Relationship between microspatial population genetic structure and habitat heterogeneity in *Pomatias elegans* (O.F. Müller 1774) (Caenogastropoda, Pomatiasidae)

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In the present study the population genetic structure of the terrestrial snail *Pomatias elegans* was related to habitat structure on a microspatial scale. The genetic variability of 1607 individuals from 51 sampling sites in five different populations in Provence, France, was studied with an allozyme marker using population genetic methods, Mantel tests and spatial autocorrelation techniques were applied to different connectivity networks accounting for the structural features of the landscape. It is suggested that the population structure is, to a large extent, a function of the habitat quality, quantified as population density, and of the spatial arrangement of the habitat in the landscape and not of the geographical distance *per se*. In fragmented habitats, random genetic drift was the prevailing force for sampling sites separated by a few hundred meters.

ADDITIONAL KEYWORDS: allozymes – dispersal – gene-flow – land snails – landscape structure – random genetic drift.

INTRODUCTION

Population genetic theory predicts that population structure at selectively neutral loci is determined by gene flow, neutral genetic drift and historical processes (e.g. Slatkin, 1993). The smaller the spatial scale considered, however, the less likely are historic processes, such as mutations, population bottlenecks, fragmentation or range expansions, to play a significant role in shaping the genetic population structure (Hutchison & Templeton, 1999). It is then predominantly determined by the dynamic equilibrium between neutral genetic drift and gene flow (Slatkin, 1987). Everything else being equal, the strength of genetic drift depends mainly on the effective size of the population, while gene flow is governed by the dispersal capacity of the organism. In natural populations gene flow and drift are, however, dependent on the qualitative and spatial structure of the habitat. The habitat quality is thought to be an important factor for the density of a population and therefore for its total size in a given area (e.g. Fahrig & Paloheimo, 1988; Partridge, Britton & Franks, 1996). Suitable habitat for an organism is not always arranged in a continuous fashion, but may be organized in a complex network of corridors or patches in the landscape. The spaces between suitable habitat patches may be more or less appropriate for dispersal of the organism not only due to the absolute distance between them but also due to their physical, geological and/or biological properties (Hansson, 1991). Discontinuity in the spatial arrangement of suitable habitat presents thus a more or less penetrable barrier to gene flow. It can consequently be suspected that both structural and qualitative heterogeneity in habitat structure has a profound influence on the genetic population structure, especially on the microspatial scale. A landscape-based approach in search of gene flow paths has only recently received attention in animal taxa (e.g. Keyghobadi, Roland & Strobeck, 1999; Rowe, Beebee & Burke, 2000; Michels *et al.*, 2001; Vos *et al.*, 2001).

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While the microspatial arrangement of genetic variability has been studied in molluscs (e.g. Lamotte, 1951; Selander & Kaufman, 1975; Pfenninger, Bahl & Streit, 1996; Arnaud *et al.*, 1999), only Arter (1990) has explicitly addressed the effect of habitat heterogeneity on population structure.

The present study focuses on the terrestrial caenogastropod *Pomatias elegans* (O.F. Müller 1774). The species range of *P. elegans* has its centre in the western mediterranean area and reaches its northern limits in Britain, the Benelux states and Germany (Kerney, Cameron & Jungbluth, 1983). *P. elegans* is strictly bound to calcareous substrates, and can be found in various types of vegetation (Boycott, 1921; Kilian, 1951). The species has separate sexes and is an obligate outcrosser. A mark recapture study has shown that *P. elegans* is a poor active disperser with a dispersal distance of 15.95 ± 12.35 cm per 42 days in suitable habitat (M. Pfenninger, unpublished data). Moreover, the species has a habit of penetrating deeply into the soil (Kilian, 1951), which makes passive dispersal with biological vectors unlikely. *P. elegans* is therefore a particularly suitable organism for the investigation of the effects of habitat heterogeneity on genetic population structure on a microspatial scale. I have addressed this issue with an analysis of the habitat and the genetic allozyme variation in five populations.

MATERIAL AND METHODS

SAMPLED POPULATIONS

Five locations from different types of habitat in Provence, France were chosen for the analysis (Fig. 1; Table 1). Most were surrounded by absolute barriers to any active dispersal. Within these areas, dispersal, and hence gene flow, were *a priori* possible for *P. elegans*. Therefore, these locations are referred to as populations. Within populations, the samples are referred to as sampling sites or sites. In some cases, they represent more or less localized demes or subpopulations (especially RIOU and RAT), in others (e.g. LUB) they are randomly chosen sampling sites in a more or less continuously colonized area. Within each population 3–16 sites were sampled.

The Luberon population (LUB) is situated in a densely wooded area on the steep northern slope of the Grand Luberon. Entremont (ENT) was sampled in and around the Oppidum d'Entremont, north of Aix-en-Provence. The area comprises patches of woodland and shrubs, agricultural fields, meadows, gardens and archaeological excavations. It is entirely surrounded by roads and housing areas, which represent absolute barriers to active dispersal. Resquiadou (RES) is situated close to the coast in the northern part of the Bay of Marseille. A limestone cliff with a strongly ridged and rutted surface constitutes the

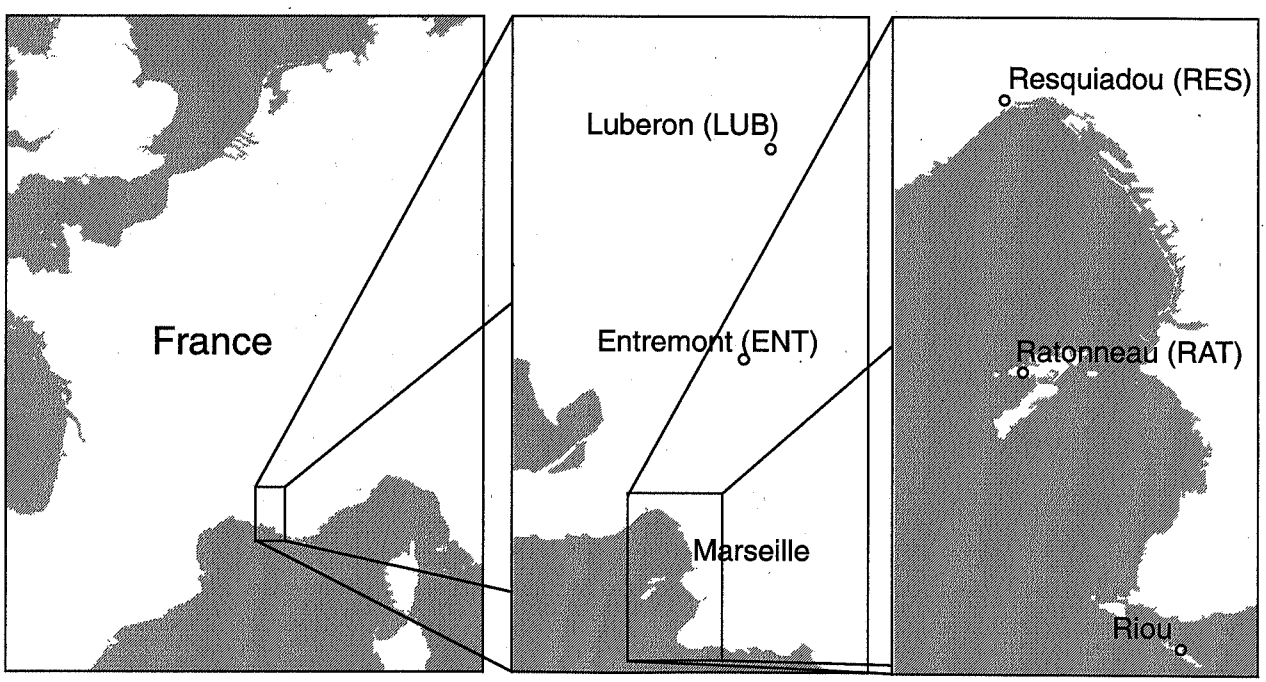

Figure 1. Sampled populations in south-east France.

Table 1. Populations sampled and sampling sites. N is the number of individuals sampled per site. Density denotes the number of living individuals per 0.0625 m^2 at the respective sampling site. F_{IS} refers to the inbreeding coefficient within sampling sites; values significantly different from zero at the 5% level are labelled with an asterisk. H_O denotes the observed level of heterozygosity

Population/sampling site	N	Density	F_{IS}	H_O
Luberon (LUB)				
LUB_I	35	56	+0.012	0.542
LUB_II	31	45	−0.005	0.607
LUB_III	34	67	+0.047	0.538
LUB_IV	30	53	−0.061	0.610
LUB_V	31	50	−0.124	0.607
LUB_VI	26	48	−0.048	0.627
LUB_VII	31	35	−0.008	0.538
LUB_VIII	32	58	−0.057	0.585
Oppidum d'Entremont (ENT)				
ENT_I	34	34	+0.003	0.561
ENT_II	30	16	−0.137	0.617
ENT_III	33	15	+0.071	0.622
ENT_IV	32	19	−0.085	0.634
ENT_V	30	6	−0.016	0.630
ENT_VI	31	13	−0.207	0.667
ENT_VII	32	31	−0.021	0.590
ENT_VIII	32	25	−0.244	0.535
ENT_IX	32	16	*-0.144	0.631
ENT_X	32	28	−0.262	0.597
ENT_XI	42	42	*-0.314	0.637
ENT_XII	31	25	+0.067	0.622
ENT_XIII	36	20	−0.280	0.634
ENT_XIV	30	10	−0.319	0.635
ENT_XV	32	16	+0.094	0.585
ENT_XVI	32	17	+0.026	0.649
Chemin de Resquiadou (RES)				
RES_I	30	10	−0.243	0.538
RES_II	32	33	−0.099	0.627
RES_III	30	21	+0.092	0.513
RES_IV	29	27	+0.002	0.519
RES_V	33	5	+0.116	0.613
RES_VI	33	7	−0.038	0.170
RES_VII	31	10	−0.034	0.593
RES_VIII	32	5	+0.064	0.467
RES_IX	31	7	+0.006	0.389
RES_X	39	10	−0.136	0.287
RES_XI	32	9	−0.069	0.299
RES_XII	30	2	+0.102	0.488
RES_XIII	34	7	+0.003	0.295
RES_XIV	40	3	−0.078	0.182
RES_XV	32	17	−0.158	0.532
RES_XVI	32	8	−0.005	0.591
Île Ratonneau (RAT)				
RAT_I	33	3	−0.149	0.635
RAT_II	30	2	−0.003	0.532
RAT_III	32	4	+0.134	0.534
RAT_IV	32	3	−0.048	0.417
RAT_V	33	3	+0.096	0.649
RAT_VI	35	4	−0.115	0.417
RAT_VII	22	2	−0.083	0.464
RAT_VIII	36	2	+0.064	0.598
Île de Riou (RIOU)				
RIOU_I	32	1	−0.016	0.062
RIOU_II	31	<1	−0.093	0.236
RIOU_III	31	1	−0.034	0.094
Total	1607			

area. The vegetation of shrubs and trees is sparsely scattered between areas of bare rock and rock debris. Like ENT, the area is enclosed by barriers to active dispersal, in this case roads and a burnt patch left behind after an ancient bush-fire. RAT was sampled on a peninsula of the Île de Ratonneau in the Bay of Marseille. The structure of the limestone rock is similar to that of RES, but with cover consisting of low shrubs and herbs. The eight sites sampled represent all the subpopulations on this peninsula. The Île de Riou (RIOU), due to overpopulation by seagulls and rats, is an ecologically degraded island south of Marseille (Vidal *et al.*, 1998). Structurally, it is similar to the previous two areas although it is covered by even less vegetation. Like all islands off the coast of Marseille, it was part of the mainland until the rise of the sea level during the Holocene (Laborel *et al.*, 1994). Only three sites with living individuals were found on this island.

Habitat analysis

The study of the influence of habitat heterogeneity on population structure requires detailed knowledge of the suitable habitat on a small spatial scale. Subjective impressions from preliminary surveys indicated that the presence of the species in the research area is tightly linked to the occurrence of well aerated organic substrates, like loose soil, foliage or other organic debris of at least 5 cm thickness. To test this hypothesis, between eight and 26 randomly chosen patches of 0.0625 m^2 within each population (85 in total) were checked for the presence or absence of living specimens and aerated organic substrate. The

organic substrate was classified as aerated when a metal ruler of 5 cm width could penetrate at least 5 cm without effort. The association between the presence of living specimens and aerated organic substrate was tested with *G*-test of fit (Sokal & Rohlf, 1981). In addition, to estimate the habitat quality of each patch where living *P. elegans* were present, the density of living individuals was recorded. Maps showing the suitable habitat were created by marking the presence of snails on scanned aerial images of the sampling areas. Habitat patch size was quantified in arbitrary units from these maps (see Fig. 2) with the program Scion Images v. beta 4.0.2.

Allozyme electrophoresis and genetic data analysis

Between 29 and 42 individuals per site were screened for genetic variation. In total, 1607 individuals from 51 sites in 5 populations were assayed. The spatial arrangements of the sampling sites within populations and dispersal barriers for *P. elegans* are shown

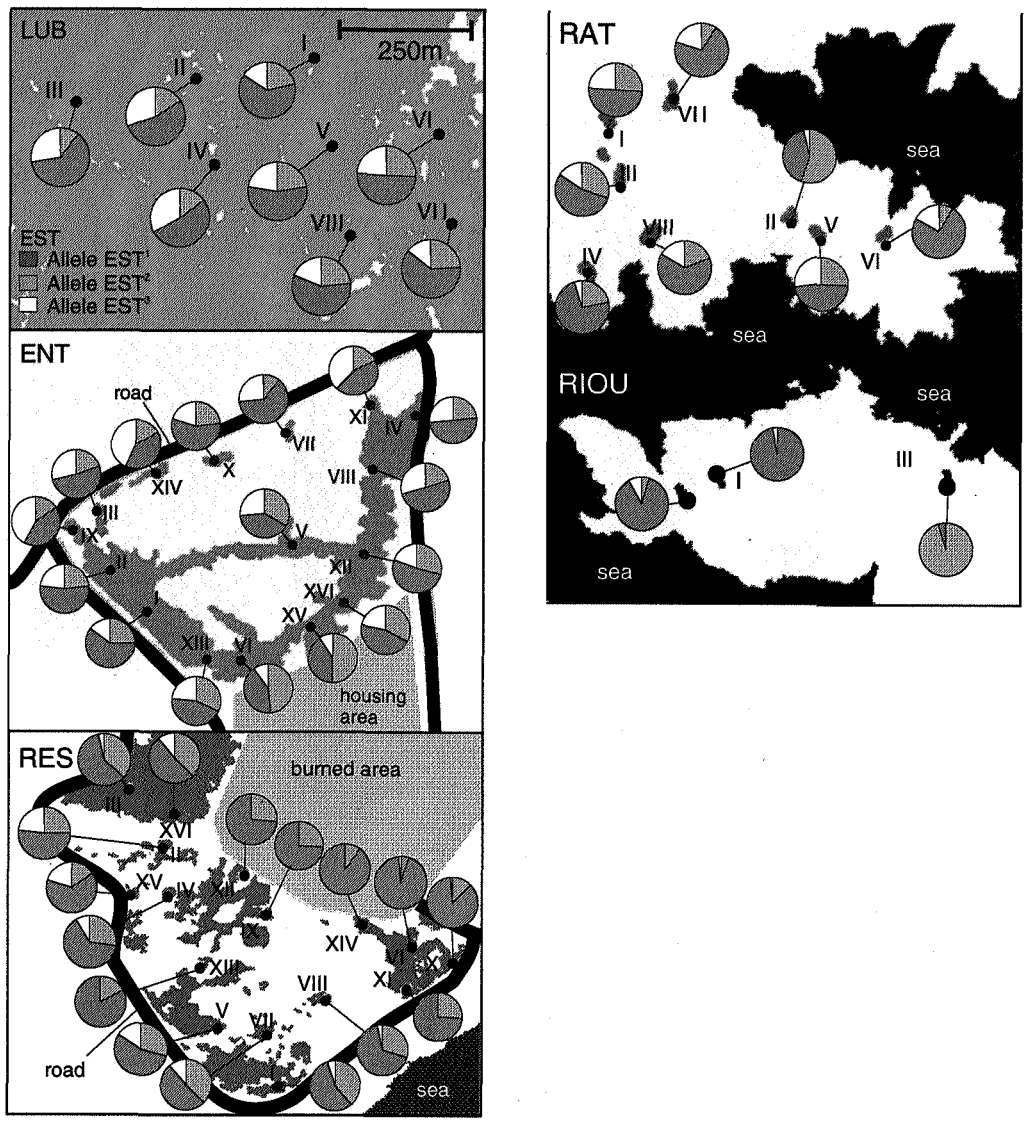

Figure 2. Sampling sites within populations, habitat structure and allele frequencies. Sampling sites within populations are marked by a solid black spot. Grey shading marks suitable habitat for *Pomatias elegans*. Barriers to dispersal such as roads or the sea are indicated. All maps are drawn to the same scale, indicated in the LUB map. The allele frequencies at the EST locus for each sampling site are shown as pie charts. Dark grey shading indicates allele Est^1, light grey Est^2 and white Est^3.

in Figure 2. Individuals from a sampling site destined for genetic analysis were sampled within 0.0625 m^2 during the density estimation (see above). Whenever the density was too low to obtain at least 29 individuals, the immediate surrounding area was searched until this number was found. However, all sampling was performed within the estimated neighbourhood of max. 20 m^2 (unpublished data). The neighbourhood is the area in a continuous population wherein random mating can be assumed, given the dispersal capacity, average lifespan and population density (Wright, 1946).

Sampled snails were crushed with their shells in Eppendorf tubes and immediately subjected to cellulose acetate gel electrophoresis. All electrophoretic protocols followed methods detailed in Hebert & Beaton (1989). We checked for polymorphisms in 31 enzymes, but only an unspecific esterase locus (EST, EC 3.1.1.-) exhibited sufficient within-population variation and was therefore retained for the analyses. The program GENEPOP v.3 (Raymond, 1995) was used to estimate the inbreeding coefficient F_{IS} and its statistical significance as well as the observed heterozygosity H_O for each sampling site, following (Weir & Cockerham, 1984). Observed heterozygosity H_O was used as measurement of genetic variability. The arcsine transform of H_O was simultaneously regressed on the log transform of density at the sampling and the habitat patch size. Multiple regression was performed with BIOMstat (1996). The program Arlequin (Schneider, Roessli & Excoffier, 2000) was used to calculate global F_{ST} estimates for each population and Slatkin's linearized F_{ST} as pairwise distance measure among sampling sites within populations.

Definition of spatial distance matrices over different connectivity networks

To account for different potential effects of spatial habitat heterogeneity on the effectivity of dispersal, four different metric distance matrices among sampling sites within populations were constructed assuming different connectivity among sampling sites as a function of the habitat. Landscape connectivity refers to the degree to which the landscape facilitates or impedes movement among patches (Hansson, 1991). The effective distances that connect local demes can be expressed in more or less complex connectivity network matrices taking into account different aspects of the influence of landscape structure on dispersal (Hanski, 1998).

The first two considered connectivity matrices between sites, assuming random dispersal with regard to the habitat. The *Euclidean* matrix connects two sites via the direct geographical distance between them. In a *Gabriel* connected graph, it is assumed that two sites are connected if no third site lies within the circumference of a circle going through both sites (Gabriel & Sokal, 1969). This matrix connects only nearest neighbour sites. The next two distance matrices take into account features of the landscape that are potentially relevant for the dispersal of the snails. In the *Relief* matrix, the sites are connected over the fall line up to the ridges (RES, RAT) or a line on the altitude of the highest sampling site (LUB). This distance matrix assumes that most of the gene flow is due to snails being washed down the slope by heavy rain. The landscape of ENT is essentially flat, so that the *Relief* matrix could not be constructed for this population. The *Habitat* matrix assumes that dispersal between sites occurs as far as possible within suitable habitat and only adjacent patches are connected via the shortest route. In LUB the habitat turned out to be practically continuous, so this matrix coincided with the *Euclidean* matrix.

Tests of association between genetic divergence and connectivity networks were investigated separately for each population using the Mantel test option (Mantel, 1967) implemented in NTSYSpc v. 2.0 (Applied Biostatistics Inc.). The statistical significance of the calculated correlation coefficient *rz* was evaluated by 9999 random permutations (Smouse, Long & Sokal, 1986). It is possible that more than a single connection matrix contributes to the observed population structure. To account for this statistical interdependence, a multiple Mantel regression was computed using all connectivity networks as independent variables with Permute 3.4 (Legendre, Lapointe & Casgrain, 1994). Both forward selection and backward elimination of variables was conducted to select variables that contributed significantly to the regression equation. The statistical significance of variables was assessed by 9999 permutations.

Additionally, the spatial patterns of genetic variation were investigated using spatial autocorrelation techniques. Spatial autocorrelation is the association of a geographically distributed variable with the values of the same variable at other localities (Sokal & Oden, 1978; Sokal, Oden & Thompson, 1997). In evolutionary biology, these techniques are used to recognize spatial patterns produced by different processes (Slatkin & Arter, 1991; Sokal *et al.*, 1997). These methods have the advantage that they allow inference of spatial genetic population structure free of the often violated assumptions of classic population genetic methods. The Excel add-in RookCase v.0.96 (Sawada, 1999) was employed to construct spatial correlograms. The degree of spatial autocorrelation for several distance classes was quantified using Moran's *I* (Sokal & Oden, 1978), a product moment correlation coefficient. As most information about spatial processes can be gained from alleles that are least

influenced by the random effects of sampling (Sokal, Jacquez & Wooten, 1989), the gene-frequency of the most common allele (*Est*^{1}) was used for computation. The significance of each individual Moran's *I* was determined by comparison of the observed value against a distribution obtained by random permutation of gene-frequencies against localities under the null hypothesis of no spatial arrangement. The Euclidean distance matrix was used for computations because this connectivity network accounts only for geographical distance among sampling sites. Because the spatial scale is comparable in all analyses, the use of this matrix permits direct comparison of the differences in spatial genetic structure due to different gene-flow patterns. Any difference in spatial genetic structure among populations is therefore most likely due to differences in habitat structure and not solely to geographical distance.

RESULTS

HABITAT ANALYSIS

The incidence of living *P. elegans* in the areas studied was closely associated with the occurrence of friable, aerated organic substrates (χ^2 = 97.38, df = 1, $P < 0.001$), regardless of other structural or vegetation features of the patch. Such a substrate was therefore classified as a suitable habitat and its presence mapped on the aerial photographs during thorough inspections of the study areas. However, the habitat quality, as expressed in differences of population density varied significantly among populations (ANOVA, $F = 46.07$, $P < 0.001$). It was highest in LUB (51.5 ± 9.5 individuals per $0.0625\,m^2$) and decreased logarithmically down to 0.8 ± 0.2 in RIOU (Table 1).

GENETIC VARIATION WITHIN POPULATIONS

The EST locus exhibited three alleles that were present in the majority of the sampling sites. Allele frequencies at each sample site are shown in Figure 2. Except for two sampling sites, there were no significant deviations from Hardy–Weinberg expectations at this local scale, as evidenced by the F_{IS} estimates (Table 1) and an exact HW test (data not shown).

The degree of genetic structure varied strongly among populations. In LUB, the F_{ST} estimate was not significantly different from zero and increased over ENT, RAT, RES to RIOU, where it attained a value of 0.807 (Table 2). Substantial genetic heterogeneity among sampling sites, however, was observed in all populations, as evidenced by Fisher's exact test of differentiation between sampling sites (results not shown). The range of genetic diversity within sampling sites, measured as heterozygosity *H*, ranged from 0.062 to 0.667. Arcsine transformed heterozygosity within sampling sites was positively correlated both to the logarithmic transformed habitat patch size ($r = 0.42$; $P = 0.002$) and the equally transformed density ($r = 0.56$; $P < 0.001$). However, the significant multiple regression (arcsine $H = 0.34 + 0.009$ log patchsize $+0.073$ log density; $r^2 = 0.32$; $P = 0.0001$) revealed that only the contribution of the density was significant ($P = 0.003$). A scatterplot of arcsine transformed heterozygosity against *log* density is

Table 2. Mantel tests of different connectivity networks and estimates of genetic divergence for sampled populations. The overall correlations *rz* between Slatkin's linearized F_{ST} between sampling sites within populations and different connectivity networks along with the associated probability *P* of being different from zero are given (Mantel-test with 9999 permutations). For details of the connectivity networks see text. Genetic divergence among sampling sites within populations was calculated as F_{ST} according to Weir & Cockerham (1984). Their significance *P* was tested by a non-parametric permutation approach (9999 permutations, Excoffier & Smouse, 1992). For details see text. $^*P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.001$

	Distance									
	Euclidean		Gabriel		Relief		Habitat			
Population	*rz*	*P*	*rz*	*P*	*rz*	*P*	*rz*	*P*	F_{ST}	*P*
LUB	–0.314	n.s.	0.151	0.032*	0.274	0.0122*	–	–	0.002	n.s.
ENT	0.198	n.s.	0.175	0.037*	–	–	0.108	n.s.	0.029	>0.000***
RES	0.411	0.004**	0.378	0.007**	0.209	0.039*	0.458	0.004**	0.088	>0.000***
RAT	–0.149	n.s.	–0.172	n.s.	–0.105	n.s.	0.020	n.s.	0.075	>0.000***
RIOU	–	–	–	–	–	–	–	–	0.807	>0.000***

shown in Figure 3. The average density per population was negatively correlated to the within population overall F_{ST} ($r = -0.81$, $P < 0.05$).

Spatial arrangement of genetic variation

In LUB the genetic distance matrix was, in increasing order, significantly correlated with the *Gabriel* network and the *Relief* matrix. Only the *Gabriel* network showed a significantly positive association with genetic distance in ENT. All connectivity matrices exhibited a significantly positive correlation in RES. However, the *Habitat* matrix explained more genetic variation than the *Euclidean* distances, the *Gabriel* network and the *Relief* matrix, in decreasing order (Table 2). No connectivity network explained the spatial arrangement of genetic variation in RAT. Other measures of genetic divergence showed very similar results and are therefore not presented.

Multiple regression included the variables *Habitat* and *Relief* within a significant model for RES ($F_{ST} = -0.33\ Relief + 0.65\ Habitat$; $r^2 = 0.19$, $P = 0.001$). In all other populations only a single variable was retained. In all cases, it was the variable that showed the highest correlation in the Mantel test. Correlograms of Moran's I showed that significantly positive correlations of the frequency of the most abundant allele with distance could be detected in ENT and RES (Fig. 4). The spatial autocorrelation coefficient tends to be high (up to $I = 0.86$) on shorter scales and decreases with increasing distance. The spatial autocorrelation vanished with distances greater than 400 m (RES) or 500 m (ENT) between sampling sites, respectively. In LUB and RAT, no significant positive correlation could be found, regardless of spatial scale. Almost identical results were obtained using distograms (Vendramin *et al.*, 1999) and Mantel correlograms (Oden & Sokal, 1986) that account for all three alleles simultaneously (data not shown).

DISCUSSION

Level of genetic drift depends on habitat quality

The genetic allozyme diversity of *P. elegans* within the populations investigated was found to be very low, thus confirming the findings of Jordaens, Platts & Backeljau (2001) in a survey throughout the species range. However, assuming selective neutrality of allelic variation and a low mutation rate, it can be assumed that all neutral loci are uniformly affected by gene flow and drift (Hedrick, 2000).

A consequence of random genetic drift is the decrease of genetic diversity due to the loss of alleles by random fixation and the increase of populational subdivision. It could be shown that heterozygosity decreases with patch size and population density. Both

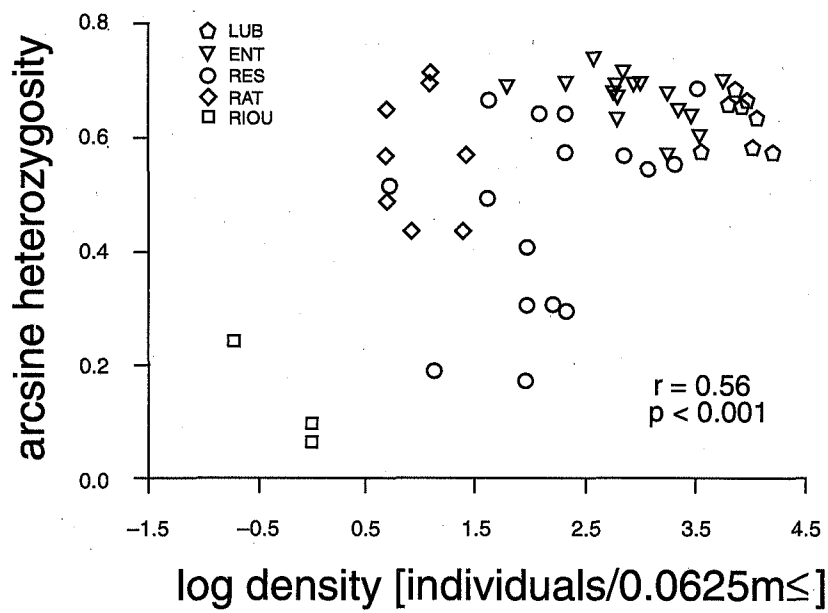

Figure 3. Scatterplot of logarithmic transformed population density against arcsine transformed heterozygosity within sampling site.

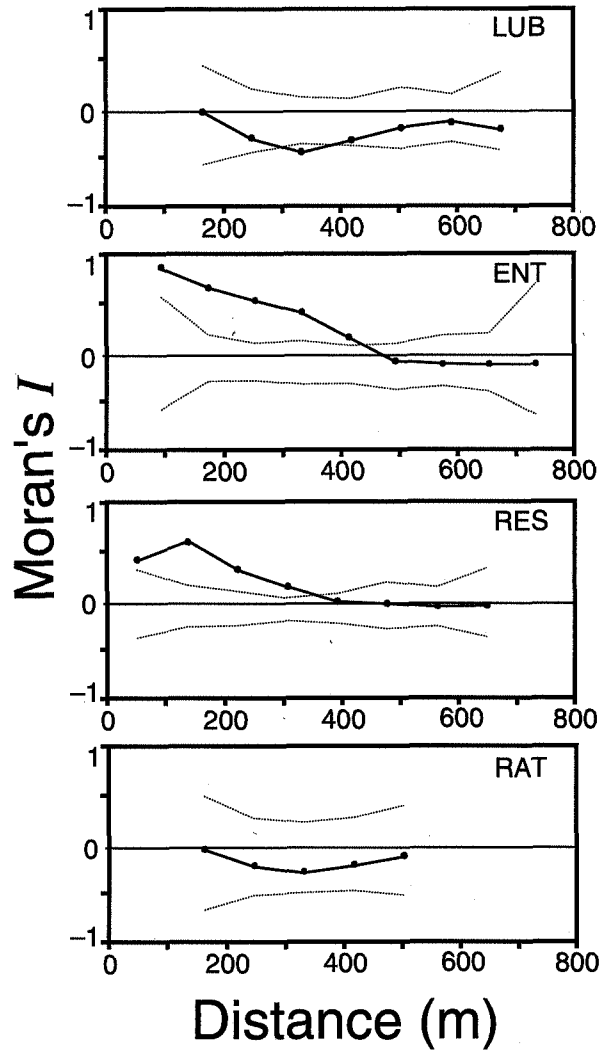

Figure 4. Correlograms of Moran's I for increasing geographical distance in LUB, ENT, RES and RAT. The estimates of spatial autocorrelation are based on the frequency of the most frequent allele Est^1, the significance was tested with 9999 permutations (Sawada, 1999). The dashed lines indicate the 95% confidence intervals of the simulated distribution.

variables tend to decrease effective population size and, hence, are expected to increase drift. However, multiple regression has shown that the contribution of the patch size is not significant. If we accept the density of individuals as an indicator of habitat quality, this result indicates that it is habitat *quality* rather than habitat *size* that determines the effective population size and, hence, the level of drift experienced by the sampling sites and the total population. Even though the characteristics which contribute to the quality of the habitat for *P. elegans* were not investigated in detail , my subjective impression was that the thickness of the organic substrate layer, its humidity and the availability and abundance of plant detritus are the crucial features determining the population density. As can be seen in Figure 2, the density of individuals decreases from LUB over ENT, RES and RAT to RIOU. Consequently, the global F_{ST} within population increases with the decrease of population density. Therefore, we can conclude that the populations investigated experience different levels of random genetic drift due to different habitat quality alone, regardless of the level or pattern of gene flow. If this finding can be generalized for other taxa with low dispersal capacities, it has consequences for the type of conservation strategies applied to endangered taxa. If the preservation of genetic diversity is at a premium, conservational efforts should be directed towards habitat quality rather than absolute habitat size or connectivity among populations.

Population structures depend on spatial habitat arrangement

In LUB, the global F_{ST} is not significant (Table 2) and the spatial correlograms show no significant positive values (Fig. 4). The absence of a significant population structure suggests that the practically continuous habitat does not limit dispersal due to geographical distance alone on the spatial scale considered in this study. LUB acted as a kind of control in this study, showing that the chosen spatial scale is not sufficient to induce a population structure in *P. elegans*. Divergent results from populations with less continuous habitat structure are therefore most likely to be due to other factors than pure geographical distance between sampling sites. However, the significant positive correlation of the *Relief* connectivity network indicates that gene flow occurs preferentially down the steep slope. As there are traces of water erosion visible in the landscape, it is likely that flowing water after heavy rainstorms washes the snails straight down the slope. Passive dispersal down the slope seems to play a simlar role in *Arianta* species as well (Baur, Ledergerber & Kothbauer, 1997). Overall, the genetic structure in the LUB population seems to be governed mainly by high levels of gene flow among adjacent sampling sites, which is reflected in the slight positive correlation of the genetic distances with the *Gabriel* network.

The suitable habitat in ENT is also more or less continuous, but arranged in a rather one-dimensional fashion. The sampling sites are sufficiently differentiated to obtain a weak, but significant population structure (see Table 2). The genetic distances are significantly correlated to the *Gabriel* network, indicat-

ing that the neighbouring sampling sites in particular are genetically similar. In the *Gabriel* network, the connections between populations do not account for the presence of suitable habitat. This suggests that *P. elegans* uses the landscape between the suitable habitats – mostly lawn, meadows and farmland – at least occasionally for dispersal. In this kind of habitat structure, the homogenizing effect of gene flow is detectable up to 500 m (Fig. 4). Above that distance, the 'resistance' of the heterogeneous habitat to active dispersal is so strong that random genetic drift becomes the prevailing force.

This effect is even more pronounced in the stronger structured RES population, where the null hypothesis for random divergence among sampling sites cannot be rejected for distances greater than 400 m (Fig. 4). The *Habitat* network explains most of the genetic variation among sampling sites (Table 2), which suggests that the gene-flow pattern in this population is influenced by the patchy habitat structure with the intervening space between the patches not being suitable for active dispersal. However, multiple regression indicates that the *Relief* matrix, and therefore passive dispersal by water erosion, also contributes to the observed gene-flow pattern in this population.

The level of the overall sampling site divergence in RAT is about the same order of magnitude as in the previous population. However, neither a correlation with a connectivity network (Table 2) nor a significant spatial autocorrelation could be detected (Fig. 4). Even though these results must be interpreted with caution due to the low number of available sampling sites, causing potential statistical problems, this indicates that the sampling sites are more or less completely isolated from each other and random genetic drift is the prevailing force shaping the population structure on the RAT peninsula. The average distance and the nature of the landscape between patches of suitable habitat seem to prevent effective, regular gene flow. Another factor likely to influence levels of gene flow among sampling sites is the climate. The amount of precipitation and the duration of periods of high humidity have a major impact on the duration of activity and thereby of dispersal distance in *P. elegans* as preliminary experiments have suggested (data not shown). Such a relation of activity to environmental conditions has already been demonstrated for other snails (e.g. Baur & Baur, 1993; Bailey, 1975; Rollo, 1982). The average annual amount of precipitation as well as the number of rainy days decreases from the most northern (LUB) to the most southern (RIOU) population, in that way accounting for at least some of the variation in gene flow within the populations investigated. The duration of periods of favourable or unfavourable weather could in particular limit the extent of unsuitable habitat that can be crossed.

The scarceness of sites where *P. elegans* could be found prevented a detailed spatial analysis on RIOU. The almost complete fixation of different alleles at the sampling sites resulting in very high F_{ST} (Fig. 3; Table 2) suggested that the demes are completely isolated. Such a pattern could be as well obtained by independent colonization events with strong founder effects. However, subfossil shells of *P. elegans* can be found in several Holocene deposits all over the island (Benjamin Kabouche, pers. comm.), suggesting that the species was more widespread and that the present day populations are indeed relics of a larger population.

CONCLUSIONS

Taking *P. elegans* as an example of a typical ground-dwelling organism with restricted dispersal capacities, this study suggests that the population structure of, and evolutionary consequences for, such species (Harrison & Hastings, 1996) depends to a large extend on the spatial arrangement and quality of the suitable habitat. Such a functional connectivity among populations, although on a much larger scale, was also found by Arter (1990) in *Arianta arbustorum*, where the inferred dispersal routes followed the drainage system. However, in contrast to other gastropods like *Arianta arbustorum*, *Helix aspersa* and *Cepaea nemoralis*, which occur preferentially in aggregations regardless of suitability of the habitat (Lamotte, 1951; Baur, 1993 Arnaud *et al.*, 2001), *P. elegans* is distributed continuously over the suitable area. This might be the reason why Arnaud *et al.* (2001) found spatial autocorrelation of neutral markers among colonies of *H. aspersa* on the same spatial scale as in *P. elegans*, despite the presence of suitable habitat between colonies and the better dispersal capacity of *H. aspersa*.

Genetic correlations due to gene flow among allele frequencies of allozymes in the present example ceased to exist with distances over a few hundred meters in fragmented habitat. Without knowledge of the exact microspatial population structure, it is impossible to know whether the observed allele frequencies in two geographically separated sampling sites are correlated by gene flow or by chance. Consider for example the allele frequencies of the EST locus of sampling site VI in LUB, II in ENT, II in RES and I in RAT (Fig. 2). The allele frequencies are almost identical, which would have suggested a high amount of gene flow among the populations if these had been the only sites that were sampled. This indicates that random drift on neutral allozyme markers may be too strong in land snails to preserve any signature of past or present gene flow over a distance of more than a few hundred meters.

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