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RESEARCH ARTICLE

Linking movement behavior and fine-scale genetic structure to model landscape connectivity for bobcats (*Lynx rufus*)

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Abstract Spatial heterogeneity can constrain the movement of individuals and consequently genes across a landscape, influencing demographic and genetic processes. In this study, we linked information on landscape composition, movement behavior, and genetic differentiation to gain a mechanistic understanding of how spatial heterogeneity may influence movement and gene flow of bobcats in the agricultural landscape of Iowa (USA). We analyzed movement paths of 23 animals to parameterize landscape resistance surfaces, applied least cost path analysis to generate measures of effective geographic distance between DNA collection locations of 625 bobcats, and tested the correlation between genetic distance and the different models of geographic distance. We found that bobcats showed a strong preference for forest over any other habitat type, and that incorporating information on habitat composition both along the path and in the surrounding landscape provided the best model of movement. Measures of effective geographic distance were significantly correlated with genetic distance, but not once the effects of Euclidean distance were accounted for. Thus, despite the impact of habitat composition on movement behavior, we did not detect a signature of a landscape effect in genetic structure. Our results are consistent with the issue of limiting factors: the high uniformity of forest fragmentation across southern Iowa, the primary study area, results in a landscape resistance pattern virtually indistinguishable from the isolation-by-distance pattern. The northern portion of the state, however, is predicted to pose a high level of resistance to bobcat movement, which may impede the regional genetic connectivity of populations across the Midwest.

Keywords Landscape genetics · Least cost path analysis · Telemetry · Resource selection function · Effective distance · Movement

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Introduction

Dispersal, be it the movement of individuals or genes, is one of the key forces shaping the ecology and evolution of natural populations. Early theoretical models of dispersal in population ecology (Karieva and Shigesada 1983; Turchin 1998) and population genetics (Wright 1943; Kimura and Weiss 1964) assumed the underlying environment is spatially homogeneous.

The landscapes natural populations actually inhabit, however, depart markedly from the idealized world. Environmental heterogeneity, whether natural or anthropogenic, can impose physical and behavioral constraints on the movement of individuals/genes across a landscape, influencing demographic and genetic processes. Landscape connectivity, the term used to describe the interaction between landscape structure and the movement response of organisms (Taylor et al. 1993), has been a focus in population ecology for more than two decades (Fahrig and Nuttle 2005). Only recently, however, has landscape connectivity been explicitly incorporated into population genetics (Manel et al. 2003).

Landscape genetics, the emerging research area that integrates landscape ecology, spatial statistics, and population genetics, provides a framework for quantitatively assessing the relationship between gene flow and landscape structure (Manel et al. 2003). One of the most common approaches for evaluating the importance of organism-environment interaction in regards to gene flow has been the correlation of genetic distances between individuals or populations with that of Euclidean (assuming a spatially homogenous landscape) or effective (assuming movement is influenced by landscape heterogeneity) geographic distances (Spear et al. 2010; Storfer et al. 2010). The approach is based on isolation-by-distance (IBD) theory, which predicts that genetic similarity among individuals decreases as the geographic distance between them increases (Wright 1943). This pattern results from spatially limited dispersal; individuals living nearby to one another are more likely to interbreed than geographically distant ones. In heterogeneous landscapes, however, straight-line geographical distances may not adequately reflect the true pattern of movement, leading to a break-down in the predicted correlation between genetic and geographic distance. Recent studies have demonstrated that measures of geographic distance which reflect landscape connectivity often explain a greater proportion of the genetic variability than simple Euclidean distance (Keyghobadi et al. 1999; Coulon et al. 2004; Cushman et al. 2006; Stevens et al. 2006; Schwartz et al. 2009).

With the proliferation of GIS data and tools, effective geographic distances are increasingly being quantified via resistance grids (a.k.a. cost or friction grids), spatially explicit representations of the landscape where each grid cell is associated with a cost reflecting the degree to which the area facilitates or impedes movement of the study organism (Sork and Waits 2010; Spear et al. 2010). Given a resistance surface, researchers can generate effective measures of geographical distance by proceeding to, for example, least-cost path analysis (Adriaensen et al. 2003), which calculates the pathway between two locations resulting in the least accumulated cost. One of the most challenging but vital steps in this process is the assignment of resistance values to different landscape components (Spear et al. 2010).

For most studies to date, the assignment of resistance values has been largely subjective, often relying on expert opinion (Beier et al. 2008; Spear et al. 2010; Zeller et al. 2012). The way in which species perceive and interact with their environment, however, may not correspond to our assumptions, and the use of empirical data offers a more robust approach than subjective methods (Pullinger and Johnson 2010). Many researchers have turned to static detection data, such as presence-only and presence-absence data obtained from sightings or other methods, as readily available or easily-acquired sources of empirical data for resistance modeling (Zeller et al. 2012). Since these data involve single point locations, movement is inferred rather than measured directly. In contrast, pathway data consisting of sequential locations offer the advantage of directly measuring movement through landscapes. In a recent review, Zeller et al. (2012) argue that path-level analysis offers the most powerful approach for estimating resistance to movement as a function of the landscape, and recommend combining multiple data types (e.g., movement and genetic) to independently validate resistance surfaces. Few studies, however, have used pathway movement data to develop resistance grids (but see Cushman and Lewis 2010; Richard and Armstrong 2010; Cushman et al. 2011), or tested an empirically derived resistance surface with an independent empirical data set (but see Stevens et al. 2006; Hagerty et al. 2011).

In this study, we parameterized landscape resistance models using a resource selection function (RSF), a widely utilized concept in the ecological literature that models resource selection by comparing landscape variables at sites used by an animal to those that were unused or potentially available (Manly et al. 2002), and then evaluated these models using genetic data. Our aim was to link information on landscape composition, movement behavior of individuals, and

fine-scale genetic structure (i.e., non-random spatial distribution of genotypes *within* populations) to gain a mechanistic understanding of how spatial heterogeneity may influence movement and ultimately gene flow of bobcats (*Lynx rufus*) in the fragmented landscape of Iowa (USA). Specifically, our objectives were to: (1) analyze empirical movement paths collected via radio telemetry using the resource selection function approach to test whether landscape composition at different spatial extents influences movements of bobcats in Iowa; (2) use the resource selection models to parameterize landscape resistance surfaces; (3) use least-cost path analysis to generate measures of effective geographic distance between DNA collection locations of individual bobcats based on the landscape resistance models, as well as two reference models: a pre-existing habitat suitability model derived from static presence-absence data and an isolation-by-distance model; and (4) use an individual-based genetics approach (where individual genotypes rather than populations are the unit of analysis) to test whether landscape connectivity influences gene flow in bobcats and leads to a departure from a pure isolation-by-distance pattern.

Methods

Study system

The landscape of Iowa has been dramatically altered since European settlement. Once comprised of a diverse array of land cover types including prairies, forests, and wetlands, the state is now dominated by annual row crop agriculture (Fig. 1a). This large scale landscape change, coupled with unregulated harvest, resulted in the extirpation of bobcats from Iowa for much of the twentieth century (Deems and Pursley 1978). Sightings of bobcats increased in the early 1990s, and during the past two decades they have naturally recolonized a portion of the state. By modeling habitat suitability with relative abundance and presence-absence data, Linde (2010) found that forest-grassland associations figured prominently in predicting favorable bobcat habitat and that intensely cultivated areas, primarily in the northern two-thirds of the state, are essentially unsuitable for bobcats. Tucker et al. (2008) found that home range selection in Iowa bobcats was closely tied to patches of forest associated with other perennial habitat like grassland, whereas row crops were generally avoided. Together, these findings predict that individuals may be less prone to disperse across expanses of agricultural lands and other unsuitable habitat, which would lead to a departure from a pure isolation-by-distance pattern of genetic variation.

Movement data

To improve our understanding of bobcat movement behavior, we followed 23 bobcats (11 females, 12 males) fitted with very high frequency (VHF) radio collars between February 2008 and March 2009. Our sample included the following age classes, as determined by cementum analysis (see Tucker et al. 2008): 4-years (i.e., born April 2004; $n = 1$), 3-years ($n = 14$), 2-years ($n = 6$), 1-year ($n = 2$). These animals were located in south-central Iowa (Fig. 1b), where bobcats are most prevalent (Linde 2010). Animal-handling and telemetry procedures were as described in Tucker et al. (2008). All animal handling followed approved Iowa State University Institutional Animal Care and Use Committee protocol #5-03-5447-W under Iowa DNR Scientific Permit SC 126.

We monitored individual movements for a total of 144 sessions (range = 1–10 sessions per individual), where each session represents a ~6-h sampling period with locations obtained approximately every 20 min. Locations consisted of visuals (i.e., animal was spotted by an observer and the actual location was recorded with GPS after the animal left; $n = 57$), biangulations (2 bearings; $n = 8$), and triangulated locations ($\geq$3 bearings; $n = 2{,}632$). From the triangulated locations, we calculated the distribution of the error ellipse polygons and removed locations with errors greater than 3.0 times the interquartile range above the 75 % quantile (extreme outliers; $n = 26$). We also removed biangulated locations since there is no way to evaluate location error. We calculated the time interval between successive locations (i.e., steps) remaining in this reduced data set ($n = 2{,}663$). The time interval was 20 min for nearly all ($n = 2{,}485$) steps, and in only 4 instances did the time lapse exceed 60 min. To minimize uncertainty regarding the true trajectory between successive locations (Zeller et al. 2012), we omitted the >60 min steps, thus splitting each of these 4 paths into 2 and resulting in a total of 148 paths.

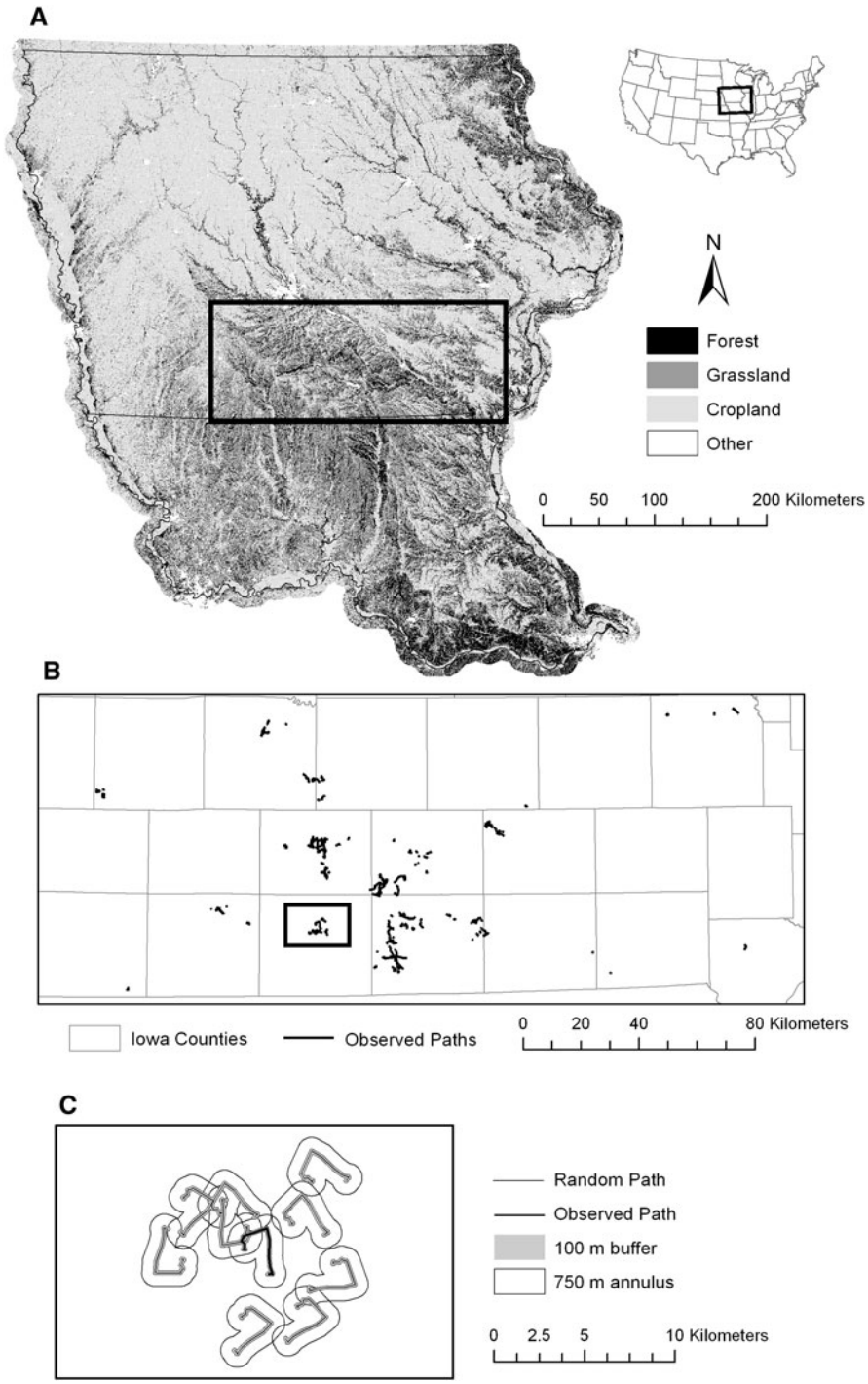

Fig. 1 Spatially nested maps showing: **a** land cover classes in the study area; **b** locations of actual bobcat movement paths; and **c** an actual path with 9 of its associated random paths, each surrounded by a 100 m buffer and 750 m annulus. Note the spatial extent of the maps in **b** and **c** are shown as *rectangles* in each preceding map

Case–control design

We used a case–control design common in resource selection studies, focusing on entire paths as the unit of analysis (Bruggeman et al. 2007; Cushman and Lewis 2010; Cushman et al. 2011) as advocated by Zeller et al. (2012). The data set consisted of 148 cases, each consisting of an observed path paired with 39 random paths (Fig. 1c). We generated the random paths using ArcGIS 10 (ESRI) and Geospatial Modelling Environment (GME; Spatial Ecology, LLC) to shift the X and Y coordinates of each of the actual paths by a random value between −5,000 and 5,000 m and to simultaneously rotate it by a random value between 0 and 360°. The 5 km radius was based on the radius of a circle with an area equivalent to the mean annual male home range in this area (Gosselink et al. 2011). The choice of 39 random paths was based on a compromise between adequately sampling the available landscape area while limiting overlap among the paths.

Landscape parameters

We downloaded the 2006 National Land Cover Data (Xian et al. 2009) at 30 m resolution for the region within a 10 km buffer around the entire state of Iowa and northern Missouri (i.e., north of the Missouri River). We collapsed the original 15 land cover classes into 6 habitat classes that we hypothesized would be functionally relevant to bobcats: (1) Water (open water); (2) Development (developed areas of low, medium, and high-intensity; barren); (3) Forest (deciduous, evergreen, and mixed forest; woody wetlands); (4) Grassland (shrub/scrub; grassland/herbaceous, pasture/hay, emergent herbaceous wetlands); (5) Cropland (cultivated crops); (6) Open space development (primarily road ditches). We applied a 100 m buffer around each path, reflecting the approximate radius of a circle with an area equivalent to the mean error ellipse associated with the telemetry data (see Results). We used GME to calculate the proportion of each land cover type within each of the buffers. To test whether movement paths of bobcats are also influenced by the landscape context of the habitat, we created an annular ring (hereafter "annulus") extending 650 m around the existing 100 m buffer and again calculated proportions of land cover types. The 750 m total buffer radius is equivalent to 3× the median step length, an arbitrary value reflecting the area an animal could potentially move into during its next 3 steps.

Conditional logistic regression

We employed fixed-effects conditional logistic regression using PROC LOGISTIC in SAS 9.2 (SAS Institute, Inc.) as our general approach to compare the landscape composition of observed and random paths. This study consisted of a 1:N matched case–control design, with one observed path matched to 39 available paths. Conditional logistic regression accounts for this clustering in the data, and results of the model are conditional upon each stratum (i.e., matched set). Thus, no intercept is estimated.

Specifically, for each of the 12 individual land cover variables (6 land cover classes times 2 buffer distances), we calculated univariate descriptive statistics (mean, standard deviation, range). We conducted univariate conditional logistic regression analyses and estimated coefficients (β) to contrast the observed and random paths. We then tested three different multivariate models: the "Path" model was constructed with each of the land cover proportions within the 100 m buffers as predictor variables; the "Annulus" model was constructed with land cover proportions within the 750 m annulus as predictor variables; the "Combined" model was constructed by combining both the 100 m buffer and 750 m annulus variables. In all multivariate models, we considered only the main effects and no interactions. Because the 6 land cover variables are proportions and sum to 1, only 5 variables are needed to capture the information. We chose to omit Grassland and use it as the baseline reference class, since it is abundant and nearest to neutral with regards to bobcat preference (Hosmer and Lemeshow 2000). Thus, the regression coefficient (β) for Grassland was set to 0, and the coefficients of all other classes were interpreted *relative* to this reference class.

We screened each model for multicollinearity between predictor variables by calculating variance inflation factors (VIFs), intending to disregard models containing predictors with VIFs > 10 (Belsey et al. 1980). We ranked the models by AIC values (Burnham and Anderson 2002) and obtained parameter estimates for each. Because the sampling design was unbalanced, with a varying number of paths per individual, and because response to landscape could vary among individuals (Gillies et al. 2006), we performed a

jackknife procedure to calculate unbiased coefficients (Abdi and Williams 2010). The procedure was accomplished by repeating the conditional logistic analysis 23 times, removing a different individual bobcat each time, to obtain partial predictions. We then computed 23 pseudo-values for each variable by finding the difference between the whole sample estimate and each partial estimate. We obtained the unbiased jackknife estimates as the mean of the pseudo-values.

We then estimated a "path selection function" (PathSF) of the form $w(x) = \exp(\beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p)/1 + \exp(\beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p)$, where β_i is the unbiased jackknife coefficient for variable x_i. The PathSF score, $w(x)$, is an index of selectivity for a path and ranges from 0 (low selectivity) to 1 (high selectivity).

Resistance surfaces

We reclassified the land cover layer such that each 30 m × 30 m grid cell of a given habitat class had a value of 1 and all other classes a 0. Thus, we created 6 new layers, one for each of the land cover types. For each of these layers, we then used the ArcGIS Spatial Analyst Neighborhood tool to use a moving window approach to calculate the proportion of the given habitat present within a 100 m radius of each focal grid cell and to create new raster layers reflecting these proportions. We repeated these calculations but using for the window an annulus with an inner radius of 100 m and outer radius of 750 m.

We used the PathSF scores, $w(x)$, from the Path, Annulus, and Combined models to generate three landscape resistance surfaces using the formula: grid cell cost = 100 * (1 − $w(x)$). Thus, grid cell values could potentially range from 0 to 100, and grid cells with the highest predicted selectivity resulted in the lowest costs of movement. We used ArcGIS Spatial Analyst Raster Calculator to apply the formula and create each resistance layer. Given the large spatial extent of the study area, we resampled the rasters to 90 m resolution using bilinear interpolation to reduce the number of grid cells from ∼380 million to ∼40 million. The resampling was necessary to reduce computation times in the subsequent least-cost path analysis, however it should have modest effects on the subsequent landscape genetic analyses (Cushman and Landguth 2010a).

In addition to the three landscape resistance models based on dynamic movement data, we created a resistance surface based on a habitat suitability model. We used the 5-variable model developed at the 12-digit hydrological unit level by Linde (2010). In this model, each watershed was given a value between 0 and 1 indicating the probability of bobcat presence (*Pr*). In this model, grid cell cost = 100 * (1 − *Pr*), such that

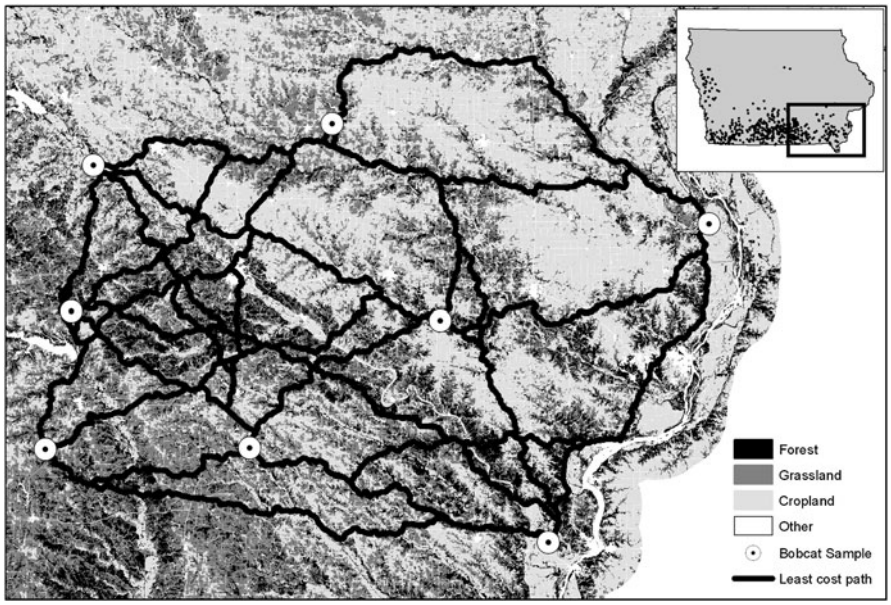

Fig. 2 Examples of least-cost paths, derived from the combined model of landscape resistance, between eight bobcat samples in southeast Iowa. The *inset* map shows the locations of $n = 625$ geo-referenced bobcat DNA samples collected in Iowa from 2001–2008

Table 1 Summary of microsatellite loci characteristics

Locus	Species	Repeat motif	Chr.	*N*	Size range (bp)	No. alleles	H_E	H_O	F_{IS}
BCE5T[a]	Bobcat	Tetra	Unknown	600	257–318	7	0.704	0.693	0.0159
Lc109[b]	Canada lynx	Di	Unknown	620	182–202	10	0.759	0.784	–0.0328
Lc110[b]	Canada lynx	Di	Unknown	622	92–104	8	0.714	0.735	–0.0290
Lc111[b]	Canada lynx	Di	Unknown	618	157–217	7	0.667	0.647	0.0299
FCA008[c]	Domestic cat	Di	A1	621	132–160	9	0.773	0.763	0.0120
FCA023[c]	Domestic cat	Di	B1	615	151–163	6	0.792	0.779	0.0164
FCA026[c]	Domestic cat	Di	D3	619	143–159	8	0.645	0.638	0.0106
FCA031[c]	Domestic cat	Di	E3	620	238–258	11	0.721	0.702	0.0269
FCA043[c]	Domestic cat	Di	C2	622	130–140	6	0.754	0.712	0.0560
FCA045[c]	Domestic cat	Di	D4	609	166–178	7	0.561	0.557	0.0071
FCA077[c]	Domestic cat	Di	C2	620	152–168	9	0.717	0.708	0.0119
FCA082[c]	Domestic cat	Di	E1	603	246–266	11	0.844	0.828	0.0200
FCA090[c]	Domestic cat	Di	A1	619	117–129	7	0.762	0.746	0.0206
FCA096[c]	Domestic cat	Di	E2	622	191–219	11	0.836	0.826	0.0117
FCA126[c]	Domestic cat	Di	B1	603	132–154	8	0.770	0.760	0.0131
FCA132[c]	Domestic cat	Di	D3	612	182–196	8	0.850	0.822	0.0331
FCA149[c]	Domestic cat	Di	B1	622	138–158	10	0.805	0.822	–0.0209
FCA391[c]	Domestic cat	Tetra	B3	609	210–236	8	0.667	0.655	0.0179
FCA559[c]	Domestic cat	Tetra	B1	618	105–141	8	0.793	0.777	0.0200
FCA740[d]	Domestic cat	Tetra	C1	618	334–358	6	0.727	0.739	–0.0170
FCA741[c]	Domestic cat	Tri	D1	616	170–188	7	0.546	0.563	–0.0318
FCA742[d]	Domestic cat	Tetra	D4	622	115–135	6	0.640	0.613	0.0424

Chromosomal location (Chr.) was determined from the NCBI Map Viewer for the domestic cat genome, available at http://www.ncbi.nlm.nih.gov/mapview. The number of genotypes (N), size range of alleles, number of alleles, expected (H_E) and observed heterozygosity (H_O), and F_{IS} were based on analysis of 625 Iowa bobcat samples

Locus name, species developed from, and repeat motif (*tetra* tetranucleotide, *di* dinucleotide, *tri* trinucleotide) were taken from the cited reference: [a] Faircloth et al. (2005); [b] Carmichael et al. (2000); [c] Menotti-Raymond et al. (1999); [d] Menotti-Raymond et al. (2005)

watersheds with high predicted probability of bobcat presence resulted in the lowest costs of movement. Again, the final raster had a 90 m grid cell size.

Finally, we created a 90 m resolution isolation-by-distance "IBD" model where each grid cell was given a cost of 1. This model assumes a homogeneous landscape across the entire region, and was viewed as the null model.

Genetics

We collected tissue samples from 625 geo-referenced individuals (Fig. 2) that were either live-captured animals ($n = 159$) or carcasses from road-killed, legally harvested, or incidentally trapped animals ($n = 466$) collected from within the state of Iowa in 2001–2008. We extracted DNA using DNeasy (Qiagen) or IDPure (IDLabs) purification kits following manufacturer protocols. We used the M13-tailed primer method (Boutin-Ganache et al. 2001) to individually amplify 22 microsatellite markers (Table 1) that were developed from the bobcat, Canada lynx (*Lynx canadensis*), or domestic cat (*Felis catus*). Total PCR volume was 10 µl, including 1× PCR buffer with 2 mM $MgSO^4$ (IDLabs), 0.2 mM dNTPs, 0.3 µM fluorescently labeled M13 primer, 0.3 µM reverse primer, 0.02 µM M13-tailed forward primer, 0.4 U ID*PROOF* DNA Polymerase (IDLabs), and 10–20 ng of template DNA. The PCR profile was 95 °C/5 min, (95 °C/20 s, 47–50 °C/20 s, 72 °C/30 s) × 30–35 cycles, 72 °C/20 min. We combined PCR products from each sample into gel sets, analyzed them on an ABI 377 sequencer at Iowa State University's DNA Facility, and scored alleles using the software Genotyper 3.7 (ABI).

We used program Microchecker (van Oosterhout et al. 2004) to evaluate whether any loci showed evidence of scoring errors such as null alleles, large allele dropout, and stuttering (1,000 iterations, Bonferroni-adjusted confidence interval). We used program GenePop 4.0 (Rousset 2008) to estimate number of alleles, observed and expected heterozygosities, and Weir and Cockerham's (1984) F_{IS} for each locus. In addition, we tested for deviation from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium (LE) for each locus (10,000 dememorization, 500 batches, 5,000 iterations), applying a sequential Bonferroni adjustment (nominal $\alpha = 0.05$) to account for multiple tests (Rice 1989). To determine whether significant population subdivision exists across Iowa, we: (1) used GenePop to perform a global test (i.e., across loci) of heterozygote deficiency and (2) used BAPS 5.2 (Corander et al. 2008) software to perform spatial clustering of individuals, with five runs each for the maximum number of populations (K) set to $K = 2$–8 since we anticipated few discrete clusters. We calculated Rousset's a_r (Rousset 2000) as a measure of individual-level genetic distance using program Spagedi (Hardy and Vekemans 2002). This metric, in which higher values indicate dissimilarity, is analogous to $F_{ST}/(1 - F_{ST})$ but for pairs of individuals rather than pairs of populations. We chose this metric because it was developed specifically to study IBD at the individual level (Rousset 2000) and is commonly used in individual-based landscape genetic studies (Coulon et al. 2004; Latch et al. 2011).

Least-cost path analysis

We used the landscape connectivity cost-distance tool in the Landscape Genetics ArcToolbox (Etherington 2011) to calculate matrices of cost-distances between each of the 625 samples for each of the 5 models (Path, Annulus, Combined, Suitability, IBD). These cost-distance values reflect the minimum possible combination of the distance that would be traveled and the cost of the landscape that would be traversed between two points. To reduce processing time, we first clipped the rasters to omit central Missouri and northern Iowa/southern Minnesota, since preliminary analyses based on 30 individuals from across the study area indicated paths did not extend far south or north.

Mantel tests

To evaluate the hypothesized resistance models, we used Mantel tests (Mantel 1967) in the R software package Vegan (Oksanen et al. 2010) to calculate the correlation between genetic and geographic distance. We used a permutation procedure (999 replicates) to evaluate the statistical significance of the relationships. We considered the most supported model to be the one with the highest significant correlation. We performed partial Mantel tests (999 replicates) to determine whether the effective geographic distances obtained from the landscape resistance models retained a significant relationship with genetic distance after partialling out the effects of Euclidean distance (i.e., IBD model). To implement a causal modeling framework (Cushman et al. 2006), we also partialled out the effects of each landscape model from Euclidean distance to test for independent support of the IBD model.

Results

Following the removal of 26 extreme outliers (error ellipse area > 215,646 m^2) and 8 biangulated locations, the telemetry data set consisted of $n = 2{,}663$ points. The mean position error of the remaining triangulated locations was 112 m. The 148 routes consisted of an average of 18 points (range 4–20) and a total of 2,515 steps (i.e., vectors connecting two successive locations). Among steps in which the animal moved (i.e., step length >0 m) and time lapse did not exceed 20 min, mean step length was 246.6 m ($n = 1{,}520$; range 1–2,632 m).

Univariate tests revealed that observed movement paths had a higher proportion of forest, but a lower proportion of all other land cover types, in the immediate surroundings (100 m buffer) compared to the random paths (Table 2). In the broader spatial context (750 m annulus), observed movement paths were located in areas containing a higher proportion of both forest and grassland, but lower proportion of cropland and water, than expected by chance (Table 2).

None of the 3 a priori path selection models (Path, Annulus, Combined) contained predictor variables with VIFs exceeding 10, and thus all were considered as candidate models in the conditional logistic regression analyses. Based on the relative coefficients

Table 2 Univariate descriptive statistics including mean, standard deviation, and range of habitat proportions found within the 100 m buffer and 750 m annulus of observed and random movement paths

Variable	AB	Observed paths			Random paths			β	*P*
		$\bar{x}$	SD	Range	$\bar{x}$	SD	Range		
Water (100 m buffer)	W100	0.0052	0.0204	0.0000–0.2042	0.0243	0.1098	0–1.0000	−8.443	0.041
Development (100 m buffer)	D100	0.0046	0.0111	0.0000–0.0633	0.0202	0.0684	0–0.9688	−25.179	<0.001
Forest (100 m buffer)	F100	0.3967	0.2414	0.0000–1.0000	0.1936	0.2090	0–1.0000	3.918	<0.001
Grassland (100 m buffer)	G100	0.3727	0.2338	0.0000–0.9269	0.4357	0.2603	0–1.0000	−1.122	0.002
Cropland (100 m buffer)	C100	0.1978	0.2289	0.0000–1.0000	0.2869	0.2880	0–1.0000	−1.617	<0.001
Road (100 m buffer)	R100	0.0229	0.0316	0.0000–0.2330	0.0393	0.0588	0–0.9378	–10.429	<0.001
Water (750 m annulus)	W750	0.0076	0.0193	0.0000–0.1281	0.0242	0.0948	0–0.9936	−9.495	0.034
Development (750 m annulus)	D750	0.0132	0.0237	0.0000–0.1895	0.0207	0.0530	0–0.7283	−6.492	0.076
Forest (750 m annulus)	F750	0.2325	0.1323	0.0036–0.7749	0.1961	0.1498	0–0.9788	1.999	<0.001
Grassland (750 m annulus)	G750	0.4647	0.1568	0.0316–0.8964	0.4371	0.1763	0–0.9297	1.352	0.022
Cropland (750 m annulus)	C750	0.2465	0.1801	0.0000–0.8107	0.2823	0.2159	0–0.9805	−1.324	0.011
Road (750 m annulus)	R750	0.0354	0.0224	0.0000–0.1876	0.0396	0.0349	0–0.3684	−5.177	0.119

The estimated coefficients (β) and associated probabilities (*P*) testing the null hypothesis ($\beta = 0$), which were obtained from univariate conditional logistic regression, are provided to illustrate the differences between observed and random paths for each of the habitat types. *AB* abbreviation of variable name

from the Path model (Table 3; AIC = 958.0), bobcats strongly selected for forested habitat over grassland and other land cover classes in their immediate surroundings. The Annulus model indicated, however, that at the broader landscape scale, forest cover was not significantly favored over grassland, though both were favored over water and cropland (Table 3; AIC = 1,072.0). The Combined model, which incorporates habitat use at both spatial scales, had the lowest AIC score and thus was identified as the best approximating model for these data (AIC = 913.7). Results of the jackknife procedure indicated the unbalanced sampling design likely did not introduce bias, as the jackknife parameter values were similar to the estimates from the whole sample (Table 3).

The Microchecker analysis revealed no significant problems with null alleles, large allele dropout, or stuttering in any of the microsatellite loci. Furthermore, none of the markers were out of HWE after applying the Bonferonni correction (Table 1). However, 8 pairs of loci exhibited significant linkage disequilibrium. Given that some of the locus-pairs suspected of linkage are known to be located on different chromosomes in the domestic cat (Table 1), and that many factors other than physical linkage (e.g., population growth or admixture) can also lead to nonrandom association of alleles (Weir 1996), we chose to retain data from all loci in this analysis. We did detect an overall heterozygote deficiency among the samples ($P = 0.002$), which may be indicative of internal population structure (Wahlund effect) such as isolation-by-distance or discrete clusters. BAPS results, however, indicated the most likely number of populations present in the data set was 1 (posterior probability = 1.0), indicating the sampled individuals came from a single population.

Cost distance values derived from the 3 movement-based resistance models (Path, Annulus, and Combined; Fig. 3) and the Suitability model were highly correlated with those obtained from the IBD null model (Fig. 4), with the Annulus distances being the most similar to Euclidean distances ($r = 0.985$, $P = 0.001$) and the Suitability model the least ($r = 0.810$, $P = 0.001$). Indeed, least-cost paths between samples were not very divergent from straight-line paths (Fig. 2). All 5 geographic distance models showed weak but significant correlations with genetic distances (Table 4; Fig. 5). However, none of the landscape models were significantly correlated with genetic distance once the effects of Euclidean distance were partialled out (Table 4). The IBD model was also not independently supported by the partial Mantel tests, except for the test involving the Path model (Table 4).

Table 3 Estimated coefficients (β), standard errors (SE), lower and upper 95 % Wald confidence limits, and unbiased jackknife coefficients (β_{jack}) of land cover variables included in the three candidate logistic models (Path, Annulus, Combined) describing path selection by bobcats in south-central Iowa

Variable	Path					Annulus					Combined				
	β	SE	Lower	Upper	β_{jack}	β	SE	Lower	Upper	β_{jack}	β	SE	Lower	Upper	β_{jack}
Water (100 m)	−8.86	4.21	−17.11	−0.62	−4.08	–	–	–	–	–	−2.19	4.83	−11.67	7.28	1.87
Development (100 m)	−13.52	7.06	−27.36	0.32	−11.78	–	–	–	–	–	−17.46	7.54	−32.24	−2.68	−15.96
Forest (100 m)	3.58	0.43	2.74	4.42	3.57	–	–	–	–	–	6.29	0.61	5.10	7.48	6.26
Cropland (100 m)	−0.04	0.47	−0.95	0.88	−0.02	–	–	–	–	–	0.30	0.65	−0.97	1.57	0.28
Road (100 m)	−3.56	2.96	−9.35	2.23	−3.19	–	–	–	–	–	−2.48	3.06	−8.47	3.51	−1.80
Water (750 m)	–	–	–	–	–	−10.14	4.38	−18.72	−1.56	−7.57	−10.81	5.21	−21.02	−0.61	−7.86
Development (750 m)	–	–	–	–	–	−5.60	4.13	−13.70	2.50	−3.12	4.22	4.71	−5.00	13.44	6.29
Forest (750 m)	–	–	–	–	–	0.96	0.77	−0.55	2.46	0.97	−6.73	1.11	−8.91	−4.55	−6.64
Cropland (750 m)	–	–	–	–	–	−1.33	0.66	−2.63	−0.04	−1.30	−1.39	0.92	−3.19	0.41	−1.34
Road (750 m)	–	–	–	–	–	0.32	4.23	−7.98	8.61	0.26	3.28	4.63	−5.79	12.35	3.25

Discussion

Our analysis of movement paths indicated that bobcats respond to spatial heterogeneity when moving through the landscape, and the response depends not only on the habitat composition in the immediate surroundings but the broader landscape context as well. Along movement paths, bobcats showed a strong preference for forest over any other land cover type. This preference is still evident but diminished over a greater spatial extent (750 m), indicating that in this landscape bobcats prefer forest surrounded by additional forest or grasslands. Relative to availability, cropland was avoided at both scales. This finding is concordant with the results of Tucker et al. (2008) and Linde (2010), which also supported a preference for forest associated with perennial habitats like grasslands. However, despite the influence of habitat composition on bobcat movement behavior, we were unable to detect a signature of a landscape effect in fine-scale genetic structure. Although measures of effective geographic distance from the three movement-based landscape models (Path, Annulus, Combined) and the habitat suitability model were significantly correlated with genetic distances, these effective distances did not account for genetic variation any better than the pure isolation-by-distance model that assumes a homogeneous environment. Indeed, the relationships between effective geographic distances and genetic distances were no longer significant after accounting for the effect of Euclidean distance.

Even within a population, recent studies have shown that habitat fragmentation can have a substantial effect on landscape connectivity and the dispersal of animals, reducing gene flow and generating fine-scale patterns of genetic differentiation (Coulon et al. 2004; Wasserman et al. 2010). Gaining knowledge of how landscape features facilitate or impede dispersal and gene flow is therefore vital for understanding many ecological and evolutionary processes and is a key goal of landscape genetics. Many current landscape genetics approaches, however, rely on expert opinion or static detection data for estimating landscape resistance to movement (Spear et al. 2010; Zeller et al. 2012). This study contributes to the developing field of landscape genetics by demonstrating how pathway movement data—arguably a more direct and valid measure of how animals move through

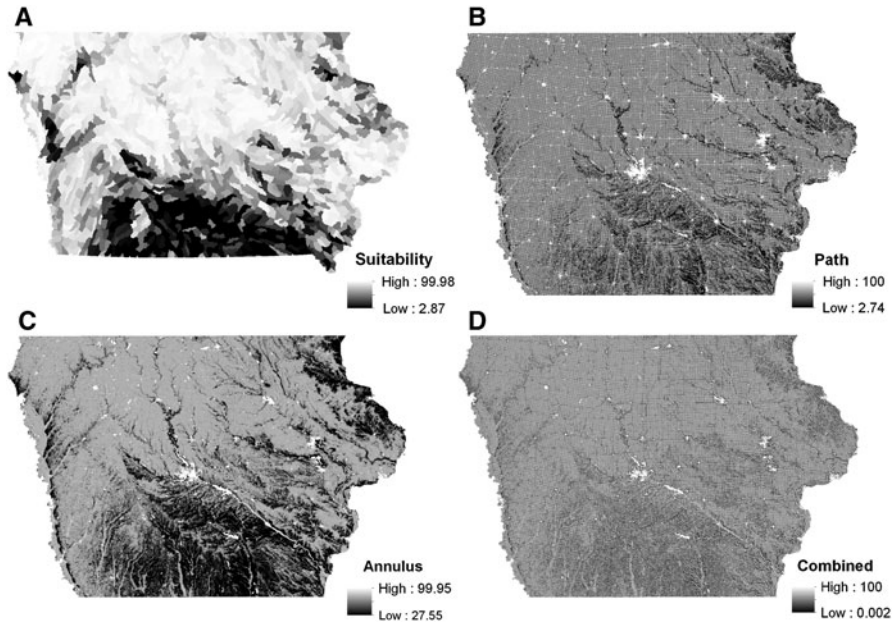

Fig. 3 Resistance surfaces for bobcat movement based on the **a** Habitat Suitability model; **b** Path model; **c** Annulus model; and **d** Combined (Path + Annulus) model

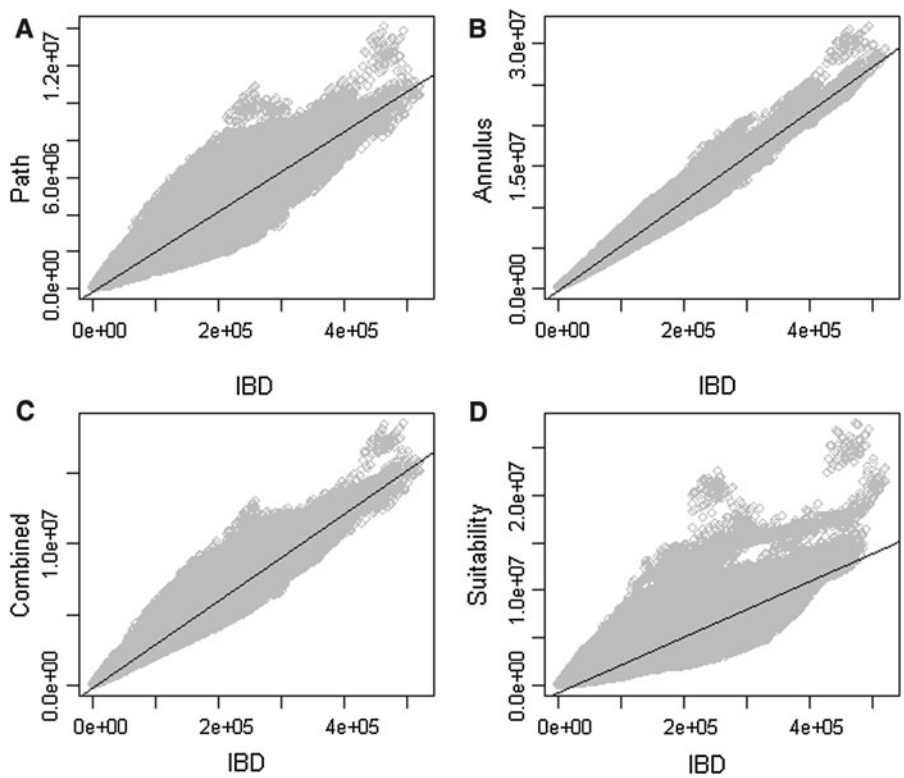

Fig. 4 Relationship between Euclidean distances (IBD model) and each of the four measures of effective geographic distances: **a** Path model; **b** Annulus model; **c** Combined (Path + Annulus) model; **d** Habitat Suitability model

a landscape—can be used in resistance surface modeling. We reasoned that movement-based models should outperform not only the IBD model in explaining fine-scale genetic structure, but also the habitat suitability model. The static detection data used to derive the habitat suitability model, though

Table 4 Mantel and partial Mantel correlations (r) between spatial and genetic pairwise distances among 625 individual bobcats in Iowa

Mantel or partial Mantel test	r	P
IBD	**0.05766**	**0.001**
Path	**0.04959**	**0.009**
Annulus	**0.05805**	**0.002**
Combined	**0.05606**	**0.005**
Suitability	**0.06082**	**0.009**
Path.IBD	−0.00392	0.564
Annulus.IBD	0.00730	0.356
Combined.IBD	0.00355	0.435
Suitability.IBD	0.02413	0.151
IBD.Path	**0.02971**	**0.048**
IBD.Annulus	0.00273	0.468
IBD.Combined	0.01394	0.210
IBD.Suitability	0.01437	0.203

Spatial distances are based on a null model of distance only (IBD), three different models of landscape resistance developed from analysis of movement paths (Path, Annulus, Combined), and a model of landscape resistance based on habitat suitability (Suitability)

A period separates the main spatial matrix on the left from the covariate matrix on the right that is partialed out in the partial Mantel test. Significant values (P) are based on 999 permutations. Bold values indicate $P < 0.05$

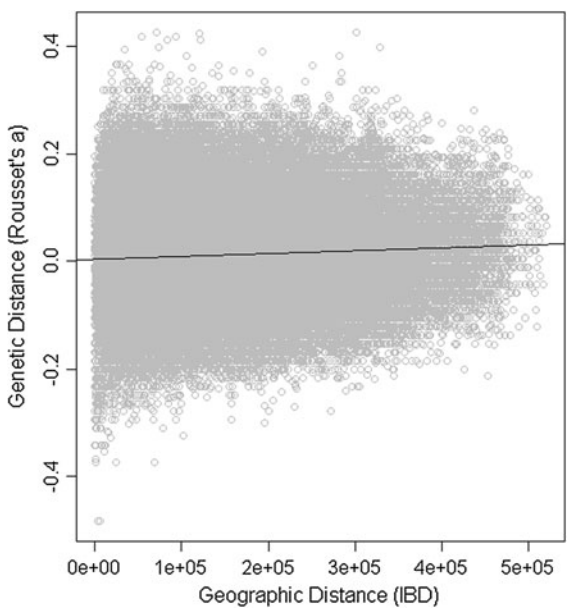

Fig. 5 Relationship between the IBD geographic model and genetic distance (a_r) for 625 bobcats

potentially easier and cheaper to collect than movement data, may not adequately reflect the decisions animals make when moving through a landscape (Spear et al. 2010; Zeller et al. 2012). Our results unfortunately did not allow us to draw any strong conclusions regarding whether movement-based models offer substantial improvements over detection-based models, given the similar but relatively weak performance of all of our geographic models in explaining genetic variation. However, this study demonstrates the feasibility of the approach and indicates that its use in other systems could allow for greater insights into the relationships between behavior, landscape connectivity, and gene flow. Very few studies other than ours have attempted to evaluate models derived from different data sources (Cushman and Lewis 2010; Pullinger and Johnson 2010), and additional work is greatly needed to assess the most appropriate and cost-effective approaches to modeling landscape resistance (Zeller et al. 2012).

Several factors may have contributed to the discordance we observed between movement behavior (upon which we detected a landscape effect) and genetic structure (upon which we detected no effect) in bobcats. First, and perhaps most likely, is that given the high correlations between the measures of effective geographic distance to Euclidean distance, our landscape models may be too similar to the IBD null model to be able to separate out their effects from pure distance effects. In landscape genetic studies, alternative geographic distance hypotheses are very commonly correlated (Cushman and Lewis 2010; Wasserman et al. 2010; Klug et al. 2011). Cushman and Landguth (2010b) demonstrated through simulations that causal modeling with partial Mantel tests—the approach used in this study—had very high power to correctly identify the driving process of genetic structure and reject correlated but incorrect alternatives in complex landscapes. Recent simulation studies, however, have shown that the ability to detect a landscape effect on gene flow can depend on the characteristics of the landscape (Cushman et al. 2012a, b; Graves et al. 2012). For example, Cushman et al. (2012b) found that homogeneous landscapes, with high levels of suitable habitat and low fragmentation, are less likely to have detectable effects on gene flow.

Although the landscape of Iowa is highly altered, composition and configuration metrics are relatively

consistent across the south-central region in which the majority of the samples were collected, with forest and grassland patches interspersed throughout the landscape (Linde 2010). Cushman et al. (2012b) explained that uniformly unfragmented landscapes do not limit gene flow because the extensive connectivity results in very little difference in movement cost (as a function of landscape) among alternative paths. In south-central Iowa, the uniform pattern of fine-scale fragmentation may have a similar effect on bobcat dispersal. Because of the 'stepping stone' pattern of perennial habitat across much of the landscape, least-cost paths between samples often approximated straight lines, despite the observed difference in movement resistance among habitat types.

These results do not imply that spatial heterogeneity is unimportant to bobcat dispersal, but rather that the relationship between gene flow and landscape connectivity was undetectable for this species within the sampled landscape. A few empirical studies have demonstrated how the landscape characteristics of a study area can greatly influence the statistical inferences about which landscape features influence movement and gene flow. For example, by investigating landscape resistance hypotheses in several study areas, Short Bull et al. (2011) discovered that landscape features were statistically supported in landscape-genetic relationships for black bears only when the features were highly variable across the area. Similarly, Cushman et al. (2011) found that, although American marten are a forest-dependent species, the influence of forest cover on movement behavior was weak in unharvested landscapes because—in contrast to areas perforated by clear-cutting—these areas were continuously forested and thus did not limit movement. The relatively consistent habitat fragmentation in south-central Iowa, combined with the bobcat's ability to disperse long (>100 km) distances (Johnson et al. 2010; Gosselink et al. 2011), may explain the weak connection between landscape characteristics and gene flow that we observed at this location and scale. Perhaps, however, more of a landscape effect would be observed if we examined a broader spatial area for which habitat fragmentation levels were more variable.

A second point to consider is that the bobcat population in Iowa is relatively new to the landscape, and it may simply take more time for the IBD pattern and landscape effects to strengthen (Anderson et al. 2010). Based on simulations, Landguth et al. (2010) found that distance and landscape effects can be detected almost immediately in spatial genetic patterns (1–15 generations), but do not reach equilibrium for several hundred generations. Furthermore, when stipulating an isolation-by-landscape resistance process, Cushman and Landguth (2010b) discovered that simple Mantel tests based on Euclidean distance produced strong, spurious correlations of similar magnitude to those produced by the correct effective distances, and that it took more than 40 generations for partial Mantel tests to be able to identify the correct process with power greater than 0.90. Assuming a generation time of $\sim$2.3 years (Gosselink et al. 2011), we estimate that bobcats began repopulating Iowa's landscape only approximately 7–20 generations ago. Genetic structure has already begun to emerge, evidenced by the weak but significant Mantel correlations we detected between geographic and genetic distance. However, a temporal lag in the buildup of a genetic response may be contributing to the inability to disentangle a landscape effect from a pure distance effect.

It is also important to point out that the movement data were taken during normal home range use, and we made the assumption that these movements are representative of the type of dispersal movements leading to gene flow. Although the movement paths we observed represent the best available data for bobcats, it is unclear how well such local behaviors link up with dispersal movements. For example, Newby (2011) found that habitat preference of dispersing pumas was similar to that of adult residents, suggesting data on within-home-range habitat selection may translate into valid resistance values for dispersal movement. However, within-home-range movements of roe deer which suggested avoidance of highly forested areas (Coulon et al. 2008) contradicted genetic patterns which indicated preferential gene flow along wooded corridors (Coulon et al. 2004). To clarify the link between movement and gene flow, movement models may need to incorporate behavioral complexity, such as accounting for "state switching" between foraging versus migrating behavior (Schick et al. 2008), as well as consider the potential differences in spatial scale of the processes involved. Longer, more directional movement paths, or ones taken during specific temporal periods, may provide a stronger fit to gene flow for many species. In our case, however, observations of dispersal events of bobcats from south-central Iowa generally support our conclusions based on local movement paths, as

dispersal was found to be biased in an east–west direction and only rarely did animals attempt to disperse north into areas of high landscape resistance (Gosselink et al. 2011).

Our results join a small subset of landscape genetics studies to use empirical movement data to model landscape resistance. By comparing habitat composition of observed paths to available paths, we found that bobcats preferentially move through forested areas associated with perennial habitats. Models of landscape resistance based on these habitat selection results indicate a large portion of Iowa's landscape, namely the northern 2/3 of the state, is predicted to generate a high level of resistance to movement for bobcats. Although we could not discern a landscape effect in fine-scale genetic structure within Iowa, these large blocks of high resistance may impede connectivity with bobcat populations in neighboring states. Thus, agricultural modifications to the Midwest's landscape may lead to genetic subdivision at the regional scale and have important consequences for the ecology, evolution, and conservation of this carnivore.

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