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RESEARCH ARTICLE

Landscape genetics and limiting factors

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Abstract Population connectivity is mediated by the movement of organisms or propagules through landscapes. However, little is known about how variation in the pattern of landscape mosaics affects the detectability of landscape genetic relationships. The goal of this paper is to explore the impacts of limiting factors on landscape genetic processes using simulation modeling. We used spatially explicit, individual-based simulation modeling to quantify the effects of habitat area, fragmentation and the contrast in resistance between habitat and non-habitat on the apparent strength and statistical detectability of landscape genetic relationships. We found that landscape genetic effects are often not detectable when habitat is highly connected. In such situations landscape structure does not limit gene flow. We also found that contrast in resistance values between habitat and non-habitat interacts with habitat extensiveness and fragmentation to affect detectability of landscape genetic relationships. Thus, the influence of landscape features critical to providing connectivity may not be detectable if gene flow is not limited by spatial patterns or resistance contrast of these features. We developed regression equations that reliably predict whether or not isolation by resistance will be detected independently of isolation by distance as a function of habitat fragmentation and contrast in resistance between habitat and non-habitat.

Keywords Landscape genetics · Limiting factors · CDPOP · Fragmentation thresholds · Simulation modeling

Introduction

Population connectivity is centrally important to many ecological processes and conservation goals, and may often be a key factor affecting regional viability of populations (Flather and Bevers 2002; Hanski 2005; Cushman 2006). Habitat loss and fragmentation affect population density (Wiegand et al. 1999, 2005; Revilla and Wiegand 2008) and distribution (Fahrig and Merriam 1985; Fahrig and Paloheimo 1988; Hanski and Ovaskainen 2000), and can decrease dispersal (Gibbs 1998), reduce genetic diversity (Reh and Seitz 1990; Wasserman et al. 2012a, b), and increase mortality (Fahrig et al. 1995). Populations may decline and habitats may not be recolonized following local extinction if immigration is prevented (Brown and Kodric-Brown 1977; Harrison 1991). Thus, the ability of individual animals to move across complex landscapes is critical for maintaining regional populations (Fahrig 2003; Cushman 2006; Lindenmayer and Fischer 2007).

Rigorous evaluation of fragmentation thresholds and connectivity limiting factors is critical to understand effects of habitat loss and fragmentation on population connectivity. Landscape genetics provides a particularly powerful approach

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for estimating effects of multiple landscape features on population connectivity (Manel et al. 2003; Holderegger and Wagner 2008; Balkenhol et al. 2009; Segelbacher et al. 2010). The genetic characteristics of many individuals sampled across large extents can be directly associated with hypotheses of connectivity (Spear et al. 2005; Cushman et al. 2006; McRae and Beier 2007; Pérez-Espona et al. 2008; Lee-Yaw et al. 2009; Shirk et al. 2010; Wasserman et al. 2010; Murphy et al. 2010). Individual-based landscape genetics methods have recently been shown to be highly powerful (Cushman and Landguth 2010) and sensitive to recent landscape change (Landguth et al. 2010), enabling researchers to test how specific landscape features and environmental conditions affect population connectivity.

However, little is known about how the extent, fragmentation and relative resistance of habitat in the landscape influences how strongly populations are spatially structured and the power of statistical methods to detect this structure. It is possible for certain landscape features to be essential for population connectivity, while at the same time not producing detectable effects. The detectability of landscape genetic relationships is related to whether or not each landscape feature limits movement and gene flow in a given landscape (e.g. Cushman et al. 2011; Jaquiéry et al. 2011). For example, recent empirical work has suggested that there may be threshold relationships between landscape complexity and genetic differentiation (Short Bull et al. 2011). Short Bull et al. (2011) found that landscape features such as forest cover, roads and elevation were consistently important but inconsistently detected drivers of genetic differentiation in American black bear (*Ursus americanus*). Similar results have also been seen in studies modeling movement based on telemetry (e.g. Cushman and Lewis 2010) and snow tracking (e.g. Cushman et al. 2011).

The empirical examples mentioned above indicate the existence of fragmentation thresholds, below which a critical dependence upon certain landscape features may be invisible to analysis. However, such empirical studies are limited in their abilities to unequivocally identify the drivers of observed patterns (Shirk 2012). Simulation modeling provides explicit control over pattern-process relationships (Epperson et al. 2010), which enables rigorous attribution of the causes of observed differentiation, which is not possible in empirical studies (Shirk 2012). By varying functional parameters, environmental characterization, and organism attributes, simulation modeling can investigate hypotheses about the relative influence of different landscape factors, their interactions, and organism life history characteristics. This provides a means to evaluate complexes of factors that would be impossible to investigate directly in the field, and to quantify how reliably the effects of landscape structure on gene flow can be detected as a function of habitat extent and fragmentation (e.g. Cushman et al. 2012).

Simulation modeling has been recently used to evaluate effects of landscape complexity on genetic differentiation. Bruggeman et al. (2010) used simulation modeling to quantify both the influence of patch size and patch isolation on abundance, effective population size and *F*st in the red-cockaded woodpecker. Their work is one of the first to provide an explicit link between population genetic processes, habitat area and critical thresholds of fragmentation affecting those processes. Their results suggest that population genetic structure is more strongly affected by habitat fragmentation than habitat patch size. However, this study did not explore how the relative importance of habitat area versus fragmentation may depend on the relative degree of resistance contrast between habitat and non-habitat. Nor did they evaluate relationships across a sufficient range of habitat area and fragmentation to quantitatively explore thresholds and limiting factors.

Cushman et al. (2012) used an individual-based spatially explicit landscape genetics simulation model to evaluate the relative influences of habitat area and fragmentation on genetic differentiation in complex landscape mosaics. They found that both habitat area and fragmentation affected genetic differentiation, and that several measures of habitat extensiveness and fragmentation were stronger predictors of genetic differentiation than habitat area alone.

An area of particular current research importance is the evaluation of how well different analytical approaches perform in identifying the correct driving process governing gene flow in complex landscapes, and under what conditions they perform reliably (Segelbacher et al. 2010; Balkenhol et al. 2009). Recently, Jaquiéry et al. (2011) compared the power and accuracy of three methods for inferring the relationship between landscape resistance and genetic differentiation, and found that the power and accuracy of all three methods are good when there is strong contrast between the permeability of different landscape elements. They found that power and accuracy decrease drastically when landscape complexity increases and the contrast between permeability of landscape elements decreases.

The goal of this paper is to explore the effects of landscape complexity and varying landscape resistance on the ability of partial Mantel tests to detect the effects of isolation by landscape resistance independently of isolation by distance. There has been confusion in the literature about the appropriateness of Mantel testing in landscape genetics. Raufaste and Rousset (2001) questioned the use of partial Mantel tests in micro-evolutionary studies. Subsequently, Castellano and Balletto (2002) attempted to rehabilitate the use of the partial Mantel test in genetic analysis. Recently, Legendre and Fortin (2010) clarified this confusion. They show that Raufaste and Rousset (2001) raised a valid point about a situation requiring a particular permutation procedure, but made unwarranted claims that partial Mantel tests are a

biased testing procedure, while Castellano and Balletto (2002) attempted to refute this, but advocated an inappropriate testing procedure. Legendre and Fortin (2010) note that distance-based regression approaches, such as the Mantel test, have lower power than traditional linear models and tend to underestimate the true magnitude of relationship. They conclude that Mantel and partial Mantel testing is the appropriate framework when the hypotheses are explicitly defined in terms of distances, as they are landscape genetic analyses of the effects of landscape resistance on neutral genetic differentiation.

Recently, Cushman and Landguth (2010) evaluated the power of partial Mantel testing in a causal modeling framework (Legendre and Troussellier 1988; Legendre 1993) and found that the method performs very well in identifying the drivers of genetic distances in complex landscapes and rejecting incorrect and correlated alternatives. In addition, the Mantel test applied to individual-based genetic data is very effective in detecting recent landscape changes (Landguth et al. 2010). However, both empirical (Short Bull et al. 2011) and simulation (Jaquiéry et al. 2011; Cushman et al. 2012) studies have shown that ability to identify the drivers of genetic differentiation is highly sensitive to the composition and configuration of landscape structure.

Quantitative information about the effects of habitat amount, configuration and contrast in landscape resistance between habitat and non-habitat is essential to guide interpretation of landscape genetic analyses in complex landscapes. It is critical to know when landscape patterns begin to limit gene flow and thus become detectable to landscape genetic analysis. In this paper, we used neutral landscape models to produce a factorial of landscapes with a controlled range of habitat area and fragmentation. We then used an individual-based simulation model to produce spatially explicit predictions of genetic differentiation that would be expected in each landscape. Our work was designed to evaluate four hypotheses. We expected that (1) landscape genetic effects would often not be detectable when habitat extensiveness is high, because when there is extensive habitat movement will not be limited by patterns of non-habitat. Second, we hypothesized that (2) landscape genetic effects would often not be detectible when habitat is not fragmented, as spatial patterns in unfragmented landscapes would not limit gene flow. Further, we expected that (3) the strength and detectability of landscape effects on genetic differentiation will be positively related to the relative resistance of non-habitat compared to habitat. Finally, we hypothesized that (4) it would be possible to develop predictive regression equations that reliably predict whether or not partial Mantel tests would identify a significant effect of landscape resistance independent of geographical distance as a function of habitat extent, fragmentation and relative resistance.

Methods

Factorial modeling experiment

We designed a factorial modeling experiment to explore how variation in habitat extensiveness, fragmentation and relative resistance of habitat and non-habitat affected the detectability of landscape genetic relationships. We used the neutral landscape model QRULE (Gardner 1999) to produce simulated binary landscape maps (habitat vs. non-habitat) which varied across controlled levels of habitat area and fragmentation (Fig. 1). QRULE controls fragmentation through the H parameter, which affects the aggregation of pixels into patches. All maps were 256 × 256 pixels in size. We varied habitat area across five levels (15, 35, 55, 75, 95 %), and H across five levels (0.2, 0.4, 0.6, 0.8, 1.0). We varied the ratio of resistance of non-habitat relative to habitat across five levels (1.5, 2, 4, 8, 16). Finally, we produced 5 replicates of this 3-way factorial to assess variation and improve statistical power, producing a total of 625 landscapes for analysis.

Selection of landscape fragmentation metrics

We selected a suite of landscape metrics to measure habitat extensiveness and fragmentation in the simulated maps. This selection was based in part on past work which assessed the strength and shape of relationship between a large number of landscape metrics and the H level in QRULE landscapes (Neel et al. 2004). Also, several landscape configuration metrics have previously been shown to be sensitive indicators of the effects of landscape structure on spatial population processes (e.g. Patch Cohesion, Schumaker 1996) and gene flow (Correlation length; Cushman et al. 2010; Short Bull et al. 2011). Cushman et al. (2012) showed that Patch Density, Patch Cohesion, Correlation Length and Aggregation Index are among the strongest predictors of the strength of landscape genetic relationships. Based on these considerations, we chose Patch Density, Correlation Length, CLUMPY, Patch Cohesion, and Aggregation Index as our suite of landscape fragmentation metrics. Patch Density is a measure of the density of habitat patches in the landscape. Correlation Length is the area-weighted mean patch radius of gyration, and measures the distance one can move in a random direction and remain within habitat if dropped into a random habitat pixel. CLUMPY is an index of habitat aggregation that is standardized relative to habitat amount, and reflects the degree to which habitat is non-randomly aggregated into contagious patches. The Aggregation Index measures the degree of aggregation of habitat into contagious patches, without being standardized relative to habitat amount. Patch Cohesion (COHESION) was proposed

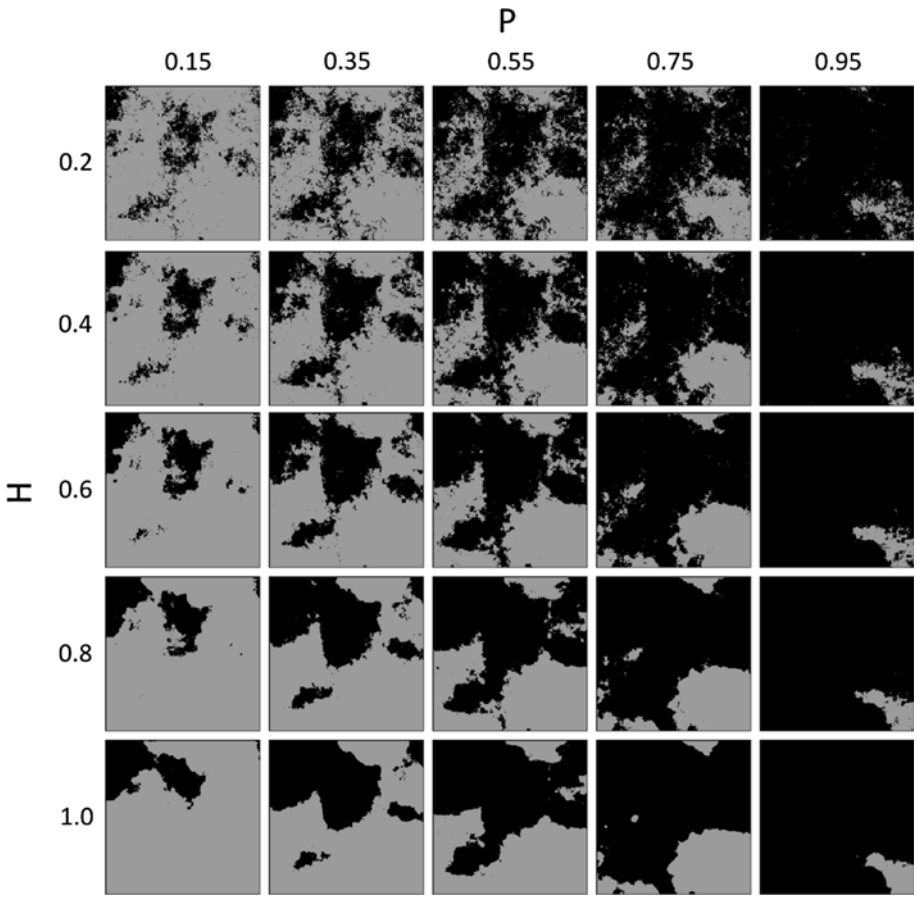

Fig. 1 Schematic of the simulation modeling experiment. We created 10 replicate landscapes at each combination of levels of habitat area (P—five levels; 0.15, 0.35, 0.55, 0.75, 0.95) and habitat fractal aggregation (H—five levels; 0.2, 0.4, 0.6, 0.8, 1.0). In the figure above habitat is *black* and non-habitat is *gray*

by Schumaker (1996) to quantify the connectivity of habitat as perceived by organisms dispersing in binary landscapes. Patch Cohesion is proportional to the area-weighted mean perimeter-area ratio divided by the area-weighted mean patch shape index (i.e., standardized perimeter-area ratio). All metrics were calculated using FRAGSTATS (McGarigal et al. 2002).

Landscape genetic simulation with CDPOP

We used CDPOP version 0.84 (Landguth and Cushman 2010) to simulate the processes of mating and dispersal as functions of the spatial patterns of habitat and non-habitat on these 625 simulated landscapes. CDPOP is an individual-based, spatially explicit, landscape genetic model that simulates birth, death, mating and dispersal of individuals in complex landscapes as probabilistic functions of movement cost among them. The model represents landscape structure flexibly as resistance surfaces whose value represents the stepwise cost of crossing each location. The model simulates mate selection and dispersal as probabilistic functions of cumulative cost across these resistance surfaces. Breeding is simulated with Mendelian inheritance and k-allele mutation, a commonly used mutation model for microsatellite loci (Balloux 2001; Manel et al. 2007). The user specifies the locations and genotypes of the initial population and the model simulates spatially explicit population genetic change through time as functions of individual-based movement (mate choice and dispersal), mating, mutation and mortality on a continuous cost surface.

In each of the 625 landscape maps, we randomly placed 500 individuals in habitat pixels. We simulated gene flow among these locations for 500 non-overlapping generations. Previous research has shown that the relationship between genetic structure and landscape resistance equilibrates relatively rapidly, generally within 100 simulated generations (e.g. Cushman and Landguth 2010; Landguth et al. 2010; Jaquiéry et al. 2011). We stipulated the population to have 10 diploid loci, with 10 alleles per locus, initially randomly assigned among individuals. These loci were treated as selectively neutral and simulated with a k-alleles mutation rate of 0.0005 (Balloux 2001). We used an inverse square mating and dispersal probability function, with maximum dispersal cost-weighted distance of 7,680 m (the diameter of the simulated landscapes) in ideal habitat (i.e. a resistance value of one). Reproduction was sexual with non-overlapping generations, and the number of offspring was based on a Poisson probability draw with mean of four. For each of the

Table 1 Matrix of Pearson product-moment correlations among independent variables

	GYRATE_AM	COHESION	CLUMPY	PD	AI
GYRATE_AM	x	**0.873**	0.064	−0.405	0.638
COHESION		x	0.323	−0.564	**0.828**
CLUMPY			x	−0.686	0.642
PD				x	**−0.908**

GYRATE_AM correlation length of habitat, *COHESION* patch cohesion of habitat, *CLUMPY* clumpy metric of habitat aggregation, *PD* density of habitat patches, *AI* aggregation index of habitat aggregation

Bold indicates pair-wise correlations of landscape metric values over 0.7. Only one of each pair of variables with correlation of this magnitude are included in candidate regression models

625 landscape maps, we ran 10 Monte Carlo replicate runs in CDPOP to assess stochastic variability. This resulted in 6250 CDPOP simulations.

Partial mantel tests to assess pattern-process relationships

CDPOP calculated a matrix of pairwise genetic distances between all 500 simulated individuals based on the proportion of shared alleles at generation 500 (Bowcock et al. 1994). We calculated a matrix of pairwise effective landscape distances between all individuals based on accumulated least-cost distance using the COSTDISTANCE function in ArcGIS (ESRI 1999–2008). To assess the relationship between the genetic and landscape distance matrices, we used Mantel tests (Mantel 1967), implemented in the ECODIST package in *R* (Goslee and Urban 2007). We calculated partial Mantel *r* (correlation between genetic distance and cost distance, partialling out Euclidean distance) for all 6,250 simulated populations at generation 500, and assessed statistical significance with 999 permutations.

Predicting significance of partial mantel *r* as functions of landscape metrics

We used logistic regression implemented in *R* (function glm) to predict whether or not a significant partial Mantel *r* correlation (at a $p < 0.05$ significance level) was detected as functions of the selected landscape metrics and relative landscape resistance (*R* Development Team 2009). We adopted an information theoretic modeling approach (Burnham and Anderson 2002). We developed a pool of candidate models including all combinations of the set of landscape metrics except those whose Pearson correlations exceeded 0.7 (Table 1). This produced an a priori set of 15 candidate models (Table S1). We evaluated this set of models at each of the five levels of relative resistance of habitat and non-habitat (R1.5, R2, R4, R8, R16). Given that it is possible that non-linear relationships may exist between landscape metrics and probability of significant partial Mantel *r* value, we explored a range of non-linear transformations of the independent variables (log, inverse, power, polynomial). No non-linear transformations improved model performance, and we retained the original linear forms for analysis.

We ranked models based on AIC and calculated AIC model weights. We evaluated the performance of the most supported model at each level of relative resistance in seven ways. First, we calculated Somers' Dxy rank correlation between predicted probabilities and observed responses. Dxy is the difference between concordance and discordance probabilities. When Dxy = 0, the model is making random predictions. When Dxy = 1, the predictions are perfectly discriminating. Second, we calculated the generalized R^2 *N* index of Nagelkerke. This has the same general interpretation as the usual R^2 but is more appropriate for a binary logistic regression model. Third, we calculated the percent of observations correctly classified by the most supported regression model at a cutpoint that optimized the Kappa statistic. Fourth, we calculated model sensitivity, which is the proportion of significant landscape genetic effects predicted to be significant. Fifth, we calculated model specificity, the proportion of non-significant landscape genetic effects predicted to be non-significant. Sixth, we calculated Kappa, the chance corrected improvement on random assignment to significant or non-significant classes. Seventh, we calculated the area under the receiver operating characteristic curve (AUC).

Overfitting is the major cause of unreliable models in logistic regression (Hosmer and Lemeshow 1989). Bootstrapping has proven to be the superior method for evaluating whether overfitting has reduced predictive accuracy (Harrell 2001). The bootstrapping provides an estimate of the expected value of the optimism, which when subtracted from the original index, provides an estimate of the expected bias-corrected index. Thus, a robust model is one in which the corrected index is nearly the same as the original index. We calculated the bootstrap optimism estimates for nine model attributes: (1) Somers' Dxy rank correlation between predicted probabilities and observed responses. (2) Generalized R^2 *N* index of Nagelkerke. (3) Maximum absolute difference in predicted and calibrated

Table 2 Frequency of significant partial mantel *r* correlations between genetic differentiation and landscape cost distance across the five levels of relative resistance

Relative resistance	Frequency non-significant	Frequency significant
R1.5	1,049	201
R2	861	389
R4	785	465
R8	732	518
R16	745	505

The five levels of relative resistance are R16 (non-habitat 16 times as resistant as habitat), R8 (non-habitat 8 times as resistant as habitat), R4 (non-habitat 4 times as resistant as habitat), R2 (non-habitat 2 times as resistant as habitat), and R1.5 (non-habitat 1.5 times as resistant as habitat

probabilities. (4) Discrimination index (model LR $(\chi^2 - 1)/n$). (5) Unreliability index; the difference in −2 log likelihood between un-calibrated X beta and X beta with overall intercept and slope calibrated to test sample/*n*. (6) Overall quality index (logarithmic probability score) $Q = D - U$. (7) Brier or quadratic probability score. (8) The intercept and (9) slope of the regression equation.

We evaluated variable importance for all variables in the most supported model at each level of relative resistance by calculating the change in log-odds for a change in the variable between the 25th and 75th percentile of its sample distribution. We held all other variables constant at their medians when calculating change in log-odds between the inter-quartile range.

Results

Significance of partial mantel *r* as functions of habitat extensiveness and fragmentation

There was a large difference in the frequency of significant partial Mantel *r* values at each level of relative resistance (Table 2). When non-habitat was 1.5 times as resistant as habitat, only 16 % of the simulated landscape patterns produced significant correlations between genetic differentiation and cost distance, after partialling out the effects of Euclidean distance. This proportion increased to 31 % when non-habitat was twice as resistant as habitat, 37 % when it was four-times as resistant, and approximately 40 % when non-habitat was eight or 16 times as resistant as habitat.

When non-habitat was 1.5 times as resistant as habitat, the top model had an AIC weight of 0.454 (Table 3; Table S1). The AIC weight of the top model increased with relative resistance, indicating higher confidence in the top model at higher levels of contrast in movement cost between habitat and non-habitat. When non-habitat was twice as resistant as habitat, the top model that had an AIC weight of 0.7. When non-habitat was four or more times as resistant as habitat the top model had an AIC rank between 0.95 and 1, indicating near complete support for it as the best model explaining differences between significant and non-significant partial Mantel correlations.

Table 3 Parameter estimates of the supported model, and min, median and max values for the landscape variables included in the model

	Parameter	Standard error	Pr (>|z|)
R16			
(Intercept)	148.1774	14.23572	$<2.00 \times 10^{-16}$
COHESION	−1.59123	0.13963	$<2.00 \times 10^{-16}$
CLUMPY	9.78702	1.7776	$3.68E^{-8}$
PD	0.34718	0.05545	$3.83E^{-10}$
R8			
(Intercept)	8.67E	1.29	1.87×10^{-11}
GYRATE_AM	-1.42×10^{-3}	1.17×10^{-4}	$<2.00 \times 10^{-16}$
AI	-6.39×10^{-2}	1.44×10^{-2}	9.08×10^{-6}
R4			
(Intercept)	−3.125	1.20	0.009213
GYRATE_AM	-8.71×10^{-4}	9.47×10^{-5}	$<2.00 \times 10^{-16}$
CLUMPY	4.46	1.18	0.000154
PD	1.66×10^{-1}	3.88×10^{-2}	1.88×10^{-5}
R2			
(Intercept)	7.037419	1.230802	1.08×10^{-8}
GYRATE_AM	−0.00072	0.000143	4.24×10^{-7}
CLUMPY	3.220334	1.309376	0.0139
AI	−0.09789	0.02435	5.82E−05
R1.5			
(Intercept)	−2.0676	0.6014	0.000586
CLUMPY	0.4751	0.6807	0.485233

COHESION patch cohesion of habitat, range 0–100, units unitless index, min 95.215, Max 99.99, median 99.974; *GYRATE_AM* correlation length of habitat, range 0—unbounded, units meters, min 422.9, max 2909.3 median 2431.6; *PD* patch density of habitat, range 0—unbounded, units patches per 100 hectares, min 0.017, max 12.16, median 0.8308; *AI* habitat aggregation index, range 0–100, units unitless index, min 68.88, max 99.92, median 97.65; *CLUMPY* clumpy index of habitat aggregation, range 1–1, units unitless index, min 0.5488, max 0.9956, median 0.9213

When non-habitat was 1.5 times as resistant as habitat, the top model included only a single variable (CLUMPY). Correlation Length, CLUMPY and Aggregation Index were included in the top model with non-habitat was twice as resistant as habitat. When non-habitat was four-times as resistant as habitat Correlation Length, CLUMPY, and Patch Density were the variables in the top model. At R8 only two variables (Correlation Length and Aggregation Index) were included in the best model. When non-habitat was 16 times as resistant as habitat, three variables (Patch Cohesion, CLUMPY and Patch Density) were included in

Table 4 Model performance statistics

Relative resistance	Dxy	R^2	PCC	Sensitivity	Specificity	Kappa	AUC
16	0.690	0.408	0.769	0.887	0.689	0.545	0.845
8	0.611	0.358	0.749	0.708	0.777	0.484	0.805
4	0.465	0.193	0.681	0.662	0.691	0.341	0.733
2	0.497	0.273	0.762	0.478	0.891	0.399	0.749
1.5	0.060	0.001	0.425	0.652	0.281	0.015	0.530

Dxy-Somers' Dxy rank correlation between predicted probabilities and observed responses, by the identity Dxy = $2(c - 0.5)$. Dxy is the difference between concordance and discordance probabilities. When Dxy = 0, the model is making random predictions. When Dxy = 1, the predictions are perfectly discriminating

R^2 generalized R^2 *N* index of Nagelkerke, *PCC* percent observations correctly classified at the max-kappa cutpoint, *Sensitivity* proportion of significant landscape genetic effects predicted to be significant, *Specificity* proportion of non-significant landscape genetic effects predicted to be non-significant, *Kappa* chance corrected improvement on random assignment to significant or non-significant classes, *AUC* area under the receiver operating characteristic curve

the model with the lowest AIC value. Parameter estimates for the most supported model at each level of relative resistance are given in Table 3. Digital animations of 3-dimensional scatter plots of the simulated landscapes in *x*, *y*, *z* space corresponding to the variables included in the most supported model at each level of relative resistance are shown in Figure S1.

Model performance

Model performance improved dramatically as relative resistance increased. When non-habitat was 1.5 times as resistant as habitat the most supported model provided virtually no discrimination between presence and absence of significant relationship between genetic differentiation and landscape cost distance. Performance improved dramatically when non-habitat was twice as resistant as habitat, and continued improving at higher levels of contrast (Table 4). When non-habitat was 16 times as resistant as habitat model performance was quite good, with an R^2 of 0.408, Kappa of 0.545, and AUC of 0.845.

The bootstrap validation showed that model reliability improved with increasing contrast in resistance between non-habitat and habitat (Table S2). At a relative resistance level of R1.5, the bootstrap results indicate that the model had poor fit and relatively large optimism values for most parameters. Model performance was much better at R2, R4, and R8 with relatively low optimism values for all parameters. When resistance of non-habitat was 16 times higher than that of habitat (R16) the bootstrap analysis indicated very high precision in estimating parameters, with very low optimism values.

Variable importance

The change in log-odds between the 25th and 75th percentile of each predictor variable, while holding all other variables constant at their medians, provides a measure of variable importance (Table 5). At resistance level R1.5 the single variable in the model (CLUMPY) had very little effect on log-odds, indicating little effect size. In contrast effect sizes were consistent and large at higher levels of resistance contrast. Patch Cohesion and Correlation Length are both measures of habitat extensiveness or connectivity across the landscape. Given their high correlation, only one of the pair was included in a given model. Correlation Length was the most influential variable at R2, R4, and R8. At R16 Patch Cohesion, CLUMPY and Patch Density had similar effect sizes.

Detection of landscape genetic effects as functions of landscape structure and relative resistance

We used the most supported regression equations to calculate the probability of significant landscape genetic relationships across all combinations of predictor variable values for each level of relative resistance (Fig. 2). As relative resistance of non-habitat compared to habitat increases, larger portions of the parameter space have high probability of producing significant landscape genetic relationships. For example, at R2 less than half of the parameter space produces more than a 50 % probability of significant landscape genetic relationships, while at R16 over half of the parameter space has more than a 90 % chance of producing significant landscape genetic relationships.

Discussion

Our results have several important implications. First, the ability to predict whether or not a given landscape configuration will significantly affect genetic differentiation is highly related to the degree of contrast in resistance to gene flow in habitat as compared to non-habitat. When the

Table 5 Change in log-odds measure of effect size

	Low	High	Effect	SE
R16				
Cohesion	99.186	99.876	−1.1	0.1
Clumpy	0.8067	0.9674	1.57	0.29
PD	0.373	4.2555	1.35	0.22
R8				
GYRATE_AM	1562.9	2691.1	−1.6	0.13
AI	92.363	99.005	−0.42	0.10
R4				
GYRATE_AM	1562.9	2691.1	−0.98	0.11
Clumpy	0.8067	0.9674	0.72	0.19
PD	0.373	4.2555	0.64	0.15
R2				
GYRATE_AM	1562.9	2691.1	−0.82	0.16
Clumpy	0.8067	0.9674	0.52	0.21
AI	92.363	99.005	−0.65	0.16
R1.5				
Clumpy	0.8067	0.9674	0.08	0.11

Effect—log-odds for change from the 25th percentile (low) to the 75th percentile (high) of each variable

contrast is low (for example 1.5 times) there is very low ability to predict whether or not landscape structure will affect genetic differentiation. As contrast in landscape resistance between non-habitat and habitat increases so too does ability to predict if a given landscape configuration will significantly affect genetic differentiation. For example, when the resistance of non-habitat is 16 times greater than habitat there is very strong ability to predict whether or not a given landscape configuration will affect genetic differentiation, as measured by model R^2, AUC and Kappa. Jaquiéry et al. (2011) also found that contrast in landscape resistance had a major influence on ability to identify the process governing gene flow, with increasing reliability of predictions as resistance contrast increased. Empirical studies of landscape resistance have generally found relative resistance of habitat compared to non-habitat of 5 times or more (e.g. Cushman et al. 2006; Wasserman et al. 2010; Spear and Storfer 2008) with some studies finding resistance contrast as high as 25,000 times (e.g. Shirk et al. 2010). This is encouraging, as it suggests in most real-world contexts there should be strong ability to predict a priori whether or not landscape genetic effects could be detected in a landscape when they are in fact driving gene flow.

The second major implication is that genetic differentiation will only be significantly related to landscape structure (independent of Euclidean distance) when habitat is highly fragmented. We simulated a large range of landscape structure using neutral models. Previous work has shown that this range of habitat area and configuration captures the range of landscape structures found in most real landscapes (Neel et al. 2004; Cushman et al. 2008). In the majority of the simulated landscapes it was not possible to identify landscape effects of gene flow independently of Euclidean distance, due to the propensity of habitat to be highly aggregated into a relatively few patches that have extensive connectivity across the landscape. This suggests that in many real landscapes it may not be possible to identify the influences of landscape resistance on genetic differentiation, even when a species is highly dependent on particular landscape features and cover types for movement and dispersal.

Our results on this point appear to differ from those of Jaquiéry et al. (2011) who reported that ability to identify landscape effects on gene flow decreased with increasing landscape complexity. Jaquiéry et al. (2011) compared the power and accuracy of partial correlations, regressions and approximate Bayesian computations in identifying simulated landscape resistance effects on gene flow. Their simulation was based on a grid of 49 populations embedded in a landscape composed of several land-uses. Importantly, their landscape resistance maps were spatially random and varied in composition (number of classes) and resolution (cell size), but not in spatial pattern. Thus, their results pertain to the effects of the number of patch types with different resistance levels, but not to the effects of spatial patterns. Our results here show that the probability of significant effects of landscape resistance on genetic differentiation increases with increasing spatial complexity (fragmentation), in interaction with degree of resistance.

Recent work has suggested that individual-based landscape genetic analysis using partial Mantel tests in a causal modeling framework have high power to correctly identify landscape resistance as a driving process and reject spurious correlations with isolation by distance and barriers (Cushman and Landguth 2010). However, the Cushman and Landguth (2010) study was in a landscape that had high habitat subdivision (by major river valleys) and in which the contrast between optimal habitat and the most resistant non-habitat was high (1 compared to 63; Cushman et al. 2006). Our simulation results suggest that landscape effects on genetic differentiation will be strongly distinguishable from isolation by distance under such conditions, but will not be separable when habitat is more uniformly connected or resistance contrast is lower.

This idea is supported by recent meta-replicated landscape genetics studies. For example, Short Bull et al. (2011) investigated how variation in landscape structure affected the apparent relationships between landscape features and genetic differentiation of American black bears (*U. americanus*) in 12 study areas in the northern U.S. Rocky Mountains. They found that landscape resistance hypotheses were highly supported in comparison to

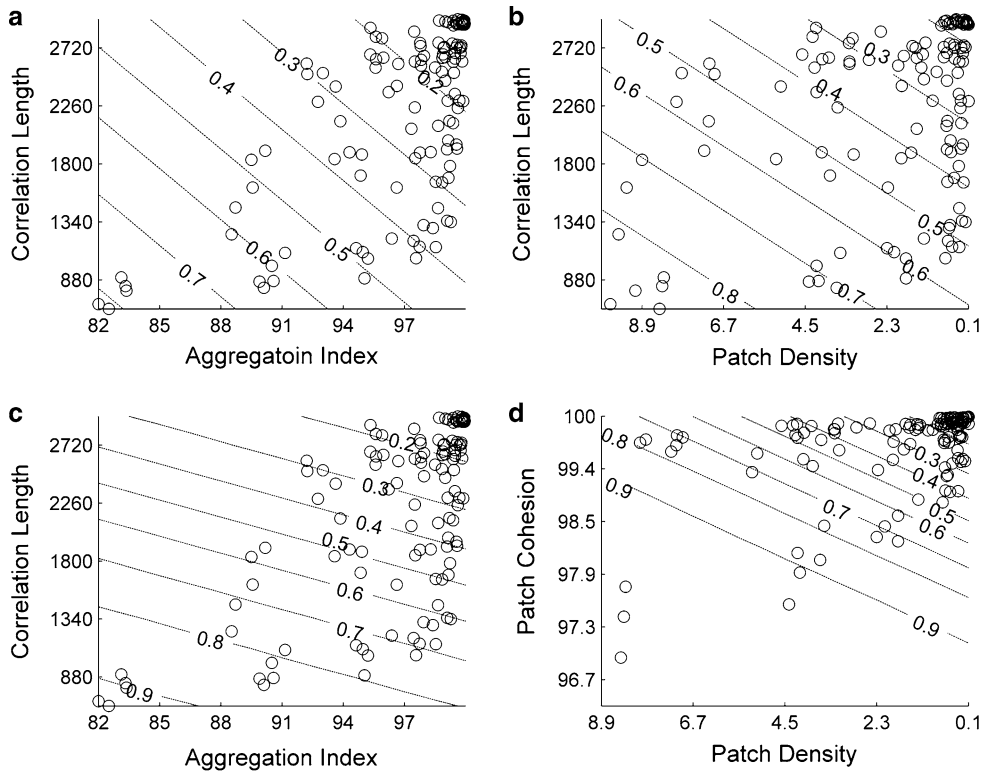

Fig. 2 Contour plots of probability of significant partial mantel *r* correlation between genetic distance and landscape cost distance, across four levels of relative resistance of habitat and non-habitat. **a** non-habitat 2 times as resistant as habitat; **b** non-habitat 4 times as resistant as habitat; **c** non-habitat 8 times as resistant as habitat; **d** non-habitat 16 times as resistant as habitat. The predictions are made using the logistic regression equations in Table 4. For plots (**a**, **b** and **d**) the logistic equation involves three variables. The prediction here is made across the values of the two variables on the *x* and *y* axes, holding the third variable constant at its median. For plot **c** there are only two variables in the most supported model. The scale of the *x* and *y* axes are from the 5th to the 95th percentile of the values of the variables in the distribution of simulated landscapes. The *circular markers* indicate locations of simulated landscapes in the parameter space

isolation by distance when there was high landscape heterogeneity in elevation, forest cover and roads. However, they also found that in homogenous and unfragmented landscapes isolation by distance and isolation by landscape resistance were equally well supported and could not be distinguished.

It is critical to know when landscape patterns begin to limit gene flow and thus become detectable to landscape genetic analysis. Our results provide quantitative guidance as to the effects of habitat amount, configuration and contrast in landscape resistance between habitat and non-habitat on genetic differentiation in complex landscapes. The regression models predicting detectability of relationships as functions of landscape metrics and contrast in landscape resistance can be useful to guide the design of studies and to compare effects sizes among different studies in different landscapes. For example, a study of a species in one landscape may find a certain landscape variable to have a large effect on genetic differentiation, while in another its effect may appear much weaker and relatively less important. Our simulation shows that this can be explained by differences in the degree to which the landscape composition and configuration limits gene flow.

For example, our results offer an explanation for the disparate results reported by American marten (*Martes americana*) landscape genetics studies. Marten populations in the Yukon and Northwest Territories (Kyle et al. 2000), Ontario (Broquet et al. 2006; Koen et al. 2012) and across large areas of mainland Canada (Kyle and Strobeck 2003) appear only weakly differentiated as a function of isolation by distance. These authors generally attributed the low level of genetic structure to a combination of large effective population sizes, high gene flow through uniform habitats and low sensitivities of the species to habitat fragmentation. In contrast, substantial marten genetic structure has been found in archipelago systems where dispersal between island habitats is limited (e.g. Small et al. 2003) and in mountainous ecosystems with complex topography (e.g. Wasserman et al. 2010, Wasserman et al. 2012a, b). If marten gene flow is related to climate or habitat connectivity, our simulation results suggest that one would not be able to detect this independently of isolation

by distance in uniform or homogeneous systems, such as much of boreal Canada. In contrast, in systems with strong gradients of resistance due to complex topography or high habitat fragmentation it will be possible to reliably detect the effects of landscape resistance independently of distance (e.g. Small et al. 2003; Wasserman et al. 2010). Wasserman et al. (2012b) applied the relationships reported in this paper between detectability of landscape genetic effects and landscape structure to determine where in the United States northern Rocky Mountains marten gene flow is likely to be governed by isolation by distance, and where isolation by landscape resistance is likely to dominate.

A general explanation of our results can be proposed based on movement ecology. Animals utilize movement behaviour to maximize fitness by optimizing access to critical resources and minimizing risk of predation. In a uniformly unfragmented landscape, there would be little heterogeneity to drive differential path selection, and movement would generally follow a random walk, resulting in patterns of isolation by distance, and no support for isolation by resistance (e.g. Cushman et al. 2011). In contrast, in landscapes that are highly fragmented by unsuitable cover types, movement paths will be chosen primarily to avoid the unsuitable conditions. This may limit the organism's ability to optimally select paths to maximize foraging success, and result in strong patterns of isolation by resistance in path selection (Cushman et al. 2011) and gene flow (Cushman and Lewis 2010). Thus, different resistance models may be supported under changing landscape conditions due to threshold effects, even when a species' relationship with landscape variables is constant.

Our prediction of fragmentation thresholds affecting genetic differentiation is a particular example of a general issue that is important in all studies of how landscape structure affects ecological processes. For example, a number of studies have explored the effects of habitat loss and fragmentation on the connectivity of habitat mosaics. Many of these studies have used neutral landscape models to assess threshold relationships in landscape patterns and the physical connectivity of patch mosaics (Gardner et al. 1987; Gardner et al. 1989; Gardner and O'Neill 1991; Pearson and Gardner 1997). These studies have described so-called percolation threshold relationships where habitat mosaics abruptly transition from fragmented to connected as habitat extent and aggregation increase. Coupling neutral landscape models with generalized population models has enabled incorporation of complex spatial patterns in population models (With 1997), identification of species' perceptions of landscape structure (With 1994), determination of landscape connectivity (With and Crist 1995; With and King 1999), evaluation of the consequences of habitat fragmentation for population subdivision, and prediction of the occurrence of extinction thresholds (With and King 1999). This paper, along with related work by Bruggeman et al. (2010), Jaquiéry et al. (2011) and Cushman et al. (2012), extends this tradition to explore fragmentation thresholds and limiting factors affecting the genetic consequences of habitat fragmentation.

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