populations (538 individuals) of the butterfly *Polyommatus coridon*, a monophagous habitat specialist with a patchy distribution. The butterfly and its larval food plant *Hippocrepis comosa* reach their northern distribution margin in the study region (southern Lower Saxony, Germany). Butterfly population size increased with host plant population size. The genetic differentiation between populations was low but significant (F_{ST} = 0.013). No isolation-by-distance was found. Hierarchical *F*-statistics revealed significant differentiation between a western and an eastern subregion, separated by a river valley. The combination of genetic and ecological data sets revealed that the expected heterozygosity (mean: 18.5%) decreased with increasing distance to the nearest *P. coridon* population. The population size of *P. coridon* and the size of larval food plant population had no effect on the genetic diversity. The genetic diversity of edge populations of *P. coridon* was reduced compared to populations from the centre of its distribution. This might be explained by (i) an increasing habitat fragmentation towards the edge of the distribution range and/or (ii) a general reduction of genetic variability towards the northern edge of its distribution.

Keywords: allozymes, calcareous grasslands, genetic diversity, habitat area, isolation-by-distance, population size

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Introduction

Habitat fragmentation is a major threat to biodiversity. Two main components of habitat fragmentation produce population effects: reduced fragment size and reduced connectivity (Krebs 2001). Smaller habitats cause smaller populations and increasing isolation leads to reduced colonization rates, therefore enhancing the risk of extinction (Rosenzweig 1995). Genetic diversity of populations decreases with increasing habitat fragmentation (e.g. Young *et al*. 1996; Buza *et al*. 2000; Pedersen & Loeschcke 2001; Keller & Largiadèr 2003; Williams *et al*. 2003). Decreasing genetic diversity, an indicator of inbreeding, increases the extinction risk of populations due to a decline in fitness of individuals (Saccheri *et al*. 1998; Reed & Frankham 2003). Therefore, conservation biologists pay increasing attention to the effects of habitat fragmentation on genetic diversity. However, most studies do not consider habitat isolation separately and focus on population size or combined effects only. Few published studies on genetic consequences of habitat fragmentation focus on butterflies or moths (Descimon & Napolitano 1993; Meglécz *et al*. 1997; Berwaerts *et al*. 1998; Williams *et al*. 2003), and only two take an independent isolation factor into account (Van Dongen *et al*. 1998; Schmitt & Seitz 2002a).

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The intensification of agricultural land-use in Central Europe has increased during the last decades, thereby reducing low-impact, seminatural habitats, such as calcareous grasslands (WallisDeVries *et al*. 2002). This habitat type is considered as endangered in Germany (Riecken *et al*. 1994). In southern Lower Saxony chalk bedrocks are scarce so that the fragmentation in our study region is considerably higher than in southern Germany. Calcareous grasslands are one of the most species-rich habitats for plants and butterflies in Central Europe (van Swaay 2002; WallisDeVries *et al*. 2002), resulting in high conservation interest. Apart from their ecological importance, these grasslands provide a useful habitat type to study the effects of habitat fragmentation, as they can be distinguished clearly from the surrounding landscape matrix.

To study the effects of fragmentation on the genetic structure of calcareous grassland specialists, the butterfly *Polyommatus coridon* (Poda) was chosen as a model organism. Its larval food plant *Hippocrepis comosa* L. is specialized on calcareous grasslands (Asher *et al*. 2001). Interestingly, both species reach their northern distribution limit in the study region (Kudrna 2002; Hennenberg & Bruelheide 2003). Fragmentation effects are generally supposed to be strongest at the edge of the distribution of a species (Thomas *et al*. 2001; Bourn & Thomas 2002). The genetic variability at the edge of the distribution range might be reduced, as genetic bottlenecks during range expansion decrease genetic variability with increasing distance from the native range (Stone & Sunnucks 1993; Lammi *et al*. 1999; Schmitt *et al*. 2002).

In this context, the aim of our study was to determine the genetic structure and genetic diversity of *P. coridon* at the edge of its distribution. We focus on the following questions:

1 Do *P. coridon* populations at the northern range margin show reduced genetic variability?
2 Has *P. coridon* a significant genetic structure between the studied (sub)population(s)?
3 Has a system of isolation-by-distance established in this species?
4 Are the *P. coridon* population groups of two subregions, divided by a river valley, genetically differentiated?
5 Is the genetic diversity of this species influenced by population size, habitat area, distance to the nearest neighbouring *P. coridon* population and the size of the food plant population?

Materials and methods

Study region and study sites

The study region is located in the Leine-Weser mountains. We selected the calcareous grasslands in the vicinity of the city of Göttingen (southern Lower Saxony, Germany) as study sites. The landscape is a structurally rich mosaic of diverse habitat types. Calcareous grasslands can be distinguished clearly from the surrounding landscape and cover only 0.26% of the surface of the study region. Further details of the sites and region are given in Krauss *et al*. (2003a,b; 2004). The valley of the river Leine, with intensive agricultural and urban land use and no naturally occurring calcareous bedrock, separates two regions, a western region with a higher density of calcareous grasslands and an eastern region with more isolated chalk hills (see Fig. 1).

Study species

The chalk-hill blue *Polyommatus coridon* (Poda) (Lycaenidae, Lepidoptera) is an univoltine habitat specialist of calcareous grasslands (Asher *et al*. 2001; Van Swaay 2002), distributed over major parts of southern and Central Europe (Kudrna 2002). The only larval food plant in the study region is the horseshoe vetch *Hippocrepis comosa* L. (Fabaceae). Only one recent *P. coridon* population is known north of our study area (50 km from the most northern population of our study region). This is also the distribution limit of *H. comosa* (Hennenberg & Bruelheide 2003). There is a gap of at least 30 km with no populations of *P. coridon* to the east and west of the study region, while it seems to be well connected to populations further south (unpublished data following the *Niedersächsisches Landesamt für Ökologie* and personal observations of J. Krauss).

Population densities of adult *P. coridon* are generally high and often reach levels of 500–1000 individuals per hectare (Bink 1992; Weidemann 1995). The dispersal ability of adult *P. coridon* is expected to be moderate, compared to other butterflies (Bink 1992; Asher *et al*. 2001; Cowley *et al*. 2001). Mark–release–recapture experiments in the United Kingdom suggest that adults disperse between colonies 1–2 km apart with 1–2% of the populations, while single adults have been seen 10–20 km from known colonies (Asher *et al*. 2001). Differences of dispersal abilities between male and female *P. coridon* are not known, but for other butterfly species Mark–release–recapture data revealed no significant differences in dispersal distances between sexes (e.g. Gutierrez *et al*. 1999; Roland *et al*. 2000).

Climate change might affect species at the northern distribution range and shift their distribution northwards (Parmesan *et al*. 1999; Warren *et al*. 2001). This effect is found mainly for habitat generalists, while habitat specialists such as *P. coridon* are more affected by habitat changes (Warren *et al*. 2001). It is known from the study region that the occurrence and population density of most habitat specialist butterflies are triggered by the size of available habitats (Krauss *et al*. 2003a). The limitation of available food plant populations further north clearly limits a northward distribution shift of *P. coridon*.

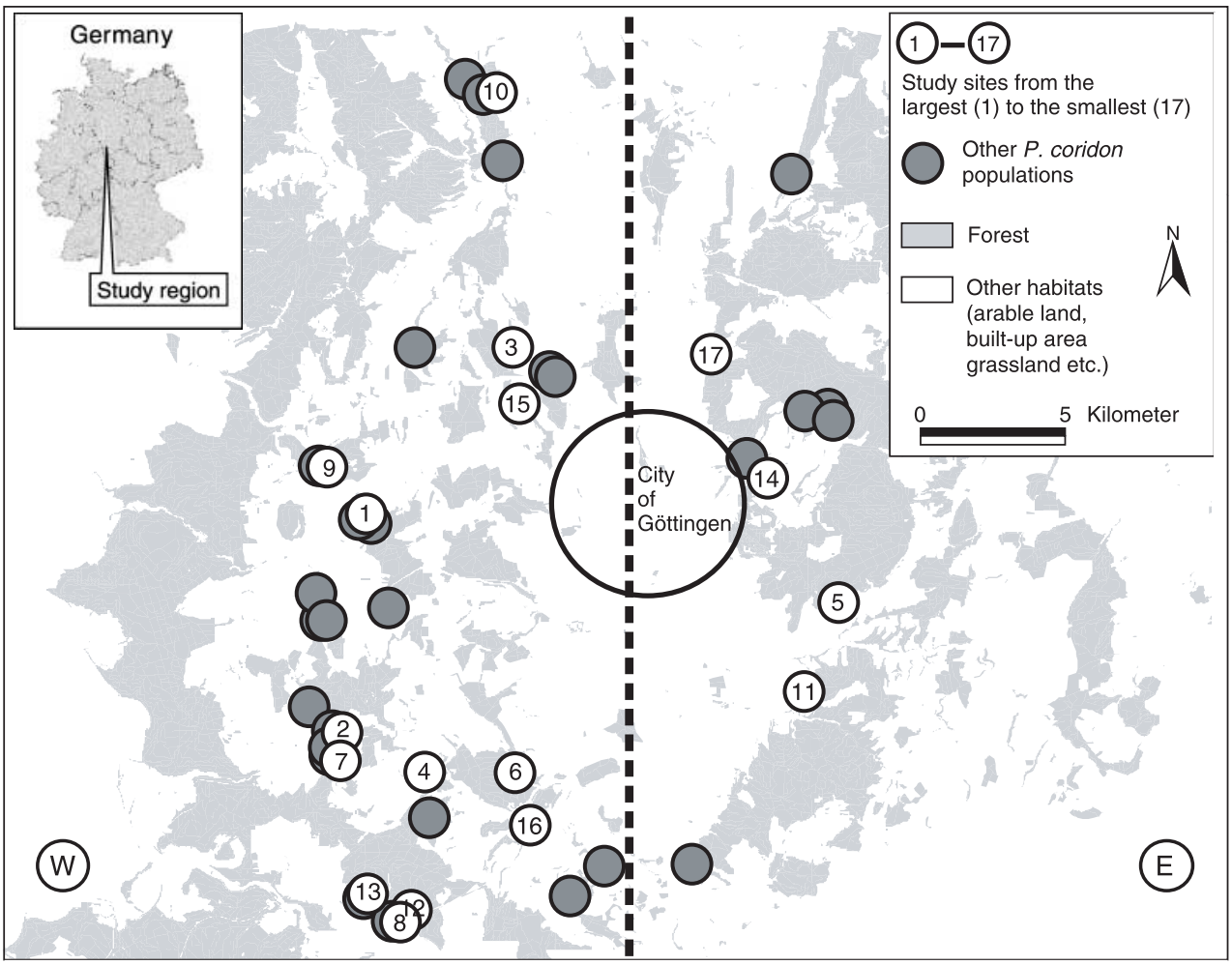

Fig. 1 Location of the 17 studied *P. coridon* populations and all further populations in the study region. The region is divided by a river valley (dashed line) into a western (W) and an eastern subregion (E). The study sites are numbered from the largest (1) to the smallest (17). Details about the study sites are given in Table 1.

Population size, habitat isolation and food plant availability

We selected 17 populations of *P. coridon*, differing in population size, habitat area, habitat isolation and the population size of larval food plant (see Table 1).

The habitat area of each of the 17 study sites was measured with a differential GPS GEOmeter 12 L (GEOsat GmbH, Wuppertal, Germany) in 2000 or 2001. *P. coridon* individuals were counted during transect walks in 2001 (19 July–02 August) and in 2002 (23 July–02 August). Each transect walk was 20 min and was conducted from 10.00 a.m. to 5.00 p.m. (only twice from 5.00 p.m. to 6.00 p.m.), when weather conditions were suitable for butterfly activity to avoid diurnal and weather effects according to Pollard (1977) and Erhardt (1985). To reduce effects of sampling schedule and between-year differences in management, we used the higher individual number of the two years to calculate population sizes. Transect distance was measured with a step counter and individuals were counted within an area of 2.5 m to each side, thus allowing an estimation of population density per hectare. By multiplication with habitat area, we obtained a relative estimation of the population size on each site.

We performed a simple Mark–release–recapture experiment with 656 captured and 391 recaptured individuals (Lincoln-Petersen) for one of the study sites (Kleiner Knüll) in 2002. Hereby, we estimated the highest day populations for males and females to be 6.1 times higher than in our calculation based on the transect count (unpubl. data J. Krauss). Therefore, we multiplied all population sizes with this factor to obtain more realistic population sizes.

Habitat isolation was defined as the shortest distance to the nearest *P. coridon* population including 50 independent populations in the region. All sites with the larval food plant *H. comosa* in the study region were visited in summer

Table 1 Habitat factors of the 17 study sites and the genetic variability measures for *P. coridon*

Locality	No.	Subregion West (W) or East (E)	Habitat area (m²)	Population size of *P. coridon* (individuals)	Distance to nearest *P. coridon* population (m)	Population size of *H. comosa* (m²)	Mean no. alleles per locus *A*	H_E %	H_O %	Polymorphic loci (total) P_{tot} (%)	Polymorphic loci (95% level) P_{95} (%)
Mühlenberg	1	W	51 400	6570	70	280	2.58	18.4	18.2	78.9	42.1
Huhnsberg	2	W	38 780	6810	130	4600	2.16	17.3	14.9	68.4	47.4
Aschenburg	3	W	30 800	12 470	1180	5000	2.63	20.1	18.9	78.9	52.6
Mackenrodt	4	W	20 900	3950	1500	700	2.42	18.5	18.3	68.4	52.6
Lengder Burg	5	E	16 000	7780	3430	2000	2.53	17.5	15.8	73.7	42.1
Tiefetal	6	W	12 600	2070	1900	200	2.32	16.3	16.9	63.2	52.6
Meenser Heide	7	W	7440	800	360	70	2.58	19.5	17.6	78.9	47.4
Hackelberg	8	W	6340	1580	190	1500	2.42	20.6	19.3	63.2	52.6
Östlicher Riesenberg	9	W	6250	650	130	100	2.58	22.6	21.5	63.2	52.6
Fläche 9	10	W	5050	510	150	150	2.37	18.9	19.5	68.4	42.1
Kleiner Knüll	11	E	3840	770	3430	700	2.37	17.9	15.6	73.7	52.6
Vor dem roten Berge	12	W	3760	620	580	20	2.47	16.9	17.6	73.7	47.4
Weinberg	13	W	3580	1760	180	1000	2.47	19.9	17.8	78.9	57.9
Luttertal	14	E	3240	550	860	600	2.32	16.7	14.6	78.9	63.2
Kuhberg	15	W	3210	630	1470	810	2.47	18.1	17.0	68.4	42.1
Wetenborn	16	W	3130	350	1900	250	2.37	19.3	19.3	68.4	52.6
Schweineberg	17	E	2290	690	3850	650	2.11	16.8	18.1	68.4	42.1

H_E: expected heterozygosity; H_O: observed heterozygosity.

Table 2 Locus-specific F_{ST}, F_{IS} and F_{IT} values, standard deviation and significances (calculations: G-STAT version 3 (Siegismund 1993) ($n = 17$) and P-values ARLEQUIN 2.000 (Schneider *et al*. 2000)

Locus	EC-No.	F_{ST}	P	F_{IS}	P	F_{IT}	P
Idh1	1.1.1.42	0.021 ± 0.012	0.041	0.067 ± 0.035	0.056	0.086 ± 0.033	0.026
Idh2	1.1.1.42	0.033 ± 0.025	< 0.0001	−0.020 ± 0.056	0.389	0.014 ± 0.037	0.294
Mdh1	1.1.1.37	0.004 ± 0.005	0.731	−0.028 ± 0.007	0.662	−0.024 ± 0.006	1.000
Mdh2	1.1.1.37	0.013 ± 0.008	0.158	−0.004 ± 0.044	0.531	0.009 ± 0.046	0.422
6-Pgdh	1.1.1.44	0.000 ± 0.005	0.951	0.036 ± 0.063	0.154	0.036 ± 0.063	0.265
G-6-Pdh	1.1.1.49	0.012 ± 0.006	0.190	0.085 ± 0.104	0.162	0.096 ± 0.107	0.065
G-3-Pdh	1.2.1.12	undefined	undefined	undefined	undefined	undefined	undefined
Pgi	5.3.1.9	0.016 ± 0.011	0.048	0.035 ± 0.030	0.124	0.050 ± 0.028	0.068
Pgm	5.4.2.2	0.009 ± 0.005	0.404	0.087 ± 0.032	0.004	0.096 ± 0.031	< 0.0001
Pep D $_{(Phe\text{-}Pro)}$	3.4.11	0.014 ± 0.008	0.163	0.113 ± 0.036	0.001	0.125 ± 0.034	< 0.0001
AAT1	2.6.1.1	0.000 ± 0.004	0.980	0.004 ± 0.041	0.486	0.004 ± 0.041	0.642
AAT2	2.6.1.1	0.023 ± 0.009	< 0.0001	−0.010 ± 0.038	0.526	0.014 ± 0.036	0.365
Gpdh	1.1.1.8	0.035 ± 0.035	0.001	−0.038 ± 0.038	1.000	−0.002 ± 0.002	1.000
Fum	4.2.1.2	0.014 ± 0.008	0.109	−0.027 ± 0.010	1.000	−0.012 ± 0.005	1.000
Me	1.1.1.40	0.026 ± 0.017	0.001	−0.060 ± 0.024	1.000	−0.033 ± 0.008	1.000
Apk	2.7.3.3	−0.000 ± 0.001	1.000	−0.000 ± 0.000	1.000	−0.000 ± 0.001	1.000
Pk	2.7.1.40	0.013 ± 0.010	0.133	−0.023 ± 0.013	1.000	−0.010 ± 0.004	0.933
Hbdh	1.1.1.30	0.009 ± 0.009	0.482	0.111 ± 0.030	0.003	0.119 ± 0.028	0.004
Acon	4.2.1.3	0.006 ± 0.008	0.517	−0.020 ± 0.010	1.000	−0.014 ± 0.004	1.000
All loci	—	0.013 ± 0.004	0.006	0.046 ± 0.016	< 0.0001	0.058 ± 0.015	< 0.0001

2001 and 2002 to count *P. coridon* during 20-min transect walks. Only patches with at least 10 individuals found in one of the two study years were considered as reproductive colonies. This helps to reduce misinterpretations of dispersing individuals. None of these populations was extinct or newly populated between the two study years. All populations of *P. coridon* are shown in Fig. 1.

Food plant availability and their total population size per habitat were measured in May and June 2001 by transect walks (covering the whole habitat) of all known calcareous grasslands in the study region ($n = 298$). We assume to have mapped all the *H. comosa* populations in the study region.

Sampling, genetic and statistical analyses

P. coridon butterflies were caught at the 17 study sites from 6 to 9 August 2001. Only males were collected to avoid damaging of populations. In total, 538 individuals (31–33 per study site) were netted. The butterflies were kept in a cold box until the evening. They were then frozen in liquid nitrogen and stored in a freezer (−80 °C) until allozyme analysis.

Nineteen allozyme loci (see Table 2) were studied by means of cellulose acetate electrophoresis using the protocol of Schmitt & Seitz (2001a). In contrast to earlier publications Acon was buffered in Tris-citrate and not in Tris-maleic acid.

Statistical analyses were performed using the software G-STAT version 3 (Siegismund 1993) for the calculation of the mean number of alleles per locus (A), the expected heterozygosity (H_E), the observed heterozygosity (H_O), the percentage of polymorphic loci with the most common allele not exceeding 95% (P_{95}), the percentage of polymorphic loci (P_{tot}), the F statistics for single loci and the genetic distances (Nei 1978). GENEPOP version 3.3 (updated version of GENEPOP version 2.1, Raymond & Rousset 1995) was used for tests on linkage disequilibrium and Mantel tests, and ARLEQUIN 2.000 (Schneider *et al*. 2000) for Hardy–Weinberg disequilibrium, hierarchical variance analyses and P-values of F-statistics. Neighbour-joining (Saitou & Nei 1987) and UPGMA phenograms were calculated from Nei (1978) genetic distances, using the package PHYLIP version 3.5c (Felsenstein 1993).

We compared the genetic diversity of *P. coridon* populations of our data set with recalculated data from Schmitt *et al*. (2002) from the distribution centre of *P. coridon* in southern France. Thereby, we reduced their sample size to 32 individuals per population and excluded the locus LDH to obtain a comparable adjusted data set. Genetic analyses and the laboratory were identical in both studies.

Statgraphics Plus for Windows 3.0 (Statgraphics 1995) was used for standard statistical procedures as t-tests, simple and multiple regressions and Pearson correlations. We chose stepwise backward elimination for multiple regressions (Sokal & Rohlf 1995). All dependent variables satisfy the assumptions of normal distribution. Habitat factors were $\log_{10}$ transformed. Arithmetic means of non-transformed data ± 1 standard deviation (SD) are given in the text.

Table 3 Genetic diversity of *P. coridon* populations at the distribution centre in southern France (literature data: $n = 11$ populations) compared to populations at the range margin (our data: $n = 17$ populations). The literature data from Schmitt *et al.* 2002) are adjusted to our sample size of 32 individuals and equal loci. Comparison of means: *t*-test

	Distribution centre	Range margin	*t*-value	*P*
A	2.80 ± 0.27	2.42 ± 0.15	4.89	< 0.0001
H_E (%)	20.2 ± 1.5	18.5 ± 1.7	2.65	0.014
P_{tot} (%)	80.4 ± 6.7	71.5 ± 5.9	3.70	0.001
P_{95} (%)	53.1 ± 4.4	49.5 ± 6.2	1.66	0.108

A: mean number of alleles per locus, H_E: expected heterozygosity, P_{95}: percentage of polymorphic loci with the most common allele not exceeding 95%, P_{tot}: percentage of polymorphic loci.

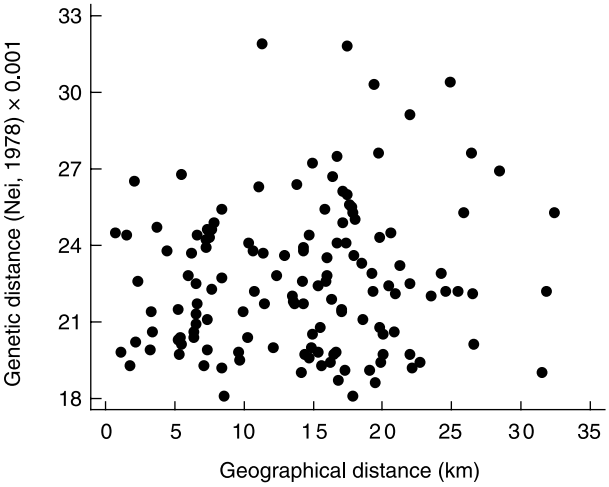

Fig. 2 Relationship between geographical and genetic distance (Nei 1978) for 17 *P. coridon* populations in southern Lower Saxony (Germany). Mantel test with 2000 permutations (GENEPOP version 3.3), genetic distance (Nei 1978) = 0.0210 + 0.0002 geographical distance, but correlation was not significant ($P = 0.251$). In addition, pairwise F_{ST} against geographical distance was not significantly correlated (Mantel test: $P = 0.189$).

Results

Eighteen of the 19 loci analysed were polymorphic in the study region. G-3-Pdh was the only monomorphic locus. Based on the allele frequencies, we calculated different population genetic parameters (A, H_E, H_O, P_{95}, P_{tot}) for all 17 populations, representing 538 individuals (Table 1). We excluded H_O from statistical analyses.

For six of the 17 populations, significant deviation from Hardy–Weinberg equilibrium was observed for one to three loci. However, none of the populations showed a significant deviation from Hardy–Weinberg equilibrium after Bonferroni correction. No significant linkage disequilibrium was found after applying the Bonferroni correction. Therefore, further analyses were performed applying standard methods of population genetics.

We compared our data of individuals from the range margin to adjusted literature data from the distribution centre in southern France (Schmitt *et al.* 2002; see Material and methods). The genetic diversity was significantly lower at the range margin compared to populations from the distribution centre (Table 3).

The F_{ST} value, taking into account all studied populations of our study region, was 0.013 ± 0.004 ($P = 0.006$). Locus-specific F_{ST} values are given in Table 2. The genetic distances (Nei 1978) among the studied populations were on average 0.023 ± 0.003, ranging from 0.018 to 0.032. No significant isolation-by-distance could be found between the populations in the study region (Fig. 2). This is also supported by cluster analyses based on genetic distances (Nei 1978), where no clear differentiations between groups were found. No considerable differences were obtained between the neighbour joining and the UPGMA tree; however, neither is shown due to lack of significance.

Hierarchical F_{ST} analysis, comparing the study sites of the eastern ridge ($n = 4$) with those of the western ridge ($n = 13$), revealed 35.2% of the difference among habitats between these two subregions ($F_{RT} = 0.006$; $P = 0.003$), while 64.8% of the differentiation between sites was within the subregions ($F_{SR} = 0.011$, $P < 0.0001$). In a separate analysis of the two subregions, the eastern region showed a marginally significant F_{ST} (0.015, $P = 0.074$), while there was no significant difference between the samples in the western region ($F_{ST} = 0.010$, $P = 0.245$). The populations in the eastern region were significantly more isolated than the populations of the western region (east: 2890 ± 1370 m, west: 750 ± 730 m to the nearest *P. coridon* population, *t*-test: $t = 4.20$, $P = 0.0008$), while the other habitat factors, population size of *P. coridon*, habitat area and population size of *H. comosa* were not significantly different (all $P > 0.1$).

The inbreeding coefficient F_{IS} was significant, 0.046 ($P < 0.0001$), in the study region. This is due mainly to the high values of two loci (Pep: 0.113; Hbdh: 0.111). None the less, these loci had also low F_{ST} values (Pep: 0.014; Hbdh: 0.009) (see Table 2).

In a Pearson correlation matrix of the independent habitat factors, the population size of *P. coridon* depends significantly upon the population size of its larval food plant population ($r = 0.620$, $P = 0.008$). Other habitat factors were not correlated ($P > 0.1$). For multiple regressions we used the habitat factors population size of *P. coridon*, population size of its larval food plant and distance to the nearest *P. coridon* population. The distance to the nearest *P. coridon* population was the only factor that explained the expected heterozygosity in multiple regression models (22.6% of variance, $P = 0.054$) (Fig. 3), whereas the population size of

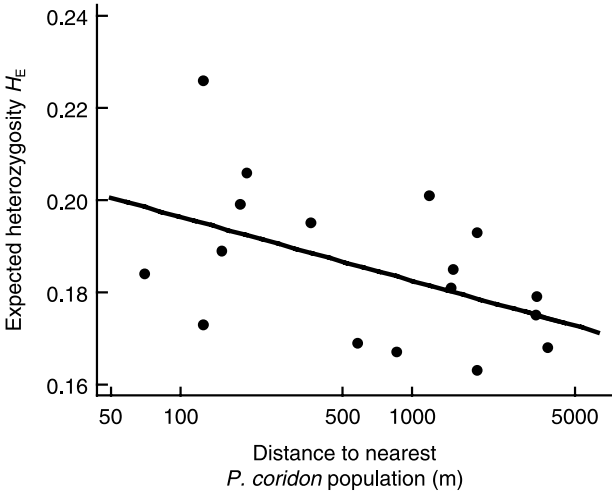

Fig. 3 Relationship between expected heterozygosity of 17 *P. coridon* populations and the distance to the nearest *P. coridon* population (isolation); $y = 0.223 - 0.014 \log_{10} \times (F = 4.38, r = -0.475, P = 0.054)$.

P. coridon and of the food plant *H. comosa* did not contribute significantly to the model.

Neither the average number of alleles per locus nor the proportion of polymorphic loci (at the 95% level or in total) showed any significant correlation in multiple regression models with the three factors (i) distance to the nearest *P. coridon* population, (ii) *P. coridon* population size and (iii) *H. comosa* population size. The multiple regression models with habitat area instead of the population size of *P. coridon* showed similar but no further significant results.

Discussion

The main objective of this study was to analyse the genetic structure and diversity of *P. coridon* populations at its range margin in relation to population size and isolation. We found significant regional genetic differentiation and reduced heterozygosity of the most isolated populations.

Regional differences of the genetic structure

The observed genetic diversity of the studied *P. coridon* populations ($H_E = 18.5\%$, $A = 2.4$) is high in comparison to other allozyme analyses of butterflies; e.g. for *Euphydryas* sp. $A = 1.2$–2.1 (Debinski 1994; Britten *et al*. 1995), for *Heliconius charithonia* $A = 1.5$ (Kronforst & Fleming 2001) and for *Mitoura* sp. mean $H_E = 14.4\%$, $A = 2.1$ (Nice & Shapiro 2001), but see the higher genetic diversity for *Aglais urticae* $H_E = 24.8\%$, $A = 2.84$ (Vandewoestijne *et al*. 1999). Previous investigations on a European scale show that *P. coridon* populations in other study regions have an even higher genetic diversity (Schmitt & Seitz 2001a,b, 2002a,b; Schmitt *et al*. 2002). We verified this statistically in a comparison of an adjusted data set from Schmitt *et al*. (2002). The lower genetic diversity of our populations might be due to their marginal position at the north of the distribution range. Possible explanations for a loss of genetic variability are bottlenecks during range expansion (Stone & Sunnucks 1993; Schmitt *et al*. 2002) and/or higher fragmentation of habitats and therefore smaller populations at the distribution border. As populations at the range margin are often the smallest, a distinction between both effects is not possible in most cases (Lammi *et al*. 1999).

Genetic differentiation between populations

The genetic diversity within populations is high. However, the percentage of differentiation between populations of the total genetic variance is comparatively low ($F_{ST} = 1.3\%$). Nevertheless, the differentiation is significant within our small spatial scale (0.7–32.4 km) and supports earlier findings for *P. coridon* on a somewhat larger geographical scale ($F_{ST} = 1.4\%$, 2.3–147.2 km) in southwestern Germany (Schmitt & Seitz 2002a). Similarly low F_{ST} values are typically observed for mobile butterfly species (Goulson 1993; Vandewoestijne *et al*. 1999; Wood & Pullin 2002).

Isolation-by-distance

We could not detect an isolation-by-distance system between the analysed populations of *P. coridon*. Other patchily distributed butterflies developed genetic isolation-by-distance systems at regional and local spatial scales (Britten *et al*. 1995; Johannesen *et al*. 1997; Keyghobadi *et al*. 1999). However, species of high dispersal ability need greater spatial scales for the development of an isolation-by-distance system than poor dispersers (Peterson & Denno 1998). The spatial scale of our study region is quite small, so that dispersal and gene flow of our study species might not be sufficiently limited. Nevertheless, even a broader scale in southwestern Germany did not reveal an isolation-by-distance system for *P. coridon* (Schmitt & Seitz 2002a). In conclusion, the dispersal ability of *P. coridon* might be underestimated, or the species-specific high population sizes of *P. coridon* populations limit genetic drift and therefore prevent the development of an isolation-by-distance system. In addition the structural diversity within the study region might counteract an isolation-by-distance system (see below).

Differences between subregions

Hostile areas often impede gene flow. Thus the genetic structures of butterfly and moth species of open habitats are often influenced by the distribution of forests (Johannesen *et al*. 1996; Meglécz *et al*. 1997; Keyghobadi *et al*. 1999; Schmitt *et al*. 2000), whereas forest species are influenced by the distribution of open land structures (Berwaerts *et al*. 1998;

van Dongen *et al*. 1998). Our study area consists of two chalk ridges, which are separated by a 5–10-km-wide floodplain with no suitable habitats for *P. coridon*. We found more than one-third (35.2%) of the genetic variance between populations between these two ridges. The western ridge with a higher density of suitable habitat patches showed no significant genetic differentiation between the 13 populations analysed, whereas a marginally significant differentiation was detected between the four samples from the eastern ridge, with more isolated habitats. Similarly, *P. coridon* populations in southwestern Germany had lower genetic differentiation in regions with high densities of suitable habitats than in regions with more scattered habitats (Schmitt & Seitz 2002a). This phenomenon can be also observed on the interspecific level, where species with very scattered and isolated populations mainly show higher differentiation between populations (Britten *et al*. 1994, 1995; Debinski 1994), while widespread and mobile species often lack major differentiation over greater areas (Goulson 1993; Porter & Geiger 1995; Vandewoestijne *et al*. 1999).

Effects of habitat fragmentation on genetic diversity

Genetic diversity is known to be an indicator for population fitness (Reed & Frankham 2003). Our results indicate a reduced expected heterozygosity in isolated *P. coridon* populations explaining 22% of the variance. These isolated populations occur mainly in the eastern subregion of our study area with naturally isolated chalk hills. We found no impact of population size, habitat area or food plant availability on the genetic diversity of the populations. Thus, only isolation but not population size may affect local population fitness negatively in our region.

While most studies of habitat fragmentation focus only on population size or habitat area, the effects of habitat isolation on the genetic diversity are often neglected. Other studies did not separate habitat isolation and population size (Berwaerts *et al*. 1998; Williams *et al*. 2003), or found nonsignificant relations (Buza *et al*. 2000; Jäggi *et al*. 2000; Schmitt & Seitz 2002a). Our results are comparable to the findings of Van Dongen *et al*. (1998), that the expected heterozygosity of the moth *Operophtera brumata* was related negatively to isolation of woodlands but not to their area. Past bottlenecks in combination with reduced gene flow might have contributed to the reduction of genetic diversity in isolated habitats in our study region, but this explanation still remains speculative. Therefore, further studies of butterflies, with different ecological traits, are needed for a better understanding of the genetic consequences of habitat fragmentation for this mobile species group.

Contrary to expectations, we could not confirm the prediction that fragmentation effects are more significant at the range margin (Thomas *et al*. 2001; Bourn & Thomas 2002). Thus, genetic variability was affected more strongly by habitat area or population size (although it was by isolation) in comparison to data from southwestern Germany (Schmitt & Seitz 2002a). The missing correlation between population size and genetic diversity in our study might be explained by relatively small differences in size between the studied populations. Other studies show that decreasing population size (or habitat area) often results in reduced genetic diversity for several taxonomic groups (Ellstrand & Elam 1993; Frankham 1996; Young *et al*. 1996; Amos & Harwood 1998).

Conclusions

We were able to show that fragmentation, even in a well-connected metapopulation system, acts on the genetic diversity of single subpopulations, as we found decreasing expected heterozygosity with increasing distance towards the nearest *P. coridon* population. While the connection of grasslands in the western subregion still seems to be sufficient for dispersal and gene flow, the populations of the eastern region might start to suffer from reduced heterozygosity and possible fitness loss. Therefore, for species conservation, we suggest stopping further habitat fragmentation of the remaining calcareous grasslands in southern Lower Saxony.

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Supplementary material

Supplementary material is available from:
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2072/MEC2072sm.htm

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