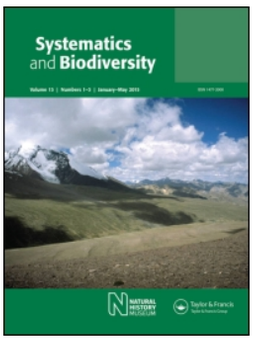

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Research Article

Colonization and divergence: phylogeography and population genetics of the Atlantic coast beach mice

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Barrier island taxa provide an opportunity to investigate recent evolutionary processes, such as colonization and isolation of recently diverged taxa, and provide important insights into understanding contemporary diversity and the assessment of conservation units. Using rapidly evolving genetic markers (mitochondrial DNA and microsatellites), we studied the Atlantic coast beach mouse subspecies (*Peromyscus polionotus decoloratus*, *P. p. niveiventris*, and *P. p. phasma*). Our data indicate that each of the extant coastal subspecies (*P. p. niveiventris* and *P. p. phasma*) is comprised of unique haplotypes indicative of their isolation, while the extinct subspecies, *P. p. decoloratus*, contain a single haplotype, which was shared with *P. p. phasma*. Moreover, all the coastal haplotypes originate from a single mainland haplotype found in central Florida, USA. The microsatellite data indicated high levels of genetic structure among our sampled populations. Additionally, these data group the populations into three distinct genetic clusters, with each of the extant coastal subspecies belonging to their own cluster and the mainland individuals forming a separate cluster. The extant Atlantic coast beach mice are on separate evolutionary trajectories, thus representative of separate taxonomic units. Therefore, the data support that two extant subspecies on the Florida Atlantic coast fit the Distinct Population Segment designation and should be managed and conserved as two separate independent units.

Key words: cytochrome b; DPS; endangered and threatened taxa; *Peromyscus polionotus*; STRUCTURE

Introduction

Islands, especially oceanic islands, have been widely used to evaluate consequences of colonization (Cowie & Holland, 2006; Losos & Ricklefs, 2010; MacArthur & Wilson, 1967). Most prior studies have been limited to oceanic islands with diverse geological origins, long periods of isolation from the mainland of continents and a history of colonization–extinction dynamics. In contrast, few studies have considered barrier islands in the context of colonization–extinction dynamics. These landforms are found closely associated with a mainland and originate from wave and wind actions. Barrier islands manifest as relatively narrow bands of sand, which are formed parallel to mainland coastlines (Johnson & Barbour, 1990). Barrier islands in North America (Bryan, Scott, & Means, 2008; Davis, 1997) and Europe (Kolditz et al., 2012; Madsen, Murray, Andersen, & Pejrup, 2010) have their origins during the Holocene. These islands are impacted by high rates of sea level change interrupting patterns of sand deposition (Rosati & Stone, 2009). With the rapid sea level rise that occurred after the last glacial maximum in North America, conditions were thus unfavourable for barrier islands formation (Davis, 1997). As sea level stabilized around 6,000 years before present, near current levels, conditions were again favourable for the formation of barrier islands (Davis, 1997; MacNeil, 1950). Therefore, species inhabiting barrier islands could only have colonized these islands very recently and offer novel opportunities to investigate the impacts of colonization on the evolutionary history of recently diverged taxa.

A species that has been greatly influenced by the recent formation of barrier islands is *Peromyscus polionotus* (old field mouse), which primarily occupies habitat with sandy soil in south-eastern USA (Whitaker & Hamilton, 1998). Much attention has been given to subspecies occupying coastal barrier islands of Alabama and Florida, with emphasis on spatial variation of

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morphological characters (Bowen, 1968; Hoekstra, Hirschmann, Bundey, Insel, & Crossland, 2006; Mullen, Vignieri, Gore, & Hoekstra, 2009; Sumner, 1926). These subspecies are collectively called beach mice and each subspecies exhibits lighter pelage colour compared with mainland conspecifics (Bowen, 1968; Hoekstra et al., 2006). The phenotypic variation in pelage colour has been attributed to different selective pressures for crypsis, based on correlations between pelage and soil colour found on the different barrier islands (Mullen & Hoekstra, 2008). The origins of the beach mice subspecies are hypothesized to be recent events associated with the Holocene formation of the barrier islands (Hoekstra et al., 2006). For the subspecies occupying the Gulf coast barrier islands, Bowen (1968) hypothesized, based on pelage colour, that the diversity was the result of multiple colonization events from mainland populations after the stabilization of the barrier islands when the Gulf was near current levels. This hypothesis was challenged by molecular data that supported an older establishment of Gulf coast taxa. It was postulated that beach mice tracked the receding shore line and became isolated (Van Zant & Wooten, 2007). Others, however, have found evidence of a single colonization of Gulf coast beach mice, but at a much more recent time than previously claimed (Domingues et al., 2012). In comparison to the multiple studies conducted and hypotheses generated for Gulf coast beach mice, the evolutionary history and colonization patterns of beach mouse subspecies occupying the Atlantic coast have received less attention in the literature. However, for the Atlantic coast beach mice Bowen (1968) proposed a single colonization event from a mainland source, with subsequent isolation on the barrier islands.

In this study we aimed to determine the evolutionary history of the beach mice occupying the Atlantic coast of Florida, using rapidly evolving genetic markers. First, we sought to evaluate lineage differentiation on the Atlantic coast barrier islands. There are three recognized subspecies occupying the Atlantic coast (Hall, 1981): *P. p. phasma* (Anastasia beach mouse), *P. p. decoloratus* (pallid beach mouse – although recently extinct), and *P. p. niveiventris* (south-eastern beach mouse). Subspecies are often used as a means of partitioning within species variation, and are frequently based on phenotypic variation (O'Brien & Mayr, 1991). However, several studies have shown that phenotypic variation may not represent independent evolutionary trajectories, especially on islands (e.g., Burbrink, Lawson, & Slowinski, 2000; Culver, Johnson, Pecon-Slattery, & O'Brien, 2000; Hull et al., 2008; Tursi, Hughes, & Hoffman, 2013) and therefore the biological validity of these subspecies should be tested. A study of Gulf Coast beach mice supported the correlation between phenotypic variation as indicated by subspecies designation and genetic differentiation (Mullen et al., 2009). We tested the hypothesis that the three Atlantic coast subspecies each maintain independent evolutionary trajectories, and can be considered as separate taxonomic units. Then, we evaluated the colonization patterns of the Atlantic coast beach mice using sequence and genotype data. We tested Bowen's (1968) single colonization hypothesis, where the Atlantic coast was colonized from a single mainland source, with subsequent processes shaping current diversity. Alternatively, the Atlantic coast beach mice could have colonized the barrier islands from multiple mainland sources, where differences among sources impacted current diversity. Finally, we tested whether the genetic diversity of Atlantic coast beach mice follows the assumptions of island populations, with founder effects and smaller effective population sizes resulting in lower diversity compared with mainland lineages (Frankham, 1997). We hypothesized that the Atlantic coast beach mouse subspecies should have lower genetic diversity compared with mainland conspecifics. Of the three Atlantic coast beach mice, one is recently extinct (Humphrey, 1992) and the extant subspecies are listed as endangered or threatened (U.S. Fish and Wildlife Service, 1989). Therefore, we conclude by discussing implications for conservation efforts related to these taxa and the areas they inhabit.

Materials and methods

Sampling and DNA extraction

We obtained a total of 492 specimens for this study from 20 locations representing the distribution of *P. polionotus* on peninsular Florida and that of extant Atlantic coast beach mice (Fig. 1). Of those, 490 live specimens were captured in the field using Sherman live traps as described by Degner et al. (2007). Traps on grids and transects were baited with large sunflower seeds and opened in the afternoon and checked early the next morning. Sterilized scissors were used to excise a portion of the tail tip (2–4 mm) of each new capture and then the animal was released. We trapped 69 individuals that we expected to represent two mainland *P. polionotus* subspecies; *P. p. subgriseus* (SRWEA and ONF), and *P. p. rhoadsi* (LARA, LLSP, APAFR, and ABS) (Table S1, see supplemental material online). We collected 77 individuals from four locations representing the range of the Anastasia beach mouse, *P. p. phasma* (Table S1, see supplemental material online). We collected 344 individuals across the current range of the other extant south-eastern beach mouse, *P. p. niveiventris* (Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online

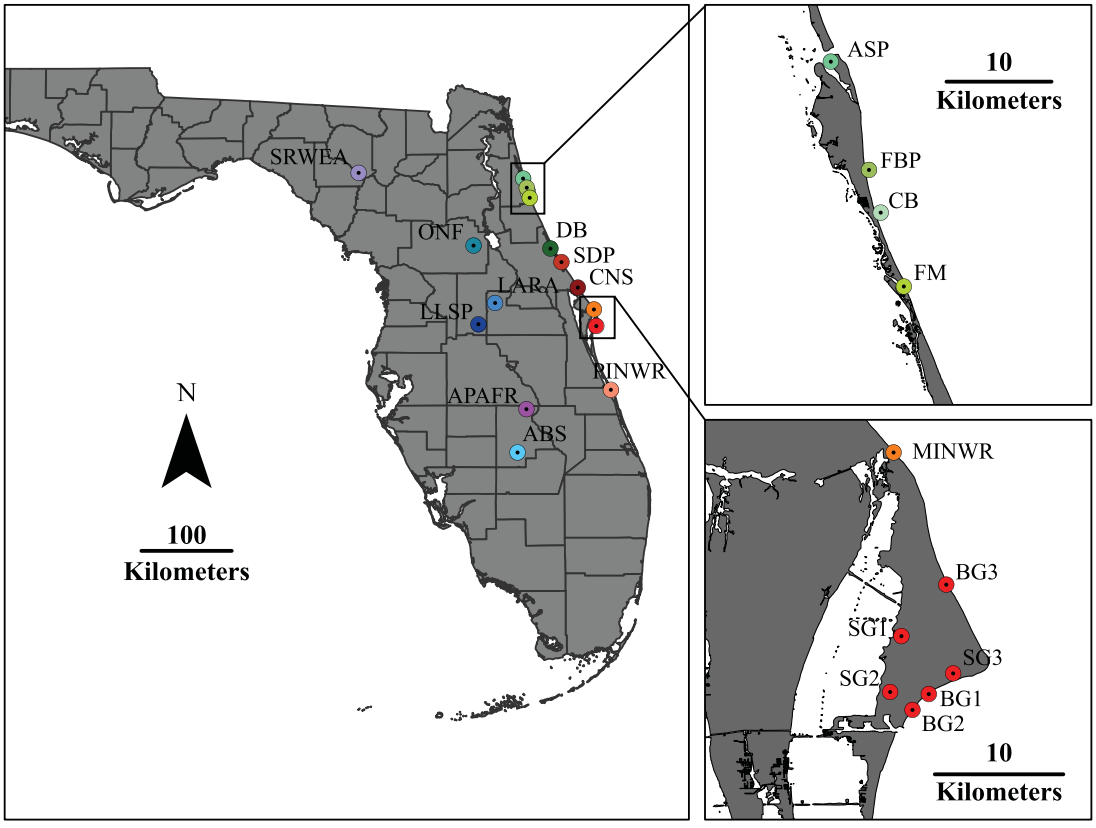

Fig 1. Localities of specimens acquired for this study. Circles designate a collection site, and are colour coded by taxonomic grouping. Mainland subspecies were represented as blue and purple: *P. p. subgriseus* (SRWEA and ONF), *P. p. rhoadsi* (LARA, LLSP, APAFR, ABS). Atlantic coast beach mice collected were: *P. p. phasma* (ASP, FBP, CB, FM – green), *P. p. decoloratus* (DB – dark green), and *P. p. niveiventris* (SDP, CNS, MINWR, BG1-3, SG1-3, PINWR – red-orange). See Table S1 (see supplemental material online) for location abbreviations.

page at http://dx.doi.org/10.1080/14772000.2018.1486339). Tissue from each individual was stored in 95% ethanol at −20 °C prior to DNA extraction. We also acquired two museum specimens of the extinct pallid beach mouse, *P. p. decoloratus*, from the Museum of Southwestern Biology (MSB 64761 and MSB 64762). These two specimens were collected on Daytona Beach (DB) in 1946. For each museum specimen, we collected tissue as a $4 \times 4\,mm^2$ section of skin taken from the venter.

We extracted genomic DNA from all tissue samples using a DNeasy tissue purification kit (Qiagen Inc.), following the manufacturer's protocols. Before extraction, the fresh tissue was lysed for 2–4 hours, until dissolved. The two museum tissues were soaked in 95% ethanol at 4 °C for 24 hours to remove any PCR inhibitors (Mullen & Hoekstra, 2008), then lysed for 24 hours until dissolved.

Genetic markers

We amplified and sequenced 1100 base pairs (bp) of the mitochondrial cytochrome *b* (*cyt b*) gene for all fresh tissue specimens following the protocol described in Herron et al. (2004). The DNA from the *P. p. decoloratus* museum specimens was degraded; therefore, we followed the procedures given in Kalkvik et al. (2012), and amplified seven 200–300 bp amplicons to generate the complete gene sequence. All sequences were processed on an ABI 3730 DNA analyser by the Nevada Genomics Center (Reno, NV). Sequences were edited in Sequencher v.4.8 (Gene Codes, Ann Arbor, MI) and aligned by eye in GeneDoc v.2.7 (Nicholas, Nicholas, & Deerfield, 1997).

We included rapidly evolving nuclear markers by genotyping 10 microsatellite loci for mainland and Atlantic coast subspecies. We utilized the following 10 microsatellite loci: pml-02, pml-06, pml-11 (Chirhart, Honeycutt, & Greenbaum, 2000); PO-25, PO-71, PO-105, PO3-68, PO3-85 (Prince, Glenn, & Dewey, 2002); and ppa-01 and ppa-46 (Wooten, Scribner, & Krehling, 1999). We conducted the PCR reactions in 25 µL volumes containing 1–10 ng DNA, 2.5 µL PCR buffer, 0.3 units *Taq* polymerase (Proligo), 0.2 µM of forward and reverse primer, 0.8 mM combined concentration of DNTPs, and 1.5–2.5 mM MgCl. We sized the PCR

products using a CEQ 8000 Genetic Analysis System (Beckman-Coulter, Fullerton, CA). We scored allele sizes using the CEQ 8.0 software and 400 bp standards (Beckman-Coulter). We tested our microsatellite data for Hardy–Weinberg equilibrium (HWE) and linkage equilibrium in GenePop v.4.0 (François, 2008). Significance was estimated using a Markov chain approach (Dememorization $=10^4$, Number of batches $=10^3$, Number of iterations per batch $=10^4$) for each locus and population.

Data analysis: taxonomic designation

To test our first hypothesis regarding the evolutionary relationships and taxonomic designations of the Atlantic coast beach mouse subspecies we used both phylogenetic approaches and haplotype networks. We estimated the phylogenetic relationship among unique haplotypes using Bayesian inference (BI; MrBayes v.3.1.2; Huelsenbeck & Ronquist, 2001) and Maximum likelihood (ML; Garli v.2.0; Zwickl, 2006). We rooted our phylogenetic analyses using *cyt b* sequences from *P. melanotis* obtained from GenBank (DQ385626; Dragoo et al., 2006), as this species has been found to be sister to *P. polionotus* and its closest relatives (Kalkvik, Stout, Doonan, & Parkinson, 2012). In order to evaluate the relationship of *P. polionotus* to its closest relatives we included *cyt b* sequences from each major lineage of *P. maniculatus* (DQ385632, DQ385706, DQ385717, DQ385756, DQ385816, DQ385825; Dragoo et al., 2006) and *P. keeni* (DQ385716; Dragoo et al., 2006) identified by Kalkvik et al. (2012). To provide a complete sampling of *P. polionotus*, we included published *cyt b* sequence data for *P. p. sumneri*, *P. p. albifons*, and *P. p. polionotus*, representing mainland subspecies from the Florida panhandle, Georgia, and Alabama respectively (EU140776, EU140779, EU140770, EU140781, EU140757, EU140767; Van Zant & Wooten, 2007). We also included four Gulf coast beach mouse subspecies (*P. p. peninsularis* [EU140791], *P. p. tryssyllepsis* [EU140784], *P. p. leucocephalus* [EU140789], *P. p. allophrys* [EU140778]; Van Zant & Wooten, 2007) for complete representation. Following the methods outlined by Brandley et al. (2005) we used Bayes factors to determine the best partitioning strategy for our data set. We determined the best substitution model for the *cyt b* data in MrModelTest v.2.4 using the Akaike information criterion (AIC: Nylander, 2004). For the BI we completed two independent Markov chain Monte Carlo (MCMC) runs in MrBayes, with each run having four chains, for 2×10^6 generations and sampling every 1,000 generations. We determined stationarity for our runs using Tracer v.1.5 (Rambaut & Drummond, 2007) and discarded the first 200,000 generations as burn-in. We used the default parameter settings in Garli to estimate ML topology. Following the recommendations by the program author (Zwickl, 2006), we initiated four runs to ensure convergence, where each run was terminated after 20,000 generations with no improvement in the likelihood score of the topology. We assessed the nodal support using bootstrapping, with 1,000 replications. Each replicate was terminated after 10,000 generations with no improvement in likelihood score of the topology. Nodes were considered supported if their posterior probability was above 0.95, as it measures probability of a node representing a true phylogenetic divergence (Huelsenbeck, Ronquist, Nielsen, & Bollback, 2001). Supported nodes had bootstrap values above 70% due to conservative estimates of inferring correct clades (Hillis & Bull, 1993). We estimated the geographic distribution and frequency of the unique haplotypes for the *cyt b* sequences by constructing a haplotype network following the 95% statistical parsimony method (Templeton, Routman, & Phillips, 1995). The network was constructed using TCS (Clement, Posada, & Crandall, 2000).

Data analysis: colonization patterns

We tested Bowen's single colonization hypothesis of the Atlantic coast barrier islands using a haplotype network based on mitochondrial sequence data and measures of genetic structure based on microsatellite data from the Atlantic coast beach mice and mainland subspecies. To test for sequence of events in the colonization of the Atlantic coast we evaluated the pattern of genetic differentiation among the Atlantic coast and mainland subspecies. Greater levels of genetic differentiation would suggest longer time isolated between the subspecies compared. For analysis of genetic structure using microsatellites we included only sample locations with more than five individuals to provide sufficient population level sampling (Table S1, see supplemental material online). In order to measure genetic structure, we estimated genetic differentiation based on microsatellite data among and between mainland and beach mouse subspecies. We determined whether allele size (R_{ST}) or allele state (F_{ST}) best fit our data. In cases where loci are following a stepwise mutation model and have high mutation rates, R_{ST} is expected to be larger than F_{ST} (Hardy, Charbonnel, Fréville, & Heuertz, 2003). We estimated genetic differentiation using SPAGeDi v.1.3 (Hardy & Vekemans, 2002). We tested the null hypothesis of no contribution of allele size on genetic differentiation ($F_{ST} = R_{ST}$) using a permutation test in SPAGeDi, where we created a null distribution of R_{ST}

Table 1. Genetic differentiation measured as pair-wise R_{ST} values for sample locations of *P. polionotus* spp., based on 10 microsatellite loci (below diagonal).

	Mainland subspecies					**Atlantic coast beach mice**											
	P. p. subgriseus		***P. p. rhoadsi***			***P. p. phasma***		***P. p. niveiventris***									
	SRWEA	**ONF**	**LLSP**	**APAFR**	**ABS**	**ASP**	**FM**	**SDP**	**CNS**	**MINWR**	**CC BG3**	**CC IG1**	**CC IG3**	**CC IG2**	**CC BG1**	**CC BG2**	**PINWR**
SRWEA	—	136.7	203.8	308.1	345.5	158.2	166.6	218.8	246.4	272.6	281.5	281.3	285.9	283.3	285.5	285.4	342.9
ONF	0.133	—	87.9	188.6	233.0	87.7	74.9	86.6	110.8	136.1	144.8	144.6	149.2	146.6	148.8	148.7	208.5
LLSP	0.092	0.034	—	104.8	146.3	167.2	148.5	106.1	104.8	113.6	116.1	112.9	116.5	112.1	114.8	113.7	148.3
APAFR	0.189	0.169	0.081	—	48.4	255.1	233.7	166.2	143.5	128.7	121.9	116.9	116.6	112.9	114.3	112.6	85.2
ABS	0.122	0.036	0.076	0.140	—	302.7	281.4	214.6	191.4	174.9	167.3	162.3	161.5	158.1	159.3	157.6	114.5
ASP	0.326	0.169	0.167	0.355	0.176	—	22.3	99.3	131.3	159.6	170.5	172.9	177.0	176.7	177.8	178.5	248.9
FM	0.693	0.719	0.554	0.778	0.535	0.319	—	77.2	109.3	137.7	148.6	150.9	155.0	154.6	155.8	156.5	226.7
SDP	0.302	0.442	0.184	0.249	0.366	0.432	0.854	—	32.2	60.9	71.6	73.8	77.9	77.4	78.6	79.3	149.6
CNS	0.221	0.239	0.197	0.336	0.253	0.322	0.617	0.199	—	28.7	39.4	41.6	45.7	45.4	46.5	47.2	118.1
MINWR	0.283	0.343	0.194	0.274	0.346	0.380	0.732	0.046	0.131	—	10.9	14.4	17.7	18.7	19.0	20.1	91.1
CC BG3	0.241	0.304	0.171	0.210	0.302	0.370	0.719	0.032	0.149	0.022	—	5.1	6.9	9.2	8.6	10.0	80.3
CC SG1	0.180	0.245	0.130	0.174	0.239	0.326	0.641	0.074	0.169	0.078	0.014	—	4.6	4.4	4.9	5.8	76.9
CC SG3	0.223	0.294	0.171	0.251	0.289	0.356	0.633	0.029	0.087	0.018	0.013	0.038	—	4.6	2.3	4.0	73.4
CC SG2	0.213	0.268	0.129	0.186	0.268	0.341	0.647	−0.002	0.128	0.022	0.007	0.027	0.006	—	2.7	2.1	72.9
CC BG1	0.291	0.358	0.206	0.206	0.351	0.424	0.741	0.028	0.201	0.040	−0.002	0.044	0.040	0.023	—	1.7	72.1
CC BG2	0.300	0.360	0.206	0.238	0.364	0.423	0.733	0.004	0.176	0.012	0.007	0.070	0.031	0.013	0.002	—	71.2
PINWR	0.137	0.353	0.194	0.301	0.252	0.375	0.751	0.244	0.134	0.185	0.194	0.161	0.105	0.136	0.230	0.221	—

Geographic distance between sample locations are shown as Euclidean distance measured in kilometres (above diagonal).

values. We estimated the distribution using 20,000 permutations. We used R_{ST} for all downstream analyses because we found R_{ST} to be a better predictor across our sample locations as our observed R_{ST} values were significantly larger than the R_{ST} null distribution (one-tailed test) (Hardy et al., 2003; see Results). To test if genetic differentiation is associated with geographic distance (i.e. isolation by distance) we conducted a Mantel test using IBDWS v.3.15 (Jensen, Bohonak, & Kelley, 2005) where significance was estimated using 30,000 permutations. We measured geographic distance as Euclidean distance in kilometres between sample locations using the *dist* function in R v.2.12 (R Core Team, 2016), and genetic differentiation as pair-wise R_{ST} as determined in SPAGeDi described above.

As an additional test of our colonization hypothesis we determined the number of genetically distinct clusters (K) using a Bayesian admixture approach (STRUCTURE v.2.2; Pritchard, Stephens, & Donnelly, 2000). STRUCTURE estimates likelihood values [Pr(X|K)] by fitting data to the given K through minimizing HWE and linkage disequilibrium. STRUCTURE also estimates the proportional association for each individual for each inferred K, measured as a membership coefficient. Using the membership coefficient, we can identify potential recent migrants or gene flow. This approach typically identifies the highest order of genetic structure across samples, so we applied a hierarchical approach to test for genetic structure and genetic isolation among the mainland and extant Atlantic beach mouse subspecies. The initial analysis included all individuals, and subsequent clusters were separately analysed in STRUCTURE to evaluate any lower level genetic structuring.

As we had uneven sampling among our subspecies, which was dominated by *P. p. niveiventris* individuals ($n = 344$), we tested for the impact of sample bias on our STRUCTURE analyses using 10 randomized runs. For each run we included all samples of *P. p. phasma* ($n = 73$) and all mainland subspecies samples ($n = 67$). For *P. p. niveiventris* we picked 75 individuals using the random function in Excel 2010 (Microsoft) for each randomized run, ensuring all sample locations were included. To evaluate the sensitivity of STRUCTURE to sample bias we also conducted additional STRUCTURE runs where we included randomly chosen sets of 100, 150, and 200 *P. p. niveiventris* individuals for separate analyses. For all our STRUCTURE analyses we determined the best fit K for our data using the best Pr(X|K), and the procedure of Evanno et al. (2005) based on the second order derivative of Pr(X|K). The second order derivative, called ΔK, shows the rate of change in likelihood between subsequent K. The highest ΔK has been shown to be a good estimate of best K based on given data (Evanno et al., 2005). Most of the parameters were kept at default values as suggested for the STRUCTURE admixture model. Each run had an initial 2×10^4 generations of burn-in with a subsequent run of 5×10^5 generations used to estimate parameters; we ran 10 independent runs per K. Each analysis was run with K values from one to the number of sample locations included in the analysis. To evaluate geographic distinctiveness, we plotted the membership coefficient values for each individual for the K determined to provide the best fit to the data. Migrants or recent gene flow was inferred when individuals had membership coefficients more associated to clusters found outside of their sample locations.

Data analysis: island vs. mainland genetic diversity

We tested our last hypothesis of reduced genetic diversity on barrier islands compared with the mainland by determining genetic diversity measures using both *cyt b* and microsatellite data. To compare genetic diversity using our *cyt b* data, we estimated gene diversity (h) and nucleotide diversity (π) using DnaSP v.5.10 (Librado & Rozas, 2009) for sequences collected from mainland subspecies and for each Atlantic coast subspecies (i.e. mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*) versus island subspecies *P. p. phasma*, *P. p. decoloratus*, and *P. p. niveiventris*). To identify significant differences between mainland and island geographic sites of mtDNA diversity, we performed Welch's t-tests in R (R Core Team, 2016). For the microsatellite data we conducted pair-wise comparisons of genetic diversity based on samples from mainland and Atlantic coast subspecies, i.e. mainland subspecies (*P. p. subgriseus* and *P. p. rhoadsi*), versus island subspecies (*P. p. phasma*, and *P. p. niveiventris*). We estimated genetic diversity for each sampled location as allelic richness (*AR*) using FSTAT v.2.9.3 (Goudet, 2001) and expected heterozygosity (H_E) for each sample location in GenAlEx v.6.4 (Peakall & Smouse, 2006). To identify significant differences between mainland and island geographic sites for differences in microsatellite genetic diversity, we ran a two-way analysis of variance (ANOVA) in R (R Core Team, 2016), testing for the effects of locus and diversity.

Results

Genetic markers

For the mainland subspecies we successfully generated *cyt b* sequence data from 56 individuals representing all mainland sample locations (Table S1, see supplemental

material online). For *P. p. phasma*, we acquired sequence data for 31 individuals representing the four sample locations, which is a subset of the total number of collected individuals (Table S1, see supplemental material online). We acquired 36 sequences of *P. p. niveiventris* that represented all sample locations (Table S1, see supplemental material online). To ensure that we had sequenced a sufficient number of individuals we conducted an individual-based rarefaction analysis (Gotelli & Colwell, 2001). The resulting haplotype accumulation curves confirmed that we had sample sizes sufficient to identify a majority of the *cyt b* haplotypes because the curves plateaued at the observed number of haplotypes before reaching the sample sizes of our different taxonomic groups.

We generated microsatellite data for all individuals from sample locations where we had collected more than five individuals (Table S1, see supplemental material online). We genotyped a total of 484 individuals that represented five mainland locations ($n = 67$; Table S1, see supplemental material online), two locations for *P. p. phasma* ($n = 73$; Table S1, see supplemental material online), and 10 locations for *P. p. niveiventris* ($n = 344$; Table S1, see supplemental material online). We did not include *P. p. decoloratus* in microsatellites analyses, because of low sample size and failure to obtain microsatellite data as a result of amplification failure. We found a total of 10 loci by population comparisons to be out of HWE from seven different sample locations after Bonferroni corrections for multiple comparisons. Because we did not observe a clear pattern of specific loci being consistently out of HWE across sample locations, we did not expect null alleles to be a major problem for our microsatellite dataset. Of all comparisons between loci within a sample location (765 total comparisons), we determined after Bonferroni correction that only two locus pairs showed significant linkage. However, because each of these locus pairs was found in only a single population comparison, we do not have strong evidence to suggest actual linkage disequilibrium among any of our loci. Due to the low number of locus-by-population deviations in HWE and lack of linkage, we did not exclude any populations or loci from our analysis.

Taxonomic designation

The aligned sequence data consisted of 1103 bp with 93 (8.4%) parsimony informative characters. Across our samples we identified a total of 23 unique haplotypes that corresponded to published haplotypes in Degner et al. (2007) and Kalkvik et al. (2012). Preliminary analysis showed that a non-partitioned model was best for estimating phylogenetic relationships from our data set (harmonic mean likelihood [unpartitioned] = −2200.13; harmonic mean likelihood [partitioned] = −2128.39; $2 \times \ln$ (Bayes factor) = 0.066). AIC chose GTR + I + Γ (Tavaré, 1986) as the best nucleotide substitution model for our data, and it was implemented into both the Bayesian (BI) and Maximum likelihood (ML) analyses.

Both BI and ML approaches resulted in similar topologies, with little resolution (Fig. 2). The tree estimated from the BI and ML approach resolved a highly supported monophyletic *P. polionotus* lineage (BI =1.00, ML =100; Fig. 2), but *P. polionotus* haplotypes formed an extensive polytomy (Fig. 2). Among the sampled haplotypes the phylogenies inferred a *P. p. niveiventris* lineage with low support (BI =0.89, ML =66; Fig. 2). We also identified a single haplotype representing both *P. p. phasma* and *P. p. decoloratus*, and we found no resolution among mainland haplotypes (Fig. 2). We identified a strongly supported clade of four haplotypes that represented *P. p. rhoadsi* in part (Ppr7–9; BI =1.00, ML =94; Fig. 2). These haplotypes were found in south Florida (APAFR and ABS) and in central Florida (LLSP). An additional lineage representing *P. p. rhoadsi* haplotypes from APAFR and LLSP (Ppr4–6) was only supported by BI (BI =1.00, ML =67; Fig. 2). Finally, we recovered a lineage representing three haplotypes of *P. p. subgriseus* found in ONF (Pps3–5); this was also supported by BI only (BI =0.97, ML =67; Fig. 2). Overall, there was a lack of resolution using the *cyt b* sequence data to resolve the phylogenetic relationships among *P. p. niveiventris*, *P. p. phasma*, *P. p. decoloratus*, and mainland *P. polionotus* spp. with confidence.

We gained additional insight into subspecies relationships through haplotype network analysis of our *cyt b* sequence data (Fig. 3). Despite a low number of informative characters leading to low resolution in the BI and ML phylogenies described above, we observed no overlap in haplotypes between mainland subspecies and those found on Atlantic coast barrier islands. We identified a total of 19 unique haplotypes among mainland subspecies. Of these, nine haplotypes represented sample locations within the distribution of *P. p. subgriseus* (Pps1–9; Fig. 3), and the remaining 10 mainland haplotypes were found in *P. p. rhoadsi* sample locations (Ppr1–10; Fig. 3). While the two mainland subspecies do not share haplotypes, we found most *P. p. subgriseus* haplotypes to be more closely associated to the *P. p. rhoadsi* haplotypes Ppr1, than to each other (Fig. 3). Additionally, there is no overlap of haplotypes between the two sample locations designated as *P. p. subgriseus* (Fig. 3). Of the haplotypes identified among the *P. p.*

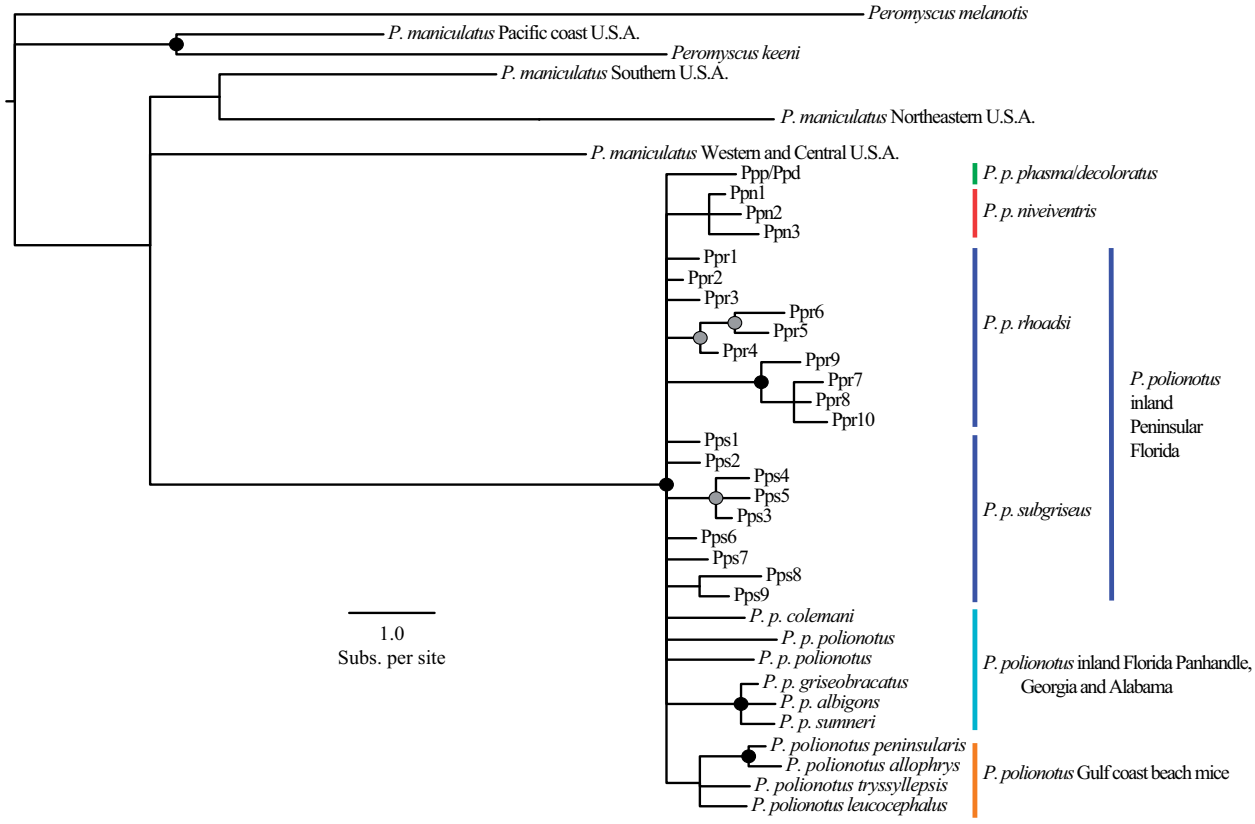

Fig 2. Phylogram based on Bayesian inference of *cyt b* haplotypes for Florida peninsula and Atlantic coast beach mice *P. polionotus*. Phylogeny included published haplotypes of Gulf coast beach mice subspecies and mainland *P. polionotus* from Florida panhandle, Alabama and Georgia. Black circles designate strong nodal support based on bootstrap values (> 70), and posterior probability from Bayesian inference (>0.95). Grey circles mark nodes that are strongly supported by posterior probability (>0.95) only.

rhoadsi sample locations, three were shared among some of the sample locations (Ppr1–2, Ppr8; Fig. 3). Haplotype Ppr1 is shared between the two sample locations in central Florida (LARA, LLSP), while Ppr2 is found in central Florida (LARA and LLSP) and in south Florida (ABS) (Fig. 3). Most of the individuals with haplotype Ppr8 were found in south Florida (APAFR and ABS), but two individuals in central Florida (LLSP) also exhibited this haplotype (Fig. 3). All the haplotypes found on the mainland link up to haplotype Ppr1 with one to seven mutational steps; this haplotype also connects to the haplotypes found in the Atlantic coast beach mice. The beach mice subspecies share a common unsampled or extinct haplotype with the mainland Ppr1 haplotype. For *P. p. niveiventris* we observed three haplotypes with two to three mutational steps from the most similar mainland haplotype. The three haplotypes in *P. p. niveiventris* were unique to the subspecies (Fig. 3). The two northern most Atlantic coast beach mouse subspecies, *P. p. phasma* and *P. p. decoloratus*, shared a single haplotype (Fig. 3). Our parsimony analysis of the haplotype network indicated that the two extant coastal subspecies are each closely related to but distinct from the mainland subspecies.

Colonization patterns

In measuring genetic differentiation, we found the observed global R_{ST} value was significantly larger than the permutation distribution (P-value =0.003). Based on the results of the permutation test, R_{ST} is a better predictor for describing genetic structure across our samples compared with F_{ST}. The global R_{ST} value indicated a high level of genetic structure among mainland *P. polionotus* locations (Global $R_{ST} = 0.266 \pm 0.050$ 95% CI). We found the pair-wise R_{ST} values to range from −0.002 (CC IG2 and SDP; CC BG1 and CC BG3) to 0.854 (SDP and FM) (Table 1). Pair-wise R_{ST} values among and between Atlantic subspecies and mainland sample locations show that the lowest amount of

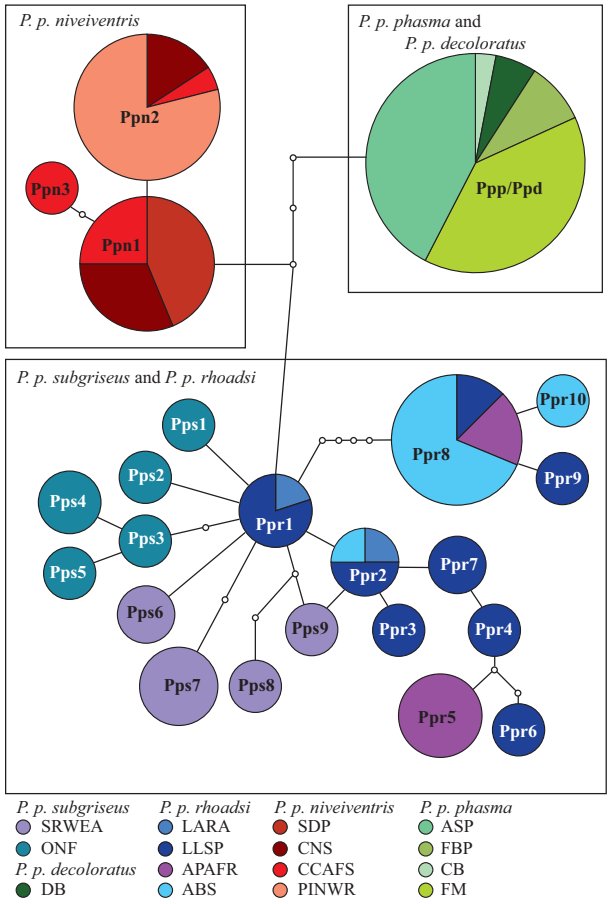

Fig 3. Haplotype network based on *cyt* b sequence data from peninsular Florida *P. polionotus* spp. (*P. p. subgriseus* and *P. p. rhoadsi*) and the three Atlantic coast beach mouse subspecies (*P. p. niveiventris*, *P. p. phasma*, and *P. p. decoloratus*). Each circle designates a unique haplotype, and size corresponds to frequency of individuals carrying haplotype. Colour corresponds to sample locations seen in legend. Small white circles designate unsampled or extinct haplotypes. See Table S1 (see supplemental material online) for location abbreviations.

structure is among *P. p. niveiventris* locations (average $R_{ST} = 0.080$). Among the mainland locations we observed an average $R_{ST} = 0.107$, with $R_{ST} = 0.133$ between the two *P. p. subgriseus* locations and an average of $R_{ST} = 0.099$ among the *P. p. rhoadsi* locations. We observed the greatest pair-wise structure within *P. p. phasma* (average $R_{ST} = 0.319$). We found pair-wise R_{ST} values for *P. p. niveiventris* to be lower when compared with mainland locations (average $R_{ST} = 0.257$) than compared with the *P. p. phasma* locations (average $R_{ST} = 0.540$). Between the mainland locations and *P. p. phasma* the average pair-wise R_{ST} was 0.447 (Table 1). We found a significant positive relationship between genetic differentiation and geographic distance (Mantel test; $P = 0.043$; Fig. S1, see supplemental material online), but the geographic distance did not explain a majority of the variation in genetic differentiation ($R^2 = 0.174$; Fig. S1, see supplemental material online).

Due to our high sample number of *P. p. niveiventris* relative to the other subspecies we tested if numerical sample bias could impact the STRUCTURE results. Thus we included 10 STRUCTURE analyses with randomized sampling of *P. p. niveiventris* samples for more balanced sampling between Atlantic coast and mainland subspecies. Numerical sampling bias did influence the hierarchical structure, with mainland subspecies and *P. p. niveiventris* individuals clustering together, and a separate cluster contained the *P. p. phasma* individuals (K = 2; Fig. 4.1). In contrast when all individuals were included in the analyses we found that *P. p. niveiventris* was in a separate cluster, while the mainland subspecies and *P. p. phasma* were associated to the same cluster (K = 2; Fig. 4.2). With approximately even sample size all independent runs split out *P. p. phasma* as its own cluster. Additional simulations showed that as the number of *P. p. niveiventris* samples increased, the likelihood increased that *P. p. niveiventris* grouped as its own cluster. When we increased the *P. p. niveiventris* sample to 100 individuals (vs. $n = 67$ and $n = 73$ for mainland subspecies and *P. p. phasma* respectively) we found 10% of the STRUCTURE runs with *P. p. niveiventris* as its own cluster (Table 2). When we increased *P. p. niveiventris* sample to over twice as many as the other two groups ($n = 200$), all runs supported *P. p. niveiventris* forming its own cluster in the STRUCTURE analyses (Table 2). Based on our findings the following cluster analyses were performed given even sampling among the mainland subspecies and the two Atlantic coast beach mouse subspecies.

Our STRUCTURE analyses indicated there is little gene flow between the clusters identified. When we had even sample sizes, all individuals were strongly associated with their respective clusters with membership coefficients over 75% for their respective cluster. Further, over 98% of the individuals had membership coefficients greater than 90% for their respective cluster (Fig. 4.1). We conducted hierarchical analyses that included the mainland subspecies individuals and the randomly picked individuals of *P. p. niveiventris*. We determined again K = 2 as best fitting the data, where the two clusters are geographically associated with one cluster containing *P. p. niveiventris* individuals, and the second cluster associated with individuals captured on the mainland (Fig. S2.1, see supplemental material online). Almost all individuals were highly associated with their geographic cluster, with 92% of the individuals having a membership coefficient over 95%,

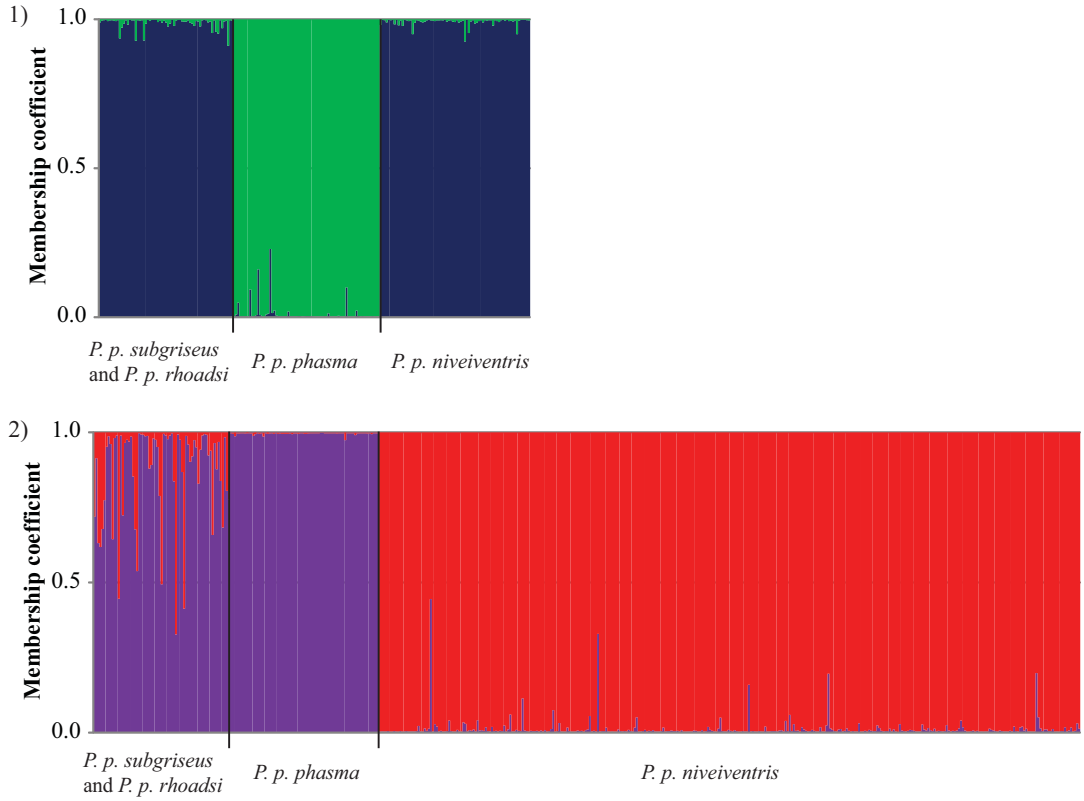

Fig 4. Estimated membership coefficients from STRUCTURE analysis based on 10 microsatellite loci for *P. polionotus* spp. from peninsular Florida. **(1)** includes all individuals of mainland spp. (*P. p. subgriseus* and *P. p rhoadsi*) and *P. p. phasma*, and membership coefficient for *P. p. niveiventris* represented 10 independent randomized subsamplings. **(2)** includes all individuals of mainland spp. (*P. p. subgriseus* and *P. p rhoadsi*) and extant Atlantic coast beach mouse spp. (*P. p. phasma* and *P. p. niveiventris*). Colours indicate different clusters with K = 2 for **(1)** and **(2)**.

suggesting little gene flow between the two clusters (Fig. S2.1, see supplemental material online). We ran STRUCTURE on individuals captured at mainland locations to evaluate genetic structure among these populations. STRUCTURE determined K = 3 fit the data best suggesting cluster membership was primarily determined by geographic location. One cluster mainly comprised of individuals captured in *P. p. subgriseus* sample locations (SWEA and ONF). The remaining clusters consisted of *P. p. rhoadsi* sample locations, with one cluster in central Florida (LLSP), and a second cluster consisting of primarily individuals from the southern range of *P. p. rhoadsi* (APAFR and ABS) (Fig. S2.2, see supplemental material online). A few individuals were associated with clusters different from their geographic location, which could indicate current or recent gene flow between populations and between mainland subspecies (Fig. S2.2, see supplemental material online). We determined that there is a lack of gene flow between the coastal subspecies and mainland, and lack of gene flow between the two extant coastal subspecies, in all analyses; we also suspect that local geography supports this assertion. We found *P.p. phasma* to have greater level of genetic differentiation relative to the other subspecies, both based on R_{ST} values and clustering patterns in our STRUCTURE analyses.

Island vs. mainland genetic diversity

We found the greatest amount of mitochondrial genetic diversity within the mainland locations (Table S2, see supplemental material online). The group with the lowest genetic diversity included *P. p. phasma* and *P. p. decoloratus*, with a single haplotype identified across all samples and locations. At all locations within *P. p. niveiventris*, we detected limited genetic diversity (Table S2, see supplemental material online). Each mainland sample location contained more than one haplotype, even with small sample sizes (LARA; $n = 2$; Table S2, see supplemental material online). We found two locations with more than one haplotype within the *P. p. niveiventris* locations (CNS and CC; Table S2, see supplemental material online), while the remaining

Table 2. Impact of skewed sample size incorporated into STRUCTURE, with different sample sizes for *P. p. niveiventris* (*Ppn*) compared to *P. p. phasma* (*Ppp*) and mainland subspecies, *P. p. subgriseus* and *P. p. rhoadsi*.

Sample size				Percent of runs with specific clustering pattern		
P. p. n.	***P. p. p.***	**Mainland**	**Max ΔK**	***Ppp* + *Ppn*/mainland**	***Ppn* + *Ppp*/mainland**	**Mainland + *Ppn*/*Ppp***
75	73	67	2	100%	0%	0%
100	73	67	2	90%	10%	0%
150	73	67	2	30%	70%	0%
200	73	67	2	0%	100%	0%
344	73	67	2	0%	100%	0%

Best K was determined based on Evanno et al. (2005) criteria. Reported was percent of runs for specific clustering patterns, with '/' indicating same cluster and '+' indicating separate cluster.

locations were fixed for a single haplotype (SDP and PINWR; Table S2, see supplemental material online).

Based on our haplotype and genetic structure data we pooled the mainland samples when comparing genetic diversity between mainland *P. polionotus* and the Atlantic coast subspecies (see Discussion). With regard to the estimated patterns of genetic diversity, we found π was not significantly different between mainland and island sites ($t = -1.512$, df $=6.28$, $P = 0.179$), whereas h was significantly greater in the mainland sites than the island sites ($t = -2.620$, df $=8.20$, $P = 0.030$).

For our microsatellite data we observed some of the lowest genetic diversity within the *P. p. phasma* sample locations, however, two of the *P. p. niveiventris* locations (SDP and PINWR; Table S3, see supplemental material online) also exhibited comparable low genetic diversity. Average allelic richness (*AR*) of microsatellites in mainland geographic sites was estimated to be 7.01 versus 4.357 for island sites (Table S3; Fig. 5). Additionally, expected heterozygosity (H_E) among mainland sites averaged 0.809 versus 0.606 for island sites averaged. The two-way ANOVA results indicated that mainland genetic diversity was significantly higher than island diversity for both *AR* and H_E (*AR*: $P < 0.001$; H_E: $P < 0.001$, Fig. 5).

Discussion

In this study, we identified divergence and isolation consistent with rapid evolution in a lineage invading novel coastal habitat. First, we found that the two extant Atlantic coast beach mouse subspecies, *P. p. phasma* and *P. p. niveiventris*, represent distinct lineages, supporting the hypothesis that these subspecies constitute unique taxonomic units. The relationship of the extinct subspecies *P. p. decoloratus* to the other subspecies remains unclear, as our limited sample sizes showed it to contain a single haplotype which was shared with *P. p. phasma*. Second, we found that all recognized subspecies on the Atlantic coast appear to have originated from a single mainland source. The haplotypes found on the barrier islands originate from a single haplotype from central Florida. These findings support Bowen's single colonization hypothesis, but we cannot distinguish between a single colonization event with subsequent diversification or multiple colonization events from that shared source. We found *P. p. phasma* to have the greatest pair-wise R_{ST} values with the other subspecies, and were found in their own cluster at the highest hierarchical level in our STRUCTURE analyses. These patterns of genetic structure suggest that *P. p. phasma* diverged from the mainland populations prior to *P. p. niveiventris*. Regardless of the route and dynamics of barrier island colonization, the two extant subspecies each have unique phylogenetic trajectories and limited genetic diversity. Third, as expected for recently established and narrowly distributed subspecies, we found low genetic diversity in *P. p. phasma* and *P. p. niveiventris* in both mitochondrial and nuclear markers, compared with mainland conspecifics (Fig. 5), potentially due to large founder populations or multiple founder events. Finally, our results indicated that the Bayesian clustering algorithm used here (i.e. the computer program STRUCTURE) is biased based on uneven sample sizes. Here, we recovered different clustering patterns depending on the amount of sample bias included in the analysis (Table 2). Overall, this study reveals important implications with regard to the evolutionary history of Atlantic coast beach mice.

Taxonomic designation: In evaluating the taxonomic status of the Atlantic coast beach mouse subspecies we found evidence that the two extant subspecies, *P. p. phasma* and *P. p. niveiventris*, show clear genetic differentiation from each other and from the mainland subspecies, with unshared *cyt b* haplotypes and lack of gene flow estimated from microsatellite data. As expected with recently diverged taxa, our phylogenetic tree provided little information for discerning evolutionary relationships among *P. polionotus* spp. In recently isolated taxa, divergence can be difficult to detect as a result of incomplete lineage sorting and insufficient time for evidence of phenotypic and genotypic differentiation to manifest in sampled characters (Maddison & Knowles,

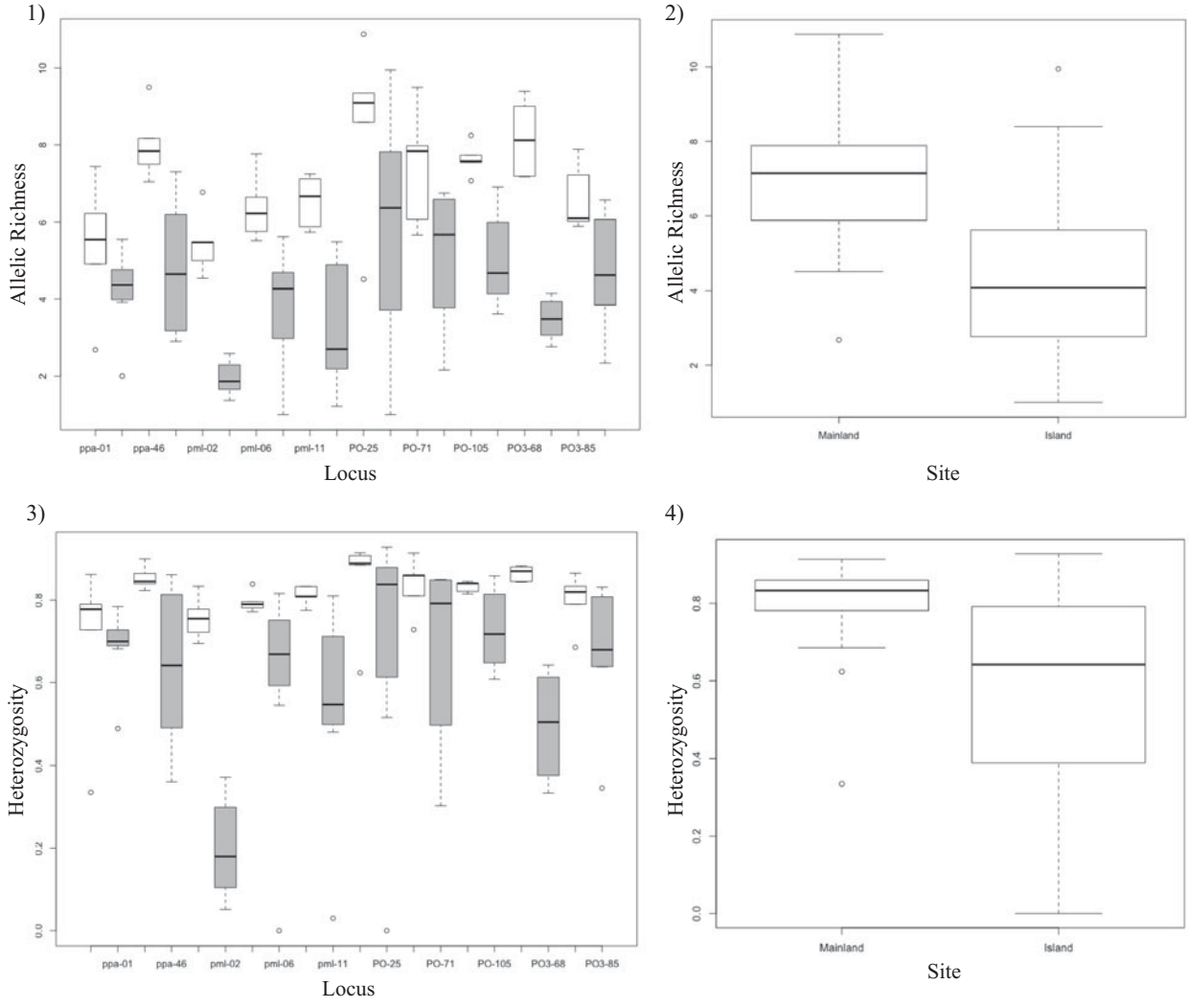

Fig 5. Measures of genetic diversity based on microsatellite loci comparing mainland (*P. p. subgriseus* and *P. p. rhoadsi*) versus island subspecies (*P. p. phasma* and *P. p. niveiventris*). In panels **1** and **3**, white boxes are diversity estimates of mainland populations while filled boxes are diversity estimates of island populations. Panel **1** shows site-by-locus comparisons for allelic richness (AR), Panel **2** shows summary comparison of island versus mainland for AR. Panel **3** shows site-by-locus comparisons for heterozygosity (H_E), Panel **4** shows summary comparison of island versus mainland for H_E. All mainland versus island comparisons showed that mainland populations exhibited significantly greater genetic diversity than island populations (see Results).

2006). The remaining analyses suggested that *P. p. phasma* and *P. p. niveiventris* belong to their own distinct taxonomic units. Lack of gene flow was documented among the Gulf coast beach mouse subspecies (Domingues et al., 2012; Mullen et al., 2009), suggesting that the recognized beach mouse subspecies all represent their own evolutionary trajectories. The only subspecies without support for evolutionary independence was *P. p. decoloratus*, where both specimens examined had the same haplotype found in *P. p. phasma*. This result suggests these two subspecies may represent the same evolutionary lineage. The different subspecies are identified by their pelage colour (Bowen, 1968), and this seems to be a strong predictor for identifying evolutionary lineages for beach mice both on the Atlantic coast, as we have shown here, as well as on the Gulf coast (Domingues et al., 2012; Mullen et al., 2009).

Variation within species is widely recognized through the use of a subspecies designation. However, many studies have shown discrepancies between evolutionary lineages and taxonomic groupings within species (Burbrink et al., 2000; Daza, Smith, Páez, & Parkinson, 2009; Mulcahy, 2008; Newman & Rissler, 2011; Tursi et al., 2013; Zink, 2004). Such deviation could reflect the influence of clinal or other environmental variation in morphological characters used for identification

(Grieco & Rizk, 2010; Myers, Lundrigan, Gillespie, & Zelditch, 1996; Svanbäck & Schluter, 2012; van Valen, 1973), rather than reflecting evolutionary history. Colour patterns have been found to be poor predictors of intraspecific variation (e.g., Burbrink et al., 2000; Trujano-Alvarez & Álvarez-Castañeda, 2007). However, in beach mice pelage-defined colour patterns are an ideal character state for determining evolutionary lineages (this study, Domingues et al., 2012; Mullen et al., 2009). The possible causal explanations for the correspondence of pelage colour to evolutionary lineage in this system may be the evolutionary processes that impact the beach mice. Extensive research has documented selective differentiation in beach mice, with a selective advantage to matching pelage colour to sand substrate (Mullen & Hoekstra, 2008; Vignieri, Larson, & Hoekstra, 2010). The genes influencing pelage colour variation in beach mice differ between Gulf and Atlantic coast subspecies (Hoekstra et al., 2006; Steiner, Rompler, Boettger, Schoneberg, & Hoekstra, 2009; Steiner, Weber, & Hoekstra, 2007), but beach mice differentiation seems to be driven by similar selective pressures on different populations. Comparable results have been reported in *Anolis* species, where dewlap colours denote intraspecific variation (Glor & Laport, 2012). In this case dewlap colours may be under selective pressure for species recognition, sexual selection, or both (Losos, 1985; Vanhooydonck, Herrel, van Damme, & Irschick, 2005).

Differential selective pressure for background matching seems to be driving coastal speciation in *P. polionotus*. However, correspondence of taxonomic units to evolutionary lineages is not as clear on the mainland. Mainland subspecies of *P. polionotus* are also recognized by phenotypic variation, with eight recognized subspecies (Hall, 1981). Our sample locations fell within the distributions of *P. p. subgriseus* and *P. p. rhoadsi*, but our genetic data do not support the existence of separate evolutionary trajectories for these taxonomic units. Although the two subspecies do not share haplotypes, most of the *P. p. subgriseus* haplotypes were closely related to central Florida *P. p. rhoadsi* haplotypes (Fig. 3). This suggests *P. p. subgriseus* haplotypes are more closely associated with a *P. p. rhoadsi* haplotype than to other *P. p. subgriseus* haplotypes. Additionally, genetic structure does not support clear differentiation between the two subspecies (Fig. S2.1, see supplemental material online). In order to resolve mainland intraspecific variation further sampling would be needed.

Colonization patterns

Dispersal to islands has become increasingly recognized as an important process affecting the distribution of biodiversity on islands (Cowie & Holland, 2006; de Queiroz, 2005), but how islands are initially colonized has received little attention (Cowie & Holland, 2006). Several studies have found that diversity of a focal taxon on islands was the result of colonization from a single source that gave rise to adaptive radiations currently seen among island taxa (Böhle, Hilger, & Martin, 1996; Burns, Hackett, & Klein, 2002; Filardi & Moyle, 2005; Grant, 1981). Barrier islands are much more closely associated with continental landmasses than oceanic islands, and could provide a greater opportunity for colonization from multiple sources. Any variation observed among islands could then be a result of variation from different sources. Our study provided an opportunity to test hypotheses of the colonization patterns of *P. polionotus* on to recently formed barrier islands.

A comprehensive study of beach mouse evolution was based on pelage colour (Bowen, 1968). He proposed hypotheses for the establishment and evolution of Gulf and Atlantic coast beach mouse subspecies. On the Gulf coast, more recent studies have rejected Bowen's multiple colonization hypothesis (Domingues et al., 2012; Van Zant & Wooten, 2007). On the Atlantic coast, Bowen (1968) hypothesized that the diversity observed is the result of a single colonization event. Our haplotype network indicated that the extant subspecies of Atlantic coast beach mice originated from the same source, based on inferred lineages to a haplotype currently found in central Florida (Fig. 3), and therefore supports Bowen's single colonization hypothesis. However, our data cannot distinguish between single colonization of the barrier islands or multiple colonization events from the same gene pool.

Our data do suggest a sequence of events regarding the formation of extant Atlantic beach mouse diversity. We found that *P. p. phasma* seems to have been isolated from mainland *P. polionotus* and *P. p. niveiventris* the longest, with the greatest amount of genetic differentiation from other subspecies based on R_{ST} values. When we included even sample sizes we also found support for an initial isolation of *P. p. phasma* from a *P. p. niveiventris*/*P. p. subgriseus*/*P. p. rhoadsi* cluster (Fig. 4.1). These findings suggest that *P. p. phasma* was isolated from other *P. polionotus* populations before *P. p. niveiventris* was isolated from the mainland.

Island genetic diversity

Colonizing islands is often associated with the reduction of effective population size through bottlenecks and founder effects. The colonization process can lead to

loss of genetic diversity through genetic drift and inbreeding (Frankham, 1997). Reduced genetic diversity has been reported in many studies of island populations (e.g., Boessenkool, Taylor, Tepolt, Komdeur, & Jamieson, 2007; Eldridge et al., 1999; Jones, Paetkau, Geffen, & Moritz, 2004). While barrier islands are often closely associated with the mainland, and therefore may avoid loss of diversity through maintenance of gene flow, we found our island subspecies conform to the hypothesis of isolation and lower genetic diversity for island populations (Fig. 5).

STRUCTURE sample bias

Our numerical sampling bias of *P. p. niveiventris* raises an important question on the sensitivity of STRUCTURE analyses using skewed sample sizes. STRUCTURE is widely used in population genetics studies, e.g., Pritchard et al. (2000) have been cited over 21,000 times (Scholar Google, accessed 11 January 2018). However, the impact of sample size has not been sufficiently addressed in the use of STRUCTURE. With greater trapping intensity of *P. p. niveiventris*, we had over three times more samples for this subspecies than for the other subspecies (Table S1, see supplemental material online). With increased sample bias, STRUCTURE tended to attribute all members of the geographic locality with the most samples as being a unique genetic population. When we reduced sample bias (i.e., used equal sample sizes from all populations) we increasingly observed a tendency of *P. p. phasma* to form its own cluster, while *P. p. niveiventris* and the mainland subspecies formed a separate cluster. Even with smaller bias (*P. p. niveiventris*; $n = 100$) we observed runs with contradictory results (90% of runs *P. p. phasma* form its own cluster, 10% of runs *P. p. niveiventris* form its own cluster; Table 2). Our findings emphasized the importance of including independent runs for STRUCTURE analysis, but also of considering the possible effects of sampling bias. We found conflicting results due to sampling bias, and our inferences must take such biases into consideration.

In summary, we found pelage colour to correspond to evolutionary lineages in the Atlantic coast beach mouse subspecies. We also found that the Atlantic coast subspecies originated from the same mainland source. With a large numerical sample bias we were able to show that STRUCTURE analyses can be influenced by numerical sampling bias. And finally, we found *P. p. phasma* and *P. p. niveiventris* to follow the predicted pattern of lower genetic diversity in island populations.

Conservation implications

Our understanding of the evolutionary history and population genetics of beach mice can greatly impact conservation efforts. Among the extant beach mouse subspecies, only one is not federally listed as either threatened or endangered. For the extant Atlantic coast beach mice, *P. p. phasma* is listed as endangered and *P. p. niveiventris* is listed as threatened (U.S. Fish and Wildlife Service, 1989). In order to protect specific segments of a species, the Endangered Species Act has since 1978 provided protection to populations of terrestrial vertebrates that are considered 'distinct population segments' (DPSs) (Pennock & Dimmick, 1997). Prior research has defined *P. p. niveiventris* as an evolutionarily significant unit (Degner et al., 2007); however, our findings support defining both extant Atlantic coast beach mouse subspecies as DPSs based on the criteria given by the US Fish and Wildlife Service and National Marine Fisheries Services (USFWS NMFS, 1996). The criteria for being defined as a DPS is 'discrete', 'significant', and endangered compared with other conspecifics. 'Discrete' refers to being disconnected from conspecifics such as by a lack of gene flow, and 'significant' relates to the use of unique habitat (USFWS NMFS, 1996). The two subspecies are 'discrete' by showing lack of gene flow to mainland conspecifics or between subspecies. The Atlantic coast beach mice are 'significant' as they occupy unique coastal habitat compared with mainland conspecifics. Finally, the Atlantic coast beach mice are federally listed, while the mainland conspecifics are considered of least concern.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplemental data

Supplemental data for this article can be accessed here.
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