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Spatial genetic structure in a metapopulation of the land snail *Cepaea nemoralis* (Gastropoda: Helicidae)

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Abstract

Habitat fragmentation is a major force affecting demography and genetic structure of wild populations, especially in agricultural landscapes. The land snail *Cepaea nemoralis* (L.) was selected to investigate the impact of habitat fragmentation on the spatial genetic structure of an organism with limited dispersal ability. Genetic and morphological patterns were investigated at a local scale of a 500 m transect and a mesoscale of 4 × 4 km in a fragmented agricultural landscape while accounting for variation in the landscape using least-cost models. Analysis of microsatellite loci using expected heterozygosity (H_E), pairwise genetic distance ($F_{ST}/1 - F_{ST}$) and spatial autocorrelograms (Moran's I) as well as shell characteristics revealed spatial structuring at both scales and provided evidence for a metapopulation structure. Genetic diversity was related to morphological diversity regardless of landscape properties. This pointed to bottlenecks caused by founder effects after (re)colonization. Our study suggests that metapopulation structure depended on both landscape features and the shape of the dispersal function. A range of genetic spatial autocorrelation up to 80 m at the local scale and up to 800 m at the mesoscale indicated leptokurtic dispersal patterns. The metapopulation dynamics of *C. nemoralis* resulted in a patchwork of interconnected, spatially structured subpopulations. They were shaped by gene flow which was affected by landscape features, the dispersal function and an increasing role of genetic drift with distance.

Keywords: effective distance, gene flow, habitat fragmentation, isolation by distance, Moran's *I*, stepping stone model

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Introduction

Habitat fragmentation is a major force affecting wild populations, especially in agricultural landscapes. Species inhabiting the mosaic of semi-natural habitats may experience population subdivision and reduced gene flow, which may contribute to local extinction (Fahrig & Merriam 1994; Saccheri *et al*. 1998; Reed & Bryant 2000). The metapopulation theory predicts regional persistence if local extinction is compensated by recolonization (Hanski & Gilpin 1997). A metapopulation structure is accompanied by gene flow and may be reflected in the spatial genetic structure (Bohonak 1999; Hutchison & Templeton 1999). The effects of a metapopulation structure on genetic patterns mainly depend on dispersal ability and the landscape context. These effects might be most pronounced in species with low dispersal abilities that are living in highly fragmented landscapes. In this case, local extinction will result in empty patches and (re)colonization might be accompanied by genetic bottlenecks if the number of founders is low and if they originate from only one or few source subpopulations (Wade & McCauley 1988). The resulting reduction in effective population size facilitates enhanced drift and reduces genetic diversity within recently founded subpopulations. Thus, repeated extinction and recolonization events may enhance differentiation between distinct subpopulations compared with a continuous population (Harrison & Hastings 1996; Pannell & Charlesworth 2000). Furthermore, dispersal affects metapopulation genetics and may result in spatial structuring as a consequence of the opposite forces of gene flow and drift (Hutchison &

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Templeton 1999). In a spatially realistic metapopulation model, dispersal is a function of the landscape context (Hanski & Gilpin 1997). This underlines the importance of considering functional or effective distance measures for investigations of dispersal (Sork *et al*. 1999; Adriaensen *et al*. 2003; Chardon *et al*. 2003).

Land snails have been considered as ideal organisms for studies of ecological genetics over a wide range of spatial scales (see References in Davison 2002). They are characterized by low active dispersal ability, a high cost of locomotion (Denny 1980), an explicit homing behaviour (Taylor 1902; Edelstam & Palmer 1950; Rollo & Wellington 1981; Chelazzi 1990) and they reside in patchy habitats likely to promote geographical structuring.

We selected the land snail *Cepaea nemoralis* (L.) as a model organism of a species with limited dispersal (about 10 m per year; Lamotte 1951 in Cook 1998). *Cepaea nemoralis* can colonize a wide range of habitats such as deciduous woodlands, hedgerows or grasslands, almost all of which have been subject to massive alteration as a result of changes in anthropogenic land use. *Cepaea nemoralis* is not found on arable land (Cain & Currey 1963; Kerney *et al*. 1983) which will act as a barrier for dispersal and gene flow. However, a wide range of population sizes has been observed ranging from 'a handful to a few thousand' individuals (Cook 1998). These conditions make it quite possible that *C. nemoralis* exhibits a metapopulation structure in a heterogeneous landscape dominated by arable land.

First studies about the population structure of *C. nemoralis* focused on the wide range of shell polymorphisms. The genetic control of the major forms is relatively simple (Lang 1904; Lamotte 1951 in Cook 1998; Cain *et al*. 1960, 1968). However, dominance of alleles and interacting loci rendered genotyping impossible in the field (Cook 1998). Cain & Sheppard (1950) showed that colour and banding pattern frequencies are influenced by the habitat and the corresponding selective pressure by visual predators, notably song thrushes (*Turdus philomelos*). In contrast, Cain & Currey (1963) reported on spatial patterns of shell polymorphism, so called area effects, where particular colour and banding types predominate in distinct areas regardless of both habitat and lack of selection by song thrushes. There is continuing discussion on whether selection or historical events explain the area effects (for a review of ideas see Cook 1998; Davison 2002). However, most studies supported historical reasons including random processes like founder effects during bottlenecks or the colonization of new areas (Goodhart 1963; Davison & Clarke 2000; Bellido *et al*. 2002).

Combining phenotypic patterns of shell polymorphism with that of selectively neutral molecular markers analysed within and among subpopulations might provide valuable insights into processes of metapopulation structuring. Therefore, microsatellites represent ideal instruments, as they are highly polymorphic with high resolution at fine scales and they can cope with decreased genetic variability caused by population turnover (Jarne & Lagoda 1996; Gaggiotti 2004).

In the present study we investigated phenotypic and selectively neutral genetic patterns at two scales: (1) a local scale within a continuous local population and (2) a mesoscale among different local populations of the land snail *C. nemoralis* in a fragmented agricultural landscape. We assumed isolation by distance according to a stepping stone model of population structure (Kimura & Weiss 1964). This was applied at both spatial scales as Arnaud *et al*. (2001) observed small-scale genetic substructuring even within a continuous population of the land snail *Helix aspersa*. At the mesoscale, population subdivision was indicated by the fact that distinct habitat patches and subpopulations of *C. nemoralis* were separated by arable fields. Under the stepping stone model, gene flow is limited by dispersal and is most likely to occur between neighbouring sites. Consequently, more closely located populations are expected to be more similar, whereas remote populations should underlie the stochastic influence of drift.

We addressed the following questions: (1) Can spatial genetic structuring be observed on a local scale within a continuous population of *C. nemoralis*? (2) Does *C. nemoralis* exhibit a metapopulation structure at a mesoscale in fragmented landscapes? Which landscape properties influence the genetic structure and diversity? (3) Can area effects be observed? Are selectively neutral genetic and phenotypic properties related?

Materials and methods

Study site and sampling design

Specimens of *C. nemoralis* (L.) were collected at two spatial scales within a 4 × 4 km test site located in an agricultural landscape near the village of Friedeburg in Saxony-Anhalt, Germany (51°37′ N, 11°42′ E). At the local scale, 11 samples of a continuous local population within a hedgerow were taken at regular intervals of 50 m along a transect of 500 m. The hedgerow was adjacent to a field path and a cultivated field, respectively. Within the local population the samples are referred to as sites. At the mesoscale, 21 locations in distinct potential habitat patches within the test site were surveyed. *Cepaea nemoralis* was present in nine locations. Empty shells indicated the former presence and hence possibly extinction events of *C. nemoralis* in at least four of the remaining 12 patches (Fig. 1). To increase the number of samples to a total of 11, the first and last location of the transect were included in the mesoscale analysis. Since group discreteness depends upon limited mixing through dispersal and the minimum distance between the sample points (500 m) exceeded the average yearly movement of

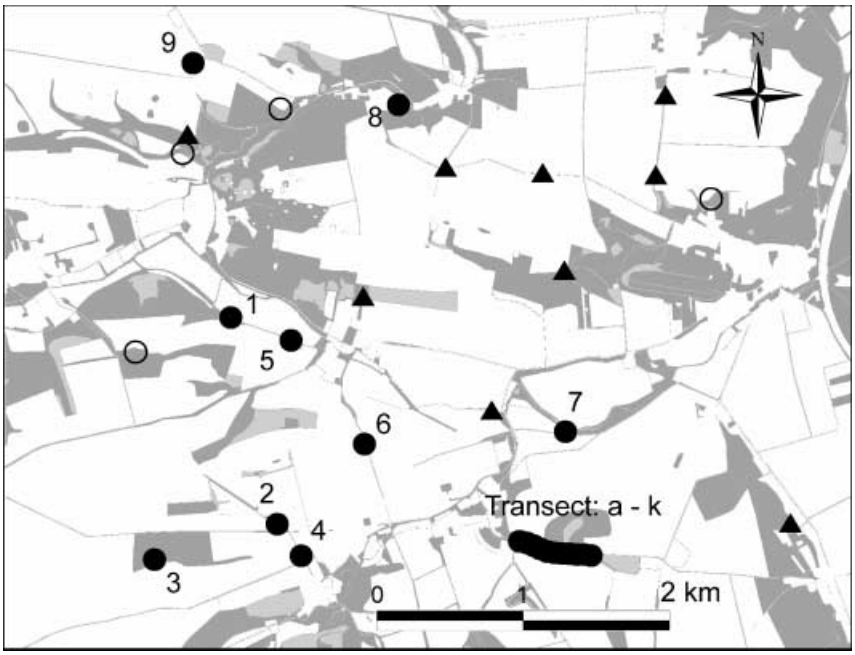

Fig. 1 Map of test site and sample sites. Dark grey, optimal habitat; light grey, suboptimal habitat; white, unsuitable habitat. Filled circles, *Cepaea nemoralis* was present; open circles, empty shells indicated local extinction; triangles, *C. nemoralis* was absent.

about 10 m by at least one order of magnitude, the specimens of each sample point were considered to represent single local populations of a metapopulation. Further on they are referred to as subpopulations. Genotypes were obtained from a total of 361 snails (transect, 213; test site, 184). Sample sizes ranged from 7 to 20 individuals. Snails were collected on a single day in September 2002 by searching the vegetation including the litter layer within a radius of 5 m. Specimens were stored at –40 °C until DNA extraction.

Microsatellite analysis

For genetic analyses, DNA was extracted from approximately 50 mg of tissue that was cut from the snail foot while still frozen. DNA was extracted using the DNeasy plant mini kit (Qiagen) according to manufacturers' instructions with the following modifications. The tissue was lysed for 3 h with 20 µL proteinase K (Qiagen) in 180 µL buffer AP1 (Qiagen) at 55 °C, and the DNA was eluted with 100 µL of AE buffer (Qiagen). Polymerase chain reaction (PCR) was performed in a total volume of 8 µL with 2 µL of DNA solution diluted 1 : 10 and 2 pmol of each fluorescence labelled forward and unlabelled reverse primers using the Qiagen multiplex PCR kit and applying a hot-start thermocycling protocol. Four trinucleotide and one tetranucleotide microsatellite loci previously published were analysed (Davison 1999). The loci *Cne*1 (label: JOE), *Cne*11 (FAM), *Cne*15 (FAM) and *Cne6* (TAMRA) were developed in a multiplex PCR reaction at an annealing temperature of 57 °C while locus *Cne*10 was run in a second PCR with a 65 °C annealing temperature. PCR products were separated on an ABI 310 genetic analyser (Applied Biosystems) with MapMarker 1000 (BioVentures Inc.) as internal size standard. Individuals were genotyped using GENOTYPER version 2.0 software (Applied Biosystems).

Genetic data analysis

The locus *Cne*10 did not produce any PCR product in more than 50% of the individuals and because of this abundant null allele, it was excluded from further analysis. To determine whether underlying assumptions for further statistical analysis of the remaining four loci were violated, deviations from Hardy–Weinberg equilibrium and genotypic linkage disequilibrium between loci were tested with GENEPOP (Raymond & Rousset 1995) with the Markov chain Monte Carlo method. Estimation of inbreeding coefficients (F_{IS}) followed the method of Weir & Cockerham (1984). *Cne*1 and *Cne*6 showed significant deviations from Hardy–Weinberg equilibrium in the majority of sites, whereas *Cne*11 and *Cne*15 did not. The heterozygote deficiency could have arisen if the sampling areas had exceeded those of Wrightian neighbourhoods ('Wahlund effect'), or because of rare null alleles. However, such inconsistent deviations from Hardy–Weinberg equilibrium among some of these loci were reported before (Davison & Clarke 2000). Since we also observed individuals that did not produce any PCR product (homozygous for null allele), we followed the assumption of Davison & Clarke (2000) that the sites were in Hardy–Weinberg equilibrium and that deviations in *Cne*1 and *Cne*6 were caused by null alleles. Null allele frequencies of both loci

Table 1 Habitat types, habitat suitability and the sets of resistance values for the different least cost models (*r*1–*r*3)

Habitat types	Habitat suitability	Resistance value		
		*r*1	*r*2	*r*3
Mesic grasslands, grassy margins	Optimal	1	1	1
Tall forb habitats	Optimal	1	1	1
Woodland fringes and scrub habitats	Optimal	1	1	1
Deciduous woodlands and hedgerows	Optimal	1	1	1
Coniferous woodlands and hedgerows	Suboptimal	1	2	10
Pastures	Suboptimal	1	2	10
Dry grasslands	Suboptimal	1	2	10
Transport networks (soft-surfaced)	Suboptimal	1	2	10
Arable land, streets, urban areas, water bodies	Unsuitable	100	10	100

were estimated under the assumption of Hardy–Weinberg proportions according to Brookfield (1996) with the software MICRO-CHECKER (van Oosterhout *et al*. 2004). Total allele frequencies were adjusted. The microsatellite loci are supposed to be selectively neutral except for *Cne*11, which showed evidence of linkage to shell banding (Davison 1999; Davison & Clarke 2000). As statistical prerequisites were satisfied, Fisher's exact tests of population differentiation and estimators of Wright's *F*-statistics following Weir & Cockerham (1984) were performed using GENEPOP. Genetic diversity corrected for sample size was computed as expected heterozygosity (H_E) (Nei 1973) with the software MSA (Dieringer & Schlötterer 2003).

Phenotypic data analysis

The specimens were scored for shell colour and banding, using slightly modified criteria of Cain & Sheppard 1950). The frequencies of white, yellow, pink, brown and the number of bands (0–5) were used to calculate the Shannon–Wiener index (Shannon 1948; Wiener 1948) for colour and banding patterns separately and combined as a measure of phenotypic diversity.

Spatial and environmental statistics

Landscape elements were digitized from orthophotos and mapped together with the sample points in a Geographic Information System (GIS) using ARCVIEW software (ESRI 1996). Two different measures of distances between the sample points were used. The first was simply the linear geographical distance. The underlying assumption is that snails disperse uniformly in any direction, regardless of landscape features. Although this is indeed suitable for the transect conditions, it is un-realistic in a heterogeneous landscape. Therefore, we used measures of functional or effective distance in order to account for differences in resistance of particular habitats to movement. The effective distances were computed using least-cost modelling (Adriaensen *et al*. 2003 and references therein) in a GIS. The habitats were classified as optimal, suboptimal and not suitable according to the habitat re-quirements of *C. nemoralis* (Cain & Currey 1963; Kerney *et al*. 1983). These classes were assigned to resistance values that determine the relative costs of *C. nemoralis* moving between patches. Since we had no detailed knowledge on the movement behaviour, three model scenarios were used that differed in the resistance of suboptimal and unsuitable habitats (Table 1). Cost values in optimal habitats were held constant at 1. The models yielded least-cost paths between the sampling points. The effective distances were obtained by moving along these paths and summing up the resistance values according to a grid of 1×1 m. Thus, they represent a product of distance and estimated resistance values. The effective distances were given in units of metre and are abbreviated 'm*' to avoid confusion with geographical distance. The underlying assumption is that the dispersal of snails in an optimal habitat may be estimated by the linear geographical distance, whereas the probability of successfully crossing barriers decreases with distance according to the resistance of the barrier.

The degree of genetic population differentiation at the two scales was compared using global F_{ST} values across all loci and the corresponding 95% confidence intervals (CI), which were determined by jackknifing. To test for isolation by distance, pairwise ($F_{ST}/1 - F_{ST}$) matrices were related to geographical distance as well as effective distances following Rousset (1997). Regressions were drawn for means of visualization only, as pairwise distance values are not independent. Mantel tests were used to test for significance (1000 permutations). A nonlinear (e.g. logarithmic) relationship between geographical or effective distance would reflect the differing roles of gene flow and genetic drift over different spatial scales as expected by the stepping stone model. Within short distances gene flow and drift were expected to be equal, whereas more distant subpopulations were expected to be relatively more influenced by drift (Hutchison & Templeton 1999).

Spatial autocorrelation of genetic variability was analysed with correlograms of Moran's *I* statistics (Moran 1950; Sokal & Wartenberg 1983; Hardy & Vekemans 1999). Spatial autocorrelation techniques have the advantage that they allow inference of spatial genetic structure independent from the often violated assumptions of classic *F*-statistics, such as absence of selection and mutation or complete random migration of a constant number of individuals between the subpopulations (Whitlock & McCauley 1999). Distance classes of geographical and effective distances were created following Sturge's rule (Legendre 1998), disregarding the last two classes because of too few replicates. As sampling was performed within a radius of 5 m, the first class represented a maximum distance between individuals of 10 m. The mean distance per distance class was used to draw correlograms. Moran's *I* statistics of individual distance classes were tested for significance by a resampling procedure (1000 permutations). The global significance of the entire correlograms was evaluated using the progressive Bonferroni technique by dividing the significance level ($P = 0.05$) according to the rank of each subsequent distance class (Hewitt *et al*. 1997). If at least one distance class remained significant the entire correlogram is deemed to be globally significant (Legendre 1998). If global significance was shown, the range of genetic autocorrelation was estimated by the first *x*-intercept of the correlogram (Sokal & Wartenberg 1983; Sokal *et al*. 1997; Escudero *et al*. 2003). The *x*-intercept gives the average distance at which the similarity of any two sites is equal to the region-wide similarity expected by chance alone. This was termed 'genetic patch size' by Sokal & Wartenberg (1983). The *x*-intercept was shown to be positively related to parent vagility and neighbourhood size and therefore to average distance of gene flow per generation (Epperson 1993; Epperson & Li 1997; Sokal *et al*. 1997). To obtain an impression of the variability of individual pairwise Moran's *I* coefficients and the *x*-intercept we calculated 95% confidence intervals by bootstrapping 10 000 times within each distance class. All spatial autocorrelograms were calculated with the software SPAGEDI (Hardy & Vekemans 2002).

The analysis of phenotypic data was similar to the genetic analysis for means of comparison. The shell morphology is known to be determined by an allelic series, with darker colours being dominant over paler ones and unbanded dominant over banded (Lang 1904; Lamotte 1951 in Cook 1998; Cain *et al*. 1960, 1968). However, dominance of alleles and interacting loci rendered genotyping impossible in the field (Cook 1998). Therefore, we relied on phenotypic data that were treated as two artificial loci (colour and banding) with four alleles (white, yellow, pink, brown) and six alleles (0–5 bands), respectively. Pairwise phenotypic distances were calculated as χ^2 distance. Population differentiation at both scales was compared by the average values of the pairwise χ^2 distances. Their 95% confidence intervals were retrieved by bootstrapping 10 000 times. The analysis of isolation by distance was performed in the same way as for microsatellites but using χ^2 distance. Spatial structuring was analysed with autocorrelograms of Moran's *I*.

To investigate the role of landscape structure at the mesoscale, circular areas were defined around each sample site. Within the circular areas the following landscape variables were calculated: local patch size, number of habitat types, number of patches, sum of patch size, average patch size, area of single habitat types and area of habitats aggregated into optimal, suboptimal and unsuitable habitat classes. To account for scale-dependent differences in landscape structure, we calculated areas of two diameters around each sample site. The diameters were derived from the *x*-intercept of the autocorrelation analyses at both spatial scales. The *x*-intercept is an approximation of the genetically homogenous surface (Sokal & Wartenberg 1983; Epperson 1993; Arnaud *et al*. 2001). Hence, it reflected the range of genetic influence at a smaller and a larger scale.

Selective pressure due to visual predation by song thrushes (*T. philomelos*) contributes to spatial structuring in snails. To account for this, we determined bird density using the point-stop-method (Bibby 2000). At 20 points on a grid of 500 m laid over the test site the birds were recorded during a stop of 10 min on three monthly visits from April to June 2001.

Results

Genetic diversity and global population differentiation

All microsatellite loci were polymorphic, having between 2 and 13 alleles per sample site. Mean expected heterozygosity (H_E) per sample site ranged from 0.73 to 0.80 at the local scale and from 0.56 to 0.80 at the mesoscale (Table 2). No significant genetic linkage disequilibrium between loci was detected. Tests of population differentiation showed significant heterogeneity for all loci among sites of the entire test site ($P < 0.001$) and the transect ($P < 0.049$), indicating spatial structuring at both scales.

Local scale: genetic differentiation within a continuous subpopulation

The field survey showed that the subpopulation of the transect along the hedgerow could be regarded as continuous as no major gap between single individuals was observed. Nevertheless, a minor but significant level of differentiation occurred at the local scale (global $F_{ST} = 0.012$, 95% CI 0.0010–0.0228). A positive correlation between genetic ($F_{ST}/(1 - F_{ST})$) and geographical distance evidenced isolation by distance within the 500 m of the transect ($R^2 = 0.052$, $P = 0.015$, Mantel test). The correlogram of the average Moran's *I* statistics over all loci indicated positive autocorrelation that ended between 50 and 100 m (Fig. 2a).

Table 2 Sample size, number of alleles and expected heterozygosity at the sample sites

Scale	Site	Sample size	No. of alleles	Mean alleles	H_E
Mesoscale	1	20	28	7.0	0.74
	2	14	27	6.8	0.80
	3	7	12	3.0	0.63
	4	18	21	5.3	0.56
	5	20	34	8.5	0.78
	6	20	24	6.0	0.76
	7	20	29	7.3	0.79
	8	10	24	6.0	0.71
	9	19	30	7.5	0.73
Local scale	a	17	26	6.5	0.75
	b	20	29	7.3	0.79
	c	20	29	7.3	0.79
	d	20	26	6.5	0.79
	e	19	28	7.0	0.77
	f	20	24	6.0	0.73
	g	20	27	6.8	0.79
	h	19	30	7.5	0.80
	i	17	25	6.3	0.76
	j	20	33	8.3	0.76
	k	18	35	8.8	0.79

No. of alleles, the total number of alleles over all four microsatellite loci; mean alleles, the mean number of alleles per locus; H_E, mean expected heterozygosity over all loci after adjustment for null alleles under assumption of Hardy–Weinberg equilibrium.

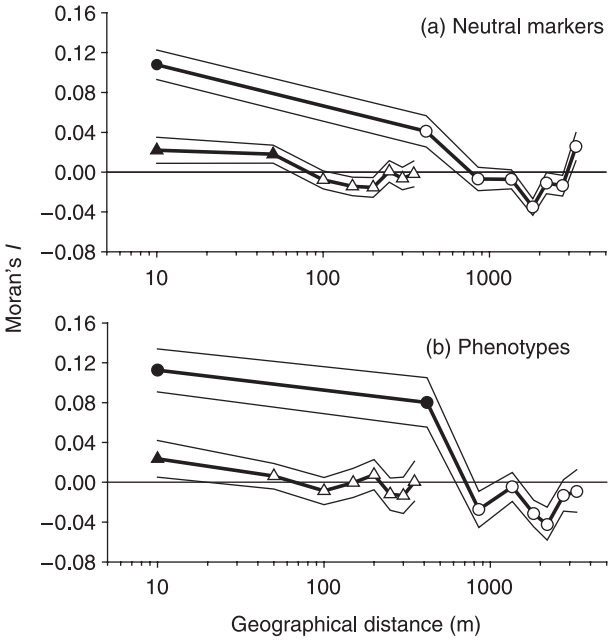

Fig. 2 Spatial autocorrelograms based on average Moran's I coefficients for all microsatellite loci (a) and phenotypic data (b). All correlograms were globally significant. Closed symbols indicate significant individual autocorrelation coefficients after progressive Bonferroni correction. The range of autocorrelation can be assessed by the x-intercept. Triangles, autocorrelation at the local scale; eight continuous distance classes of 50 m along the transect of 500 m were used. Circles, autocorrelation at the mesoscale; eight continuous distance classes of 500 m for the subpopulations of the test site were used (average distance within a class given). Thin lines, 95% confidence intervals of individual pairwise Moran's I coefficients per class.

Mesoscale: genetic differentiation among distinct subpopulations

At the mesoscale, the subpopulations of the entire test site exhibited a significantly greater amount of differentiation (global F_{ST} = 0.076, 95% CI 0.053–0.100) than the sites at the local scale, as both confidence intervals did not overlap. Significant pairwise F_{ST} values between neighbouring sample sites indicated discrete subpopulations. However, no significant correlation between genetic ($F_{ST}/(1 - F_{ST})$) and geographical distance (untransformed or logarithmic) was detected with the Mantel test, while correlograms revealed significant spatial structuring. Average Moran's I statistics over all loci indicated positive autocorrelation that ended between 416 m and 854 m (mean distance per distance class; Fig. 2a). This was significantly larger than at the local scale and pointed to different modes of dispersal at both scales.

In addition to the geographical distance we considered landscape properties and evaluated the effective distances among the sampling points with three alternative models (Table 1). The correlation between genetic distance ($F_{ST}/(1 - F_{ST})$) and the logarithm of effective distance was only significant in models $r1$ and $r3$. The explained variance of $r1$ was quite similar (R^2 = 0.14) to r3 (R^2 = 0.13). We chose to use $r1$ because of higher R^2 and the fact that fewer assumptions were made about the resistance values (see Crawley 2002). Correlations between genetic distance and untransformed effective distances were not significant, whereas significant results were obtained using the logarithm of effective distances (R^2 = 0.14, P = 0.007, Mantel test; Fig. 3a). Spatial autocorrelation analysis in terms of effective distance revealed significant autocorrelation up to the distance class with a mean of 2358 m* and an upper class border of 3500 m* effective distance. Below this effective distance of 3500 m* gene flow and drift were in equilibrium as the linear regression of ($F_{ST}/(1 - F_{ST})$) on effective distance was significant (R^2 = 0.47, P = 0.001, Mantel test). Above this threshold, drift was dominating as no significant correlation was shown.

Mesoscale: genetic and phenotypic diversity within subpopulations

The diversity of colour and banding patterns (Shannon–Wiener index) was highly correlated (R^2 = 0.51, P < 0.001). This indicated that forces affecting their diversity did not

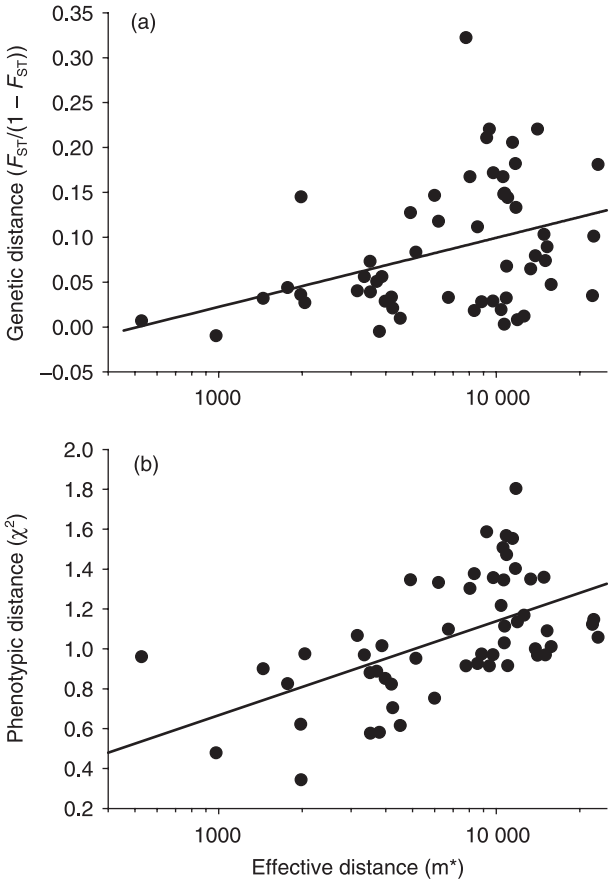

Fig. 3 Isolation by distance at the mesoscale. Effective distance was calculated according to least-cost modelling and represents a product of distance (m) and an estimated resistance value of the particular habitat class. (a) Pairwise genetic distance ($F_{ST}/(1 - F_{ST})$) showed a logarithmic relation to pairwise effective distance over the entire test site ($R^2 = 0.14$, $P = 0.007$, Mantel test). (b) Pairwise phenotypic distance (χ^2) exhibited a similar distance dependence ($R^2 = 0.31$, $P < 0.001$, Mantel test).

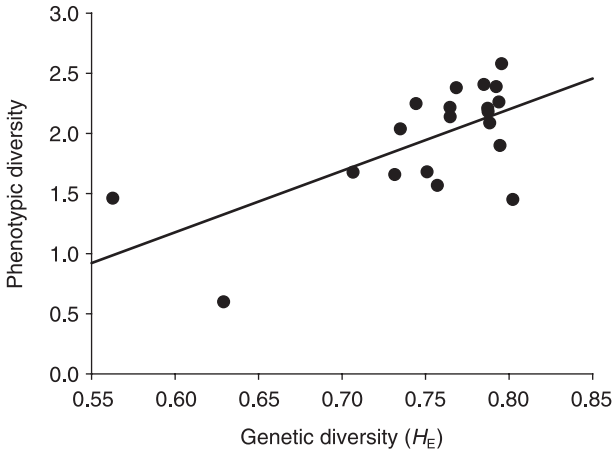

Fig. 4 Genetic (H_E) and phenotypic diversity (Shannon–Wiener index) were positively correlated ($R^2 = 0.44$, $P = 0.001$). The two outliers to the left are supposed to have recently undergone severe demographic bottlenecks (see text).

differ significantly between both characteristics. Therefore both characteristics were combined as morphological diversity. Bird surveys showed that the density of song thrushes was negligibly low within the test site and zero around all sample points. Hence, the absence of selection by predation on phenotypic patterns was assumed.

No significant relationship between genetic (H_E) and morphological diversity (Shannon-Wiener index) was encountered at the local scale of the transect, whereas the correlation was significant at the mesoscale of the test site ($R^2 = 0.44$, $P = 0.001$; Fig. 4). However, the correlation was influenced by two sites with both reduced morphological and genetic diversity, so was not significant when these two points were omitted from the analysis ($R^2 = 0.16$, $P = 0.102$).

To analyse the effect of landscape structure on genetic diversity within the subpopulations of the mesoscale we related the landscape structure variables to H_E. For a small-scale analysis, a radius of 80 m was chosen according to the local range of genetic autocorrelation (*x*-intercept in Fig. 2a). In a stepwise multiple regression including all landscape variables, only the area of field paths significantly enhanced genetic diversity ($R^2 = 0.41$, $P = 0.002$). Neither local patch size nor other local variables had significant effects on genetic diversity. For a larger scale analysis, a radius of 500 m was chosen from the correlogram of the test site (Fig. 2a), but there was no significant correlation between H_E and any of the landscape variables.

Genetic related to phenotypic differentiation, area effects

The analysis of the phenotypic data yielded results similar to the genetic data. The subpopulations exhibited a greater amount of differentiation at the mesoscale (mean χ^2 distance = 1.06, 95% CI 0.98–1.14) compared with the local scale (mean χ^2 distance = 0.72, 95% CI 0.68–0.76).

Local scale. In contrast to the genetic analysis the pairwise phenotypic distances (χ^2) were not correlated with geographical distance (Mantel test). However, average Moran's *I* correlograms for colour and banding indicated a globally significant spatial autocorrelation (Fig. 2b). The *x*-intercept was between 50 m and 100 m, but 95% CIs indicated a potential restriction of spatial autocorrelation to the first distance class, which is in fact the diameter of the sample sites.

Mesoscale. The spatial analyses were consistent with results obtained from the microsatellites. No correlation between phenotypic (χ^2) and geographical distance was detected (Mantel test). However, spatial structuring was indicated by significant Moran's *I* correlograms for colour and banding separately with *x*-intercepts between 416 m and 854 m. The

average correlogram of both characteristics combined corresponded to that of the genetic analysis and indicated positive autocorrelation that ended between 416 m and 854 m (Fig. 2b). When landscape properties were considered, correlations were significant between the logarithm of effective distance and pairwise phenotypic distances (χ^2) of colour ($R^2 = 0.16$, $P = 0.009$) and banding ($R^2 = 0.21$, $P = 0.013$) as well as for both characteristics combined ($R^2 = 0.31$, $P < 0.001$, Mantel test; Fig. 3b).

The spatial structuring of colour and banding patterns revealed by Mantel tests and correlograms indicated area effects (Cain & Currey 1963), as closer subpopulations were morphologically more similar than distant ones (Figs 2b and 3b). However, the spatial arrangement of these patterns differed between colour, banding and genetics since the respective pairwise distances were not significantly related to one another (Mantel test).

Discussion

Local scale genetic structure within a continuous subpopulation

Cepaea nemoralis exhibited spatial structuring at both scales of the investigation. Even within a continuous subpopulation and in absence of barriers genetic patterns revealed a deme-like structure that was panmictic internally, but isolated from adjacent demes to some extent. This reflected the marked homing behaviour and limited dispersal abilities. The monotonic increase of pairwise genetic distance ($F_{ST}/(1 - F_{ST})$) evidenced isolation by distance over at least 500 m. The average Moran's I correlogram indicated a range of genetic autocorrelation that ended between 50 m and 100 m (Fig. 2a). This corresponded well to previous studies of the land snail *H. aspersa*, where the length of a panmictic unit was found to range between 50 m and 80 m by indirect (Arnaud *et al*. 1999) and direct methods (Madec 1989 in Arnaud *et al*. 1999).

Mesoscale metapopulation structure

In our study we found evidence for a metapopulation structure of *C. nemoralis* in a fragmented landscape. A first hint was that suitable habitat patches were not colonized and empty shells indicated local extinction in some of them (Fig. 1). The two major forces shaping metapopulation structure are demographic processes and dispersal. Demography can be affected by a drastic reduction in population size owing to a reduction in patch size or colonization by a low number of migrants. Depending on dispersal ability and landscape properties empty habitat patches may be (re)colonized. However, both reduction in population size and colonization can cause severe bottleneck effects. As a consequence of random drift, bottlenecks are characterized by a drastic reduction in genetic diversity. This should affect both diversity of selectively neutral markers as well as phenotypic diversity regardless of possible differences in selection, mutation or drift. In the present study, recent bottlenecks were indicated by Fig. 4 at the mesoscale. As the proportion of subpopulations that have undergone recent bottlenecks might be low, the two points to the left in Fig. 4 should not be regarded as outliers but as an indication of recent drastic bottlenecks. This is corroborated by the fact that in these two sample points both shell colour and banding diversity were reduced. As the bottlenecks were unlikely to be caused by selective predation, we assumed that they were caused by common, namely demographic processes.

To investigate whether the demographic processes reflected a metapopulation structure that was created by local extinction and (re)colonization or if the observed patterns merely reflected the influence of landscape patterns on a more or less static population, we related the genetic diversity to landscape and patch characteristics. We found that genetic diversity at the mesoscale was not related to landscape or patch characteristics. This was consistent with previous studies of the land snail *Pomatias elegans* (Pfenninger 2002). Here it was reported that the density of the individuals rather that patch size contributed significantly to genetic diversity. As neither reduction of patch size nor connectivity reduced genetic diversity, we assumed that the population bottlenecks were a consequence of founder effects after colonization of empty patches indicating a metapopulation structure. Multiple regression revealed that the presence of field paths increased the genetic diversity of a particular subpopulation. This could be either due to migration along the grassy margins of field paths or by passive displacement by animals or even agricultural implements (Dorge *et al*. 1999).

The consequences of a metapopulation structure on genetic structure may depend on the dispersal function. *Cepaea nemoralis* is known to display a leptokurtic dispersal distribution (Davison 2000), where most individuals short distances and only a few disperse over intermediate and long distances (Ibrahim *et al*. 1996). This was reflected in our study by the patterns of genetic and phenotypic variability across the scales. The higher number of short distance dispersers was indicated by a comparably low degree of genetic differentiation ($F_{ST} = 0.012$) and a low range of spatial autocorrelation at the local scale (50–100 m; Fig. 2). Consequently, a low number of long-distance dispersers was indicated by a higher degree of genetic differentiation ($F_{ST} = 0.076$) and a higher range of spatial autocorrelation (416–854) at the mesoscale. This was in accordance with expectations from the leptokurtic dispersal function and suggested different structuring processes at both spatial scales. Davison (2000) reported that the ability of even a small number of individuals to disperse over long

distances makes *C. nemoralis* an efficient colonizer of vacant habitats. Thus, within a fragmented anthropogenous landscape, *C. nemoralis* may be able to establish a metapopulation with an equilibrium of local extinction and colonization.

The range of positive spatial autocorrelation corresponded well with previous studies of the land snail *Pomatias elegans* in moderately fragmented landscapes, where spatial genetic autocorrelation ended at distances greater than 500 m (Pfenninger 2002). As active migration of land snails in general is restricted to a few meters per year (Pfenninger *et al*. 1996; Arnaud *et al*. 1999), passive displacement by abiotic, biotic and anthropogenic vectors is the most likely mechanism for long-distance dispersal and appears to be more common than previously thought (Dorge *et al*. 1999).

Despite the importance of passive displacement, gene flow between subpopulations of land snails is significantly affected by landscape features. (Arnaud 2003) found evidence that *H. aspersa* uses functional migration pathways such as canal embankments, road verges and hedgerows. In the present study we considered resistance values of particular habitat classes in order to evaluate functional aspects of the landscape context. Thereby we revealed a positive correlation between genetic and effective distance over the range of the whole test site, whereas the linear geographical distance failed. Effective distances mostly increased by the influence of barriers, while they were less affected by the resistance values of optimal and suboptimal habitats such as woodlands or grassy margins. Such sites may have served as habitat for *C. nemoralis* in the past or as active or more likely as passive migration pathway. Gene flow and drift were shown to be in equilibrium at the mesoscale but the importance of genetic drift increased with effective distance (Fig. 3a). However, the logarithmic relation indicated a shift in the importance from gene flow to drift. Furthermore, the x-intercept of a Moran's I correlogram (not shown) provided evidence for a threshold. Below this threshold of an effective distance of 3500 m* the regression of genetic distance on effective distance was linear and significant. This indicated an equilibrium between gene flow and drift up to this effective distance (Hutchison & Templeton 1999). Above the threshold the regression was not significant, indicating the increased importance of drift. Thus, with least cost modelling and adequate setting of resistance values, a more realistic landscape model was developed.

Area effects

The spatial structuring of shell colour and banding morphs revealed significant area effects at the mesoscale (Figs 2b and 3b). The observed patterns were unlikely to be caused by selection of visual predators or by habitat features. However, equal ranges of phenotypic and genetic spatial autocorrelation supported common structuring processes such as dispersal (Fig. 2).

Assuming a metapopulation structure and leptokurtic dispersal, the pronounced spatial structuring is most likely the consequence of bottlenecks. These bottlenecks produced three sets of area effects, one in genetic population structure and two in shell morphology (banding and colour). This was also suggested by Davison & Clarke (2000) for *C. nemoralis* studied in the Marlborough Downs in Wiltshire, UK. They reported that the differences in the spatial patterns of genetic and morphological structure were only marginal. In the present study these differences were more pronounced, although the observed spatial autocorrelation was similar. This is most likely due to the fact that gene flow and phenotypic exchange are driven by the same dispersal function. The observed decoupling of the spatial genetic and phenotypic patterns might be a consequence of metapopulation dynamics in fragmented landscapes and the stochastic nature of colonization and drift. Low numbers of founders colonizing empty patches establish unique allele frequencies. Subsequently neighbouring patches are colonized or become genetically mixed, thereby establishing area effects. In the absence of visual predators colour and banding morphs may develop independent spatial patterns similar to neutral genetic markers.

Conclusions

Taking *C. nemoralis* as an example of an organism with limited dispersal capabilities, our study suggests that the metapopulation structure of such species in fragmented landscapes depends on both the landscape features and the shape of the dispersal function. *Cepaea nemoralis* exhibited spatial structuring at two scales. At the local scale a deme-structured subdivision of a continuous subpopulation corresponded to the limited active dispersal ability. At the mesoscale rare dispersal events, most likely driven by passive displacement, are suggested to lead to metapopulation persistence in a fragmented landscape. Under absence of visual predators the observed area effects of shell morphs may reflect the historical events structuring the metapopulation. The stochastic nature of drift and colonization in fragmented landscapes is suggested to cause a decoupling of genetic and phenotypic spatial patterns.

Further research may focus on expectations of metapopulation theory concerning genetic patterns over a broad range of landscapes. Comparative investigations including continuous, subdivided and highly isolated populations may provide detection, understanding and discrimination of metapopulation effects on population genetics.

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Supplementary material

The following material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2357/MEC2357sm.htm

Appendix S1. Sample coordinates (X, Y, in Gauss–Krüger coordinate system) and allele frequencies at four microsatellite loci for nine sample sites at the mesoscale and 11 sample sites at the local scale. Frequencies were adjusted for estimated null allele frequencies (Null)

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This work is part of Oliver Schweiger's PhD thesis on the effects of landscape structure in agricultural landscapes on biodiversity at various scales and levels. He is supervised by Roland Brandl at the University of Marburg, Germany. Mark Frenzel is an animal ecologist with a focus on biogeographical patterns. Walter Durka is population geneticist with major interest in processes determining population structure. The authors work together at the UFZ — Centre for Environmental Research in the Department of Community Ecology, which focuses on the merging of plant and animal ecology with population genetics and community ecology.
