MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2015) **15**, 512–525 doi: 10.1111/1755-0998.12332

Performance of partial statistics in individual-based landscape genetics

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Abstract

Individual-based landscape genetic methods have become increasingly popular for quantifying fine-scale landscape influences on gene flow. One complication for individual-based methods is that gene flow and landscape variables are often correlated with geography. Partial statistics, particularly Mantel tests, are often employed to control for these inherent correlations by removing the effects of geography while simultaneously correlating measures of genetic differentiation and landscape variables of interest. Concerns about the reliability of Mantel tests prompted this study, in which we use simulated landscapes to evaluate the performance of partial Mantel tests and two ordination methods, distance-based redundancy analysis (dbRDA) and redundancy analysis (RDA), for detecting isolation by distance (IBD) and isolation by landscape resistance (IBR). Specifically, we described the effects of suitable habitat amount, fragmentation and resistance strength on metrics of accuracy (frequency of correct results, type I/II errors and strength of IBR according to underlying landscape and resistance strength) for each test using realistic individual-based gene flow simulations. Mantel tests were very effective for detecting IBD, but exhibited higher error rates when detecting IBR. Ordination methods were overall more accurate in detecting IBR, but had high type I errors compared to partial Mantel tests. Thus, no one test outperformed another completely. A combination of statistical tests, for example partial Mantel tests to detect IBD paired with appropriate ordination techniques for IBR detection, provides the best characterization of fine-scale landscape genetic structure. Realistic simulations of empirical data sets will further increase power to distinguish among putative mechanisms of differentiation.

Keywords: landscape genetics, Mantel test, ordination, partial statistics, redundancy analysis

Received 16 July 2013; revision received 7 September 2014; accepted 10 September 2014

Introduction

Classical population genetics and contemporary landscape genetics traditionally involve analysing genetic differentiation within and among genetically discrete groups. Populations can be challenging to delineate in continuously distributed species that do not have clearly defined population boundaries. One approach for delineating cryptic population structure is to utilize Bayesian clustering algorithms (e.g. Pritchard *et al.* 2000; Guillot *et al.* 2005; Corander & Marttinen 2006) to define populations within a landscape. However, common patterns of spatial genetic structure such as isolation by distance (IBD; Wright 1943) or weak barriers to gene flow can lead to incorrect estimates of the number of genetically discrete populations across a landscape (Latch *et al.* 2006; Frantz *et al.* 2009; Schwartz & McKelvey 2009). In these situations, individual-based analytical approaches offer a viable alternative to population-based techniques. Because individual-based statistics do not require a priori definition of populations, they offer an appealing alternative approach to investigate a wide variety of fine-scale influences on gene flow.

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Individual-based methods in landscape genetics have become particularly popular for differentiating between two models of gene flow across a heterogeneous landscape: IBD and isolation by resistance (IBR; McRae 2006). IBD occurs when geographic distance is positively correlated with genetic differentiation regardless of landscape composition and often serves as a null hypothesis in landscape genetic studies. Conversely, in IBR, landscape heterogeneity (e.g. habitat types, roads, rivers) influences gene flow. To evaluate the relative influence of habitat variables on gene flow, the landscape itself is parameterized using user-defined resistance values. These resistance values are assigned to each pixel on a raster map based on their hypothesized effect on gene flow. For example, Cushman *et al.* (2006) parameterized

landscapes for black bears in Idaho such that anthropogenic structures such as roads and agriculture inhibited gene flow (high resistance) and suitable forest habitats promoted gene flow (low resistance). Selection of appropriate resistance values requires a priori knowledge of relevant landscape features (for discussion see Zeller *et al.* 2012), so previous life history data such as telemetry (e.g. Cushman & Lewis 2010; Reding *et al.* 2013), expert opinion (e.g. O'Brien *et al.* 2006) or presence/absence data (e.g. Walpole *et al.* 2012) are often employed to derive meaningful resistance surfaces (raster maps where each pixel has a resistance value). Each parameterized resistance surface, therefore, represents a hypothesis about how landscape features affect gene flow. Hypothesis testing then involves quantifying correlations between landscape resistance and pairwise measures of genetic differentiation to identify which landscape features, if any, explain patterns of genetic differentiation. One potential challenge to quantifying IBR is that landscape parameters of interest are often tightly correlated with geography. Autocorrelation in landscape and genetic variables can then result in spurious correlations in genetic analyses unless statistics can explicitly incorporate geographic covariates (Meirmans 2012).

One of the primary remedies for spatial autocorrelation in IBR studies has been the use of partial statistics, in which the effects of geographic coordinates are separated from landscape data. Partial statistics can isolate IBR from IBD, and therefore, prevent spurious correlations observed in aspatial statistics (e.g. Cushman & Landguth 2010; Meirmans 2012). Currently, the most prominent partial statistics in individual-based landscape genetics can be broadly classified as distance-based methods. Distance-based methods typically correlate pairwise distance metrics of landscape resistance with genetic differentiation (termed genetic distance) while controlling for straight-line (Euclidean) distances between individuals. Numerous metrics exist for estimating genetic differentiation between pairs of individuals, where larger calculated values of genetic distance typically indicate higher levels of divergence. Pairwise measures of cost to gene flow through a landscape between all pixels of the landscape (hereby called cost distance; see Djikstra 1959; McRae & Beier 2007) are then calculated based on resistance surfaces. Cost distances are essentially the cumulative effect of travelling through pixels of different resistances between two individuals, irrespective of geographic distance, so like resistance values, higher cost distances represent stronger impediments to gene flow. Generally, once pairwise distance measures are derived, distance-based partial statistics test for positive correlations between genetic and cost distances indicative of IBR. Although several distance-based methods can be adapted for genetic data, the partial Mantel test (Smouse *et al.* 1986) is unquestionably the most popular in landscape genetics.

Mantel tests quantify correlations between two or more matrices, and in landscape genetics, these matrices are typically composed of pairwise genetic, Euclidean and cost distances. Based on their widespread use in landscape genetics, simulation studies have largely focused on identifying factors that affect performance of Mantel tests like resistance strength (i.e. values of pixels within raster maps; Cushman *et al.* 2012, 2013a) and landscape configuration (Landguth *et al.* 2012). Resistance strength and landscape configuration have received attention in simulation studies because both can dramatically affect conclusions about gene flow (Cushman *et al.* 2012, 2013a; Oyler-McCance *et al.* 2013). In general, Mantel tests perform best in highly fragmented landscapes with high amounts of resistant matrix habitat (Cushman *et al.* 2012, 2013a). When resistance values of matrix habitat are much higher than suitable habitat (generally 5–10 times higher), partial Mantel tests detect inhibitory effects of habitat variables on gene flow (Cushman *et al.* 2012, 2013a). Even when landscape configuration and resistance values are set to maximize statistical performance within simulated data sets, multiple authors have raised concerns about potential problems with Mantel tests including low power (Legendre & Fortín 2010; Graves *et al.* 2013) and high type I error rates (Balkenhol *et al.* 2009; Guillot & Rousset 2013). These studies have provided important caveats for empirical studies that rely on Mantel tests and elucidate the need for alternative statistical approaches to complement results from Mantel tests and improve interpretation of empirical data sets.

Few other partial statistics have received the mass utilization as Mantel tests, but ordination techniques such as redundancy analysis (RDA) have recently been suggested as a viable alternative (e.g. Balkenhol *et al.* 2009; Legendre & Fortín 2010). Ordination techniques offer considerable flexibility as compared to Mantel tests because they do not require distance-based metrics and can overcome core assumptions of Mantel tests (e.g. linear relationships between variables). RDA is an unconstrained ordination technique where genetic data can be either genetic distances (distance-based redundancy analysis [dbRDA]; Legendre & Anderson 1999) or individualistic measures of genetic variation like allele frequencies. Individualistic metrics for landscape and geographic variables (e.g. spatial coordinates, habitat type identity, precipitation measures, side of putative barrier) increase the diversity of explanatory variables that can be tested while negating the requirement for pairwise distance calculations that can result in losses of statistical power (Legendre & Fortín 2010). Also, ordination techniques such as RDA and dbRDA can utilize

transformations such as principal coordinates analysis (PCoA) to linearize genetic variables, thus removing any potential violations of linearity observed in Mantel tests (Graves *et al.* 2013; Guillot & Rousset 2013).

Another potential advantage of ordination methods is that they provide measures of variance around parameter estimates, which allows for improved interpretation of results over Mantel tests that only provide a correlation coefficient and *P*-value (Legendre & Fortín 2010). Correlation coefficients from Mantel tests can be highly variable (Graves *et al.* 2013; Guillot & Rousset 2013), and without measures of variance, researchers may have trouble distinguishing between a type I error and the correct conclusion. In contrast, RDA provides statistics typical of ANOVAs including sum of squares, variance explained by each component and measures of variance around *F*-ratios. These additional results generated by RDA provide more information upon which to base interpretations about whether and how particular landscape variables influence gene flow (i.e. partition variance among landscape variables) and the statistical validity of the entire model (Legendre & Fortín 2010). Given these characteristics—the ability to overcome linearity assumptions, flexibility in type and number of explanatory variables, and statistical outputs—ordination methods offer potentially useful alternatives to Mantel tests.

Within individual-based genetics, simulation studies have evaluated the performance of Mantel tests under different scenarios that occur in empirical data sets (e.g. Cushman *et al.* 2012, 2013a). Our goal was to use simulations to test performance of ordination techniques, a potentially viable alternative to Mantel tests for individual-based landscape genetic analyses. We quantified the performance of partial Mantel tests and ordination methods by assessing each statistics' accuracy and explanatory power for detecting IBR (alternative hypothesis where landscape parameter was significant) while controlling for underlying patterns of IBD (null hypothesis of no effect of landscape heterogeneity). Simulations were designed such that spatial genetic structure was driven by IBD in all landscapes and additionally included varying levels of resistance across the landscape (IBR). Given that RDA does not require quantification of pairwise distances and tend to be more robust in population-based analyses (Balkenhol *et al.* 2009; Legendre & Fortín 2010), RDA was predicted to be more effective at detecting the effect of landscape resistance (i.e. costs to gene flow) than Mantel tests. In addition to comparing statistical approaches, we also designed simulations to assess the role of underlying landscape structure (habitat amount and configuration) and resistance strength of matrix habitat on statistical accuracy and explanatory power of IBR models. Both landscape structure and resistance strength have been identified as important predictors of accuracy in Mantel tests (Cushman *et al.* 2012, 2013a), and we expected that these variables would likewise influence performance in ordination methods, although perhaps to a different degree. Our findings are relevant to the larger field of work investigating optimal methods for detecting IBR in individual-based landscape genetics.

Methods

Generation of landscapes

Landscapes in nature are composed of a mosaic of features and resources that each species can perceive as suitable or unsuitable based on their life history requirements. Many species utilize areas within a heterogeneous landscape in a nonrandom way, and preference for or avoidance of certain landscape variables is often assumed to promote or inhibit gene flow, respectively (e.g. Shafer *et al.* 2012). Our simulated landscapes were composed of two habitat types: suitable and unsuitable (matrix) habitats. Heterogeneity of a landscape can be expressed using many different metrics, but we controlled two aspects of landscape configuration, level of fragmentation and proportion of suitable habitat, because these aspects have been shown to impact the strength of correlations between genetic differentiation and landscape resistance (Cushman *et al.* 2012, 2013a). The level of fragmentation, in particular, seems to have direct impacts on both demographic (Revilla & Wiegand 2008) and genetic processes (Keyghobadi 2007; Bruggeman *et al.* 2010) in empirical and simulated data sets, so controlling fragmentation and proportion of habitat types should allow us to isolate the effects of different cost measures and resistance strength on partial statistics.

We manipulated habitat configuration of the simulated landscapes by creating artificial landscapes in QRULE (Gardner 1999) using the 'Multifactal Random Map' function. Multifactal random maps were created by defining the amount of aggregation of pixels or level of fragmentation (*H*) as well as the proportion of suitable habitat (*P*). We chose an *H* value of 0.50 with suitable habitat comprising either 20%, 40%, 60% or 80% of the total landscape because extreme values in *H* or *P* in either direction are probably less realistic in nature or prevent meaningful levels of IBR or IBD within simulations. Only variation in *P* was tested within fractal landscapes for two reasons. First, Cushman *et al.* (2012, 2013a) found that configuration metrics (e.g. patch cohesion; Schumaker 1996 and correlation length of habitat; McGarigal *et al.* 2002) that explained genetic differentiation were highly correlated with *P*. Metrics that are correlated with *H* (e.g. clumpy index of habitat aggrega-

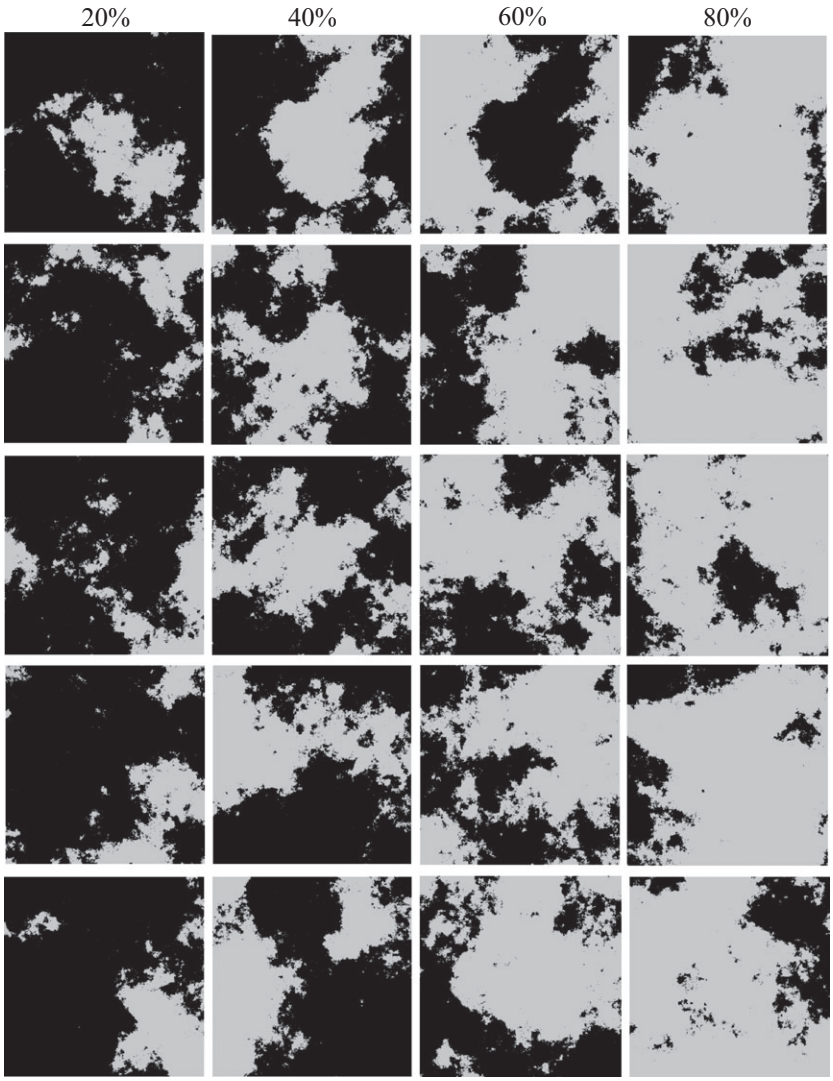

Fig. 1 Multifractal landscapes generated for this study where 400 individuals were randomly placed on each landscape. Each landscape consisted of two landscape categories: suitable (light grey) and matrix (black) habitat. The amount of suitable habitat (*P*) varied from 20% to 80% with five replicates within each *P* category. Pixels of suitable habitat were given a resistance value of 1, whereas matrix habitat was given one of four values (5, 10, 25 and 50) across simulations for a total of 80 simulated landscapes.

tion; McGarigal & Cushman 2002) also explained variation in genetic differentiation (Cushman *et al.* 2012, 2013a), but *H* itself was not important (Cushman *et al.* 2012). Second, we observed considerable variation in metrics associated with *H* by solely varying *P* because the effects of *H* and *P* are tightly linked (Cushman *et al.* 2012). Landscapes that have low habitat area also are highly fragmented and vice versa, so varying *P* produced varying levels of fragmentation even with a single *H* for all landscapes. Taken together, we decided to focus on varying *P* within our simulations to test performance in landscape genetic statistics. Five replicate landscapes were generated within each *P* category (20%, 40%, 60% or 80% suitable habitat) to evaluate variation in statistics, resulting in 20 independent landscape configurations (Fig. 1).

We calculated three configuration metrics (patch cohesion, clumpy index of habitat aggregation and correlation length of habitat) for all simulated landscapes. Configuration metrics have higher predictive power to explain how landscapes impact IBR than just suitable habitat alone (Cushman *et al.* 2012). Patch cohesion, a measure of habitat class aggregation, measures the physical connectedness of a focal habitat class as a percentage (McGarigal *et al.* 2002). Clumpy index of habitat aggregation (hereby called clumpy) also measures habitat class aggregation, but is not influenced by area of a focal habitat class (Neel *et al.* 2004). Clumpy ranges from −1 to 1 where −1 is completely disaggregated, 0 is random and 1 is maximally clumped (McGarigal *et al.* 2002). Finally, correlation length of habitat defines the average distance an individual could travel in a random direction without leaving suitable habitat when randomly placed in a habitat patch, making correlation length a measure of the extensiveness of suitable habitat (McGarigal *et al.* 2002).

By utilizing multiple landscapes from QRULE, we were able to isolate the impact of different resistance strengths in addition to suitable habitat amount and configuration on detection of IBR. Each simulated raster was 512 × 512 pixels (each pixel = 10 m × 10 m) where each pixel contained one of two cost values: suitable habitat was always set to 1 and the matrix was given a value of 1 (indicating IBD only), 5, 10, 25 or 50. Essentially, matrix habitat was parameterized such that high-valued pixels impeded gene flow, and the higher the value, the stronger the inhibitory effect. In total, five landscapes (R1, R5, R10, R25 and R50) per habitat area category (total = 100 landscapes) were utilized for gene flow simulations.

The spatial arrangement (and geographic coordinates) of individuals was identical for each population to facilitate comparison among the five resistance surfaces. Four hundred individuals were randomly generated onto the landscape using the 'Create Random Points' function in ARCMAP. In addition to spatial coordinates for each individual, pairwise cost matrices were calculated based on raster values using the 'cost matrix' function found in the landscape genetics toolbox for ARCGIS (Etherington 2011). This tool sums the resistances of pixels along a Euclidean line connecting two individuals and then produces a pairwise cost distance matrix. Euclidean distances between all pairs of individuals were also calculated for Mantel tests using the 'Euclidean Distance' function in the toolbox.

Gene flow simulations

Individual-based simulations were performed in the program CDPOP v. 1.3 (Landguth & Cushman 2010) using the cost matrices of the simulated landscapes. In total, 10 000 populations were simulated, 500 populations (5 replicates of 100 populations) for each of the five resistances (R1, R5, R10, R25, R50) across four habitat area categories (20%, 40%, 60%, 80% suitable habitat). Each simulation started with 400 individuals (200 male, 200 female) characterized at 10 polymorphic microsatellite loci (15 alleles per locus). Dispersal and mating movements occurred according to an inverse square distribution. An inverse square distribution describes a dispersal pattern in which it is far more common for individuals to remain near their birthplace than to disperse long distances. This dispersal distribution typifies many species in nature due to the risks associated with long-distance dispersal (Mayr 1963; Endler 1977). Furthermore, an inverse square dispersal distribution ensures an underlying IBD pattern in all simulated populations. Pairwise cost distance matrices constrained both mating and dispersal where the maximum movement through suitable habitat was the length of the landscape (6120 m) and decreased according to resistance strength in matrix (R5 = 1224 m, R10 = 612 m, R25 = 244.8 m, R50 = 122.4 m). Mating was sexual with replacement, and each female could have up to five offspring with an average of two. Generations were nonoverlapping and results were saved after 500 generations. Statistics were then run on 400 sampled individuals.

Statistical analysis: Mantel tests

Mantel tests require pairwise genetic distances, so we used SPAGEDI v. 1.3 (Hardy & Vekemans 2002) to calculate matrices of interindividual genetic distances (Rousset's *a*; Rousset 2000), for each simulated population. Matrices of Euclidean, cost and genetic distances (Table 1) were then utilized for full and partial Mantel tests in the R package VEGAN (functions *mantel* and *mantel.partial*; Oksanen *et al.* 2013). Simple Mantel tests compared genetic distance to Euclidean distances, and partial Mantel tests compared genetic and cost distances while controlling for Euclidean distance. This procedure was repeated for each population across resistance value categories (R1, R5, R10, R25 and R50) to quantify the ability of partial Mantel tests to separate the effects of cost and

Table 1 Summary of variables calculated for partial Mantel tests, dbRDA and RDA analyses

	Partial Mantel	dbRDA	RDA
Genetic data	Pairwise genetic distance	Pairwise genetic distance	sPCA spatially lagged scores
Landscape data	Pairwise cost distance	% Suitable habitat in buffer	% Suitable habitat in buffer
Geographic data	Pairwise Euclidean distance	Geographic coordinates	Geographic coordinates
Accuracy	Proportion of populations that had significant IBD or IBR tests when IBD or IBR was present		
Type I error rate	Proportion of populations where tests were significant for IBR when only IBD was present		
Type II error rate	Proportion of populations where tests were not significant for IBR when IBR was present		

Partial Mantel tests use pairwise distances for all variables. dbRDA uses genetic distance as the response variable and individualistic measures of ecological and geographic data as predictors. Genetic data for RDA were derived from a spatial principal components analysis (sPCA). All three tests had three indices of performance (accuracy, type I and type II error rates) calculated based on 100 simulated populations per landscape.

Euclidean distances as the relative effect of cost decreased. Statistical significance in all tests was assessed via Pearson's correlation coefficients (*r*) after 9999 permutations.

Landscape variable derivation: ordination

Ordination techniques can incorporate multiple explanatory variables into the analysis, but require individualistic measures of genetic diversity, geography and costs (Table 1). One difficulty in adapting ordination techniques to individual-based landscape genetics is quantification of landscape resistance to gene flow typically relies on interindividual distances. Multiple techniques exist to quantify cost distances (e.g. least cost path analysis; Djikstra 1959, circuit theory; McRae & Beier 2007), but cost distance calculations often require substantial life history information to properly parameterize a landscape (Spear *et al.* 2010). Information on dispersal itself instead of habitat use is very difficult to obtain, so even with habitat use data, cost distances may not reflect the factors that affect dispersal (Zeller *et al.* 2012). Therefore, we quantified habitat area around each individual to derive a landscape variable that does not depend on cost distances.

Ordination tests incorporated point estimates of habitat area surrounding each individual derived using following methodology. Circular buffers of 100, 200 and 300 m were initially drawn around each individual point using the 'Buffer' function in ArcMap v. 10. Then, the area of matrix habitat was calculated within each buffer and expressed as a proportion (area of matrix/total area in buffer) for use the landscape predictor variable. Point estimates of landscape resistance do not require a priori parameterization, which makes them an important complement to analyses that utilize cost distances derived from natural history data or expert opinion.

Statistical analysis: ordination

Calculations in dbRDA involve transforming genetic distances (Rousset's *a*) using a PCoA (Legendre & Anderson 1999) and then applying a normal RDA on the derived PCoA axes. All PCoA axes with positive eigenvalues are retained for analysis. When distances are completely linear, no axes will have negative eigenvalues. Genetic distances, however, are often not linear (Graves *et al.* 2013), so dbRDA effectively removes errors created by nonlinear distances to make them appropriate for RDA. As an alternative to PCoA, we also utilized spatial principal components analysis (sPCA; Jombart *et al.* 2008) to derive linear, spatially lagged scores as explanatory variables for a normal RDA.

In population-based analyses, population averages of genetic diversity are often employed as response variables in ordination techniques, but this method is not feasible for individual-based genetics. sPCA incorporates both genetic data and spatial autocorrelation to summarize overall genetic and spatial patterns into independent ordination axes. We chose to use sPCA because this technique removes much of the extraneous variation in genetic data to isolate important patterns and has been successful in isolating landscape effects in empirical individual-based studies (e.g. Robinson *et al.* 2012; Kierepka 2014). Unlike dbRDA that retains all positive PCoA axes, authors can choose the number of retained ordination axes from sPCA (Jombart *et al.* 2008). Typically, axes with the highest eigenvalues are retained for further analysis, but the exact number often depends on a study's focus and underlying processes affecting gene flow. For simplicity, we retained the first two sPCA axes in all calculations, and derived sPCA-lagged scores for genetic explanatory variables. Lagged scores correspond to each individual's position in ordination space and incorporate both genetic diversity and spatial autocorrelation. All sPCA calculations were performed in the R package ADEGENET (Jombart 2008).

Full and partial dbRDAs (function *capscale*) and RDAs (function *rda*) were performed in the R package VEGAN (Oksanen *et al.* 2013). Statistical significance for all ordination techniques (dbRDA and RDA) was assessed using the *anova.cca* function in VEGAN; this function is specifically designed to calculate pseudo *F*-ratios, variance components and *P*-values from ordination methods (Oksanen *et al.* 2013). As the total variance explained by ecological and geographic variables is typically very small (1.20–12.35% in our simulated data sets), we extracted pseudo *F*-ratios because like Pearson's correlation coefficients, higher *F*-ratios indicate a stronger relationship between landscape or geographic variables and genetic variation.

Statistical performance

In this study, statistical performance was first defined by counting the number of times each test correctly identified the underlying pattern of IBD or IBR (hereby termed accuracy; Table 1). Because all landscapes were characterized by an underlying IBD pattern, a correct test occurred when a full test was significant in the R1–R50 landscapes. In the IBR case (R5–R50 landscapes), accuracy of a partial test (in which geographic distances were controlled) was defined by how many significant partial tests occurred out of 100 simulated populations. Along with accuracy, we also counted how often each test did not correctly identify the appropriate mechanism driving gene flow (type I or II errors; Table 1). Type I errors

occur only in the R1 (IBD only) scenario when partial tests are significant for IBR despite no underlying IBR. In contrast, type II errors were defined as the inability to detect the effect of matrix habitat in the IBR landscapes (R5–R50).

We also assessed the effect of landscape heterogeneity (percent suitable habitat, landscape configuration metrics and resistance strength) on the strength of detected IBR relationship (i.e. explanatory power of the model). General linear models (glm) for each partial test incorporated either *F*-ratios (ordination techniques) or Pearson's correlation coefficients (Mantel tests) calculated from the IBR scenarios (R5–R50) as explanatory variables. Predictor variables included six variables: percent suitable habitat, clumpy and resistance as well as the residuals of regressions between percent suitable habitat and correlation length or patch cohesion. Separating configuration (i.e. correlation length and patch cohesion) from habitat amount (percent suitable habitat), effects can be difficult because these landscape variables are highly correlated (all Mantel $r > 0.866$; $P < 0.001$), but Cushman *et al.* (2012) showed that configuration metrics explained additional variance in Pearson's correlation coefficients. Therefore, percent suitable habitat was regressed against patch cohesion and correlation length to derive residuals that represent the variance in patch cohesion and correlation length not explained by percent suitable habitat. As our total data set contained 8000 populations (excluding IBD cases), we randomly selected 20 populations out of the total 100 within each landscape/resistance combination to minimize errors created by large sample size (final $n = 1600$).

Model selection used an information criterion approach (Burnham & Anderson 2002) to identify the best model among 31 candidate models. Model ranking occurred according to Burnham & Anderson (2002) where models with the lowest Akaike information criterion (AIC) and a ΔAIC value <2.0 were the best models. We calculated AIC weights to examine how likely the top model is the best model among all candidate models. Final parameter estimates for resistance and landscape variables were calculated through model averaging of all top models, and parameters that do not include zero explained significant variation within Mantel *r* or *F*-ratios. All glm and AIC procedures were performed in R.

Results

Both partial Mantel tests and dbRDA identified IBD in all populations (100%), whereas RDA could not explicitly test for IBD because sPCA axes explicitly incorporate both spatial autocorrelation and genetic variation, making it unable to isolate IBD in a simple test. Unlike IBD detection, where both partial Mantel tests and dbRDA consistently yielded high accuracy rates, IBR detection was highly variable across landscapes and resistance strengths. In the 20% suitable habitat landscapes, partial Mantel tests performed better (53–100% accuracy) than ordination methods (4–94% accuracy), but then accuracy in partial Mantel tests fell below ordination as suitable habitat increased. Type I error rates (i.e. detecting IBR when the resistance of matrix habitat was 1), were lower (5–26%) than type II error rates (17–96%) in Mantel tests. Ordination approaches also had higher type I errors, particularly in dbRDA (25–99%; details below; Fig. 2). Type II errors (i.e. failure to detect IBR when resistance of matrix habitat was >1) occurred in all landscapes and resistances with relatively high frequencies for all tests. High type II error rates were not unexpected given that intrapopulation levels of genetic structure are often quite small. Resistance strength, percent suitable habitat and landscape configuration influenced all statistical tests' abilities to detect the effect of matrix habitat.

The effects of landscape configuration and resistance strength on IBR detection accuracy were different for each test. Maximum performance in partial Mantel tests occurred within the 20% suitable habitat at resistance 50 where significant IBR was detected in 82–99% of populations. Correlation coefficients describing relationships between cost distances and genetic differentiation were low overall, ranging from −0.0487 to 0.125. The two top-ranked glm models (summed AIC weight = 0.9991; Table 2) included percent suitable habitat, resistance strength, correlation length and clumpy (all $P < 0.005$, Table S1). Pearson's correlation coefficients were negatively correlated with percent suitable habitat, correlation length and clumpy and positively correlated

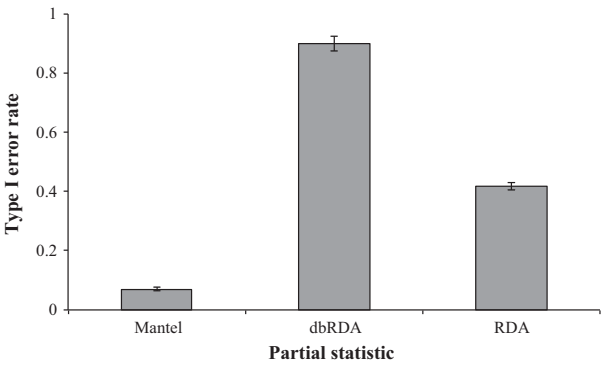

Fig. 2 Frequency of type I errors in partial Mantel tests, dbRDA and RDA in simulated landscapes. IBR was incorrectly detected most often in dbRDA tests and least often in partial Mantel tests. Error bars correspond to standard errors calculated across the 1600 simulated populations.

Table 2 Top six models that explained variation in partial Mantel *r*s (a), dbRDA pseudo *F*-ratios (b) and RDA pseudo *F*-ratios. Models are ranked according the lowest Akaike information criterion (AIC) value, and all those with a ΔAIC < 2.0 are considered top models (bold)

Model	AIC	ΔAIC	Weight
(a)			
%SH + Clumpy + Resistance + ResCL	**−7638.7**	**0.0**	**0.4996**
%SH + Clumpy + Resistance + ResCL + ResCoh	**−7638.7**	**0.0**	**0.4996**
%SH + Resistance + ResCL	−7625.4	13.3	0.0006
%SH + Resistance + ResCL + ResCoh	−7623.4	15.3	0.0002
%SH + Clumpy + ResCL	−7603.2	35.5	0.0000
%SH + Clumpy + ResCL + ResCoh	−7603.1	35.6	0.0000
(b)			
%SH + Clumpy + ResCL	**2783.6**	**0.0**	**0.4883**
%SH + Clumpy + Resistance + ResCL	**2785.0**	**1.4**	**0.2424**
%SH + Clumpy + ResCL + ResCoh	**2785.6**	**2.0**	**0.1796**
%SH + Clumpy + Resistance + ResCL + ResCoh	2787.0	3.4	0.0892
Clumpy + ResCL	2799.5	15.9	0.0002
Clumpy + Resistance + ResCL	2800.8	17.2	0.0000
(c)			
%SH + Clumpy + ResCL	**11437**	**0.0**	**0.3170**
%SH + Clumpy + Resistance + ResCL	**11438**	**1.0**	**0.1923**
%SH + Clumpy + ResCL + ResCoh	**11439**	**2.0**	**0.1167**
%SH + Clumpy + Resistance + ResCL + ResCoh	11440	3.0	0.0707
%SH + Clumpy	11440	3.0	0.0707
%SH + Clumpy + Resistance	11441	4.0	0.0429

%SH, percent suitable habitat; ResCL, residuals of linear regression between correlation length and percent suitable habitat; ResCoh, residuals of linear regression between patch cohesion and percent suitable habitat.

with resistance strength (Table 3). Taken together, these results show that partial Mantel tests detected IBR more often in landscapes with low percent suitable habitat, very high resistance of matrix habitat and high fragmentation of suitable habitat (low clumpy and correlation length).

Table 3 Model averaged parameters for the top glm models in partial Mantel tests, dbRDA and RDA. Only parameters that were shared between models are reported (i.e. models with an ΔAIC < 2.0). When 95% confidence intervals did not include zero, parameters were considered significant (bold)

	Estimate	SE	Upper CI	Lower CI
Partial Mantel tests				
%SH	**−0.0005**	**5.9953E-05**	**−0.0005**	**−0.0006**
Clumpy	−0.1379	0.0387	−0.0621	−0.2137
Resistance	**0.0002**	**3.1667E-05**	**0.0003**	**0.0001**
ResCL	**0.0006**	**0.0005**	**0.0008**	**0.0003**
Intercept	**0.1693**	**0.0341**	**0.2384**	**0.1021**
dbRDA				
%SH	**−0.9376**	**0.0013**	**−0.9351**	**−0.9401**
Clumpy	**7.1272**	**1.5802**	**10.2244**	**4.0299**
ResCL	**0.0096**	**0.0040**	**0.0174**	**0.0019**
Intercept	**−4.7656**	**1.1481**	**−2.5153**	**−7.0159**
RDA				
%SH	−0.0195	0.0132	0.0063	−0.0453
Clumpy	**59.3786**	**16.3289**	**91.3832**	**27.3739**
ResCL	0.0424	0.0412	0.1232	−0.0384
Intercept	**−49.0327**	**14.9210**	**−19.7876**	**−78.2779**

%SH, percent suitable habitat; ResCL, residuals of linear regression between correlation length and percent suitable habitat; SE, unconditional standard errors; Upper and Lower CIs, 95% confidence interval boundaries.

Overall, dbRDA had very high type I error rates where IBR was falsely detected in 62–99% of R1 populations (Fig. 2). In IBR landscapes (R5–R50), dbRDA rarely detected IBR as frequently as the R1 landscapes (Fig. 3). Accuracy within IBR landscapes was highly variable among resistances and amount of suitable habitat, but highest performance generally occurred within the 20% and 40% suitable habitat landscapes. The three top glm models (summed AIC weight = 0.9304) indicated that *F*-ratios in R5–R50 populations were positively associated with clumpy and negatively related to percent suitable habitat and correlation length (Table 3). All three variables remained significant after model averaging. Resistance and patch cohesion were not found to be significant factors in explaining *F*-ratios (Table 2; Table S2).

Like dbRDA, RDA with sPCA spatially lagged scores had higher type I errors than partial Mantel tests (Fig. 2). Type I errors in RDA ranged from 25% to 60%, which was similar to accuracy rates in IBR scenarios (20–68%; Fig. 3). However, IBR landscapes always had higher numbers of significant tests than the IBD case. The three top glm models included percent suitable habitat, clumpy and correlation length (summed AIC weight = 0.6259; Table 2). Only clumpy and the intercept remained significant after model averaging (Table 3), which is likely due to over half of competing models

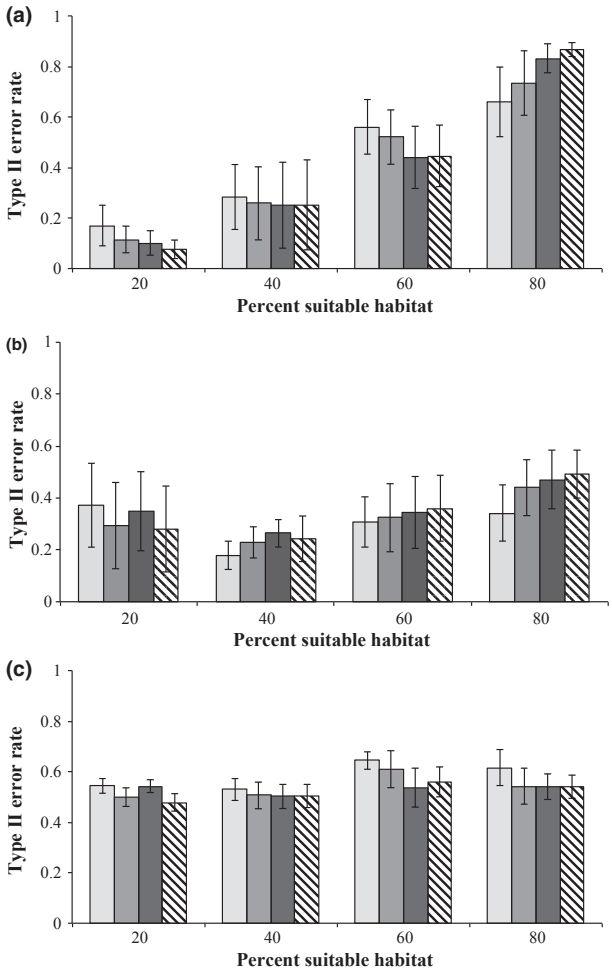

Fig. 3 Frequency of type II errors in partial Mantel tests (a), dbRDA (b) and RDA (c) in simulated landscapes. Five replicate landscapes were simulated for each of the four percent suitable habitat categories (20%, 40%, 60%, 80%) (R5—light grey bars; R10—grey bars; R25—dark grey bars; R50—striped bars). One hundred populations were simulated for each of the 80 total landscapes. Type II errors were calculated as the proportion of populations (of 100) in which IBR was not detected in our simulated landscapes, and corresponding error bars indicate standard errors calculated across five replicate landscapes. In general, type II errors increased with amount of suitable habitat but were similar across resistance levels for all tests.

having ΔAICs from 3.0 to 9.0 (Table S3). Clumpy was positively associated with *F*-ratios, whereas percent suitable habitat and correlation length were negatively correlated with *F*-ratios in RDA. Taken together, the results for dbRDA and RDA are concordant and show that IBR detection is highest in fragmented landscapes with a few clumped patches (low correlation length and high clumpy) and low–intermediate amounts of suitable habitat.

Discussion

Ordination techniques possess numerous characteristics that were predicted to enhance differentiation between IBD and IBR models of gene flow in individual-based studies over more common distance-based methods like Mantel tests. Ordination methods are able to overcome the linearity assumptions that characterize distance-based methods, offer flexibility in the type and number of explanatory variables and provide a suite of statistical outputs that permit thorough interpretation of results. Despite predictions that ordination methods would outperform Mantel tests, our data show that no one test was uniformly superior to any other. Across all landscapes, both partial Mantel tests and dbRDA were effective at detecting IBD regardless of the strength of IBR. The need to transform genetic data for RDA prevented complete isolation of IBD and IBR, making it impossible to explicitly test for IBD. In landscapes that were characterized by both IBD and IBR, IBR detection was highly variable across tests. Mantel tests use pairwise distances that have considerable noise, likely resulting in decreased power to detect IBR. In contrast, ordination techniques are more powerful tests (i.e. can detect IBR more often), but suffer from false detections of IBR likely created by variable transformations. Landscape composition also had a powerful impact on all test statistics. Maximum performance occurred in landscapes where IBR was strongest (highly fragmented with low suitable habitat). Ordination methods were more robust to changes in the underlying landscape than Mantel tests, suggesting that ordination methods might provide more meaningful conclusions about gene flow when little is known about the resistance of the landscape.

Performance of Mantel tests and ordination techniques

We found that both Mantel tests and dbRDA were highly effective at detecting IBD because they always detected IBD when it was present regardless of the strength of IBR. These high accuracy rates indicate that investigators can be confident that significant Mantel tests or dbRDA is strong evidence for IBD in a natural population. RDA, in contrast, could not test for IBD because sPCA does not explicitly control for IBD (Jombart *et al.* 2008), resulting in spatially lagged scores that reflect both IBR and IBD. IBD and IBR typically occur simultaneously in nature, so verification of IBD is a critical step before isolating the effects of IBR. Unlike the high accuracy and power in IBD tests, IBR detection was highly variable across tests and landscape configurations.

Mantel tests, the most common test in individual-based landscape genetics, generally had low accuracy rates for IBR tests, failing to detect IBR when it was

present. These high type II error rates suggest that Mantel tests' dependence on pairwise genetic, cost and Euclidean distances may reduce power to detect IBR. Indeed, Legendre & Fortín (2010) found that Mantel tests often suffer from reduced power as compared to ordination techniques due to data transformations into pairwise distances (Legendre & Fortín 2010). Similarly, Cushman *et al.* (2013a) found high frequencies of nonsignificant tests (745–1049/1250 tests) across simulated neutral landscapes with two habitat classes, suggesting Mantel tests have low power to detect IBR. If Mantel tests inherently have reduced power to detect IBR, then the subtle genetic differentiation present in individual-based studies may be too low to consistently detect IBR. The inability of Mantel tests to detect intrapopulation levels of genetic differentiation is concerning because Mantel tests may not detect IBR in empirical studies with levels of genetic differentiation similar to this study. For example, failure to identify genetic differentiation caused by anthropogenic land use could be a critical problem when trying to inform management agencies about how fragmentation impacts populations of conservation concern. Mantel tests can detect barriers if there is adequate lag time (Landguth *et al.* 2010), but recent anthropogenic barriers may not result in sufficient genetic differentiation to detect IBR. In cases where Mantel tests do not detect a barrier, we recommend use of more powerful tests and field data to better understand anthropogenic barriers to gene flow. Alternatively, if Mantel tests do detect a barrier, researchers can be confident that IBR is present because Mantel tests rarely detected IBR in IBD only landscapes within our simulations.

Despite their low accuracy rates in IBR detection, Mantel tests rarely detect IBR when only IBD is present (i.e. type I errors). This finding illustrates the utility of Mantel tests for individual-based landscape genetics. The low type I error rates found in this study are in contrast to findings for population-based simulation studies, which have found high rates of false IBR detection using Mantel tests (e.g. Balkenhol *et al.* 2009; Guillot & Rousset 2013). High type I errors for Mantel tests in population-based studies are likely due to greater genetic differentiation between population pairs and substantially fewer pairwise comparisons than individual-based studies, both of which increase power to detect IBR (and the potential for type I errors). Individual-based simulation studies have also recorded greater type I errors than this study, especially in complex landscapes with three or more habitat types (Cushman & Landguth 2010; Graves *et al.* 2013). In such complex landscapes, a type I error can stem from false significance of an IBR test in an IBD landscape or from detection of an incorrect landscape factor driving gene flow. More ways to generate type I errors in complex landscapes and often high correlations between alternative models lead to higher overall type I error rates (Cushman & Landguth 2010; Cushman *et al.* 2013b; Graves *et al.* 2013). In contrast, more simple landscapes in our study and Cushman *et al.* (2013a) seem to produce high frequencies of insignificant tests (i.e. type II errors), which indicates that error rates in Mantel tests are highly dependent on the complexity of a focal landscape and associated landscape genetic hypotheses. Partial Mantel tests can certainly perform well in some landscapes (e.g. Schwartz *et al.* 2009; Cushman *et al.* 2013b), but the high variability in their performance makes broad application of partial Mantel tests in landscape genetics prone to errors.

Our results suggest that ordination techniques (dbRDA and RDA) detect IBR more often than partial Mantel tests based on the higher number of significant partial tests in most IBR landscapes. The lower type II error rates in ordination techniques suggest they have higher power to detect IBR patterns than partial Mantel tests, which can be highly useful in situations where genetic differentiation is low, as is common in individual-based studies. For example, anthropogenic changes to once continuous landscapes (e.g. roads or land conversion) require multiple generations of reduced gene flow to produce genetic differentiation within a population (Landguth *et al.* 2010). Ordination techniques could potentially detect such contemporary barriers faster than Mantel tests because they can detect weaker IBR. However, one critical caveat of ordination techniques is their propensity to detect IBR even in populations that only exhibit IBD (i.e. type I errors). Both dbRDA and RDA exhibited high type I errors, which indicates they have difficulty removing the effects of IBD from IBR.

Type I errors were highest in dbRDA, a likely result of the transformation of genetic distances. Pairwise genetic distances are transformed using a PCoA, and then all resultant PCoA axes with positive eigenvectors are retained for analysis. In essence, PCoA removes the often high variability in raw genetic distances between individuals, so the reduced noise within genetic data may inflate type I error rates. PCoA is based on linear distances, so as genetic distances become less linear due to IBR, negative PCoA axes will occur. Thus, the amount of variation explained by PCoA axes is maximized within the R1 landscape because genetic distances are only correlated with Euclidean distance, making the amount of variation contained within PCoA axes very high. When IBR occurs, genetic distances are no longer solely correlated with Euclidean distance, so the PCoA attempts to force the non-Euclidean genetic distances into linear axes. Any remaining variation goes into negative PCoA axes and is not used in the dbRDA, so the amount of variation explained in genetic distances is

less than in the R1 landscape. Although PCoA transformations are designed to linearize and reduce noise in genetic data, dbRDA may not produce valid results in individual-based studies because it cannot effectively distinguish between IBD and IBR due to high type I errors.

Like dbRDA, RDA with sPCA-lagged scores exhibited high type I errors, which also is likely related to transformation of genetic data into ordination axes. Further investigation is warranted to understand if including more or fewer ordination axes impact performance in sPCA (and other similar techniques that would collapse genetic variation into ordination axes). In this study, we retained the first two sPCA axes because they explained the most variance and their eigenvalues were usually well differentiated from other axes. Even though sPCA summarizes patterns in spatial autocorrelation and genetic diversity, it does not explicitly control for it, so IBD can contribute to multiple sPCA axes (Jombart *et al.* 2008). As a result, ordination axes may just reflect IBD instead of patterns in IBR, so use of sPCA and RDA requires individual inspection of each axis to identify patterns that may be associated with IBD and IBR. Screeplots from sPCA aid in this process because they provide a visual representation of the amount of spatial autocorrelation within each axis (Jombart *et al.* 2008). Essentially, there is no rule for the number of sPCA axes to retain for genetic analysis, and each axis should be inspected individually to help understand how IBD and IBR impact genetic variation.

Although ordination techniques are more powerful tests (i.e. can detect IBR more often), removing IBD from IBR is difficult when using ordination-based genetic variables. Population-based studies often do not suffer from this problem because they can utilize population-specific variables such as allele frequencies and connectivity indices (see Balkenhol *et al.* 2009; Manel & Holderegger 2013; Pflüger & Balkenhol 2014). Population-specific allele frequencies and connectivity indices cannot be calculated for individuals leaving few alternatives to partial Mantel tests in individual-based studies. To date, focus in landscape genetic methods has generally been placed on comparing or developing methods that are alternatives to partial Mantel tests in population-based studies (e.g. Robinson *et al.* 2012; Wang 2013). Many of these population-based techniques offer considerable promise in individual-based landscape genetics, but development of proper genetic variables for individual-based techniques remains problematic. Therefore, we recommend exploration into alternative individual-based variables that could better disentangle IBR from IBD to maximize the utility of ordination and regression statistics in landscape genetics.

Influence of landscape composition on partial statistics

While intrinsic factors (i.e. variable type) of each test certainly contributed to accuracy and power, the underlying landscape also had a powerful impact on each tests' ability to detect IBR. Landscapes with high fragmentation and low suitable habitat had the highest performance indices (highest accuracy and test statistics and lowest error rates) across all tests. The high performance in the most extreme cases of fragmentation and habitat amount supported our prediction that highly fragmented landscapes with low suitable habitat would produce the strongest amount of genetic differentiation, making IBR easier to detect. Despite that maximum performance occurred in the same fragmented landscapes with low suitable habitat, partial Mantel and ordination test statistics were sensitive to different landscape metrics (i.e. percent suitable habitat, correlation length, patch cohesion, clumpy and resistance strength).

Mantel correlation coefficients were significantly associated with all landscape metrics except patch cohesion, indicating Mantel tests are highly sensitive to changes in the underlying landscape. Mantel tests detected IBR more often than ordination techniques only in the landscapes with the most extreme levels of fragmentation (i.e. low correlation length), suitable habitat and matrix resistance. Correlation length was particularly important for explaining Mantel correlation coefficients because correlation length is an indicator of the extensiveness of suitable habitat. Mantel tests rely on pairwise distance calculations, so measures of landscape resistance and genetic differentiation are highly dependent on how much suitable habitat occurs between two individuals. As correlation length decreases, the amount of suitable habitat between two individuals also decreases, which in turn elevates genetic distances. Therefore, a strong, significant Mantel correlation coefficient will occur in landscapes with small correlation lengths (see Cushman *et al.* 2012, 2013a). Another important factor in explaining Mantel correlation coefficients was resistance strength of matrix habitat where a positive relationship was recorded. Higher resistance strength increases genetic differentiation and significance in Mantel tests (Cushman *et al.* 2012, 2013a), so this relationship was not surprising, but it does raise an important issue for use of Mantel tests in empirical studies. Estimating resistances can be difficult without proper life history (Spear *et al.* 2010; Zeller *et al.* 2012), so the degree of error in Mantel tests will be unknown. Based on the fairly limited situations where Mantel tests have high accuracy rates, partial Mantel tests should be paired with other analyses like ordination techniques to help distinguish between a type II error and IBD.

Maximum performance (i.e. highest accuracy rates and *F*-ratios) of ordination methods occurred within the same fragmented, low suitable habitat landscapes as Mantel tests, but clumpy was the most important landscape variable. Clumpy had the biggest impact on strength of IBR in both dbRDA and RDA, which likely reflects the buffer calculation utilized as landscape variables. In more clumped landscapes, percent suitable habitat within a buffer was similar for many individuals. One critical advantage in ordination techniques was that resistance strength of the matrix had no impact on test statistics in either dbRDA or RDA. Ordination techniques do not require accurate parameterization of landscape resistance and are robust to changes in landscape resistance, so ordination techniques could be particularly useful for species lacking relevant life history data (e.g. presence-absence, mark–recapture, path analysis; Zeller *et al.* 2012) to parameterize resistance landscapes. Ordination techniques offer considerable promise for empirical studies, and we encourage further study with more complex landscapes and natural populations to understand performance of ordination techniques in individual-based landscape genetics.

Conclusions

Investigation of IBR using ordination techniques will probably be most informative when paired with simulated populations that exhibit IBD to quantify type I errors. In particular, simulations of populations under IBD (i.e. null hypothesis) with geographic locations, landscape configuration and genetic diversity that mimic the study population can aid in characterizing how often statistics produce erroneous conclusions about gene flow (Kierepka 2014). Simulations should closely mirror the empirical data set because deviating from observed landscape configuration, genetic data parameters or sampling schemes can have drastic impacts on statistical results (e.g. Cushman *et al.* 2012, 2013a). Interpretation of simulated populations in an individual-based framework could follow several paths, but we recommend quantifying type I errors for each proposed landscape hypothesis and only accepting factors that were not detected in IBD landscapes at high frequencies (e.g. 5%). Simulating populations under IBR and comparing them to empirical data sets is another possibility, but meaningful simulations require considerable life history data (i.e. population-specific demographics) that are largely unavailable for many species.

Despite the risks of type I errors with ordination tests, they are potentially more versatile than Mantel tests based on their ability to reduce noise within data sets and utility in a wide variety of landscape genetic scenarios. Partial Mantel tests are likely to perform well in species with strong habitat specialization or patchy distribution (e.g. Schwartz *et al.* 2009; Wasserman *et al.* 2010; Shafer *et al.* 2012). Our results indicate that partial Mantel tests may not be accurate for species that utilize a variety of habitats or are continuously distributed. For these species, estimating resistance can be difficult even with the inclusion of individual movements that relate to single landscape factors (e.g. Zeller *et al.* 2012). In cases where the resistances of landscape factors are unknown and population boundaries are difficult to define, solely applying Mantel tests is unlikely to be an effective method to understand genetic connectivity. Combining Mantel tests and ordination techniques to evaluate specific appropriate hypotheses, synthesizing the results across approaches to facilitate comprehensive interpretation and using simulations to evaluate significance of test statistics may be the best way to glean meaningful conclusions about gene flow in individual-based landscape genetics.

Acknowledgements

We would like to extend our gratitude to the University of Wisconsin-Milwaukee for providing laboratory space and funding through the Distinguished Dissertator Fellowship, and Ruth Walker, Ivy Balsam, James D. Anthony and Joseph Baier Awards. Valuable insight into variable derivation and implementation was provided by F. Alberto, M. Johansson, C. Cullingham, S. Robinson and N. Keyghobadi. We also thank the anonymous reviewers for their comments that greatly improved this manuscript.

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Both authors (E.M.K and E.K.L) contributed to conceptualization and writing of the manuscript. E.M.K performed all analyses.

Data accessibility

R scripts, example input files and simulation results are available on Dryad DOI: 10.5061/dryad.sc88q.

Supporting Information

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

Table S1 All ranked glm models relating partial Mantel test *r* and landscape metrics (percent suitable habitat, landscape configuration metrics, and resistance).

Table S2 All ranked glm models between *F*-ratios from dbRDA and landscape metrics (percent suitable habitat, landscape configuration metrics, and resistance).

Table S3 All ranked glm models between *F*-ratios from RDA and landscape metrics (percent suitable habitat, landscape configuration metrics, and resistance).