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Research article

Metapopulation genetic structure and migration pathways in the land snail *Helix aspersa*: influence of landscape heterogeneity

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Abstract

The spatial genetic structuring of the land snail *Helix aspersa* was investigated for 32 colonies within an intensive agricultural area, the polders of the Bay of Mont-Saint-Michel (France). Given the habitat patchiness and environmental instability, the setting of *H. aspersa* colonies meets the broader view of a metapopulation structure. The identification of extrinsic barriers to migration and their impact on the genetic distribution was addressed through the genotyping of 580 individuals using a combined set of enzyme and microsatellite loci. To evaluate the distance as well as the direction over which the spatial genetic arrangement occurs, two-dimensional spatial autocorrelation analyses, Mantel tests of association and multivariate Mantel correlograms were used. Different connectivity networks and geographical distances based on landscape features were constructed to evaluate the effect of environmental heterogeneity and to test the adequacy of an isolation by distance model on the distribution of the genetic variability. Genetic divergence was assessed using either classical IAM-based statistics, or SMM-based genetic distances specifically designed to accommodate the mutational processes thought to fit microsatellite evolution (IAM: Infinite Allele Model; SMM: Stepwise Mutation Model). Genetic distances based only on genetic drift yielded the most plausible biologically meaningful interpretation of the observed spatial structure. Applying a landscape-based geographical distance which postulates that migration arises along roadside verges, hedges or irrigation canal embankments gave a better fit to an isolation by distance model than did a simple Euclidean distance. The progressive decline of genetic similarity with physical distance appeared to be environmentally induced, leading to functional migration pathways.

Introduction

Many species are geographically structured into arrays of local populations interconnected with varying degrees of dispersal, leading to the so-called metapopulation concept (McCauley 1995; Harrison and Taylor 1997; Hanski 1999). The study of metapopulation structures and dynamics has become a central theme and a common framework in population ecology since natural populations can be often considered as a transient set of ephemeral populations whose average lifespan is much shorter than that of the whole network. Thus, the metapopulation persistence is generally viewed as the result of a balance between recurrent extinction and recolonisation events with high population turn-over (e.g., Ingvarsson et al. 1997). From a genetic point of view, the effects of population subdivision on the distribution of genetic variability have drawn the interest of evolutionary biologists. Indeed, metapopulation structure allows microevolu-

tionary processes to occur, which is not possible in large undivided and panmictic populations (McCauley 1995; Pannell and Charlesworth 2000). When assuming the selective neutrality of genetic markers under consideration, the observed patterns of genetic structure are primarily determined by the interaction of the opposing forces of gene flow and genetic drift. Hence, investigations of dispersal patterns have been greatly facilitated by the use of molecular markers because different ecological and evolutionary processes can leave distinct genetic signatures in current spatial structuring of neutral genetic distribution (Slatkin 1994; McCauley 1995; Bohonak 1999; Hutchison and Templeton 1999). Even at very fine geographical and temporal scales of observations, many studies of genetic differentiation have allowed greater understanding of close interactions among interconnected populations (e.g. Ingvarsson et al. 1997, Viard et al. 1997, Rowe et al. 2000, Arnaud et al. 2001).

A classical genetic pattern studied in population genetics is the well-known 'isolation by distance' model, a process by which geographically-restricted gene flow generates, under migration-drift equilibrium, a substantial increase of genetic divergence with geographical distances among populations (Wright 1942). However, few populations persist long enough to reach this equilibrium and many factors can obscure such a pattern of genetic structure (McCauley 1995; Hutchison and Templeton 1999). An ecological determinant rarely taken into account in population genetic structure analyses is the influence of environmental heterogeneity. Indeed, barriers to migration imposed by structural features of landscape are particularly important, and the identification of such barriers as well as the prediction of their impact in shaping the spatial arrangement of genetic diversity remain a major challenge in population ecology (Bohonak 1999; Sork et al. 1999; Castric et al. 2001). Although many studies have investigated the influence of geographical separation on the degree of inter-population connectivity, relatively few of them (e.g. Arter 1990; Keyghobadi et al. 1999; Rowe et al. 2000; Michels et al. 2001; Vos et al. 2001) explicitly address the relative influence of landscape textures on the spatial genetic variation. Patterns of dispersal between geographical locations is often viewed as a linear process, assuming that the dispersal area that connects two populations is a featureless environmental matrix of unsuitable habitat (Wiens 2001). However, pathways to gene flow through a mosaic of patches is not likely to be linear, and subtle differences in environmental characteristics can influence the patterns of genetic differentiation.

The present study focuses on population genetic structure in the simultaneously hermaphroditic garden snail *Helix aspersa* (Gastropoda, Helicidae) within a highly intensive agricultural area: the polders of the Bay of Mont-Saint-Michel (France). Land snails are particularly suitable organisms to investigate the effect of spatially-restricted dispersal on genetic structure as they live in aggregated discrete colonies connected by little ongoing gene flow owing to their low dispersal capabilities (e.g., Arter 1990; Baur 1993; Guiller et al. 1994; Bahl et al. 1996; Arnaud et al. 1999a, b; Davison and Clarke 2000). The polders area combines a mixture of farmlands, pastures and cultivated fields delimitated by a complex network of hedges and dikes, the last of which was built only sixty years ago (Arnaud 2000). Such a rural landscape is relevant for testing a metapopulation genetic structure involving ephemeral local colonies since agricultural practices are often the main cause of destruction of these patchily distributed colonies of *H. aspersa*. By combining a set of enzyme and microsatellite markers, the present study aims at investigating the influence of landscape constraints on spatial genetic connectivity among *H. aspersa* colonies. Classical indirect estimates of gene flow (*Nm*) were not computed because they rely on unrealistic assumptions that do not hold in natural populations (for reviews, see McCauley 1995; Pannell and Charlesworth 2000). Instead, two-dimensional spatial autocorrelation analyses, as well as Mantel tests of association and multivariate Mantel correlograms, were used to depict the spatial scale and the direction over which the apportionment of genetic variability is structured. By using different geographical descriptors of populations connectivity, we evaluated whether the spatial heterogeneity of the studied area may contribute to the genetic divergence through a classical isolation by distance process.

Materials and methods

Study area

The 'polders site' (Figure 1A), located in Brittany (48°36′ N; 1°32′ W, France), is an intensive agricultural area delimited by a complex network of dikes and hedges used as hospitable refuges by most animal species (Paillat and Butet 1996). Ninety percent of the polder area (for an area of $\sim$3050 ha) is under intensive agriculture. The first dike was created

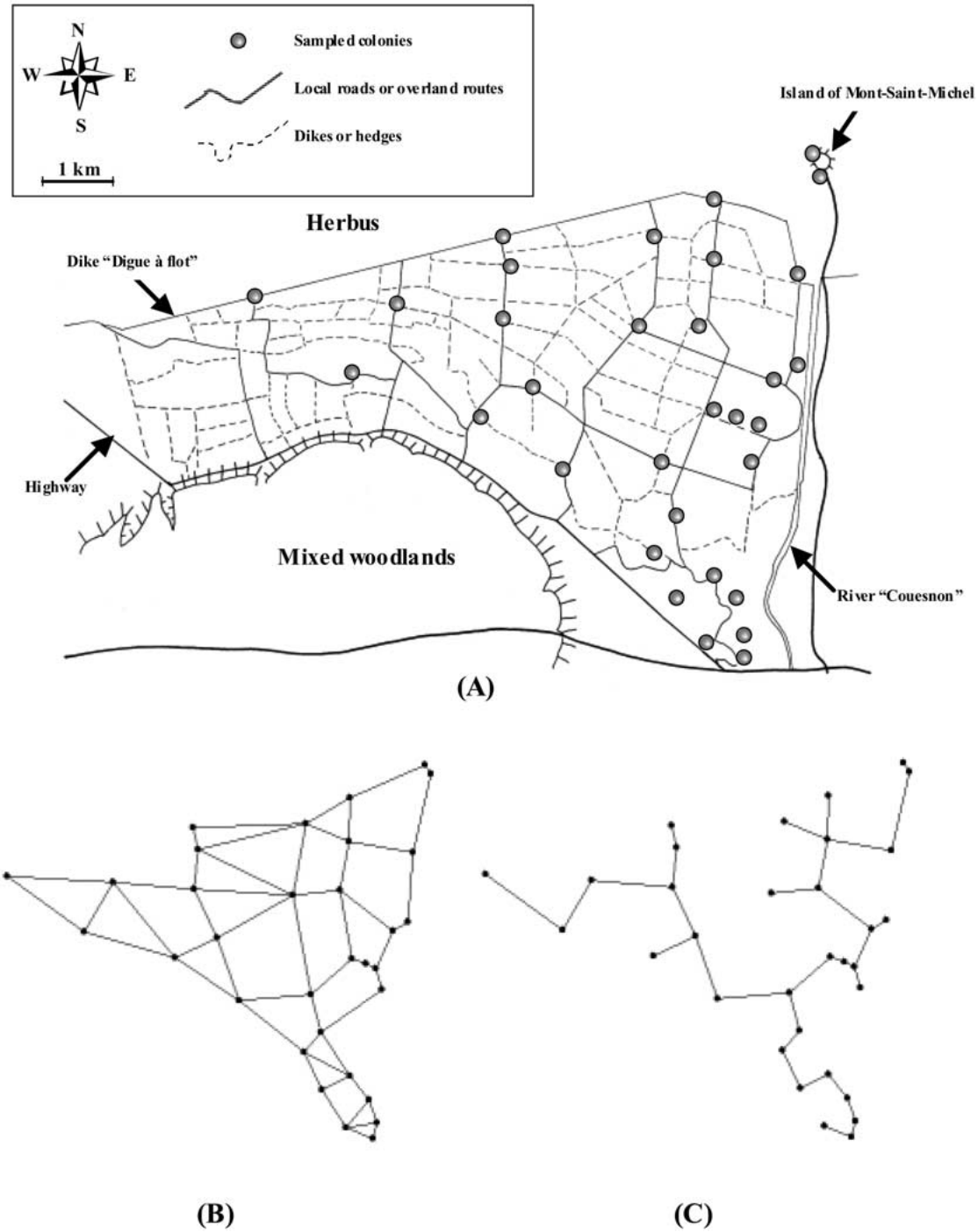

Figure 1. Map of sampled area and geographical location of 32 colonies of *Helix aspersa* (A); Gabriel-connected graph superimposed on colonies location (B); Minimum Spanning Tree-based graph superimposed on colonies location (C).

in 1054, the building of the polders started in 1851, and the last dike completed in 1933. The last dike ('Digue à Flot') protects the polders area against the sea. The 'Herbus' area is a salt meadow periodically covered by the sea. This recent man-made environment has been successfully colonised by *H. aspersa* despite herbicide and pesticide treatments, human predation and occasional burning (Madec et al. 2000; Arnaud 2000). Such a landscape, characterised by rapid anthropogenic changes and frequent local extinction events, is relevant to investigate the effect of habitat fragmentation on a metapopulation genetic structure. A total of 580 individuals was collected from 32 locations (Figure 1A). To minimise genetic artefacts due to substructuring within samples (i.e., Wahlund effect), individuals were collected over a very restricted surface (< 5 m^2).

Genetic data

A combination of four polymorphic enzyme loci (*Lap-2, Est-3, Aat-1* and *Mdh-1*) and five hypervariable microsatellite markers (*Ha2, Ha5, Ha10, Ha11* and *Ha13*) was used to examine the spatial distribution of genetic variability. Detailed procedures of enzymatic electrophoresis and microsatellite DNA genotyping are given in Guiller et al. (1994, 2000). Linkage disequilibrium, agreement of genotypic proportions with Hardy-Weinberg expectations and overall differentiation among colonies are treated elsewhere (Arnaud 2000).

Spatial analysis

Univariate analysis. Patterns of genetic variation were first investigated using spatial autocorrelation techniques. The degree of spatial autocorrelation is the dependence of the value of one geographically distributed variable (gene frequency in our study context) on the values of the same variable at other locations, and is usually quantified by Moran's I (Sokal and Oden 1978; Barbujani 1987). Detailed definitions and statistical properties of this autocorrelation coefficient can be found in Rosenberg (2000) (see also Arnaud et al. 1999b, 2001). To separate spatial trends of genetic variation in different compass directions, directional (two-dimensional) spatial correlograms were computed following the technique first introduced by Oden and Sokal (1986). Two-dimensional correlograms allow one to depict not only the scale over which spatial processes occur but also the direction in which they occur (e.g. Sokal et al. 1986, 1987; Sokal and Thomson 1998; Rosenberg et al. 1999; Rosenberg 2000). The significance of individual autocorrelation coefficients was determined by comparing their observed values against expected values of $-1/(n-1)$ under the null hypothesis of no spatial arrangement (Sokal and Oden 1978). Overall significance of each correlogram was assessed using a Bonferroni test as suggested by Oden (1984). All two-dimensional correlograms were constructed using the software PASSAGE (Rosenberg 2001). Moran's I were additionally estimated for strict nearest-neighbour pairs of colonies using a system of assigning weights w_{ij} based either on a Gabriel connected graph (Gabriel and Sokal 1969) or a Minimum-Spanning-Tree graph (Figures 1B and 1C). These calculations were carried out with AUTOCOR, a program written by A. Bellido (University of Rennes 1). In order to maintain statistical independence of tests, only the most common alleles were used for spatial analysis (e.g., Arnaud et al. 1999a, b 2001).

Multivariate analysis. Tests of association between genetic divergence and geographical variables were investigated using either 'overall' Mantel tests or unidirectional Mantel correlograms (Oden and Sokal 1986; Smouse et al. 1986, see also Sokal et al. 1986, 1987 and Arnaud et al. 1999a). The construction of Mantel correlograms has two advantages. First, it describes spatial pattern for more than one variable simultaneously (i.e., not only the frequency of one allele), and, as such, integrate *all* the genetic information. Second, it applies concepts of genetic distances to measure dissimilarities among populations (Vendramin et al. 1999). To construct a Mantel correlogram (multivariate analogue of standard correlograms), a normalised Mantel statistic (correlation coefficient *rz*, Smouse et al. 1986) is calculated for each of binary connection matrix against the genetic distance matrices to be analysed. Because pairwise elements of distance matrices are not independent, and thus violate the underlying assumptions associated with standard tests of significance, significance of the Mantel correlation was evaluated by a classical nonparametric Mantel test (10000 permutations). All multivariate Mantel correlograms were constructed using AUTOCOR.

As there is a limited number of sampling locations, and consequently a small number of distance-direction classes with reasonable sampling size, the overall spatial distribution of genetic variation was also investigated by testing for the direction of maximum

genetic change through the bearing procedure (see Sokal and Thomson 1998; Rosenberg 2000). A Mantel correlation was estimated for 36 weighted geographical distance matrices (defined by a strictly east-west reference line), the results being plotted as a function of the fixed bearing angle (degrees north of due east). This plot indicates in general terms the direction of greatest overall genetics-geography correlation.

Geographical distances and connectivity networks

To examine the connectivity among sampled colonies and to test whether isolation by distance can explain the apportionment of genetic variation, relationships between genetic divergence and physical isolation of colonies were further investigated using different measures of geographical distance. Commonly used in most molecular ecology studies, the first one was simply the *linear geographical distance* measured 'as the crow flies', which is straight-line distances between any pair of locations (as used for univariate spatial autocorrelation analyses described above). The underlying assumption is that snails disperse uniformly in any direction, regardless of the spatial heterogeneity of sampled area. However, given the reality of habitat patchiness, this criterion presumably lacks of realism. The second geographical distance used was a distance accounting for possible barriers of dispersal: cultivated fields as well as irrigation canals were considered as insurmountable obstacles for snails. Roads were not taken into account because microgeographical patterns of genetic variation between opposite road populations suggested no barrier effects on individual dispersal (Arnaud et al. 1999b, 2001). Hence, geographical separation of colonies was estimated by measuring the minimal distance connecting two localities following the borders of hedges and roads, as well as irrigation canal embankments that constitute stretches of hospitable environment for snail dispersal. In such a case, the underlying assumption is that snails move preferentially along environmentally-induced pathways possibly implicated in the genetic structuring. Consequently, two colonies may be geometrically located nearer than others, but their connecting paths through this modified distance can be longer and maybe more correlated with genetic divergence (e.g., Arter 1990; Michels et al. 2001; Vos et al. 2001). This distance will be called '*landscaped distance*' throughout this study.

Furthermore, to investigate possible genetic connectivity among sampled colonies, two connectivity descriptors were used: (i) a *Gabriel-connected graph* where two localities A and B are considered as connected if no other locality C lies on or within the circle of diameter AB (Gabriel and Sokal 1969), the underlying assumption being that colonies connectivity follow a step-by-step relationship between immediate neighbours (see Figure 1B); (ii) a *Minimum-Spanning-Tree* (MST) network (Figure 1C) where colonies are connected according to the minimum path necessary to join all the sampled localities, i.e., similar to a stepping-stone model of spatial relationship. Using AUTOCOR, binary connectivity matrices (called also adjacency matrices) were then constructed by connecting localities that are considered to be nearest neighbours through Gabriel or MST-based connectivity graphs (e.g., Sokal 1979; Madec et al. 1996; Arnaud et al. 1999a, b). In addition, both straight-line geographical distances and landscaped distances defined above were also recalculated with AUTOCOR following the minimal distance necessary to connect any pair of locality along the Gabriel and MST networks.

Choice of genetic distances

As there is currently no clear consensus about the relative performance of existing genetic distances using microsatellite markers (e.g., Takezaki and Nei 1996; Goodman 1997; Lugon-Moulin et al. 1999; Rowe et al. 2000; Rousset 2001), classical measures of population differentiation or divergence (initially developed for allozymic data and based on the infinite allele model, IAM) were used, as well as new alternative methods accounting for the mutational processes thought to fit the microsatellite evolution, i.e., the so-called stepwise mutation model, SMM (reviewed in Estoup and Cornuet 1999). As measurements of genetic differentiation, classical Wright's F-Statistics (F_{ST}) were estimated following the Weir and Cockerham (1984) ANOVA procedure using an updated version of FSTAT (Goudet 1995). An unbiased derivative of F_{ST}, based on variance in allele size and called ρ_{ST}, was also calculated for microsatellite loci with RSTCALC 2.2 (Goodman 1997). To assess the genetic divergence among colonies, the chord distance DCE (Cavalli-Sforza and Edwards 1967) and the Rogers' (1972) distance DR were estimated using POPULATIONS version 1.2.19, a program written by O. Langella and available at http://www.cnrs-gif.fr/pge/bioinfo. Such distances make no assumptions regarding different mutation rates among loci

and assume that genetic drift is the most likely evolutionary factor influencing population divergence (Takezaki and Nei 1996). Specifically developed for microsatellite data, the DSW distance (Shriver et al. 1995) and the DZ distance (Zhivotovsky 1999) were also calculated using POPULATIONS. DSW is a derivative of Nei's standard genetic distance, relying on both allele frequencies and difference in allele size, whereas DZ is a pure SMM-based distance based on the between-locus correlation in the mean repeat scores and accounting for constraints in allele size (for detailed comments on microsatellites evolution, see Estoup and Cornuet 1999; Schlötterer 2000).

Results

Spatial statistics (univariate approach) based on pairwise straight-line geographical distances, weighted or not by connectivity networks

Results of the two-dimensional spatial autocorrelation analysis are presented on Figures 2 and 3. Out of 352 individual autocorrelation coefficients, 70 (19.8%) were significant at $P < 0.05$. After Bonferroni correction, more than half (59.1%) of the 22 allele frequency surfaces yielded overall significant ($P < 0.05$) windrose correlograms (Figures 2 and 3). Although there was substantial genetic heterogeneity over the studied area, this was not related with obvious spatial arrangements inferred by spatial autocorrelation. Whereas no substantial patterns were depicted for some alleles (e.g., *Lap-2*^{100}, *Lap-2*^{90}, *Aat-1*^{100}, *Ha2*^{304}, *Ha2*^{306}, *Ha5*^{191} or *Ha11*^{231}), some others displayed a patchy genetic distribution (e.g., *Mdh-1*^{100}, *Mdh-1*^{90}, *Ha2*^{308}, *Ha13*^{218}) with sectors characterised by high positive Moran's *I* independently of physical distances between pairwise localities. In general, positive and significant autocorrelation occurred for the first distance-direction class, indicating a clumping of alleles for at least the shortest scales (e.g., *Mdh-1*^{100}, *Est-3*^{95}, *Ha5*^{187}, *Ha5*^{195}, *Ha11*^{229}, *Ha13*^{170} or *Ha13*^{188}) with apparent random fluctuation of Moran's *I* values at longer distances. In summary, excepted for alleles *Est-3*^{95}, *Ha10*^{233}, *Ha10*^{235}, and to a lesser extent for alleles *Est-3*^{100} and *Ha13*^{218}, which showed a southeast-northwest gradient pattern, most of allelic distributions seemed to reflect heterogeneous patches without any clear spatial trends.

Global Moran's *I* values based either on a Gabriel-connected network or a MST-based network are visualised in Table 1. Although not always significant,

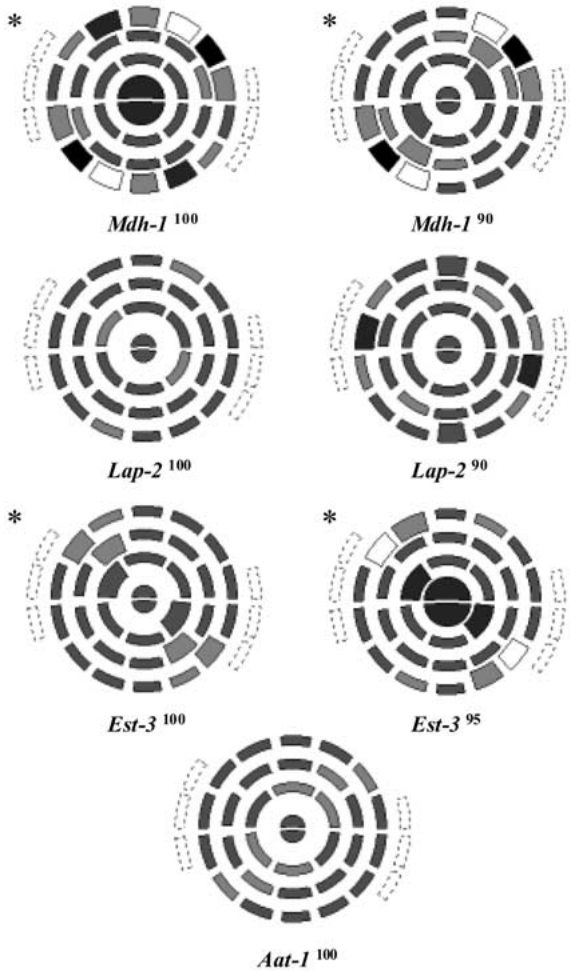

Figure 2. Two-dimensional (windrose) correlograms for the most common alleles of 4 enzyme markers in the land snail *Helix aspersa*. The distance-direction classes are arranged as sectors of circular annuli, each defining a distance class by taking into account both the distance and compass direction between colony samples. Upper limits of distance class annuli: 1000, 2600, 5200, 8800 meters. Shading represents approximate quintiles of the overall distribution of autocorrelation coefficients. Upper limits of *I* values: white: −0.699; pale grey: −0.186; medium grey: 0.325; dark grey: 0.837; black: 1.349. Sectors with dashed outlines represent distance-direction classes with insufficient pairwise replicates to report consistent autocorrelation for them. Statistically significant sectors ($P < 0.05$) are shown in full whereas non-significant ones are drawn at half-width. An asterisk denotes an overall significant windrose correlogram ($P < 0.05$) following the Bonferroni procedure.

Moran's *I* values were generally positive for both networks, indicating an overall trend of genetic affinities arising according to a 'nearest-neighbours' relationship. Only the locus *Aat-1* showed substantial (although non significant) negative autocorrelation coefficients, indicating either a random distribution of

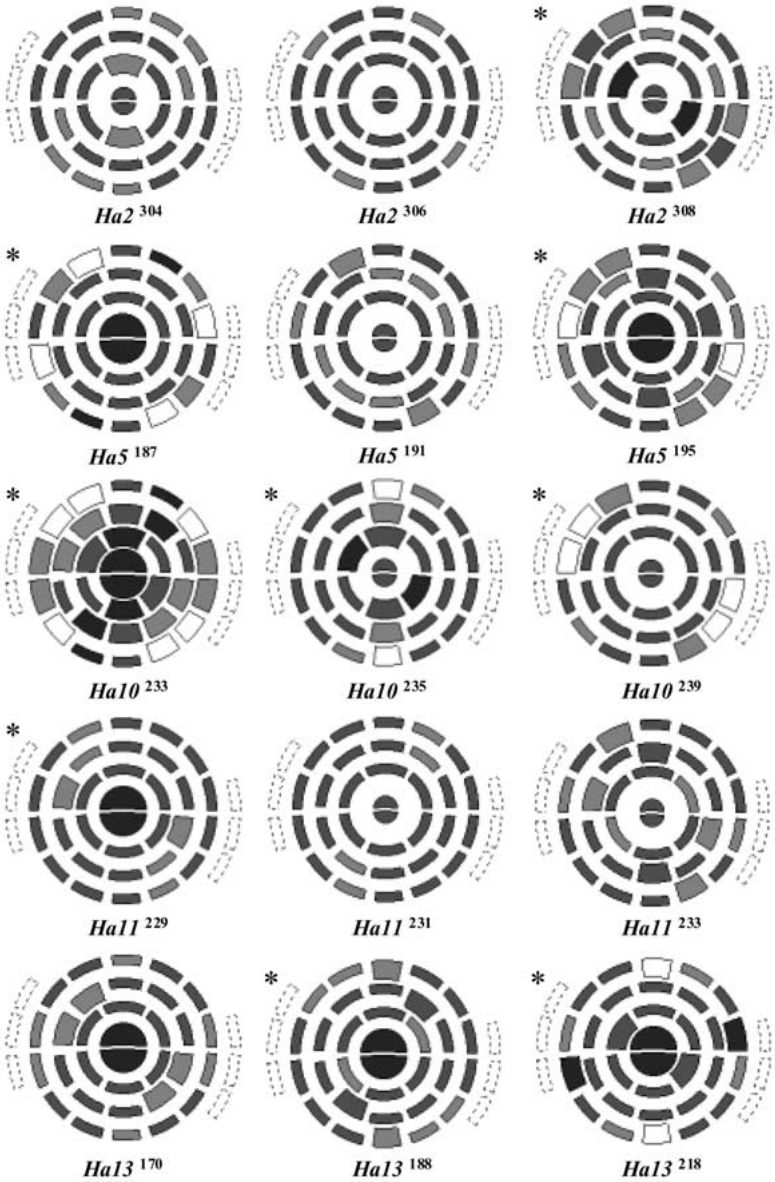

Figure 3. Two-dimensional (windrose) correlograms for the most common alleles of 5 microsatellite loci in the land snail *Helix aspersa* (see Figure 2 for symbolic conventions and legends).

allelic frequencies or an inadequacy of both networks used.

Global Mantel correlation and multivariate Mantel correlograms: comparison of straight-line and landscaped distances combined with the integration of connectivity-networks

The plot of genetic-geography correlation versus the compass direction (bearing procedure) is illustrated in Figure 4. Although the amplitude of correlation variation was very limited, the pattern of matrix associations suggested the direction of greatest correlation to be at about 40° (northeast to southwest) whereas the least correlation occurred at about 140°, indicating a slight southeast-northwest gradient. This agrees with trends previously depicted from windrose correlograms of alleles *Est-3*^{95} or *Ha10*^{233}. Figure 4 is based on the genetic distance DCE (Cavalli-Sforza and Edwards 1967) and very similar results are obtained

Table 1. Moran's I values for 32 colonies of *Helix aspersa* based either on a Gabriel-connected graph or a Minimum-Spanning-Tree (MST) graph. Only the most common alleles of enzyme and microsatellite loci are analysed. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS: not significant.

Markers	Alleles	Gabriel network		MST network	
Allozymes					
	Lap-2^{100}	0.06	NS	0.12	NS
	Lap-2^{94}	0.24	NS	0.17	NS
	Est-3^{100}	0.18	NS	0.17	NS
	Est-3^{95}	0.36	**	0.26	NS
	Mdh-1^{100}	0.31	**	0.45	**
	Mdh-1^{90}	0.32	**	0.32	*
	Aat-1^{100}	−0.14	NS	−0.12	NS
Microsatellites					
	Ha2^{304}	0.06	NS	−0.08	NS
	Ha2^{306}	0.07	NS	−0.18	NS
	Ha2^{308}	0.21	NS	0.08	NS
	Ha5^{187}	0.31	**	0.31	*
	Ha5^{191}	0.06	NS	0.04	NS
	Ha5^{195}	0.35	**	0.48	**
	Ha10^{233}	0.43	***	0.55	***
	Ha10^{235}	0.22	NS	0.25	NS
	Ha10^{239}	0.15	NS	0.37	**
	Ha11^{229}	0.28	*	0.21	*
	Ha11^{231}	−0.06	NS	0.13	NS
	Ha11^{233}	0.14	NS	−0.06	NS
	Ha13^{170}	0.19	NS	0.29	NS
	Ha13^{188}	0.18	NS	0.21	NS
	Ha13^{218}	0.26	*	0.26	NS

using pairwise estimates of genetic differentiation (F_{ST}).

Results of global Mantel correlation for combination of different estimates of genetic divergence and geographical descriptors are presented in Table 2. DCE performed better than other estimates of genetic divergence in term of strongest correlation with modified or unmodified geographical distances. Whatever the genetic distance employed, the use of landscaped distance always yielded a better fit to an isolation by distance model compared to the simple linear geographical distance with, for instance, an overall correlation of 0.49 ($P < 0.001$) using the DCE distance (see Table 2). The same trend was observed when linear and landscaped geographical distances were recalculated through the Gabriel and MST-connected graphs. Note that the use of connectivity networks in the calculation of linear and landscaped geographical distances did not really improve Mantel correlation, giving sometimes reduced values of matrix correlation (e.g., landscaped distance vs. MST-landscaped distances). Nonetheless, the simple consideration of population connectivity using binary matrices gave significantly positive values of matrix associations, slightly higher for the Gabriel criterion (Table 2). SMM-based genetic distances specifically designated for microsatellite data (i.e., ρ_{ST}, DSW and DZ, see Table 2) followed the same trends described above. However, values of rz were always lower than for classical IAM-based distances, except for DSW which is a compromise between classical and SMM-based distances as it accounts for both allele frequencies and variance in allele length. Although giving overall significantly positive correlation between extents of genetic divergence and geographical descriptors, SMM-based genetic distances did not translate into ac-

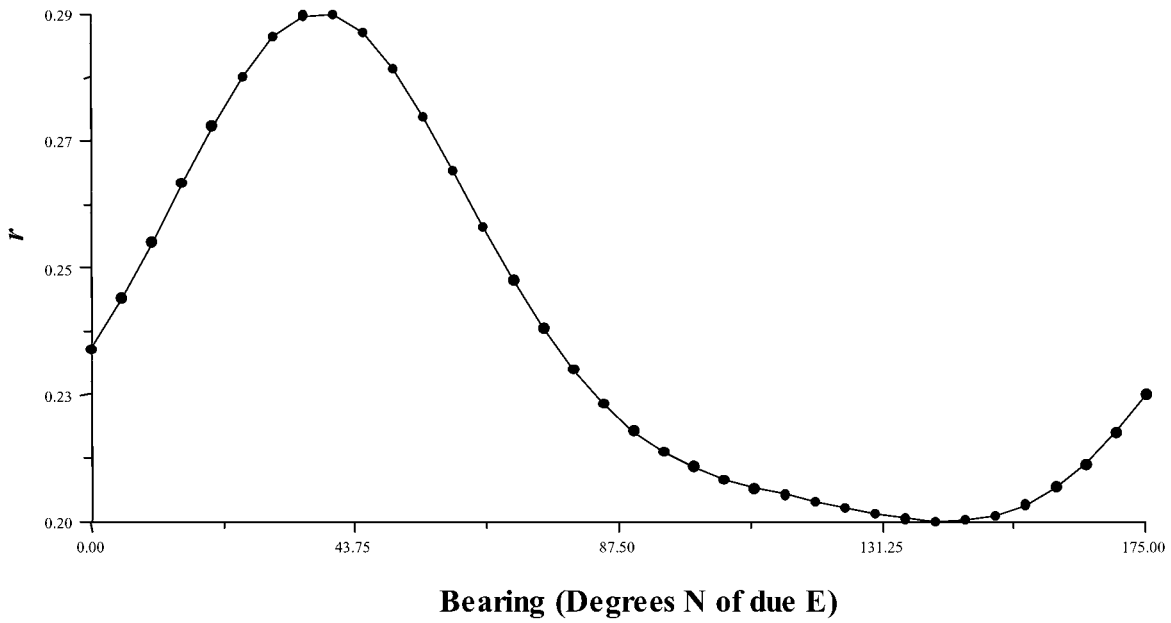

Figure 4. Bearing plot of genetic distance (DCE) among colonies of *Helix aspersa* as a periodic function of the correlation between pairwise genetic divergence and geographical distance against compass direction. Bearing: degrees north of due east; *r*: Mantel correlation coefficients. The direction of highest correlation of genetic divergence with geography occurs at approximately 40° north of east, i.e. a northeast-southwest trend.

curate and realistic population trees as did more classical distances like F_{ST}, DCE, or DR which rely only on genetic drift as dominant evolutionary process (dendrograms not shown). Generally, using microsatellite data alone yielded highly significant correlation and the incorporation of allozyme data did not really provide additional information, as shown by the quite similar *rz* values when both data are combined (Table 2).

Multivariate Mantel correlograms based either on the linear or landscaped geographical distance are illustrated in Figure 5. Generally, all correlograms displayed a progressive decrease of genetic similarities with increasing geographical distances. However, stochastic fluctuations were more pronounced when linear geographical distance was used (Figure 5a), whereas the correlogram shape based on the landscaped distance (Figure 5d) was suggestive of a pure genetic isolation by distance pattern, i.e., with the characteristic form of appreciable positive short-distance correlations followed by fast decline in *rz* values, generally coupled with non significant long-distance correlations (see Barbujani 1987; Sokal and Thomson 1998). Curiously, a strongly similar correlogram shape was depicted using the linear geographical distance calculated through the MST network (Figure 5c). Altogether, such results may confirm the influences, not mutually exclusive, of both connectivity paths and geographical isolation of colonies in the spatial genetic structuring. It is worth noting that, excepted for Figure 5c, the superimposition of Gabriel or MST networks to either linear or landscaped geographical distances did not really lead to a better vision of a continuous decrease of genetic affinities with physical colony isolation (see Figure 5b,e,d). All results were based on the F_{ST} estimates from both information of allozyme and microsatellite data. Separating the two categories of nuclear markers yielded similar results for microsatellites, by contrast to allozyme-based correlograms which seemed more affected by random fluctuations. Furthermore, very similar Mantel correlograms were found when employing related genetic distances like DCE or DR, whereas SMM-based distances did not improve the correlograms resolution whose shapes illustrated erratic fluctuations of *rz* values (correlograms not presented).

Discussion

Owing to habitat fragmentation and anthropogenic disturbances, local extinction and recolonisation processes are ubiquitous phenomena in natural popu-

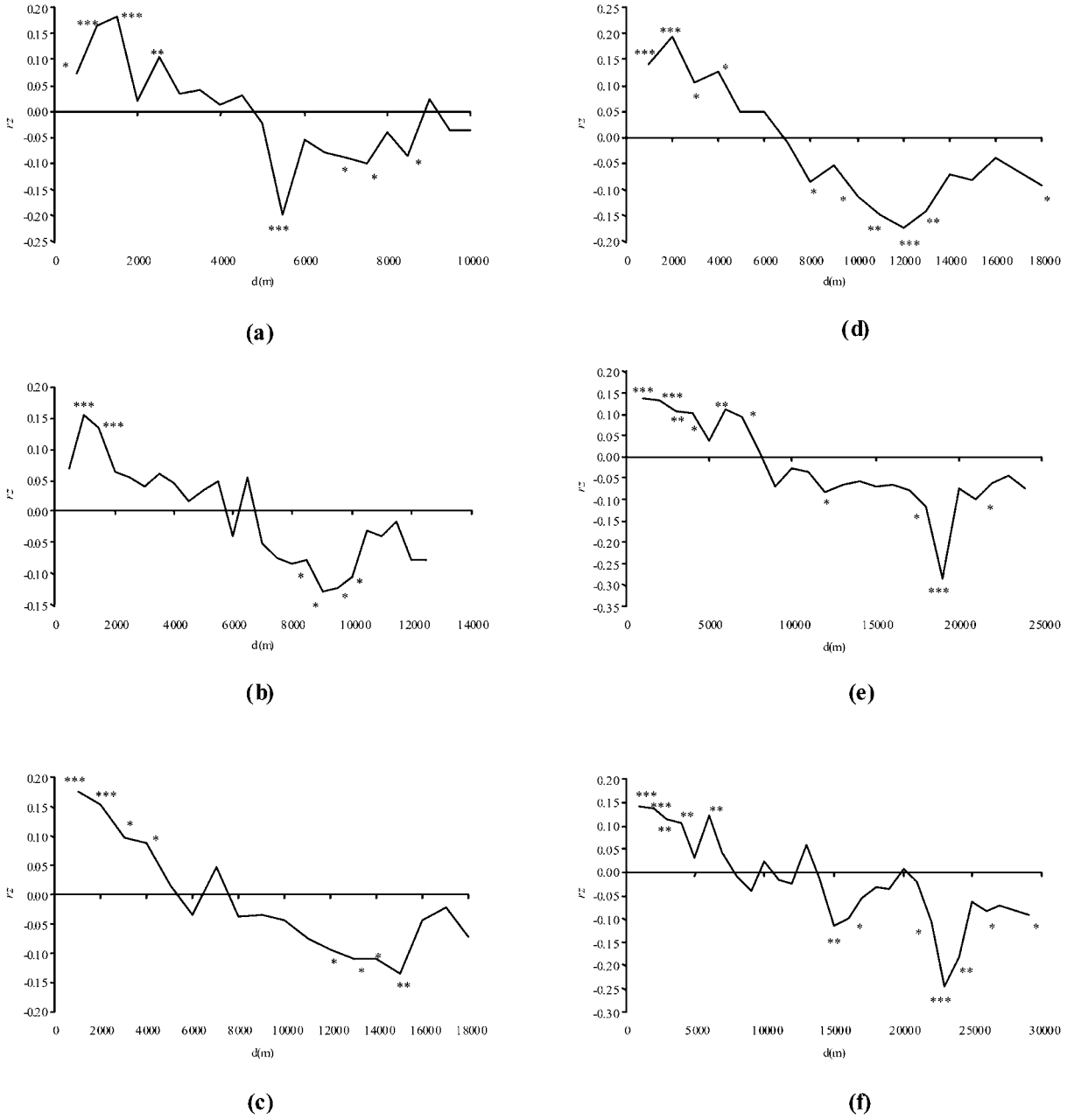

Figure 5. Mantel correlograms depicting the relationships between estimates of genetic divergence and geographical descriptors among 32 colonies of the land snail *Helix aspersa*. The genetic distance matrix is based on pairwise estimates of genetic differentiation (F_{ST}) using both information of allozyme and microsatellite loci. Binary matrices describing the geographical relationships among colonies are based on (a) the pairwise geographical distance measured as a straight-line, (b) the pairwise geographical distance measured as a straight-line following a Gabriel-connected graph, (c) the pairwise geographical distance measured as a straight-line following a Minimum Spanning Tree-based graph, (d) the pairwise landscaped distance (accounting for environmental heterogeneity, see text for explanations), (e) the pairwise landscaped distance but following a Gabriel-connected graph, and (f) the pairwise landscaped distance following a Minimum Spanning Tree-based graph. *rz*: normalised Mantel statistic; d(m): distance in meters; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ (Mantel test with 10000 permutations).

Table 2. Overall correlations (*rz*, Smouse et al. 1986) of genetic and geographical descriptors within the study area for 32 colonies of *Helix aspersa*. Estimates of genetic divergence or differentiation used are the classical *F*-Statistics (F_{ST}) following Weir and Cockerham (1984), the chord distance (DCE) of Cavalli-Sforza and Edwards (1967), the distance DR of Rogers (1972). SMM-based distances accounting for variance in allele sizes, specifically developed for microsatellite loci, are additionally used: the differentiation index ρ_{ST} (Goodman 1997), the DSW distance (Shriver et al. 1995) and the DZ distance (Zhivotovsky 1999). Geographical descriptors of colony relationships are based on (i) the linear distance measured as the straight line between each colony, superimposed or not to a Gabriel or MST-connected graph, (ii) a landscaped distance (accounting for spatial features of environmental heterogeneity) superimposed or not to a Gabriel or a MST-based graph, and (iii) binary matrices connecting the colonies according to the Gabriel or MST criterion (see text for further explanations). Correlations are based on both microsatellites and allozymes (upper number) and on either allozymes/microsatellites (lower number). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; NS: not significant (Mantel test with 10000 permutations).

Descriptor of geographical relationships among colonies	Genetic distances					
	F_{ST}	DCE	DR	ρ_{ST}	DSW	DZ
Linear geographical distance	0.32^{***} (0.15^{NS}/0.31^{***})	0.43^{***} (0.23^{**}/0.41^{***})	0.34^{***} (0.15^{NS}/0.35^{***})	0.23^{**}	0.30^{**}	0.20^{*}
Gabriel-connected linear geographical distance	0.34^{***} (0.13^{NS}/0.34^{***})	0.44^{***} (0.24^{**}/0.43^{***})	0.36^{***} (0.14^{*}/0.38^{***})	0.24^{**}	0.35^{***}	0.24^{*}
MST-connected linear geographical distance	0.35^{***} (0.16^{NS}/0.34^{***})	0.43^{***} (0.25^{**}/0.40^{***})	0.35^{***} (0.13^{NS}/0.36^{***})	0.21^{**}	0.34^{***}	0.22^{*}
Landscaped geographical distance	0.43^{***} (0.27^{**}/0.36^{***})	0.49^{***} (0.32^{***}/0.43^{***})	0.44^{***} (0.24^{*}/0.39^{***})	0.27^{**}	0.43^{***}	0.31^{**}
Gabriel-landscaped geographical distance	0.43^{***} (0.33^{**}/0.33^{***})	0.50^{***} (0.36^{***}/0.41^{***})	0.45^{***} (0.29^{**}/0.36^{***})	0.23^{*}	0.48^{***}	0.39^{**}
MST-landscaped geographical distance	0.40^{***} (0.28^{**}/0.32^{***})	0.46^{***} (0.32^{**}/0.39^{***})	0.41^{***} (0.24^{*}/0.35^{***})	0.20^{*}	0.43^{***}	0.33^{**}
Binary connectivity according to the Gabriel network	0.21^{***} (0.10^{**}/0.20^{***})	0.30^{***} (0.13^{***}/0.31^{***})	0.24^{***} (0.10^{**}/0.24^{***})	0.13^{***}	0.16^{***}	0.09^{**}
Binary connectivity according to the MST network	0.17^{***} (0.06^{*}/0.18^{***})	0.25^{***} (0.10^{**}/0.27^{***})	0.19^{***} (0.06^{*}/0.22^{***})	0.11^{***}	0.13^{***}	0.06^{*}

lations (Bahl et al. 1996; Harrison and Taylor 1997; Ingvarsson et al. 1997; Hanski 1999). However, it remains often unclear under what circumstances various patterns of genetic variation are involved with metapopulation structures in the wild (e.g., Rowe et al. 2000). In a landscape-based view on metapopulations, the dispersal pathways among populations and the probability that dispersing individuals successfully reach suitable patches are affected by the spatial arrangement and the degree of connectivity among habitat patches through the surrounding landscape (Michels et al. 2001; Vos et al. 2001; Wiens et al. 2001). However, identifying a relationship between landscape features and the distribution of genetic variation is generally not taken into account in the search of genetic isolation by distance (Keyghobadi et al. 1999; Castric et al. 2001).

The present study first reveals that the lack of two-dimensional spatial autocorrelation for some loci (e.g., *Aat-1, Lap-2, Ha2*) cannot preclude a strong genetic drift coupled with a low amount of gene flow among sampled colonies. Despite a significant patchy genetic structuring (e.g., alleles $Mdh\text{-}1^{100}$ or $Ha13^{218}$), a slight spatial trend along a northwest-southeast direction was nevertheless observed using either single-allele spatial autocorrelation and bearing procedure (Figures 3 and 4). Such a spatial genetic arrangement may be suggestive of a directional stepwise range expansion of individual colonists coming from the adjacent mixed woodlands. However, the clear establishment of this hypothetical population spread genetic signature is possibly confounded by concomitant multiple human-introduction related to occasional snail farming (polders' farmers, pers.

com.). Given the overall patchily distributed genetic variation, I could conclude that there is no isolation by distance within the studied area, except for surfaces with positive and significant autocorrelation coefficients in the first distance class (e.g., alleles $Ha13^{170}$, $Ha13^{188}$, $Ha5^{187}$). Significant Moran's I at short distances may reflect effective short-range gene flow between nearby colonies (Sokal et al. 1986, 1987; Barbujani 1987; Sokal and Thomson 1998). This apparent lack of long-distance structuring could partly reflect departures from a mutation-drift equilibrium state at broader spatial scale, as increasingly documented in various taxa (e.g., Slatkin 1994; McCauley 1995; Bohonak 1999, Hutchison and Templeton 1999; Keyghobadi et al. 1999; Castric et al. 2001). However, two-dimensional spatial analysis was based on simple straight-line distances calculated from spatial coordinates of sampling locations, and consequently did not account for potential dispersal barriers regarding the role of historical and contemporary landscape components.

In that respect, the spatial genetic structure measured by connectivity networks and different paths between collected sites gave some interesting results. Highest correlation occurred between IAM-based genetic distances and the landscaped distance. Mantel correlation against binary connectivity matrices based on Gabriel and MST graphs lead essentially to a test of low order spatial autocorrelation (see Sokal 1979). When dispersal pathways among colonies were restricted to the Gabriel network, positive and significant correlations occurred, a pattern suggestive of a genetic connectivity among nearest-neighbours. By integrating all the genetic information with multivariate analyses (multilocus analyses), a step-by-step pattern of migration was also reflected by the Mantel correlogram shape based on the MST network. Hence, when geographical distances other than the simple Euclidean distance were considered, Mantel correlograms generally displayed a more pronounced decrease of genetic similarities among colonies compared to the univariate analysis. This suggests an isolation by distance along gene flow paths determined by the spatial configuration of suitable habitat for snail dispersal, i.e., along canal embankments, roadside verges and hedges. These guidelines could then act as 'corridors' connecting any isolated colonies in a functional network. In another Helicid, *Arianta arbustorum*, Arter (1990) also clearly demonstrated that the genetic distribution of isozyme variation was best explained by a functional isolation by distance model over a drainage system with streams as main gene flow pathways. Although the role of corridors in connecting isolated habitat patches has attracted the attention of conservation biologists, it should be noted that their utility appears quite controversial in land snails (Baur 1993). Simulations and studies of daily movements established that the dispersal behaviour of land snails is likely to result in shorter distances of dispersal in linear habitats than in two-dimensional habitats (Baur and Baur 1992, 1993). Nevertheless, linear hospitable habitats like roadsides ditches or hedges may impose a directionality in genetic exchanges between local colonies of *H. aspersa*. Thus, such habitats allow a moderate but effective genetic connectivity as shown by spatial autocorrelation as well as by Mantel correlation based on nearest-neighbours networks. Such a hypothesis is reinforced by trees of relationships that also suggest a neighbourhood diffusion along roadside verges acting as gene flow pathways (Arnaud 2000).

Microsatellite loci have become the markers of choice in ecological genetic studies and many developments have been made to improve statistical analyses using such markers (see Estoup and Cornuet 1999). However, a word of caution is needed concerning the appropriate estimator of population differentiation for studying a metapopulation genetic structure (e.g., Viard et al. 1997; Lugon-Moulin et al. 1999; Rowe et al. 2000). In the present work, classical IAM-based measures of genetic distances like F_{ST}, DCE or DR performed better than do SMM-based distances when looking for isolation by distance (this study), as well as in depicting genetic affinities of colonies with trees of relationships (Arnaud, unpublished results). Firstly, this suggests that genetic divergence among the studied colonies was more likely to be generated by genetic drift rather than mutation processes (Slatkin 1995; Goodman 1997; Rousset 2001). Secondly, evidence is accumulating that sequences changes observed at microsatellite loci do not always follow a strict symmetrical, stepwise mutation model. Instead, it involves more complex mutation events like large insertions/deletions, a dependence of mutation rates on the structure of motifs repeated in tandem, allele size constraints or biased mutation towards microsatellite expansion (reviewed by Schlötterer 2000). Such departures from a simple SMM are likely to dramatically increase the variance of microsatellite-based statistics specifically developed for estimating population divergence (Rousset 2001). This could explain the significant but trivial genetic/geography correlations observed in this study, that do not translate into bi-

ologically meaningful population trees in *H. aspersa* (Arnaud 2000). However that may be concerning the choice of genetic distances, microsatellite markers yielded a more informative resolution than did enzyme loci which were presumably more affected by stochastic variations at the present scale of observation (see Table 2).

In many cases, recent human-caused habitat fragmentation can confound the interpretation of observed population genetic structure because current spatial genetic distribution may not reflect current levels of gene flow (Slatkin 1994; McCauley 1995; Vos et al. 2001). However, in the present study, genetic drift presumably acts enough to result in significant isolation by distance even in a landscape recently altered by human. In such a system, the Euclidean distance may not be the appropriate measure for interpopulation connectivity. The simple assumption of linear connections between pairwise populations, like in an island model, does not hold in such a system and has to be distinguished from a genetic isolation by distance arising, at least partially, along a landscape-based connectivity network. Correlation between landscape/connectivity variables and genetic divergence largely reflect a substantial effect of environmental heterogeneity on snails dispersal, and suggest a confounding effect of landscape features when studying a metapopulation genetic structure in highly fragmented habitats.

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