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Microspatial genetic structure in the land snail *Helix aspersa* (Gastropoda: Helicidae)

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The microspatial genetic structure of allele frequencies at seven isozyme loci was examined for 15 populations of the land snail *Helix aspersa* sampled in a village from Brittany (north-western France). Spatial heterogeneity of allele frequencies was highly significant ($P < 0.001$). Fixation indices reflected nonrandom mating within neighbourhoods and a slight but consistent differentiation between colonies ($F_{ST} = 0.044$; $P < 0.01$). Analyses of gene flow or genetic distances failed to reveal a significant relationship with geographical distance, probably because of the complexity of environmental heterogeneity. However, matrix comparisons between genetic distances and connectivity networks among adjacent colonies (Gabriel-connected graph) yielded a significant correlation in every case, indicating a 'step-by-step' relationship between neighbouring localities. Moreover, most of the allozymes were spatially structured and showed (i) a gradual isolation of colonies with increasing geographical distances, and (ii), for some correlogram profiles, a circular gradient illustrating a multidirectional colonization of the village. The probable existence of disperser individuals allowed us to suggest a metapopulation model which would explain the maintenance of such animals in fragmented habitats where anthropogenic disturbances and extinction/recolonization events are commonly observed.

Keywords: allozymes, *F*-statistics, genetic structure, land snail, metapopulation, spatial autocorrelation.

Introduction

Land snails typically live in discrete populations, often isolated from one another. Because of their sedentary nature and a very high cost of locomotion, snails and slugs are characterized by a low dispersal ability (Denny, 1980; Fearnley, 1993). This suggests that land snails are prone to the effects of population subdivision with reduced gene exchanges between demes, leading presumably to strong local differentiation (Schilthuizen & Lombaerts, 1994; Pfenninger *et al*., 1996). Moreover, habitat fragmentation and instability of man-disturbed environments may impose severe restrictions on gene flow and increase random genetic drift. Extinction and recolonization dynamics in local populations may also modify the distribution of genetic variability, leading to a decrease or an increase of variation among populations (Schilthuizen & Lombaerts, 1994; Ruckelshaus, 1998). Furthermore, some studies on intraspecific genetic variation over heterogeneous habitats suggest that genetic differentiation can result from specific habitat-related effects at local or even microscales (Johannesson & Tatarenkov, 1997). In this context, microevolutionary processes will drive the amount and organization of genetic variability within natural subdivided populations.

Attention has been given for a long time to geographical patterns of genetic variation in the terrestrial Gastropod *Helix aspersa* (Selander & Kaufman, 1975; Madec, 1989; Guiller *et al*., 1994, 1996). Past geological and historical events such as disjunction of populations by plate tectonics during the Tertiary, isolation of populations during the last glacial period or human introduction during the Neolithic period have been proposed to explain the disjunct distribution of *H. aspersa* observed in Maghreb and Europe (Guiller *et al*., 1994, 1996). In addition, a preliminary study showed a patchy genetic differentiation along a linear transect in agricultural habitats (the polders of the Bay of Mont-St-Michel) over distances of less than 5 km: a pseudoclinal variation involving several immigration events was detected for some alleles, whereas the others exhibited

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no significant pattern (Arnaud *et al*., 1999). However, to our knowledge, only one study, improved in an autocorrelation analysis by Sokal & Oden (1978a), deals with microgeographical processes that would take place among microsubdivided demes in *H. aspersa* (Selander & Kaufman, 1975). In that work, there were no clear patterns that could explain intercolony variation. Historical demographic factors like migratory events followed by diffusion 'mimicking' an adaptive cline were nevertheless suspected (Sokal & Oden, 1978a). More recently, Fearnley (1993) observed a sexual asymmetry, i.e. a trade-off involving a beneficial increase of adult size linked to a reduced development of the female reproductive system. This asymmetry is associated with a polymorphism for dispersal leading to the distinction of sedentary (smaller and characterized by a faster reproduction) and disperser (bigger) individuals. Despite the limited locomotion ability of these snails (Denny, 1980), 'dispersers' have the potential to colonize new habitats and promote gene flow between demes (Fearnley, 1993). As genetic differentiation is not related to inbreeding in *H. aspersa*, a low level of interdemic mixing should be ensured by such 'dispersers' (Fearnley, 1993), corresponding to differences between demes found in only a few allozyme frequencies by Selander & Kaufman (1975) (mean F_{ST} ranging from 0.026 to 0.040 for five enzyme loci studied). Such a hypothesis could lead to a metapopulational equilibrium occurring in a fragmented habitat.

The present investigation describes the microgeographical spatial pattern of genetic variation among natural populations of *H. aspersa* living in a human-disturbed environment, namely a small village located in Brittany. Because the distribution of genetic variability is inherent to the spatial context, several studies have examined spatial autocorrelation analysis as a tool for making inferences on microevolutionary processes which potentially generate observed spatial patterns of variation in allele frequencies (Sokal & Wartenberg, 1983; Epperson, 1990; Epperson & Li, 1996; Sokal *et al*., 1997). Although it may be unlikely that spatial autocorrelation could assess all different evolutionary mechanisms, this approach seems quite appropriate to describe the main forces affecting a population structure and has been successfully applied to data sets from animals (Sokal & Oden, 1978a,b; Sokal *et al*., 1987), plants (Epperson & Clegg, 1986; Campbell & Dooley, 1992; Peakall & Beattie, 1995; Shapcott, 1995; Ruckelshaus, 1998) and humans (Sokal *et al*., 1986). In this study, we employed a suite of statistics, including spatial autocorrelation analysis (i) to examine the genetic differentiation at seven enzyme loci in 15 colonies of *H. aspersa*, and (ii) to detect some substantial localized spatial structures of allozymes.

Materials and methods

Sample collection

Fifteen populations were sampled in a village located in the region of the Bay of Mont-St-Michel (Brittany, north-western France) (Fig. 1). All snails with a diameter greater than 5 mm were collected by searching through surface vegetation and under leaf litter, boards, bricks and the like. To obtain an exhaustive sampling (i.e. including all the snails within the chosen size class), the collecting procedure was repeated three times at 2-day intervals. A total of 455 snails was collected and sample sizes ranged from 10 to 43 individuals per population.

Electrophoretic analysis

Electrophoresis was performed on polyacrylamide gels. Details of procedures and the genetic interpretation of zymograms are given in Guiller *et al*. (1994). Seven isozyme loci were scored for each individual in all samples: four alleles each for leucine aminopeptidase (LAP, EC 3.4.11.1), aspartate aminotransferase (AAT, EC 2.6.1.1) and malate dehydrogenase (MDH, EC 1.1.1.37); three alleles for esterase (EST, EC 3.1.1.1); two alleles each for phosphoglucomutase (PGM, EC 5.4.2.2) and phosphoglucoisomerase (PGI, EC 5.3.1.9); and only one allele for superoxide dismutase (SOD, EC 1.15.1.1).

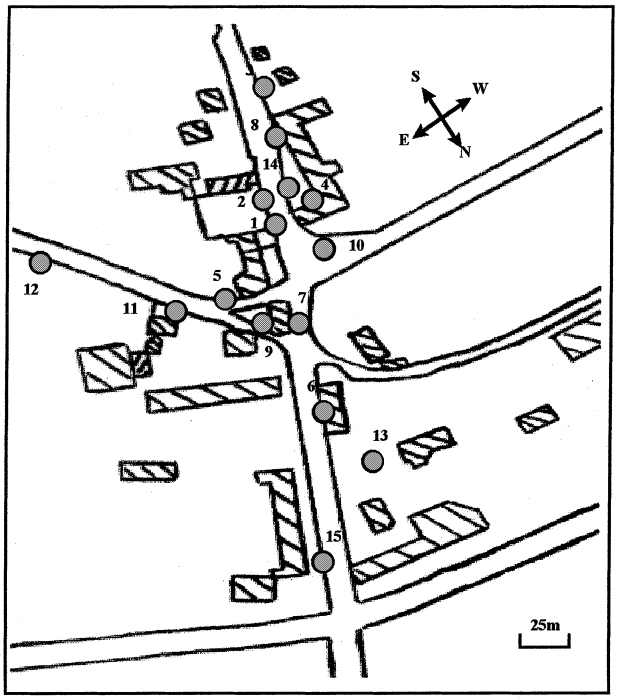

Fig. 1 Sample locations and their codes.

Statistical analysis of electrophoretic data

Allele frequencies and heterozygosity estimates were calculated using BIOSYS-1, release 1.7 (Swofford & Selander, 1989). Estimates of Hardy–Weinberg disequilibrium were obtained for each locus in each sample with GENEPOP version 1.2 (Raymond & Rousset, 1995). Using methods based on Markov processes and two-way tables, GENEPOP also provided some information on the statistical population differentiation by performing a Fisher exact test. Estimators of Wright's *F*-statistics following Weir & Cockerham (1984) were provided by FSTAT (Goudet, 1995). To quantify the effect of migration on the genetic structure, gene flow was estimated between each pair of demes by converting F_{ST} to amounts of gene flow (Nm), according to an island model under neutrality and negligible mutation (Slatkin, 1993). Moreover, values of F_{ST} and Nei's (1978) genetic distances were computed between pairwise populations and used for the construction of matrices of genetic distances.

To determine if genetic heterogeneity corresponded to a spatial structure, three kinds of analyses were conducted.

Spatial autocorrelation

Spatial autocorrelation is defined as the association of the values of one geographically distributed variable with the values of the same variable at other localities (Sokal & Oden, 1978a). Spatial autocorrelation occurs for a variable if the observed value at one locality is dependent on the values at neighbouring localities (Sokal & Oden, 1978a).

The spatial relationship of sampling locations was indicated by the specification of 'real distance' (in m for instance) or 'functional distance' that can be a graph of arbitrary connections (Sokal & Oden, 1978a,b; Sokal & Wartenberg, 1983). Then, spatial autocorrelation coefficients are the expression of similarities between neighbouring locations (Sokal *et al.*, 1997). Here, we used Moran's *I*:

$$I_k = \frac{n \sum_{i \neq j} w_{ij}^{(k)} (x_i - \bar{x})(x_j - \bar{x})}{\sum_{i \neq j} w_{ij}^{(k)} \sum_i (x_i - \bar{x})^2} \quad ,$$

where n is the number of locations sampled, $\bar{x}$ is the average value of x_i, k is the distance class, and $w_{ij}^{(k)} = 1$ if i and j are both in class k and 0 otherwise. A test for a significant autocorrelation was obtained by comparing the observed value of I with the expected value $E(I) = -(n-1)^{-1}$ under the null hypothesis of no spatial structure (Sokal & Oden, 1978a).

Mantel test

A second approach was used with the multivariate Mantel test by comparing the distance matrices describing genetic and geographical relationships among the sampling locations (Oden & Sokal, 1986). We computed the normalized Mantel statistic as $rz = [1/(n-1)] \times \sum_i \sum_j [(x_{ij} - \bar{x})/s_x][(y_{ij} - \bar{y})/s_y]$ for $i \neq j$, where i and j are row and column indices, x_{ij} and y_{ij} are the corresponding elements of the two matrices **X** and **Y**, and n is the number of distances in one of the matrices (Smouse *et al.*, 1986). Significance of rz values was tested by randomly permuting (10 000 permutations) rows and columns of one matrix while keeping the other constant, which gave the sampling distribution of rz under the H_o (Smouse *et al.*, 1986).

Choice of spatial relationship between sampling locations

The linear distance criterion (*dm*) was used, where all pairs of points located in the same geographical distance class were connected. Correlograms were based on alleles from the six different polymorphic loci analysed, but the most infrequent ones (in this case *Mdh-1*^{115}, *Est-3*^{105}, *Aat-1*^{80} and *Aat-1*^{110}) were excluded in order to maintain statistical independence of our tests (Campbell & Dooley, 1992). Correlograms were constructed using the two statistics described above, I and rz, the latter one ('multivariate Mantel correlogram') according to Oden & Sokal (1986). The significance of a correlogram was assessed using the Bonferroni technique (Oden, 1984).

A second criterion (*cg*) was based on the Gabriel network which can be regarded as a 'functional distance' (Gabriel & Sokal, 1969). In a Gabriel-connected graph, any two localities A and B are considered as connected if no other locality lies on or within the circle whose diameter is the line AB ($d^2_{AB} < d^2_{AC} + d^2_{BC}$). A binary matrix was then constructed, pairs of connected populations having a weight w_{ij} of 1, and 0 otherwise.

A third criterion (*cgr*) was also created according to the Gabriel-connected graph, but this time by taking into account the possible barrier effect of the main road that isolated western and eastern populations (see Fig. 1).

The last criterion (*dcg*) was also derived from the Gabriel network: the distance between two points is the least number of edges that must be traversed in the Gabriel-connected graph to connect these points (Sokal & Oden, 1978a). In other words, the distance between any pair of localities was determined by counting the

smallest number of connections to get from one population to the other through this network of near-neighbours (see Arnaud *et al*., 1999).

Analysis of gene flow from F-*statistics*

Slatkin (1993) suggested that plotting estimates of gene flow (*Nm*) against geographical distance on a log–log scale provides a method to test for isolation by distance without knowing if populations are at equilibrium or not. Hence, the relationship between *Nm* and geographical distance (*d*) between pairwise populations was evaluated by a linear regression of log(*Nm*) on log(*d*). As pairs of values are not independent of each other, the significance of the relationship was obtained by a Mantel randomization test.

Results

Genetic variation within and among demes

The population structure analysis is presented in Tables 1 and 2. Mean F_{IS} among all loci and all populations was significantly positive (0.056 ± 0.047; $P < 0.01$), reflecting a general nonrandom mating within populations. However, only *Pgi-2* and *Mdh-1* showed a significant heterozygote deficiency ($F_{IS} = 0.447$ and 0.228, respectively). Significant deviations from Hardy–Weinberg genotypic proportions also occurred at the *Lap-2* locus, but for three populations with opposite disequilibrium (Table 2). No linkage disequilibria were observed, except for population 4 between *Pgi-2*/*Aat-1* ($P < 0.001$). With regard to this sample, all rare alleles were found at very high occurrences. Mean F_{ST} was significantly different from zero (0.044 ± 0.006; $P < 0.01$), indicating a weak but significant differentiation among populations. This was confirmed by a highly significant spatial heterogeneity of allele frequencies for all loci over the whole area (exact test of differentiation: $P < 0.001$).

Pattern of gene flow and spatial arrangement

The test for an isolation by distance with the log–log regression model showed no significant relationship between *Nm* and geographical distance ($rz = -0.22$; $P = 0.34$) (Fig. 2). In the same way, the shape of the 'Mantel correlogram' illustrated random fluctuations of *rz*, which were not significantly different from zero for any distance class (Fig. 3). In addition, there was no significant correlation between genetic and geographical distances (Table 3).

In contrast to real geographical distances, connections according to the Gabriel network modified (*cgr*) or not (*cg*) led to highly significant correlations (Table 3). However, Moran's *I* approach based on these connectivity networks showed that only three allozyme frequencies seemed to be characterized by a significant spatial structure (Table 4).

All correlograms indicated a spatial structure at an overall significance level of $P < 0.05$ when the Bonferroni procedure was applied (see Fig. 4). However, the last distance class (200–220 m) had to be interpreted with caution because it contained less than 5% of all pairs of localities. Even though none of these correlograms was strictly monotonic, the previous results concerning alleles *Lap-2*^{100} and *Lap-2*^{92} were reinforced by the corresponding correlograms because of a strong positive autocorrelation at short distances and a progressive decline of Moran's *I* for the following distance classes. Alleles *Lap-2*^{96}, *Est-3*^{100} and *Mdh-1*^{90} had similar profiles but exhibited an increase of *I* at far distances. Quite different results occurred for *Aat-1*^{100}, *Aat-1*^{90} and *Pgm-2*^{100} which reflected a consistent

Table 1 *F*-statistics estimated over all the 15 populations of *Helix aspersa*

Locus	F_{IS} (f)	F_{IT} (F)	F_{ST} (θ)
Lap-2	0.010 (0.053) NS	0.044 (0.053) NS	0.034 (0.015)**
Aat-1	0.015 (0.046) NS	0.057 (0.054) NS	0.042 (0.023)**
Pgm-2	−0.013 (0.039) NS	0.023 (0.035) NS	0.036 (0.019)**
Est-3	0.016 (0.067) NS	0.059 (0.088) NS	0.042 (0.035)**
Pgi-2	0.447 (0.245)*	0.642 (0.329)**	0.271 (0.156)**
Mdh-1	0.228 (0.056)**	0.266 (0.051)**	0.049 (0.026)**
Mean	0.056 (0.047)**	0.098 (0.049)**	0.044 (0.006)**
C I (95%)	(0.002; 0.162)	(0.039; 0.207)	(0.037; 0.066)

Mean values per locus and standard deviates calculated by jackknifing among the samples; average indices and standard deviates computed by jackknifing over all loci; 95% confidence intervals obtained by bootstrapping. Bonferroni correction: *$P < 0.05$; **$P < 0.01$.

Table 2 Observed (H_o) and expected (H_e) heterozygosities under the Hardy–Weinberg equilibrium in *Helix aspersa*. The sample size (N; italics) appears under each population number

		Populations															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
		24	*39*	*30*	*30*	*41*	*25*	*43*	*40*	*25*	*16*	*42*	*27*	*35*	*10*	*28*	
Locus																	Fisher test
Lap-2	H_o	0.500	0.692	0.367	0.733	0.683	0.600	0.558	0.400	0.520	0.313	0.429	0.481	0.600	0.800	0.321	
	H_e	0.571	0.616	0.518	0.744	0.554	0.450	0.502	0.563	0.520	0.506	0.506	0.476	0.543	0.763	0.328	
	D	NS	NS	**	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	**
Aat-1	H_o	0.375	0.410	0.200	0.300	0.439	0.440	0.349	0.250	0.520	0.313	0.405	0.444	0.429	0.400	0.321	
	H_e	0.403	0.346	0.188	0.442	0.397	0.393	0.452	0.297	0.510	0.280	0.402	0.435	0.421	0.337	0.275	
	D	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Pgm-2	H_o	0.417	0.359	0.267	0.300	0.390	0.360	0.558	0.450	0.480	0.625	0.429	0.630	0.343	0.500	0.429	
	H_e	0.422	0.386	0.235	0.259	0.470	0.429	0.506	0.425	0.509	0.484	0.477	0.484	0.358	0.479	0.416	
	D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Est-3	H_o	0.167	0.103	0.367	0.267	0.195	0.160	0.093	0.300	0.280	0.375	0.357	0.370	0.171	0.400	0.107	
	H_e	0.156	0.189	0.481	0.264	0.178	0.150	0.090	0.303	0.246	0.315	0.297	0.307	0.161	0.337	0.223	
	D	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS
Pgi-2	H_o	0.000	0.000	0.000	0.200	0.000	0.040	0.023	0.000	0.000	0.000	0.000	0.037	0.086	0.000	0.179	
	H_e	0.000	0.000	0.000	0.452	0.000	0.040	0.023	0.000	0.000	0.000	0.000	0.037	0.135	0.000	0.166	
	D	—	—	—	**	—	—	—	—	—	—	—	—	NS	—	NS	***
Mdh-1	H_o	0.167	0.205	0.300	0.500	0.171	0.080	0.326	0.450	0.280	0.125	0.310	0.481	0.343	0.300	0.429	
	H_e	0.265	0.293	0.373	0.584	0.369	0.347	0.309	0.516	0.344	0.181	0.382	0.540	0.549	0.268	0.397	
	D	**	*	*	NS	***	***	NS	*	**	NS	**	NS	**	NS	NS	***
Fisher test		NS	NS	**	**	***	**	NS	**	NS	NS	NS	NS	*	NS	NS	***

D: deviations from Hardy–Weinberg expectations; Fisher's test used to combine information over loci and over populations.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

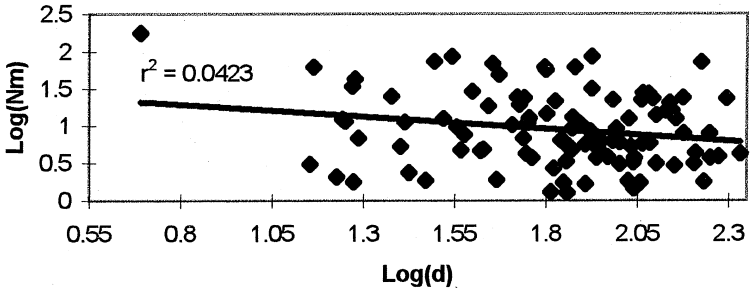

Fig. 2 Log–log regression of gene flow (*Nm*) against geographical distance (*d*) where gene flow was estimated from mean θ between each pair of populations of *Helix aspersa*. The regression equation is $y = -0.327x + 1.544$.

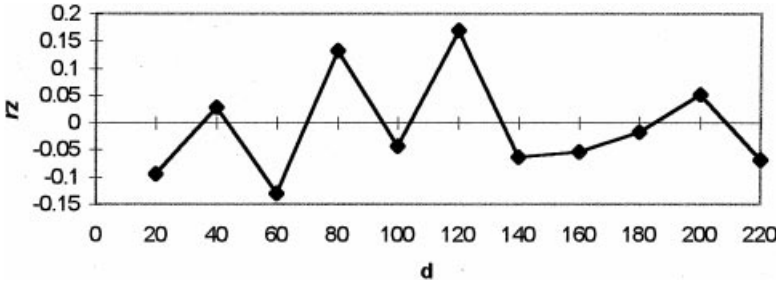

Fig. 3 Mantel correlogram for pattern of variation of Nei's genetic distance for the 15 populations of *Helix aspersa*; *rz*, normalized *Z*; *d*, geographical distance (m).

Table 3 Mantel tests of association for populations of *Helix aspersa*

	Nei's distance	F_{ST}
Geographical connection	*rz*	
dm	0.036 NS	0.084 NS
cg	−0.158**	−0.191**
cgr	−0.098*	−0.137**
dcg	0.060 NS	0.128 NS

Geographical connections were based on the geographical distance (*dm*), the Gabriel network modified (*cgr*) or not (*cg*) (see text for explanations) and the minimal distance between two locations according to the Gabriel-connected graph (*dcg*) (*rz*: normalized *Z*; *$P < 0.05$; **$P < 0.01$).

Table 4 Moran's *I* for populations of *Helix aspersa*

Alleles	*cg*	*cgr*
Lap-2^{100}	0.478*	0.438*
Lap-2^{92}	0.447*	0.679**
Lap-2^{94}	−0.114 NS	−0.223 NS
Lap-2^{96}	−0.047 NS	0.024 NS
Aat-1^{100}	0.196 NS	0.249 NS
Aat-1^{90}	0.339 NS	0.417 NS
Est-3^{100}	0.149 NS	0.215 NS
Pgm-2^{100}	0.225 NS	0.190 NS
Pgi-2^{100}	−0.047 NS	−0.135 NS
Mdh-1^{100}	−0.084 NS	−0.331 NS
Mdh-1^{90}	0.501*	0.364 NS
Mdh-1^{110}	0.098 NS	0.293 NS

I is calculated with w_{ij} based on the Gabriel networks *cg* and *cgr* (*$P < 0.05$; **$P < 0.01$).

pattern of decreasing autocorrelation up to 100–120 m, followed by a significant increase of *I* up to the higher distances. Such a trend could be observed less clearly for *Mdh-1*^{100} and *Mdh-1*^{110}: Moran's *I* showed a marked peak for the [140–160] distance class with a decreased autocorrelation for the most distant pairs of populations. *Pgi-2*^{100} and *Lap-2*^{94} showed Moran's *I* close to 0 with very slight fluctuations.

Discussion

The present study shows that a substantial degree of heterogeneity and populational structure can be detected at a microgeographical scale in *H. aspersa* populations, as in the work of Selander & Kaufman (1975). Similar observations have been made on microlocal differentiation in other Gastropod species by Schilthuizen & Lombaerts (1994), Pfenninger *et al.* (1996) and Johannesson & Tatarenkov (1997). In our investigation, significant departures from Hardy–Weinberg equilibrium were principally observed at two loci (*Mdh-1* and *Pgi-2*). Such deviations from panmixia have been frequently observed in *Helix aspersa* (Guiller *et al.*, 1996) and other simultaneously hermaphrodite land snails like *Cepaea* spp. (Guiller & Madec, 1993). They are interpreted as a result of sampling in genetically differentiated microlocal demes, rather than a consequence of selective pressures. Thus, a colony would be subdivided into interbreeding neighbourhoods (Shapcott, 1995). Samples formed by an admixture of local populations with differing allelic frequencies and/or

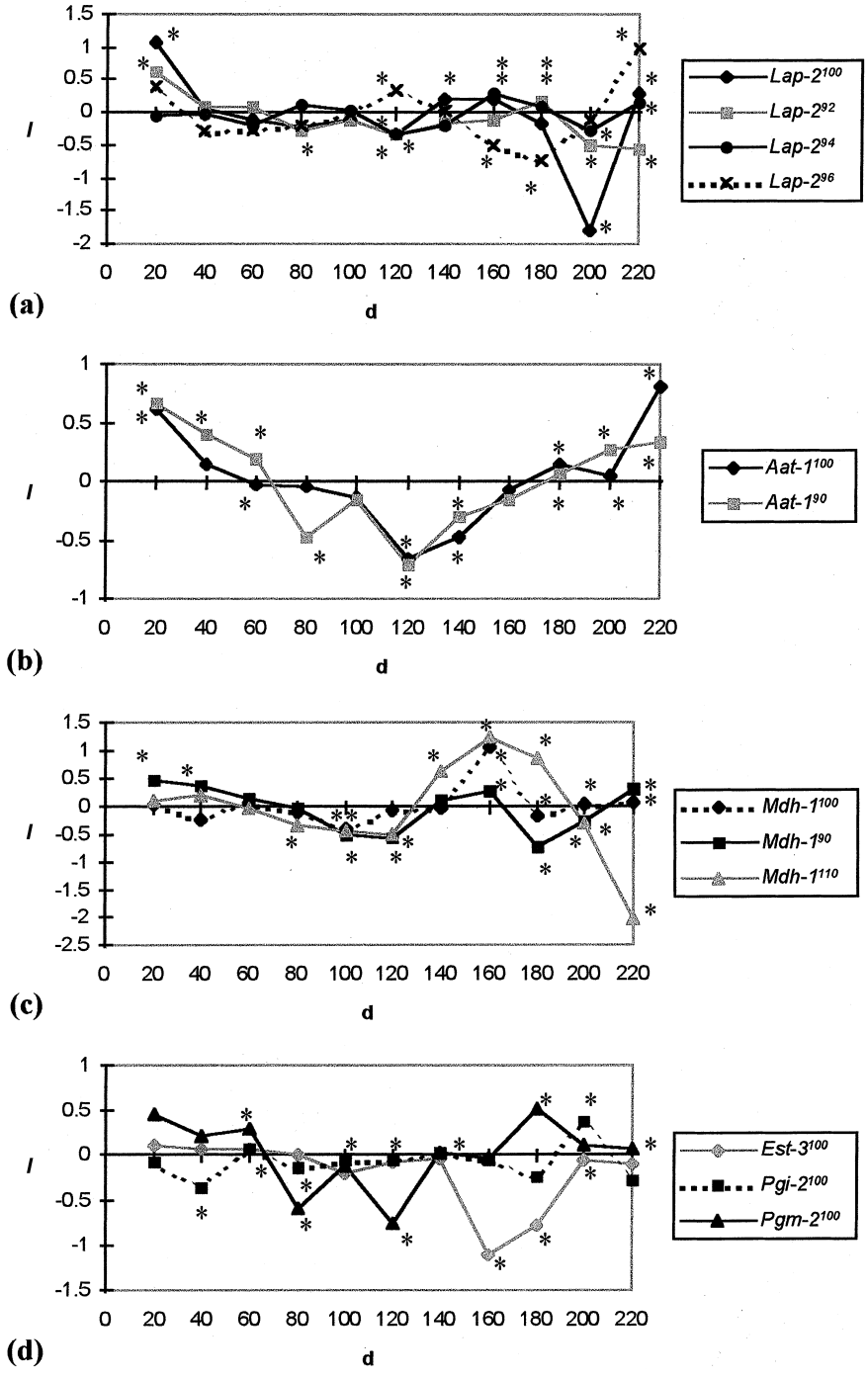

Fig. 4 Correlograms for microgeographical patterns of variation of allozyme frequencies for the 15 populations of *Helix aspersa*; (a) (b) (c) and (d) for loci *Lap-2*, *Aat-1*, *Mdh-1* and [*Est-3* + *Pgi-2* + *Pgm-2*], respectively. *I* is the Moran's index and *d* the geographical distance (m); significant individual autocorrelation coefficients ($P < 0.05$) are indicated by an asterisk.

juveniles which possibly came from the same family (intrademe Wahlund effect) could be additional factors explaining deviations from Hardy–Weinberg expectations. Furthermore, individuals tend to 'home' from their foraging expeditions to former resting sites, such a behavioural trait presumably facilitating the development of local genetic structure because of matings between related individuals.

Average F_{ST} (0.044; $P < 0.01$) revealed a moderate but consistent pattern of differentiation among colonies. Similarly, Selander & Kaufman (1975), Campbell & Dooley (1992) and Peakall & Beattie (1995) found only slight but not negligible differentiation of allele frequencies. Because of habitat fragmentation over our sampled area, the spatial arrangement of suitable patches presumably determined the genetic structure of demes,

as observed by Schilthuizen & Lombaerts (1994) and Pfenninger *et al*. (1996). Indeed, populations surrounded by an inhospitable environment (terraces overlaid with gravel or tar) are clearly isolated from neighbouring colonies. Possible subtle characteristics in the micro-environment resulting in habitat-specific variation (Johannesson & Tatarenkov, 1997), jointly with restricted dispersal and marked homing behaviour of snails, could be the main causes in determining genetic differentiation among the demes.

Overall spatial differentiation among demes

With the help of Mantel's statistics, Sokal *et al*. (1986, 1987), Pfenninger *et al*. (1996) and Arnaud *et al*. (1999) found that average genetic differentiation clearly reflected a significant relationship with geographical distance. More precisely, several studies have shown a decline of genetic similarity with increasing geographical distance as expected under an isolation-by-distance model (e.g. Campbell & Dooley, 1992). However, in the present work, values of *rz* and the Mantel correlogram failed to reveal any spatial structure, and consequently no genetic arrangement based on an isolation-by-distance model. This pattern is consistent with the lack of correlation observed between gene flow and geographical distance separating subpopulations (Fig. 2), suggesting that populations are not at equilibrium (with regard to the genetic structure) and would have recently invaded the studied area (Slatkin, 1993; Ruckelshaus, 1998). Moreover, occasional extinction of demes (human disturbances) and subsequent recolonization may constitute an important form of gene flow acting as a homogenizing force counteracting the differentiation of demes (Schilthuizen & Lombaerts, 1994; but see Ruckelshaus, 1998). In their study on *H. aspersa*, Selander & Kaufman (1975) implicated an isolation-by-distance model because the genetic identity between colonies was higher for the most neighbouring colonies. Schilthuizen & Lombaerts (1994) and Pfenninger *et al*. (1996) showed, in other land snails, that most of the geographical change in genetic composition can be essentially explained by dispersal-limited gene flow. As *H. aspersa* seems to be characterized by an ability to colonize successfully a large range of human-disturbed habitats, gene flow would not be the main determinant of genetic differentiation among subpopulations. Indeed, in our work, the 'real' distance was not related to the genetic differentiation of demes and the connectivity network seemed to take a more important role in shaping the spatial genetic structure, as proved by comparisons of genetic distances vs. Gabriel-connected graphs (*cg* and *cgr*) matrices. Moreover, Moran's *I* based on these networks of near-neighbours reflected a nonrandom distribution in space for some allozymes. This suggests a 'step-by-step' relationship pattern between nearest-neighbouring colonies (Sokal & Oden, 1978a), which would be relevant in land snails (Selander & Kaufman, 1975; Schilthuizen & Lombaerts, 1994; Arnaud *et al*., 1999).

Although the Gabriel-connected graph integrating the main road (*cgr*) does not show the clear pattern expected if roads acted as barriers on individual dispersal, the environmental heterogeneity leads to unusual patterns of differentiation among populations, as shown by some neighbouring colonies which display a peculiar allelic diversity (e.g. population 4).

Fine-scale spatial structure

Overall spatial structure, reflected in the Mantel correlogram profile, would be strongly affected by the nature and complexity of environmental heterogeneity because of numerous physical obstacles (houses, walls, roads). However, in spite of this 'noisy background', which has probably altered the genetic structure, correlograms based on allozyme frequencies showed that most loci were spatially structured. Thus, some allozymes underlie the role played by geographical distance in the differentiation of demes, i.e. a gradual isolation of colonies with increasing distances. Epperson & Clegg (1986), Campbell & Dooley (1992) and Shapcott (1995) showed that allele frequencies were spatially and positively autocorrelated at very short distances in plant species (< 5 m to < 20 m). For many alleles analysed in the present work, the distance at which correlograms cross the *X*-axis is between 50 and 80 m. This distance, called 'patch size' by Sokal & Wartenberg (1983), corresponds to the average size of a homogeneous area and could reflect parameter values of neighbourhood size and mating system (Sokal & Wartenberg, 1983; Epperson & Clegg, 1986; Epperson, 1990). However, in most cases, patch size cannot be equal to a panmictic unit because the *X*-intercept is relative to the total area of study and cannot easily be compared across studies involving unequal geographical distances or different connection weights (Sokal & Wartenberg, 1983). Nevertheless, the patch size found in our study seems to be quite similar to the mark–recapture results of Madec (1989), who defined the length of a panmictic unit as being equal to 70 m in *H. aspersa*.

Spatial analyses of allozyme frequencies have led to the recognition of several mechanisms to explain patterns of genetic variation, such as stochastic effects (Epperson & Clegg, 1986; Sokal *et al*., 1986; Arnaud *et al*., 1999) or restricted gene flow by limited dispersal (Peakall & Beattie, 1995; Shapcott, 1995). Selander &

Kaufman (1975) and Sokal & Oden (1978a) have proposed two different scenarios to explain the spatial arrangement observed among subpopulations in *H. aspersa*: founder effects and genetic drift on the one hand, and migratory events followed by diffusion on the other. In the present study, correlogram profiles related to alleles $Aat\text{-}1^{90}$, $Aat\text{-}1^{100}$ and $Pgm\text{-}1^{100}$ could be explained by a circular or symmetrical gradient where high positive autocorrelations occur in short- and long-distance classes (Sokal & Oden, 1978b). The larger distance classes must be interpreted with caution because (i) patches with considerable internal heterogeneity will not give clearly defined correlograms and (ii) Moran's *I* statistics are more efficient in cases where the spatial scale of sampling is within the context of a larger spatial pattern (Sokal & Oden, 1978a,b; Epperson & Li, 1996). However, we speculate that this gradient may involve a multidirectional invasion where colonies coming from the adjacent mixed woodland matrix would colonize the village from outside, founder effects and genetic drift accompanying the colonization because of human disturbances (mowing and weeding).

Differences across loci in their corresponding correlograms have been used to examine a possible influence of selective pressures at various hierarchical levels (Sokal *et al.*, 1987; Epperson, 1990; Slatkin & Arter, 1991; Campbell & Dooley, 1992; Sokal *et al.*, 1997). However, there is no observable microhabitat heterogeneity in our studied area to allow us to suppose any consistent small-scale selection (but see Johannesson & Tatarenkov, 1997).

With reference to hypotheses which can be made using spatial autocorrelation, Slatkin & Arter (1991) pointed out that several factors such as stochastic variation (genetic drift) and mutation blur the observed patterns and make it difficult to infer biological processes from such techniques. However, Epperson & Li (1996) and Sokal *et al.* (1997) demonstrated that spatial autocorrelation analysis led in many cases to useful inferences about biological scenarios, even if such statistical techniques cannot detect all the evolutionary forces. Nevertheless, the consistent fine-scale spatial structure detected in our study presumably simplified the real situation. The presence of groups of empty shells (extinct colonies) in the sampled location and the heterozygote deficiencies frequently found in genetic analyses of samples of *H. aspersa* (Guiller *et al.*, 1994, 1996) could be explained because of collecting in different demes of a metapopulation (the Wahlund effect). However, these hypotheses need to be tested by an adequate experimental design involving (i) a 'direct approach' based on mark–recapture studies and (ii) markers more appropriate to this level of geographical variation such as microsatellite loci, in order to investigate thoroughly the fine-scale genetic arrangement in populations of *H. aspersa*.

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