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OPINION

The trouble with isolation by distance

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Abstract

The genetic population structure of many species is characterised by a pattern of isolation by distance (IBD): due to limited dispersal, individuals that are geographically close tend to be genetically more similar than individuals that are far apart. Despite the ubiquity of IBD in nature, many commonly used statistical tests are based on a null model that is completely non-spatial, the Island model. Here, I argue that patterns of spatial autocorrelation deriving from IBD present a problem for such tests as it can severely bias their outcome. I use simulated data to illustrate this problem for two widely used types of tests: tests of hierarchical population structure and the detection of loci under selection. My results show that for both types of tests the presence of IBD can indeed lead to a large number of false positives. I therefore argue that all analyses in a study should take the spatial dependence in the data into account, unless it can be shown that there is no spatial autocorrelation in the allele frequency distribution that is under investigation. Thus, it is urgent to develop additional statistical approaches that are based on a spatially explicit null model instead of the non-spatial Island model.

Keywords: AMOVA, FDIST, Mantel test, null model, population structure, spatial autocorrelation

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Introduction

In most species, dispersal is severely limited. Even though most animals have active locomotion, many of them rarely disperse far beyond their place of birth (Greenwood 1980). Plants have overcome the limitations of their sessile life by a dazzling array of adaptations that aid in the dispersal of their seed and pollen. Still, even in dandelions, which are well-known for the parachute-like appendages to their seeds, more than 99.5% of the seeds land within 10 metres of the mother plant (Tackenberg *et al.* 2003). This limited dispersal has important consequences on the spatial distribution of genetic variation. If dispersal distances are small, a pattern of spatial autocorrelation emerges in the distribution of genetic variation: individuals that are close to each other are likely to be more related, and therefore genetically more similar, than individuals that are farther apart. Therefore, within populations a positive relationship is expected between relatedness and geographic distance, and this has been observed frequently in nature (Vekemans & Hardy 2004). The same principle works at larger geographic scales: limited dispersal leads to a positive relationship between genetic distance and geographic distance among populations.

Such processes of isolation by distance (IBD; Wright 1943) are very well understood theoretically and there is a large body of literature describing how patterns of IBD emerge and how IBD affects the distribution of variation over populations (e.g. Rousset 1997). Following these theoretical results, most studies on the population structure of species now routinely include tests for IBD, most popularly by testing the association between genetic and geographic distance using a Mantel test (Mantel 1967). Despite the ubiquity of IBD, many popular statistical methods for analysing population genetic data are based on a null model that is completely non-spatial: the Island model (Wright 1931). Under the Island model, there is equal migration among all populations in a system, regardless of their distance. Obviously, this model is a drastic oversimplification (Whitlock & McCauley 1999) and is not applicable when a correlation has been found between genetic and geographical distance. In such cases, using the Island model as a null model is problematic because spatial autocorrelation can severely bias the results of these analyses. In this paper, I illustrate this problem by taking two popular types of population genetic analyses, testing hierarchical population structure and detection of loci under selection, and showing how their results can be biased by IBD processes. For simplicity, I will only discuss IBD at the population level, though much of the argument is equally applicable to among-individual IBD within large continuous distributions. I then argue that explicitly spatial models should be used as null models for analyses of population structure instead of the non-spatial Island model.

IBD and hierarchical population structure

There are many reasons why populations may be structured in a hierarchical way. Most importantly, postglacial recolonisation from multiple refugia can lead to distinct clusters of populations (Taberlet *et al.* 1998). In the great majority of cases, such clusters are geographically

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Table 1 Results of standard, stratified, and partial Mantel tests distinguishing the effects of isolation by distance and hierarchical population structure

			Hierarchical island		Stepping-stone	
Matrix A	Matrix B	Adjustment	Mantel's *r*	% of significance	Mantel's *r*	% of significance
Genetic	Geographical	—	0.64	100	0.86	100
Genetic	Geographical	Stratified: permuted within clusters	0.64	4	0.86	100
Genetic	Geographical	Partial: clusters as covariate	0.01	19	0.78	100
Genetic	Clusters	Partial: geography as covariate	0.80	100	−0.07	7

The values of Mantel's *r* are averaged over 100 replicate simulations of either a hierarchical Island model or a stepping-stone model.

clustered, and therefore also represent a pattern of spatial autocorrelation. Mantel tests and tests of spatial autocorrelation do not distinguish between patterns resulting from clustering and those resulting from isolation by distance (Meirmans *et al.* 2011). As a result, tests of IBD and hierarchical population structure are confounded in two ways. First, tests for IBD, including Mantel tests and correlograms (Moran 1950; Smouse *et al.* 1986), are biased by the presence of a hierarchical structure. Second, tests for a hierarchical structure, such as the analysis of molecular variance (AMOVA, Excoffier *et al.* 1992), are biased by the presence of IBD.

To illustrate these two problems, I used the program MARLIN (Meirmans 2011; see also Neuenschwander *et al.* 2008) to simulate a landscape of 5×20 populations both under a hierarchical Island model of migration and a stepping-stone model (see supplementary material for more details on the simulations). For the hierarchical Island model, the landscape was split into two clusters; for the stepping-stone model the landscape was completely uniform. To show the pattern of spatial autocorrelation for both simulations, I calculated Mantel tests and Mantel correlograms (Oden & Sokal 1986) on subsamples of 20 populations and 20 individuals per populations; sample sizes that are typical for studies of IBD. These tests were performed using the VEGAN package (Oksanen *et al.* 2009) in R (http://www.r-project.org/). For the simulations with stepping-stone dispersal, I also tested for hierarchical population structure between the left (''western'') and the right (''eastern'') side of the landscape, using an AMOVA (Excoffier *et al.* 1992) performed in GENODIVE (Meirmans & Van Tienderen 2004). The simulations and analyses were done with 100 replicates.

Tests of IBD are biased by hierarchical structure

Under a hierarchical Island model there is no spatially restricted gene flow within clusters, and therefore no IBD. Nevertheless, the Mantel tests (Table 1, first row) clearly show a positive correlation between geographical distance and genetic distance. The average value of Mantel's r was as high as 0.64, and the association between the matrices was significant at $P = 0.001$ for all 100 replicate simulations. Figure 1 (top row) schematically shows how the

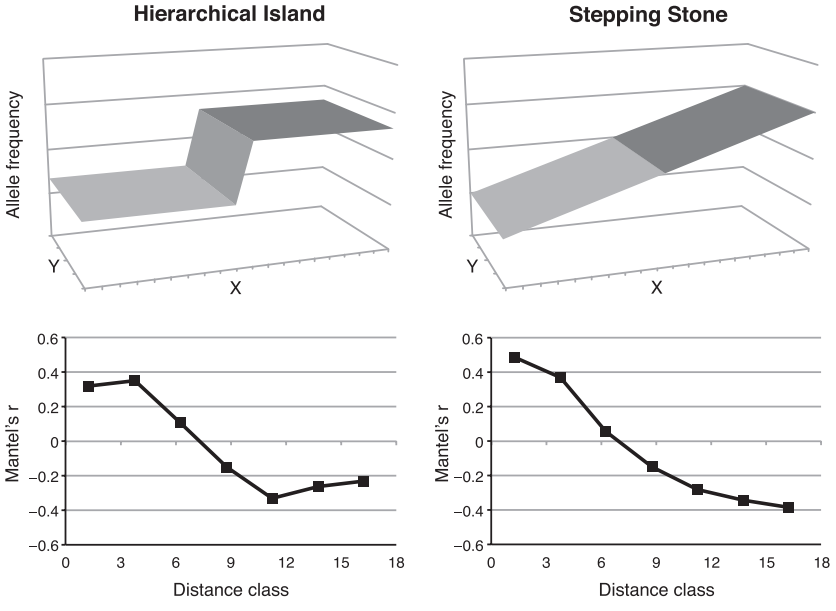

Fig. 1 Top row: simulated 5×20 landscape with a schematic representation of allele frequencies under a hierarchical Island model and under a stepping-stone model. Bottom row: Mantel correlogram showing spatial autocorrelation in allele frequencies simulated under a hierarchical Island model and under a stepping-stone model. The values of Mantel's r are averaged over 100 replicate simulations.

hierarchical Island model (left) leads to a different distribution of allele frequencies than the stepping-stone model (right). However, the Mantel correlograms (Fig. 1, bottom row) calculated from the simulations under these two models are largely comparable.

To address this bias, tests for IBD will have to be adjusted to allow for the effect of the hierarchical population structure. For example, several studies have addressed the problem by performing the Mantel tests separately for every cluster (Kuchta & Tan 2005). Though this approach does solve the problem, it has the disadvantage that it reduces statistical power because of the lower number of populations and the need for correction for multiple testing. Therefore, it might be better to directly take the effects of population structure into account in the overall Mantel test (Landguth *et al.* 2010). One way to take the hierarchical structure into account is to use a stratified Mantel test in which the permutation scheme is changed to permute the locations of populations within the clusters (Oksanen *et al.* 2009). Testing this method using the data simulated under a hierarchical Island model shows that it was unbiased and showed significance only in 4% of the cases (Table 1, second row). A second method is to use the clustering as a covariate in a partial Mantel test (Smouse *et al.* 1986). However, this test showed a clear bias, with significant results for 20% of the repeats (Table 1, third row). When used on the data simulated under the stepping-stone model, where there is IBD but no hierarchical differentiation, all analyses correctly showed 100% significance. This means that when there really is IBD the stratified and partial Mantel tests are not overly conservative.

Tests of hierarchical structure are biased by IBD

As mentioned above, a hierarchical population structure can easily be mistaken for a pattern of IBD. However, the reverse is also true, i.e. a pattern of IBD can easily be mistaken for a hierarchical population structure. The reason is that IBD readily gives rise to a gradient in allele frequencies. When such a gradient is divided into two or more parts, the presence of the gradient will cause the allele frequencies in these parts to differ even in absence of any barriers to gene flow (Fig. 1 top row). This problem is illustrated by AMOVAs performed on the simulations with stepping-stone dispersal. Despite the lack of a barrier in the simulations, the differentiation between the two sides was highly significant for all 100 repeat simulations (999 permutations, $P = 0.001$). The differentiation between the two sides as measured by F_{SC} was on average 0.037, ranging from 0.021 to 0.076. This means that simply testing for differentiation between different parts of a species' range will easily give spurious differentiation if there is also a pattern of IBD across the whole range.

To overcome this bias, it is necessary to test whether an apparent hierarchical clustering is in fact caused by IBD. As with any statistical problem, data visualisation can be a great tool for this. As a first step, plotting allele frequencies over the landscape can help to distinguish clusters from gradients. Furthermore, barrier analyses and clustering analyses can be used to see whether breaks in allele frequencies coincide with certain geographical features (see e.g. Jaramillo-Correa *et al.* 2010). However, the most popular clustering method, STRUCTURE (Pritchard *et al.* 2000), is sensitive to patterns of IBD and may discern multiple clusters where there is only a single large area with IBD (Frantz *et al.* 2009). Therefore it is advised to use clustering approaches that take the geographical position of the samples into account (e.g. François *et al.* 2006). A partial Mantel test can also be of help here, testing the association between the matrix of genetic distances and a model matrix of cluster membership with the matrix of geographical distances as a covariate (Drummond & Hamilton 2007). Using such a partial Mantel test on the simulations with stepping-stone dispersal showed that it correctly accounts for the pattern of IBD: only 7% of the tests showed a significant association between the clusters and genetic distance after correction for the geographical distances (Table 1, bottom row). This is only slightly higher than the 5% that is expected at $\alpha = 0.05$. Furthermore, when the same test was applied to the simulations with a hierarchical Island model, which does have a barrier but no IBD, all 100 replicates were significant, showing that the test was not overly conservative.

IBD and the detection of loci under selection

Modern genetic tools for the first time allow us to gain a detailed understanding of how selection shapes genetic variation at a genomic level (Barrett & Hoekstra 2011). Even for non-model organisms, it is now possible to perform so-called genome-scan analyses, where one samples genotypes within a population at a large number of loci and then screens for loci that are putatively under selection. There are two basic approaches to this: correlative methods that look for associations between genetic variation and environmental variables (Foll & Gaggiotti 2006; Joost *et al.* 2007; Eckert *et al.* 2010), and methods for the detection of outlier loci that show unexpectedly high or low differentiation (Beaumont & Nichols 1996; Foll & Gaggiotti 2008; Excoffier *et al.* 2009). For both approaches it is possible to take the spatial population structure into account in the analysis to avoid bias deriving from IBD processes (Coop *et al.* 2010; Eckert *et al.* 2010). However, this is only rarely done and methods that ignore these confounding effects are in fact more widely used, possibly leading to serious errors in the interpretation of the results.

To illustrate these problems, I used MARLIN (Meirmans 2011) to simulate a landscape based on the map of the Scandinavian Peninsula. This map was divided into grid cells, with migration between cells following a stepping-stone model (see supplementary material). For testing the bias in the association between the simulated allele frequencies and ecological data, I applied the SAM method (Joost *et al.* 2007) on the simulated datasets. SAM works by calculating logistic regressions between binary allele presence-absence for individuals and environmental variables

of their sampling locations (Joost *et al.* 2007). Since it is based on simple logistic regressions, the method implicitly assumes a complete spatial independence in both the genetic and the environmental data. As environmental data from the Scandinavian Peninsula, I selected four bioclimatic variables from the WorldClim database (Hijmans *et al.* 2005): temperature, temperature seasonality, precipitation, and precipitation seasonality. SAM was performed on random samples of 100 and 200 individuals from different grid cells. For testing the bias in the detection of outlier loci, I applied the FDIST method (Beaumont & Nichols 1996) on the simulated datasets. I randomly sampled 20 populations and analysed these with the FDIST function implemented in ARLEQUIN (Excoffier & Lischer 2010), which uses the Island model as a null model. The sample sizes were deliberately chosen to be relatively low, to show that the bias presents itself also at small sample sizes. However, they are within the range that is typically used for genetic studies. The simulations and analyses were done with 100 replicates.

Associations between genetic variation and environmental variables

When testing for associations between individual genotypes and their local environment, it is important to correct for any present spatial population structure to avoid bias deriving from the overlap between the spatial component in the genetic and environmental data. Most environmental data is spatially structured at multiple scales. At large continental scales there are various climatic gradients, e.g. in temperature, seasonality and precipitation. On smaller scales there are spatial patterns in other variables such as elevation, vegetation zones, soil, bedrock, and hydrology. Therefore, environmental data is almost invariably spatially autocorrelated (Legendre 1993; Borcard *et al.* 2011). However, such autocorrelation presents a problem for many statistical tests as the assumption of independence of the samples is violated (Borcard *et al.* 2011). This problem is exacerbated when analysing the correlation between two independent variables that are both spatially autocorrelated, for example the frequency of an allele and a climatic variable. Overlap between the spatial patterns of the two variables can easily lead to significant, but spurious, correlations. This will often be the case when testing for associations between alleles and ecological variables in the face of IBD with a method that is based on a non-spatial null model or an assumption of spatial independence.

Using SAM to directly test the associations between the neutral markers and the four bioclimatic variables resulted in many significant associations despite the fact that the genetic data was simulated completely independently of the climatic data. With only 100 sampled individuals, the distribution of *P*-values was heavily biased towards small values (Fig. 2). Averaged over the four climatic variables, 24% of the tests had *P*-values smaller than 0.05. The results differed between climatic variables, with 32% of the *P*-values for temperature seasonality being significant, compared to 10% for precipitation seasonality. These differences are probably due to differences in spatial patterns between variables. Temperature seasonality had a strong east-west gradient, corresponding mostly to the distance from the sea. In contrast, precipitation seasonality showed more patchy geographical patterns. Increasing the number of sampled individuals also increased the bias of the test. When 200 individuals were randomly sampled from the population, the percentage of significant associations was on average 36%. These results confirm that IBD processes can easily lead to spurious correlations and one should therefore be careful in the *interpretation* of the results. Therefore, when IBD is detected in a study, it is important to always use methods that account for the spatial population structure when testing the associations between the markers and environmental variables.

Detection of outlier loci

Besides adaptation to local conditions, there are other ways in which selection can affect allele frequency differences among populations. For example, strong differences in allele frequencies may be driven by genetic incompatibilities at certain genomic regions, such as those deriving from inversions or chromosomal rearrangements. Conversely, balancing selection (overdominance) may result in allele frequencies being evened out across populations. It is therefore of interest to look for outlier loci that show particularly high or low differentiation. Several methods exist for such outlier analyses, of which the FDIST method (Beaumont & Nichols 1996;) is most widely used. In an FDIST analysis, F_{ST}-values are calculated for every locus and then compared to a distribution of F_{ST}-values that is obtained through coalescent simulations. Loci that fall outside of the confidence interval of the simulated values are deemed to be outlier loci that are under selection. In the standard implementation of the method, the performed simulations are based on the Island model, but other simulation models can be used, e.g. assuming a hierarchical population structure (Excoffier *et al.* 2009) or a model of postglacial recolonisation (Eckert *et al.* 2010). Previously, it has been shown that the use of the Island model can lead to a bias when the population is in fact hierarchically structured, and the use of a hierarchical Island model is advocated in such situations (Excoffier *et al.* 2009). However, the method is actually equally biased when the assumption of non-spatial migration is violated.

When FDIST was applied to the simulations of the Scandinavian Peninsula, there was a clear bias towards small *P*-values with 21% of the *P*-values being smaller than 0.05 (Fig. 3). Increasing the sample size also increased the bias: when 30 populations were sampled from the Scandinavian Peninsula, the percentage of significant tests increased to 25%. When FDIST was applied to the simulations of the 5×20 landscape, the results were largely similar with 23% of the *P*-values below 0.05 when 20 populations were sampled (not shown). This shows that deviations from the model assumed by FDIST can lead to a large number of false positives.

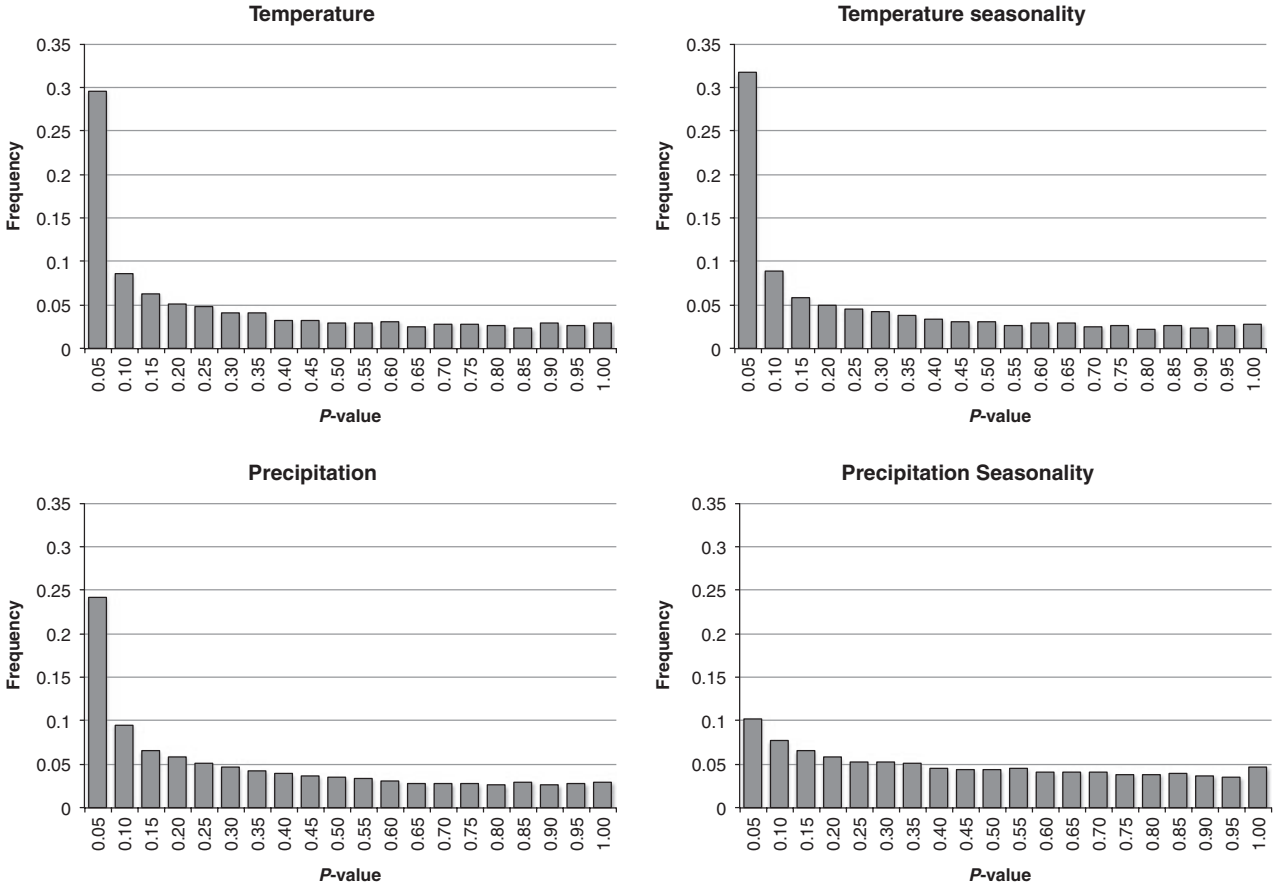

Fig. 2 Distribution of *P*-values from SAM (Joost *et al*. 2007), testing the association between simulated neutral genetic markers and actualclimatic variables of the Scandinavian Peninsula. The bias towards small *P*-values is due to overlapping patterns of spatial autocorrelation between the two datasets.

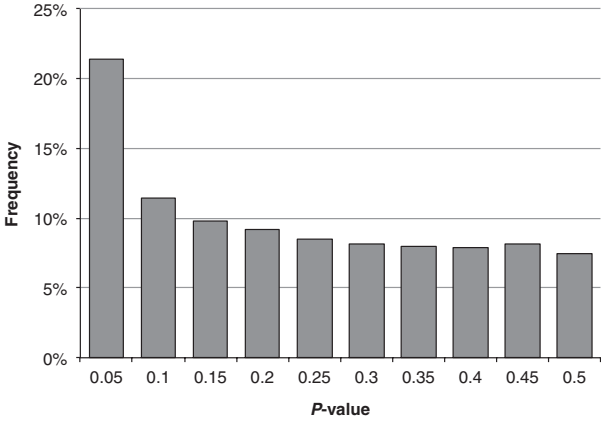

Fig. 3 Distribution of *P*-values for the detection of outlier loci using the FDIST method (Beaumont & Nichols 1996). The genetic markers were fully neutral and simulated based on the geography of the Scandinavian peninsula with stepping-stone dispersal.

Using null models based on isolation by distance

Despite their limited scope, the above simulations clearly show how the presence of isolation by distance can bias the results of statistical inferences. All four methods tested here, AMOVA, Mantel tests, SAM, and FDIST, showed a large number of false positives under IBD. This means that applying these tests on genetic datasets that are spatially autocorrelated will often lead to spurious results. These biases are not due to sampling artefacts; for all tests, increasing the number of sampled populations only exacerbated the problem. The biases are also not due to multiple testing. Both SAM and FDIST perform many significance tests that require adjustment of the significance level. However, the bias in these tests is that the distribution of *P*-values gets enriched for low values, and Bonferroni correction does not change this. The actual problem is that these analyses assume spatial independence of the data, or are explicitly or implicitly based on a null model such as the Island model that is inherently non-spatial. These results show that this represents a severe oversimplification.

The use of null models in genetics

Population genetics has a strong tradition of using null models for the distribution of neutral genetic variation. For studies at the molecular level, the null model used is generally the neutral theory of molecular evolution by Motoo

Kimura (1968). Though the introduction of the neutral theory has sparked a large and heated discussion in the past (Kimura 1983), it is now generally agreed that the availability of such a null model has greatly helped the field progress. For studies at the population level, the most widely used null model is Wright's (1931) Island model. The most important use for these null models is that they form a baseline against which hypotheses of selection can be tested. The main insight that we have gained from these models is that genetic variation at the genomic and the population level is not only shaped by selection, but also to a very large extent by neutral and near-neutral processes. Even though in genetics there has historically been a great awareness of the importance of spatial processes owing to the work of Wright (1943) and Malécot (1948), these insights never got incorporated into the standard null model, which is mostly the (non-spatial) Island model.

The ubiquity of IBD

Isolation by distance is a widespread phenomenon and is found in the majority of cases in which it is tested. I reviewed all papers published in Molecular Ecology in 2011 that used a Mantel test to test for IBD (assessed December 13). Of the 72 papers found, 50 (69%) reported a significant correlation between genetic and geographic distance. However, most of these studies, but certainly not all, in their further testing neglected the observed IBD and performed further tests that assume spatial independence. This means that some of the results of these studies may be spurious and solely the result of the bias caused by spatial autocorrelation. Though this bias has been reported earlier (Vasemägi 2006; Barrett & Hoekstra 2011), it has received relatively little attention and the extent of the bias has never been properly researched. The simulations presented here provide a starting point for such studies. The problem is also getting more imminent with the advent of new molecular techniques that allow a larger scale for studies of genetic diversity, and the availability of global GIS layers with climatic, geological, and land-use information.

Patterns of IBD readily arise from limited dispersal as shown in the simulations above. In addition, IBD can also arise from historical demographical processes such as postglacial recolonisation. Since postglacial recolonisation mostly happened in a poleward direction, the resulting allele gradients will largely overlap with major climatic gradients, e.g. in temperature. For example, when the above simulations of the Scandinavian Peninsula were changed to a recolonisation scenario with a single refugium in the South (results not shown), the percentage of significant tests in SAM rose to 44%, compared to 24% for the equilibrium scenario. Such a strong overlap between the spatial genetic and climatic gradients means that actual local adaptation may be indistinguishable from recolonisation patterns (Holliday *et al.* 2010). In such cases, genome scans may not be suitable to uncover local adaptation and experimental approaches are required.

Because of the ubiquity of IBD, any study of population structure should systematically include a detailed analysis of the spatial patterns in the data. A good starting point is the creation of geographical allele distribution maps and other plots that visualise the allele frequency distribution. Furthermore, though a standard Mantel test can be helpful to test for the presence of spatial dependency, it does not provide a means to visualise the spatial structure. Correlograms (Moran 1950; Smouse *et al.* 1986) provide a much better tool for this and allow a better interpretation of the scale and strength of the spatial structure. However, it is important to note that correlograms are susceptible to the same bias as standard Mantel tests for IBD. Both multilocus and single locus correlograms can be produced; the latter are a good means to get an overview of the range of autocorrelation patterns and may be helpful in the identification or verification of outliers.

Integrating the spatial data in the analysis

In practice, the observed bias means that all analyses in a study should incorporate the spatial dependence in the data, unless it can be demonstrated that there is no spatial autocorrelation in the genetic data. Therefore, there is a clear need for the development of additional statistical tools that can account for the spatial structure in the data. One important way in which this may be achieved is to make use of null models that are spatially explicit. There are already several tools available that show how this can be done (Wasser *et al.* 2004; François *et al.* 2006; Coop *et al.* 2010; Eckert *et al.* 2010). TESS (François *et al.* 2006) uses so-called hidden Markov Random Fields to model spatial dependencies in allele frequencies. It then detects geographical discontinuities in the allele frequencies and uses this to find clusters, providing a spatially explicit alternative to STRUCTURE. The program SCAT (Wasser *et al.* 2004) uses a smoothing algorithm to estimate allele frequencies across the entire range of a species and used these as the basis of an assignment test. The FDIST approach can be used with different null models besides the standard Island model, such as a hierarchical Island model (Excoffier *et al.* 2009) or a recolonisation scenario (Eckert *et al.* 2010). Above, I have shown that there can be similarities between a hierarchical population structure and a pattern of IBD. However, this does not mean that the hierarchical Island model can be used to correct for the presence of IBD in an FDIST analysis, since there is significant spatial genetic variation that is not captured by this model (Table 1).

Though using a spatially explicit null model will help to avoid much of the bias resulting from IBD processes, it is important to realise that any null model presents an abstraction and will not capture the complete genetic process. Therefore, any null model will be an oversimplification and may lead to a bias when carelessly applied. There are many ecological, geographical, demographic, and stochastic processes operating that affect the distribution of genetic variation at different spatial scales. All these processes affect the validity of the null model (Wiegand & Moloney 2004).

As always, it is therefore important to pay close attention to the biological relevance of the results and, if possible, look for independent confirmation. Such confirmation can e.g. be done using experimental approaches such as common garden experiments or crossing experiments.

It is important to note that the spatial autocorrelation in the allele frequencies can also be accounted for without explicitly defining a spatial null model. Dyer *et al*. (2010) used conditional genetic distances to remove the effect that the phylogeographic history may have on the correlation between the genetic variation and ecological factors. Lee & Mitchell-Olds (2011) recently used a combination of multivariate analysis and multiple regression to analyse genetic diversity. However there is a trade-off with the multilocus approach that they used. Though it did allow them to quantify the relative contributions of IBD and environmental variables on population differentiation, they were unable to pinpoint any loci that are putatively under selection. There are many other ways in which the spatial dependency in the data can be incorporated into the statistical analysis and the statistical toolbox for such analyses is constantly expanding (Guillot *et al*. 2009; Borcard *et al*. 2011).

Outlook

The ubiquity of IBD and the effects this has on the statistical analysis of genetic data mean that we have to rethink some basic elements of our approach to genetic data analysis. Several fundamental questions arise: Which null model is most suitable? How close should the selected null model be to the unknown (or even unknowable) true model? Which summary statistics best describe the spatial genetic structure? How do these summary statistics perform with techniques such as Approximate Bayesian Computations? How do we interpret the overlap in spatial genetic and ecological patterns? What modifications can be made to existing techniques to avoid bias? Is such a modification necessary and even possible for all techniques? Answering these questions will take a substantial research effort, but will be fundamental in order to avoid the serious misinterpretations of the data that can result from IBD.

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Data accessibility

The full simulated datasets of all 100 replicates for the three simulation scenarios are available on: DRYAD entry doi:10.5061/dryad.jh0gs15j.

Supporting information

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

Fig. S1 The used 5 × 20 landscape, divided into two halves for the hierarchical Island model.

Fig. S2 Simplified map of the Scandinavian Peninsula Map of simulated Scandinavian populations. The greyscale indicates the population size, with black cells having a size of 30 individuals and white cells being empty.

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