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Geographical patterns of gene frequencies in Italian populations of *Ornithogalum montanum* (Liliaceae)

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Summary

Geographic variation was studied at 15 electrophoretic loci (40 alleles) in Italian populations of *Ornithogalum montanum* Cyr. ex Ten. (Liliaceae). Homogeneity of allele frequencies was assessed by *G* tests; gene-frequency patterns were described by spatial autocorrelation statistics; matrices of genetic and environmental distance were compared through a series of Mantel's tests, and the zones of highest overall gene-frequency change per unit distance (steep multi-locus clines, or genetic boundaries) were identified. Nineteen allele frequencies appear heterogeneously distributed, but only 3 of them show significant spatial structure. Only 2 allele frequencies are correlated with 1 environmental parameter. Large genetic differences are observed between spatially close populations. These findings support a model of differentiation in which the genetic relationships between isolates do not depend on their spatial distances, but reflect mainly population subdivision and restricted gene flow.

1. Introduction

Inferences about the mechanisms maintaining genetic polymorphism in populations can be made on the basis of spatial patterns of gene frequencies (Gould & Johnston, 1972; Felsenstein, 1982; Endler, 1982, 1986; Manly, 1985). Spatial autocorrelation measures and similar indices have been widely used to summarize gene-frequency distributions. In addition, these statistics allow testing of hypotheses on the causes of genetic divergence of populations when the geographical patterns determined by evolutionary pressures and demographic processes can be predicted (Douglas & Endler, 1982; Manly, 1986; Barbujani, 1987; Epperson, 1990).

In a previous work (Barbujani & Pigliucci, 1989) we studied karyotype polymorphism in a bulbous perennial lilid, *Ornithogalum montanum* Cyr. ex Ten., which is an extremely variable species, both at the phenotypic (Terracciano, 1906; Cullen & Ratter, 1967; Pigliucci *et al.* 1991) and at the karyotypic level (Capineri *et al.* 1979). The main conclusions of that work were: (i) geographic variation of karyotypes is not random; (ii) environmental factors associated with karyotype polymorphisms (e.g. winter temperatures) do not seem variable enough to exert a major selective effect; (iii) local population differentiation is consistent with isolation by distance, roughly within a range of 50 km; but (iv) isolation by distance cannot account for long-distance patterns of karyotype variation, which, conversely, are interpretable as due to subdivision of the population in partly independent units. We suggested this could result from independent colonization of three regions of Italy by stocks of *Ornithogalum* with different karyotype-banding patterns

The present study deals with enzyme polymorphism in the same species and considers a wider area, including Sicily. Geographic variation of 15 electrophoretic markers (40 alleles overall) in 13 populations is summarized by spatial autocorrelation indices. The association between genetic variation and some environmental parameters is assessed through a series of Mantel's (1967) tests between matrices of genetic and environmental distance. Finally, areas of abrupt genetic change, which suggest population subdivision or response to abrupt environmental change (Endler, 1977; Nagylaki, 1988), are identified by mapping the zones where the ratio of genetic to geographic distances is maximum.

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2. Materials and methods

(i) *The data*

Five of the 13 populations of this study (Table 1) coincide with populations considered in the previous paper (Barbujani & Pigliucci, 1989), although not exactly because of the extensive resampling carried out after 1980. Eight new sampling sites have been added, but four sites that provided chromosome banding data have been discarded because of lack of sufficient specimens. Standard starch gel electrophoresis was employed to determine the enzyme types at 15 loci. Details on the techniques and a description of the sampling procedure are in Pigliucci *et al.* (1990*b*). Samples sizes ranged from 16 to 45 individuals (Table 1). The loci studied were: glutamic oxalacetic transaminase (*GOT*) *1, 2, 3* and *4*; esterase (*EST*); aldolase (*ALD*), phospho-glucoso isomerase (*PGI*) *1* and *2*; 6-phosphatase (*6P*) *1* and *2*; malate dehydrogenase (*MDH*) *1, 2, 3* and *4*; and shikimic dehydrogenase (*SKDH*). On the whole, 40 alleles were identified. *GOT-4, MDH-1, MDH-2* and *MDH-3* turned out to be monomorphic in all populations. The results we present are based on the 11 polymorphic loci (36 alleles).

(ii) *Testing of gene-frequency homogeneity*

The first step of this study was to test for homogeneity of allele frequencies, i.e. the hypothesis that the observed data represent random samples from a population with a single set of gene frequencies. For this purpose, *G* tests of independence among samples were applied to the 36 alleles (Sokal, 1979a), and only the spatial patterns of the allele frequencies showing significant heterogeneity across localities were analysed.

(iii) *Spatial autocorrelation analysis*

Spatial autocorrelation statistics quantify the genetic similarity between pairs of isolates as a function of their distance in space (Sokal & Oden, 1978*a*, *b*). A 13×13 matrix of geographic distances was computed between pairs of localities, which subsequently were grouped in four distance classes whose limits were 0–104·6, 104·6–6–233·5, 233·5–290·4 and 290·4–458·7 km. These limits were chosen so as to define equally informative classes (with between 18 and 20 pairs of localities included in each). An autocorrelation index, Moran's *I*, was then calculated for each allele showing significant heterogeneity in space, for each distance class. The formula employed was:

$$I = N\Sigma\Sigma w_{ij}(q_i - q)(q_j - q)/W\Sigma(q_i - q)^2,$$

where q_i and q_j are the frequencies of the allele of interest at the *i*th and *j*th locality, q is their mean across the N (13 in the present case) localities, w_{ij} is equal to 1 for all the pairs of localities falling in the distance class studied and equal to 0 for all other pairs, and W is the sum of all w_{ij} values in that distance class.

Moran's *I* tends to 1 when the gene frequencies are similar (i.e. depart from the mean in the same direction); it tends to -1 when gene frequencies are dissimilar, and is expected to be equal to $-1/(N-1)$, under a randomization hypothesis. Significance levels were calculated according to Sokal & Oden (1978*a*). The plots of autocorrelation coefficients versus distance are referred to as *correlograms*; their overall significance was assessed by a Bonferroni test (Oden, 1984). Finally, the correlograms computed for the various alleles were clustered by the UPGMA technique, on the basis of Manhattan distances between correlograms (Sneath & Sokal, 1973).

Table 1. *A list of the localities studied*

Code	Population	Lat.	Long.	Elev.	Sample sizes
V1	Vico 1	40·72	15·73	670	16–40
Vico 1	40·72	15·73	670	16–40	
V2	Vico 2	40·72	15·76	1000	24–38
SE	Monte Serranetta	40·79	15·57	1100	20–35
SI	Monte Sirino	40·13	15·84	1050	22–45
PO	Monte Pollino	39·86	16·13	1050	16–36
LU	Lungro	39·74	16·12	440	24–40
SL	Sila	39·27	16·69	1210	16–40
CI	Ciricilla	39·20	16·63	1350	20–38
SN	Monte S. Nicola	38·54	16·51	1050	22–44
PZ	Piano Zucchi	37·82	14·00	1250	23–30
GS	Geraci Siculo	37·86	14·16	1200	17–35
PM	Portella Mandarini	37·95	14·51	1200	22–42
PB	Piano Battaglia	37·90	14·00	1600	22–40

Lat., latitude North; Long., longitude East; Elev., elevation. Minimum and maximum size of the samples used to estimate gene frequencies are given.

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(iv) *Detection of genetic boundaries*

Spatial autocorrelation analysis assumes stationarity of the gene-frequency distributions, that is to say, constancy of the rate of genetic change per unit distance all over the region studied. The identification of regions where this assumption is not fulfilled can allow for evolutionary inferences (Womble, 1951; Barbujani *et al.* 1989). We refer to the zones where several gene frequencies vary abruptly as *genetic boundaries*. In order to detect them we chose an approach not requiring prior interpolation of data (see Barbujani *et al.* 1990; Pigliucci *et al.* 1990*a*). Initially, the 13 localities were connected by a Delaunay network (Fig. 1; Green & Sibson, 1977; Brassel & Reif, 1979). Nei's (1972) genetic distances were calculated between all pairs of directly connected localities, based on 36 alleles, and were divided by the respective geographic distances. Six genetic boundaries were then identified as follows. Consider the pair of localities between which the ratio of genetic to geographic distance is maximum. The line that can be traced perpendicular to the network edge connecting them was recognized as the origin of a genetic boundary, which was extended along the adjacent edge showing the highest rate of genetic change. This procedure was continued until the boundary reached the limits of the network or, for boundaries other than the first one, another pre-existing boundary (Monmonier, 1973), and was repeated 6 times, thus yielding a map showing as strings the 6 zones where the rate of genetic change between localities is maximum.

(v) *Association between genetic and environmental diversity*

In the third part of this study we tested whether factors other than distance can account for the observed genetic population structure of *O. montanum*. The problem here was that both allele frequencies and environmental parameters such as temperature, elevation and rainfall, are spatially autocorrelated, because their local values depend on the values at neighbouring localities, violating the assumption of independence (see Legendre *et al.* 1989 and Sokal *et al.* 1989*b*). As a consequence, a greater percentage of observations tends to fall in the rejection region of the test distributions than the nominal percentage of the critical values (Sokal, 1990). In other words, autocorrelation of data tends to inflate the significance of statistical tests, including those on Pearson's correlation coefficient (Richardson & Hémon, 1981). To avoid that bias, we chose to employ a nonparametric test of association, Mantel's test (Mantel, 1967; Sokal, 1979*a*; Manly, 1985; Smouse *et al.* 1986). This test compares two distance or similarity matrices by means of a Z statistic obtained as follows:

$$Z = \Sigma\Sigma(X_{ij}\, Y_{ij}),$$

where X_{ij} and Y_{ij} are the elements of two square, not

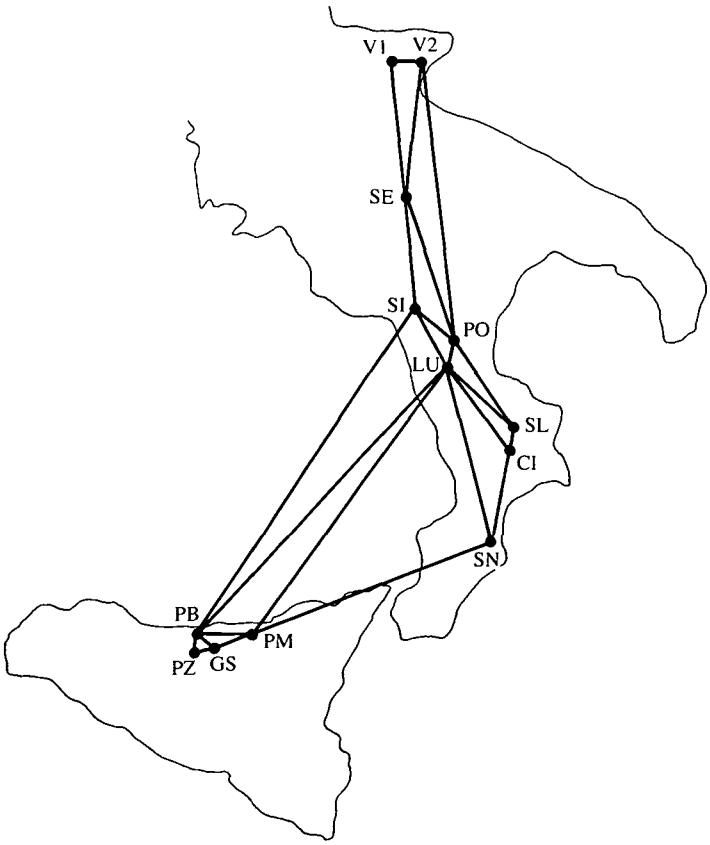

Fig. 1. Delaunay connection graph between 13 localities in Southern Italy. Locality codes as in Table 1.

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Table 2. *Frequencies of 33 alleles analysed (only one allele is given for the biallelic loci* PGI-1, PGI-2 *and* MDH-4)

	Locus...	*GOT-1*			*GOT-2*				*GOT-3*				*EST*				
Pop.	Allele...	*1*	*2*	*3*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*1*	*2*	*3*	*4*	*5*
V1		1·00	0·00	0·00	0·58	0·42	0·00	0·00	0·14	0·82	0·04	0·00	0·00	0·54	0·38	0·08	0·00
V2		1·00	0·00	0·00	0·67	0·33	0·00	0·00	0·22	0·78	0·00	0·00	0·03	0·51	0·42	0·04	0·00
SE		1·00	0·00	0·00	0·48	0·52	0·00	0·00	0·02	0·98	0·00	0·00	0·03	0·53	0·28	0·16	0·00
SI		0·94	0·06	0·00	0·41	0·56	0·00	0·03	0·16	0·84	0·00	0·00	0·12	0·53	0·28	0·07	0·00
PO		0·97	0·03	0·00	0·41	0·58	0·01	0·00	0·24	0·74	0·00	0·02	0·03	0·53	0·41	0·03	0·00
LU		0·97	0·03	0·00	0·22	0·78	0·00	0·00	0·15	0·85	0·00	0·00	0·07	0·39	0·49	0·05	0·00
SL		0·98	0·02	0·00	0·44	0·56	0·00	0·00	0·30	0·70	0·00	0·00	0·04	0·59	0·28	0·09	0·00
CI		1·00	0·00	0·00	0·60	0·40	0·00	0·00	0·23	0·77	0·00	0·00	0·06	0·49	0·36	0·09	0·00
SN		0·71	0·26	0·03	0·45	0·55	0·00	0·00	0·34	0·66	0·00	0·00	0·00	0·12	0·15	0·53	0·20
PZ		0·99	0·01	0·00	0·73	0·27	0·00	0·00	0·31	0·62	0·07	0·00	0·13	0·47	0·39	0·01	0·00
GS		0·93	0·07	0·00	0·70	0·30	0·00	0·00	0·14	0·82	0·04	0·00	0·13	0·39	0·44	0·04	0·00
PM		1·00	0·00	0·00	0·63	0·37	0·00	0·00	0·07	0·89	0·04	0·00	0·04	0·17	0·71	0·08	0·00
PB		1·00	0·00	0·00	0·85	0·15	0·00	0·00	0·07	0·82	0·11	0·00	0·19	0·42	0·20	0·19	0·00

	Locus...	*ALD*			*PGI-1*	*PGI-2*	*6P-1*			*6P-2*			*MDH-4*	*SKDH*				
Pop.	Allele...	*1*	*2*	*3*	*1*	*1*	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*1*	*2*	*3*	*4*	*5*
V1		0·08	0·92	0·00	0·09	0·00	0·27	0·73	0·00	0·84	0·14	0·02	0·00	0·93	0·00	0·00	0·07	0·00
V2		0·00	0·74	0·26	0·06	0·02	0·02	0·92	0·06	0·69	0·10	0·21	0·08	0·79	0·11	0·02	0·04	0·04
SE		0·02	0·98	0·00	0·13	0·08	0·02	0·98	0·00	0·96	0·02	0·02	0·10	0·93	0·02	0·00	0·05	0·00
SI		0·00	0·88	0·12	0·05	0·00	0·00	1·00	0·00	1·00	0·00	0·00	0·96	0·69	0·17	0·02	0·12	0·00
PO		0·06	0·85	0·09	0·03	0·07	0·06	0·88	0·06	1·00	0·00	0·00	0·93	0·67	0·10	0·02	0·19	0·02
LU		0·00	0·90	0·10	0·06	0·02	0·02	0·80	0·18	0·80	0·18	0·02	0·04	1·00	0·00	0·00	0·00	0·00
SL		0·00	1·00	0·00	0·00	0·00	0·00	1·00	0·00	0·78	0·03	0·19	0·11	0·87	0·05	0·08	0·00	0·00
CI		0·06	0·92	0·02	0·08	0·02	0·02	0·94	0·04	0·71	0·27	0·02	0·14	0·50	0·00	0·00	0·00	0·50
SN		0·00	0·89	0·11	0·09	0·00	0·00	1·00	0·00	0·74	0·26	0·00	0·17	0·45	0·05	0·00	0·05	0·45
PZ		0·00	0·94	0·06	0·06	0·04	0·10	0·87	0·03	0·53	0·10	0·37	0·04	0·40	0·04	0·08	0·35	0·13
GS		0·03	0·92	0·05	0·05	0·03	0·09	0·72	0·19	1·00	0·00	0·00	0·03	0·82	0·05	0·10	0·03	0·00
PM		0·00	1·00	0·00	0·07	0·09	0·04	0·70	0·26	0·98	0·00	0·02	0·09	0·38	0·07	0·17	0·10	0·28
PB		0·00	0·98	0·02	0·35	0·02	0·02	0·98	0·00	0·62	0·09	0·29	0·04	0·52	0·07	0·18	0·05	0·18

necessarily symmetrical, matrices. The value of Z is maximum when there is positive association of the corresponding elements of X and Y, and minimum when the association is negative. A null distribution of Z' values (the prime denotes these are *not* observed values) can be obtained by randomly permuting rows and columns of one matrix while keeping the other constant, and computing Z' each time (Mantel, 1967; Manly, 1986); the observed Z is then tested against its empirical null distribution. However, because there are $13! \approx 6 \times 10^9$ possible permutations of the matrices we were dealing with in this study, it was simpler to compute the expected mean, $E(Z)$, and variance, $\mathrm{var}(Z)$ of that null distribution (Mantel, 1967); from them a standardized statistic g was obtained, which is Normally distributed with variance = 1:

$$g = [Z - E(Z)]/\sqrt{[\mathrm{var}(Z)]}.$$

We compared 4 matrices of pairwise genetic distances based on different criteria (referred to as **NEI**, **AUT**, **NAU**, and **BND**, and described in detail in the legend to Table 4), a matrix of geographic distances (**GEO**), and a synthetic matrix of environmental distances (**ENV**) based on 13 mostly climatic variables. In addition, two of the aforementioned matrices of genetic distances, calculated on the frequencies of spatially autocorrelated alleles (**AUT**) and on the frequencies of all other alleles (**NAU**), respectively, were compared with 13 matrices of environmental dissimilarity (listed in the legend to Table 5), each one based on a single variable.

3. Results

The loci *PGI-1*, *PGI-2* and *MDH-4* appeared strictly biallelic; to avoid redundancy of information, only one allele was considered for each of them. The frequencies of 14 of the remaining 33 alleles (Table 2; some figures differ from the ones in Pigliucci *et al.* (1990*b*) because they are based on larger samples) were not significantly heterogeneous among sampling localities (Table 3, where the F_{st} values are also given) and were excluded from spatial autocorrelation analysis. For most of the 19 spatially heterogeneous allele frequencies, positive but insignificant short-distance autocorrelation is observed, followed by negative insignificant Moran's I values at greater distances. Only the correlograms of *Got-2*^{1}, *Got-2*^{2} and *Skdh*^{3} are overall significant (Fig. 2), and form a cluster in the lower part of the dendrogram calculated by UPGMA (Fig. 3). The cophenetic correlation

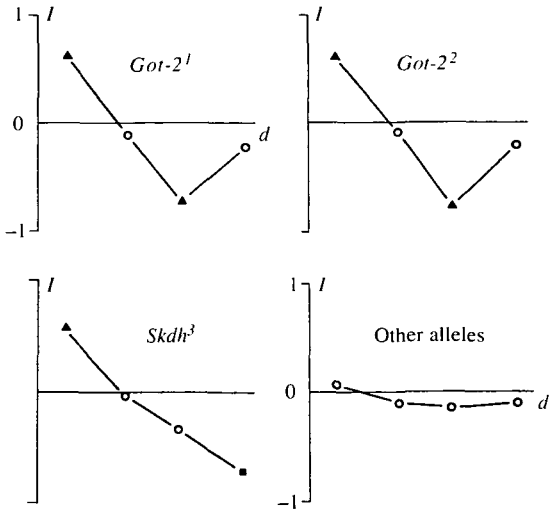

Fig. 2. Spatial correlograms of 3 individual allele frequencies, and average correlogram for 16 heterogeneously distributed alleles. I = Moran's I; d = distance. Open circles, insignificant coefficients; solid squares, $P < 0{\cdot}01$; solid triangles, $P < 0{\cdot}001$.

Table 3. *Standardized gene-frequency variances* (F_{st}; *Pigliucci* et al. *1990*b), *and* G *tests for the heterogeneity of allele frequencies across localities* (*12* D.F.)

Locus	F_{st}	Alleles (*G* value)		
GOT-1	0·157	*1* (33·7, ***)	*2* (29·3, **)	*3* (5·1, N.S.)
GOT-2	0·127	*1* (38·3, ***)	*2* (35·6, ***)	*3* (2·8, N.S.)
		4 (5·1, N.S.)		
GOT-3	0·057	*1* (19·2, N.S.)	*2* (16·3, N.S.)	*3* (14·8, N.S.)
		4 (2·8, N.S.)		
EST	0·119	*1* (21·8, *)	*2* (29·7, **)	*3* (32·1, ***)
		4 (40·0, ***)	*5* (26·1, *)	
ALD	0·061	*1* (11·0, N.S.)	*2* (17·3, N.S.)	*3* (20·7, N.S.)
PGI-1	0·090	*1* (18·4, N.S.)		
PGI-2	0·061	*1* (14·7, N.S.)		
6P-1	0·118	*1* (20·3, N.S.)	*2* (34·9, ***)	*3* (33·7, ***)
6P-2	0·144	*1* (48·0, ***)	*2* (34·2, ***)	*3* (48·7, ***)
MDH-4	0·620	*1* (141·9, ***)		
SKDH	0·180	*1* (67·1, ***)	*2* (13·2, N.S.)	*3* (22·7, *)
		4 (30·9, **)	*5* (69·7, ***)	

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

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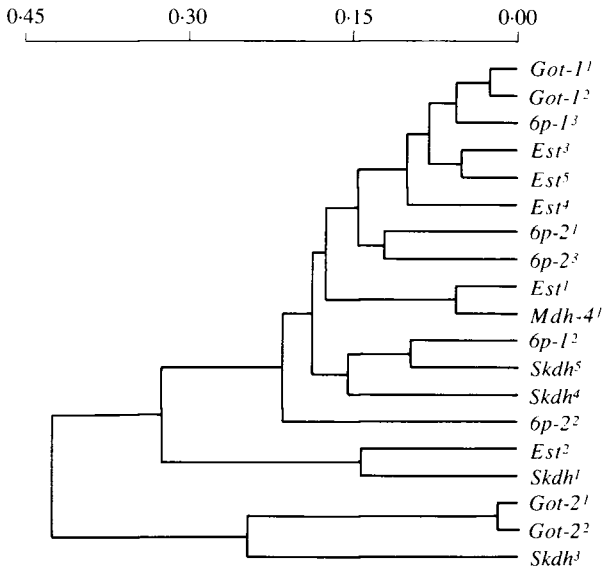

Fig. 3. UPGMA clustering of the 19 geographically-differentiated alleles. *x* axis: Manhattan distances between correlograms.

coefficient of the dendrogram (0·88) is high, thus indicating that the clustering obtained accounts for a large fraction of the variation between correlograms (Sneath & Sokal, 1973). Maps describing spatial variation for four representative alleles are in Fig. 4.

Two alleles of the *GOT-2* system show decreasing autocorrelation coefficients at short and intermediate distances, followed by a nonsignificant value at large distance. This type of correlogram describes a circular cline (Sokal, 1979*b*) or, in the quasi-linear habitat we are dealing with, two clines converging about the centre of the region studied. The width of such clines may be inferred from the intercept of the correlogram with the *x*-axis (Sokal, 1979*b*), i.e. roughly 250 km. The autocorrelation coefficients for the third allele whose variation is spatially structured, $Skdh^3$, decrease monotonically with distance, which is evidence for a gradient of gene frequencies.

The sharpest genetic boundary detected (Fig. 5) separates a northwestern region including V1 and SE from the rest of the area studied. Two other large regions appear distinct, and they correspond to a northeastern area, and a southern area that includes most of Sicily and Calabria. However, most zones of abrupt genetic change tend to surround single isolates, such as PZ, PB, GS, and CI (three of which are in Sicily, i.e. at the extremities of the Italian range of *O. montanum*), rather than subdividing the area studied in wide sectors.

In the pairwise comparisons of the six distance matrices described above (Table 4), only four associations are significant. Geographical and environmental distances are correlated (spatially close sites have also similar climates), as are the **NEI**, **AUT** and **NAU** matrices. Actually, **AUT** and **NAU** are based on subsets of the alleles considered in calculation of the

Table 4. *Mantel's test for the association of genetic, geographic and environmental distances between populations*

	NEI	AUT	NAU	BND	GEO	ENV
NEI	—					
AUT	2·54*	—				
NAU	5·05*	2·31*	—			
BND	1·32	1·35	1·11	—		
GEO	0·23	0·28	0·13	1·48	—	
ENV	−0·53	0·54	−0·45	−0·25	2·23*	—

The statistic *g* is given; * $P < 0{\cdot}05$ (all tests are one-tailed). Matrices are coded as follows: **NEI**, Nei's distances based on all 36 allele frequencies; **AUT**, Nei's distances based only on the 3 alleles that resulted significantly autocorrelated; **NAU**, Nei's distances calculated on the basis of the 33 non-autocorrelated alleles; **BND**, a binary matrix, in which pairs of populations were assigned distance 1 if separated by a genetic boundary, and distance 0 if not; **GEO**, geographic distances (great circle distances); **ENV**, distances based upon elevation and 12 climatic variables (described in the legend to Table 5); these variables were standardized and were given equal weight in the computation of the overall index.

Table 5. *Mantel's tests* (g *values*) *for the association between genetic distances and environmental differences*

Environmental parameters	NAU	AUT
1	−0·26	−0·16
2	−0·65	−0·70
3	−0·78	−0·94
4	−0·57	−0·98
5	0·64	1·81*
6	0·45	0·89
7	−0·18	1·09
8	−0·38	1·28
9	−0·40	0·58
10	−0·63	0·57
11	0·58	0·67
12	−1·26	−0·20
13	−0·38	−0·90

NAU and **AUT** defined as in Table 4; environmental variables as follows: 1, max. winter temp.; 2, min. winter temp.; 3, mean winter temp.; 4, max. summer temp.; 5, min. summer temp.; 6, mean summer temp.; 7, total winter rainfall; 8, max. winter rainfall; 9, days of winter rainfall; 10, total summer rainfall; 11, max. summer rainfall; 12, days of summer rainfall; 13, elevation.
* $P < 0{\cdot}05$ (all tests are one-tailed).

NEI matrix, and should not be expected to be independent from it. Conversely, the variation of both autocorrelated and not autocorrelated alleles seems to be independent of the overall environmental distances and of the presence of genetic boundaries, and the overall genetic distances (**NEI**) are not associated with either geographic or environmental distances. Finally, the relationship between the **BND** and the **ENV** matrices is insignificant and negative, thus showing

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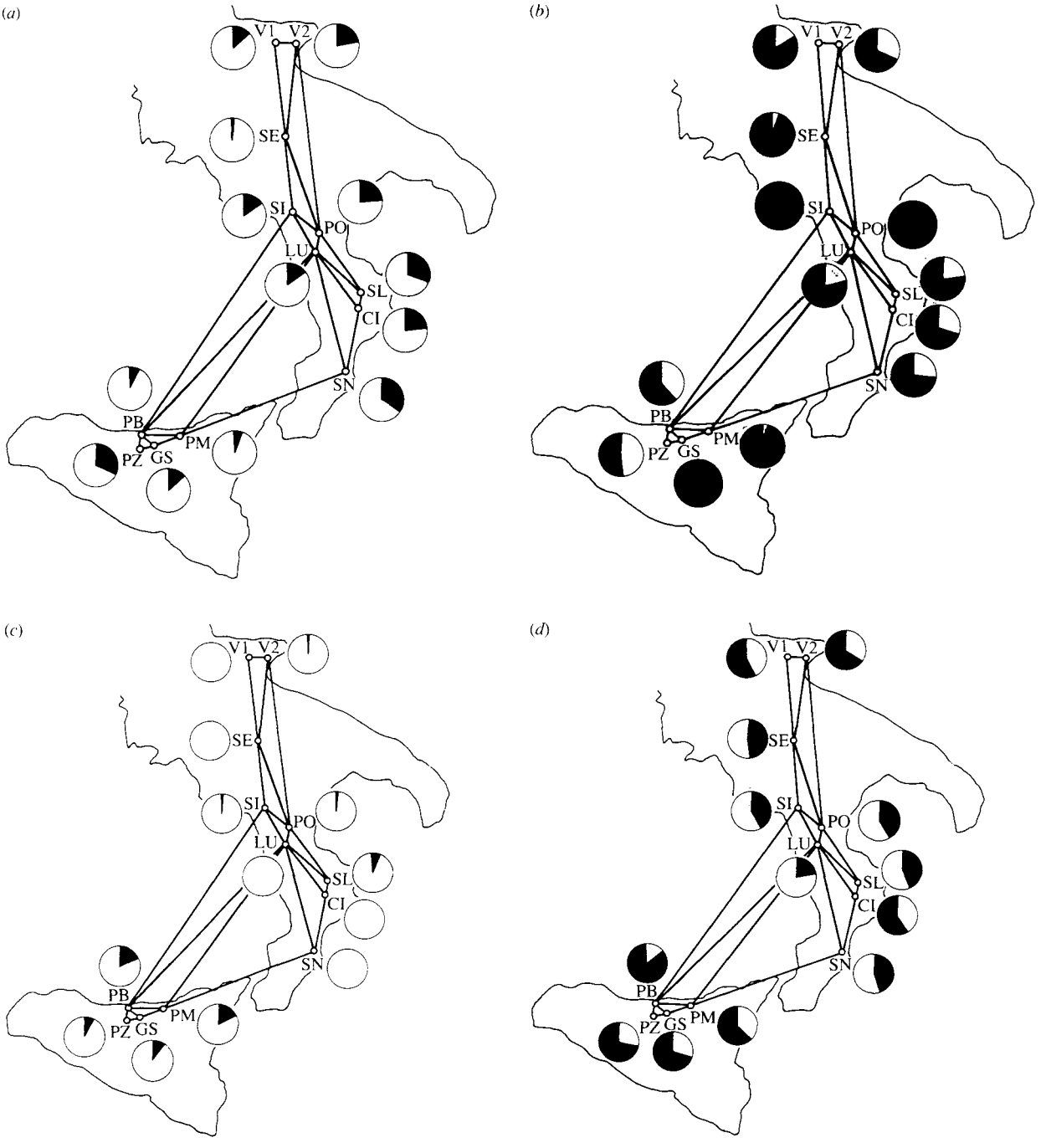

Fig. 4. Distributions of 4 alleles showing the four basic modes of spatial variation observed. Shaded areas of the pies represent the frequency of the allele of interest. (*a*) *Got-3*^{1} (insignificant differentiation); (*b*) *6P-2*^{1} (significant differentiation, no significant spatial structure); (*c*) *Skdh*^{3} (significant differentiation, significant cline); (*d*) *Got-2*^{1} (significant differentiation, significant non-clinal structure). Locality codes as in Table 1.

that abrupt genetic change is not associated with abrupt change of the environmental indices we were able to measure.

Only 1 out of 26 comparisons of genetic distances with individual environmental variables is significant (Table 5), namely **AUT** versus minimum summer temperatures. Exclusion of *Skdh*^{3} from the **AUT** matrix does not result in a decrease of Mantel's correlation statistic, whereas both the *Got-2* alleles considered contribute substantially to the association; *Got-2*^{1} is more frequent where minimum summer temperatures are higher.

4. Discussion

The frequencies of 19 alleles differ significantly among populations of *O. montanum* (as do the frequencies of Q bands in their chromosomes: Barbujani & Pigliucci, 1989). However, a significant geographic structure is apparent for only three alleles. Only for *Skdh*^{3} is the

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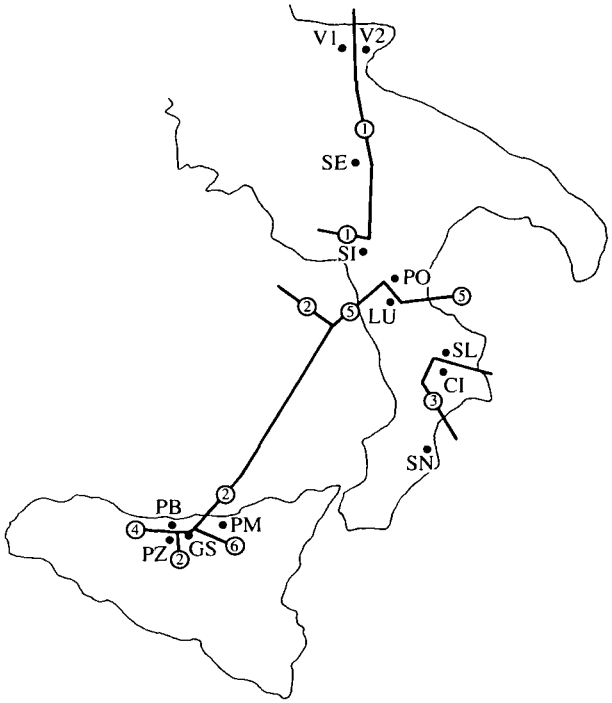

Fig. 5. Genetic boundaries between localities, ranked from the sharpest (number 1), on the basis of Monmonier's (1973) procedure. Locality codes as in Table 1.

variation clinal, whereas allele frequencies at the *GOT-2* locus correlate with minimum summer temperatures. The frequencies of most alleles, conversely, are random in the geographical space, and do not appear associated with any of the environmental factors considered in this study.

The significant differences in various allele frequencies at different sites indicate that the Italian population of *O. montanum* is not panmictic; its isolates evolved to a certain degree independently of one another. What then is the cause of their diversity? Several genetic boundaries (i) separate localities whose climatic characteristics appear similar in this study, and (ii) originate between spatially close isolates. The former does not argue for sharp selective gradients as the cause of rapid genetic change, and the latter implies that, in addition to geographic distance, other factors have limited gene flow between local populations. As a consequence, subdivision and isolation, as opposed to adaptation, seem the major forces determining at least two aspects of the genetic structure of this population, namely the spatially random patterns of several markers and the distribution of zones of sharp multi-locus differentiation. This is in agreement with the analysis of karyotype polymorphism, which suggested that subdivision contributes to maintenance of genetic differentiation among three regions of the Italian range of the species (Barbujani & Pigliucci, 1989).

Whatever the role of selection (discussed below), subdivision and isolation point to the existence of barriers that *Ornithogalum* is unable to cross. In fact, its pollen is dispersed only by raindrops, and is known to travel not more than a few hundred metres (Van Raamsdonk, 1985). Therefore, physical obstacles (such as the valleys between SL and CI, PO and LU, and among PZ, PB, GS and PM, all associated with genetic boundaries) are expected to act as reproductive barriers; biological barriers may be envisaged as well (Barton & Hewitt, 1985). As a consequence, gene flow is locally restricted, and random fluctuations of gene frequencies can accumulate across time (Wright, 1931; Slatkin, 1985, 1987), leading to substantial genetic divergence of isolates. Since the distribution of physical barriers does not follow any spatial pattern, genetic variation in space appears correspondingly random; only few allele frequencies show the positive and decreasing short-range autocorrelation expected under *isolation by distance*, i.e. when genetic drift is balanced by dispersal (Barbujani, 1987).

In summary, the spatial patterns of chromosome bands and enzyme polymorphisms suggest that subdivision and isolation are important in maintaining genetic differences among demes; such differences seem caused mainly, if not necessarily only, by genetic drift. Indeed, both the decrease of $Skdh^3$ frequencies with latitude (and hence temperature), and the association of $Got\text{-}2^1$ and $Got\text{-}2^2$ frequencies with one climatic variable may in principle be adaptive. However, one significant cline over 40 alleles studied, and one significant Mantel's test out of 26 performed, are compatible with the effects of chance. In addition, neither different activities of *GOT* isoenzymes, nor effects of temperature on *GOT* activity are reported in recent biochemical studies on nitrogen fluxes in plants (Beck & Renner, 1990). In conclusion, we cannot rule out that gene frequencies are as they are because the plants responded adaptively to variation of environmental factors we did not consider, but there is no direct evidence for existence of such factors either. Testing for selection by spatial autocorrelation analysis (as in Sokal *et al.* 1987, 1989*a*; Epperson, 1990) would require a greater number of gene frequencies, and data on the distribution of the putative selective agent.

Conversely, there is evidence supporting a different hypothesis. Very little genetic variation has been observed within two isolates, SI and PO, and this has been interpreted as a result of a recent population bottleneck (Pigliucci *et al.* 1990*b*). Moreover, SI and PO (i) occupy the central section of the Italian range of the species, where karyotypes common in other zones appeared to be absent (Barbujani & Pigliucci, 1989); (ii) have similar allele frequencies, so much so that they form a cluster in discriminant analysis (Pigliucci *et al.* 1990*b*); and (iii) are separated from most other isolates by genetic boundaries 1 and 5 (Fig. 5). To account for all these findings it is sufficient to assume either that the population of SI and PO underwent a drastic bottleneck (which, however, would not fully explain their genetic similarity), or

that both sites were colonized recently by few seeds coming from an adjacent isolate, perhaps after the pre-existing local populations became extinct.

Be that as it may, the overall picture emerging is one in which the extant genetic population structure basically reflects demographic events in the history of the species. Small effective population sizes, subdivision and restricted gene flow are all likely to have created the conditions for genetic drift to act and lead populations to diverge. In addition, bottlenecks and/or local extinctions and recolonizations should have occurred at least around SI and PO, and possibly elsewhere. Subdivided populations subject to such processes evolve quickly, as their effective sizes are much less than their actual average size (Wright, 1970). In this way, large genetic differences may have arisen even between close sites (see Slatkin, 1989). A series of studies on the giant toad *Bufo marinus* in Australia shows that extensive population differentiation and some clines may indeed result from range expansion (Easteal, 1981, 1985). These findings cannot be mechanically transposed to a far less mobile species, such as *O. montanum*, but argue for the *possibility* of the process outlined above. In such a context, drift and selection are not mutually exclusive. It makes little difference if the genetic variation observed is entirely due to drift, or if a small part of it did evolve as a response to unidentified selective pressures.

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