

FORUM

Dismantling the Mantel tests

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Summary

1. The simple and partial Mantel tests are routinely used in many areas of evolutionary biology to assess the significance of the association between two or more matrices of distances relative to the same pairs of individuals or demes. Partial Mantel tests rather than simple Mantel tests are widely used to assess the relationship between two variables displaying some form of structure.
2. We show that contrary to a widely shared belief, partial Mantel tests are not valid in this case, and their bias remains close to that of the simple Mantel test.
3. We confirm that strong biases are expected under a sampling design and spatial correlation parameter drawn from an actual study.
4. The Mantel tests should not be used in case autocorrelation is suspected in both variables compared under the null hypothesis. We outline alternative strategies. The R code used for our computer simulations is distributed as supporting material.

Key-words: landscape ecology, landscape genetics, phylogeography, geographic epidemiology, spatial structure, isolation by distance, isolation by resistance, autocorrelation, type I error, *Loa loa*

Introduction

For the detection of clustering of cancer cases in space and time, Mantel (1967) introduced a test based on permutations. He concluded his article by claiming that this method was general – a claim later relayed by Sokal (1979) – and could be used whenever one has to assess the significance of the correlation between the entries of two square matrices containing ‘distances’ relative to pairs of individuals. Dietz (1983) discussed the efficiency of various measures of correlation and Smouse, Long & Sokal (1986) proposed an extension of the test, referred to as partial Mantel test, and aimed at assessing the dependence between two matrices of distances while controlling the effect of a third distance matrix. The latter may contain phylogenetic distances or plain geographical (Euclidean) distances between pairs of sampling sites, but it may alternatively contain values that attempt to reflect the actual cost for an individual to move across the geographical area (accounting e.g. for the presence of barriers or hostile areas). In the latter case, the distance is known in ecology as ‘cost distance’. It may not enjoy the properties of a mathematical distance (lacking the triangular inequality property), but it is in general correlated with the Euclidean distance.

Since the original papers of Mantel (1967) and Sokal (1979), and despite the fact that (or perhaps because) none of them stated the null hypothesis explicitly, the simple and partial Mantel tests have known to have a tremendous popularity. The Mantel tests are for example used routinely to assess the significance of the association between two matrices of phenotypic or genetic distances. They are also extensively used to assess how a matrix of genetic or phenotypic distances relates to a matrix of geographical distances (see e.g. Legendre & Fortin 2010; and references therein), or to test if such distances can be explained by phylogenetic relatedness. Another classical analysis consists in assessing the significance of the dependence between genetic (or phenotypic) distances and cost distances while ‘controlling for the effect’ of geographical distances through the partial Mantel test (see e.g. Cushman *et al.* 2006; Storfer *et al.* 2007; Balkenhol, Waits & Dezzani 2009). In view of the various tasks above, the Mantel tests have a number of appealing features. First they allow one to synthesize information contained in multivariate data in a single index and hence in a single test; secondly, they allow one to deal with the case outlined above where the ‘distance’ between individuals cannot be expressed as a difference (or combination of differences) between one or several variables (e.g. case of a cost distance); finally, they do not seem to rely on any parametric assumption.

It is well known that the data displaying some form of structure or autocorrelation are ubiquitous in ecology. Their analy-

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sis brings up some statistical issues (Diniz-Filho, Bini & Hawkins 2003) because structure or autocorrelation violates many assumptions made by standard statistical methods.

Regarding the Mantel tests, some concerns regarding the type I error rate and power have been raised. Oden & Sokal (1992) reported a problem for the partial Mantel test for spatially autocorrelated data. Lapointe (1995) found problems with the simple Mantel test when used for the comparison of dendrograms. Raufaste & Rousset (2001) and Rousset (2002) gave an example where the partial Mantel test leads to the wrong conclusion. Lastly, Nunn, Borgerhoff Mulder & Langley (2006) and Harmon & Glor (2010) expressed concerns about the simple and partial Mantel tests when used for phylogenetic comparative analyses. Essentially, all the issues reported by these studies relate to inflated type I error rate or low power.

In a recent review article, Legendre & Fortin (2010) discussed some of these issues, but on the basis of simulations concluded that the simple and partial Mantel tests were valid statistical methods without any recommendation about the conditions of validity. In another study, Cushman & Landguth (2010) carried out a simulation study of performances of the simple and partial Mantel tests in a set up typical of landscape genetics studies. Under the simulation condition considered, they claimed that, in contrast with the simple Mantel test, the type I error rates of the partial Mantel test was not inflated. However, they only reported average correlation coefficients, not actual error rates of the tests.

There is therefore a great confusion about these tests and despite the various criticisms expressed about them, they are still routinely used in many branches of evolutionary biology. In the present article we (i) clarify what assumptions are involved in the use of the simple and partial Mantel tests, (ii) investigate by simulation the effect on the tests of structure (autocorrelation) in the data and show that under some widely encountered conditions and a broad range of parameter values, the simple and partial Mantel tests do not achieve the targeted type I error rate, (iii) analyse theoretically the source of the problem, in particular emphasizing that it results from the permutation procedure common to all variants of partial Mantel tests (iv) outline how existing methods for structured data could be used when the Mantel tests are not valid.

Materials and methods

THE SIMPLE AND PARTIAL MANTEL TESTS

The procedure introduced by Mantel (1967) is as follows: for a data set $(x_i, y_i)_{i=1, \dots, n}$ (i) compute the set of pairwise distances $D_{ij}^x = \text{dist}(x_i, x_j)$ and $D_{ij}^y = \text{dist}(y_i, y_j)$; (ii) compute the scalar product $r = \sum D_{ij}^x D_{ij}^y$; (iii) for a large number of times: (iii-a) draw a random permutation of $\{1, \dots, n\}$ uniformly, (iii-b) compute the set of pairwise distances \tilde{D}_{ij}^x for the vector of permuted x_i s, (iii-c) compute the scalar product $\tilde{r} = \sum \tilde{D}_{ij}^x D_{ij}^y$; (iv) derive a P -value by comparing r to the collection of \tilde{r} values obtained in (iii).

The partial Mantel test introduced by Smouse, Long & Sokal (1986) aims at controlling the effect of a third distance matrix D^S . In this method, the test statistic r is the partial correlation coefficient of D^x and D^y given D^S , the permutation procedure remains the same.

MODELLING FRAMEWORK

A model for one variable structured in space

In a first class of widely encountered problems, x_i is a coordinate that locates individual or deme i (hereafter referred to as unit i for short) in the geographical space or on a phylogenetic tree and y_i is a set of genetic or phenotypic observations relative to unit i . In this case, x can be treated as a deterministic variable and y as a (random) function of the variable x . Testing the dependence of y on x can be done by testing whether $y(x)$ is a random function that displays some form of autocorrelation. This is not the only way to model the dependence between two variables, but it is one way which is relevant to many studies in evolutionary biology.

A model for two variables structured in space

In a second set of problems, both x_i and y_i are phenotypic or genetic observations about unit i . In this case, there is no reason to give a different status (random vs. deterministic) to x and y as they play the same role in the analysis. In this second setting, it is more natural to model both x and y as random functions. These random functions depend on a third variable s which is deterministic, again typically, a coordinate locating an individual on the geographical space or on a phylogenetic tree. In this case, testing the dependence between x and y amounts to testing the dependence between two random functions that are both potentially autocorrelated.

A key point of the present article is that under the two-random-function model and in the presence of autocorrelation, both the simple and partial Mantel tests fail to return the targeted type I error rate. This will be illustrated by a simulation study and then analysed from a more theoretical point of view.

An explicit model to simulate under H_0

A rich and parsimonious family of random functions: the Gaussian random field model. The present simulation study is concerned with evaluating the type I error rate of the Mantel tests for two autocorrelated random functions. Defining H_0 as ' X and Y are independent' is not enough. To be able to analyse the statistical properties of the tests, we need a statistical model allowing us to compare r to the distribution of \tilde{r} . We need a model that is simple enough to be simulated and analysed easily, but rich enough to encompass the main features encountered in real life. A widely used model for autocorrelated variables whose variation is observed throughout the geographical space is the Gaussian random field (GRF) model. A GRF $x(s)$ is a function of the geographic coordinate s such that for any set of locations (s_1, \dots, s_n) , $x = (x(s_1), \dots, x(s_n))$ forms a multivariate Gaussian random vectors, i.e. has a probability density function (pdf) proportional to $\exp[-\frac{1}{2}(x - \mu)' \Sigma^{-1}(x - \mu)]$ (Mardia, Kent & Bibby 1979). In informal terms: x has the well-known bell-shaped pdf centred around μ , that can be easily visualized in one or two dimensions.

Simplifying the Gaussian random field model. The variation in a random field can be described by its mean function $\mu(s)$ and its covariance function $\text{Cov}[x(s), x(s')]$ between arbitrary locations s and s' . The mean function represents the expected value at geographical site s . If the random field models a variable which can be observed repeatedly (e.g. annual cumulative rainfall at site s), $\mu(s)$ can be thought of as the average value across multiple observations at site s . If the random field

represents a variable that is essentially unique (e.g. elevation) then $\mu(s)$ is introduced for modelling convenience and is often assumed to have a simple parametric form (e.g. linear trend). For the sake of model parsimony, we assume here that $\mu(s)$ is constant or linear in s . The covariance function quantifies the linear statistical dependence between $x(s)$ and $x(s')$ as a function of s and s' . For s and s' far apart, it is reasonable to assume that $x(s)$ and $x(s')$ become independent. The covariance function allows us to model at which rate and which distance this decorrelation occurs. Again, for the sake of model parsimony, we assume that $\text{Cov}[x(s), x(s')]$ depends only on the geographical lag $s - s'$ between s and s' and not on the direction of $s - s'$. This set of assumptions about $\mu(s)$ and $\text{Cov}[x(s), x(s')]$ is known as second-order stationarity. Furthermore, we assume that $\text{Cov}[x(s), x(s')]$ depends only on the geographical distance $h = |s - s'|$ and not on the orientation of the vector $s - s'$. This property is known as isotropy. To summarize, we have here a stationary, isotropic GRF model. This is widely considered as a parsimonious, yet flexible and powerful model to study variables structured in space (Chilès & Delfiner 1999; Lantuéjoul 2002; Diggle, Ribeiro & Christensen 2003; Wackernagel 2003; Gelfand *et al.* 2010). Among the broad family of parametric models of covariance functions currently used, we chose the exponential model, i.e. we assume that the statistical dependence between $x(s)$ and $x(s')$, as measured by the covariance, decays exponentially as a function of the geographical distance between

s and s' : $\text{Cov}[x(s), x(s')] = \exp(-|s - s'|/\kappa)$. In the above, κ is a scale parameter and has the dimension of a geographical distance. We assume a similar model for y and we assume that x and y are independent.

Graphical examples of two independent stationary isotropic GRFs

An example of realizations of our model on a fine grid is shown on Fig. 1. For all pairs of sites (s, s'), $\text{Cov}[x(s), x(s')]$ and $\text{Cov}[y(s), y(s')]$ are non-zero. For this reason, the variations of x and y both display a clear structure in space. Each variable could represent a genetic variable (e.g. the logit transform of an allele frequency for a population with continuous spread in space), a phenotypic variable or an environmental variable. If $y(s)$ represents an environmental variable such as the elevation or the temperature at sites s , then a matrix of pairwise differences D^y could be interpreted as a matrix of ecological cost distances.

Simulation study design

Biological or environmental variables are often not observed in the continuum, but rather at a limited number of irregularly spaced sites.

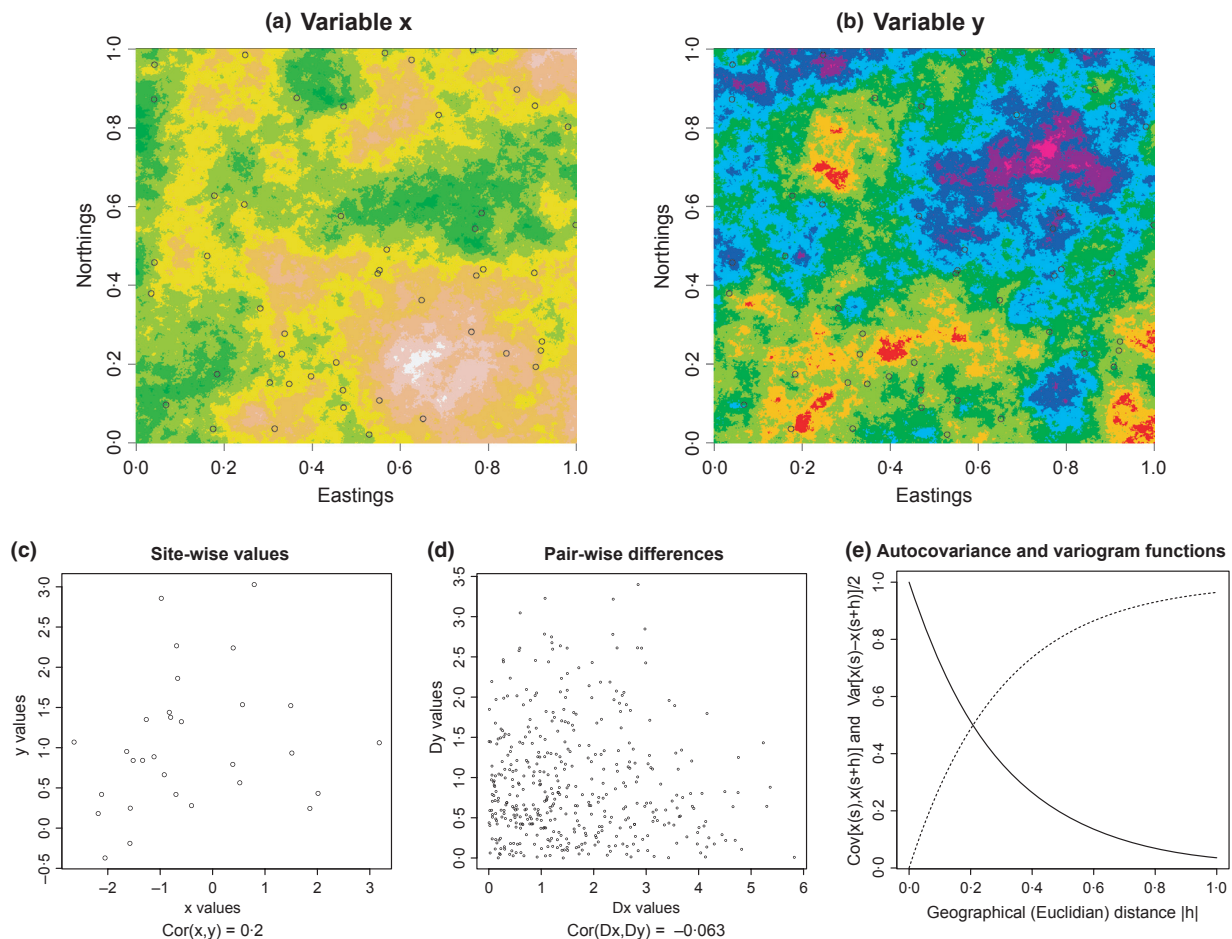


Fig. 1. Top: two independent random fields with an exponential covariance function with a common scale parameter $\kappa = 0.3$ and the locations of 50 sampling sites; Because the covariance function decays slowly, values taken at pairs of close sites are similar. For a spatial lag $s - s'$ large enough the value at a site s does not say much about that value at site s' , there is statistical decorrelation. Bottom: scatter plot of individuals' x values vs. y values at the 50 sampling sites (c), scatter plot of pairwise distances $|x_i - x_j|$ vs. $|y_i - y_j|$ values (d), common theoretical covariance and variogram functions of x and y (e).

We considered here the case where x and y are observed at the same set of $n = 50$ sites or $n = 200$ sites. We considered the cases $\kappa = 0$, $\kappa = 0.3$ and $\kappa = 0.7$. These values are meaningful only relative to the diameter of the sampling window. With $\kappa = 0.3$, decorrelation is approximately reached at one distance unit which is the window width here.

For each value of κ , we simulated 200 data sets (x_1, \dots, x_n) , (y_1, \dots, y_n) , computed the Mantel statistics r with 10000 permutations and computed the associated ' P -value' following Mantel's algorithm. In case autocorrelation is suspected, a recommended strategy consists in entering the matrix D^s of geographical distances in a partial Mantel test between D^x and D^y with the aim of 'controlling the effect of distances'. We implemented this strategy with the same simulation design as above. We also carried out the same experiment with random fields displaying a linear spatial trend β^t s. Such a trend could arise from the presence of large scale geographical features (e.g. distance to the sea) in the spatial variation of temperature. We sampled the two-component parameter β of the linear trend from an independent two-dimensional normal $N(0, 1)$ distribution. Because some authors have discussed the effect of various permutation procedures (Smouse, Long & Sokal 1986; Legendre 2000), we implemented four different permutations strategies, referred to as Methods 1–4 in Legendre (2000) and in Figs 2 and 3. There procedures differ in the nature of the statistic computed and

exchanged among pairs of locations, but are all instantiations of the same permutation procedure originally defined by Mantel.

Results

The proportion of false positives for a test at level α should be α for any $\alpha \in [0, 1]$. In other words, the distribution of the P -values obtained should be uniformly distributed. The distribution of the P -values obtained is illustrated in Fig. 2. Misalignment with the diagonal line shows a departure of the P -values with the expected uniform distribution hence a problem in the test investigated. For $\kappa = 0$, i.e. in the absence of spatial autocorrelation in both variables, the simple and partial Mantel tests perform well (top row). If spatial structure is present in the data in the form of a deterministic linear trend only (no autocorrelation), this can be controlled by introducing a third matrix of geographical distances in a partial Mantel test (cf. Fig. 2 top right panel). As soon as spatial structure is present in the form of autocorrelation (spatial scale parameter $\neq 0$), the simple Mantel test fails to achieve the targeted type I error

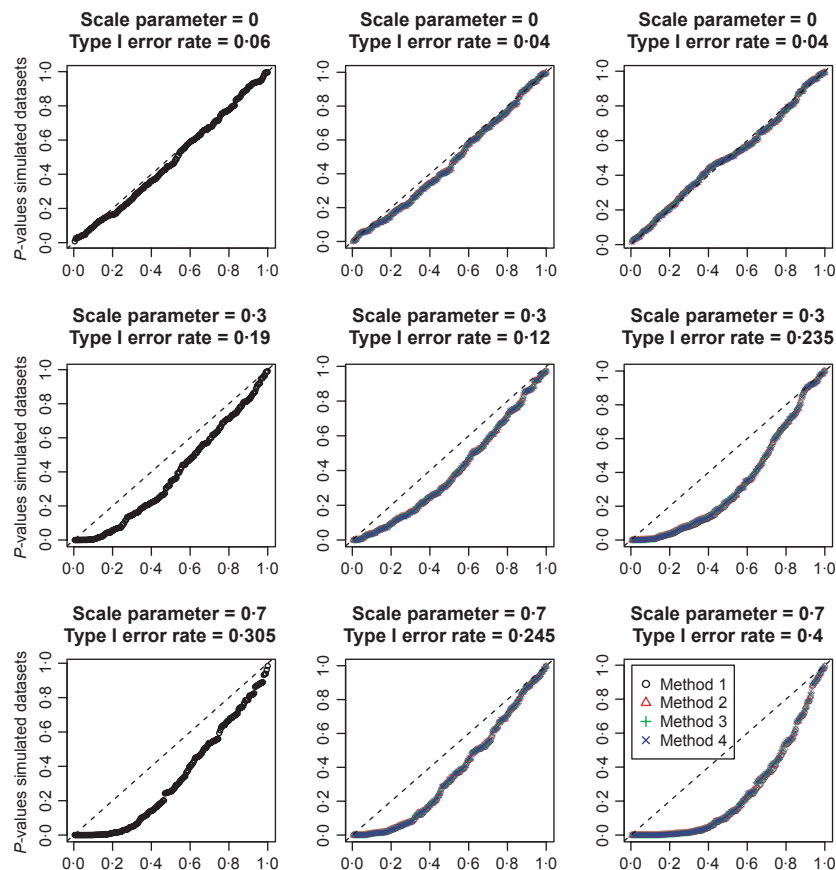


Fig. 2. Quantile–quantile plots of P -values obtained on simulated data with $n = 50$ sampling sites. Each point corresponds to a simulated data set. The y-axis is the P -value returned by the simple Mantel test and the x-axis is the corresponding quantile for a uniform distribution on $[0, 1]$. The null hypothesis tested is the independence between x and y . Left column: simple Mantel test, the matrices D^x and D^y are obtained from independent random fields with zero mean (no deterministic spatial trend). Middle column: partial Mantel test, the matrices D^x and D^y are obtained from independent random fields with zero mean (no deterministic spatial trend). Right column: partial Mantel test, the matrices D^x and D^y are obtained from independent random fields with a deterministic linear spatial trend. For the partial Mantel test (middle and right columns), each dataset was analysed by four permutation methods. The P -values should be aligned along the diagonal. The type I error rate reported is achieved for a targeted level of $\alpha = 0.05$. See also main text for references about methods 1–4.

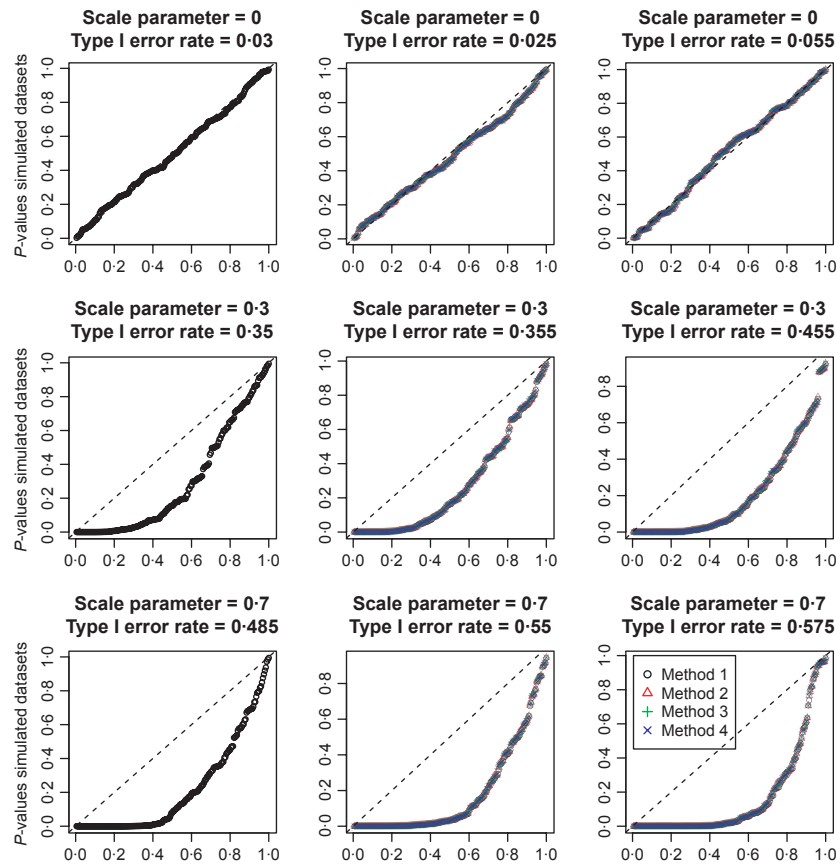


Fig. 3. Quantile–quantile plots of P -values obtained on simulated data with $n = 200$ sampling sites. See Fig. 2 for details.

rate. It produces indeed a considerable excess of small P -values i.e. the simple Mantel test rejects the null hypothesis of independence too often and produces a much higher number of false positives than what it should do. In the presence of autocorrelation, including the matrix of geographic distances in a partial Mantel test does not ‘control’ autocorrelation as the excess of small P -values remains substantial. Besides, the curves for the four methods investigated by Legendre (2000) are perfectly overlapping (cf. Fig. 2 middle and right columns). This shows that the failure of the partial Mantel test is not inherent in the choice of one of the particular variants of the permutation procedure discussed earlier in the literature. The magnitude of the excess of small P -values increases with the magnitude of autocorrelation in the data and for the highest spatial scale parameter value considered here ($\kappa = 0.7$), the type I error rate effectively achieved for a test targeting $\alpha = 5\%$ ranges between 25% and 40%. It increases with the sample size, up to 55% when $n = 200$ (Fig. 3).

The realism of the design of the simulation study can be questioned. For example, the correlation among the most distant samples remains large when $\kappa = 0.7$ in our simulations. However, large biases of the tests are also observed in realistic conditions. Few biological studies provide estimates of κ , and we here consider Diggle et al. (2007) who investigated the effect of elevation and a vegetation index on the prevalence of infection by the filarial nematode *Loa loa* (involved in oncho-

cerciasis) in Cameroon. We have therefore assessed the performance of a partial Mantel test of the effect of elevation on a response variable having the same autocorrelation as reported in this work ($\kappa = 0.7$ in units of longitude and latitude, but with more distant samples in these units than in our previous simulations), the same number (196) and positions of sampled locations (which were more clustered than in our previous simulations), and the same observed values of elevation. Of the 200 replicate simulations, the realized error rate was 27.5% at the nominal 5% rate (Fig. 4), showing that large biases can be observed in real conditions.

Discussion

WHY ARE THE MANTEL TESTS RETURNING ERRONEOUS P -VALUES?

Simple Mantel test

Let us define H_0 as ‘ x and y are uncorrelated’. The question is now: does the simple Mantel permutation procedure produce correlation coefficient values according to the right distribution? The distribution of the correlation coefficient involves not only the dependence structure between x and y but also the joint distributions of (x_1, \dots, x_n) and that of (y_1, \dots, y_n) . So the answer depends on what these joint distributions are. If

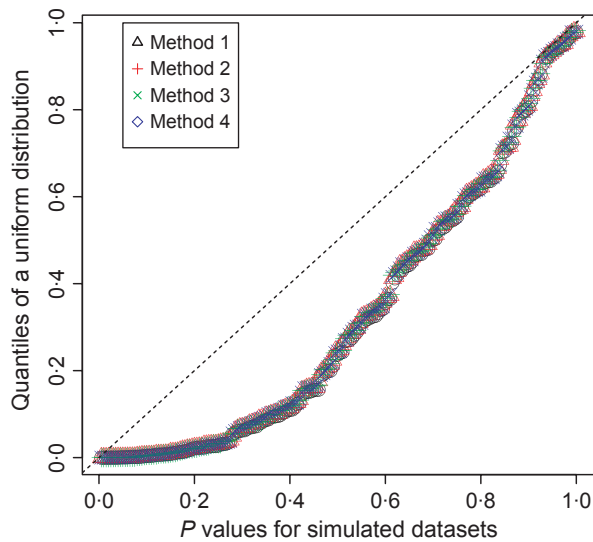


Fig. 4. Quantile–quantile plots of P -values obtained when the x variable is the elevation observed at $n = 196$ sites in a $500 \text{ km} \times 500 \text{ km}$ area in Cameroon and the y variable is simulated independently from x , but autocorrelated with the same spatial characteristic scale as the prevalence of *Loa loa* infection in this area (as per parameter estimates by Diggle *et al.* 2007). The type I error rate is 27.5% at the nominal $\alpha = 5\%$.

(x_1, \dots, x_n) and (y_1, \dots, y_n) are both independent and identically distributed (i.i.d.), then permuting the entries of (x_1, \dots, x_n) breaks the potential dependence between x_i and y_i while leaving the joint distribution of (x_1, \dots, x_n) unchanged. We note here for the sake of completeness that the i.i.d. assumption above is not strictly required and the assumption of exchangeability, i.e. invariance of the distribution under permutation of its variables (Kingman 1978) is sufficient.

In the case of correlated data, the consequences of the permutation are different. For a small spatial lag $|s_i - s_j|$ and because of the spatial structure, $x_i - x_j$ and $y_i - y_j$ tend to have the same order of magnitude (typically $x_i - x_j$ and $y_i - y_j$ are both small), even though the random fields x and y are independent.

The effect of a permutation amounts to substitute index k to index j for various pairs (j, k) . After permutation, the correlation coefficient computed involves the pair of sites (i, j) in a term which is actually $(x_i - x_k)(y_i - y_j)$ (up to some centring). The term $(x_i - x_k)$ has no reason to be of the same order of magnitude as $(y_i - y_j)$. In other words, the permutation not only breaks the potential dependence between x and y but also breaks the spatial structure among the entries of the variable subject to permutation.

The permutation procedure has no reason to produce values from the distribution of the test statistics under the null hypothesis. The simple Mantel test produces values typical of the correlation coefficient between two independent variables when one of them is not structured. What is required for a proper test is the distribution of the correlation coefficient between two independent variables being both structured. In landscape genetics, one is often inter-

ested in testing whether a genetic data set x for a species subject to isolation-by-distance (IBD) display variation that can be explained by environmental variable(s) y . The Mantel test will produce pseudo-genetic data by re-sampling. These pseudo-genetic data will be (as targeted) independent of the environmental conditions y , but there will no longer be representative of IBD data. There will be more likely to look like data arising from an island model.

The simple Mantel permutation procedure produces values that display far less dispersion than what *autocorrelated data* should do under the null hypothesis (as illustrated in Fig. 5). In the presence of autocorrelation, the feature of the data that is implicitly rejected is not the independence of x and y , but rather the absence of spatial structure of x and y .

Partial Mantel test

In the partial Mantel test, the test statistic is the partial correlation coefficient of D^x and D^y given D^s . This coefficient is defined as the usual correlation coefficient between the residuals of D^x and D^y after a linear regression on D^s . The variation of a random field is far more complex than a linear trend (cf. Fig. 1d). The regressions of D^x on D^s and of D^y on D^s fail to capture most of the variability in the data. The residuals of these regressions still display some spatial structure with an intensity that is close to that present in the site-wise values. The procedure consisting in permuting sampling units and computing correlation of residuals is subject to the same issue as in the simple Mantel test. The correlation coefficient values obtained by permutations do not display enough variability.

WHEN ARE THE MANTEL TESTS VALID STATISTICAL METHODS?

Testing the existence of structure of one variable in space

Let us consider the model outlined in the section ‘One random function’ above and let us assume that the random function $y(x)$ is stationary (i.e. the statistical distribution of any finite sequence of $y(x_i)$ values is invariant by spatial translation of the x_i values). Under this model, the Mantel permutation procedure produces correlation coefficient values from the distribution under the null hypothesis. The stationarity assumption above is a reasonable assumption in the case of genetic mutation–migration–drift equilibrium. The simple Mantel test is therefore suitable to test the absence of IBD from population genetic data in this case.

Testing the dependence between two random variables

When x and y are two random functions of a third variable, the simple Mantel test is valid if both x and y are stationary and either x or y is non-autocorrelated. The latter situation has been partially studied by Legendre, Brocard & Peres-Neto (2005), but we note that none of the above assumptions is clearly relevant in evolutionary biology studies.

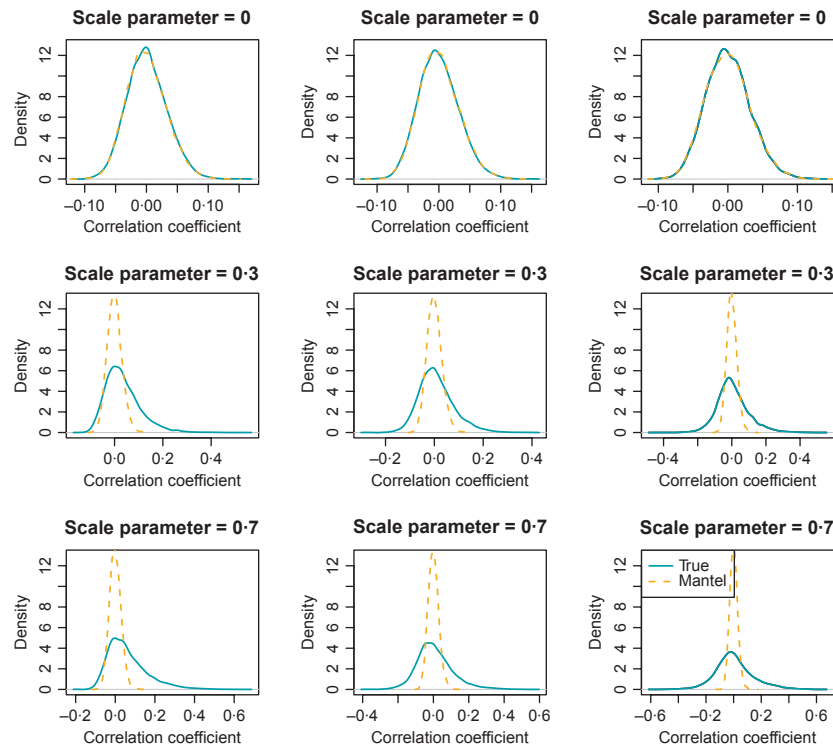


Fig. 5. Estimated density functions for the true distribution of the correlation coefficient and the Mantel correlation coefficient between two variables at $n = 200$ sampling sites as in the section ‘Simulation study design’ and Fig. 3. In the presence of autocorrelation, the Mantel distribution is erroneous and displays far less dispersion than the true distribution.

Reconciling our results with the findings of Legendre & Fortin (2010)

In their recent review, Legendre & Fortin (2010) discussed at length the Mantel tests. In the section ‘Multivariate, spatially structured data’, they write: ‘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’. Our results are in stark contrast with this claim. So what is going on?

This section above in Legendre & Fortin (2010) reports findings from an earlier study by Legendre, Brocard & Peres-Neto (2005) summarized in Appendix 2 of Supplementary Material to Legendre & Fortin (2010). They do not give enough detail about the simulation study, but our analysis suggests unambiguously that either (i) the model simulated by Legendre, Brocard & Peres-Neto (2005) does not produce spatially autocorrelated data at all or (ii) the parameters used (not clear from the original publication) are such that the data are structured at a spatial scale that is much smaller than the sampling window.

Also, Legendre & Fortin (2010) conclude their abstract by writing: ‘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’. Potential users are easily misled by such claims to consider that their problem ‘can only be formulated

in terms of distances’ and then feel allowed to use the (partial) Mantel tests. Yet, it is not clear what these claims means. Indeed, it is proper to simulate the per-location data out of which matrices are computed, which means that hypotheses are de facto not expressed ‘only in terms of distances’ but in terms of per-location data. This emphasizes that hypotheses are better described as features of probability distributions (e.g. a correlation function), and that distances matrices are only a way of representing data, not a way of specifying hypotheses. Thus, no set of conditions where partial Mantel tests are valid is identified by such claims.

Reconciling our results with the findings of Cushman & Landguth (2010)

In their study, Cushman & Landguth (2010) simulated genotypes from a model accounting explicitly for the landscape or the presence of a barrier and concluded that the Partial Mantel test could accurately identify the proper scenario and was not prone to any false discovery as reported in this study.

However, they only reported average ‘Mantel’ correlation coefficients in 100 simulations, which is not sufficient to make conclusions about the validity of the partial Mantel test. For example, in the conditions where partial Mantel tests performed worst in Fig. 2, the average partial correlation coefficient was 0.005 (see also Fig. 5). This emphasizes that the whole distribution of correlation coefficients, not only its mean, matters for the validity of the test. We do not know how

partial Mantel tests would actually perform in their simulations. Their study includes 300 Monte Carlo simulations, but they are all drawn from a single landscape resistance surface (identified by Cushman *et al.* 2006 as the most supported out of 110 alternative models for black bear *Ursus americanus* gene flow in northern Idaho, USA). Rather than rehabilitating the partial Mantel tests, such a study would rather suggest a method of investigating *ex-post* how realistic Mantel *P*-values are. However, this involves intensive simulations and a similar computational burden should allow scientists to perform better inferences along the lines suggested below, and estimate directly key ecological parameters explaining genetic variation, without resorting to the Mantel tests.

ALTERNATIVE STRATEGIES

Testing independence between two point processes

We first note that the original problem of Mantel (1967) amounts to detecting the dependence between the marks and locations of a marked point process. This problem is not chiefly relevant to evolutionary biology data as information about the sampling effort is unfortunately often not available. However, we note that this question has been addressed in a rigorous way by Schlather, Ribeiro & Diggle (2004).

Shift permutation

We recall that if at least one of the variables to be compared is observed at the nodes of a regular grid (as it may occur in landscape genetics and phylogeography with Geographic Information System data), one can apply shift permutations of this variable (Upton & Fingleton 1995).

*Adapting the *t*-test*

Assessing the significance of the correlation between two random fields is a question that has been considered by Clifford, Richardson & Hemon (1989), Richardson & Clifford (1991) and Dutilleul *et al.* (1993) for quantitative continuous variables and Cerioli (2002) for categorical variables. The methods proposed there can be readily used when the data are available as site-wise univariate values. They can be also adapted when the data are multivariate. The case where data are pairwise distance matrices that are not obtained as differences between site-wise values requires further work.

Fixing the Mantel test for nonlinear trends

The incentive for developing the partial Mantel test was the intuition that one variable can have a confounding effect when analysing the dependence between two other variables. The method fails to fulfil its promise because the method was not based on an explicit statistical model and the implicit underlying model (a simple regression) is most often not appropriate. However, the idea of filtering out some (possibly nonlinear) deterministic trend to retrieve some transformed data (under

which the Mantel test is appropriate) could be further explored. This could lead to methods for data with spatial structure in the form of deterministic trend only.

Inference and testing in a hierarchical model

Linear mixed models with autocorrelated errors are correctly specified models for analysing data simulated by GRFs, so that the likelihood ratio tests based on such models should be at least asymptotically valid. Analysing the dependence between two variables can be considered in the broader framework of a generalized linear mixed model (GLMM), allowing non-Gaussian response variables. Inference and testing in such models can be made e.g. with Monte Carlo methods (Robert & Casella 2004), or adjusted profile likelihood methods (Lee & Nelder 2009). Simulations-based methods akin to Approximate Bayesian Computation (e.g. Marin *et al.* 2011) could also be useful. The framework of hierarchical models coupled with modern numerical inference and testing methods is promising, but it has not been investigated as an alternative to the Mantel tests so far. Although procedures for fitting spatial GLMM models have been described in the literature (e.g. Diggle *et al.* 2007; Lee & Lee 2012), there is a dearth of computer implementations to performs such analyses in an automated way. Most GLMM softwares, for example, do not include procedures for specifying and/or estimating spatially structured correlation matrices. Therefore, such implementations, and investigation of their small-sample performance, are required.

IMPLICATIONS OF OUR STUDY

Spatial autocorrelation is widespread in evolutionary biology data and it is likely that many studies based on the partial Mantel tests who concluded to the existence of an association between two sets of variables were based on an erroneously small *P*-value. Spatial autocorrelation is perhaps the most common form of autocorrelation in evolutionary biology, but any form of autocorrelation would lead to the same issue. In particular, the case of two variables displaying some form of phylogenetic signal is subject to the same issue. The range of questions where using the partial Mantel test instead of the simple Mantel test alleviates the statistical issues discussed here is very limited (presence of a deterministic linear trend and no other form of spatial structure). Outside the situations enumerated in the section *When are the Mantel tests valid statistical methods?* above, the Mantel tests are not validated statistical methods and any result based on them is dubious. Mantel tests are widely used in the scientific community and while it has become increasingly obvious that they are not well-grounded statistical methods, no computer program implementing alternative methods have been developed. This relates presumably to the several reasons: most users of the method have long been considering the partial Mantel test as a 'safe' alternative to the simple Mantel test, the problem is not trivial, useful methods should be able to deal with all kinds of data (multivariate and categorical as well as quantitative data) and lastly,

‘mainstream’ spatial statistical methods (which have known a tremendous development in the last 20 years, most notably with the advent of hierarchical models and simulation methods) are traditionally more concerned with prediction than with testing. Our study stresses the need for a general and well-validated computer program. As a final note, we stress that the geostatistical modelling framework taken here is not the only meaningful framework for the analysis of evolutionary biology data in this context. It is taken here mostly because it allowed us to make statements about statistical distributions *under some well-defined assumptions*, which is often the missing corner stone of analyses based on the Mantel tests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Data S1. R script and data used in simulation study.