

Ecological resistance surfaces predict fine-scale genetic differentiation in a terrestrial woodland salamander

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Abstract

Landscape genetics has seen tremendous advances since its introduction, but parameterization and optimization of resistance surfaces still poses significant challenges. Despite increased availability and resolution of spatial data, few studies have integrated empirical data to directly represent ecological processes as genetic resistance surfaces. In our study, we determine the landscape and ecological factors affecting gene flow in the western slimy salamander (*Plethodon albagula*). We used field data to derive resistance surfaces representing salamander abundance and rate of water loss through combinations of canopy cover, topographic wetness, topographic position, solar exposure and distance from ravine. These ecologically explicit composite surfaces directly represent an ecological process or physiological limitation of our organism. Using generalized linear mixed-effects models, we optimized resistance surfaces using a nonlinear optimization algorithm to minimize model AIC. We found clear support for the resistance surface representing the rate of water loss experienced by adult salamanders in the summer. Resistance was lowest at intermediate levels of water loss and higher when the rate of water loss was predicted to be low or high. This pattern may arise from the compensatory movement behaviour of salamanders through suboptimal habitat, but also reflects the physiological limitations of salamanders and their sensitivity to extreme environmental conditions. Our study demonstrates that composite representations of ecologically explicit processes can provide novel insight and can better explain genetic differentiation than ecologically implicit landscape resistance surfaces. Additionally, our study underscores the fact that spatial estimates of habitat suitability or abundance may not serve as adequate proxies for describing gene flow, as predicted abundance was a poor predictor of genetic differentiation.

Keywords: amphibian, circuitscape resistance, compensatory movement, landscape genetics, *Plethodon albagula*, resistance optimization

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Introduction

Since its formal introduction, landscape genetics has sought to combine landscape ecology, population genetics and spatial statistics (Manel *et al.* 2003; Manel & Holderegger 2013). Significant advances have been made in analytical methods and frameworks (e.g. Cushman *et al.* 2006; Murphy *et al.* 2008; Dyer *et al.* 2010;

Van Strien *et al.* 2012), simulation environments (Landguth & Cushman 2009; Rebaudo *et al.* 2013) and methods to optimize resistance surfaces (Wang *et al.* 2009; Shirk *et al.* 2010; Graves *et al.* 2013). Despite these advances, there is still a paucity of studies utilizing ecologically explicit, empirically derived resistance surfaces to test landscape genetics hypotheses (Spear *et al.* 2010). Nearly all landscape genetic studies assess spatial factors that are believed to have an effect on the ecology of their study species (e.g. temperature, moisture, land cover), but these are generally indirect proxies for the

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actual ecological processes underlying gene flow. The development of spatial surfaces that explicitly measure ecological processes (e.g. rate of dispersal, foraging time, rate of reproduction, survival probability) has the potential to offer novel insights into functional connectivity and a deeper understanding of specifically how the landscape affects gene flow (Michels *et al.* 2001; Stevens *et al.* 2006b). Moving towards greater integration of ecological data to test hypotheses of landscape resistance is particularly pertinent given the evidence that expert opinion rarely performs satisfactorily (Shirk *et al.* 2010; Charney 2012) and that resistance surfaces are sensitive to incorrect parameterizations (Beier *et al.* 2009; Rayfield *et al.* 2010).

Given the increasing availability and resolution of spatial data, and advances in methods for making spatial projections of species abundance or occupancy (e.g. Royle *et al.* 2007; Fiske & Chandler 2011; Sillett *et al.* 2012), there is great potential for researchers to develop resistance surfaces that meaningfully relate to the ecology of their study organism. Methods to parameterize ecological resistance surfaces have been reviewed by Spear *et al.* (2010) and can include telemetry or tracking data (e.g. Driezen *et al.* 2007), presence-absence data (e.g. Wang *et al.* 2008) or movement studies (e.g. Stevens *et al.* 2006b). The majority of these studies seek to identify habitat features that are conducive to species occurrence or to determine the relative effects of habitat features on movement; the resulting resistance surfaces generally represent a single landscape feature and assign resistance values to land cover types. However, novel composite resistance surfaces have been created by combining multiple landscape features through the use of habitat suitability models (e.g. Wang *et al.* 2008), resource selection functions (e.g. Chetkiewicz & Boyce 2009) or multiple regression on distance matrices (Garroway *et al.* 2011).

Regardless of how resistance surfaces are developed, an important assumption is that these surfaces contribute to the processes underlying genetic differentiation. Spatial variation in local population dynamics, survival, reproduction and successful dispersal measured over several generations all contribute to genetic differentiation between populations. Therefore, ecological surfaces derived from empirical data that describe a single component of an organism's ecology (e.g. abundance, habitat use) may not adequately describe the long-term, multivariate processes driving gene flow. There are also potential pitfalls to using ecological measures that do not directly measure movement, which is a complex and highly variable individual trait (Baguette & Van Dyck 2007). Compensatory movement, wherein an organism modifies the directionality and speed of its movement depending upon the local landscape, may

lead to unexpected patterns in dispersal and connectivity in taxa ranging from insects, birds, amphibians, reptiles and small mammals (reviewed by Knowlton & Graham 2010). For example, compensatory movement may result in decreased movement through highly suitable habitat and accelerated movement through suboptimal or low quality habitat (e.g. Goodwin & Fahrig 2002), resulting in a negative relationship between habitat suitability and resistance.

Another important consideration when developing spatial resistance surfaces is that of scale; the spatial extent and resolution of the landscape must match the biology of the study organism. Small, terrestrial animals are often closely associated with temperature and moisture microclimates (Baur & Baur 1995; Peterman & Semlitsch 2013), which may lead to differential survival, movement and abundance across the landscape. A close dependence on local microclimate may make these taxa particularly relevant for investigating the effects of fine-scale ecological processes on gene flow. Unique among terrestrial vertebrates, plethodontid salamanders are lungless and respire cutaneously (Whitford & Hutchinson 1967). As a result, their skin must remain moist and permeable, imposing physiological and ecological constraints. These salamanders exhibit minimal dispersal (Liebgold *et al.* 2011) and can show significant genetic differentiation at distances of 200 m within continuous forest habitat (Cabe *et al.* 2007). These life history characteristics suggest that genetic differentiation is likely to occur over fine spatial scales and in relation to local habitat features.

We conducted a fine-scale landscape genetic assessment of a terrestrial woodland salamander, *Plethodon albagula* (western slimy salamander), with specific emphasis on building upon empirical ecological data acquired through field surveys and experimentation. *Plethodon albagula* are large plethodontid salamanders of the *P. glutinosus* species complex (Highton 1989) that live in forested habitats throughout the Ozark and Ouachita Mountains of Missouri, Arkansas, eastern Oklahoma and northeastern Texas, USA. In Missouri, Peterman and Semlitsch (2013) found that abundance of *P. albagula* is greatest in ravines with dense canopy cover, high moisture and low solar exposure. As a potential mechanism shaping patterns of abundance on the landscape, Peterman (2013) estimated rates of water loss across the landscape finding that water loss was most affected by topographic position, solar exposure, canopy cover, maximum daily temperature and time since rain. Further, the resulting water loss landscape was highly correlated ($r = 0.68$) with predicted abundance on the landscape.

Although Peterman (2013) found water loss to be a significant predictor of the spatial distribution of abundance, the effect of water loss as a physiological

mechanism affecting functional connectivity across the landscape remains unknown. Using our observations of the landscape features affecting abundance and water loss, as well as the spatial representations of these ecological processes, we designed this study to determine the factors affecting gene flow in *P. albagula* across the landscape. We hypothesized that (i) fine-scale population genetic differentiation would be evident for *P. albagula*; (ii) gene flow would be best predicted by resistance surfaces directly representing local population dynamics (abundance; Peterman & Semlitsch 2013) and physiology (rate of water loss; Feder & Londos 1984; Peterman 2013), which can affect the amount of time a salamander can be surface active, foraging, dispersing or searching for a mate; and (iii) gene flow would be greatest through landscape and ecological features conducive to salamander reproduction and survival (i.e. ravines, high moisture, low solar exposure, high canopy, low maximum temperature).

Methods

Study site and species

Our study took place in east-central Missouri within the River Hills Ecoregion (Chapman *et al.* 2002) at Daniel Boone Conservation Area (DBCA; 38.78° N, 91.39° W; 157–280 m a.s.l.; Fig. 1a). This physiographic region and conservation area border the Missouri River and are characterized by forested ridges and valleys with slopes that are frequently covered by exposed rock or rock outcrops. DBCA encompasses 1424.5 ha of mature (80–100 years old) second-growth forest (Semlitsch *et al.* 2008).

Population sampling

Tissue was collected from 10 to 25 *P. albagula* at each of 22 sample locations by taking 0.5 cm of tail tissue, which was stored in 95% EtOH at –20 °C until DNA extraction. At each location, samples were collected within a 25-m² area. When possible, adult salamanders were preferentially chosen over juvenile or hatchling salamanders in an attempt to minimize the number of sibling pairs captured. The centre of each sample location was marked with a handheld GPS (Garmin 60sc) with repeated measurements until the estimated precision was ≤3 m. The minimum and maximum distances between sample locations were 75 and 3978 m, respectively (mean = 1725 ± 947 m).

Population genetic analyses

DNA was extracted using the Wizard SV 96 Genomic DNA Purification System (Promega, Madison, WI,

USA) according to the manufacturer's protocols. Twenty-four tetra- and penta-nucleotide microsatellite loci were amplified using PCR; primers were fluorescently labelled and arranged into two multiplex reactions as described in Spatola *et al.* (2013). Amplification products were sized on an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using Liz 600 size standard at the University of Missouri DNA Core Facility, and results were scored using GENE-MARKER (v.1.97; Softgenetics, State College, PA, USA). Before proceeding with analyses, we tested for, and removed, full-siblings from our data set using COLONY (Jones & Wang 2010). Following removal of siblings, we had 360 individuals in our data set (mean = 16.36/site; Table 1).

Genepop 4.2 (Raymond & Rousset 1995; Rousset 2008) was used to test whether loci conformed to expected heterozygosity values under Hardy–Weinberg equilibrium (HWE) and to test for linkage disequilibrium among pairs of loci. Both tests were conducted using 250 batches with 2500 iterations following a burn-in of 2500. We tested for presence of null alleles using Micro-Checker (Van Oosterhout *et al.* 2004). Rarefied allelic richness and private alleles were calculated using HP-RARE (Kalinowski 2005). Observed and expected heterozygosity as well as F_{ST} , and chord distances (D_C) were calculated using GenoDive (Meirmans & Van Tienderen 2004), Jost's D (D_{est} , corrected for sample size; Jost 2008) was calculated using DEMETics (Gerlach *et al.* 2010) in the R statistical environment (v3.0.2; R Core Team 2013), and the proportion of shared alleles (D_{PS} ; Bowcock *et al.* 1994) was calculated using microsat (–ln (proportion shared)). We chose to assess D_{PS} and D_C as alternative metrics to F_{ST} because they rely on fewer assumptions and, as such, may provide less biased estimates of differentiation. D_{est} was included as a bias-corrected differentiation metric to account for variable sample size.

Landscape resistance surfaces

Eleven continuous landscape resistance surfaces, which were hypothesized to affect survival or movement of *P. albagula*, were created in ArcGIS 9.3 (ESRI, Redlands, CA, USA) (Fig. 1b–i). Topographic wetness index (TWI; Fig. 1b), topographic position index (TPI; Fig. 1c), distance from ravine (Fig. 1d) and potential relative radiation (PRR; Fig. 1e) were all derived from 1/9 arc second Nation Elevation Dataset (~3-m resolution; <http://seamless.usgs.gov/products/9arc.php>), while normalized difference vegetation index (NDVI = canopy cover; Fig. 1f) was calculated from Landsat 7 satellite imagery and had an original resolution of 30 m. Additionally, maximum temperature on the landscape

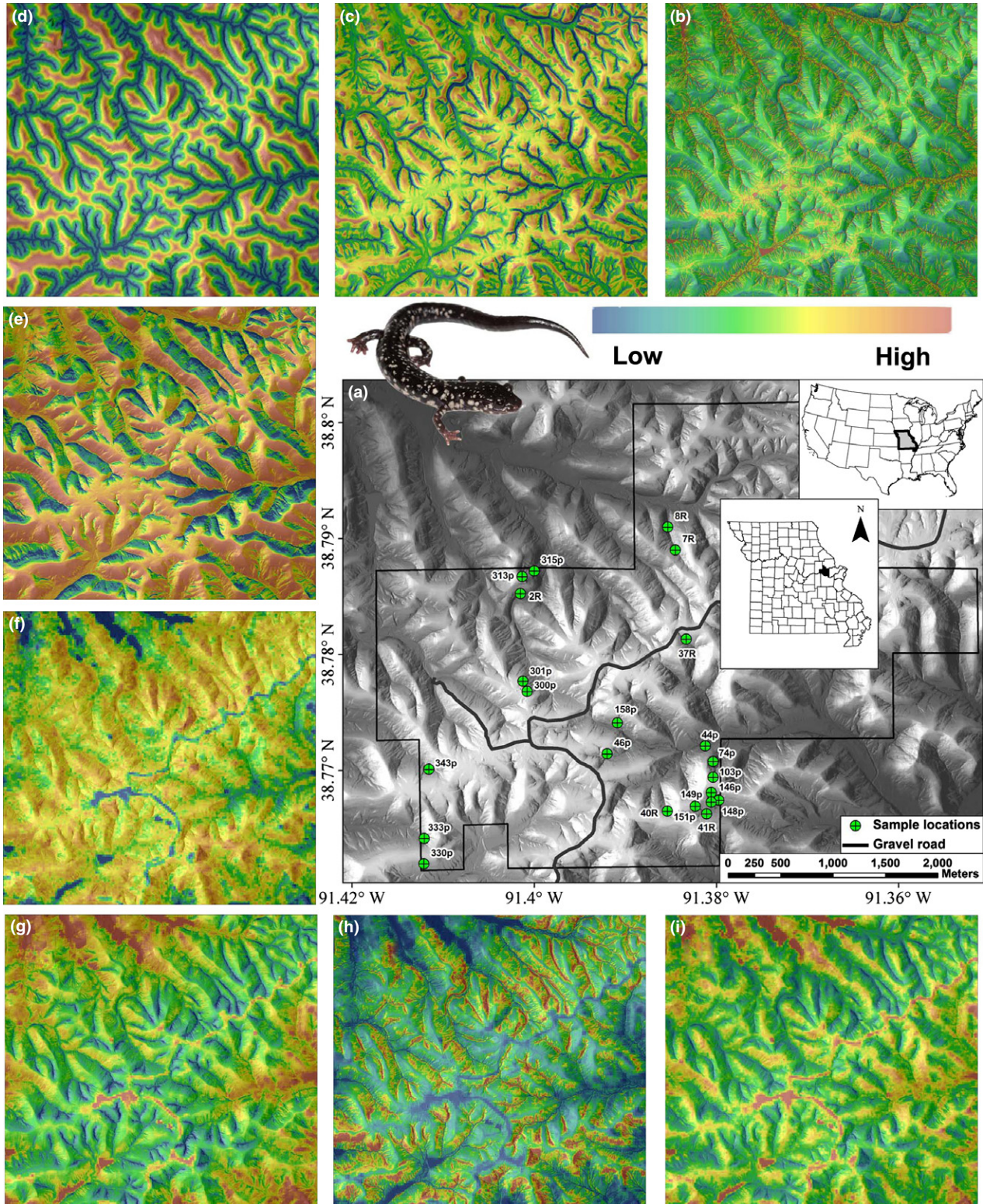


Fig. 1 Map of the Daniel Boone Conservation Area in Warren County, MO, USA. The 22 sample locations, hillshade relief and conservation area boundaries (thin black line) are depicted in panel (a). (b) topographic wetness (TWI), (c) topographic position index (TPI), (d) distance from ravine, (e) potential relative radiation (PRR), (f) canopy cover (NDVI), (g) maximum surface temperature, (h) predicted abundance and (i) estimated rate of water loss for adult *P. albagula* in the summer, respectively. Blue represents low, green intermediate and red high values for each respective surface.

Table 1 Population genetic summary statistics for 22 sample sites in the Daniel Boone Conservation Area, MO, USA

Popu- lation	Northing	Easting	N	H_E	H_O	A_R	A_P
103p	4292372.86	640720.62	15	0.50	0.47	3.88	0
146p	4292226.41	640701.14	21	0.49	0.42	3.93	0.15
148p	4292152.06	640774.62	14	0.49	0.51	3.85	0.04
149p	4292134.41	640701.42	20	0.49	0.50	3.67	0.09
151p	4292093.46	640550.17	14	0.52	0.53	3.93	0.03
158p	4292894.26	639806.07	19	0.51	0.51	3.95	0.05
2R	4293194.32	638944.23	12	0.50	0.48	3.73	0.07
300p	4293291.61	638904.60	24	0.48	0.50	3.73	0.04
301p	4294348.96	639012.25	22	0.49	0.47	3.79	0.06
313p	4294294.32	638897.69	17	0.50	0.50	3.93	0.05
315p	4291542.88	637959.14	20	0.52	0.51	3.84	0.05
330p	4291785.98	637962.56	11	0.47	0.46	3.69	0
333p	4292449.62	638006.56	12	0.49	0.48	3.68	0.03
343p	4292672.86	640645.62	15	0.45	0.45	3.55	0.04
37R	4292597.86	639708.12	18	0.52	0.46	3.61	0.02
40R	4292522.85	640720.62	20	0.56	0.53	4.08	0.08
41R	4294131.57	638880.38	19	0.51	0.51	3.7	0.02
44p	4293694.71	640463.13	10	0.51	0.48	3.95	0.02
46p	4292049.02	640282.59	11	0.46	0.47	3.68	0.02
74p	4292022.60	640657.48	16	0.48	0.46	3.84	0.05
7R	4294549.24	640359.22	13	0.50	0.49	3.64	0.11
8R	4294768.24	640286.79	17	0.51	0.50	3.71	0.06
Avg.			16.36	0.50	0.49	3.79	0.05

Avg.: average; N : is the number of samples after removal of full-siblings; H_E : is expected heterozygosity; H_O : is observed heterozygosity, and A_R and A_P : are the mean rarefied allelic richness and frequency of private alleles.

surface (Fig. 1g) was estimated using a hierarchical mixed-effects model as described by Fridley (2009) from 61 Thermochron iButton data loggers (Maxim) deployed across our landscape (Peterman & Semlitsch 2013). The maximum temperature surface is a composite surface that incorporates all of the previously described resistance surfaces to predict surface soil temperature at 3-m resolution. We also included a predicted salamander abundance surface (Fig. 1h), which was estimated from a hierarchical binomial mixture model that incorporated TPI, TWI, PRR and NDVI as independent variables (Peterman & Semlitsch 2013; Appendix S1, Supporting information). Finally, we included four different water loss surfaces representing predicted rates of water loss for adult and juvenile salamanders in both spring and summer (adult summer water loss shown in Fig. 1i). These surfaces were generated from linear mixed-effects models that included different combinations of TPI, PRR, NDVI and distance from ravine as independent variables (Peterman 2013; Appendix S1, Supporting information). We predicted that abundance, NDVI and

TWI would promote gene flow (i.e. higher values would have lower resistance), while all other surfaces would limit gene flow (i.e. higher values would have increased resistance).

To modify resistance surfaces, we used the monomolecular and Ricker functions as described in Bolker (2008) as well as a rescaling function. Details of the functions used and their implementation can be found in Appendix S2 (Supporting information). Using these three functions, a wide range of data transformations can be realized, and nonlinear transformations can be explored (Fig. 2). We chose to use the Ricker and monomolecular functions to transform resistance surfaces because their shape and maximum values are dictated by only two parameters (Appendix S2, Supporting information). Previous studies have utilized a Gaussian transformation to model a peaked response (Cushman *et al.* 2006; Shirk *et al.* 2010; Graves *et al.* 2013), but this requires optimization of three parameters. See Appendix S2 (Supporting information) for the full equations used for each transformation, as well as an example transformation of a resistance surface.

Statistical modelling and optimization

We measured the resistance distance between sample locations using CIRCUITSCAPE (v.4.0-Beta; McRae 2006). This approach assesses all possible pathways between any two points and may better represent gene flow that occurs over multiple generations (McRae 2006). For this analysis, we assessed connectivity based on average resistances using an eight neighbour connection scheme. To make the Circuitscape analyses tractable, all resistance surfaces were resampled to a resolution of 15 m. Previous research has shown inferences to be robust to changes in the resolution of landscape surfaces (McRae & Beier 2007).

To evaluate the relative support for each resistance surface, we fit linear mixed-effects models using the maximum-likelihood population effects (MLPE) parameterization to account for the nonindependence of values within pairwise distance matrices (Clarke *et al.* 2002; Van Strien *et al.* 2012). Mixed-effects models were fit by maximum likelihood using *lme4* (Bates *et al.* 2013) in the. Pairwise genetic distance (linearized F_{ST} , D_C , D_{PS} , D_{est}) was used as the dependent variable, while scaled and centred effective resistance between populations was the independent variable. Because distance is implicitly incorporated into the effective resistance measure calculated by Circuitscape, Euclidean distance was not included as an additional factor in our models.

Using AIC as our objective criteria to evaluate model fit and optimize parameter values, we utilized a two-step optimization procedure. First, we fit 17

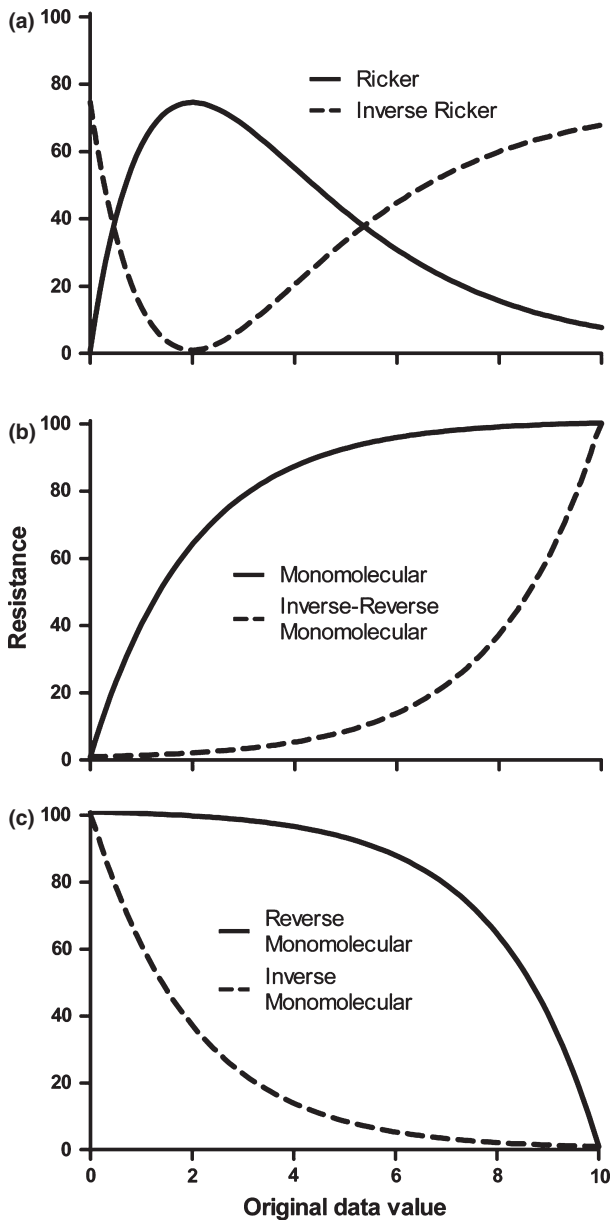


Fig. 2 Example data transformations with the two-parameter Ricker and monomolecular functions. Using a combination of reflection over the x - and y -axes and rescaling, a diversity of data transformations can be realized to optimize resistance surfaces. All examples have the maximum value parameter set to 100, and the shape parameter set to 2. See Appendix S2 (Supporting information) for details on these transformation functions.

models with the maximum value (b) fixed at 100 and then varied the shape parameter (c) from 0.2 to 1 by 0.2 and from 1.75 to 10 by 1.75. The value of c that minimized AIC among these 17 models was then used as the starting value in the nonlinear minimization algorithm (nlm function; Dennis & Schnabel 1983;

Schnabel *et al.* 1985). Nonlinear minimization uses a Newton-type algorithm to search parameter space to select the optimal parameter values that minimize the objective criteria (AIC). We implemented this two-step approach because preliminary analyses revealed the presence of a linear trough in parameter space extending away from the optimal shape parameter (Appendix S3, Supporting information). As such, our two-step approach allowed us to conduct a restricted grid search of parameter space to provide informed starting values for optimization. For each of the eleven resistance surfaces, we optimized the Ricker, inverse Ricker, monomolecular, inverse monomolecular, reverse monomolecular and inverse-reverse monomolecular equations (Fig. 2). Although we had a priori predictions concerning the relationships of resistance surfaces to gene flow, our optimization procedure made no a priori assumptions about the direction or magnitude of the relationship between each resistance surface and genetic distance.

After each surface was optimized with each of the six functions, we determined the function that minimized AIC for each of the eleven resistance surfaces and then ranked models and calculated the delta AIC for the included models. To assess the robustness of our model selection and optimization, we conducted a bootstrap resampling of our data. Using the function and parameter values that optimized each surface, we randomly selected 17 of our 22 populations without replacement and then fit the optimized model to these selected populations. Following 10 000 iterations, we determined the frequency that each surface was selected as the top model as well as the average rank of each model. Code to implement our optimization procedure using Circuitscape, as well as model bootstrapping, is provided in Appendix S4 (Supporting information).

Results

Population genetic analyses

Three microsatellite loci were monomorphic (PLAL_791, PG_RIH and PG_3XI), and PLAL_EIXNY had a high error rate and evidence of null alleles. The remaining 20 loci had 2–27 alleles (mean = 8.20 ± 5.76) across all samples. All loci and populations conformed to HWE expectations, and there was no evidence for linkage between pairs of loci. Observed heterozygosity at each sample location ranged from 0.42 to 0.53 (mean = 0.49; Table 1). Pairwise estimates of F_{ST} ranged from 0 to 0.0529, D_C ranged from 0.185 to 0.423, D_{PS} ranged from 0.128 to 0.307 and D_{est} ranged from 0 to 0.093 (Tables S1–S2, Supporting information).

Comparison of genetic and landscape distances

Optimization and model selection results were largely congruent for each of the four genetic distances, with the rate of water loss experienced by adult salamanders in the summer being the best-supported resistance surface (Tables 2 and S3, Supporting information). Using F_{ST} and D_{estv} the summer water loss and maximum surface soil temperature surfaces were more equally supported (Table S3, Supporting information). However, models using D_{PS} and D_C as the response variable resulted in strong support for the adult summer water loss surface, with minimal support for any other resistance surface. Because D_{PS} is a genetic measure with fewer assumptions than F_{ST} , we focus on the results from D_{PS} in the rest of the paper. The best-supported functional form for the adult summer water loss surface was the inverse Ricker function (Table 2). The linear mixed-effects model fit the data well (Appendix S5, Supporting information), and adult summer water loss is a significant predictor of genetic distance in the generalized linear mixed-effects model (Appendix S6, Supporting information). Further, the optimized adult summer water loss surface was selected as the top model 62% of the time and had an average model ranking of 1.90 based on 10 000 bootstrap resamples (Table 2). This optimized surface assigns high resistance to regions of the landscape where the predicted rate of water loss is low, with decreasing resistance as the rate of water loss increases to 3.72%/h. Resistance then increases as the rate of water loss increases beyond this threshold (Appendix S7, Supporting information). While rates of water loss observed on the landscape

ranged from 1.22 to 13.40%/h, only 6% of the landscape had rates >3.72%/h. As such, there is a small fraction of the landscape with high rates of water loss, but these areas may impede movement of salamanders or affect survival or recruitment.

For each of the eleven resistance surfaces, the function that optimized the surface was either the Ricker or inverse Ricker (Table 2). We note that these functions are highly flexible, and in instances where the Ricker was the optimal model, the peak in resistance is strongly positively skewed (Appendix S7, Supporting information). This results in a negative exponential-like decay over the majority of parameter space. Contrary to our predictions, higher values of abundance, TWI and NDVI corresponded with higher resistance, while high values of all the other surfaces corresponded with low resistance.

Discussion

We found clear support for the landscape resistance surface that represented the rate of water loss in adult salamanders during the summer. This surface directly represents an important physiological process for salamanders that affects several key drivers of genetic differentiation. Specifically, water loss will affect the amount of time salamanders can be surface active (Feder & Londos 1984), which has been shown to influence foraging, dispersal, survival and fecundity (Grover 1998; Milanovich *et al.* 2006). Unexpectedly, this resistance surface, and all other resistance surfaces assessed, affected gene flow contrary to our predictions. Previous

Table 2 Model selection results for linear mixed-effects models optimized on D_{PS} . AIC is the Akaike information criterion, ΔAIC is the difference in AIC between the best model and each competing model, and w_i is the Akaike weight, representing the probability that a model is the best in the model set. The average rank and top model frequency for each model was calculated based on 10 000 bootstrap iterations using a random resampling of 75% of the sampled populations. Adult summer water loss was the best-supported model based on AIC, received the majority of the model weight, had the highest average model rank and was most frequently ranked as the top model in 10 000 bootstrap iterations

Surface	Equation	AIC	ΔAIC	w_i	Avg rank	Frequency top model
Ad summer water loss	Inverse Ricker	-1071.31	0.000	0.666	1.90	0.620
Jv summer water loss	Inverse Ricker	-1068.03	3.278	0.129	2.99	0.077
Max Temp	Inverse Ricker	-1066.55	4.757	0.062	4.65	0.075
Stream Dist	Inverse Ricker	-1065.92	5.389	0.045	5.07	0.053
Jv spring water loss	Ricker	-1065.28	6.031	0.033	4.68	0.050
Ad spring water loss	Inverse Ricker	-1064.95	6.360	0.028	5.03	0.104
Abundance	Inverse Ricker	-1062.98	8.325	0.010	7.48	0.000
TWI	Inverse Ricker	-1062.85	8.461	0.010	8.16	0.010
TPI	Ricker	-1062.52	8.785	0.008	8.10	0.008
PRR	Ricker	-1061.92	9.384	0.006	8.50	0.005
NDVI	Inverse Ricker	-1060.82	10.483	0.004	9.94	0.000
Euclidean distance	NA	-1056.91	14.397	0.000	11.80	0.000
Null	NA	-1050.50	20.806	0.000	12.71	0.000

research has shown that abundance of salamanders is significantly greater in cool, moist regions of the landscape where desiccation rates are the lowest (Peterman 2013; Peterman & Semlitsch 2013). Results from our current study clearly demonstrate that genetic resistance across most of the landscape decreases as the rate of water loss increases. However, resistance does increase again when rates of water loss exceed 3.72%/h (Appendix S7, Supporting information).

Our finding that gene flow is relatively low through seemingly favourable habitat might be explained by the complex movement behaviour of salamanders. Plethodontid salamanders are active on the ground surface almost exclusively at night, and activity is greatest during or immediately following rain (Petranka 1998). Under these conditions, water loss may not be a limiting factor for dispersing salamanders. However, if salamanders fail to completely traverse inhospitable habitat in a single night, or if water loss rates are too high, they must settle, potentially increasing their risk of mortality. To minimize the need to settle in unfavourable habitat, salamanders may exhibit different dispersal behaviours depending upon local landscape features. Movement ability and behaviour can be significantly affected by landscape context (Goodwin & Fahrig 2002), and Semlitsch *et al.* (2012) found that the rate of movement and straightness of movement paths increased when salamanders (*P. metcalfi*) were placed on roads or exposed ground. Similar compensatory movement behaviours have been experimentally observed in another terrestrial salamander, *Ensatina escholtzii*, which increased velocity and were less likely to settle when traversing bare soil (Rosenberg *et al.* 1998). The experimental findings of Semlitsch *et al.* (2012) and Rosenberg *et al.* (1998) suggest that the motivation and subsequent pattern of dispersal for salamanders can vary depending upon the local landscape. Individuals inhabiting cool, moist habitats may make more exploratory movements and move only as far as necessary to establish a territory, while individuals in hot, dry habitats may make more rapid and directed movements when environmental conditions permit. Moving directly and rapidly through inhospitable habitats may be a general evolutionary dispersal strategy that reduces mortality risk, and exploratory movements within suitable habitat may best maximize fitness (Knowlton & Graham 2010).

Despite the patterns of dispersal observed in this study, we emphasize that our study focused on a small region of continuously forested habitat, with only minimal influence of anthropogenic alteration (gravel road, Fig. 1a). However, the gravel road and other nonforested regions of our landscape generally had rates of water loss that exceeded 3.72%/h, suggesting that these features may limit salamander movement or increase

mortality (Appendix S7, Supporting information). If we assume that salamanders are able to lose 10% of their body water before seeking refuge to rehydrate (Feder & Londos 1984), then surface activity in these nonforested areas would be limited to <2.7 h a night. In an experimental displacement study, Connette and Semlitsch (2013a) found that salamanders never moved more than 15.5 m over a three-hour period. These findings suggest that even small areas with high rates of water loss may pose formidable challenges to dispersing salamanders. Further, land uses such as logging, agriculture or urbanization would undoubtedly increase rates of water loss on the landscape, and the scale of such land uses may alter dispersal and connectivity and result in different patterns of resistance than measured in our study. Previous research at DBCA has found that abundance and apparent recruitment of *P. albagula* is significantly reduced in clear-cut logged habitats for up to 7 years (Hocking *et al.* 2013), and Connette and Semlitsch (2013b) found that populations of *P. shermani* in North Carolina, USA, may take >100 years to fully recover following logging. Because rates of water loss will be increased following land uses such as logging, larger-scale anthropogenic disturbances may have profound impacts on local genetic diversity and affect the level genetic differentiation.

Other genetic-based studies have also revealed unexpected movement patterns of animals. Keller and Holderegger (2013) found that short-distance movements of damselflies were confined to stream corridors, but long-distance dispersal was best described by straight line paths across agricultural matrix. A study of *Ambystoma californiense*, a grassland-associated species, found that dispersal costs were greatest through grassland habitat (Wang *et al.* 2009). Stevens *et al.* (2006b) used behavioural experiments with natterjack toads (*Epidalea calamita*) to parameterize alternate resistance models that reflected relative resistance values of habitat type (Stevens *et al.* 2004) and habitat boundary permeability (Stevens *et al.* 2006a). They determined that habitat permeability was significantly correlated with genetic distance, while speed of movement through habitats was not. In our study, estimated abundance was a poor predictor of genetic differentiation on the landscape, and although summer water loss was the best predictor of genetic differentiation, the optimized surface had an unexpected relationship with genetic distance. All of these examples highlight the potential shortcomings of expert opinion and even empirical observation to inferring factors relevant to multigenerational processes affecting gene flow.

Our optimization framework allows researchers to extensively explore relationships between resistance surfaces and genetic distances without making a priori

assumptions. This is a critical advancement over methods that iteratively optimize, combine and reoptimize resistance surfaces by varying parameters that alter the shape and magnitude of resistance (Shirk *et al.* 2010). Such procedures are generally conducted over a limited parameter space and the direction of the effect must be predetermined. This is particularly problematic given the growing consensus that expert opinion often poorly describes the ecological processes being modelled (Shirk *et al.* 2010; Charney 2012). Although optimization of univariate resistance surfaces is relatively tractable, biological realism may be better captured by using multivariate resistance surfaces, which can become exceedingly complex to parameterize (Spear *et al.* 2010).

We used the Ricker and monomolecular functions to transform resistance surfaces, which gave us great flexibility to explore parameter space in an unbiased manner (Fig. 2). When paired with linear mixed-effects models, we found that AIC was an effective objective criteria to optimize each resistance surface using our optimization algorithm. A recent simulation and optimization study by Graves *et al.* (2013) found that the Mantel correlation surface can be very flat over large regions of parameter space, which can pose significant challenges to optimization. In this study, we did not find excessively flat response surfaces. In contrast, we found one clear trough in parameter space (Appendix S3, Supporting information) that indicates the strong identifiability of a nonlinear relationship between gene flow and water loss rates. Given the flexibility of our utilized functions and the pronounced topography of the resultant response surfaces (Appendix S3, Supporting information), we are confident that we have correctly identified water loss, a physiologically based, ecologically explicit resistance surface, as a primary driver of genetic differentiation in *P. albagula* at the scale we assessed.

A primary goal in our study was to develop spatial resistance surfaces with direct ecological meaning and to test their relationship with spatial genetic differentiation. In finding that the rate of water loss best described genetic differences between population, we have made a clear, mechanistic link between the observed genetic pattern and the process underlying it. Importantly, each of our ecological resistance surfaces was a composite of multiple landscape features, estimated using field-collected data and a variety of statistical models (Peterman 2013; Peterman & Semlitsch 2013). Previous studies have converted habitat suitability models to resistance surfaces (Wang *et al.* 2008; Richards-Zawacki 2009; Koen *et al.* 2012; Wang 2012), and this approach represents a step towards testing hypotheses concerning how habitat use and species occurrence on the landscape relate to genetic differentiation. Although these methods

generally integrate multiple habitat features into a single resistance surface, they are often used as 'black box' methods (Yackulic *et al.* 2013) and the resultant surface lacks a clear connection to ecological processes (but see Laiolo & Tella 2006; Wang *et al.* 2008; Koen *et al.* 2012). Garroway *et al.* (2011), through their use of multiple regression on distance matrices, made a significant advancement by combining landscape features using regression coefficients, and while the combined surface best explained genetic distance between populations, direct ecological meaning is still unknown.

We have gone beyond correlation of landscape features that influence connectivity and specifically tested hypotheses concerning how ecological processes affect population connectivity. By conducting relevant field research and experimentation, we were able to derive composite resistance surfaces representing aspects of population dynamics (abundance) and physiology (water loss). Directly combining multiple resistance surfaces in a coherent manner poses significant challenges with no clear analytical framework. In our study, we only assessed individual and composite resistance surfaces in isolation, and it is possible that other secondary factors could contribute to movement and connectivity of *P. albagula* beyond water loss alone. The use of surfaces that have a direct ecological meaning allows researchers to formulate more specific hypotheses about how and why observed patterns of spatial genetic structure have emerged, a significant advancement beyond simply concluding that certain combinations of landscape features affect dispersal. However, caution must still be taken, as there is no guarantee that surfaces representing one aspect of an organism's ecology (e.g. abundance) will be a relevant predictor of genetic differentiation across the landscape. While methods for optimizing and weighting multivariate resistance surfaces continue to be sought, we advocate that, regardless of future developments in optimization procedures, greater emphasis be placed on developing resistance surfaces with clear and direct ecological meaning. To date, landscape genetics has been a predominantly exploratory field (Manel & Holderegger 2013), seeking correlations with habitat and landscape features, with plausible ecological explanations being built around these results. While novel insight into cryptic or hard to study species will always be a strength of landscape genetics, it should not preclude the inclusion of empirical, field-derived ecological data.

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W.E.P. conceived and designed the study, collected and analysed data, and wrote the manuscript; G.C.M. contributed to analyses; G.C.M., R.D.S. and L.S.E. contributed to meaningful discussion and helped write the manuscript.

Data accessibility

Original resistance surfaces: Dryad (doi:10.5061/dryad.m4f17). Microsatellite genotypes: Dryad. R code for optimizing resistance surfaces using Circuitscape can be accessed at GitHub. <https://github.com/wpeterman/ResistanceOptimization> (directions in Appendix S4, Supporting information).

Supporting information

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

Table S1 Pairwise genetic distances measured using F_{ST} and D_C .

Table S2 Pairwise genetic distances measured using D_{PS} and D_{est} .

Table S3 Model selection and bootstrap results using F_{ST} , D_C , and D_{est} as the response variable.

Table S4 Parameter estimates for functions fit to adult summer water loss.

Appendix S1 Description of models used to derive ecological resistance surfaces.

Appendix S2 Full description of functions used to transform and optimize resistance surfaces.

Appendix S3 AIC response surface for adult summer water loss.

Appendix S4 Instructions to access and implement methods used in this study.

Appendix S5 Diagnostic plots showing linearity and normal distribution of residual error.

Appendix S6 Parameter estimates and significance for mixed effect model fit to adult summer water loss.

Appendix S7 Resistance transformations for optimized surfaces.