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METHODOLOGICAL ADVANCES - INFERENCE OF SPATIAL STRUCTURE

Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data

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Abstract

The Mantel test is widely used to test the linear or monotonic independence of the elements in two distance matrices. It is one of the few appropriate tests when the hypothesis under study can only be formulated in terms of distances; this is often the case with genetic data. In particular, the Mantel test has been widely used to test for spatial relationship between genetic data and spatial layout of the sampling locations. We describe the domain of application of the Mantel test and derived forms. Formula development demonstrates that the sum-of-squares (SS) partitioned in Mantel tests and regression on distance matrices differs from the SS partitioned in linear correlation, regression and canonical analysis. Numerical simulations show that in tests of significance of the relationship between simple variables and multivariate data tables, the power of linear correlation, regression and canonical analysis is far greater than that of the Mantel test and derived forms, meaning that the former methods are much more likely than the latter to detect a relationship when one is present in the data. Examples of difference in power are given for the detection of spatial gradients. Furthermore, the Mantel test does not correctly estimate the proportion of the original data variation explained by spatial structures. The Mantel test should not be used as a general method for the investigation of linear relationships or spatial structures in univariate or multivariate data. Its use should be restricted to tests of hypotheses that can only be formulated in terms of distances.

Keywords: multiple regression, numerical simulations, permutation test, power, redundancy analysis, spatial analysis, type I error, variation partitioning

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Introduction

Brought to the attention of evolutionists, systematists and geneticists by Sokal (1979), the Mantel test (Mantel 1967; Mantel & Valand 1970) allows linear or monotonic comparisons between the elements of two distance matrices. The Mantel statistic is usually tested by permutation although it can also be tested using an asymptotic normal approximation when the number of observations, n, is large. The space-time clustering procedure of Mantel (1967) was originally designed to relate a matrix of spatial distances and a matrix of temporal distances in a generalized regression approach. Since Mantel & Valand (1970), the procedure, known as the Mantel test in the biological and environmental sciences, includes any analysis relating two distance matrices or, more generally, two resemblance or proximity matrices.

Sokal (1979) pioneered the use of Mantel test by comparing phenetic distances among local populations to geographic distances expressed in various ways. An equivalent test is the quadratic assignment procedure (QAP) developed by psychometricians (Hubert & Schultz 1976). Clarke (1988, 1993) developed a form of Mantel test called the ANalysis Of SIMilarities (ANOSIM) computed on ranked distances, which is widely used in marine

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biology. Smouse *et al.* (1986) proposed to compute partial correlations on distance matrices as the basis for partial Mantel tests. They used that method to test alternative hypotheses about the factors (geographic and linguistic distances) responsible for genetic variation among the Yanomama Indians of lowland South America. Because of the ease of representation of spatial relationships by a distance matrix, Legendre & Troussellier (1988) used the partial Mantel test (described in Appendix S4) to control for the effect of spatial distances in ecological studies. Since then, Mantel tests have been used in various fields of biology: morphology (van Schaik *et al.* 2003), behaviour (Cheverud 1989), ecology (Leduc *et al.* 1992; Fortin & Gurevitch 2001; MacDougall-Shackleton & MacDougall-Shackleton 2001; Wright & Wilkinson 2001) and phylogenetics (van Buskirk 1997). In a micro-evolutionary study, Le Boulengé *et al.* (1996) used partial Mantel tests to test alternative hypotheses about the cause of morphological variation among local muskrat populations in a river catchment: straight-line distances, swimming distances along the river network and 'decisions' distances along the network. In population genetics, Mantel tests have been used to determine whether local populations that are geographically close are either genetically or phylogenetically similar (e.g., Lloyd 2003). This question can be reformulated as follows: is the spatial genetic variation spatially organized? If so, what are the significant spatial patterns and at what scale(s) are they found?

In landscape genetics recently, several studies used Mantel tests to include landscape features in the analysis of genetic variation in a spatially explicit way (Vignieri 2005; Cushman *et al.* 2006; Wang *et al.* 2008). The tested hypotheses may include physical or biotic environmental conditions, or else species-dependent processes such as seed dispersal limitation. The latter processes are more likely to affect neutral genes than non-neutral loci that are differentially selected by environmental conditions.

The objective of this study is to evaluate the performance of the Mantel test in spatial population genetic and landscape genetic analysis in comparison with alternative statistical approaches (correlation, canonical analysis) not based on distances. In spatial population genetics, landscape genetics and spatial community ecology, statistical analyses are often undertaken to 'explain' (in the statistical sense) the spatial variation of a response variable (**y**, univariate) or data table (**Y**, multivariate). Several processes can generate spatial genetic structures (Fig. 1), and several regression and distance-based methods can be used to relate these structures to landscape and environmental conditions. In this study, we will show that the Mantel test is not equivalent to either a correlation or regression analysis in the univariate case, or a canonical analysis in the multivariate case. We will also show that there is a great difference in power between these alternative tests, and that for spatial analysis, more powerful alternatives are available, unless the hypothesis to be tested strictly concerns distances. Moreover, Mantel tests underestimate the coefficient of determination estimating the variation explained by the spatial structure.

Mantel test and derived forms

The statistic used by Mantel (1967) was the cross-product of the distances in the two matrices under analysis. Nowadays, most if not all computer programs offer a Mantel correlation statistic r_M, which is the cross-product between the standardized distances divided by $(d - 1)$, where d is the number of distances in the upper-triangular portion of each matrix when the statistic is computed from symmetric matrices. This transformation has no effect on the probability obtained by permutation tests, and r_M is conveniently bounded between −1 and +1. Equations for the Mantel statistic will be given further down after the presentation of the sum-of-squares (SS) statistics. In the rare cases where r_M is computed over asymmetric matrices, d is the total number of distances in each matrix, except the diagonal elements, which trivially take the value 0.

In its most simple form in spatial analysis of genetic data, the Mantel test is used to compare a matrix of geographic distances to a matrix representing relevant dissimilarities among the same objects computed from another data table (phylogenetic, genetic, environmental, etc.). Derived forms include the partial Mantel test carried out using partial correlation statistics computed on distances (Smouse *et al.* 1986), the Mantel correlogram (Oden & Sokal 1986; Sokal 1986), multiple regression on distance matrices (Hubert & Golledge 1981; tests of significance described in Legendre *et al.* 1994), as well as the test of congruence among several distance matrices (CADM, Legendre & Lapointe 2004). A few years ago, a controversy arose in the literature about the validity of the permutation procedure used in partial Mantel tests. The issue is summarized in Box 1. A description of the various permutation methods proposed for partial Mantel tests is given in Appendix S4, together with recommendations about the use of three of these procedures.

When the spatial structure of gene frequencies or genetic diversity (Fig. 1a) at the sampling locations is of interest per se, more powerful methods of analysis have been developed for modelling gradients or other forms of broad-scale spatial structures created in univariate or multivariate data as a response to forcing environmental variables (induced spatial dependence), as well as finer-scaled patterns corresponding to autocorrelation generated in response variables by dynamic processes (Diniz

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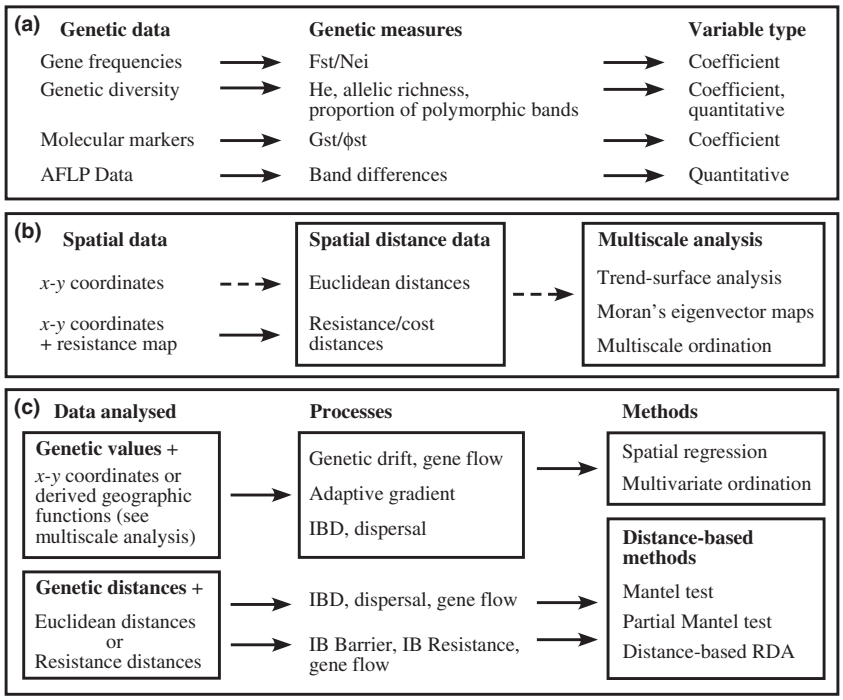

Fig. 1 Spatial genetic analyses. Selection of the appropriate statistical methods depending on data types and derived measures of (a) genetic and (b) spatial distances. (c) Methods of analysis according to the process under study. (a) Genetic data can be either adaptive or neutral genes depending on the markers used from which are derived either distance data (based on various coefficients: Fst, Nei) or quantities (number of alleles, band differences). (b) Spatial data can be only *x-y* coordinates leading to either (as indicated be dashed arrows) Euclidean distances or multiscale analysis (see text for brief description of these methods). Then (c), the selection of methods depends on whether the genetic data being used are (i) quantitative values variables (quantities such as number of alleles, proportion of polymorphic bands or band length differences), in which case-specific processes can be studied using spatial regression (SAR, CAR, autologistic, GWR, etc.), multiscale analysis (methods listed in section b: polynomial, Moran's eigenvector maps, multiscale ordination) and/or multivariate ordination methods (PCA, RDA, CCA); or (ii) they are in the form of genetic distances, in which case distance-based methods should be used to test spatial population and landscape genetics questions. Boxes, which contain lists of methods that can be used or processes to be tested, indicate that any of the methods or of the processes within them could be used or tested. IB ... : isolation by ...; IBD: isolation by distance.

Box 1 Controversy about the validity of the partial Mantel test

Two articles appeared in the journal *Evolution* in 2001–2002 about the use of partial Mantel tests in micro-evolutionary studies: Raufaste & Rousset (2001) and Castellano & Balletto (2002). These articles ignored previously published work in which the properties of different forms of partial Mantel tests, with or without spatial autocorrelation, had been spelled out. The first article raised a valid point about a situation requiring a particular permutation procedure, but it left readers with the impression that the partial Mantel test is, in general, an inadequate testing procedure, which is not the case. The second article tried to rehabilitate the partial Mantel test, but advocated an inappropriate testing procedure. Comments presented in Appendix S3 attempt to clarify some of the underlying concepts. Appendix S4 gives recommendations about appropriate testing procedures for partial Mantel tests. This issue is addressed here because there is still some confusion among users about the validity of the permutation test used in partial Mantel tests.

et al. 2009; Guillot *et al.* 2009). These methods involve transforming the geographic coordinates of the sites into derived geographic functions (Fig. 1b): polynomial of the geographic coordinates (leading to polynomial canonical trend-surface analysis, Legendre 1990), Moran's eigenvector maps (mentioned in Table 1, specifically called PCNM or MEM spatial eigenfunctions analysis: Borcard & Legendre 2002; Borcard *et al.* 2004; Dray *et al.* 2006) or asymmetric eigenvector maps (AEM: Blanchet *et al.* 2008). These analyses are carried out by using the derived geographic functions as explanatory variables in multiple regression, canonical analysis or variation partitioning among environmental and spatial components (Borcard *et al.* 1992; Borcard & Legendre 1994). Moran's eigenvector maps can also be used in multiscale ordination (Wagner 2004). In landscape genetic studies, landscape features between sampling locations are often of interest; they can be incorporated in the analysis as resistance or

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Table 1 Representation of environmental and spatial data to test different types of hypotheses. 'Factor' is a generic term for multistate qualitative (or categorical) variables. Distance matrices **D** (right column) can easily be computed from rectangular data tables (left column). One can also go from a **D** matrix to a rectangular data table by principal coordinate analysis (metric, or classical, multidimensional scaling).

Linear models	Mantel test and derived forms
Landscape genetics hypothesis: response data are related to environmental variables or experimental factors	
Environmental variables (quantitative, binary, or factor)	**D** matrix computed from the environmental variables
Experimental (ANOVA) factor	Design matrix **D** describing the factor
Spatial population genetic: response data are related to 'space'	
Geographic regions (factor)	Design matrix **D** representing regions
Geographic coordinates (quantitative)	**D** matrix of Euclidean geographic distances or log of geographic distances
Polynomial of geographic coordinates	**D** matrix from polynomial of geographic coordinates
Moran's eigenvector maps (spatial eigenfunctions) computed from connection diagrams (e.g., Delaunay triangulation) or geographic coordinates	Design matrix **D** containing connecting links between neighbouring sites. The links may be binary (absence of link = 0, presence = 1) or weighted by any appropriate form of distance on a map (Euclidean, or least-cost, or along practicable paths)

cost values and assembled in resistance/cost distances that are used instead of Euclidean geographic distances (Fig. 1b).

Specialized statistical packages used by evolutionary biologists offer Mantel test modules, distance-based RDA and multiscale analysis to help answer these questions (e.g., Genepop: Raymond & Rousset 1995; Arlequin: Excoffier *et al.* 2005; GENALEX: Peakall & Smouse 2006; NTSYSpc: Rohlf 2009; in the R language: ade4: Dray *et al.* 2007; vegan: Oksanen *et al.* 2010; ape: Paradis *et al.* 2009; ncf: Bjørnstad 2009).

Statistical aspects

Equivalence of rectangular data and distance matrices for sum-of-squares statistic

Whether the data form a vector **y** or a rectangular data table **Y** about objects (individuals or local populations), the total variance of the data can be computed. For a single variable **y** measured about n objects, the SS can be computed as $\mathrm{SS}(\mathbf{y}) = \sum_{i=1:n} (y_i - \bar{y})^2$ or, after computing a Euclidean distance matrix $\mathbf{D} = [D(y_i, y_h)] = [D_{ih}]$ among the values of **y**, $\mathrm{SS}(\mathbf{y}) = \left(\sum_{i \neq h} D(y_i, y_h)^2\right)/n$, which can be written in the more compact form $\left(\sum_{i \neq h} D_{ih}^2\right)/n$. Only the D_{ih} values in the upper-triangular portion of **D** are used in this formula. The equivalence of the two formulas can easily be checked using any set of numerical values.

In the multivariate case, where matrix **Y** contains p variables, the formula for the SS computed from the raw data is:

$$\mathrm{SS}(\mathbf{Y}) = \sum_{j=1:p} \sum_{i=1:n} (y_{ij} - \bar{y}_j)^2 \quad (1)$$

The formula computed from Euclidean distances is as above:

$$\mathrm{SS}(\mathbf{Y}) = \left(\sum_{i \neq h} D_{ih}^2\right)/n \quad (2)$$

Note that the division is by the number of objects n, not by the number of distances involved in the calculation. The values from eqns 1 and 2 for SS(**Y**) are equal (Appendix S1; Legendre & Legendre 1998, Eqs. 8.5 and 8.6). The ordinary unbiased estimate of the total variance in the data is obtained by dividing SS(**y**) or SS(**Y**) by $(n - 1)$. The equality of eqns 1 and 2 only holds for Euclidean distances. If the distance matrix has been computed using another formula than the Euclidean distance function, the SS obtained from eqn 2 is no longer equal to the SS of the original data (eqn 1). Equation 2 can be used in least-squares algorithms that compute statistics in the distance world, e.g. for *K*-means partitioning of objects based upon a question-specific distance matrix.

Null hypotheses

A test of statistical significance involves three main components: a null hypothesis, a test statistic and a reference distribution under the null hypothesis to assess the significance of the statistic with respect to that hypothesis. The null hypothesis (H_0) in a test of the correlation coefficient states that the correlation (linear or monotonic) between the variables in the reference population is zero ($\rho = 0$); the formulation of H_0 in terms of a linear or

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monotonic relationship determines the choice of a linear or rank correlation coefficient as the test statistic. In simple or multiple linear regression, the null hypothesis states that the explanatory variables used in the analysis explain no more of the response variable's variation than would random variables with the same distributions. The test statistic is the F statistic derived from the coefficient of determination (R^2 statistic) of the regression. For multivariate response data, the same type of null hypothesis can be tested using canonical redundancy analysis (RDA); the same R^2 and F statistics as in linear regression are used for testing.

Users of the Mantel test often overlook the fact that the test assumes a linear relationship between the *distances* in the two **D** matrices under study. The null hypothesis of the Mantel test states that the distance matrices are unrelated in some specified way (linear relationship). This assumption can be relaxed to that of a monotonic relationship by using the Spearman instead of the Pearson correlation to compute r_M, as suggested by Mantel (1967) and Dietz (1983). Distances can also be transformed using logs or other simple functions, but more complex forms of nonlinearity cannot easily be handled by the Mantel test. Splines and other nonlinear smoothing methods can, however, be used on distance–distance or distance-similarity dispersion diagrams to fit a curve to the plot, helping the eye see the shape of the relationship (e.g., Figs 2 and 3 in 'Spatial gradients' section on spatial gradients). A relationship which is linear for the raw data may become nonlinear when using distance matrices, as will be shown in the 'Spatial gradients' section. The Mantel statistic can be tested by an appropriate form of permutation (the 'matrix permutation' briefly described in the 'Bivariate case' section) or, if n is large, transformed into a statistic called t by Mantel (1967) and tested with reference to the standard normal distribution.

Correspondence between data, question and method of analysis

Before using a statistical model or a testing method to interpret observational or experimental data, the following questions must be carefully examined and answered:

1 Is the chosen statistic appropriate to the data and to the hypothesis to be tested (Fig. 1, Table 1)? We will show in 'Different sum-of-squares statistics' that the statistics computed in correlation and Mantel analysis are fundamentally different. Correlation statistics (linear or monotonic) are appropriate to test a hypothesis of correlation between variables. Mantel statistics (linear or monotonic) are appropriate to test hypotheses that only concern and can only be formulated in terms of distances.

2 Does the method have a correct type I error rate? Simulation studies can be used to assess the rate of type I error of statistical methods in situations where there is no effect ($\rho = 0$ in correlation analysis) corresponding to the tested hypothesis.

3 Given the data, is the method the most powerful among those available? Here again, simulation studies can be used to compare the power of statistical methods in situations where there is an effect corresponding to the tested hypothesis ($\rho \neq 0$ in correlation analysis).

To test different types of hypotheses in spatial population genetics (spatial data) and landscape genetics (spatial and environmental data) about the underlying processes affecting genetic spatial structures (Fig. 1), Table 1 shows how environmental and spatial data can be represented in linear models and in distance-based Mantel tests.

Different sum-of-squares statistics

When rectangular tables of raw data are transformed into distance matrices, a Mantel test between the two distance matrices is not equivalent to a test of the simple correlation between two vectors (for two rectangular tables of size $n \times 1$) or the canonical correlation between two multivariate data tables: the null hypotheses ('Null hypotheses' section) and the test statistics differ. Let us now focus on the test statistics.

Consider two variables $\mathbf{y}$ and $\mathbf{x}$. The formula for the Pearson correlation coefficient between the variables $\mathbf{y_c}$ and $\mathbf{x_c}$ centred on their respective means is:

$$r_{\mathbf{y},\mathbf{x}} = \frac{\sum_{i=1:n}(y_{i\mathbf{c}} \times x_{i\mathbf{c}})}{\sqrt{\sum_{i=1:n}(y_{i\mathbf{c}}^2)}\sqrt{\sum_{i=1:n}(x_{i\mathbf{c}}^2)}} = \frac{\mathbf{y_c}'\mathbf{x_c}}{\sqrt{\mathrm{SS}(\mathbf{y_c})}\sqrt{\mathrm{SS}(\mathbf{x_c})}} \quad (3)$$

In the framework of the linear regression of $\mathbf{y}$ on $\mathbf{x}$, the coefficient of determination $R^2_{\mathbf{y}|\mathbf{x}}$ is the SS of the fitted values (SS of vector $\hat{\mathbf{y}}$) divided by the total sum-of-squares of $\mathbf{y}$ (SS($\mathbf{y}$) at the beginning of section 'Equivalence of rectangular data and distance matrices for sum-of-squares statistic'):

$$R^2_{\mathbf{y}|\mathbf{x}} = \frac{\mathrm{SS}(\hat{\mathbf{y}})}{\mathrm{SS}(\mathbf{y})} \quad (4)$$

so that the bivariate correlation coefficient is:

$$r_{\mathbf{y},\mathbf{x}} = (\mathrm{sign})\sqrt{\frac{\mathrm{SS}(\hat{\mathbf{y}})}{\mathrm{SS}(\mathbf{y})}} \quad (5)$$

where (sign) is the sign of the covariance between $\mathbf{y}$ and $\mathbf{x}$. Equations 3 and 5 produce the same result for r. In the multivariate case, canonical redundancy analysis (RDA) of a matrix $\mathbf{Y}$ by $\mathbf{X}$ produces an R^2 statistic constructed in the same way:

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$$R^2_{\mathbf{Y}|\mathbf{X}} = \frac{\mathrm{SS}(\hat{\mathbf{Y}})}{\mathrm{SS}(\mathbf{Y})} \quad (6)$$

The important point here is that in the univariate and multivariate cases, the sum-of-squares in the denominator of R^2 are SS(**y**) and SS(**Y**), respectively. This is the SS that is partitioned by the regression or RDA into a SS of fitted values SS($\hat{\mathbf{y}}$) or SS($\hat{\mathbf{Y}}$) and a residual sum-of-squares SS($\mathbf{y}_{\mathbf{res}}$) or SS($\mathbf{Y}_{\mathbf{res}}$).

Consider now two distance matrices, $\mathbf{D}_\mathbf{Y}$ and $\mathbf{D}_\mathbf{X}$, computed from vectors **y** and **x** or data tables **Y** and **X**. For the present demonstration, assume that the Euclidean distance function has been used to compute the distances, as in eqn 2. String out the upper-diagonal portions of the two distance matrices as long vectors $\mathbf{d}_\mathbf{Y}$ and $\mathbf{d}_\mathbf{X}$, each of length $n(n-1)/2$. The Mantel correlation, r_M, is the correlation coefficient computed from these two vectors, as shown in Legendre & Legendre (1998, Fig. 10.19) and other textbooks. It is also the square root of the coefficient of determination R^2_M of the linear regression of $\mathbf{d}_\mathbf{Y}$ on $\mathbf{d}_\mathbf{X}$:

$$R^2_\mathrm{M} = R^2_{\mathbf{dY}|\mathbf{dX}} = \frac{\mathrm{SS}(\hat{\mathbf{d}}_\mathbf{Y})}{\mathrm{SS}(\mathbf{d}_\mathbf{Y})} \quad (7)$$

so that

$$r_\mathrm{M} = (\mathrm{sign})\sqrt{\frac{\mathrm{SS}(\hat{\mathbf{d}}_\mathbf{Y})}{\mathrm{SS}(\mathbf{d}_\mathbf{Y})}} \quad (8)$$

where (sign) is the sign of the covariance between $\mathbf{d}_\mathbf{Y}$ and $\mathbf{d}_\mathbf{X}$. Again, we have the SS of the vector of distances $\mathbf{d}_\mathbf{Y}$ in the denominator of the R^2 equation. What is that value? We can compute it as:

$$\mathrm{SS}(\mathbf{d}_\mathbf{Y}) = \sum_{i\neq h}(D_{ih\mathbf{Y}} - \bar{D}_\mathbf{Y})^2 = \sum_{i\neq h}(D_{ih\mathbf{Y}})^2 - \frac{\left(\sum_{i\neq h} D_{ih\mathbf{Y}}\right)^2}{n(n-1)/2} \quad (9)$$

This formula is written using D_{ih} values to make it comparable to eqn 2. For symmetric distance matrices, only the D_{ih} values in the upper-triangular portion of **D** are used. The main point here is that SS($\mathbf{d}_\mathbf{Y}$) in eqn 9 is not equal to, is not a simple function of, and cannot be reduced to SS(**Y**) in eqn 2.

To summarize, the sum-of-squares SS(**Y**) (eqns 1 or 2) and SS($\mathbf{d}_\mathbf{Y}$) (eqn 9) are different statistics. As a consequence, the coefficients of determination constructed with SS(**Y**) or SS($\mathbf{d}_\mathbf{Y}$) in the denominator ($R^2_{y|\mathbf{x}}$ for two variables or $R^2_{\mathbf{Y}|\mathbf{X}}$ for two matrices analysed by RDA on the one hand, and $R^2_{\mathbf{dY}|\mathbf{dX}}$ for two distance matrices analysed by the Mantel test on the other), and the corresponding coefficients of correlation (Pearson r for two variables, Mantel r_M for two distance matrices), are also different statistics: they do not measure the same relationship.

Consider the numbers 1–10 for example. Their total sum-of-squares, SS(**y**), is 82.5 (eqn 1). Now compute a Euclidean distance matrix **D** among these 10 numbers: the sum-of-squares SS($\mathbf{d}_\mathbf{Y}$) from eqn 9 is 220. So R^2, the coefficient of determination or the square of the Pearson correlation between two vectors, which represents a fraction of eqn 1, cannot be equal to R^2_M, the square of the Mantel correlation between the derived distance matrices, which is a fraction of eqn 9.

The two families of statistical methods will also diverge, perhaps more, when the raw data tables are transformed into distance matrices **D** using non-Euclidean distance functions that are specific to the field of application, such as genetic or ecological distances. It should now be clear that testing the relationship between two variables and rectangular data tables is not equivalent to testing the relationship between distance matrices derived from them.

Empiricists who frown upon theoretical justifications should be interested in the fact that the values of R^2_M of a Mantel test or a regression on distance matrices are always much lower than those of the R^2 of a (multiple) regression or canonical analysis computed on the raw data (when it is possible to do so), as will be seen in the simulation results reported in section the 'Bivariate case'. This was one of the results reported by Dutilleul *et al.* (2000, Table 2) who worked out the relationships between the theoretical correlation between two simple variables and the expected value of the Mantel statistic for distance matrices computed from these two variables, under the assumption of normality. So, R^2_M cannot be used as a measure of explained variance for the original data.

Summary of findings – The Pearson correlation r and Mantel r_M statistics are based on different sums-of-squares that are not equal, are not a simple function of, and cannot be reduced to each other. The Pearson correlation is a statistic describing the linear relationship between the variables (monotonic relationship in the case of the Spearman correlation), whereas the Mantel statistic based on the Pearson formula describes the linear relationship between distances (or a monotonic relationship if the Spearman formula is used).

Bivariate case

Monte Carlo simulations allow researchers to compare statistical methods in situations where they know the exact relationship between the variables. No doubt exists as to which, of H_0 or H_1, is true in each particular simulated data set (Milligan 1996). Legendre (2000) simulated bivariate data to compare the power of the Pearson corre-

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lation and Mantel tests in situations where the correlation coefficient between two simple variables was appropriate. We completed and expanded these simulations here. Two vectors of random normal deviates, **y** and **x**, each of length n = {10, 30, 50, 100}, were generated and correlated by a predetermined population value $\rho(\mathbf{y},\mathbf{x})$ = {−0.5, 0, 0.5}. H_0 was true when $\rho = 0$ and false when $\rho(\mathbf{y},\mathbf{x}) = -0.5$ or 0.5. One-tailed tests (parametric and permutational using 999 random permutations) of Pearson's r correlation coefficient were conducted in both the lower and upper tails; only the test in the tail corresponding to the sign of ρ made sense in each case because we wanted to assess the power of the tests to detect the imposed population correlation. Note that a permutation test using Pearson's r as the test statistic, as was carried out here, is strictly equivalent to a test based on the pivotal t-statistic derived from r; this is because t is a strictly monotonic function of r for any constant value of n (Legendre & Legendre 1998, Section 1.2.2). The two vectors were transformed to distance matrices $\mathbf{D}_\mathbf{y}$ and $\mathbf{D}_\mathbf{x}$, and a permutational Mantel test was run using 999 random permutations of the objects identifying the rows and columns in one of the distance matrices; this form of permutation is called 'matrix permutations' in Legendre (2000). As in the case of Pearson's r, one-tailed tests were conducted in both the lower and upper tails. Power was the rejection rate of H_0 at the 5% significance level after 10,000 independent simulations.

The simulation results (Table 2) lead to the following observations:

1 To be valid, a test of significance should have a rate of rejection of the null hypothesis not larger than the significance level α, for any value of α, when H_0 is true (Edgington 1995). The results in Table 2 (first 4 lines, $\rho = 0$) show that, for data with normal error, the parametric and permutation tests of the Pearson correlation coefficient had correct levels of type I error, with rejection rates near the significance level $\alpha = 0.05$. This was also the case for the permutational Mantel test. Similar results had been found by Legendre (2000, Fig. 1a–b). The additional information provided in Table 2 is that the means of the Pearson correlations and Mantel statistics were both near 0. However, Dutilleul *et al.* (2000) described a few cases where the values of the Mantel statistics are negative, whereas the Pearson correlation is strictly 0; their Table 4 also shows cases, for real bivariate data, where the signs of the Mantel statistics varied but were unrelated to the signs of the Pearson correlations.

2 In the simulations where the data vectors were positively correlated ($\rho = 0.5$), the parametric and permutational tests of the Pearson correlation in the upper tail always had greater power (the rejection rate of H_0 was higher) than the Mantel test. The difference in power between the test of Pearson's r and the Mantel test is because of the fact that the two tests use different statistics, as explained in the section 'Different sum-of-squares statistics'.

3 In the simulations where the data vectors were negatively correlated ($\rho = -0.5$), the tests of Pearson's r that made sense were those conducted in the lower tail because we wanted to estimate the power of the test to detect a negative correlation. The test in the lower tail showed power that increased with n, as expected. Tests conducted in the upper tail seldom detected a

Table 2 Comparison of power of the tests of the Pearson correlation (parametric and permutation tests) and the simple Mantel test (permutation test) in simulations where the correlation coefficient between two simple variables was appropriate. n is the number of objects in each simulation; $\rho(\mathbf{y},\mathbf{x})$ is the population correlation value imposed to the two data vectors. Power is the rejection rate of H_0 at the 5% significance level after 10000 independent simulations. 't-test of r' = parametric test of the t-statistic associated with Pearson's r; 'perm. test r' = permutational test of r; 'lower tail' and 'upper tail' refer to the opposite tails in one-tailed tests.

n	$\rho(\mathbf{y},\mathbf{x})$	Mean of Pearson r	Power of t-test of r lower tail	Power of t-test of r upper tail	Power of perm. test r lower tail	Power of perm. test r upper tail	Mean of Mantel r	Power of Mantel test lower tail	Power of Mantel test upper tail
10	0	−0.0022	0.0447	0.0495	0.0476	0.0497	−0.0029	0.0498	0.0469
30	0	0.0013	0.0497	0.0491	0.0487	0.0485	−0.0008	0.0503	0.0489
50	0	0.0001	0.0508	0.0504	0.0507	0.0505	−0.0006	0.0503	0.0516
100	0	−0.0003	0.0515	0.0480	0.0522	0.0485	0.0002	0.0518	0.0517
10	0.5	0.4772	0.0011	0.4554	0.0010	0.4529	0.1907	0.0145	0.2792
30	0.5	0.4935	0.0000	0.8976	0.0000	0.8963	0.2142	0.0021	0.6283
50	0.5	0.4949	0.0000	0.9833	0.0000	0.9834	0.2164	0.0003	0.8071
100	0.5	0.4967	0.0000	0.9999	0.0000	0.9999	0.2194	0.0001	0.9679
10	−0.5	−0.4814	0.4591	0.0019	0.4568	0.0016	0.1971	0.0128	0.2828
30	−0.5	−0.4926	0.8995	0.0000	0.8982	0.0000	0.2133	0.0013	0.6279
50	−0.5	−0.4954	0.9855	0.0000	0.9854	0.0000	0.2181	0.0003	0.8039
100	−0.5	−0.4991	1.0000	0.0000	1.0000	0.0000	0.2217	0.0000	0.9691

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significant correlation (and this only happened with $n = 10$), also as expected.

4 An interesting point here is that the mean of the Mantel statistics was positive for data vectors generated with $\rho = -0.5$. Because Euclidean (i.e. 'unsigned') distances had been used, the Mantel test only detected positive relationships between distances despite the fact that the original data vectors were negatively correlated. Even in that case, one expects the distances in the two matrices to increase together if any effect at all is detected. Mantel tests in the lower tail nearly never detected a significant relationship. Note that the parametric and permutational tests of the Pearson correlation in the lower tail always had greater power than the Mantel test in the upper tail.

5 The mean of the Pearson correlations was always close to the imposed population mean ρ. The small difference when $n = 10$ is because of the distributions of simulated correlations, which is highly skewed to the left when n is small and $\rho > 0$, and to the right when n is small and $\rho < 0$. The mean of the Mantel statistics was always much smaller than $|\rho|$, a phenomenon that was noted and explained by Dutilleul *et al.* (2000) for the bivariate normal case.

Summary of findings – Users of the Mantel test should be aware of three facts when they are analysing data and testing a bivariate correlation hypothesis: (i) the test of the Pearson correlation has much greater power than the Mantel test to detect a linear relationship between data vectors; this means that the test of Pearson's r is more likely than the Mantel test to detect a relationship when it is present in the data. (ii) When the correlation between the two original vectors is negative, the Mantel test cannot detect its sign: it finds a positive relationship in the world of distances. Using a Mantel two-tailed test to detect a relationship among distances whatever its sign is not a good solution either because two-tailed tests have less power than one-tailed tests. (iii) The value of the Mantel statistic is always much smaller than the population correlation, so it cannot be used as an estimate of that correlation.

Spatial gradients

Spatial gradients can arise in genetic data as a result of several types of processes. They can appear, for example, during secondary contact between distant and temporarily isolated populations that have diverged, or as a response of non-neutral alleles to environmental gradients. Spatial gradients can also result from processes such as sequential colonization events. For example, the colonization of the world by humans has led to a pattern where genetic distance between the ancestral and descendant populations increases with geographical distance (Ramachandran *et al.* 2005). Spatial patterns can therefore be observed in the heterozygosity, which decreases as the distance from the ancestral population increases (Prugnolle *et al.* 2005; Ramachandran *et al.* 2005; Foll & Gaggiotti 2006). Other gradients that mimic those

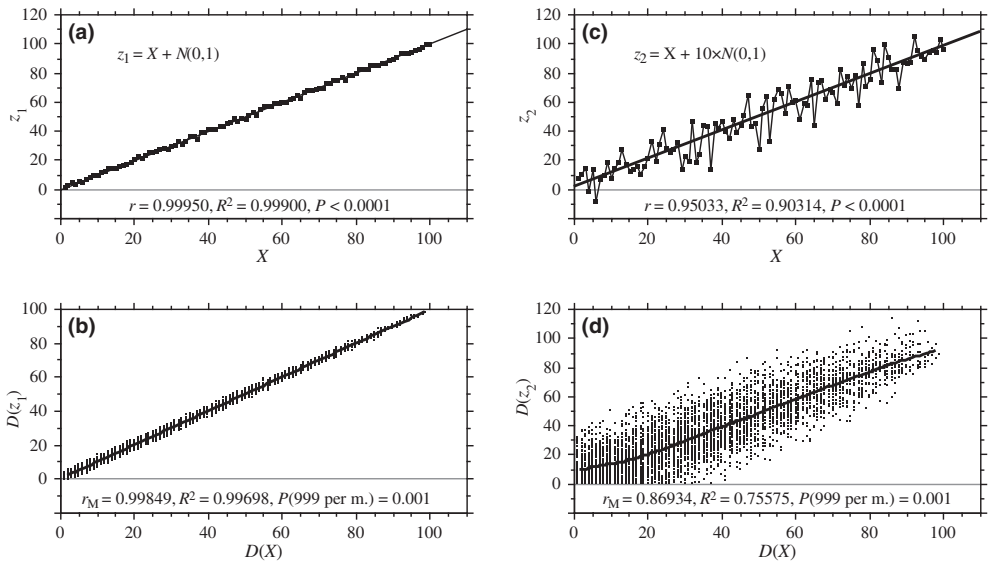

Fig. 2 One hundred points forming a linear gradient in a single geographic dimension (transect X), (a,b) with a small amount of noise, (c,d) with more noise. (a,c) One-dimensional maps of the gradients, with regression lines; X is the geographic axis, z the response variable. (b,d) Scatter plots of the distances in the response variable $D(z)$ compared to the geographic distances $D(X)$, with a smoother function ('supersmoother'). r is the Pearson correlation coefficient, r_M the Mantel statistic.

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generated by selection may arise through allele surfing (Edmonds *et al.* 2004). All these processes can also be strongly influenced by genetic drift.

Some other types of genetic processes are expected to produce autocorrelation in the genetic data, but not gradients per se: genetic drift, gene flow, dispersal, isolation by distance, isolation by resistance (McRae 2006), etc. (Fig. 1c). Autocorrelation can be studied through univariate correlograms and multivariate Mantel correlograms, or modelled by regression or canonical analysis using Moran's eigenvector maps (spatial eigenfunction analysis: Introduction, penultimate paragraph) or multiscale ordination (Wagner 2004) (Fig. 1b).

Using a simple example, we will now show that in population genetic studies of adaptive genes along spatial gradients, a significant correlation between vectors and matrices of raw data does not guarantee that a significant correlation will be identified by the Mantel test in the corresponding distance matrices. We simulated a variable z forming a linear gradient in one or two geographic dimensions, with random normal error $\varepsilon = N(0,1)$, using the equation $z = f(X,Y) + k\varepsilon$; X and Y are the geographic coordinates of the points. The amount of error, k, will vary from 1 to 10.

In Fig. 2, the simulated structure is a linear gradient along a transect (100 points). In Fig. 3, we simulated a gradient running diagonally across the map. The equations used for generating the response variable z are shown on, or on the side of the maps. These simulations will illustrate the fact that (i) the amount of error affects the power of the Mantel test more than it does the test of the correlation coefficient in this simple form of trend-surface analysis, and (ii) the Mantel test is more likely to identify the gradient along a transect than on a map.

The basic amount of error was $k = 1$ in Figs 2a,b and 3a,b; in other words, a local innovation was generated by adding a random normal deviate $N(0,1)$ to the gradient value at each point, creating values with a standard deviation near 3 along 10 points of the gradient in the two spatial structures. In the case of the transect, the Mantel test (Fig. 2b) successfully identified the gradient. So we created a more difficult problem in Fig. 2c,d where the noise parameter k was 10. There is more dispersion around the smoother line in Fig. 2d than in Fig. 2b, but the gradient remains mostly linear, as shown by the smoother line, and the Mantel test still identifies it successfully.

A gradient with low noise ($k = 1$) running diagonally across the map was easily detected by regression on the X and Y geographic coordinates (trend-surface analysis, Fig. 3a: $\sqrt{R^2} = r = 0.92$) and by the Mantel test (Fig. 3b: $r_M = 0.49$). Increasing the amount of noise to $k = 2$, the relationship between distance matrices **D** lost monotonicity and became more difficult to detect (Fig. 3d: $r_M = 0.30$), although it was easily detected by regression (Fig. 3c: $\sqrt{R^2} = r = 0.76$). The power of the Mantel test

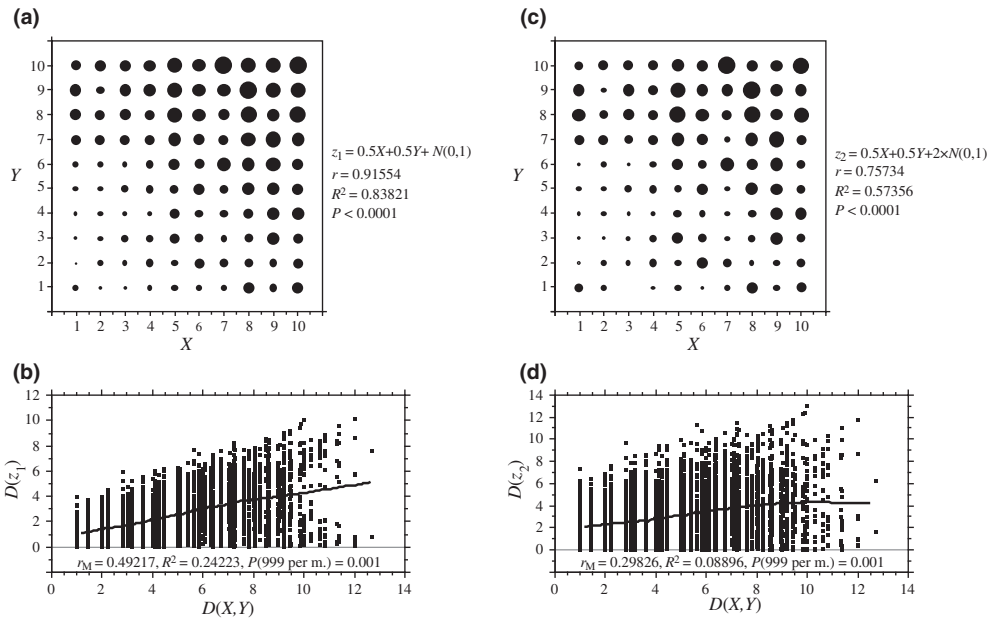

Fig. 3 One hundred points forming a linear gradient running diagonally across a surface (X,Y), (a,b) with a small amount of noise, (c,d) with more noise. (a,c) Bubble plot maps of the gradients: X and Y are the geographic coordinate axes, z the response variable; the size of the circles is proportional to the value of z. (b,d) Scatter plots of the distances in the response variable $D(z)$ compared to the geographic distances $D(X,Y)$, with a smoother function ('supersmoother').

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was affected by the loss of monotonicity of the distance relationship as k increased. Figure 4 reports the results of the tests carried out by trend-surface analysis and by the Mantel test, for k varying from 1 to 10. Trend-surface analysis (circles) produced a significant equation for all values of k in the graph, whereas the Mantel test lost significance, for an α-level of 0.05, from $k = 6$ and on. The one-tailed test of R^2 would be equivalent to a two-tailed test of r in the simple linear regression case (e.g. a transect). Because a one-tailed test has more power than a two-tailed test, the comparison in Fig. 4 should have given advantage to the one-tailed Mantel test had the two tests had equivalent power for detecting the gradient in the data.

Summary of findings – These simulated examples show that the power of the Mantel test is lower than that of trend-surface analysis for the detection of noisy spatial gradients. Because of its omnidirectional nature, the Mantel test is not ideal for the detection of directional spatial structures on two-dimensional maps, although it behaves well along transects for testing hypotheses formulated in terms of distances.

Multivariate, spatially structured data

Appendix S2 summarizes the results, published elsewhere (Legendre *et al.* 2005), of numerical simulations conducted to empirically compare the power of canonical redundancy analysis (RDA) and Mantel tests to detect environmental signature and autocorrelated spatial structures in multivariate response data. Autocorrelation is the hypothesized outcome of many spatial processes in population genetic data. This is one of the types of question addressed in landscape genetics where researchers are trying to understand genetic variation in terms of neutral genetic processes and selective responses to environmental conditions.

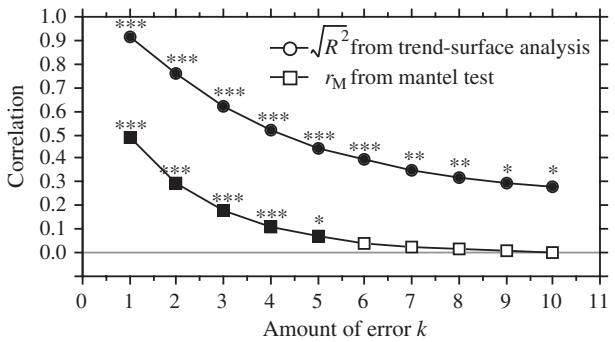

Fig. 4 Analysis of surfaces with different amounts of noise k, generated using the equation $z = 0.5X + 0.5Y + k\varepsilon$ where ε is a random standard normal deviate. R^2 is the coefficient of determination of the trend-surface regression equation; r_M is the standardized Mantel statistic. Closed symbols: statistics significant at ***, $P \leq 0.001$; **, $0.01 \geq P > 0.001$; *, $0.05 \geq P > 0.01$. Open symbols: $P > 0.05$. Trend-surface regressions: the one-tailed test of R^2 would be equivalent to a two-tailed test of r in the simple linear regression case. Mantel correlations: one-tailed tests.

Summary of findings – (i) Permutation tests used in the analysis of rectangular data tables by regression or canonical analysis, or in the analysis of distance matrices by Mantel tests, all have correct levels of type I error; so they are all statistically valid. For regression analysis, this has been shown by a number of authors including Anderson & Legendre (1999); for canonical redundancy analysis, by Legendre *et al.* (2005); for Mantel tests, by Legendre (2000) and Legendre *et al.* (2005). (ii) For the detection of multivariate species-environment relationships, linear analysis by RDA has far greater power than methods based on distance matrices. This means that when a relationship is present in data, one is much more likely to detect it by RDA than by Mantel test or regression on distance matrices. The differences in power reported in Table A2.1 (Appendix S2) between RDA and Mantel test results are in line with the differences found in the section 'Bivariate case' between Pearson r and Mantel r_M (Table 2). (iii) For autocorrelated data, each method (RDA and Mantel) had variants that did better than using the X and Y coordinates only, but RDA always outperformed the distance-based Mantel tests. The differences in power reported in Table A2.1 between RDA and Mantel test results are in line with the differences shown in Fig. 4 (univariate response data) between the R^2 statistic of trend-surface equations and Mantel r_M. (iv) These conclusions should apply to all types of response data. The allele frequency and other types of frequency data analysed in the frameworks of spatial population genetics and landscape genetics are of the same type as the species abundances that served as the reference for the simulations reported in Table A2.1.

Other statistical aspects

A major handicap of the raw data approach (i.e., the analysis of rectangular data tables) has been lifted in recent years for the linear analysis of multivariate frequency data, such as allele frequencies and community composition data: Legendre & Gallagher (2001) have shown how to transform community composition data in such a way that distances that are of interest in genetics and community ecology (e.g., the chord, chi-square and Hellinger distances) are preserved in the analysis. These simple transformations of the frequency data make them suitable for linear analyses such as PCA, RDA and K-means partitioning. The first step of these transformations is to standardize by row (in different ways, depending on the transformation), thus removing from the analysis the dif-

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ferences in row sums, which correspond to the total number of individuals per site included in the genetic analysis, or the total productivity of the sites in community ecology. The chord distance has a long history of application in genetic analysis. The Hellinger distance is the chord distance computed on square-root-transformed frequencies.

Our simulation results (Table 2, Figs 2–4, Appendix S2) have shown that the Pearson r and Mantel r_M statistics are quite different in values. For that reason, the Mantel R^2_M statistics that serve as the basis for variation partitioning based on distances are not equal to the R^2 statistics from linear regression or canonical analysis that serves as the basis for linear variation partitioning. So the fractions of variation obtained by variation partitioning in the distance world are incorrect when they pretend to represent fractions of the original data variation. Their exact meaning has never been explained by the proponents of that method.

Another way of looking at this problem is the following: the square of the Mantel r statistic may be called an R^2, but it is not a coefficient of determination as known in linear models (regression, canonical analysis), and it cannot be interpreted as the proportion of the response variables' (**Y**) variance explained by **X**, but only as a measure of fit of a linear model to the paired sets of distances. The distances used to compute $SS(\mathbf{d_Y})$ and the fitted values used to compute $SS(\hat{\mathbf{d}}_\mathbf{Y})$ are not independent of one another within each set, as they are in a linear regression R^2.

A Mantel test only produces an r statistic and a P-value. Canonical analysis produces results that are much richer: biplots are produced, and the contribution of each response and explanatory variable is computed and can be examined in biplots. This is another reason to prefer canonical analysis to analyse the variation of multivariate response variables such as allele frequencies.

Another drawback is that an adjusted R^2 (R^2_{adj}) cannot be computed from Mantel statistics: no equation has been proposed and demonstrated to produce an unbiased adjusted R^2 (R^2_{Madj}) in Mantel-type regression. An adjusted R^2 is required to obtain unbiased estimates of the fractions in variation partitioning (Peres-Neto *et al.* 2006). Those who insist on interpreting the square of the Mantel r_M as a coefficient of determination are left with, at best, a biased estimate.

Additivity is a nice property of linear variation partitioning: an identical total amount of explained variation of **Y** is obtained, whether all explanatory variables are put in a single table **X** or they are divided into any number of tables. The effects of the explanatory variables are thus additive. This is not the case in partitioning on distances: different total amounts of explained variation for the response **D**(**Y**) are obtained if one includes all explanatory variables in a single distance matrix **D**(**X**) or if separate distance matrices are computed for the groups of explanatory variables $\mathbf{D}(\mathbf{X}_1)$, $\mathbf{D}(\mathbf{X}_2)$, ..., $\mathbf{D}(\mathbf{X}_k)$ (Legendre *et al.* 2008). Likewise, transforming **Y** and **X** into distance matrices $\mathbf{D_Y}$ and $\mathbf{D_X}$ and carrying out a Mantel test does not produce a test equivalent to that of multiple regression or canonical analysis, as we saw earlier on theoretical bases and in the simulation results of sections 'Bivariate case', 'Spatial gradients' and 'Multivariate spatially structured data'. Furthermore, if **X** is divided into various subsets $\mathbf{X}_1, \mathbf{X}_2, \ldots, \mathbf{X}_k$, and each of these explanatory subsets is transformed into a distance matrix $\mathbf{D}_{\mathbf{X}_1}, \mathbf{D}_{\mathbf{X}_2}, \ldots, \mathbf{D}_{\mathbf{X}_k}$, a Mantel test of $\mathbf{D_Y}$ against $\mathbf{D_X}$ is not equivalent to, and does not produce the same result as, a test of the statistic of a multiple regression of $\mathbf{D_Y}$ on the set of distance matrices $\mathbf{D}_{\mathbf{X}_1}, \mathbf{D}_{\mathbf{X}_2}, \ldots, \mathbf{D}_{\mathbf{X}_k}$. The latter method should only be used when the hypothesis to be tested clearly involves a subdivision of the explanatory variables into precise subsets. Until all these statistical points have been cleared, variation partitioning should not be performed on distance matrices.

Discussion

In this study, we are concerned with the power of the Mantel test in situations where the primary question or hypothesis involves relationships between raw data. We are warning population geneticists that in these cases, statistical analyses based on distances lead to a large loss of statistical power; power is the ability of a statistical method to detect an effect when one is present in the data. We have shown the loss of power in studies of relationships between variables and rectangular data tables, which are turned into distance matrices, with special emphasis on the situation where one of the data tables represents spatial relationships among the study sites. In situations where the question or hypothesis is clearly formulated in terms of the raw data (vectors or rectangular data tables), Mantel tests should not be used. Legendre *et al.* (2005, pp. 438–439) give several examples of such misuses in the community ecology literature.

In spatial population genetics and landscape genetics, many research questions involve distance relationships. For example, the effect of landscape structure on movement, mating and gene flow among individuals or populations is usually studied by making predictions about relationships between matrices of genetic distance and landscape cost and testing these predictions by Mantel tests, whereas adaptive variation along environment gradients is studied using regression and canonical analysis of raw data tables. These statistical methods seem to be interchangeable (Table 1) because of the easiness with which a distance matrix can be computed from a raw data table, or the opposite – going from a distance matrix

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to a rectangular data table – using principal coordinate analysis. The following questions will have to be addressed by future research and articles:

1 Consider the isolation by distance hypothesis for example: it makes predictions about relationships between genetic and geographic distance, and these predictions can be tested using Mantel tests or regression on distance matrices. It also predicts that autocorrelation should be present in the genetic response data; this prediction can be tested using univariate or multivariate correlogram analysis, or by regression or canonical analysis using Moran's eigenvector maps (spatial eigenfunction analysis).
2 Predictions of relationships between distance matrices can be tested using Mantel tests, but the same relationships could also be tested by canonical analysis after transforming the distance matrices into rectangular matrices through principal coordinate analysis; this is called distance-based (db) canonical analysis (db-RDA, db-CCA: Legendre & Anderson 1999; example of application to genetic distance data: Geffen *et al.* 2004) (Fig. 1c). For questions formulated in terms of distances, which statistical method has the highest power remains to be determined. This question should be examined by numerical simulations carried out by working groups of population geneticists.

Scientists should use multiple regression (for a single response variable) or canonical redundancy analysis (RDA) when investigating response-environment relationships or spatial structures, unless the hypothesis to be tested is strictly formulated in terms of distances (or involves the variance of the distances). The reasons are the following: (i) the null hypothesis of the Mantel test involves distances, whereas those of correlation analysis, regression analysis and RDA involve the original variables (rectangular data tables); and (ii) correlation analysis and RDA lead to higher R^2 statistics and offer a more powerful test than Mantel analysis in tests of hypothesis involving relationships among the original variables.

The second point is supported by the results of simulations reported in this study. The section 'Bivariate case' showed that for testing a bivariate correlation hypothesis, e.g. between a response and an environmental variable, the test of the Pearson correlation has much greater power than the Mantel test to detect a linear relationship between data vectors; this means that the test of Pearson's r is more likely than the Mantel test to detect a relationship when it is present in the data. Using examples, we showed in the section 'Spatial gradients' that the power of the Mantel test is lower than that of trend-surface analysis for the detection of noisy spatial gradients. In the section 'Multivariate spatially structured data' and Appendix S2, we reported the results of extensive simulations showing that for the detection of species-environment relationships or spatial structures in the multivariate response data (e.g. several species, several alleles), linear analysis by RDA has far greater power than methods based on distance matrices. This means that when relationships of these types are present in the response data, one is much more likely to detect them by RDA than by Mantel test or regression on distance matrices. These empirical findings are not surprising given the fact that the SS involved in the denominator of the Pearson correlation (or partitioned by multiple regression or RDA), and that of the Mantel test and derived forms (such as linear regression on distance matrices), are not equal, are not a simple functions of, and cannot be reduced to each other (section 'Different sum-of-squares statistics').

The domain of application of the methods of comparison based on distance matrices (Mantel test, QAP, partial Mantel test, ANOSIM, multiple regression on distance matrices) is the set of {evolutionary, genetic, ecological, etc.} questions that are originally formulated in terms of distances. Testing the distance predictions of a hypothesis of isolation by distance in genetics is one of these questions. Isolation by distance also predicts the presence of spatial autocorrelation in the response data, however, and that prediction should best be tested using other methods, including univariate or multivariate correlogram analysis, regression or canonical analysis using spatial eigenfunctions.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1 Two ways of computing SS(Y)

Appendix S2 Simulations involving multivariate, spatially structured data

Appendix S3 Controversy about the validity of the partial Mantel test

Appendix S4 Tests of significance for partial Mantel tests

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