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ARTICLE

Differing, multiscale landscape effects on genetic diversity and differentiation in eastern chipmunks

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Abstract

Understanding how habitat loss and fragmentation impact genetic variation is a major goal in landscape genetics, but to date, most studies have focused solely on the correlation between intervening matrix and genetic differentiation at a single spatial scale. Several caveats exist in these study designs, among them is the inability to include measures of genetic diversity in addition to differentiation. Both genetic metrics help predict population persistence, but are expected to function at differing spatial scales, which requires a multiscale investigation. In this study, we sampled two distinct spatial scales in 31 independent landscapes along a gradient of landscape context (i.e., forest amount, configuration, and types of intervening matrix) to investigate how landscape heterogeneity influences genetic diversity and differentiation in the forest-associated eastern chipmunk (*Tamias striatus*). Overall, quality of intervening matrix was correlated with genetic differentiation at multiple spatial scales, whereas only configuration was associated with regional scale genetic diversity. Habitat amount, in contrast, did not influence genetic differentiation or diversity at either spatial scale. Based on our findings, landscape effects on genetic variation appears to differ based on spatial scale, the type of genetic response variable, and random variation among landscapes, making extrapolation of results from single scale, unreplicated studies difficult. We encourage landscape geneticists to utilize multiscale, replicated landscapes with both genetic diversity, and differentiation to gain a more comprehensive understanding of how habitat loss and fragmentation influence genetic variation.

Introduction

With increasing human pressures on wild populations, landscape genetics methodologies are increasingly employed to assess how habitat loss and fragmentation influence genetic variation. Landscape genetic methods typically correlate measures of landscape connectivity and genetic differentiation among individuals or sampling sites to infer how intervening habitat features influence gene flow. Linking landscape genetic results to observed habitat loss and fragmentation becomes difficult, however, because habitat loss and fragmentation are inherently correlated in nature. Thus, both processes are likely to contribute to the composition and configuration remnant and matrix habitats on a landscape, which in turn influence effective population size (N_e), a direct correlate of genetic drift, and gene flow. On one extreme, landscapes with high habitat loss and fragmentation support few individuals that experience little migration between remnant habitats (e.g., Gustafson and Gardner 1996; Bender and Fahrig 2005; Haddad and Tewksbury 2005; Ewers and Didham 2006; Prevedello and Vieira 2010; Gebauer et al. 2013; Cushman et al. 2012, Kierepka and Latch 2015), resulting in low genetic diversity and high differentiation. Outside this situation, isolating the independent effects of habitat loss and fragmentation is nearly impossible when focusing solely on intervening habitats. Therefore, several authors have suggested that current landscape genetic frameworks may require more complexity to fully capture

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landscape effects on genetic variation (Pflüger and Balkenhol 2014; DiLeo and Wagner 2016).

One current shortcoming of landscape genetics is that authors rarely assess genetic diversity and differentiation simultaneously across a landscape, despite both being important for population persistence (DiLeo and Wagner 2016). Disparate attributes of a landscape may drive changes in genetic diversity and differentiation because these metrics reflect different aspects of genetic variation. Genetic diversity reflects all the available variation within a population, which is expected to be strongly linked to N_e within a population (Frankham 1996). In contrast, genetic differentiation is a measure of observed dissimilarity between two populations where gene flow homogenizes genetic variation within two populations. Based on the differing processes that underlie genetic diversity and differentiation, landscape effects are not likely to be identical. Indeed, simulations have shown that habitat amount is the strongest predictor of genetic diversity (Jackson and Fahrig 2014) while fragmentation and resistance of intervening matrix are strong predictors of genetic differentiation (e.g., Cushman et al. 2012; Kierepka and Latch 2015). Therefore, studies which seek to evaluate both genetic diversity and differentiation across a landscape necessitate more complex study designs to fully capture potential differing scale and landscape effects on genetic variation.

Effectively incorporating genetic diversity into study designs for differentiation is not straightforward because scale is expected to have a substantial impact on the usefulness of landscape genetic analyses. Genetic diversity and differentiation among populations in a landscape are expected to reach migration–drift equilibrium at different rates (Varvio et al. 1986) with changes in genetic differentiation arising faster than changes in genetic diversity. Therefore, landscape effects on genetic diversity should be apparent at a larger spatial scale than differentiation. Indeed, prior work demonstrates that landscape heterogeneity may not influence genetic differentiation and diversity equally (Lange et al. 2012; Balkenhol et al. 2013; Taylor and Hoffman 2014; Da Silva Carvalho et al. 2015), which at least partially is an artifact of the differing spatial scales over which these genetic parameters are expected to change in response to habitat fragmentation.

Assessing landscape effects on genetic diversity and differentiation simultaneously would best be accomplished via multiscale designs that are more commonly used in nongenetic landscape ecology studies (e.g., Meyer and Thuiller 2006; McGarigal et al. 2016; but see Murphy et al. 2010; Millete and Keyghobadi 2015). Unfortunately, the replication across landscapes needed to perform such assessments is relatively rare in landscape genetics, owing to the difficulty in sampling individuals across multiple landscapes (DiLeo and Wagner 2016). Nonetheless, multiscale studies incorporate the inherent hierarchical nature of landscape pattern (Wu 2004) with replication across multiple spatial scales, which provides several benefits over simpler designs. First, using multiscale designs can help identify at which scale landscape heterogeneity is most important for a focal variable ("scale of effect"; Jackson and Fahrig 2012). Since genetic diversity and differentiation likely respond differentially across spatiotemporal scales, a multiscale design is more likely to detect the scale of effect for both metrics. With replication, multiscale studies include gradients of fragmentation and habitat loss that aids in extrapolation across landscapes, a commonly cited concern in landscape genetics (Short Bull et al. 2011; Hand et al. 2016). Taken together, multiscale studies represent a suitable method to increase complexity to examine genetic diversity and differentiation, but to date, such designs are rarely implemented in landscape genetics.

Here, we utilized a multiscale study design to examine landscape effects on genetic diversity and differentiation in eastern chipmunks (*Tamias striatus*). Our study area, Indiana's Upper Wabash River Basin (UWB), has been subject to long-term studies of fragmentation (e.g., Nupp and Swihart 1998; Nupp and Swihart 2000; Goheen et al. 2003; Moore and Swihart 2005; Swihart et al. 2006; Dharmarajan et al. 2009; Beatty et al. 2012; Anderson et al. 2015; Kierepka et al. 2016). Importantly, the UWB was sampled in 35 replicate 23 km^2 study cells (i.e., landscapes) to investigate how biotic communities respond to forest fragmentation. Eastern chipmunks provide an ideal organism to investigate how landscape heterogeneity influences genetic variation, because previous field (Moore and Swihart 2005; Rizkalla and Swihart 2012) and genetic studies (Anderson et al. 2015; Kierepka et al. 2016) demonstrated that chipmunks exhibit strong responses to heterogeneity of forested habitats in the UWB. For example, Kierepka et al. (2016) found that clumpiness of forest patches and intervening matrix drive genetic differentiation between 23 km^2 landscapes sampled across the entire UWB, while Anderson et al. (2015) found forest amount within corridors influenced genetic differentiation among study cells. Forest amount is also critical for chipmunk abundance and occupancy within the UWB (Moore and Swihart 2005; Rizkalla and Swihart 2012), so it appears that heterogeneity in forest habitats could result in complex patterns of genetic variation.

Our study extends previous work in the UWB by assessing how landscape effects vary according to genetic parameters (diversity vs. differentiation) and scale to examine if additional complexity in landscape genetic study design better captures the effects of landscape heterogeneity. We expected genetic diversity to be

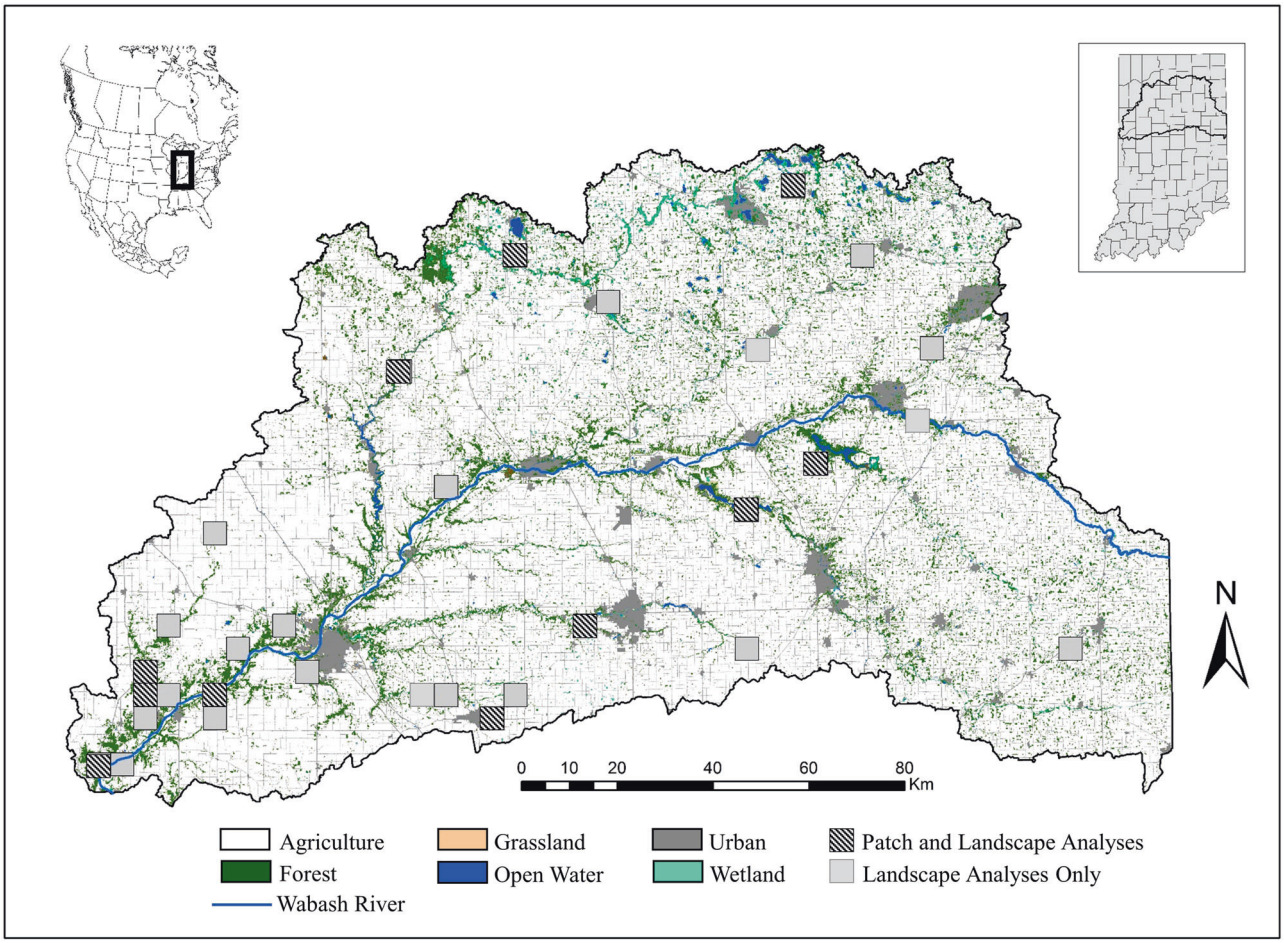

Fig. 1 Geographic distribution of 31 study cells (23 km^2) across the Upper Wabash River Basin (UWB) where eastern chipmunks were sampled from 2001–2003. All 31 study cells were included in the study-cell level of genetic diversity, whereas the subset of 12 study cells (dotted lines) were used in the patch-level analyses. The subset of 12 had at least four sampled patches with ≥5 individuals chipmunks per patch.

correlated with both the amount and configuration of forest habitats as observed in field studies (Moore and Swihart 2005; Rizkalla and Swihart 2012). In addition, spatial scale should be particularly important for genetic diversity where at the larger, landscape scale, we expect much stronger landscape effects than the local scale because genetic diversity operates at a broader spatial scale than individual patches (Jackson and Fahrig 2014). In contrast, landscape effects on genetic differentiation are expected to be similar across our two spatial scales. Genetic differentiation directly relates to successful dispersal between landscapes or patches, so the same factors that prevent movement should be present regardless of scale. Therefore, genetic differentiation between patches should exhibit similar relationships to Kierepka et al. (2016) where differentiation was correlated with resistance of intervening matrix and to a lesser extent, measures of fragmentation (i.e., configuration metrics).

Materials and methods

Study area

Our study occurred in the UWB (Fig. 1), an area encompassing >2 Mha and eight major watersheds in north-central Indiana, USA. The UWB has undergone substantial conversion to agriculture following European settlement, and current forest cover is ~8% across the UWB. The remaining forests (mainly *Quercus-Carya-Acer*) are highly fragmented and tend to be clustered around the major drainages within the UWB because floodplains or topography were not suitable for agriculture. Approximately 96% of the UWB is privately owned with 88% designated as agriculture.

Sample collection

A full description of sampling methods can be found in Moore and Swihart (2005) and Urban and Swihart (2009).

During the summers of 2001–2003, eastern chipmunks were live-trapped within 35 study cells (23 km^2 each; Fig. 1). Locations of trapping grids were chosen based on a stratified random design that emphasized natural land cover types (i.e., grassland, forest, and wetland; 27.8% of grids for each type) over urban and agriculture (13.9% and 2.8% of grids, respectively). A maximum of 45 trapping grids were placed within each study cell with 1–3 grids within any individual patch of habitat (Swihart and Slade 2004; Moore and Swihart 2005; Urban and Swihart 2009).

Trapping grid design differed slightly in 2001 compared with 2002 and 2003 because 2001 had two grid sizes: 3 × 3 grids of Fitch live traps placed 15 m apart in small to medium forest patches and 7 × 7 grids in large forest patches. In 2002 and 2003, we utilized a mix of Fitch and Sherman live traps in either 3 × 3 or 5 × 5 trapping grids. All other trapping methods were identical across years where 5 × 2 grids were placed in habitat corridors (i.e., habitats < 30 m wide). Each trapping session included a pretrapping period where traps were opened for 3 days followed by 5 days of active trapping. Traps were baited with black-oil sunflower seeds, and while active, checked twice daily. We collected ear or toe clips from all captured chipmunks, treated sampled individuals with ferric subsulfate if bleeding occurred, and subsequently released each animal. All handling procedures were approved by the Purdue Animal Care and Use Committee under protocol #01–024. All tissues were stored in a −80 °C freezer until DNA extraction.

Microsatellite genotyping

All laboratory methods are previously described in Anderson et al. (2015) and Kierepka et al. (2016). Briefly, we amplified all extracted chipmunk samples at 12 (EACH01-12; Anderson et al. 2007) microsatellite loci in four multiplex PCR reactions (locus-specific information and reactions given in Anderson et al. 2015, Kierepka et al. 2016). The amplification conditions consisted of initial 94 °C for 2 min, 35 cycles of 94 °C for 30 s, primer specific annealing temperatures for 30 s, 72 °C for 30 s, then a final extension of 72 °C for 10 min, and a soak at 60 °C for 45 min. We sized PCR products on an Applied Biosystems 3730 automated sequencer, and genotyped all individuals via GeneMapper 3.7 (Applied Biosystems).

To ensure accurate genotypes, we applied four quality control methods. First, all 96-well plates contained a negative control, two preamplified positive controls, and a concurrently amplified positive control. Second, we attempted to resolve ambiguities and missing genotypes by rerunning samples up to two times in either a multiplex or singly. If missing genotypes remained following reamplification, samples were reextracted and genotyped at all loci. Finally, any individuals with greater than 30% missing genotypes were removed from the final dataset. Following quality control, we removed four study cells because less than 15 individuals were captured within each cell, resulting in 1368 total chipmunks (125/16,416 or 0.761% missing genotypes) within our dataset.

Genetic diversity

Regional scale: genetic variables

For genetic diversity, we calculated allelic richness (A_R), observed and expected heterozygosity (H_O and H_E), and F_{IS} for the 31 study cells in the R package *diveRsity* (function "divBasic"; Keenan et al. 2013). The number of genotyped individuals within each study cell ranged from 18 to 144 individuals, so we focused on A_R as the genetic diversity variable. Allelic richness corrects for unequal sample sizes through rarefaction, so all A_R values were calculated based on the smallest sample size ($n = 18$).

Regional scale: landscape variables

Landscape variables included measures of forest amount and configuration of forest within each study cell. Each 23 km^2 study cell plus a 1.6 km buffer was digitized from 1998 digital aerial ortho quads and converted to 3 m resolution land cover rasters (Moore and Swihart 2005; Rizkalla and Swihart 2012). We utilized the program FRAGSTATS v. 4.2 (McGarigal et al. 2002) to calculate the following landscape metrics: proportion of forest within each study cell (prForest), density of forest patches (PD), and clumpiness of forest habitat (Clumpy; Table 1). Proportion of forest is a measure of habitat amount within each study cell while the other two are measures of habitat configuration (PD and Clumpy). All of these factors exhibited a positive correlation with occupancy or simulated abundances within the UWB (Moore and Swihart 2005; Rizkalla and Swihart 2012), so we expected a similar positive relationship with genetic diversity.

In addition to PD and Clumpy, we calculated correlation length of forest habitats, another measure of configuration. Correlation length is considered a metric of physical connectedness of a landscape (McGarigal et al. 2002), and in simulations has been a major predictor of landscape genetic patterns (Cushman et al. 2012; Kierepka and Latch 2015). Unlike the other landscape metrics, proportion of forest and correlation length were highly correlated ($r = 0.712$, $p < 0.001$; Supplementary Table S1). To control for this correlation, we utilized the residuals of a linear regression between correlation length and proportion of forest instead of correlation length (hereby called CorrL.Res). In total, the study-cell level analysis contained four landscape variables as predictors: prForest, PD, Clumpy, and CorrL.Res (Table 1).

Table 1 Description of all predictor and response variables used in this study.

Variable	Definition	Type	Analysis
Study cell A_R	Allelic richness of a study cell	Response variable	Regional genetic diversity
prForest	Proportion of forest within each study cell	Habitat amount	Regional genetic diversity
PD	Patch density, density of forest patches within each study cell	Habitat configuration	Regional genetic diversity
Clumpy	Measure of aggregation of forest patches	Habitat configuration	Regional genetic diversity
CorrL.Res	Residuals of a linear regression between prForest and correlation length, measure of connectivity of forest habitats	Habitat configuration	Regional genetic diversity
mIBD	Mean of all resistance distances between individuals within a study cell; calculated based on isolation-by-distance resistance surface[a]	Intervening matrix	Regional genetic diversity
mMort	Mean of all resistance distances between individuals within a study cell; calculated based on mortality resistance surface[a]	Intervening matrix	Regional genetic diversity
Patch A_R	Allelic richness of a forest patch	Response variable	Local genetic diversity
m.patchIBD	Mean of all resistance distances connected to a focal patch, calculated based on isolation-by-distance resistance surface[a]	Intervening matrix, fixed effect	Patch genetic diversity and differentiation
m.patchMort	Mean of all resistance distances connected to a focal patch, calculated based on mortality resistance surface[a]	Intervening matrix, fixed effect	Local genetic diversity and differentiation
AREA	Area of a focal forest patch	Habitat amount, fixed effect	Local genetic diversity and differentiation
ENN	Euclidean nearest neighbor distance between forest patches	Habitat configuration, fixed effect	Local genetic diversity and differentiation
ID	ID of study cell where focal forest patch is located	Random effect	Local genetic diversity and differentiation
GU_{FST}	Genetic uniqueness for F_{ST} values: average of all pair-wise F_{ST} values involving a focal patch within a study cell	Response variable	Local genetic differentiation
GU_{JD}	Genetic uniqueness for Jost's D values: average of all pair-wise Jost's D values involving a focal patch within a study cell	Response variable	Local genetic differentiation

We conducted three analyses at differing spatial scales: a linear regression at the regional scale (regional genetic diversity) and two linear mixed models with allelic richness (local genetic diversity) and genetic differentiation (local genetic differentiation). Response variables are allelic richness across a study cell (regional genetic diversity), allelic richness in a patch (local genetic diversity), genetic uniqueness based on F_{ST} (GU_{FST}) or Jost's D (GU_{JD}; local genetic differentiation). Landscape predictor variables differ between analyses, but were either calculated from FRAGSTATS 4.2 (prForest, PD, Clumpy, CorrL.Res, AREA, and ENN) or CIRCUITSCAPE (mIBD, mMort, m.patchIBD, or m.patchMort). Each landscape variable describes one of three features of a study cell or patch: amount of forest habitat (prForest and AREA), configuration of forest habitat (PD, Clumpy, CorrL.Res, and ENN), or the resistance of the intervening matrix between patches (mIBD, mMort, m.patchIBD, or m.patchMort)

[a]See Kierepka et al. (2016) for derivation of IBD and Mort resistance surfaces

For the intervening matrix, Kierepka et al. (2016) found that landscape resistance between study cells was highly correlated with genetic differentiation in chipmunks across the UWB. Therefore, we included the average landscape resistance among all individuals within a study cell as a fifth predictor for our analysis of genetic diversity. Resistance distances essentially represent the difficulty of moving between two patches within each study cell, and are calculated via parameterized raster maps in the program CIRCUITSCAPE v. 3.5 (McRae and Shah 2011). We parameterized each landscape raster based on two previously described models: isolation-by-distance (IBD) and mortality (MortH). Specific details on how resistance values were assigned to each land cover type and statistical assessment of alternative models can be found in (Kierepka et al. 2016; described in Supplementary Material 1). Briefly, IBD is a null model where every pixel in the raster is assigned a resistance value of 1. The mortality model (termed MortH in Kierepka et al. 2016) assumes that chipmunks experienced heightened probabilities of mortality on nonforested land cover types, and each pixel was assigned a resistance value based on mortality probabilities described in Rizkalla and Swihart (2012). Specifically, wetlands/open water and urban habitat had a resistance of 5 (i.e., five times more resistant to gene flow than forest) and roads had a resistance of 30. Resistance distances are inherently correlated, so landscape genetic models either included resistance distances from the IBD or MortH model, but not both.

Regional scale: statistical analysis

We tested the relationship between allelic richness and our six landscape predictor variables (prForest, PD, Clumpy, CorrL.Res, mIBD, or mMort) via multiple regression. Multiple regression assumes normality and equal variances in error terms, so we tested these assumptions via a Wilks–Shapiro and Levene's test, respectively. Neither test was significant, so all variables remained untransformed (all $p > 0.212$). Each study cell represented a data point ($n = 31$), and all regression methods were performed in R (R Development Core Team 2014). We assessed model fit via adjusted R^2 and conducted model comparison via AIC_C (Burnham and Anderson 2002). Top models were determined based on established protocols where all models with a $\Delta AIC_C \leq 2$ are considered top models (Burnham and Anderson 2002).

Local scale: genetic variables

To warrant inclusion in our patch-level analysis of genetic diversity, we required that a study cell have ≥5 genotyped chipmunks within each of ≥3 forest patches. These requirements ensured sufficient sample sizes for accurate genetic diversity estimates and power to test the effect of study cells on patch-based metrics. Based on our requirements, the local scale dataset consisted of 50 patches distributed across 12 study cells ($n = 756$ animals; Fig. 1). We then calculated the same genetic diversity metrics (A_R, H_O, H_E, and F_{IS}) for each patch ($n = 50$) in *diveRsity*.

To examine if the lowest sample size ($n = 5$) had an impact on statistic results, we performed a sensitivity analysis on the number of chipmunks sampled. Since A_R is calculated based on the smallest sample size through rarefaction, all statistical analyses were run for study cells that had ≥10, ≥15, and ≥20 genotyped chipmunks within forest patches. Thus, A_R values would be calculated based on $n = 5$, $n = 10$, $n = 15$, and $n = 20$ to examine how statistical results changed based on sample sizes.

Local scale: landscape variables

Like the regional scale predictor variables, we utilized FRAGSTATS to calculate patch-specific metrics identified as important for occupancy and simulated abundances within patches (Moore and Swihart 2005; Rizkalla and Swihart 2012). Patch metrics included area of a focal forest patch (AREA), weighted sum of area forest patches <400 m away (proximity; PROX), perimeter of a forest patch relative to the maximally compact forest patch (shape index; SHAPE), and the Euclidean distance to nearest patch (ENN; Table 1). PROX and ENN are both measures of configuration (i.e., isolation between patches) whereas AREA and SHAPE are patch-specific metrics of habitat amount. All patch metrics excluding ENN were highly correlated (all $r > 0.7$, all $p < 0.001$; Supplementary Table S1), so we only included AREA and ENN as predictor variables in subsequent models.

We included landscape resistance as a measure of matrix quality for local scale analysis. However, the local scale analysis needed resistance distances between patches, not individuals, so we reran CIRCUITSCAPE to calculate resistance distances between focal patches within each study cell. All calculation methods in CIRCUITSCAPE were identical to the regional scale analysis. To be comparable with genetic diversity, resistance distances between patches (IBD and Mort) had to be transformed into point estimates for each patch. Therefore, we calculated the mean of all resistance distances connected to a patch to derive a point estimate of resistance for each patch (m.patchIBD or m.patchMort). This method of transformation also controls for the effects of differing numbers of sampled patches across study cells ($n = 3$–16 patches per landscape). In total, the patch-level analysis included three landscape predictor variables (AREA, ENN, and m.patchIBD or m.patchMort).

Local scale: statistical analysis

The genetic diversity response variable for the local scale analysis was allelic richness (A_R) within patches. We then used linear mixed models in the R package lmerTest (function "lmer"; Kuznetsova et al. 2013) to examine the relationship between our landscape predictors (AREA, ENN, and m.patchIBD, or m.patchMort) and A_R. All landscape predictor variables were mean center transformed and coded as fixed effects, while study cell ID was considered a random effect. Model selection was conducted via conditional AIC (cAIC), an extension of AIC appropriate for mixed models (Saefken et al. 2014). Conditional AICs were calculated in the R Package cAIC4 (Saefken et al. 2014), and top models had a ΔcAIC ≤ 2 as described in the study cell methods.

Unlike least squares regression, linear mixed models do not provide an obvious statistic (e.g., p values and R^2) to assess model fit, but a number of methods exist to calculate analogous values (e.g., Edwards et al. 2008; Nakagawa and Schielzeth 2013). The lmer function in lmerTest provides p values based on Satterthwaite's approximation for degrees of freedom, but does not calculate R^2 values. Instead, we calculated a marginal and conditional R^2 in the R package MuMIn (Bartón 2015) based on suggestions from Nakagawa and Schielzeth (2013). Essentially, marginal and conditional R^2 describe the variance explained by the fixed effects alone (marginal) and the total model (conditional).

Genetic differentiation

Local scale: genetic variables

Kierepka et al. (2016) found that both habitat configuration and intervening matrix had a strong impact on genetic differentiation between UWB study cells, and so we did not perform additional analyses of genetic differentiation at the regional scale. For genetic differentiation between patches, we calculated both F_{ST} (Weir and Cockerham 1984) and Jost's D (Jost 2008) in *diveRsity* to facilitate comparison with Kierepka et al. (2016). Like the genetic diversity analyses, we sought a point estimate of genetic differentiation, so we utilized a per-patch measure of genetic uniqueness (Prunier et al. 2018). Genetic uniqueness for each patch corresponds to the average of all F_{ST} (GU_{FST}) or Jost's D (GU_{JD}) for a focal patch. GU values did not include F_{ST} or Jost's D values from outside a study cell. In total, our patch-based dataset for genetic differentiation consisted of 50 individual patches across 11 study cells. Like the A_R analysis, we conducted a sensitivity analysis on $n = 10$, 15, and 20 to examine the impact of sample sizes within a patch on statistical results.

Local scale: statistical analysis

Similar to the patch-based A_R analyses, we used linear mixed models in lmerTest to examine the relationship between patch genetic uniqueness (GU_{FST} and GU_{JD}) and our landscape predictor variables (AREA, ENN, m.patchIBD, and m.patchMort). All landscape predictors (AREA, ENN, m.patchIBD, and m.patchMort) were mean center transformed and coded as fixed effects, whereas study cell ID was coded as a random effect. Methods for model selection (cAIC) and model fit assessment (p values and conditional/marginal R^2) were identical to the patch-based A_R analysis described above.

Results

Genetic diversity

Regional scale

Allelic richness corrected to $n = 18$ across the 31 study cells ranged from 5.27 to 7.58 alleles/locus. After model selection, the best model contained PD, CorrL.Res, and Clumpy ($R^2 = 0.4008$, $R^2_{adj} = 0.334$, $F = 6.020$, $p = 0.003$; Supplementary Table S1). All other models had a $\Delta AIC_C \geq 2.621$, so there were no competing models (Supplementary Table S2). PD and Clumpy had a positive association with A_R whereas CorrL.Res exhibited a negative relationship with A_R (Table 2; Supplementary Fig. S1). After significance testing, both Clumpy and CorrL.Res were significant ($p < 0.05$), but PD had a marginal p value ($p = 0.072$). PrForest and mIBD/mMort were not included in any of the top models.

Local scale

When corrected to five individuals per patch, A_R ranged from 3.26 to 4.59 alleles/locus across the 50 patches. The

Table 2 Parameter estimates for the top linear regression model that quantified the relationship between Patch Density (PD), Clumpy, and residuals of correlation length and percent forest (CorrL.Res) and allelic richness (A_R) across 31 study cells in the UWB.

Variable	Estimate	Standard error	p value
Intercept	−224.5	105.2	0.042
PD	0.251	0.134	0.072
Clumpy	232.0	106.0	0.012
CorrL.Res	0.002	0.000	0.009

The top model was the same regardless of the resistance surface, so prForest, mIBD, or mMort are not included in the parameter estimates. All variables were significant except PD (p values < 0.05)

sensitivity analysis had similar results to the original dataset of 50 patches even with the smaller sample sizes. Therefore, only results for the full dataset (patches with ≥5 individuals) are presented here. There were two top models after model selection (i.e., ΔcAIC ≤ 2.0), but one of the models only included the random effect of study cell ID (Supplementary Table S3). Moreover, the other competing models (m.patchIBD + ID and m.patchMort + ID) did not explain more variance than the null model ($F = 0.7849–0.997$, $p = 0.428–0.522$). Although no landscape predictors appear to be significantly associated with A_R within patches, the null model (i.e., study cell ID) explained 22.12% of the variance (i.e., $R^2_{conditional} - R^2_{marginal}$ = variance explained by study cell alone alone).

Genetic differentiation

Local scale

GU_{FST} (range: 0.0190–0.0904) and GU_{JD} (range: 0.0059–0.1308) were highly correlated ($r = 0.972$, $p < 0.0001$), so we present statistical results for GU_{FST} only. All top models included either patchIBD or patchMort with varying combinations of the other landscape variables (Supplementary Table S4, S5). As with the A_R analyses, the sensitivity analysis identified the same top models as the full dataset, but results were weaker due to smaller sample sizes. The only significant variables in the top models were the resistance distances (m.patchIBD or m.patchMort; $t = 2.976–3.116$, $p \le 0.0039$). Genetic uniqueness was positively associated with patchIBD and patchMort (Table 3; Supplementary Fig. S3). In contrast, AREA and ENN were not significant within these top models (all $t = -1.050$ to -0.942; $p \ge 0.427$). Unlike AREA and ENN, study cell ID appeared to be important in our linear mixed models because the random effect explained more variance than all landscape variables. Within the top m.patchIBD and m.patchMort models, study cell IDs explained 21.285–31.345% of the variance.

Both m.patchIBD and m.patchMort were significant within all top models, and had very similar $R^2_{conditional}$ values (Table 3). $R^2_{conditional}$ for m.patchMort models ranged from 0.397 to 0.400 (m.patchMort + ID: $R^2_{conditional} = 0.400$) whereas m.patchIBD had $R^2_{conditional}$ of 0.400–0.403 (m.patchIBD + ID: $R^2_{conditional} = 0.403$). However, the $R^2_{marginal}$ for m.patchMort models (0.241–0.245) were higher than those for patchIBD ($R^2_{conditional} = 0.181–0.190$; Table 3). Therefore, m.patchMort resistance models explained more variance in genetic differentiation between patches than m.patchIBD (Supplementary Fig. S3).

Table 3 Parameter estimates for the mixed linear models that quantified the relationship between average resistances between patches (m.PatchIBD or m.PatchMort) and genetic uniqueness (GU_{FST} [A], GU_{JD} [B]) for 50 patches.

Variable	Estimate	Standard error	p value	$R^2_{marginal}$
(A)				
m.PatchIBD	0.084	0.029	0.006	0.181
m.PatchMort	0.031	0.009	0.003	0.245
(B)				
m.PatchIBD	0.106	0.052	0.048	0.097
m.PatchMort	0.030	0.019	0.051	0.091

Although there were competing models, the top model in all cases was the resistance only model. Therefore, we report the parameter estimates for m.PatchIBD or m.PatchMort only. For the GU_{FST} tests, the m.PatchMort model had a higher $R^2_{marginal}$ than m.PatchIBD, but results were similar in the GU_{JD} tests

Discussion

Overall, we documented differing landscape effects on genetic diversity and differentiation across the UWB. As predicted, landscape effects on genetic diversity were only found at the regional spatial scale where allelic richness was associated with all configuration metrics (PD, Clumpy, and CorrL.Res). Genetic diversity was not associated with either habitat amount (prForest) or landscape resistance (mIBD, mMort, m.patchIBD, and m.patchMort). In contrast, genetic differentiation was correlated with landscape resistance between patches (i.e., matrix quality; IBD or Mort), but not configuration (ENN) or habitat amount (AREA). Combined with results from Kierepka et al. (2016), quality of intervening matrix appears to be the most important driver of genetic differentiation across our regional and local scales. In all patch-based analyses, we found that study cell ID explained more variance than patch-based metrics or resistance, which suggests that other regional scale differences in study cells have a strong impact on both genetic metrics.

Landscape effects on genetic diversity

Based on the replicated study cells (i.e., each 32 km^2 landscape) within the UWB, landscape configuration has the strongest impact on genetic diversity, but only at the regional scale. Two configuration metrics (Clumpy and CorrL.Res) were significantly associated with allelic richness, and allelic richness was highest in the least fragmented study cells (high correlation length and high Clumpy). High correlation length often correlates with large patch size, because correlation length is the distance that an individual can travel within a single habitat type (McGarigal et al. 2002). High correlation length, therefore, implies somewhat large forest patches, so the landscapes with high genetic diversity likely have a high number of clumped, large forest patches (i.e., high PD, correlation length, and Clumpy).

Based on our regional analysis of genetic diversity, high effective sizes appear to occur within the least fragmented landscapes with highly clumped forest patches.

Local analyses found that study cell differences explained the most variance in all models. The strong impact of study cell could indicate that each area is undergoing differing rates of genetic drift created by population dynamics (e.g., disease, food availability, and predation rates) operating at the regional scale. Furthermore, low gene flow between patches may lead to strong spatial autocorrelation of allele frequencies, which may blur the effects of patch metrics. No other patch-based metrics predicted allelic richness, a surprising result given that the habitat amount and configuration metrics were selected from previous studies in UWB (Moore and Swihart 2005; Rizkalla and Swihart 2012). Instead, landscape context appears to be important at different spatial and temporal scales for abundance, occupancy, and genetic diversity (i.e., Jackson and Fahrig 2012). Indeed, Jackson and Fahrig (2014) found landscape effects on genetic diversity are strongest at a larger spatial scale than for abundance or occupancy. While local abundance certainly influences genetic diversity (i.e., the number of potential alleles available), change in genetic diversity requires many more generations than a loss of abundance due to incoming gene flow (Jackson and Fahrig 2014). Consequently, local conditions important for abundance or occupancy (e.g., patch-based landscape factors) are unlikely to influence genetic diversity, even if studies are conducted in an identical landscape. Based on the incongruence between previous demographic responses and genetic diversity, it is likely that the importance of landscape context will differ between measured responses, which in turn may complicate conservation decisions.

Landscape effects on genetic differentiation

Based on an earlier analysis of UWB data and this study, genetic differentiation was most influenced by intervening matrix habitat (i.e., resistance) at both the local and regional scale. For both scales, the Mort resistance surface explained more variance in genetic differentiation than pure distance (i.e., IBD). The Mort model included open water/wetland, urban, and roads at high resistances, habitats identified in previous studies as suboptimal habitats for movement (Rizkalla and Swihart 2007; Anderson et al. 2015). Open water (large rivers) delineate genetic clusters in chipmunks (Chambers and Garant 2010; Kierepka et al. 2016), so large rivers may be beyond the swimming abilities of chipmunks. Much of the open water in study cells consisted of the Wabash River and its tributaries, so large expanses of open water likely form strong barriers to gene flow in chipmunks within study cells. Unlike open water, limited evidence exists for roads and urban habitat causing genetic differentiation in chipmunks (Anderson et al. 2015). Multiple studies have recorded road avoidance in chipmunks (e.g., Oxley et al. 1974; Ford and Fahrig 2008; McGregor et al. 2008), but roads did not delineate genetic clusters in the UWB (Anderson et al. 2015). Instead, the accumulation of urban areas and roads between patches may eventually prevent gene flow enough to cause genetic differentiation between patches rather than a single road or urban area delineating distinct clusters.

Although resistance distances were a significant predictor of genetic differentiation between patches, study cell ID explained the most variance in the linear mixed models. Previous empirical studies also have noted that genetic patterns in one landscape do not translate across all study areas (Moore et al. 2011; Short Bull et al. 2011; Trumbo et al. 2013; Millete and Keyghobadi 2015; Castillo et al. 2016). It is unclear why study cell ID explained more variance in our linear models, but other factors may influence the interplay between genetic drift and gene flow differs across landscapes. Genetic differentiation appeared to be greater in study cells where patches were separated by mixes of urban, roads, and agriculture (i.e., greatest variability in resistance distances and corresponding fragmentation) than those separated by just agriculture, but this may only be evident in extreme cases of loss and fragmentation of forest habitats (e.g., Short Bull et al. 2011; Cushman et al. 2012). Therefore, we hypothesize that habitat configuration contributes to the significance of study cell ID, conjecture consistent with the findings that genetic differentiation was highest between study cells with low Clumpy (i.e., high fragmentation; Kierepka et al. 2016).

One difference between the regional and local scales was that configuration was only important at the regional (i.e., between study cells) scale. Configuration, in particular, was expected to impact genetic differentiation in chipmunks via its effect on migration rates (DiLeo and Wagner 2016) and effective population size (Bruggeman et al. 2010). Migration rates and effective population size directly impact gene flow and drift respectively, so highly fragmented populations are expected exhibit high genetic differentiation. Indeed, both simulation and empirical studies have found that fragmented populations experience lower gene flow and higher rates of genetic drift, resulting in strong landscape effects on genetic differentiation (Lange et al. 2012; Cushman et al. 2012; Kierepka and Latch 2015; Millette and Keyghobadi 2015; Kierepka et al. 2016). Thus, the scale difference in the importance of configuration on genetic differentiation may reflect that gene flow between patches at the local scale is overtaking genetic drift.

Role of habitat amount

In surprising contrast to the intervening matrix and configuration of forest habitat, the amount of forest habitat within study cells (prForest) had little influence on either genetic metric. Habitat amount is important in explaining ecological responses to landscape heterogeneity as determined by patch occupancy (Betts et al. 2007; Quesnelle et al. 2013; Hornseth et al. 2014), species diversity (Fahrig 2013), and abundance (Flather and Bevers 2002) including studies performed on chipmunks in the UWB (Moore and Swihart 2005; Rizkalla and Swihart 2012). We suggest two nonmutually exclusive mechanisms for why prForest was unimportant in our landscape genetic models. First, we may not have had a large enough variance in forest amount to observe differences in genetic structure according to habitat amount. Hanski (2015) posited that habitat amount is only more important at small spatial scales in landscapes with high amounts of suitable habitat. Our study area likely does not fit these criteria, at least for chipmunks, because extensive habitat loss had occurred in all of our study cells; prForest ranged from 1.1 to 38.4%, with 35–216 individual patches per study cell. Effects of habitat configuration are expected to be strongest with intermediate amounts of habitat (Villard and Metzger 2014), which coincides with conditions in at least some of our study cells. In addition, distances between patches in the UWB often exceeded average dispersal distances of eastern chipmunks (Loew 1999; Chambers and Garant 2010) and previously recorded positive spatial autocorrelations between individuals (<100 m; Kierepka et al. 2016), which suggests that effects of habitat amount may be more apparent at a smaller spatial scale. Regardless, a combination of low forest cover across the UWB and spatial scale suggests habitat configuration and intervening matrix are more important for predicting genetic variation of chipmunks within this highly fragmented ecosystem.

Another explanation for the low explanatory power of prForest is that chipmunks are not completely reliant on forests. Chipmunks can use a range of habitats (Swihart et al. 2003), are fairly ubiquitous across the UWB (Moore and Swihart 2005), and perhaps more importantly, can recolonize patches via unvegetated corridors (Henderson et al. 1985; Bowman and Fahrig 2002). While abundance and occupancy certainly are highest in study cells with high prForest (Moore and Swihart 2005; Rizkalla and Swihart 2012), densities of chipmunks within less forested study cells may be sufficient to resist losses of genetic diversity or strong genetic drift. Cushman et al. (2012) found that the importance of habitat amount increased with the resistance of the matrix, so chipmunks' ability to disperse through unsuitable habitats may also depress the relationship between prForest and our genetic response variables. Similarly, genetic differentiation between genetic clusters in the UWB were associated with both forest cover and grassland habitats, which further supports chipmunk use of other habitats for dispersal (Anderson et al. 2015). Our study suggests that habitat amount is not always the most important factor explaining genetic differentiation; further exploration, particularly with simulations, is warranted to identify conditions where habitat loss drives genetic diversity and differentiation in the UWB and other human-dominated landscapes.

Conclusions

To date, most landscape genetic studies have focused on how intervening matrix between groups impacts genetic differentiation at a single spatial scale (DiLeo and Wagner 2016). Our results suggest that such an approach may be too simplistic to fully capture how landscape heterogeneity impacts genetic variation. Processes that contribute to genetic variation (e.g., dispersal ability, reproductive strategies, and abundances) are known to vary across landscape context as well as spatial and temporal scales, which likely caused the differing patterns observed in genetic diversity and differentiation in our study. However, genetic diversity and differentiation are rarely tested together in landscape genetics because independent replication of landscapes and spatial scales typically is required (DiLeo and Wagner 2016). We acknowledge that replication of landscapes and definition of appropriate spatial scales is often not feasible, especially for highly mobile or continuously distributed species (but see Short Bull et al. 2011), so authors should be cautious about extrapolating results beyond their study area. When replication is feasible, landscape geneticists should incorporate study designs much like those in landscape ecology, where data routinely are collected at multiple spatial scales along a gradient of habitat loss and fragmentation. Clearly, these study designs require alternative analytical frameworks (see DiLeo and Wagner 2016), but given the complexity in ecological responses to landscape heterogeneity, measurement of genetic responses across a number of spatial scales and landscapes is needed to gain a comprehensive picture of how populations respond to fragmentation and habitat loss.

Data availability

All landscape and genetic metrics for statistics are posted on Dryad (https://doi.org/10.25338/B86P54).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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