RESEARCH ARTICLE

Ecological metrics predict connectivity better than geographic distance

D. Grant Hokit · Marina Ascunce · Joel Ernst · Lyn C. Branch · Ann Marie Clark

Abstract We use microsatellite loci to examine genetic structure of the Florida scrub lizard (*Sceloporus woodi*) and test for the effects of landscape variables at the scale of neighboring patches. We evaluate ecological metrics of connectivity with genetics data, which to our knowledge is the first application of these particular metrics to landscape-level genetics studies in Florida scrub. Florida scrub is a highly threatened ecosystem in which habitat patches are remnants of a previously widespread xeric landscape. Analysis of mitochondrial DNA (mtDNA) has shown that landscape structure influenced the evolutionary history of the Florida scrub lizard (*S. woodi*) across its range. Our results concur with these mtDNA studies in documenting divergence between xeric ridge systems and also demonstrate divergence at very local scales. Both least-cost distance and pairwise isolation (a metric used in ecological studies that includes patch size, quality and a modified isolation index) were better predictors of genetic distance than Euclidean distance, indicating that mesic and hydric habitat influence spatial patterns in genetic variation. Our results support the need for focusing on spatial distribution of scrub habitat at the scale of neighboring patches, as well as regionally, in conservation management and restoration. Also, our study points to the value of integrating landscape ecology metrics into landscape genetics.

Keywords Landscape genetics · Landscape connectivity · Florida scrub lizard · *Sceloporus woodi* · Microsatellite · Florida scrub

Introduction

Understanding how landscape structure and processes affect gene flow is crucial to improving conservation strategies (Crandall et al. 2000; Moritz 2002; Manel et al. 2003; Allendorf and Luikart 2007). Range-wide phylogeographic studies elucidate historic biogeographic processes that shape the current genetic structure of species (e.g., Avise 1992, 2000; Osentoski and Lamb 1995; Branch et al. 2003; Habel and Assmann 2009). Such studies support conservation decisions by providing information on spatial distribution of genetic diversity at regional to continental scales (Moritz and Faith 1998). Landscape structure also influences microevolutionary and demographic processes at population and subpopulation scales (see reviews in Allendorf and Luikart 2007; Storfer et al. 2007). Landscape characteristics such as habitat patch size, isolation, and the type and quality of habitat matrix, interact with the dispersal ability of species to affect evolutionary and ecological processes such as local adaptation, spread of beneficial mutations, and population dynamics on heterogeneous or fragmented landscapes (Hanski and Gilpin 1997; Manel et al. 2003; Allendorf and Luikart 2007; Storfer et al. 2007; Holderegger and Wagner 2008).

D. Grant Hokit (✉)
Department of Natural Sciences, Carroll College,
1601 N. Benton Ave, Helena, MT 59625, USA
e-mail: ghokit@carroll.edu

M. Ascunce · J. Ernst · A. M. Clark
ICBR Genetic Analysis Laboratory, University of Florida,
1376 Mowry Road, Gainesville, FL 32610, USA

M. Ascunce
US Department of Agriculture, Agricultural Research Service,
CMAVE, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

L. C. Branch
Department of Wildlife Ecology and Conservation, University
of Florida, Gainesville, FL 32611, USA

Landscape genetics has emerged as a methodology that combines the tools of landscape ecology and population genetics to examine the relationship between landscape structure and spatial genetic variation (Manel et al. 2003; Holderegger and Wagner 2006, 2008 Storfer et al. 2007). While traditional studies in population genetics have long used isolation-by-distance (or Euclidean distance) to describe spatial patterns in genetic variation, landscape genetics extends such analyses to examine how landscape composition and configuration influence gene flow and genetic population structure. One of the primary goals of landscape genetics is to describe the functional connectivity of a landscape to better understand evolutionary and ecological processes that may influence conservation decisions.

Landscape connectivity can be generally defined as the degree to which the landscape promotes or impedes movement of individuals and, thus, results from the interaction between landscape structure and species vagility (Merriam 1984; Taylor et al. 1993; Goodwin 2003). If the matrix habitat between populations has no effect on the movement of organisms then Euclidean distance can be used to characterize landscape connectivity. However, many matrix features (e.g. streams, roads, waterways, topography) are known to restrict or prevent species movement (e.g. Keyghobadi et al. 1999; Michels et al. 2001; Funk et al. 2005; Broquet et al. 2006). In this case, understanding connectivity requires measurement of the "effective distance" between individuals or populations. One common method for measuring effective distance is the least-cost distance, which measures the path between two locations that circumvents barriers and minimizes the distance through "resistant" matrix habitat (Singleton et al. 2002; Adriaensen et al. 2003). One problem with the application of the least-cost path is that often this measure of connectivity does not include landscape features that are important to species movement such as patch size, patch quality or habitat preferences (Broquet et al. 2006). In fact, many landscape variables identified in ecological studies as important for species vagility are often not included in models of landscape genetics (Storfer et al. 2007). Currently, extensive discussion in landscape ecology focuses on the most appropriate metrics for describing landscape-level processes such as connectivity (Moilanen and Nieminen 2002; Pascual-Hortal and Saura 2006). Evaluation of these ecological metrics with genetics datasets could inform this discussion and advance the integration of landscape ecology and landscape genetics.

We examine the landscape genetics of the Florida scrub lizard (Sceloporus woodi) and explicitly test for associations between landscape variables and genetic structure and evaluate ecological metrics of connectivity with genetics data. The Florida scrub lizard provides an opportunity to study patterns of genetic variation in a species restricted to a hierarchical arrangement of habitat patches in a landscape that is highly threatened by human development. The Florida scrub lizard was surveyed previously with mitochondrial DNA (mtDNA) cytochrome b sequences across its range (Clark et al. 1999; Branch et al. 2003). These studies showed deep genetic separations among disjunct ridges and population structure within ridges. Experimental field studies also suggest that movement of lizards is limited by mesic and hydric habitat that separates scrub patches (Tiebout and Anderson 1997; Clark et al. 1999; Hokit et al. 1999). State and federal conservation initiatives for scrub habitat focus on establishment of an archipelago of reserves (United States Fish Wildlife Service 1991; The Nature Conservancy 1991). Successful reserve design and long-term management of Florida scrub will depend, in part, on understanding effects of landscape structure on distribution and dynamics of scrub organisms.

Here, in addition to corroborating the previous large-scale, mtDNA study that documents strong genetic structure, we test for genetic variation among neighboring scrub patches, which is the scale relevant to local land management. We then compare alternative landscape models to test for the effects of patch size, quality, isolation and matrix habitat on genetic variation. Finally we discuss the conservation implications of our findings.

Methods

Model system, study area and sampling

Scrub lizards are endemic to peninsular Florida and restricted to xeric, scrub habitat (DeMarco 1992). In Florida, scrub occurs on elevated, well-drained, sandy soils distributed in patches along ridges that extend down the peninsula (Myers 1990). These ridges represent ancient shorelines that formed as the Florida peninsula contracted and expanded with changes in sea level (Webb 1990). During high sea levels associated with interglacial periods, the central ridges were isolated for long periods of time, resulting in many endemic species including several plants, insects, lizards, and a bird (Deyrup 1989, 1996; Huck et al. 1989; McCoy and Mushinsky 1992; McDonald and Hamrick 1996). As mesic habitat expanded during the Pliocene and Pleistocene (Watts and Hansen 1988), xeric habitat within these ridges became fragmented and shrank into archipelagos of scrub patches.

Our study was conducted on Avon Park Air Force Range (APAFR, 15 km east of Avon Park, FL) and neighboring Arbuckle State Forest (ASF). Scrub patches on APAFR and ASF are located on the Bombing Range Ridge and the Lake Wales Ridge, respectively (Fig. 1), which are

Fig. 1 Location of scrub patches where the Florida scrub lizard was sampled on the Lake Wales Ridge (Arbuckle State Forest) and the Bombing Range Ridge (Avon Park Air Force Range). Site numbers correspond to patch numbers in Table 2
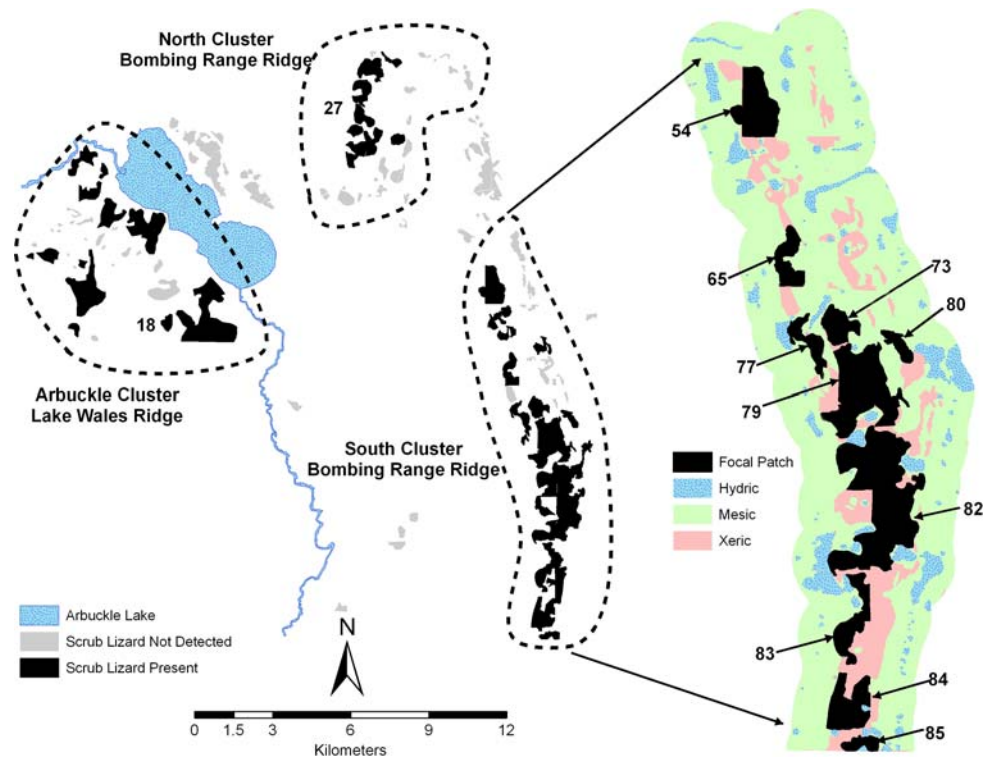
separated by several kilometers and hydric habitat (Arbuckle Lake and tributaries). Scrub patches on APAFR and ASF are isolated naturally by mesic and hydric habitat. Prior to sampling, we delineated clusters of patches within each ridge so that gaps between neighboring patches were no greater than 750 m, the estimated maximum dispersal distance for scrub lizards (Hokit et al. 1999). This resulted in one cluster of patches on ASF and two clusters, referred to as north and south, on APAFR (Fig. 1). A description of patch delineation methods and construction of GIS buffers to define patch clusters has been reported elsewhere (Hokit et al. 1999, 2001).

We sampled 10 patches in the south cluster of APAFR to conduct a within-cluster analysis of population structure. Additional samples were collected in one patch in the north cluster and in one patch on ASF to compare between clusters and between ridges. We collected tissue samples from an average of 37 individuals per patch for a total of 444 individuals. Scrub lizards were captured by noosing or by hand, a portion of the tail was removed, and animals were released at the capture site. Tissues were preserved in a salt buffer (saturated NaCl; 25 mM EDTA pH 7.5; 20% DMSO) using a protocol modified from Amos and Hoelzel (1991).

Molecular methods

DNA isolations were performed using the DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA) following protocol for animal tissue. We used a total of seven microsatellites developed for scrub lizards: *SW614-A1*, *SW614-A4*, *SW614-A6*, *SW614-A7*, *SW614-B6*, *SW614-B10*, and *SW614-B12*. Detailed laboratory protocols, repeat motifs and primer sets are presented in Ernst et al. (2004). All PCR products were run on a capillary sequencer MegaBace™ 1000 (Amersham Biosciences/GE Healthcare) using GeneScan™—500 [ROX]™ (Applied Biosystem) as size standard. Alleles were scored employing GeneMarker software version 1.5 (SoftGenetics, LLC, CA).

Landscape metrics

We tested for the effects of the total patch area, prime area of the patch (area adjusted by habitat quality) and relative isolation on genetic structure. Details of remote sensing and geographic information techniques (GIS) used in measuring landscape structure are presented in Hokit et al. 1999. To assess which landscape features were associated with genetic structure, we compared a null model using only Euclidean distance (D_E) against alternative models using two measures of effective distance: a least-cost path parameter (D_{LC}) and a pairwise isolation parameter (I) that included landscape metrics of patch size, quality, and a modified measure of relative patch isolation within a group of patches. Euclidean distance (D_E) was estimated as the shortest, straight-line distance between each pair of patches using a GIS (ArcGIS 9.2) and landcover data for APAFR. To estimate the least-cost distance (D_{LC}), habitat types

were classified relative to the movement abilities of scrub lizards. Previous studies indicate that scrub lizards move best through xeric habitat, move poorly through densely vegetated mesic habitat, and that hydric habitat acts as a barrier (Tiebout and Anderson 1997; Hokit et al. 1999). Accordingly, scrub communities (sand pine scrub, oak scrub, rosemary scrub, and scrubby flatwoods) were defined as xeric "stepping stones", moderately moist communities (pine flatwoods, prairies, oak hammock, cutthroat grass, pine plantations, and pastures) as mesic "filters", and hydric communities (marshes, swamps, wet prairies, cutthroat-wet flatwoods, lakes, and ponds) as "barriers." The least-cost distance between each pair of patches was measured as the shortest distance between two patches that (1) used intervening xeric habitat as stepping stones, (2) minimized travel through mesic habitat, and (3) circumvented hydric barriers.

Patch size, quality and isolation may affect genetic structure by influencing the number of migrants a patch receives (e.g., Merriam 1984; Gustafson and Gardner 1996; Hanski 1999). The relative isolation of a patch within a group of patches can be described as:

$$S_i = \Sigma\, p_j \exp(-d_{ij}) A_j,$$

where the sum is taken over all patches $j \neq i$, p_j is 1 for patches occupied by scrub lizards and 0 for patches unoccupied by scrub lizards, d_{ij} is the distance between patches i and j, and A_j is the area of patch j (Hanski 1994). Defined in this manner, isolation is a function of distances between a patch and other occupied patches and the area of occupied patches. Area is included because the number of individuals in a patch and the number of dispersers often are correlated with patch area (Fahrig and Paloheimo 1988; Pulliam 1988; Hanski 1994). The number of individuals in a patch also is influenced by habitat quality. To test for effects of habitat quality, we first calculated S_i using total patch areas for A_j and then again using "area of prime habitat" for A_j. Scrub lizards most often are associated with open-sandy habitat (DeMarco 1992; Hokit et al. 1999) and, thus, prime area was defined as total amount of open-sandy habitat within each patch as determined from aerial photographs and GIS (Hokit et al. 2001). Note that the isolation index for each patch does not use area of the patch itself—only area of occupied neighboring patches within 750 m. Distance between patches (d_{ij}) was measured as described for D_{LC}. To transform relative patch isolation (S_i) into a parameter that could be analyzed with respect to pairwise genetic differences between patches, we calculated a pairwise isolation parameter (I) by summing transformed S_i values for each pair of patches. More isolated patches have smaller S_i values. We transformed S_i values to $1/(1 + S_i)$ to produce a more heuristic index where isolation increases with increasing I. We predicted that two patches with small I values would be well connected to other patches in the cluster and more likely to share alleles. Conversely, two patches with large I values would not be well connected to other patches and less likely to share alleles even though the patches may be relatively close to each other by Euclidean distance measures.

Data analysis

We used the software program MICRO-CHECKER version 2.2.3 (Van Oosterhout et al. 2004) to identify the possibility of null alleles at each locus and FeeNA (Chapuis and Estoup 2007) to estimate the frequency of null alleles. We calculated allele frequencies, expected and observed heterozygosities, and exact probabilities for Hardy–Weinberg and genotype disequilibrium using GENEPOP version 3.4 (Raymond and Rousset 1995). Allelic richness (number of alleles corrected for sample size, Petit et al. 1998), F statistics, and exact probabilities of differentiation in allele frequencies among patches were estimated using FSTAT version 2.9.3.2 (Goudet 1995). We used STATISTICA version 5.1 (StatSoft, Inc.) to perform linear regression analyses to test for effects of patch characteristics (total area, prime area, and relative isolation) on allelic richness and expected heterozygosity.

We investigated genetic structure at two scales. First, we used analysis of molecular genetic variance (AMOVA, Excoffier et al. 1992) with F_{ST} to examine broad scale structuring between ridges and between clusters. Using ARLEQUIN version 3.01 (Excoffier et al. 2005), we compared the amount of variation explained by a model that lumped the clusters together with a model that split the data into respective clusters.

Second, we used two methods to investigate small-scale structuring within the south cluster on APAFR. We examined pairwise F_{ST} and their associated exact probability of differentiation in allele frequencies when all loci are combined (Sokal and Rohlf 1981) to determine which patches were genetically distinct. Then we used a Bayesian clustering approach with the program STRUCTURE version 2.0 (Pritchard et al. 2000) to estimate the number of randomly mating populations (K) in the south cluster. We used the admixture model, which assumes gene flow between populations, to assign a proportion of each individual genome to each of the K populations. We ran a series of simulations that calculated the probability that there are from $K = 1$ to 10 populations in the south cluster. We performed simulations both with and without the assumption that allele frequencies are correlated, using a burn-in length of 50,000 and a run length of 10^5. For each K, we completed five independent simulations and then averaged the results. We estimated average contribution of

each individual genome to each K within each patch. Each patch was assigned to the population that contained the greatest proportion of the genome for individuals from the patch.

We examined associations between genetic distance (F_{ST}) and four alternative models of functional connectivity: Euclidean distance (D_E), least-cost distance (D_{LC}), pairwise isolation using total patch area (I_{TA}), and pairwise isolation using prime patch area (I_{PA}). Using patches from the south cluster of the Bombing Range Ridge, we first tested for significant associations using simple Mantel tests (Mantel 1967) in FSTAT and then compared alternative models of geographic distance using Akaike's information criterion (AIC; Burnham and Anderson 1998). The 'best' parameter for modeling geographic distance was determined by selecting the model that was significantly associated with F_{ST} and resulted in the lowest AIC score (e.g. Roach et al. 2001; Spear et al. 2005). In a post hoc analysis, we used partial Mantel tests (Smouse et al. 1986) to assess whether alternative measures of geographic distance were statistically significant after accounting for the 'best' parameter.

In all cases where multiple statistical tests were performed, we applied sequential Bonferroni corrections (Rice 1989) to α levels to account for simultaneous tests.

Results

Variation within patches

Out of 252 tests for linkage disequilibrium, none of the tests were found to be significant. Genotype frequency tests for each locus within each patch revealed that 23 out of 84 tests deviated significantly from Hardy–Weinberg proportions. Twenty-two of these deviations occurred within the same 3 loci: *SW614-B12* in 11 patches, *SW614-B6* in 7 patches, and *SW614-A7* in 4 patches. Data screening with MICRO-CHECKER suggested that these three loci had an excess of homozygotes and a high probability of null

alleles. Assuming that all non-amplification events are due to homozygous null alleles (an obvious simplification that could overestimate null allele frequencies), the results from FreeNA suggested that null allele frequencies were much higher for these three loci than for the other four loci (Table 1). The remaining test deviating from expected Hardy–Weinberg proportions was locus *SW614-A4* in patch 18. Screening results suggested a possibility of a null allele at low frequencies for this locus at this patch. Because at least three of our loci had a high probability of null alleles, we performed two sets of analyses for all subsequent tests: the first using only the four loci with rare null alleles and the second using all seven loci after using FreeNA to correct for the presence of null alleles. FreeNA statistically corrects genotype data and F_{ST} estimates for microsatellite data presumed to have null alleles (Chapuis and Estoup 2007).

Landscape and genetic characteristics for each patch are presented in Table 2. Overall genetic diversity was associated significantly with landscape structure. For the four loci data set, mean allelic richness and mean expected heterozygosity were significantly, negatively associated with relative isolation (S_i) of each patch (Table 3). Allelic richness was positively associated with prime area of a patch, although this association fell out after Bonferroni correction of alpha levels. Similar results were observed for the seven loci data set except that only expected heterozygosity was significantly associated with S_i after Bonferroni corrections.

Spatial variation across the region and among neighboring patches

Larger scale structuring was revealed between ridges and between clusters within a ridge. Alternative groupings in AMOVA showed that a model that split the three clusters was significant ($P = 0.014$ for both the four loci and seven loci analyses) and accounted for a larger portion of among group variance than did a model that combined the three clusters or combined the clusters into two groups defined

Table 1 Genetic diversity characteristics estimated for each locus pooled across all habitat patches for 444 lizards

H_E average (across all habitat patches) expected heterozygosity, *AR* allelic richness, *C* estimates corrected for the presence of null alleles

Loci	Number of alleles	H_E	AR	Non-amplification frequency	Null allele frequency	H_E^C	AR^C
SW614-A1	17	0.75	7.0	0.11	0.18	0.74	7.7
SW614-A4	9	0.69	5.9	0.09	0.16	0.75	7.3
SW614-A6	19	0.84	9.1	0.04	0.08	0.84	10.7
SW614-A7	19	0.85	9.5	0.15	0.34	0.82	11.0
SW614-B6	31	0.75	9.6	0.13	0.30	0.76	10.9
SW614-B10	18	0.80	8.6	0.05	0.12	0.83	10.2
SW614-B12	18	0.85	9.4	0.19	0.43	0.75	9.8

Table 2 Characteristics of scrub patches and genetic samples from the Lake Wales Ridge in Arbuckle State Forest (ASF) and the Bombing Range Ridge in Avon Park Air Force Range (APAFR)

Patch	Total area (ha)	Prime area (ha)	S_i	N	Alleles[4]	H_E^4	AR[4]	Alleles[7]	H_E^7	AR[7]
18	30.1	9.1	0.98	37	27	0.73	5.9	58	0.78	7.0
27	35.5	11.3	0.06	16	23	0.75	5.7	44	0.78	6.3
54	95.7	14.5	0.72	38	24	0.71	5.2	49	0.72	6.1
65	45.7	8.3	0.99	37	24	0.59	5.2	57	0.70	6.6
73	34.1	10.8	0.03	36	34	0.77	6.9	73	0.81	8.4
77	18.9	2.9	0.04	34	27	0.75	6.1	63	0.80	7.6
79	141.8	51.6	0.07	31	35	0.80	7.5	73	0.80	8.7
80	14.7	7.6	0.99	40	23	0.74	5.1	55	0.78	6.7
82	278.3	84.3	0.19	37	33	0.77	7.0	76	0.81	8.8
83	63.2	20.1	0.05	51	35	0.78	6.9	78	0.82	8.5
84	132.4	54.1	0.18	37	35	0.80	7.1	79	0.82	8.7
85	14.9	2.0	0.05	50	31	0.78	6.4	68	0.81	7.8

Two separate analyses were performed using four loci without null alleles problems (superscript = 4) and using all seven loci after correcting for null alleles (superscript = 7). Patch 18 is located on ASF, patch 27 in the northern cluster of APAFR and all other patches are in the southern cluster of APAFR

S_i relative isolation index, modified from Hanski (1994), N number of lizard samples, H_E average (across all loci) expected heterozygosity, AR average (across all loci) allelic richness

Table 3 Results of linear regression analysis testing for the effects of total area (TA), prime area (PA), and relative isolation (S_i) on allele richness (AR) and expected heterozygosity (H_E)

Bonferroni α	Test[4]	r^4	P value[4]	Test[7]	r^7	P value[7]
0.008	AR × S_i	−0.73	0.007*	H_E × S_i	−0.73	0.007*
0.010	H_E × S_i	−0.72	0.008*	AR × S_i	0.65	0.023
0.013	AR × PA	0.63	0.028	AR × PA	0.64	0.025
0.017	AR × TA	0.51	0.089	AR × TA	0.52	0.083
0.025	H_E × PA	0.41	0.179	H_E × PA	0.37	0.240
0.050	H_E × TA	0.28	0.369	H_E × TA	0.21	0.521

Two separate analyses were performed using four loci without null alleles problems (superscript = 4) and using all seven loci after correcting for null alleles (superscript = 7)

r Correlation coefficient

* Significant at sequential Bonferroni corrected alpha level

by ridge location (i.e. Lake Wales v Bombing Range Ridges). Neither the one group (combining all clusters) nor the two group (by ridge) models were statistically significant. Furthermore, F_{ST} with associated exact tests (Table 4) revealed that all pairwise comparisons with patch 18 on ASF and patch 27 in the north cluster of APAFR were highly significant, with an average F_{ST} of 0.169 using four loci and 0.123 using seven loci.

Exact tests and F_{ST} also revealed significant structuring at a finer scale within the south cluster (Table 4). For the 4 loci data set, out of 45 tests only 5 were not significant indicating that the patches in these 5 pairings could be grouped together as one population. These results suggest

that as many as 6 randomly mating populations could be represented within the 10 scrub patches. The results were even more discriminating for the seven loci data set with only one pairing that was not significant, suggesting a possible nine distinct populations.

The clustering analysis in STRUCTURE also revealed multiple populations in the south cluster but with fewer estimated populations. The four loci analysis estimated a maximum number of two randomly mating populations combining patches 54–80 into a northern population and 82–85 into a southern population. The seven loci analysis revealed three possible populations by combining patches 54–65, 73–80, and 82–85 into unique populations.

Table 4 Pairwise F_{ST} and the probability (after 66,000 iterations) that allelic distributions are identical between patches when all loci are combined

Patch	18	27	54	65	73	77	79	80	82	83	84	85
18	0	0.141***	0.147***	0.163***	0.131***	0.123***	0.108***	0.130***	0.116***	0.111***	0.112***	0.124***
27	0.230***	0	0.170***	0.215***	0.114***	0.107***	0.081***	0.130***	0.095***	0.085***	0.086***	0.101***
54	0.182***	0.165***	0	0.111***	0.102***	0.112***	0.101***	0.105***	0.125***	0.123***	0.108***	0.103***
65	0.245***	0.304***	0.165***	0	0.083***	0.106***	0.091***	0.091***	0.089***	0.108***	0.112***	0.076***
73	0.172***	0.151***	0.087***	0.108***	0	0.042***	0.015***	0.038***	0.035***	0.052***	0.039***	0.035***
77	0.182***	0.131***	0.073***	0.100***	0.033*	0	0.011***	0.029***	0.030***	0.041***	0.028***	0.030***
79	0.157***	0.101***	0.074***	0.105***	0.008[NS]	0.002[NS]	0	0.018***	0.019***	0.034***	0.023***	0.027***
80	0.190***	0.169***	0.081***	0.095***	0.017***	0.035***	0.013*	0	0.047***	0.063***	0.046***	0.044***
82	0.181***	0.131***	0.120***	0.108***	0.030***	0.030***	0.015[NS]	0.054***	0	0.005[NS]	0.022***	0.025***
83	0.176***	0.117***	0.098***	0.142***	0.054***	0.033***	0.032***	0.066***	0.009[NS]	0	0.021***	0.025***
84	0.159***	0.099***	0.071***	0.141***	0.035***	0.035***	0.021**	0.049***	0.024**	0.014**	0	0.014**
85	0.173***	0.133***	0.075***	0.083***	0.039***	0.019***	0.019***	0.035***	0.023**	0.024***	0.016[NS]	0

The estimates below the diagonal are for the four loci without significant null allele problems while the estimates above the diagonal are for all seven loci after correcting for the presence of null alleles

NS not significant

*** Significant at Bonferroni corrected alpha level of 0.00002 corresponding to a nominal alpha level of 0.001

** Significant at Bonferroni corrected alpha level of 0.00015 corresponding to a nominal alpha level of 0.01

* Significant at Bonferroni corrected alpha level of 0.00076 corresponding to a nominal alpha level of 0.05

Landscape structure and spatial variation

Mantel tests performed with south cluster patches showed that all four models of geographic distance were significantly associated with F_{ST} using either the four or seven loci data sets (Table 5). However, according to AIC scores, pairwise isolation (either I_{TA} and I_{PA}) was a better predictor of F_{ST} than either least-cost distance (D_{LC}) or Euclidean distance (D_E). The difference in AIC scores can be used to compare candidate models (Burnham and Anderson 1998) with a difference ≤2 from the lowest AIC indicating an equally viable model, less support for models with a difference between 2 and 10, and little or no support for models with a difference >10. Thus, I_{TA} and I_{PA} are equally viable models, D_{LC} has less support, and D_E can be rejected as a viable alternative model of geographic distance.

Post-hoc, partial Mantel tests confirm this conclusion. Neither D_{LC} ($P = 0.45$ and 0.16 for the four loci and seven loci data, respectively) nor D_E ($P = 0.44$ and 0.20 for the four loci and seven loci data, respectively) were significantly associated with F_{ST} after accounting for I_{TA}.

Discussion

Our microsatellite results corroborate the findings of previous studies demonstrating genetic structure on a regional scale. F_{ST} comparisons and AMOVA each detected strong, population divergence between the Lake Wales and Bombing Range Ridges and between the north and south clusters within the Bombing Range Ridge. These results are consistent with an examination of mtDNA haplotypes that found deep, evolutionary separations between scrub lizards sampled on five major ridge systems across Florida and between some patches within ridges (Clark et al. 1999). This regional scale structure most likely results from historic events (e.g. sea level changes) operating on a

temporal scale much larger than the genetic structure observed between neighboring patches.

At the neighboring patch scale, expected heterozygosity and allelic richness were associated negatively with relative isolation (S_i) of a patch as theory would predict for populations receiving a low number of migrants (Wright 1931; Young and Clarke 2000). Exact tests of differentiation of allele frequencies and the clustering approach of STRUCTURE showed significant population structure among the 10 patches within the south cluster. Estimates of population structure can be influenced by sample size, number of loci examined, amount of admixture, and magnitude in differences in allele frequencies between populations (Pritchard et al. 2000). Thus, our estimates of the number of randomly mating populations on the landscape should be viewed with caution. However, given the similarity in results between the exact tests and the clustering approach, we feel confident that more than two randomly mating populations, or demes, occur in the 10 patches in the south cluster. If we use the clustering results to define a lower boundary and exact tests to define an upper boundary as suggested elsewhere (Funk et al. 2005), we conclude that there are between three and nine demes within the south cluster when using the seven loci data set.

Connectivity among demes can be influenced by distance to neighboring patches, variation in matrix habitat, quality and size of surrounding patches, edge effects, dispersal ability of migrants, and a variety of other factors (Hanski 1994; Michels et al. 2001; reviewed in Storfer et al. 2007). In this scrub system, patch and matrix characteristics, as indicated by the pairwise isolation parameter, and measures of least-cost distance between patches, provided superior estimates of connectivity than Euclidean distance. Other studies also have reported stronger associations of genetic structure with effective distance than Euclidean distance (Keyghobadi et al. 1999; Chardon et al. 2003) and landscape genetics is defined by the analysis of such landscape features (Storfer et al. 2007; Holderegger and Wagner 2008).

Table 5 Results of simple Mantel tests investigating the relationship between genetic distance (F_{ST}), pairwise isolation (using total patch area I_{TA}, and prime area of habitat patches I_{PA}), the least-cost distance between patches (D_{LC}), and Euclidean distance (D_E) for the south cluster patches on the Bombing Range Ridge

Parameter	Bonferroni α	r^4	P value[4]	AIC[4]	r^7	P value[7]	AIC[7]
I_{TA}	0.013	0.54	<0.001*	−173.78	0.74	<0.001*	−202.17
I_{PA}	0.017	0.53	<0.001*	−172.94	0.74	<0.001*	−201.54
D_{LC}	0.025	0.48	0.001*	−169.96	0.69	<0.001*	−195.93
D_E	0.050	0.30	0.046*	−162.30	0.45	0.002*	−176.49

Two separate analyses were performed using four loci without null alleles problems (*superscript* 4) and using all seven loci after correcting for null alleles (*superscript* 7)

r Correlation coefficient

* Significant at sequential Bonferroni corrected alpha level

However, of particular note, we found that the measure of connectivity most strongly associated with genetic distance was the pairwise isolation parameter (both I_{TA} and I_{PA}), calculated using the isolation index S_i, (Hanski 1994). This index is used commonly in ecological studies (e.g., Bonte et al. 2003; Schultz and Crone 2005) but, to our knowledge, has not been applied in landscape-level genetics studies. I represents a pairwise connectivity parameter (Goodwin 2003) with potentially more explanatory power than either Euclidean distance or simple measures of least-cost distance. I explicitly attempts to model the relative number of dispersers into a patch by incorporating the least-cost distance to all occupied patches and area of these patches. Our parameter I_{PA} also considered habitat quality by including the area of open-sandy habitat of neighboring patches, a factor known to be associated with scrub lizard abundance (Hokit et al. 1999, 2001). This parameter, which incorporates all patches within dispersal distance into a connectivity measure, better describes relative isolation of a patch in the scrub system than do simple distance measures such as distance to nearest occupied neighbor (Hanski 1994). For example, although patches 65 and 54 are only a moderate distance apart (1.8 km), these two patches have the highest F_{ST} value (0.165) of any pair of patches within the south cluster which stretches over 14 km. Patches 54 and 65 also have relatively high S_i values indicating that, although they are fairly close together, neither patch is well connected to other patches in the cluster and thus, are less likely to share genetically similar migrants from other patches. Based on our results, we suggest that the pairwise isolation index I may be useful for landscape genetics studies in other systems characterized by discrete habitat patches.

In the last several decades, conversion of scrub to citrus groves and urbanization have reduced the number and size of scrub patches, increased isolation of patches, and inhibited the natural dynamics of fire, a key process affecting structure and function of this system (Myers 1990; Greenberg et al. 1994). Habitat quality for many species within scrub patches declines with time if habitat is not burned or otherwise restored. As a result of these processes, more than 50 scrub species are recognized as endangered or potentially endangered and other scrub species, such as the Florida scrub lizard, are not yet listed as threatened or endangered but are included on Florida's list of Species of Greatest Conservation Concern (McCoy and Mushinsky 1992; United States Fish Wildlife Service 1991; Stout 2001; Florida Fish and Wildlife Conservation Commission 2004, 2005). Dispersal is a key population process for many species that occupy ephemeral (e.g. fire dependent) habitats because organisms must move between habitat patches when conditions become unsuitable. Such species often exhibit a metapopulation structure in which subpopulations go extinct and persistence of the regional population is contingent upon recolonization dynamics linked to return of habitat to suitable conditions and arrival of dispersers (Harrison and Taylor 1997; Husband and Barrett 1998). The genetic differentiation observed in our study, at such small geographic distances, indicates that recolonization potential of lizards is limited and restricts the possibility for metapopulation dynamics to patches separated by at most a few hundred meters. These results corroborate field studies that found that 80% of the patches occupied by scrub lizards at our study sites occurred within 200 m of an occupied patch, and that no occupied patches were separated from another occupied patch by more than 750 m (Hokit et al. 1999). Patches separated by tens of meters of xeric habitat may behave as a "patchy" population where interchange of individuals is more frequent resulting in demographic rescue of subpopulations (Hanski and Gilpin 1997). Patches separated by as little as a few hundred meters of mesic habitat may contain isolated populations with little opportunity for recolonization if local populations go extinct.

A growing body of evidence from genetic, demographic and modeling studies indicates that landscape structure greatly limits distribution and abundance of the scrub reptile and amphibians (Tiebout and Anderson 1997; Clark et al. 1999; Hokit et al. 1999, 2001 McCoy and Mushinsky 1999; Hokit and Branch 2003a, b; McCoy et al. 2004). At the regional scale, these findings have important implications for designing reserves that encompass genetic diversity of scrub organisms (Branch et al. 2003). At a more local scale, our results can help guide programs aimed at maintaining viability of fragmented populations through habitat management and restoration. In the absence of natural fire regimes, active management (e.g. prescribed fire) is required for scrub species. Conservation of scrub organisms will require landscape-level management that takes dispersal ability into account. Although scrub habitat can be restored with fire, if populations become locally extinct in scrub patches as a result of fire suppression or other factors, recolonization is not likely to occur except across very small distances. Similarly, as scrub patches are destroyed by development, demographic rescue of remaining populations and recolonization of empty patches becomes increasingly less likely.

References

Adriaensen F, Chardon JP, de Blust G et al (2003) The application of 'least-cost' modeling as a functional landscape model. Landsc Urban Plan 64:233–247

Allendorf FW, Luikart G (2007) Conservation genetics of populations. Blackwell, Malden, MA

Amos B, Hoelzel AR (1991) Long-term preservation of whale skin for DNA analysis. Rep Int Whal Comm 13((Special Issue)):99–103

Avise JC (1992) Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge, MA

Bonte D, Lens L, Maelfait JP, Hoffmann M, Kuijken E (2003) Patch quality and connectivity influence spatial dynamics in a dune wolfspider. Oecologia 135:227–233

Branch LC, Clark AM, Moler PE, Bowen BW (2003) Fragmented landscapes, habitat specificity, and conservation genetics of three lizards in Florida scrub. Conserv Genet 4:199–212

Broquet T, Ray N, Petit E et al (2006) Genetic isolation by distance and landscape connectivity in the American marten (*Martes Americana*). Landsc Ecol 21:877–889

Burnham KP, Anderson DR (1998) Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chardon JP, Adriaensen F, Matthysen E (2003) Incorporating landscape elements into a connectivity measure: a case study for the Speckled wood butterfly (*Pararge aegeria* L.). Landsc Ecol 18:561–573

Clark AM, Bowen BW, Branch LC (1999) Effects of natural habitat fragmentation on an endemic scrub lizard (*Scelolporus woodi*): an historical perspective based on a mitochondrial DNA gene genealogy. Mol Ecol 8:1093–1104

Crandall KA, Bininda-Edmonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology: returning to the original meaning of "evolutionary significant units". Trends Ecol Evol 15:290–295

Demarco V (1992) Florida scrub lizard. In: Moler PE (ed) Rare, endangered biota of Florida: amphibians, reptiles, vol III. University of Florida Press, Gainesville, FL, pp 141–145

Deyrup M (1989) Arthropods endemic to Florida scrub. Fla Sci 52:254–270

Deyrup M (1996) Two new grasshoppers from relict uplands of Florida (Orthoptera: Acrididae). Trans Am Entomol Soc 122:199–211

Ernst JA, Branch LC, Clark AM, Hokit DG (2004) Polymorphic microsatellite markers for the Florida scrub lizard (*Scelolporus woodi*). Mol Ecol 4:364–365

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restrictions data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) ARLEQUIN (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50 (Online)

Fahrig L, Paloheimo JE (1988) Determinants of local population size in patchy habitats. Theo Pop Biol 34:194–213

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164:1567–1587

Florida Fish and Wildlife Conservation Commission (2004) Florida's Endangered Species, Threatened Species, and Species of Special Concern. http://www.myfwc.org. Cited 17 Oct 2006

Florida Fish and Wildlife Conservation Commission (2005) Florida's wildlife legacy initiative, comprehensive wildlife conservation strategy, Tallahassee, FL

Funk WC, Blouin MS, Corn PS, Maxell BA, Pilliod DS, Amish S, Allendorf FW (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. Mol Ecol 14:483–496

Goodwin BJ (2003) Is landscape connectivity a dependent or independent variable? Landsc Ecol 18:687–699

Goudet J (1995) FSTAT, (version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Greenberg CK, Neary DG, Harris LD (1994) Effect of high-intensity wildfire and silvicultural treatments on reptile communities in sand-pine scrub. Conserv Biol 8:1047–1057

Gustafson EJ, Gardner RH (1996) The effect of landscape heterogeneity on the probability of patch colonization. Ecology 77:94–107

Habel JC, Assmann T (eds) (2009) Relict species: phylogeography and conservation biology. Springer, New York

Hanski I (1994) A practical model of metapopulation dynamics. J Anim Ecol 63:151–162

Hanski I (1999) Metapopulation ecology. Oxford University Press, New York

Hanski I, Gilpin ME (1997) Metapopulation biology. Academic Press, New York

Harrison S, Taylor AD (1997) Empirical evidence for metapopulation dynamics. In: Hanski I, Gilpin ME (eds) Metapopulation biology. Academic Press, San Diego, CA, pp 27–42

Hokit DG, Branch LC (2003a) Associations between patch area and vital rates: consequences for local and regional populations. Ecol Appl 13:1060–1068

Hokit DG, Branch LC (2003b) Habitat patch size affects demographics of the Florida scrub lizard (*Scelolporus woodi*). J Herp 37:257–265

Hokit DG, Stith BM, Branch LC (1999) The effect of landscape structure in Florida scrub: a population perspective. Ecol Appl 9:124–134

Hokit DG, Stith BM, Branch LC (2001) Comparison of two types of metapopulation models in real and artificial landscapes. Conserv Biol 15:1102–1113

Holderegger T, Wagner HH (2006) A brief guide to landscape genetics. Landsc Ecol 21:793–796

Holderegger R, Wagner HH (2008) Landscape genetics. Bioscience 58:199–207

Huck RB, Judd WS, Whitten WM, Skean JD Jr, Wunderlin RP, Delaney KR (1989) A new *Dicerandra* (Labiatae) from the Lake Wales Ridge of Florida, with a cladistic analysis and discussion of endemism. Syst Bot 14:197–213

Husband BC, Barrett SCH (1998) Spatial and temporal variation in population size of *Eichhornia paniculata* in ephemeral habitats: implications for metapopulation dynamics. J Ecol 86:1021–1031

Keyghobadi N, Roland J, Strobeck C (1999) Influence of landscape on the population genetic structure of the alpine butterfly *Parnassius smintheus* (Papilionidae). Mol Ecol 8:1481–1495

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

McCoy ED, Mushinsky HR (1992) Rarity of organisms in the sand pine scrub habitat of Florida. Conserv Biol 6:537–548

McCoy ED, Mushinsky HR (1999) Habitat fragmentation and the abundances of vertebrates in the Florida scrub. Ecology 80:2526–2538

McCoy ED, Hartmann PP, Mushinsky HR (2004) Population biology of the rare Florida scrub lizard in fragmented habitat. Herpetologica 60:54–61

McDonald DB, Hamrick JL (1996) Genetic variation in some plants of Florida scrub. Am J Bot 83:21–27

Merriam G (1984) Corridors and connectivity: animal populations in heterogeneous environments. In: Saunders DA, Hobbs R (eds) Nature conservation 2: the role of corridors. J. Beatty & Sons, Surrey, pp 133–142

Michels E, Cottenie K, Neys L, De Gelas K, Coppin P, De Meester L (2001) Geographical and genetic distances among zooplankton populations in a set of interconnected ponds: a plea for using GIS modelling of the effective geographic distance. Mol Ecol 10:1929–1938

Moilanen A, Nieminen M (2002) Simple connectivity measures in spatial ecology. Ecology 83:1131–1145

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 5:238–254

Moritz C, Faith DP (1998) Comparative phylogeography and the identification of genetically divergent areas for conservation. Mol Ecol 7:419–430

Myers RL (1990) Scrub and high pine. In: Myers RL, Ewel JJ (eds) Ecosystems of Florida. University of Central Florida Press, Orlando, FL, pp 150–193

Osentoski MF, Lamb T (1995) Intraspecific phylogeography of the gopher tortoise, *Gopherus polyphemus*: RFLP analysis of amplified mtDNA segments. Mol Ecol 4:709–718

Pascual-Hortal L, Saura S (2006) Comparison and development of new graph-based landscape connectivity indices: toward the priorization of habitat patches and corridors for conservation. Landsc Ecol 21:959–967

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conserv Biol 12:844–855

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pulliam HR (1988) Sources, sinks and population regulation. Am Nat 132:652–661

Raymond M, Rousset F (1995) GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. J Hered 83:248–249

Rice W (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Roach JL, Stapp P, Van Horne B, Antolin MF (2001) Genetic structure of a metapopulation of black-tailed prairie dogs. J Mammal 82:946–959

Schultz CB, Crone EE (2005) Patch size and connectivity thresholds for butterfly habitat restoration. Conserv Biol 19:887–896

Singleton PH, Gaines WL, Lehmkuhl JF (2002) Landscape permeability for large carnivores in Washington: a geographic information system weighted-distance and least-cost corridor assessment. US Forest Service Research Paper PNW-RP-549

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst Zool 35:627–632

Sokal RR, Rohlf FJ (1981) Biometry, 2nd edn. W.H. Freeman, New York

Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (Ambystoma tigrinum melanostictum). Mol Ecol 14:2553–2564

Storfer A, Murphy MA, Evans JS et al (2007) Putting the 'landscape' in landscape genetics. Heredity 98:128–142

Stout IJ (2001) Rare plants of the Florida scrub, USA. Nat Areas J 21:50–60

Taylor PD, Fahrig L, Henein K, Merriam G (1993) Connectivity is a vital element of landscape structure. Oikos 68:571–573

The Nature Conservancy (1991) Lake Wales/Highland Ridge ecosystem Carl Project proposal. Unpublished Report, Archbold Biological Station, Florida Natural Areas Inventory

Tiebout HM, Anderson RA (1997) A comparison of corridors and intrinsic connectivity to promote dispersal in transient successional landscapes. Conserv Biol 11:620–627

United States Fish Wildlife Service (1991) Preliminary project proposal: proposed establishment of lake wales ridge national wildlife refuge. United States Fish and Wildlife Service, Atlanta, GA

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Watts WA, Hansen BCS (1988) Environments of Florida in the Late Wisconsin and Holocene. In: Purdy BA (ed) Wet site archaeology. The Telford Press, Caldwell, NJ, pp 307–323

Webb SD (1990) Historical biogeography. In: Meyers RL, Ewel JJ (eds) Ecosystems of Florida. University of Central Florida Press, Orlando, FL, pp 70–102

Wright S (1931) Evolution of Mendelian populations. Genetics 16:97–158

Young AC, Clarke GM (2000) Demography and viability of fragmented populations. Cambridge University Press, Cambridge