



Original Article

Fire Does Not Strongly Affect Genetic Diversity or Structure of a Common Treefrog in the Endangered Florida Scrub

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Abstract

Fire regimes influence natural populations of organisms in diverse ways, via direct effects on population dynamics as well as indirect effects on habitat and ecosystem processes. Although many amphibian species have evolved to persist in fire-dependent ecosystems, the effects of fire on the genetic diversity of amphibian populations remain relatively unexplored. We examined how different aspects of fire history relate to population genetic diversity and structure of an abundant anuran, *Hyla femoralis*, in a large, intact area of Florida scrub containing hundreds of seasonally inundated ponds. Specifically, we assessed the overall population genetic structure and examined whether variation in time since fire, fire intensity, or historical fire frequency at breeding sites explained spatial variation in genetic diversity. Based on our sampling of 17 breeding aggregations within the 2,100-ha study area, neither recent nor frequent fire reduce genetic diversity or restrict connectivity among ponds for *H. femoralis*. Overall, mean effective population sizes were large (average range = 68–572). We detected a positive trend between effective population size (N_e) and average intensity of the most-recent fire, with this factor explaining 42% of the variation in N_e . Our results contrast with previous studies that consistently demonstrate strong relationships between fire history and population genetic structure of scrub-associated lizard species, suggesting that *H. femoralis* is resilient to a wide range of fire regimes. More generally, our study contributes to understanding the roles of life-history characteristics and environmental unpredictability in shaping organisms' responses to fire.

Keywords: Archbold Biological Station, dispersal, fire history, genetic structure, *Hyla femoralis*

Introduction

Natural and prescribed fire regimes can profoundly affect animal populations via multiple direct and indirect mechanisms. In addition to direct effects on population dynamics (e.g., mortality rates,

dispersal), fire can indirectly affect populations and communities by increasing habitat heterogeneity, enhancing productivity, and altering wetland hydroperiods, succession, and nutrient cycling (Kozlowski and Ahlgren 1974; Pilliod et al. 2003). Further, the mosaic of

habitat heterogeneity created by fire has been shown to shape genetic diversity patterns across diverse taxa (Schrey et al. 2011a; Schrey et al. 2011b; Pereoglou et al. 2013; Pierson et al. 2013). Interspecific variation in responses to fire, and fire suppression, can arise from differences in life-history strategy, habitat preferences, and evolutionary history of exposure to fire disturbance (Kozłowski and Ahlgren 1974; Russell et al. 1999; Pilliod et al. 2003; Bond and Keeley 2005).

Many amphibian species have evolved and persisted in fire-dependent ecosystems, and thus are likely to demonstrate local adaptation to fire (Pilliod et al. 2003). However, the effects of fire on the genetic diversity of amphibian populations remain relatively unexplored (Smith et al. 2014; Porvin et al. 2017). Both frequent fire and fire suppression have the potential to shape patterns of amphibian genetic diversity. For example, both can create barriers to dispersal, and thus fragment habitat, which may reduce gene flow and genetic diversity. Alternatively, fire may create corridors for gene flow by altering habitat structure and quality, thus increasing genetic diversity. In addition, fire may create short-term pulses in soil nutrient availability (Dean et al. 2015) and primary production in wetlands (Venne et al. 2016) potentially boosting larval amphibian production and effective population sizes compared to habitats where fire has been suppressed. Empirical data supporting these alternative possibilities are lacking, yet understanding the consequences of fire on population genetic diversity and structure is important for conservation and management of target amphibian species, especially in light of population declines and anthropogenically altered fire regimes (Pilliod et al. 2003; Bury 2004; Gardner et al. 2007). Understanding amphibian responses to different fire regimes could play a critical role in maintaining stable populations of common species, as well as recovering imperiled species (e.g., dusky gopher frog, *Rana sevosa* (USFWS 2015a); frosted flatwoods salamander, *Ambystoma cingulatum* (USFWS 2009); and reticulated flatwoods salamander, *Ambystoma bishopi* (USFWS 2015b)).

We examined the genetic response to fire intensity and fire frequency for a pond-breeding amphibian in the Florida scrub, a unique, relict formation of stunted oak scrub endemic to peninsular Florida. The scrub supports among the highest concentrations of threatened and endangered species in the continental United States (Myers 1990; Turner et al. 2006). Little of this biologically diverse ecosystem remains, owing to anthropogenic habitat loss and fire suppression (Turner et al. 2006). Florida scrub is naturally maintained by intermittent (5–100 year intervals) and high-intensity lightning fires (Menges et al. 1999). Fire in the Florida scrub has major effects on nutrient cycling, plant demography and phenology, and the abundance and diversity of many species (Mushinsky 1985; Mushinsky and McCoy 1985; Menges et al. 1993; Menges et al. 1999; Menges and Quintana-Ascencio 2004; Boughton et al. 2006). Species inhabiting the Florida scrub show variable responses to fire frequencies and histories. For example, the federally listed Florida scrub-jay requires more frequent burning to maintain its preferred nesting habitat of stunted and acorn-laden scrub oak (Woolfenden and Fitzpatrick 1984), whereas the Florida sand skink is more abundant in longer-unburned areas where there is an accumulation of leaf litter (McCoy et al. 2013a; McCoy et al. 2013b). Managing fire periodicity and intensity has become a primary goal for Florida scrub restoration (Turner et al. 2006). Thus, an understanding of differences in requirements and responses of Florida scrub-dwelling organisms to fire is needed to inform management of this imperiled ecosystem.

Archbold Biological Station (ABS) is a 2,100-hectare preserve of Florida scrub located near the southern end of the Lake Wales Ridge (Figure 1). ABS is divided into approximately 200 burn units managed

with differing fire regimes, which creates a mosaic of habitat patches that vary in vegetation structure owing to differences in fire return intervals and time since most recent fire. ABS maintains detailed records of all wildfires and prescribed fires, including date, spatial extent, and fire intensity (Menges et al. 2017). Previous studies have documented fine-scale genetic structure within the geographic limits of ABS in several plant species (Dolan et al. 1999; Dolan et al. 2008), Florida scrub-jays (*Aphelocoma coerulescens*) (Aguillon et al. 2017), and in three lizards: Florida sand skinks (*Neoseps reynoldsi*), Florida scrub lizards (*Sceloporus woodi*) and six-lined racerunners (*Aspidoscelis sexlineata*) (Schrey et al. 2011a; Schrey et al. 2011b; Schrey et al. 2016). However, the relationship between fire and genetic diversity has not been explored for any of the 14 native anuran species that occur at ABS.

We used the well-documented variation in fire history across the Florida scrub habitat at ABS to test the effects of fire on genetic diversity and population connectivity of the pine woods treefrog (*Hyla femoralis*). This common and locally abundant species is found throughout the southeastern United States, ranging from Virginia to Florida and Louisiana. In southern Florida, *H. femoralis* primarily inhabits pine flatwoods, xeric hammocks, sand pine scrub, and sandhill habitat (Meshaka and Layne 2015). In the scrub habitats at ABS, *H. femoralis* commence breeding when the seasonal ponds fill, typically in June or July. There are no studies of dispersal, site fidelity, or population genetic structure of *H. femoralis* across its range. Considering the wide range of fire-prone habitats occupied by this species, it is likely resilient to varying fire regimes, in contrast to habitat specialists that depend on specific fire regimes to maintain a narrow range of preferred habitat conditions. Regardless, we would still expect some aspects of its demography and patterns of gene flow to be influenced by fire. This expectation is supported by previous observations of high abundances observed one to three years after fire and population declines over longer time-since-fire (Schurbon and Fauth 2003, 2004).

We used *H. femoralis* as a focal species for testing the effects of fire history on genetic patterns of a moderately fire-dependent anuran. If wetland colonization and/or recruitment are strongly affected by fire, we would expect genetic diversity within local *H. femoralis* populations to vary among wetlands with differing fire histories. Specifically, within-wetland genetic diversity and effective population sizes may correlate with number of fires, fire intensity, or time since most-recent fire. Alternatively, gene flow and demography may not be strongly affected by fire. In this case, we expect to find one of two scenarios: 1) patterns of genetic structure or differences in within-wetland genetic diversity are unrelated to fire history metrics, or 2) populations are panmictic, having no or very weak genetic structure across the ABS landscape. We took advantage of known historical variation in fire regime at ABS combined with information from variable genetic markers to distinguish among these scenarios for *H. femoralis*.

Methods

Sampling and data collection

We hand-captured 416 individual *H. femoralis* from 17 breeding aggregations throughout ABS in June–September 2012 and July 2013. Toe clips were collected from 2 to 40 individuals per site and stored in 95% ethanol as genetic samples. Frogs were returned to their capture locations immediately or within 18 h.

Fire data

We obtained the detailed fire history of each breeding site from the ABS Grid Database, a relational database linked to various ABS

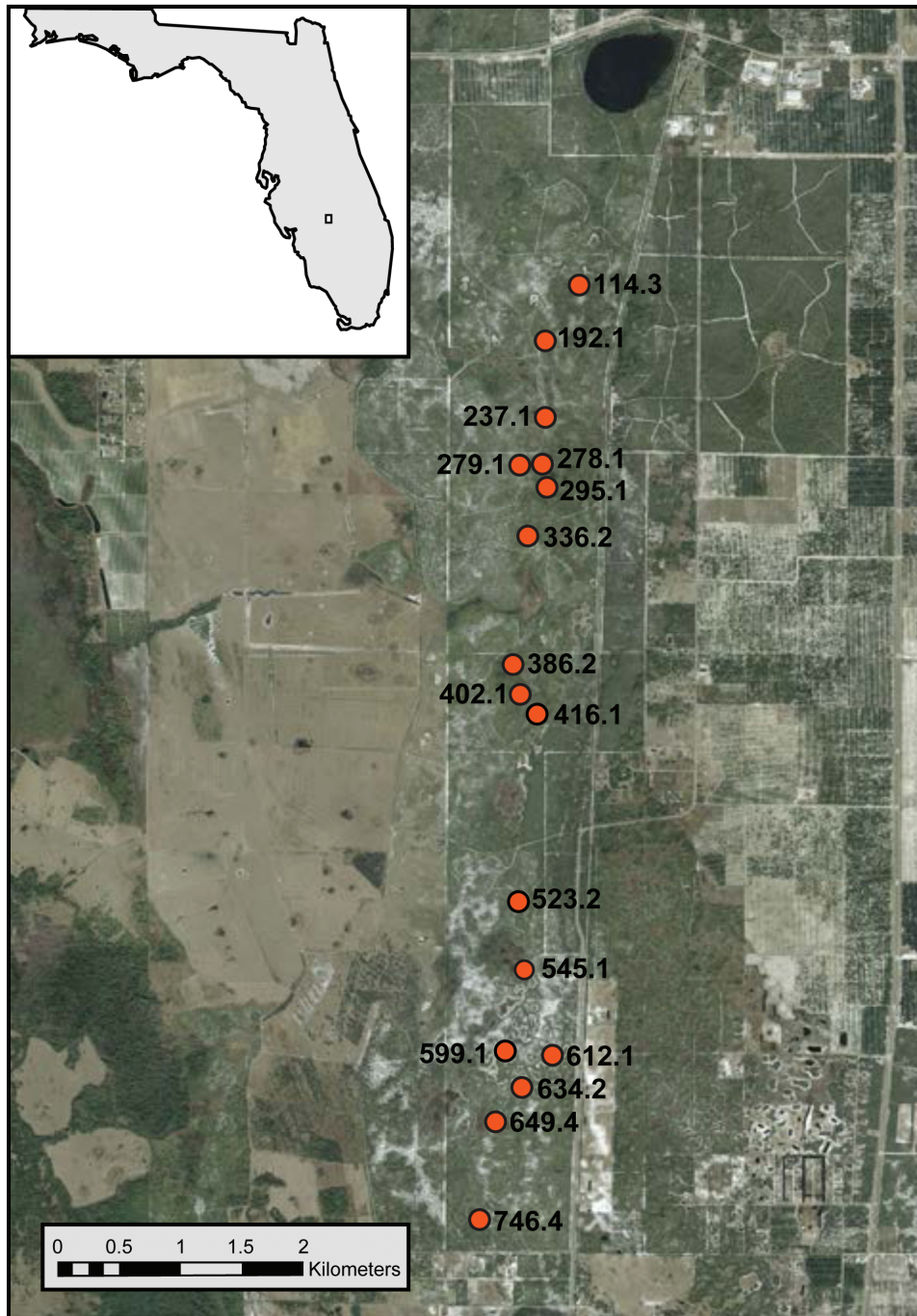


Figure 1. Sampling localities of *Hyla femoralis* at Archbold Biological Station (ABS) located in south central Florida (see inset). Wetland IDs correspond to localities listed in [Table 2](#).

spatial data layers (i.e., GIS shapefiles) converted to a common grid of 1.6 million 5×5 -m cells. First, we ran a query to select all 5×5 -m cells occurring within the 17 sampled ponds. We then queried the database to extract relevant fire-related metrics for this subset of cells, including date of the most recent fire, intensity of the most recent fire, and dates of all fires used for calculating average fire interval and total number of fires. Intensities were mapped from post-fire aerial photographs and rated on a severity scale from 0 = unburned to 3 = litter and vegetation completely consumed (Menges et al.2017). These fire history metrics

cover the period between 1989 and 2012 because 1989 is when ABS began detailed mapping of intensity following every fire.

We classified a pond as “burned” using four different criteria: if any grid cells within the pond were burned, and if at least 20%, 30%, and 40% of the grid cells were burned. For each pond, we calculated several indices to summarize the fire history for each pond under each of these burn criteria. These included number of fires since 1989, date of the most recent fire, average intensity of the most recent fire, and average time between fires.

Table 1. Characteristics of 17 microsatellite loci developed for *Hyla femoralis*

Locus	Panel	Dye	Repeat motif	Primer	Size Range (bp)	NA	H_o	H_e
1130	MP1	6-FAM	AGAT (11)	F: CGAGTTTTCCAGTCACGACAAACCTTCTTCGGCAACTTGTAG R: GTTTCCTTTGGCCAAATCGTCTTATCTATGAC	320–384	14	0.910	0.887
S21	MP1	VIC	AGAT (10)	F: CGAGTTTTCCAGTCACGACTTGAGGCTCCTTGGAGTATTGAG R: GTTTCCTTTGGTGTGCTGCCTATATTTTCATTC	180–240	15	0.621	0.718
S20	MP1	NED	AGAT (11)	F: CGAGTTTTCCAGTCACGACACCGACATGTTAAAGGGTTATCC R: GTTTCCTTTGTTTACACTGTTGCTGTTCTAC	272–364	22	0.925	0.918
1197	MP1	PET	AGAT (14)	F: CGAGTTTTCCAGTCACGACCCTGCTCCTGTTTATCGAAGTAAG R: GTTTCCTATGTAGGCTCTGAAAGTTTGGTG	372–460	23	0.912	0.934
2155	MP2	6-FAM	AGAT (11)	F: CGAGTTTTCCAGTCACGACAAACCCAGCTGACATATCACAAAC R: GTTTCCTCAACCTGTCTGCCCTTAGTAAAG	320–448	28	0.839	0.937
S13	MP2	NED	AGAT (12)	F: CGAGTTTTCCAGTCACGACATCAGCCTTATATGTGCAGAAATG R: GTTTCCTTCTACTTGCCTCACTACATCAGG	148–352	21	0.925	0.920
S26	MP2	PET	AC (14)	F: CGAGTTTTCCAGTCACGACACTCCATATTAAGACCTCCATGAG R: GTTTCCTCAACCACTACCTGTATCACAC	182–234	24	0.900	0.910
2139	MP3	6-FAM	AGAT (10)	F: CGAGTTTTCCAGTCACGACAGTACTCCTCGCTGCAATAAATG R: GTTTCCTTGATCAAGCCTTAATCCCGGTAG	314–390	18	0.928	0.891
1430	MP3	VIC	AGAT (11)	F: CGAGTTTTCCAGTCACGACAGGTGGCTAGAACTATCGGTAAG R: GTTTCCTTATACAGAGGGGTGTCACAGTG	305–373	18	0.916	0.916
S6	MP3	PET	AGAT (14)	F: CGAGTTTTCCAGTCACGACACAGACCAGTACAACATGCTTTG R: GTTTCCTAAATGGAAGGAGTATCAGAGCAC	188–272	19	0.925	0.909
373	MP4	6-FAM	AGAT (13)	F: CGAGTTTTCCAGTCACGACTGCATATTTGGATTGAGGAACCG R: GTTTCCTCAACTAAGAGGCTAAGTAACTTGC	402–522	25	0.932	0.933
124	MP4	VIC	AGAT (12)	F: CGAGTTTTCCAGTCACGACAACAGAGCAACAGACTAAGCATC R: GTTTCCTTGGTTTAGGGCTGATTAACGACTC	346–474	31	0.955	0.950
1630	MP4	NED	AGAT (11)	F: CGAGTTTTCCAGTCACGACAGGTTCTCAATGTATGGAGATGC R: GTTTCCTTGCTCCAATCCCTGAACAGTTTAG	256–308	14	0.859	0.868
1555	MP4	PET	AGAT (12)	F: CGAGTTTTCCAGTCACGACAGACCAGTACTTGTACCCTG R: GTTTCCTGATAGGCTGAAACAATTGGTGC	154–246	24	0.933	0.923
S15	MP5	6-FAM	AGAT (11)	F: CGAGTTTTCCAGTCACGACGCCACTAATTTGTCACTACTTTGG R: GTTTCCTTTGTTGCACCTTTGTTTGTTCATG	182–238	14	0.720	0.885
1163	MP5	VIC	AGAT (14)	F: CGAGTTTTCCAGTCACGACGTGATACTGATACGTGAACCTGC R: GTTTCCTTAGGCTGCTTTAAGGTCCAAATG	351–423	19	0.899	0.906
2423	MP5	NED	AGAT (13)	F: CGAGTTTTCCAGTCACGACTTCCAGGGCCAAATTATAAGCC R: GTTTCCTTGCTGAAAGAGGTCTATAAGCAG	384–448	13	0.760	0.763

Loci were amplified in five multiplex (MP) reactions and labeled with dye for visualization. Repeat motif, PCR primers, allele size range, the number of alleles (NA), and observed and expected heterozygosity provided for each locus. A tag on the 5' end of each forward primer was added during the optimization phase of microsatellite development (CGAGTTTTCCAGTCACGA).

Genetic data collection

Total genomic DNA was extracted from tissue samples using the DNeasy Animal Tissue Kit (Qiagen, Valencia, CA). We developed a new microsatellite library for this species using Illumina sequencing in collaboration with the Evolutionary Genomics Core Facility at Cornell University (Table 1). Candidate loci were tested on a set of 24 individuals to verify clean amplification, variability, and no evidence of linkage using MICROCHECKER (van Oosterhout et al. 2006) and GENEPOP (Rousset 2008).

We optimized a final set of 17 loci into five multiplex PCR reactions with dye-labeled forward primers (6-FAM, Integrated DNA Technologies; PET, VIC, and NED, Applied Biosystems). We performed multiplexed PCR reactions using the QIAGEN Type-it Microsatellite PCR kits in a total volume of 12.5 μ l, each containing 6.25 μ l of Type-it Master Mix, 0.1 μ l of BSA, 1.25 μ l of the primer mix (each primer at 2 μ M), and \sim 70 ng/ μ l template DNA. The thermocycling conditions for all reactions consisted of: 95 $^{\circ}$ C for 10 min; 35 cycles at 95 $^{\circ}$ C for 30 s, 60 $^{\circ}$ C for 30 s, 72 $^{\circ}$ C for 30 s; and a final extension at 60 $^{\circ}$ C for 30 min. PCR products were mixed with HiDi formamide and GeneScan 500 LIZ ladder (Applied

Biosystems) and read on an ABI 3730xl DNA analyzer (Life Sciences Core Laboratories at Cornell University). Fragment sizes were manually confirmed using the microsatellite plug-in with Geneious v7.17 (Kearse et al. 2012).

We assessed conformity of genotype proportions to Hardy-Weinberg equilibrium (HWE) using exact tests and tested for linkage disequilibrium (LD) across all pairs of loci using GENEPOP v4.3 (Rousset 2008). Markov chain parameters for all comparisons used 10,000 dememorization steps, 200 batches, and 10,000 iterations per batch. We also used GENEPOP to examine microsatellite loci for evidence of null alleles and scoring error due to stutter or large allele dropout.

Data analyses

The final dataset of genotypes at 17 loci fit HWE expectations and did not show evidence for linkage disequilibrium or null alleles following sequential Bonferroni correction. We used two methods to assess overall population genetic structure of *H. femoralis*. First, we used the individual-based clustering analysis in STRUCTURE v2.3.3 (Pritchard et al. 2000) to determine the number of distinct genetic

groups of *H. femoralis* throughout ABS. We conducted 10 independent runs each for a range of possible genetic clusters ($K = 1$ to 8). We used an initial burn-in of 1 million steps followed by an additional 1 million steps. Correlated allele frequencies and admixture were assumed. We identified the most likely number of genetic clusters by visual examination of the STRUCTURE plots and by using the ΔK method (Evanno et al. 2005). Second, we analyzed pairwise genetic differentiation (F_{ST}) among breeding sites using FSTAT version 2.9.3.2 (Goudet 1995). Individuals were grouped by breeding aggregation, but we omitted three sites due to low sample size (≤ 7 individuals). A non-significant F_{ST} after applying a global Bonferroni correction for 81 pairwise tests indicated a lack of allele frequency differences between any two breeding sites.

To test whether spatial differences in fire history explain landscape-level variation in genetic diversity, we estimated a set of genetic diversity metrics for the 14 breeding aggregations with more than seven sampled individuals. Observed and expected heterozygosity within breeding aggregations were estimated using ARLEQUIN 3.5.2.2 (Excoffier and Lischer 2010) for the subset of loci for which we had adequate data. Rarefied allelic richness accounting for sample size was estimated using FSTAT version 2.9.3.2 (Goudet 1995). We assumed a diploid population of seven individuals, the smallest sample included. For each breeding aggregation we also estimated the effective population size (N_e) and 95% credible limits of the estimate including all loci for which there was adequate data (between 15 and 17 loci) using ONeSAMP (Tallmon et al. 2008). We tested the effects of wetland area and a suite of fire history variables (number of years since last fire, average number of years between fires, total number of fires, and average intensity of most recent fire) on multiple genetic diversity indices (effective population size, allelic richness, and heterozygosity) using linear regression in R 3.2.3. Prior to conducting analyses, we tested variables for normality using a Shapiro–Wilk test. Effective size was natural log transformed and the average intensity of the most recent fire was squared prior to regression analyses. Significance of regressions was determined after applying Bonferroni corrections to account for the three tests involving each fire history variable (corrected $\alpha = 0.05/3 = 0.017$).

Results

We found evidence of null alleles in global tests for three loci with a frequency greater than 0.025, but less than 0.1. For two additional loci, data were missing for two of the 17 sampled ponds. Thus, we excluded these five loci when estimating within-population heterozygosity and allelic richness. Overall levels of genetic diversity were high within *H. femoralis* breeding aggregations (Table 2). Observed heterozygosity ranged from 0.85 to 0.90 and allelic richness ranged from 10.5 to 11.2 alleles per locus. Mean estimates of effective population sizes ranged from 67.8 to 571.8 individuals. N_e was not reported for two sites because estimates in ONeSAMP were not consistent across replicate runs. The multilocus genotype for each individual is available in Dryad (Robertson et al. 2017; doi:10.5061/dryad.33pm5).

Contrary to expectations, we found no evidence of fine-scale population structure among ABS ponds. Low pairwise F_{ST} estimates revealed little difference in allele frequencies among *H. femoralis* sampled from different ponds throughout ABS (Table 3). We did not recover significant differentiation between any pairs of populations in FSTAT after Bonferroni correction. For Bayesian assignment tests in STRUCTURE, the Evanno et al. (2005) method recovered $K = 2$, but this approach is not capable of recovering $K = 1$. Based on visual

inspection of the individual assignment plots (Figure 2), we interpret $K = 1$ for ABS sites.

Results from the linear regressions were not dependent on which burn criterion was used. We therefore report results based on fire metrics that were calculated from all grid cells that burned in a given fire event. We found a positive relationship between effective population size (N_e) and average intensity of the most recent fire (Figure 3D; $R^2 = 0.42$; $P = 0.024$), although this result was not significant based on the Bonferroni-corrected $\alpha = 0.017$. There was also a positive, but non-significant trend between allelic richness and the total number of fires (Figure 3J; $R^2 = 0.10$; $P = 0.169$). The remaining fire history metrics and wetland area were not strong predictors of within-pond genetic diversity (Figure 3A–O).

Discussion

Our study was the first to investigate the effects of fire history on an amphibian in the Florida scrub, and one of few studies to examine the effects of fire on genetic diversity in pond-breeding frogs (Potvin et al. 2017). We found that variation in fire history metrics had a largely neutral or slightly positive effect on genetic diversity and did not restrict gene flow among *H. femoralis* at the spatial scale examined in this study. We found no evidence of genetic structure and high levels of genetic connectivity among *H. femoralis* throughout ABS, suggesting that these breeding ponds behave as a single panmictic population.

Effects of fire on genetic diversity

We found a positive relationship between effective population size (N_e) and average intensity of the most recent fire ($R^2 = 0.42$, $P = 0.024$; Figure 3D). Although this relationship was not statistically significant, we think it reflects the effects of an extreme fire event that occurred 11 years ago. The relationship between N_e and average intensity was driven largely by five ponds that burned with high intensity in a 317-ha wildfire during the dry season in 2001. Mean N_e of these ponds averaged 283 (range 154–572) compared to 217 (range 68–555) for the remaining ponds, which were burned in prescribed fires during the wet season (late May–October). Fire intensity is greatly influenced by fuel conditions and moisture levels; during dry periods, vegetation within and adjacent to dry ephemeral ponds burns more completely, which may result in short-term increases in nutrient availability (Dean et al. 2015) and periphyton (Venne et al. 2016). Thus, juvenile recruitment of treefrogs may be higher following more intense fires, resulting in increased effective breeding sizes compared to ponds that burned with less intensity. This and alternative explanations, such as enhanced immigration into recently burned breeding sites, are interesting avenues for future research. We also found a positive trend between total number of fires and allelic richness suggesting that fire frequency has a slightly positive effect on genetic diversity. This trend is opposite to what was found in a study of two *Litoria* frogs that found reductions in diversity and effective population sizes following a fire (Potvin et al. 2017). Taken as a whole, our results point to a relatively neutral (if not slightly positive) effect of fire on genetic diversity in *H. femoralis*.

In contrast to our findings, fire history has been shown to affect patterns of genetic diversity and connectivity at Archbold Biological Station (ABS) in different ways for several other species. The Florida sand skink showed higher levels of genetic diversity and larger effective population sizes in areas with greater time since fire (Schrey et al. 2011a; Schrey et al. 2011b; Schrey et al. 2016). Additionally,

Table 2. Sample size, location, genetic diversity, and fire history metrics for each breeding aggregation

Wetland ID	N	Latitude	Longitude	Ho	He	Ar	N_e (95% CI)	Wetland area (m ²)	YSF	Int.	(N_{fires})	Intensity
114.3	30	27.191593	-81.354869	0.88	0.89	11.1	219 (132, 513)	147	3	10	3	2.06
192.1	27	27.187476	-81.357635	0.88	0.89	10.9	141 (97, 389)	141	3	5.5	3	1.65
237.1	23	27.181852	-81.357596	0.86	0.88	10.5	154 (107, 363)	474	11	NA	1	2.37
278.1	29	27.178409	-81.357825	0.90	0.89	11.0	229 (150, 643)	69	11	5	2	3.00
279.1	30	27.178318	-81.359731	0.87	0.89	11.0	271 (175, 690)	81	11	5	2	2.11
295.1	26	27.176712	-81.357457	0.86	0.89	11.1	188 (113, 440)	276	11	5	2	3.00
336.2	30	27.173160	-81.359017	0.85	0.89	10.9	572 (329, 1887)	382	11	NA	1	3.00
386.2	25	27.163691	-81.360219	0.87	0.89	11.0	161 (110, 418)	37	4	4.7	4	2.57
612.1	19	27.140384	-81.358540	0.87	0.89	10.8	68 (54, 119)	112	8	NA	1	1.18
634.2	28	27.130092	-81.361523	0.86	0.89	10.5	135 (95, 299)	164	4	NA	1	2.13
746.4	40	27.132609	-81.359373	0.87	0.88	10.9	NA ^a	183	NA	NA	0	NA
402.1	29	27.161508	-81.359619	0.89	0.9	11.2	NA ^a	351	8	7	2	0.27
649.4	30	27.134990	-81.356865	0.88	0.89	10.8	555 (346, 1745)	267	4	NA	1	2.69
545.1	34	27.141822	-81.360747	0.88	0.89	10.7	238 (140, 483)	NA	2	8.5	3	2.13
416.1	6	27.160028	-81.358277	NA	NA	NA	NA	NA	NA	NA	NA	NA
523.2	7	27.146266	-81.359760	NA	NA	NA	NA	NA	NA	NA	NA	NA
599.1	2	27.135274	-81.360822	NA	NA	NA	NA	NA	NA	NA	NA	NA

See [Figure 1](#) for locations of wetlands. The number of years since last fire (YSF), the average interval in years between fires (Int.), total number of fires (N_{fires}), and average intensity of most recent fire (Intensity) are provided for each pond.

^aEffective population size estimates were excluded because different random starting seeds produced very different estimates.

Table 3. Pairwise F_{ST} for 14 breeding aggregations of *H. femoralis* for which more than seven individuals were genotyped

Wetland ID	192.1	114.3	237.1	278.1	279.1	295.1	336.2	386.2	402.1	545.1	612.1	649.4	634.2
192.1	—												
114.3	0.0004	—											
237.1	0.0042	-0.0005	—										
278.1	0.0049	0.0001	0.0017	—									
279.1	0.0033	-0.0006	-0.0009	-0.0007	—								
295.1	0.0009	-0.0021	0.0000	0.0019	-0.0001	—							
336.2	0.0064	-0.0026	0.0001	0.0018	-0.0001	-0.0008	—						
386.2	0.0070	0.0007	-0.0013	-0.0012	-0.0013	0.0032	-0.0009	—					
402.1	0.0018	0.0008	0.0005	-0.0011	-0.0014	-0.0009	0.0018	0.0013	—				
545.1	0.0029	-0.0007	0.0049	0.0025	-0.0016	0.0010	0.0006	0.0033	0.002	—			
612.1	-0.0014	-0.0027	0.0027	0.0008	-0.0003	-0.0007	-0.0033	-0.0017	0.0021	0.0032	—		
649.4	0.0037	0.0001	0.0033	-0.0007	-0.0016	0.0036	0.0040	-0.0010	0.0005	0.0003	0.0010	—	
634.2	0.0056	-0.0009	0.0036	-0.0026	-0.0030	0.0028	0.0029	-0.0001	0.0032	0.0006	0.0010	-0.0014	—
746.4	0.0027	-0.0007	0.0019	0.0016	-0.0011	-0.0009	-0.0002	-0.0013	0.0022	0.0047	-0.0032	0.0005	0.0030

Wetland IDs correspond to [Figure 1](#). No pairwise comparisons were significantly different from zero after Bonferroni correction.

Florida sand skinks at ABS showed an overall pattern of isolation by distance, but that pattern was disrupted by patches of recently burned scrub ([Schrey et al. 2011b](#)). In contrast, Florida scrub lizards ([Schrey et al. 2011a](#)), six-lined racerunners ([Ragsdale et al. 2016](#)), and an endemic perennial plant, *Hypericum cumulicola* ([Dolan et al. 2008](#)), showed the highest levels of genetic variation in more recently burned sites. Based on these previous findings of fire-mediated variation in genetic diversity at ABS, we were surprised not to detect a strong effect of fire on population genetic patterns in *H. femoralis*. Our results suggest that taxonomic differences in life history and association with unpredictable breeding habitat (i.e., ephemeral ponds) could underlie variable responses to fire across taxa, even in strongly fire-associated landscapes like the Florida scrub.

Effective population size and genetic structure at fine spatial scales

Estimates of N_e were relatively high in our study (average N_e ranges from 68 to 572; SD = 158; [Table 2](#)) compared to most other

pond-breeding amphibians ([Frankham 1995](#); [Schmeller and Merilä 2007](#), [Wang et al. 2011](#), [Guicking et al. 2017](#)). Variation in effective population sizes can be affected by the extent of genetic isolation, reproductive skew, and asymmetry in sex ratio. Among-population variation in N_e can also be shaped by differences in evolutionary history and/or landscape features ([Phillipsen et al. 2011](#)). In the endangered California tiger salamander (*Ambystoma californiense*) estimates of effective population sizes were typically low at breeding ponds (average $N_e = 30$), but were positively related to pond area, even after accounting for genetic isolation, suggesting that pond suitability (including area) also plays a strong role in enhancing N_e ([Wang et al. 2011](#)). In our study, we did not detect an effect of pond area on N_e ([Figure 3C](#)), but this could be due to our study having fewer ponds or a narrower range of pond sizes relative to the [Wang et al. \(2011\)](#) study. Given high observed levels of genetic connectivity at spatial scales up to 6.8 km, it is likely that high gene flow is maintaining large effective population sizes for *H. femoralis* populations.

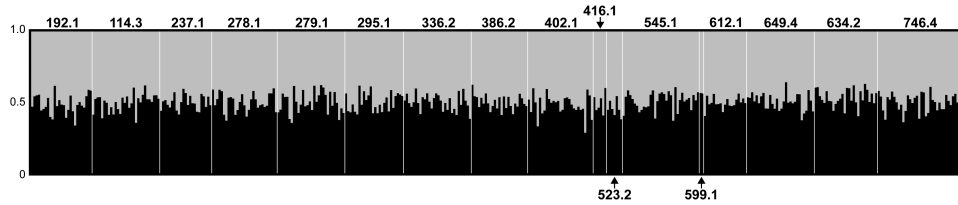


Figure 2. Assignment probabilities for individuals from 17 wetlands for $K = 2$. Bayesian clustering analyses conducted in STRUCTURE support a single genetic deme ($K = 1$) for all sampling sites.

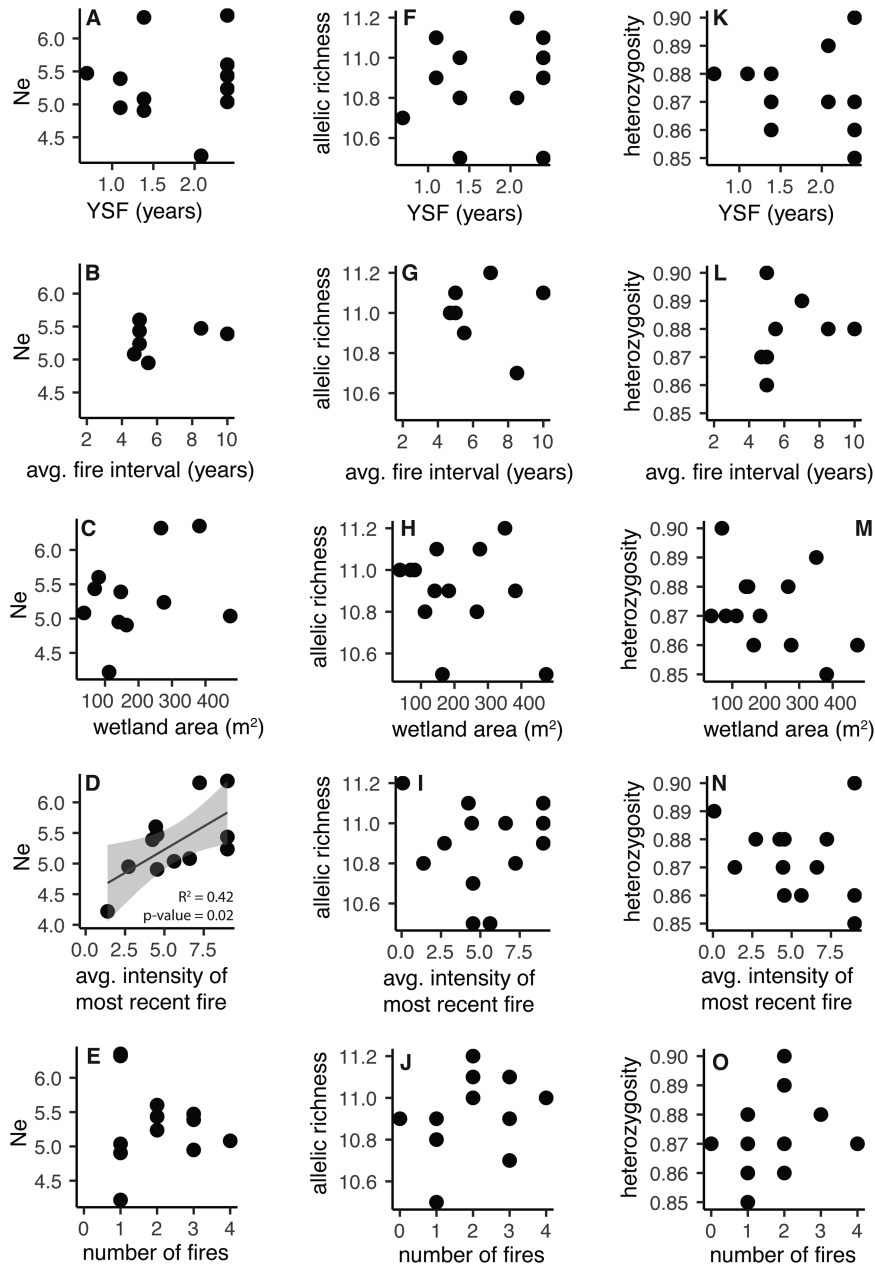


Figure 3. Panels A–O illustrate relationships between environmental variables hypothesized to affect genetic diversity (four fire metrics plus wetland area) and three genetic diversity indices (N_e , allelic richness, and heterozygosity). Results from the only significant linear regression are indicated in the bottom right corner of panel D. Time since fire (TSF) and effective population size (N_e) were log transformed and average intensity of most recent fire was squared.

We did not find evidence for genetic structure in *H. femoralis* at the spatial scale of our study, which could be explained in part by the natural history of this species. Breeding by *H. femoralis* is

prolonged throughout late spring and summer, although at ABS reproductive activity tends to be most concentrated during the early part of the rainy season in June–July. We detected no pattern of

isolation by distance for *H. femoralis* at ABS and estimates of pairwise genetic distance did not differ significantly from zero, indicating widespread genetic connectivity across this intact landscape. Although there have been no direct studies of philopatry or dispersal ability in this species, our data suggest the dominant terrestrial habitats (flatwoods and scrubby flatwoods) do not pose significant barriers to dispersal.

The conventional view for amphibians is that they are dispersal-limited and thus highly genetically structured (Rowe et al. 2000; Shaffer et al. 2000). Indeed, the spatial scale of our study (interwetland distances of 0.16–6.6 km) is comparable to the geographic range of other studies of pond-breeding amphibians that have demonstrated fine-scale population genetic structure (Burns et al. 2004; McKee et al. 2017; see review by Chan and Zamudio 2009). However, there are also many counter examples of pond-breeding frogs (Newman and Squire 2001; Brede and Beebe 2004; Nunziata et al. 2013; Coster et al. 2015; Roth and Jehle 2016) and salamanders (Wang et al. 2011) that exhibit high levels of gene flow at this scale. For example, a pond-breeding hyliid (*Hyla arborea*) colonized new ponds separated by distances of 2–5 km, even across relatively unsuitable habitat (Le Lay et al. 2015). *Litoria aurea*, a pond-breeding frog that shows substantial structuring at large spatial scales, exhibits genetic connectivity at scales < 5 km (Burns et al. 2004). These examples reinforce the notion that life history and dispersal abilities should be accounted for when examining population genetic structure of pond-breeding amphibians.

Two other factors may explain panmixia in *H. femoralis* across ABS. First, the high density of ponds (Figure 1) translates into relatively short interpond distances, which in some cases may be easily traversed by adult treefrogs in a single rainy night. Secondly, the flat topography facilitates sheet flow and hydrological connectivity during extreme rainfall events. Since 1989, when ABS began monitoring pond hydrology, there have been two extreme weather events - multiple hurricanes in 2004–2005 and El Niño in 2015–2016 - that caused prolonged (> 1 year) inundation of scrub wetlands and allowed temporary connections to form among normally isolated ponds (ABS, unpubl. environmental monitoring datasets). Under such conditions, even fully aquatic larval amphibians may be able to disperse between adjacent wetlands. Although the direct effects on dispersal have not been documented, it is likely that a combination of these environmental factors serves to homogenize allele frequencies across breeding sites.

Effects of fire on amphibians in Florida scrub

Although fire did not seem to affect patterns of diversity or gene flow in *H. femoralis*, it could still have important effects on within-site recruitment and population dynamics of this species. A recent field experiment at ABS suggested that larval oak toads have higher survivorship in more recently burned ponds (Noss and Rothermel 2015). Fire effects on juvenile recruitment of *H. femoralis* have not been investigated, but adults seem to persist and/or recolonize ponds quickly post fire. For instance, *H. femoralis* tadpoles were detected in a pond at ABS just 2 months after a high-intensity prescribed fire that burned the pond and adjacent uplands (H. Goldspiel and B. Rothermel, unpubl. data). Thus, fire frequency and intensity may play an important role in metapopulation dynamics with respect to within-patch recruitment or persistence.

Florida scrub is an unpredictable environment from the perspective of a pond-breeding anuran. Amphibians that occur in

unpredictable environments exhibit a “colonizer syndrome” (Cayuela et al. 2016) characterized by short life spans, high recruitment, and high dispersal capacity. Considering the unpredictable hydrology of breeding sites and timing of fires, populations of *H. femoralis* in Florida scrub are good candidates for the colonizer syndrome. A similar lack of population structure has been found for other amphibians in arid environments where ponds fill unpredictably (Chan and Zamudio 2009) and there is presumed selection against philopatry.

Conclusions

Our study adds to a growing body of evidence that while within-site characteristics might govern population dynamics and recruitment, environmental unpredictability could mediate patterns of population connectivity in pond-breeding amphibians. Somewhat counter to our expectations for a species endemic to a fire-dependent ecosystem, we found little evidence of an effect of fire on effective population sizes, genetic isolation among ponds, or genetic diversity in *H. femoralis*. We observed two non-significant trends: increased effective population sizes and increased genetic diversity with increased fire intensity and frequency. Thus, fire may have positive genetic effects on *H. femoralis* in some situations. Observational or experimental studies of breeding site preference, larval recruitment, and adult survival in relation to fire could provide insights regarding the mechanisms contributing to this species' high resilience to differing fire regimes. For land managers charged with conserving Florida scrub biodiversity, our results suggest that applying a wide range of prescribed fire regimes to benefit habitat specialists will not incur strong trade-offs in terms of maintaining gene flow for this common species. However, given the taxonomic and ecological diversity of amphibians, there is a need for similar studies of other anurans associated with Florida scrub.

In general, life-history traits are likely to mediate the extent to which patterns of genetic diversity and connectivity will be impacted by fire history and thus may provide better predictors of population genetic connectivity than fire history itself. For example, for the Florida scrub-jay at ABS, relatively strong philopatry and small home ranges result in strong population genetic structure at the same spatial scale as our study (Aguillon et al. 2017), whereas limited dispersal ability explains genetic subdivision of two species of scrub-associated lizards (Schrey et al. 2011a). Our results indicate that *H. femoralis*, an abundant hyliid, responds differently to fire than reptiles and other taxa in this fire-dependent ecosystem do. Overall, our results are consistent with an emerging pattern that dispersal capacity and life-history characteristics have substantial impacts on organisms' responses to fire.

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Data Availability

Microsatellite data used in this study is available at the Dryad Digital Repository doi:10.5061/dryad.33pm5

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