Fire Alters Patterns of Genetic Diversity Among 3 Lizard Species in Florida Scrub Habitat

AARON W. SCHREY, KYLE G. ASHTON, STACY HEATH, EARL D. MCCOY, AND HENRY R. MUSHINSKY

From the Department of Integrative Biology, University of South Florida, Tampa, FL 33620 (Schrey, Heath, McCoy, and Mushinsky); and the Archbold Biological Station, Lake Placid, FL (Ashton).

Abstract

The Florida Sand Skink (Plestiodon reynoldsi), the Florida Scrub Lizard (Sceloporus woodi), and the Six-lined Racerunner (Aspidoscelis sexlineata) occur in the threatened and fire-maintained Florida scrub habitat. Fire may have different consequences to local genetic diversity of these species because they each have different microhabitat preference. We collected tissue samples of each species from 3 sites with different time-since-fire: Florida Sand Skink $n = 73$, Florida Scrub Lizard $n = 70$, and Six-lined Racerunner $n = 66$. We compared the effect of fire on genetic diversity at microsatellite loci for each species. We screened 8 loci for the Florida Sand Skink, 6 loci for the Florida Scrub Lizard, and 6 loci for the Six-lined Racerunner. We also tested 2 potential driving mechanisms for the observed change in genetic diversity, a metapopulation source/sink model and a local demographic model. Genetic diversity varied with fire history, and significant genetic differentiation occurred among sites. The Florida Scrub Lizard had highest genetic variation at more recently burned sites, whereas the Florida Sand Skink and the Six-lined Racerunner had highest genetic variation at less recently burned sites. Habitat preferences of the Florida Sand Skink and the Florida Scrub Lizard may explain their discordant results, and the Six-lined Racerunner may have a more complicated genetic response to fire or is acted on at a different geographic scale than we have investigated. Our results indicate that these species may respond to fire in a more complicated manner than predicted by our metapopulation model or local demographic model. Our results show that the population-level responses in genetic diversity to fire are species-specific mandating conservation management of habitat diversity through a mosaic of burn frequencies.

Key words: conservation, fire, Florida scrub, microsatellites

Florida scrub is one of the most severely threatened habitats in Florida (Noss et al. 1995), and nearly all Florida scrub now exists as small managed preserves (Turner et al. 2006). The Florida scrub habitat is maintained by high intensity fire on an infrequent time scale (5–20 years for scrubby flatwoods and oak-palmetto scrub and 15–100 years for rosemary scrub and sand pine scrub; Laessle 1958; Myers 1990; Menges 1999). Fire generates a wide array of responses in herpetofauna in general (Russell et al. 1999); fire can influence species richness (Mushinsky 1985), alter dispersal and movement (Templeton et al. 2001; Brisson et al. 2003; McDonough and Loughry 2005), and change local abundances (Griffiths and Christian 1996; Schurbon and Fauth 2003; Brook and Griffiths 2004; Langford et al. 2007; Carrel 2008; Webb and Shine 2008). Accordingly, understanding the effects of fire on Florida scrub species assemblages is critical to their management. If effects differ across species, it may be necessary to manage scrub preserves as mosaics.

We explored the relationship between fire and genetic diversity in 3 lizards that likely have population-level response to fire, the Florida Sand Skink (Plestiodon reynoldsi), the Florida Scrub Lizard (Sceloporus woodi), and the Six-lined Racerunner (Aspidoscelis sexlineata; Figure 1). All have different microhabitat specificity (McCoy and Mushinsky 1992) and dispersal ability (Carpenter 1959; Tiebout and Anderson 1997; Gianopulos 2001; Penny 2001). Genetic diversity of these species was expected to change with time-since-fire (TSF) because of different habitat preferences (Mushinsky 1985; Tiebout and Anderson 1997; Hokit et al. 1999; McCoy et al. 1999; Tiebout and Anderson 2001), documented abundance changes with fire history (Schrey et al. 2011), and because they have fine-scale genetic
differentiation (Clark et al. 1999; Branch et al. 2003; Richmond et al. 2009; Hokit et al. 2010; Schrey et al. 2011).

The Florida Sand Skink is listed as threatened under the US Endangered Species Act (United States Fish and Wildlife Service 1999) and as a species of greatest conservation need in Florida (Florida Fish and Wildlife Conservation Commission (FFWCC) 2005). It is a sand burrower and is confined to scrub and sandhill habitats with deep sand that occur along a narrow corridor in central Florida (Christman 1992). The Florida Sand Skink prefers more overgrown areas (McCoy et al. 1999, unpublished data) and occurs in higher densities at longer TSF (Schrey et al. 2011). Dispersal is constrained because of dependence on sand for locomotion, but individuals have been recaptured at distances of 0.24 km (Penny 2001). Phylogeographic analysis found that the Florida Sand Skink is differentiated into separate lineages on the Mt. Dora Ridge, Northern Lake Wales Ridge, Central Lake Wales Ridge, and Southern Lake Wales Ridge (Branch et al. 2003), a pattern that corresponds to the geological history of Florida (Branch et al. 2003; Richmond et al. 2009). The Florida Sand Skink has genetic differentiation within and among the currently fragmented scrub patches (Richmond et al. 2009; Schrey et al. 2011), and, specifically, genetic differentiation is known to occur across Archbold Biological Station (ABS), the site of our study (Richmond et al. 2009; Schrey et al. 2011). Local populations of the Florida Sand Skink have increased variance in genetic diversity estimates in more recently burned areas, yet tend to have greater diversity at longer TSF (Schrey et al. 2011).

The Florida Scrub Lizard is listed as a species of greatest conservation need in Florida (FFWCC 2005) and is restricted mainly to upland scrub habitats. The Florida Scrub Lizard prefers recently burned areas (Tiebout and Anderson 1997, 2001) and tends to occur in higher densities with shorter TSF (Britt, E., Catenazzi, A., Mushinsky, H. R., and McCoy, E. D., unpublished data). The Florida Scrub Lizard is a poor disperser and is reluctant to cross either open sand or overgrown areas (Greenberg et al. 1994). They are not likely to move among distant scrub patches without intermediate scrub patches (Tiebout and Anderson 1997). The Florida Scrub Lizard has major phylogeographic lineages that follow the major upland ridges in Florida and has genetic differentiation within and among the ridges (Clark et al. 1999; Branch et al. 2003). Individuals are closely linked to scrub patches, and population structure occurs among scrub patches, separated by distances as small as 200 m (Clark et al. 1999; Branch et al. 2003; Hokit et al. 2010). The Florida Scrub Lizard also has population structure at ABS (Heath, S., Schrey, A. W., Ashton, K. G., Mushinsky, H. R., and McCoy, E. D., unpublished data).

The Florida Scrub Lizard is listed as a species of greatest conservation need in Florida (FFWCC 2005) and is restricted mainly to upland scrub habitats. The Florida Scrub Lizard prefers recently burned areas (Tiebout and Anderson 1997, 2001) and tends to occur in higher densities with shorter TSF (Britt, E., Catenazzi, A., Mushinsky, H. R., and McCoy, E. D., unpublished data). The Florida Scrub Lizard is a poor disperser and is reluctant to cross either open sand or overgrown areas (Greenberg et al. 1994). They are not likely to move among distant scrub patches without intermediate scrub patches (Tiebout and Anderson 1997). The Florida Scrub Lizard has major phylogeographic lineages that follow the major upland ridges in Florida and has genetic differentiation within and among the ridges (Clark et al. 1999; Branch et al. 2003). Individuals are closely linked to scrub patches, and population structure occurs among scrub patches, separated by distances as small as 200 m (Clark et al. 1999; Branch et al. 2003; Hokit et al. 2010). The Florida Scrub Lizard also has population structure at ABS (Heath, S., Schrey, A. W., Ashton, K. G., Mushinsky, H. R., and McCoy, E. D., unpublished data).

The Six-lined Racerunner is not currently imperiled and has a much wider geographic distribution (from Maryland to the Florida Keys and west to Missouri) than the Florida Sand Skink and the Florida Scrub Lizard. The Six-lined Racerunner strongly prefers open areas and avoids densely vegetated areas (Mushinsky 1985; Hokit et al. 1999). Yet, specific scrub habitat preference is not as well documented.
Table 1  Genetic diversity estimates for the FSS, the FSL, and the SR from three locations at ABS

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>TSF</th>
<th>N</th>
<th>Ar</th>
<th>pAr</th>
<th>H_S</th>
<th>f</th>
<th>MPR</th>
<th>HL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSS</td>
<td>54B</td>
<td>3</td>
<td>28</td>
<td>12.69</td>
<td>3.21</td>
<td>0.87</td>
<td>0.16</td>
<td>0.05* (0.04, 0.07)</td>
<td>0.26^A</td>
</tr>
<tr>
<td></td>
<td>58E</td>
<td>14</td>
<td>27</td>
<td>13.66</td>
<td>2.60</td>
<td>0.91</td>
<td>0.13</td>
<td>0.02* (0.01, 0.03)</td>
<td>0.21^AB</td>
</tr>
<tr>
<td></td>
<td>60D</td>
<td>21</td>
<td>18</td>
<td>14.88</td>
<td>3.81</td>
<td>0.91</td>
<td>0.07</td>
<td>0.02* (0.00, 0.04)</td>
<td>0.15^B</td>
</tr>
<tr>
<td>FSL</td>
<td>54D</td>
<td>3</td>
<td>24</td>
<td>10.58</td>
<td>3.03</td>
<td>0.80</td>
<td>0.06</td>
<td>0.01 (−0.01, 0.03)</td>
<td>0.24^A</td>
</tr>
<tr>
<td></td>
<td>58E</td>
<td>9</td>
<td>24</td>
<td>9.59</td>
<td>1.58</td>
<td>0.77</td>
<td>0.17</td>
<td>−0.02 (−0.05, 0.02)</td>
<td>0.32^AB</td>
</tr>
<tr>
<td></td>
<td>60D</td>
<td>16</td>
<td>22</td>
<td>8.76</td>
<td>2.17</td>
<td>0.69</td>
<td>0.14</td>
<td>0.14* (0.11, 0.16)</td>
<td>0.34^B</td>
</tr>
<tr>
<td>SR</td>
<td>54D</td>
<td>3</td>
<td>18</td>
<td>11.76</td>
<td>3.85</td>
<td>0.87</td>
<td>0.17</td>
<td>0.02* (−0.01, 0.04)</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>58E</td>
<td>9</td>
<td>24</td>
<td>11.81</td>
<td>3.12</td>
<td>0.88</td>
<td>0.30</td>
<td>0.02* (0.00, 0.04)</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>60D</td>
<td>16</td>
<td>24</td>
<td>13.41</td>
<td>4.62</td>
<td>0.89</td>
<td>0.23</td>
<td>−0.01 (−0.03, 0.01)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Time from last fire to sample collection (TSF) is provided in years. Estimates of Ar, pAr, He, inbreeding coefficient (f), MPR, and HL are averaged over loci. For MPR, an asterisk indicates the estimate is greater than chance and 95% confidence interval bounds are in parenthesis. For HL, different letters indicate significant t-tests between sites, shared letters indicate nonsignificant differences. Florida Sand Skink (FSS), Florida Scrub Lizard (FSL), and Six-lined Racerunner (SR).

As for the Florida Sand Skink or Florida Scrub Lizard. The Six-lined Racerunner may prefer recently burned areas (Mushinsky 1985), and the species tends to occur in higher density with shorter TSF (Britt, E., Catenazzi, A., Mushinsky, H. R., and McCoy, E. D., unpublished data). The Six-lined Racerunner likely has a greater dispersal capability than the other 2 species; however, once established in a location, individuals tend to remain in place (Clark 1976; Mushinsky 1985). To our knowledge, there is no information about genetic differentiation among populations of this species.

Our first objective was to determine if fire altered the genetic diversity of local populations for each species and to determine if the relationship between genetic diversity and TSF differed among species. We hypothesize that the genetic diversity will vary with TSF and that the pattern of change will differ among species. We expect that habitat preference will predict the change in genetic diversity for each species. Specifically, we expect that the Florida Sand Skink will have higher genetic diversity in sites with longer TSF, following their preference for more overgrown areas. Conversely, we expect the Florida Scrub Lizard and the Six-lined Racerunner will have higher genetic diversity in sites with shorter TSF because they both prefer more open areas. Because we used different microsatellite loci in the 3 species, the genetic diversity estimates are not directly comparable. However, the pattern of how genetic diversity changes with TSF is comparable.

We then tested 2 models, Metapopulation Source/Sink Model and Local Demographic Model, to investigate the potential driving mechanisms underlying the change in genetic diversity. For the Metapopulation Source/Sink Model, fire is expected to stimulate gene flow and alter genetic diversity by causing an influx of individuals into (preference for burned scrub), or out of (preference for unburned scrub), the burned area. Genetic diversity is expected to be increased in the area receiving the influx of new individuals, which brings in new genetic diversity (i.e., Kolbe et al. 2008). The influx of individuals would also increase density, which can increase genetic diversity (Xie and Zhang 2006; Dong et al. 2010). Thus, genetic diversity could be greater in the burned area or greater in the surrounding unburned areas depending on habitat preference. In either case, this mechanism would decrease the amount of local genetic differentiation.

For the Local Demographic Model, fire is expected to change the demographics of the burned area, thereby altering the standing population’s genetic diversity. The fire could decrease genetic diversity by causing a bottleneck in the burned area, or the fire could have a variable effect on genetic diversity if stochastic changes in the local population occur (Schrey et al. 2011). The fire’s effect would be limited to the area burned and would not increase gene flow. Here, fire would alter genetic diversity, yet not necessarily follow habitat preferences, and fire could increase genetic differentiation among local populations that were subdivided before the fire.

Materials and Methods
Sample Collection
Florida Sand Skinks (n = 73), Florida Scrub Lizards (n = 70), and Six-lined Racerunners (n = 66) were collected in bucket traps at ABS (Table 1; Figure 2). ABS is a 2100 ha natural preserve of Florida scrub subdivided into burn units that experience fire at different frequencies, generating a diverse and well-documented fire history. Samples for each species were collected at one location in 3 different burn units (Figure 2). All sites were within 4.37 km (Table 2). The burn units were designated 58E (Florida Sand Skink: 27.144364, −81.365036; Florida Scrub Lizard and Six-lined Racerunner: 27.140425, −81.363875), 60D (Florida Sand Skink: 27.121750, −81.364547; Florida Scrub Lizard and Six-lined Racerunner: 27.123447, −81.362044), 54D (Florida Scrub Lizard and Six-lined Racerunner: 27.159806, −81.358450), and 54B (Florida Sand Skink: 27.160900, −81.361347). We determined TSF for each location as the number of years between sample collection and most recent recorded fire. TSF differs for identical burn units (Table 1).
Genetic Data Collection

DNA was extracted from tissue samples using the DNeasy Animal Tissue Kit (Qiagen, Valencia, CA) or a high salt protocol (Watts 2001). Eight microsatellite loci were screened for the Florida Sand Skink (Nr—52.02, 52.04, 52.07, 52.11, 60.02, 60.05, 60.11, and 60.34; Reid et al. 2004). These 8 loci are known to be sufficient to detect significant genetic differentiation among Florida Sand Skinks at ABS (Richmond et al. 2009; Schrey et al. 2011). We optimized 6 microsatellite loci for the Six-lined Racerunner (Loci Acos5, A13, A33, A42, A62, and A71) from 13 loci previously developed for the genus (Crawford et al. 2008). No previous genetic information was available for the Six-lined Racerunner, so we attempted to screen a similar number of loci as for other species. We tested each locus in each site for Hardy–Weinberg Equilibrium (HWE) with population structure among Florida Scrub Lizards at a fine scale (Hokit et al. 2010), including at ABS (Heath, S., Schrey, A. W., Ashton, K. G., Mushinsky, H. R., and McCoy, E. D., unpublished data). We optimized 6 microsatellite loci for the Six-lined Racerunner (Loci Acos5, A13, A33, A42, A62, and A71) from 13 loci previously developed for the genus (Crawford et al. 2008). No previous genetic information was available for the Six-lined Racerunner, so we attempted to screen a similar number of loci as for other species. We tested each locus in each site for Hardy–Weinberg Equilibrium (HWE) with

Figure 2. ABS with sample collection locations (54B, 54D, 58E, and 60D) of the Florida Sand Skink (FSS), Florida Scrub Lizard (FSL), and the Six-lined Racerunner. Results of Bayesian clustering with STRUCTURE are provided for the 2 species with multiple groups, the Florida Sand Skink (k = 2) and the Florida Scrub Lizard (k = 3). The shaded portion indicates the proportion of individuals from a particular site that were assigned to each genetic group.
Fire Alters Genetic Diversity in Florida Scrub

**Table 2** Pairwise $\theta_{ST}$ estimates (below diagonal) and geographic distance in kilometer (above diagonal) among geographic samples of the FSS, the FSL, and the SR

<table>
<thead>
<tr>
<th></th>
<th>54B</th>
<th>58E</th>
<th>60D</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSS</td>
<td>---</td>
<td>1.88</td>
<td>4.37</td>
</tr>
<tr>
<td>54B</td>
<td>0.048</td>
<td>---</td>
<td>2.52</td>
</tr>
<tr>
<td>58E</td>
<td>0.047</td>
<td>0.025</td>
<td>---</td>
</tr>
<tr>
<td>60D</td>
<td>0.018</td>
<td>2.22</td>
<td>4.06</td>
</tr>
<tr>
<td>FSL</td>
<td>0.065</td>
<td>0.078</td>
<td>---</td>
</tr>
<tr>
<td>54D</td>
<td>0.0027</td>
<td>---</td>
<td>1.90</td>
</tr>
<tr>
<td>58E</td>
<td>0.065</td>
<td>0.078</td>
<td>---</td>
</tr>
<tr>
<td>60D</td>
<td>0.0027</td>
<td>---</td>
<td>1.90</td>
</tr>
</tbody>
</table>

All pairwise comparisons were statistically significant. Florida Sand Skink (FSS), Florida Scrub Lizard (FSL), and Six-lined Racerunner (SR).

FSTAT version 2.9.3 (Goudet 1995) to determine if there were null alleles and/or allelic dropout.

Microsatellite loci were amplified by multiplex polymerase chain reaction (PCR). PCR was performed at a final volume of 10 µl containing 1× PCR Buffer (50 mM KCl, 10 mM Tris HCl pH 9.0), 2 mM MgCl$_2$, 200 µM each deoxyribonucleotide triphosphates, 0.1 unit Taq DNA polymerase, 0.9 µM of each PCR primer (forward primers labeled with 6-Fam, NED, or HEX), and 1–20 ng template DNA. Thermal cycles were 95°C 2 min, 95°C 30 s, Annealing Temp. 30 s, 72°C 30 s, 70°C 5 min, repeated 40 times. Annealing temperatures are defined in Reid et al. (2004) for the Florida Sand Skink, in Ernst et al. (2004) for the Florida Scrub Lizard, and in Crawford et al. (2008) for the Six-lined Racerunner. Reactions were diluted 1:1 with loading buffer (deionized formamide, blue dextran EDTA, and MRK 500; The Gel Company, San Francisco, CA) and electrophoresed on an ABI 377 (Applied Biosystems, Foster City, CA) using 96-well upgrade, 36 cm well-to-read, and 4.25% polyacrylamide gels. Gel images were analyzed, and allele sizes were defined with GENESCAN 3.2.1 and GENOTYPER v 2.5 (Applied Biosystems). Raw allele sizes were scatter plotted and binned to specific allele categories.

**Genetic Diversity and TSF**

Genetic diversity estimates were calculated for each species at each site to determine the relationship with TSF. Allelic richness (Ar) and private allelic richness (pAr) were calculated by HP-Rare (Kalinowski 2005). Expected heterozygosity (H$_E$) and the inbreeding coefficient ($f$) were calculated by Genetic Data Analysis (GDA; Lewis and Zaykin 2001). Mean pairwise relatedness (MPR, Queller and Goodnight 1989) was calculated by GENALEX-6 (Peakall and Smouse 2006). For each site, MPR estimates were tested for significant departures from random expectation by permutation ($n = 999$) for each site, and bootstraps ($n = 1000$) were performed to estimate 95% confidence intervals for MPR at each site to compare sites within species. Homozygosity by locus (HL; Aparicio et al. 2006), an individual-level estimate of homozygosity weighted by the allelic variability of each locus, was calculated for each individual at each site. Within species, HL was compared pairwise among sites using a $t$-test.

**Mechanisms of Change in Genetic Diversity**

To test the Metapopulation Source/Sink Model and the Local Demographic Model, we compared the pattern of change in genetic diversity with TSF to the habitat preferences of each species. We tested each sample for the signature of a bottleneck with the program BOTTLE-NECK (Cornuet and Luikart 1996). Each sample of each species was tested for an increase in observed heterozygosity relative to HWE expectations, indicative of a bottleneck, using 3 models (infinite alleles model, stepwise mutation model, and 2-phase model). We also determined if the allele frequency distribution deviated from the expected L-shape, which would indicate a bottleneck. We then used STRUCTURE v 2.3 (Pritchard et al. 2000; Falush et al. 2003) to estimate the number of populations ($k$) among the 3 sites independently for each species and to identify individual membership in each $k$ with a $q$ value. We performed 5 runs at $k = 1–4$ for each species using the admixture model, 30,000 burn-in steps, 1,000,000 post burn-in steps and allowed correlated allele frequencies. We used the natural log probability of observing the data Ln Pr($x|k$) method of STRUCTURE and the Delta $k$ (Evanno et al. 2005) to determine the number of groups that best fit the data. Individuals were assigned to the group with the highest $q$ value.

We calculated the $\theta_{ST}$ estimate of genetic differentiation for each species and pairwise among sites with FSTAT. Statistical significance was determined by permutation, and Bonferroni correction of alpha $= 0.05$ was performed where appropriate (Rice 1989). We also performed spatial autocorrelation with GENALEX-6 to characterize the spatial relationship of genetic relatedness among individuals. GENALEX-6 calculates an autocorrelation coefficient of the squared pairwise genetic distance and pairwise geographic distance among all pairs of individuals (Smouse and Peakall 1999; Peakall et al. 2003), similar to Moran’s $I$ (Moran 1950). Our collection sites resulted in individuals being collected at 0, 1–2, 2–3, and 3–5 km. Thus, the pairwise autocorrelation comparisons were binned at 1 km distance classes ($0$–1, 1–2, 2–3, 3–4, and 4–5 km) to determine how genetic relatedness changes with geographic distance. We used 9999 permutations to test if the estimate was significantly different from zero and 9999 bootstraps to test if estimates significantly varied within species.

**Results**

All microsatellite loci screened were highly variable and varied with TSF (Table 1). The Florida Sand Skink deviated
from HWE at each site at locus Nr—60.34, which may have been caused by null alleles (Reid et al. 2004; Richmond et al. 2009; Schrey et al. 2011). Richmond et al. (2009) found estimates of genetic differentiation were not significantly altered by including this locus. We completed all subsequent analyses of the Florida Sand Skink with and without locus Nr 60.34. Including the locus did not affect our results, thus, it was retained. Three other tests of HWE were significant for the Florida Sand Skink (Nr 52.11 and Nr 60.11 in 54B and Nr 52.11 in 58E), which may have been caused by low-frequency allelic dropout or sampling error. The Florida Scrub Lizard had only 2 significant tests of HWE (SW614-A4 and SW614-B6 in 60D), which also are likely explained by low-frequency deviations, possibly caused by allelic dropout. Cross-species amplification was successful for the Six-lined Racerunner, although 8 tests of HWE showed significantly less heterozygosity than expected. The significant tests were locus A13 in 54D and 58E, locus A33 in 58E and 60D, locus A42 in 58E and 60D, and locus A71 in 58E and 60D. The number of significant tests, which consistently occurred in sites 58E and 60D, indicate that the Six-lined Racerunner may be out of HWE at these sites, likely because of inbreeding (see below).

### Genetic Diversity and TSF

Genetic diversity varied with TSF, and the pattern of change was different among the 3 lizards (Table 1). Genetic diversity was increased at longer TSF for the Florida Sand Skink and Six-lined Racerunner, and genetic diversity was decreased at longer TSF for the Florida Scrub Lizard. The Florida Sand Skink and the Six-lined Racerunner had greater Ar and He with longer TSF, whereas the Florida Scrub Lizard had greater Ar and He with shorter TSF and had larger He variation overall (Table 1). The Florida Sand Skink and the Six-lined Racerunner had intermediate pAr at short TSF, low pAr at intermediate TSF, and high pAr at long TSF (Table 1). The Florida Scrub Lizard had high pAr at low TSF, low pAr at intermediate TSF, and intermediate pAr at long TSF (Table 1). The Florida Sand Skink had decreasing f with TSF, whereas the Florida Scrub Lizard and the Six-lined Racerunner had lowest f at low TSF, highest f at intermediate TSF, and intermediate f at long TSF (Table 1).

A similar pattern occurred among species in the relationship between MPR and TSF. The Florida Sand Skink and the Six-lined Racerunner had higher relatedness at shorter TSF, and the Florida Scrub Lizard had higher relatedness at longer TSF (Table 1). The Florida Sand Skink had significantly greater MPR than expected by chance at all sites ($P = 0.001$ each). A pattern of decreasing MPR with TSF occurred; site 54B, the most recent TSF, had significantly greater MPR than site 58E, the second most recent TSF. The Florida Scrub Lizard had greater MPR with longer TSF. The site with longest TSF, 60D, was significantly greater than expected by chance ($P = 0.001$) and was significantly greater than all others. The Six-lined Racerunner had MPR significantly greater than expected by chance at the 2 more recent TSF sites (54D $P = 0.027$; 58E $P = 0.004$) and a trend of decreasing MPR with TSF, yet bootstrap values overlapped for each site.

The Florida Sand Skink and the Florida Scrub Lizard also had opposite patterns of change in HL with TSF (Table 1). The Florida Sand Skink had significantly greater homozygosity in the shortest TSF than in the longest TSF (HL = 0.26 and 0.15, $P = 0.01$). The Florida Scrub Lizard had significantly greater homozygosity in the longest TSF than in the shortest TSF (HL = 0.34 and 0.24, $P = 0.04$). For both species, neither HL for the long or short TSF was different from HL for the intermediate TSF (Table 1). The Six-lined Racerunner had no significant differences in HL over TSF (Table 1).

### Mechanisms of Change in Genetic Diversity

The change in genetic diversity with TSF followed the Florida Sand Skink’s and the Florida Scrub Lizard’s habitat preferences. In both cases, genetic diversity was higher in the preferred habitat. The Six-lined Racerunner had greater genetic diversity with longer TSF, which did not follow its preference for more open habitat.

There were no consistent results indicating a bottleneck had occurred in any sample. All samples had allele frequency distributions that followed the expected L-shape. The Florida Sand Skink had inconsistent results among the 3 models testing for an increase in observed heterozygosity compared with expectations. The infinite alleles model identified significant heterozygosity excess (as would be expected with a bottleneck) in 58E ($P = 0.002$) and 60D ($P = 0.01$). However, the stepwise mutation model had significant tests of heterozygosity deficiency in all 3 samples (all $P = 0.01$), and the 2-phase model did not have a significant result in any sample. The Florida Scrub Lizard had no evidence of a bottleneck in any population. There were 3 significant tests of heterozygosity deficiency; the stepwise mutation model in 58E ($P = 0.04$) and 60D ($P = 0.008$) and the 2-phase model in 60D ($P = 0.02$). The Six-lined Racerunner had 2 significant tests of heterozygosity excess at the infinite alleles model in 54D ($P = 0.02$) and 58E ($P = 0.01$). However, no other test was significant.

STRUCTURE identified population structure for the Florida Sand Skink and the Florida Scrub Lizard and did not identity population structure for the Six-lined Racerunner. For the Florida Sand Skink, the $\ln \Pr(x|k)$ and the Delta $K$ were maximized at $k = 2 (\ln \Pr(x|k) \ k1 = -3330, k2 = -3223, k3 = -3246, k4 = -3330, k2 = -3223, k3 = -3246, k4 = -3519; \Delta \ K, k2 = 758, k3 = -15, k4 = -16)$. The majority of individuals from 54B (68%) assigned to one group, and the majority of individuals from 58E (100%) and 60D (89%) assigned to the other group (Figure 2). For the Florida Scrub Lizard, the $\ln \Pr(x|k)$ and the Delta $K$ were maximized at $k = 3 (\ln \Pr(x|k) \ k1 = -1755, k2 = -1772, k3 = -1732, k4 = -1936; \Delta \ K, k2 = -14, k3 = 81, k4 = -17)$. All of the individuals from site 60D (100%) assigned to group 1, whereas individuals from sites 54D and 58E assigned to all 3 groups, 54D group 1 = 13%, group 2 = 46%, group 3 = 42%; 58E group 1 = 13%, group 2 =
54%, group 3 = 33%. Although individuals from sites 54D and 48E were assigned to multiple groups, the smallest number of individuals assigned to the group characterized by the individuals from 60D (Figure 2). For the Six-lined Racerunner, the $\ln Pr(\theta|k)$ was maximized at $k = 1$ ($\ln Pr(\theta|k) \approx 1 = -2085, k2 = -2343, k3 = -2685, k4 = -2284$).

The Florida Scrub Lizard and the Florida Sand Skink had more genetic differentiation than the Six-lined Racerunner, with a similar magnitude and pattern of spatial autocorrelation (Table 2, Figure 3). The Florida Scrub Lizard had the highest estimate of genetic differentiation ($h_{ST} = 0.041, P < 0.001$), with pairwise estimates ranging from 0.018 to 0.078; all were significant. The Florida Sand Skink was intermediate ($h_{ST} = 0.052, P < 0.001$), with pairwise estimates ranging from 0.025 to 0.048; all were significant. The Six-lined Racerunner had the least ($h_{ST} = 0.016, P < 0.001$), with pairwise estimates ranging from 0.010 to 0.027; all were significant. Spatial autocorrelation identified isolation by distance in all species (Figure 3). For the Florida Sand Skink and the Florida Scrub Lizard, results were significantly greater than zero at 0–1 km, significantly less than zero at 1–2 km, not different from zero at 2–3 km, and significantly less than zero at 3–5 km. The Six-lined Racerunner had less spatial autocorrelation than the others and did not show a strong isolation by distance. The 0–1 km class was significantly greater than zero and the 2–3 km class significantly less than zero; yet, values were all near zero. For all species, the autocorrelation at 0–1 km was significantly higher than for all other distance classes.

**Discussion**

Fire-related patterns of genetic diversity differed for 3 Florida scrub lizards. The different patterns indicate that these lizards have incompatible preferences to different fire periodicities. The Florida Sand Skink and the Six-lined Racerunner had higher genetic diversity in longer TSF, whereas the Florida Scrub Lizard had higher genetic diversity in shorter TSF. MPR was lower in the preferred habitat for all species, indicating that fire history may affect local mating systems. Homozygosity by locus also followed habitat preferences for the Florida Sand Skink and Florida Scrub Lizard, yet no pattern was detected for the Six-lined Racerunner.

The Florida Scrub Lizard had the greatest genetic differentiation and the strongest positive relationship between genetic differentiation and geographic distance (Figures 2 and 3, Table 2). Both findings indicated dispersal greater than 1 km is rare, and isolation by distance occurs at a fine scale. The Florida Sand Skink had similar genetic differentiation as the Florida Scrub Lizard (Figure 3), being nearly as great in magnitude (Figure 2, Table 2), and also indicating dispersal may be less than 1 km with isolation by distance. The Six-lined Racerunner had lower magnitude genetic differentiation than both the Florida Sand Skink and the Florida Scrub Lizard (Figure 3, Table 2), which indicates it has greater dispersal.

Our results indicate that these species may respond to fire in a more complicated manner than predicted by the Metapopulation Source/Sink Model or the Local Demographic Model. Results for the Florida Sand Skink and the Florida Scrub Lizard did not strictly follow either model. Both had genetic diversity that followed their habitat preferences, supporting the Metapopulation Source/Sink Model. Yet, both had population structure among the sites sampled, not supporting the Metapopulation Source/Sink Model, and neither had evidence of bottlenecks.

A slight modification of the Metapopulation Source/Sink Model, accounting for the limited dispersal capabilities of these species, may better fit the results. The Florida Sand Skink prefers long unburned habitat and likely moves away from recently burned areas into surrounding unburned areas to find ground leaf litter to forage (Telford 1959; Myers and Telford 1965; Sutton 1996). The Florida Scrub Lizard prefers open habitat (Tiebout and Anderson 1997, 2001), which would increase density and genetic diversity in more...
recently burned areas. But because both have limited dispersal capabilities, the movement may not occur over a large enough area to generate gene flow to alter genetic differentiation across ABS.

It is also possible that the Local Demographic Model operates at the same time as the modified Metapopulation Source/Sink Model, yet not at a magnitude that would cause bottlenecks. Thus, fire (Florida Sand Skink) or the absence of fire (Florida Scrub Lizard) may disrupt local populations. The disruption may alter the local populations in a stochastic manner, thereby altering genetic diversity. Here, the metapopulation dynamics associated with habitat preferences may drive the general direction of change in genetic diversity, whereas local disturbance increases variation in the response. Fire has been shown to increase the amount of variance in genetic diversity in recently burned areas for the Florida Sand Skink, yet an overall trend of greater genetic diversity with increasing TSF persists (Schrey et al. 2011). Mark-recapture studies of the movement of these species after fire could help to differentiate among the hypothesized explanations.

The Six-lined Racerunner did not follow either of the models. A trend of increasing genetic diversity in long unburned areas was found but occurred at a lower magnitude than the other species (no significant differences in HL). The increase in genetic diversity was not predicted by the strong preference for open areas and avoidance of densely vegetated areas. There was no consistent evidence of a bottleneck, and less genetic differentiation occurred than for the other species. Notably, inbreeding coefficients were greater in magnitude, and several loci significantly deviated from HWE in the longer TSF sites (58E and 60D), whereas MPR at each site was lower than observed in the other species. We hypothesize that the apparent contradiction between genetic diversity and habitat preference is explained by the Six-lined Racerunner regularly dispersing at distances greater than the areas burned during controlled fires. Thus, the Six-lined Racerunner may have a more complicated genetic response to fire or is acting on a larger geographic scale than we have investigated.

We note that our findings show genetic diversity of the 3 lizards is different with TSF, but our data do not indicate if the relationship is consistent for these species. Because fire is an inherently variable and complex event, and different local environments may apply different constraints on the species, different fires may have different consequences for genetic diversity. Thus, our data predict that the 3 species would have a different genetic diversity after any fire but do not necessarily indicate that the relationship between genetic diversity and TSF would be the same in all areas.

This study extends the information available about how fire alters genetic characteristics of local populations. In fire-maintained habitats, suppression of fire has fragmented habitat, increased genetic differentiation, and caused local extinctions (Hutchison and Templeton 1999; Templeton et al. 2001; Brisson et al. 2003). In the same habitat, fire management increases dispersal, colonization, and population sizes (Templeton et al. 2001; Brisson et al. 2003). Our results indicate that the suppression of fire in Florida scrub habitat would have different effects on different species.

Further, the different patterns of change in genetic diversity observed among the Florida Sand Skink, Florida Scrub Lizard, and Six-lined Racerunner highlight the variable effect fire can have on local populations. The broad-headed snake (Hoplocephalus bungaroides) and the small-eyed snake (Cryptophis nigrescens) responded differently to the same intense wildfire in Australia (Webb and Shine 2008). Also, the inherently variable nature of a natural fire history may alter patterns of gene flow (Stow et al. 2007) and may affect genetic characteristics by disrupting habitat and reducing population sizes (Griffiths and Christian 1996; Brook and Griffiths 2004; Ujvari et al. 2008).

Our results support the importance of controlled fire for the conservation of scrub habitat. Genetic diversity of the Florida Scrub Lizard is increased by fire, whereas genetic diversity of the Florida Sand Skink is decreased by fire. These incompatible trends with fire require management for habitat diversity, using different fire periodicity. We suggest that the current management approach of ABS, maintaining habitat diversity through a mosaic of burn frequencies, accommodates habitat preferences and may maintain genetic diversity of the focal species.

Funding

Cemex; Disney Entertainment Corporation; Archbold Biological Station; Earthwatch Institute; and the Florida Fish and Wildlife Conservation Commission.

Acknowledgments

The authors acknowledge Entrix and graduate students from the McCoy and Mushinsky lab at University of South Florida (USF) for valuable field assistance. Samples were collected under permits issued to 2 coauthors. This research followed approved protocols from the IACUC at USF (no. W2842) and ABS (no. 05-2). M. Awad, M. Cook, and M. Grippo assisted with various laboratory functions, A. Catenazzi and T. Robbins provided pictures. R. Pickert provided the aerial image of ABS. N. Schrey reviewed an early draft of the manuscript.

References


Gianopulos KT. 2001. Response of the threatened sand skink (Neoseps reynoldsi) and other herpetofaunal species to burning and clearcutting in the Florida sand pine scrub habitat [master’s thesis]. [Tampa (FL):] University of South Florida. p. 91.


Schrey et al. Fire Alters Genetic Diversity in Florida Scrub


Received May 11, 2010; Revised January 31, 2011; Accepted April 27, 2011

Corresponding Editor: Rob DeSalle