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RESEARCH ARTICLE

Unraveling natural versus anthropogenic effects on genetic diversity within the southeastern beach mouse (*Peromyscus polionotus niveiventris*)

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Abstract Anthropogenic habitat loss is among the greatest threats to biodiversity. Populations undergoing fragmentation and loss of habitat are also threatened by erosion of genetic diversity. However, contemporary genetic diversity may be the legacy of natural processes acting prior to anthropogenic influences. Measurements of genetic diversity from contemporary and historical samples can evaluate the relative impact of natural and anthropogenic effects on its status. We investigated the genetic diversity of a threatened subspecies occupying Atlantic Coast barrier islands of Florida, *Peromyscus polionotus niveiventris* (southeastern beach mouse). To test for recent loss of genetic diversity, we compared *cyt b* data from museum samples (historical—prior to human impact) with contemporary samples throughout their range. Ten microsatellite loci were genotyped for samples from the contemporary range, to determine current population interconnectedness and structure. The results using *cyt b* data revealed no statistically significant loss of genetic diversity between historical and contemporary populations of *P. p. niveiventris.* Both nuclear and mitochondrial data support our conclusion that the observed capture and conservation of historical genetic diversity is explained by the large federally protected region of continuous habitat that remains with minimal human impact. Whereas, the two disjunct populations isolated by anthropogenic habitat destruction, exhibit significant losses of genetic diversity. Collectively, these findings offer a sound basis from which to formulate a conservation strategy to maintain the genetic diversity of *P. p. niveiventris*. Furthermore, our study underscores the importance of large expanses of continuous habitat within the geographic range of species to facilitate the maintenance of genetic integrity.

Keywords Anthropogenic habitat loss · Beach mice · Cytochrome *b* · Endangered species · Genetic management · Historical genetic sampling · Microsatellite

Introduction

Habitat loss and fragmentation are among the greatest negative impacts humans have on natural populations (Groom et al. 2005; Fischer and Lindenmayer 2007). With the reduction of habitat and increased fragmentation, populations tend to become isolated from each other making them 'islands' in a landscape of unfavorable habitat. Smaller populations are subject to genetic drift and loss of genetic diversity (Lacy 1987; Frankham 1997; Thalmann et al. 2011; Tracy and Jamieson 2011). Populations that have sustained losses of genetic diversity have an increased probability of extinction (Saccheri et al. 1998; Reed and Frankham 2003; Lavergne and Molofsky 2007). Therefore levels of genetic diversity have been widely used in conservation biology as measures of human impact on taxa of interest and in assessing their future management needs (Miller and Waits 2003; Schwartz et al. 2007; Helm et al. 2009).

Several studies have shown that natural populations can exhibit low genetic diversity independent of human influences (O'Brien 1994; Hedrick 1995). Thus, low levels of contemporary genetic diversity can be explained by

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historical events acting prior to anthropogenic impacts (Miller and Waits 2003; Taylor et al. 2007; Reding et al. 2010). For example, historical population bottlenecks, gene flow restrictions, and founder effects can account for current levels of genetic structure and diversity (Culver et al. 2000; Paxinos et al. 2002; Miller and Waits 2003; Taylor and Jamieson 2008). Changes in evolutionary pressures may also result in variation in genetic diversity and structure across a species' distribution (Lawton 1993; Eckert et al. 2008) and genome-wide selection sweeps can also cause reduced genetic diversity (Amos and Harwood 1998). These historical impacts on a population can result in adaptation to local environments (Avise et al. 1987; Slatkin 1987; Åbjörnsson et al. 2004).

Continental and insular populations are well known to exhibit differences in genetic diversity (Frankham 1997; Frankham et al. 2002). In a wide range of taxa that occupy landforms such as barrier islands, patterns of reduced genetic diversity relative to congeners on the mainland have been observed (Triggs et al. 1989; Frankham 1997; Bidlack and Cook 2001; MacAvoy et al. 2007). The genetic diversity in these insular populations has been shaped by historical (natural features) forces and more recently subjected to natural and anthropogenic influences. Populations occupying islands are often founded by few individuals, with negative consequences on genetic diversity (Mayr 1942; Tinghitella et al. 2011). Taxa occupying barrier islands most likely dispersed from mainland populations and natural colonization events could account for contemporary genetic diversity. Barrier islands are also unique in that natural disturbance events such as hurricanes and floods may provide additional hardship (selective pressures) for taxa due to reoccurring bottlenecks during such events (Breininger et al. 1999; Oli et al. 2001; Scileppi and Donnelly 2007; Pries et al. 2009; Saha et al. 2011b). Anthropogenic influences manifest as land use conversion in coastal habitats and result in the loss of biotic diversity (Barbier et al. 2008; Mhemmed et al. 2008). One-third of earth's human population now occupies coastal areas (Barbier et al. 2008) and this trend seems to be increasing (Small and Nicholls 2003). Thus the genetic diversity of barrier island taxa can be shaped by historical founder events or bottlenecks acting alone, recent anthropogenic habitat loss, or a combination of historical and contemporary events.

Our purpose here is to examine genetic diversity of *Peromyscus polionotus niveiventris* (southeastern beach mouse) endemic to barrier islands subjected to diverse extrinsic factors, e.g., hurricanes and development pressures. This taxon is one of seven extant subspecies of *P. polionotus* that occupy barrier islands of the gulf coast of Alabama and Florida and the Atlantic coast of Florida (i.e. beach mice; Hall 1981). Historically, ancestral beach mouse populations were isolated from mainland conspecifics with the formation of the barrier islands (Hoekstra et al. 2006; Van Zant and Wooten 2007). Within the last few decades *P. p. niveiventris* has experienced a range contraction on the barrier islands of southeast Florida from a historical range of approximately 350 km to a contemporary range of approximately 70 km of continuous habitat as described by Stout (1992). In addition, two disjunct populations at the northern and southern end of the current distribution each occupy only a few kilometers of habitat (Fig. 1). The range contraction of *P. p. niveiventris* can be tracked over the last few decades (Stout 1992), and fits well with decreased habitat associated with increased housing development of the area (Winsberg 1992). Prior work with this subspecies did not determine if genetic diversity was compromised by recent habitat losses (Degner et al. 2007).

To understand the genetic consequences of anthropogenic impacts we must be able to compare populations of *P. p. niveiventris* sampled prior to and after extensive urban development. Recent advances in genetic analysis techniques allow the use of historical DNA derived from museum collections to compare historical and contemporary genetic diversity (Wandeler et al. 2007; Leonard 2008). Studies using this approach have been able to identify taxa where historical processes prior to human influence can explain current low genetic diversity (e.g. Hoffman and Blouin 2004; Chan et al. 2005; Reding et al. 2010), while other studies have documented a reduction in genetic diversity associated with human impacts (e.g. Hauser et al. 2002; Culver et al. 2008; Thalmann et al. 2011).

We predicted a loss of genetic diversity in mitochondrial DNA in the contemporary range of *P. p. niveiventris* compared to the historical samples, based on the extensive loss of habitat for this subspecies. This comparison will identify the degree to which historical diversity has been affected by hypothesized anthropogenic influences over recent decades. Next we examined ten microsatellite loci to describe genetic diversity and genetic structure within contemporary populations of *P. p. niveiventris*. We expected that extant genetic diversity would be higher in the contiguous tracts of habitat relative to disjunct habitats. The results we report inform future conservation strategies for this taxon based on insights into the historical and contemporary trends in its population genetics.

Methods and material

Sampling and DNA extraction

We acquired tissue as 4×4 mm^2 sections of skin taken from the venter of 78 dried museum specimens (Table S1

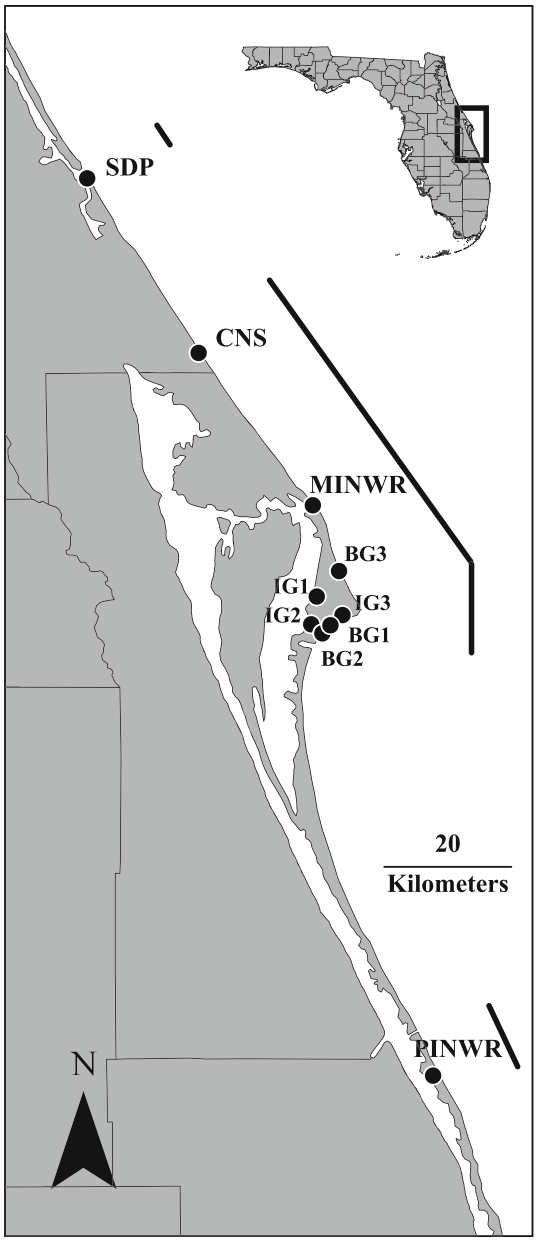

Fig. 1 Sample locations of *P. p. niveiventris* across their contemporary distribution (*black line*) along the Atlantic coast of Florida. Tissue samples were collected from Smyrna Dunes Park (SDP), Canaveral National Seashore (CNS), Merritt Island National Wildlife Refuge (MINWR), Cape Canaveral, and Pelican Island National Wildlife Refuge (PINWR). Six trapping grids were set up at Cape Canaveral, designated as Beach Grid (BG) 1 to 3 and Inland Grid (IG) 1 to 3

in Supplementary materials). These samples, categorized as historical, were collected from Volusia to Palm Beach Counties and represent the known historical range of *P. p. niveiventris* (Table 1). Our contemporary samples were from individuals collected at Smyrna Dunes Park (SDP, Volusia Co.), Canaveral National Seashore (CNS, Volusia Co.), Merritt Island National Wildlife Refuge (MINWR, Brevard Co.), Cape Canaveral (CC, Brevard Co.) and Pelican Island National Wildlife Refuge (PINWR, Indian River Co.) (Fig. 1). Samples from the current distribution were categorized as peripheral (SDP and PINWR), or central (CNS, MINWR and CC), based on location (Table 1; Fig. 1). In total, 344 contemporary individuals were included in this study. Individuals from contemporary sample locations were live trapped and tissue samples collected using methods described in Degner et al. (2007).Tissue samples were stored in 95 % ethanol at −20 °C prior to DNA extraction.

Genomic DNA was extracted from all tissue samples using a Qiagen DNeasy tissue purification kit (Qiagen Inc.). Museum tissues were soaked in 95 % ethanol at 4 °C for 24 h to remove any salts and PCR inhibitors (Mullen and Hoekstra 2008). These tissues were subsequently lysed for 24 h until dissolved. Contemporary tissue was lysed for 3–4 h until completely dissolved. All lab procedures were conducted at separate lab benches for historical and contemporary samples, to avoid any cross contamination. As an additional precaution, different lab equipment and reagents were used for historical and contemporary samples.

Evaluating loss of genetic diversity using mtDNA

We amplified and sequenced 1,100 bp of the rapidly evolving mitochondrial gene cytochrome *b* (*cyt b*), to assess the genetic diversity of contemporary and historical *P. p. niveiventris*. Because historical DNA samples were degraded, we amplified seven 200–300 base pair portions of the gene to recover the entire *cyt b* sequence. We ran two or more independent PCR reactions for amplicons that exhibited sequence variation, to ensure that variation was not caused by nucleotide disincorporation or other contamination issues (Hofreiter et al. 2001). With each PCR we also included negative controls to check for contamination. PCR were done in 25 µL volumes, containing 20–30 ng DNA, 2 mM MgCl, 1× Amplitaq Gold Buffer, 200 µM dNTPs, 0.75 units AmpliTaq Gold (Roche, NJ), and 160 nM primer. The AmpliTaq Gold is modified to improve amplification of samples with low DNA concentrations, and has been extensively used in a wide range of studies using historical and degraded DNA (e.g., Miller and Waits 2003; Vilà et al. 2003; Wilson et al. 2012). Thermocycler conditions were: 94 °C for 2 min, 45 cycles of 94 °C for 1 min, annealing temperature for 1 min, and 72 °C for 2 min. Annealing temperature and primer sequences used for historical DNA can be found in supplementary materials (Table S2). Among the contemporary samples we sequenced between six and 16 individuals for each sample location, a total of 37 individuals, following the same protocol given in Herron et al. (2004). All

Table 1 Measures of genetic diversity for *cyt b* sequence data across the contemporary and historical range of *P. p. niveiventris*

Time scale	Location	Category	N	Haplotypes	Polymorphic sites	k	π
Contemporary	Overall		37	3	3	0.62	0.00056
	Smyrna Dunes Park	C—P	7	1	0	0.00	0.00000
	Canaveral National Seashore	C—C	8	2	1	0.54	0.00050
	Cape Canaveral	C—C	6	3	3	1.00	0.00088
	Sebastian Inlet State Park	C—P	1	1	0	0.00	0.00000
	Pelican Island National Wildlife Refuge	C—P	15	1	0	0.00	0.00000
Historical	Overall		63	5	6	0.91	0.00120
	New Smyrna	H	7	3	2	0.52	0.00071
	Cape Canaveral	H	5	2	1	0.40	0.00113
	Oak Lodge	H	25	5	5	0.86	0.00130
	Jupiter Island	H	10	1	0	0.00	0.00000

For each time scale and each location we report: category (C—C: contemporary–central, C—P: contemporary–peripheral, H: historical), sample size (N), haplotypes, number of polymorphic sites, average nucleotide differences (k), and nucleotide diversity (π)

sequences were processed on an ABI 3730 DNA analyzer by Nevada Genomics Center (Reno, NV). Sequences were edited in Sequencer v.4.8 (Gene Codes, Ann Arbor, MI), and aligned by eye using GeneDoc v.2.6 (Nicholas et al. 1997).

To compare diversity between historical and contemporary samples of *cyt b*, we estimated number of haplotypes, number of polymorphic sites, average nucleotide differences (k), and nucleotide diversity (π) using DnaSP v.4.2 (Rozas et al. 2003). We did a rarefaction analysis using EcoSim v.7.0 (Gotelli and Entsminger 2001) that confirmed our sample numbers were adequate to detect genetic diversity across the historical and contemporary samples. We compared the genetic diversity among historical samples to contemporary samples using a one-tailed Welch's *t* test in R v.2.11 *stats* package to test for the loss of genetic diversity. We only included estimated genetic diversity in the *t* test for locations with more than five individuals in the sample area.

To evaluate the evolutionary relationships among haplotypes we constructed haplotype networks for both historical and contemporary *cyt b* sequences. The haplotype networks were generated using Templeton et al. (1992) methodology, following the statistical parsimony approach implemented in TCS v.1.4b1 (Clement et al. 2000). The connection limit for the haplotype network was set to 95 %.

Contemporary genetic structure and interconnectivity

We utilized microsatellite loci to assess genetic structure and infer patterns of population interconnectivity across the current distribution of *P. p. niveiventris*. Even with loci of smaller fragment sizes (100–150 bp) and using AmpliTaq Gold (Roche, NJ), we were unsuccessful in consistent amplification of microsatellite loci in a high proportion of museum samples. Lower success rates have been reported for microsatellite loci for museum samples, compared to mitochondrial DNA (Austin and Melville 2006). The higher copy number of mitochondria in each cell might explain the difference in success rates. With the inconsistent microsatellite loci amplification in historical samples we only genotyped contemporary individuals. We genotyped all 344 contemporary individuals at ten microsatellite loci: pml-02, pml-06, pml-11 (Chirhart et al. 2000), PO-25, PO-71, PO-105, PO3-68, PO3-85 (Prince et al. 2002), ppa-01, and ppa-46 (Wooten et al. 1999). PCR reactions were conducted 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. PCR products were sized using CEQ 8000 genetic analysis system (Beckman-Coulter, Fullerton, CA). Allele sizes were scored using the CEQ 8.0 software and 400 bp standards (Beckman-Coulter).

We determined if each sample location and locus was in Hardy–Weinberg equilibrium (HWE) and linkage equilibrium using GenePop v.4.0 (François 2008), where significance values were estimated using a Markov chain approach (dememorization = 10^4, number of batches = 10^3, number of iterations per batch = 10^4). We determined the distribution of genetic diversity across the sample locations in the current range by measuring diversity as the number of alleles and allelic richness, adjusted for sample size, using FSTAT v.2.9 (Goudet 2001), in addition to observed and expected heterozygosity, which were estimated using GenAlEx v.6.1 (Peakall and Smouse 2006). Significant differences in genetic diversity between populations were determined using the group comparison tool in FSTAT to test for significance at $\alpha = 0.05$ probability level based on 10^4 permutations.

We determined current genetic structure across the range of *P. p. niveiventris* by estimating global *F*-statistic

values following the methods of Weir and Cockerham (1984). To evaluate population interconnectivity we estimated differentiation between sample locations as pair-wise F_{ST} (Weir and Cockerham 1984). Both measures of differentiation were estimated in FSTAT. Genetic differentiation is predicted to increase with geographic distance (i.e. isolation by distance; Wright 1943), so to test for the relationship between genetic and geographic distances, we ran a Mantel test in IBDWS v.3.15 (Jensen et al. 2005). Significance was estimated using 3×10^4 randomizations. Pair-wise F_{ST} was used as a measure of genetic distance, and geographic distance was the Euclidean distance (km) between sample locations estimated using *dist* functions in R.

Lastly, we estimated the number of genetically distinct clusters (K) as an additional test of genetic structuring across the current range of *P. p. niveiventris* using a Bayesian admixture procedure (STRUCTURE v.2.2; Pritchard et al. 2000). With a Bayesian admixture procedure we can also identify recent gene flow between populations. The software STRUCTURE fits the data to a given K minimizing Hardy–Weinberg and linkage disequilibrium, and gives a likelihood score [Pr(X|K)] for how well the data fit a set K. We applied a hierarchical approach to test the connectivity between the peripheral sample locations and the central part of the current range. First we included all individuals, then we included only one or the other peripheral sample locations (SDP and PINWR, Fig. 1) with all central populations. Lastly, we included only central sample locations in the STRUCTURE analysis. To determine the best fit K for each analyses, we used the best Pr(X|K) score as well as the method suggested by Evanno et al. (2005), which determines the second order derivative of Pr(X|K). Most parameters were set to the defaults given by STRUCTURE, with an admixture ancestral model. The initial 2×10^4 MCMC generations were discarded as burn-in, with a subsequent 5×10^5generations used to estimate parameters. We ran the analyses for the number of clusters ranging from 1 to 10, with 25 independent runs for each cluster. We plotted the membership coefficient values for each individual included in the analysis to evaluate the genetic structure for the best fit K. Individuals with membership coefficients associated closely to clusters other than those found in its sample location were considered evidence of a migrant or recent gene flow.

Results

Evaluating loss of genetic diversity using mtDNA

Sixty-three of 78 samples from museum specimens provided sufficient (>75 %) *cyt b* sequence data to analyze (Table S1 in Supplementary materials). Five haplotypes were identified from the historical samples and two corresponded to published haplotypes of this subspecies (Fig. 2a; EF216336 and EF216337; Degner et al. 2007). Of the 63 historical samples, 45 had sufficient locality information to assign to specific trapping locations: New Smyrna, Cape Canaveral, Oak Lodge and Jupiter Island (Table 1; Fig. 2a).

A total of 37 *cyt b* sequences were generated from the contemporary sampling of *P. p. niveiventris*. Three haplotypes, corresponding to published haplotypes, were identified in the contemporary distribution (Table 1; EF216336-216338; Degner et al. 2007) and two locations had more than one haplotype (Cape Canaveral and Canaveral National Seashore; Fig. 2b). We sequenced a subset of individuals representing Cape Canaveral, including MINWR, BG1-3 and IG1-3, as there is evidence of gene flow (see Results and Degner et al. 2007). Our rarefaction simulation analyses indicate that we had sufficient sample size in order to detect historical and contemporary genetic diversity (Fig. S1 in Supplemental materials). All measures of mitochondrial genetic diversity were higher from the historical range than the current range (Table 1). However, we found no statistically significant loss of genetic diversity between historical and contemporary samples (haplotype: $t = 0.933$, $df = 5.534$, $P = 0.195$; polymorphic sites: $t = 0.677$, $df = 5.798$, $P = 0.262$; k: $t = 0.185$, $df = 5.449$, $P = 0.430$; and π: $t = 1.121$, $df = 5.575$, $P = 0.138$). We did observe a significant loss of nucleotide differences (k: $t = 2.545$, $df = 3$, $P = 0.043$) and nucleotide diversity (π: $t = 2.731$, $df = 3$, $P = 0.036$) when we excluded the central sample locations from the analysis.

The historical haplotype network showed the highest frequency of haplotype A (44 %), followed by haplotype X (36 %), which was not recovered in the contemporary distribution (Fig. 2c). Haplotype B was well represented with eight sequences found across the historical range (Fig. 2c). Two other historical haplotypes, Z and Y, were found at lower frequencies. Four of the five haplotypes identified in the historical range were each found in several sample locations (Fig. 2a). The only location with a unique haplotype was OL, where we identified the Z haplotype (Fig. 2a). The resolution of the haplotype network was poor, and does not inform the relationship between the different haplotypes (Fig. 2c). All of the haplotypes differed by one to two base pair changes. The contemporary samples show a majority of individuals were assigned to haplotype B (54 %) followed by haplotype A (43 %), with only one individual of haplotype C (Fig. 2d). Haplotypes A and B differ by one base pair, whereas A and C have two base pair differences separating them (Fig. 2d).

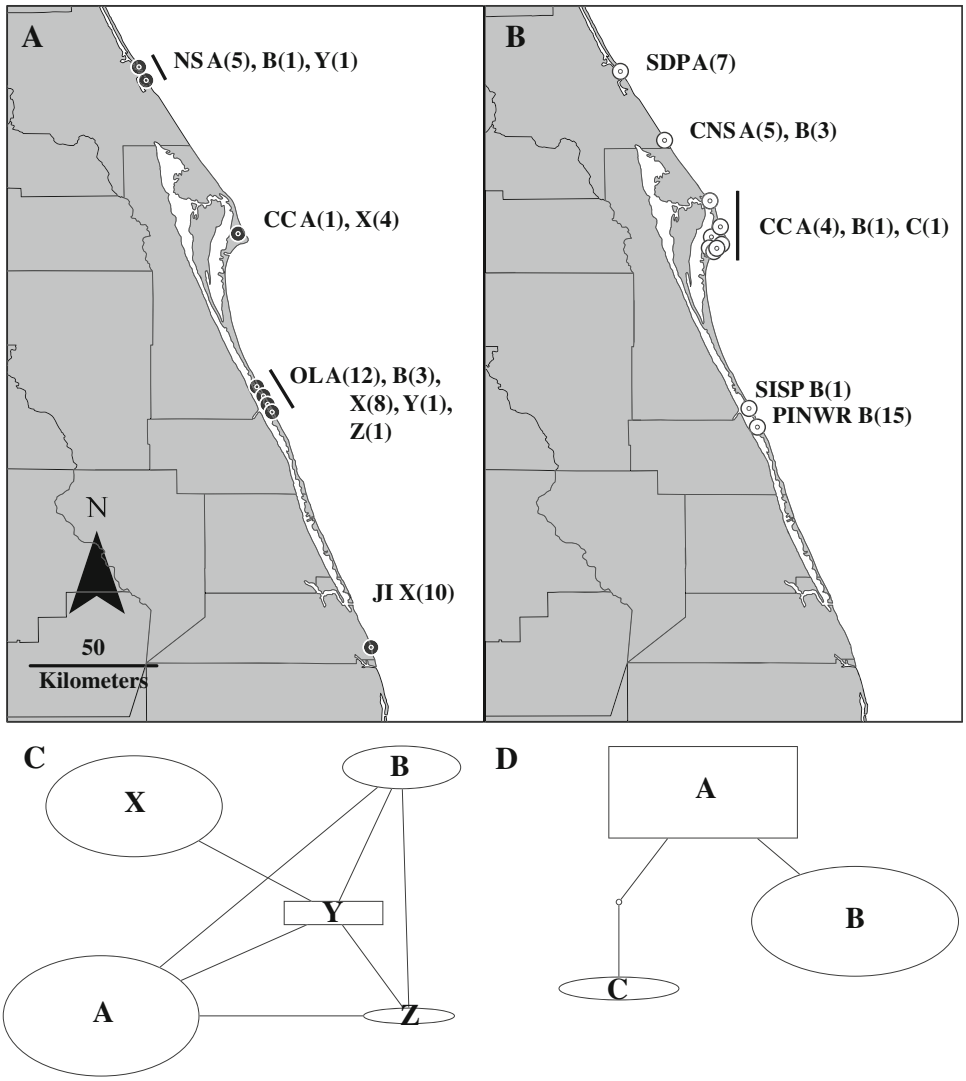

Fig. 2 Sampling locality of *cyt b* sequence data across the historical (**A**) and contemporary (**B**) range of *P. p. niveiventris* with haplotypes labeled by each location (haplotype frequencies are stated in parentheses). Historical samples were collected from four areas; New Smyrna (NS), Cape Canaveral (CC), Oak Lodge (OL), and Jupiter Island (JI). Contemporary samples were collected from Smyrna Dunes Park (SDP), Canaveral National Seashore (CNS), Cape Canaveral (CC), Sebastian Inlet State Park (SISP), and Pelican Island National Wildlife Refuge (PINWR). The relationship among the historical (C) and contemporary (D) haplotypes is shown as a haplotype network, where the relative size of the box or oval illustrates frequency of the haplotype and lines illustrate proposed mutational steps

Contemporary genetic structure and interconnectivity

Three of the locus-by-location comparisons deviated from HWE for the microsatellite data set after the Bonferroni correction for multiple comparisons was applied. The deviation in HWE was found in three separate populations. In estimating linkage disequilibrium among all loci within each sample location, two loci combinations significantly deviated from linkage disequilibrium after Bonferroni correction. Based on the limited number of locations with loci out of HWE (N = 3), and only two locus pairs in linkage disequilibrium, we included all populations and loci in our analysis.

We found similar genetic diversity among the sample locations from the central part of the current distribution of *P. p. niveiventris* (Fig. 1; Table 2). The two locations found on the periphery of the range (SDP and PINWR; Fig. 1; Table 2) had significantly lower allelic richness (P = 0.024) and expected heterozygosity (P = 0.024) than values found for the remaining range of *P. p. niveiventris*.

Global F_{ST} estimated across the current distribution of *P. p. niveiventris* was 0.042 ± 0.004 SE. The pair-wise F_{ST} estimates ranged from 0.004 between the geographically closest locations (BG3 and IG3) to 0.213 between the most distant locations (SDP and PINWR; Table 3). We did uncover a significant relationship between geographic distance and genetic distance throughout the dataset, with increased genetic differentiation following increased geographic distance (Mantel test; $P < 0.0001$, $R^2 = 0.855$; Fig. S2 in Supplemental Material).

When we included all sample locations in the Bayesian admixture model we observed an increase in likelihood scores [P(X|K)] until reaching a mode of K = 8 (Fig. S3A in Supplemental Material). Whereas, based on the procedures by Evanno et al. (2005), the best K value for explaining our microsatellite data was K = 2 (Fig. S3A in

Table 2 Summary data for ten microsatellite loci across the contemporary range of *P. p. niveiventris* represented by 344 individuals

Sample location	N	A	AR	Ho	He
Cape Canaveral					
Beach Grid 1	35.0 ± 0.00	9.4 ± 2.99	8.0 ± 2.54	0.706 ± 0.193	0.759 ± 0.180
Beach Grid 2	40.8 ± 0.42	9.5 ± 3.95	7.7 ± 2.96	0.716 ± 0.270	0.738 ± 0.240
Beach Grid 3	27.6 ± 0.52	8.9 ± 3.98	7.7 ± 3.21	0.709 ± 0.191	0.738 ± 0.185
Inland Grid 1	43.0 ± 0.00	8.6 ± 3.53	7.1 ± 2.90	0.707 ± 0.261	0.731 ± 0.242
Inland Grid 2	44.0 ± 0.00	9.6 ± 4.35	7.8 ± 3.09	0.691 ± 0.222	0.691 ± 0.222
Inland Grid 3	56.0 ± 0.00	9.8 ± 4.26	7.9 ± 2.85	0.711 ± 0.206	0.731 ± 0.242
Canaveral National Seashore	30.5 ± 0.53	7.7 ± 3.30	6.6 ± 2.45	0.598 ± 0.232	0.673 ± 0.251
Merritt Island National Wildlife Refuge	31.7 ± 0.67	7.6 ± 3.41	6.7 ± 2.70	0.671 ± 0.263	0.718 ± 0.201
Smyrna Dunes Park	18.9 ± 0.32	4.2 ± 1.32	4.1 ± 1.34	0.588 ± 0.237	0.525 ± 0.212
Pelican Island National Wildlife Refuge	15.0 ± 0.00	4.2 ± 1.62	4.2 ± 1.62	0.600 ± 0.229	0.583 ± 0.188

Sample location, sample size (N), number of alleles (A), allelic richness (AR), and observed (H_o) and expected (H_e) heterozygosity. AR is adjusted for sample size. Values are reported as averages and standard deviations

Table 3 Geographic and genetic distance between sample locations of *P. p. niveiventris*

	BG1	BG2	BG3	CNS	IG1	IG2	IG3	MINWR	SDP	PINWR
BG1	–	4.6	4.6	45.7	4.0	6.9	2.3	17.7	77.1	73.4
BG2	0.012	–	4.4	41.6	5.8	5.1	4.9	14.4	72.9	76.9
BG3	0.005	0.009	–	45.4	2.1	9.2	2.7	18.7	76.6	72.9
CNS	0.032	0.026	0.028	–	47.2	39.4	46.5	28.7	31.4	118.1
IG1	0.021	0.027	0.024	0.046	–	10.0	1.7	20.1	78.5	71.2
IG2	0.012	0.013	0.013	0.033	0.017	–	8.6	10.9	70.8	80.3
IG3	0.009	0.010	0.004	0.023	0.019	0.015	–	19.0	77.8	72.1
MINWR	0.020	0.019	0.013	0.022	0.035	0.019	0.020	–	60.1	91.1
SDP	0.127	0.108	0.109	0.118	0.148	0.116	0.107	0.122	–	148.9
PINWR	0.112	0.125	0.125	0.147	0.149	0.138	0.119	0.130	0.213	–

Below diagonal is pair-wise F_{ST} estimated in FSTAT based on ten microsatellite loci, while above diagonal is Euclidean distance between sample locations measured in kilometers. Sample location abbreviations are defined in Fig. 1

Supplemental Material). For K = 2 there was no clear geographic association of the clusters among individuals collected in the central sample locations. Individuals had membership coefficients which placed them in either cluster equally, further supporting no specific geographic association (Fig. 3a). All individuals from the two peripheral locations (SDP and PINWR) were associated with the same cluster, suggesting the peripheral sample locations are isolated compared to the central sample locations (Fig. 3a). The geographic structure among our sample locations is more evident for K = 8. We found evidence of admixture in the central populations, with individuals variably associated with the different clusters (Fig. 3b). With K = 8 the two peripheral sample locations had strong membership coefficients for their respective clusters, again indicating isolation. This also indicates a lack of gene flow among the respective peripheral locations and the central portion of the current distribution (Fig. 3b). These findings were further supported in our hierarchical analyses. We ran three separate STRUCTURE analyses, where two analyses included only one peripheral sample location together with the central sample locations per run, and a final analysis included only individuals from the central sample locations. Based on Evanno et al. (2005) procedures, we determined best K = 3 when only one or the other peripheral location was included, and K = 7 when only central sample locations were included (Fig. S3B–D in Supplemental Material). When we only included one of the two peripheral sample locations in our analyses, individuals in the peripheral sample locations showed high membership coefficients to a single cluster, while the central sample locations showed high levels of admixture (Fig. S4A, B in Supplemental Material). If the analysis was restricted to central sample locations, all showed admixture, with individuals showing an association to multiple clusters and no clear geographic separation in membership coefficients (Fig. S4C in Supplemental Material).

Discussion

Evaluating loss of genetic diversity using mtDNA

Understanding current anthropogenic impact on taxa and ecosystems is central to conservation biology; however, it is vital to understand the historical processes and patterns that have affected these taxa prior to human settlements. Anthropogenic habitat loss has resulted in the recent extirpation or reduction in numbers of *P. p. niveiventris* populations (Stout 1992). Low genetic diversity prior to anthropogenic impacts is also observed. Compared to mainland relatives, where 22 haplotypes have been identified (Degner et al. 2007; Kalkvik et al. 2012), we recovered a total of six haplotypes among both historical and contemporary samples. It is thought that beach mice were isolated by lagoonal systems, when barrier islands became separated from the mainland (Hoekstra et al. 2006; Van Zant and Wooten 2007). It has been suggested that the divergence between beach mice and mainland populations occurred around 6,000 ybp with the formation of the barriers (MacNeil 1950; Hoekstra et al. 2006), but other evidence suggests this divergence occurred around 200,000 ybp (Van Zant and Wooten 2007). An initial founder effect or bottleneck in the early stages of the formation of *P. p. niveiventris* may be the most parsimonious explanation for the historical loss of genetic diversity. Such events can affect taxa over a long period of time with subsequent bottlenecks having little additional impact on the genetic diversity (Taylor and Jamieson 2008). Several studies have found that current, low levels of genetic diversity are explained by historical bottlenecks and founder effects dating to times prior to anthropogenic impacts (Hoffman and Blouin 2004; Chan et al. 2005; Calvignac et al. 2008; Reding et al. 2010).

We were unable to demonstrate a statistically significant loss of genetic diversity in *P.p. niveiventris* over the last century based on *cyt b* sequence data obtained from museum specimens. However, when we excluded the contemporary sample locations found in the continuous habitat from the analysis we observed a significant loss of nucleotide differences (k) and nucleotide diversity (π) compared to historical data (Fig. 2b). The sample locations within the central range are the only locations that currently exhibit location level genetic variation among *cyt b* sequences (Table 1; Fig. 2b). These findings indicate that this remaining continuous habitat serves as a refuge of historical genetic diversity. The two areas with the highest historical genetic diversity (New Smyrna and Oak Lodge; Fig. 2a), are currently fixed for their haplotypes (described as SDP and PINWR in current distribution; Fig. 2b). Thus it seems that animals in these areas have undergone a recent genetic loss as a result of isolation and population declines, which is most likely due to anthropogenic induced habitat destruction.

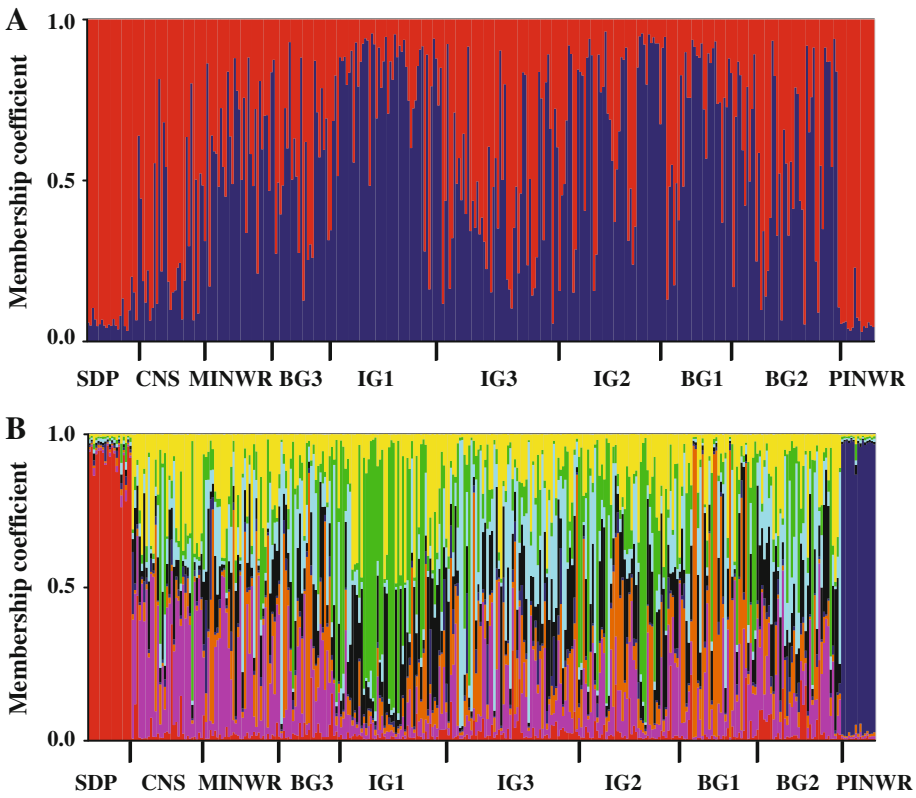

Fig. 3 Estimated membership coefficients for individuals within the ten sampled locations across the contemporary distribution of *P. p. niveiventris* (based on ten microsatellite loci), for K = 2 (**A**) and K = 8 (**B**) clusters. Sample locations are arranged by decreasing latitude and abbreviations are defined in Fig. 1

Contemporary genetic structure and interconnectivity

Studies using temporal sampling have shown that human exploitation and habitat alteration can result in loss of genetic diversity within natural populations of a diverse set of organisms over short periods of time (Pichler and Baker 2000; Hauser et al. 2002; Culver et al. 2008; Thalmann et al. 2011). We predicted a loss of genetic diversity in *P. p. niveiventris* after an approximate 80 % reduction in its range over the last few decades. Contrary to our predictions, we observed no statistically significant loss of *cyt b* diversity over the current distribution (Table 1), although this locus was not hyper-variable in either sample. We do find a strong geographic pattern of contemporary genetic diversity, which correlates to habitat richness. The central portion of the contemporary range is the most genetically diverse, whereas, the northern and southern disjunct sample locations exhibit statistically lower levels of genetic variation. The importance of continuous habitat for the persistence of biodiversity has been addressed both theoretically and empirically by Fahrig (2002, 2003) who has shown that continuous habitat is favored for persistence. This has been supported when measured both genetically and demographically in a wide range of taxa, such as reptiles (Johnson et al. 2007; Dixo et al. 2009), birds (Coulon et al. 2010), and mammals (Haag et al. 2010; Holland and Bennett 2010).

Contemporary genetic structure of *P.p. niveiventris* provides insight into the processes that most likely explain the conservation of historical genetic diversity. Central to the outcome of these processes is the continuous, linear configuration of the habitat. We found indirect evidence of dispersal within the 70 km extent of coastal dunes currently occupied by *P. p. niveiventris*. For example, pairwise F_{ST} values estimated from microsatellite data showed low levels of genetic structuring (Table 3), and the STRUCTURE analyses indicated high levels of admixture among these locations (Fig. 3, Fig. S4 in Supplemental material). These levels of genetic admixture may explain how *P. p. niveiventris* is able to maintain the highest level of genetic diversity observed among beach mice subspecies with an average of 8.0 alleles per loci (Table 2), compared to 4.3–6.8 alleles per loci for subspecies on the Gulf coast that typically occupy more fragmented habitat (Mullen et al. 2009).

We identified two peripheral sample locations, SDP and PINWR (Fig. 1), as being disjunct from the current central distribution of *P. p. niveiventris*. These sample locations are isolated by urbanized zones that represent barriers to gene flow. The two peripheral sample locations do not share haplotypes, indicating isolation. Further evidence for the lack of connectivity comes from the absence of genetic admixture with the central sample locations (Fig. 3, Fig. S4A and B in Supplemental material) and higher levels of genetic differentiation for the disjunct locations compared to the central sample locations (Table 3). A lack of gene flow may further reduce genetic diversity through genetic drift. We did observe a pattern of isolation by distance (IBD; Fig. S2 in Supplemental Material), that is explained by an equilibrium between migration and genetic drift across the range (Wright 1943; Hutchison and Templeton 1999). A lack of gene flow to some of the sample locations would explain a deviation from IBD. However, we may observe IBD across the current range of *P. p. niveiventris* as a result of collinearity between habitat availability and geographic distance, or that the isolated populations are peripheral in the current distribution.

Consequences for management

The relatively undeveloped 70 km stretch of preserved continuous coastal habitat now occupied by *P. p. niveiventris* came under Federal ownership and protection as an indirect result of the cold war and U.S. space program. Coastal habitats south of Cape Canaveral, with the exception of isolated parcels, have lost beach mice as a component of these ecosystems. A combination of land conversion, beach erosion, tropical storms and hurricanes, feral animals, and human activities explain this loss (Stout 1992). In the short term, the sequestered Federal lands should continue to support *P. p. niveiventris.* Long term, climate disruption poses an unknown threat given the fact that the critical habitat lies at the interface of land and the Atlantic Ocean (Barbier et al. 2008; Mawdsley et al. 2009).

Overall, our *cyt b* data indicate that genetic diversity has generally been maintained over the past 100 years, even with extensive loss of habitat. However, we postulate that this diversity is only maintained because of the presence of a long section of undeveloped coastal dune habitat, found on protected and managed Federal lands. These findings illustrate the importance of preserving continuous habitat or larger areas for organism to inhabit, to reduce the overall impacts of human interference and allow persistence of the taxon (Breininger et al. 1998; Fischer and Lindenmayer 2007; Medina-Vogel et al. 2008). This area has also been recognized as significant for the conservation of several sea turtle species (Schmid 1995). Going forward, the conservation of the approximately 70 km coast line of intact habitat will be essential to ensure the genetic integrity of *P. p. niveiventris*, and presumably the genetic integrity of other taxa occupying this area.

Given the isolation and associated lower genetic diversity of the two peripheral populations (SDP and PINWR), we believe these populations (Fig. 1) are of immediate conservation concern. Lower genetic diversity can be expected in peripheral populations (Lawton 1993; Eckert

et al. 2008), but historical data indicate that these two populations had historically higher genetic diversity and the recent range contraction and isolation seem to have resulted in a loss of genetic material from these areas.

Peromyscus polionotus niveiventris is a taxon that most likely will be increasingly impacted by current global warming and sea level rise, as seen and projected in other taxa (Geselbracht et al. 2011; Maschinski et al. 2011; Saha et al. 2011a). Adaptation in response to these changing environmental conditions depends in part on the genetic variation represented in the population at risk (Lavergne and Molofsky 2007). With no evidence of overall loss of mitochondrial genetic diversity over the last few decades, and the highest current nuclear diversity observed among beach mice, *P. p. niveiventris* seems to have endured little genetic impact from human encroachment into the currently occupied coastal habitats. However, we show the importance of evaluating changes in the distribution of genetic diversity and isolation of peripheral populations from a historical perspective. By including historical and contemporary information we show that the persistence of coastal taxa may dependent on connected habitat with low anthropogenic impacts.

Conclusions

Genetic diversity is associated with persistence of populations (Reed and Frankham 2003; Lavergne and Molofsky 2007), and is therefore an important metric in our conservation of species. Both historical and anthropogenic impacts may be responsible for current levels of genetic diversity and structure. We determined that historical forces are probably responsible for current low levels of genetic diversity rather than recent anthropogenic impacts on habitat of *P. p. niveiventris*. These results are supported by similar studies in different taxonomic groups (e.g. Hoffman and Blouin 2004; Chan et al. 2005; Reding et al. 2010). We determined that *P. p. niveiventris* has maintained historical levels of genetic diversity in the large federally protected continuous habitat as opposed to the two peripheral populations that have reduced diversity. The 70 km long federally protected coastal habitat functions as a refuge for genetic diversity, while lands outside of this area are undergoing anthropogenic change. This study illustrates the importance of evaluating historical genetic diversity in a landscape influenced by both historical events and recent anthropogenic influence. Our results indicate the importance of maintaining continuous habitat for the future persistence of genetic diversity.

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