Museum Genomics Provide Evidence for Persistent Genetic Differentiation in a Threatened Seabird Species in the Western Atlantic

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Synopsis Connectivity among wildlife populations facilitates exchange of genetic material between groups. Changes to historical connectivity patterns resulting from anthropogenic activities can therefore have negative consequences for genetic diversity, particularly for small or isolated populations. DNA obtained from museum specimens can enable direct comparison of temporal changes in connectivity among populations, which can aid in conservation planning and contribute to the under- standing of population declines. However, museum DNA can be degraded and only available in low quantities, rendering it challenging for use in population genomic analyses. Applications of genomic methodologies such as targeted sequencing ad- dress this issue by enabling capture of shared variable sites, increasing quantity and quality of recovered genomic information. We used targeted sequencing of ultra-conserved Elements (UCEs) to evaluate potential changes in connectivity and genetic diversity of roseate terns (*Sterna dougallii*) with a breeding distribution in the northwestern Atlantic and the Caribbean. Both populations experienced range contractions and population declines due to anthropogenic activity in the 20th century, which has the potential to alter historical connectivity regimes. Instead, we found that the two populations were differentiated historically as well as contemporaneously, with little evidence of migration between them for either time period. We also found no evidence for temporal changes in genetic diversity, although these interpretations may have been limited due to sequencing artifacts caused by the degraded nature of the museum samples. Population structuring in migratory seabirds is typically reflective of low rates of divergence and high

connectivity among geographically segregated subpopulations. Our contrasting results suggest the potential presence of ecological mechanisms driving population differentiation, and highlight the value of targeted sequencing on DNA derived from museum specimens to uncover long-term patterns of genetic differentiation in wildlife populations.

Introduction

Standing genetic diversity is a critical component of fit- ness in wildlife populations (Reed and Frankham 2003; Hughes et al. 2008). Anthropogenic activity has been empirically demonstrated to negatively affect diversity in wildlife populations (Frankham 1996; Allendorf et al. 2008; Li et al. 2016), resulting in an estimated 6% loss of genetic variation across animal taxa in the 20th century (Leigh et al. 2019). Human-mediated loss of genetic diversity can occur in wildlife populations through multiple pathways. Disruption of dispersal patterns among geographically distinct populations has in particular been established to have a critical negative effect on genetic diversity (Johnson et al. 2004; Peery et al. 2010). Connectivity via dispersal of breeding individuals facilitates exchange of genetic material between populations, introducing variation and potentially offset- ting loss of alleles resulting from population declines (Reding et al. 2010; Welch et al. 2012; Ramírez et al. 2013; Lonsinger et al. 2018) and genetic drift. When populations become fragmented via habitat loss and range contractions, movement of individuals among them can be compromised, reducing exchange of genetic material and enhancing genetic differentiation between populations (Peery et al. 2010; Athrey et al. 2011). For small populations, these reductions in gene flow from external populations can lead to inbreeding depression and loss of adaptive potential as genetic diversity erodes (Frankham 1996; O'Grady et al. 2006; Charlesworth and Willis 2009). Evaluating changes in rate of gene flow over time among and changes in genetic variation within extant populations can therefore provide insight into the factors contributing to declines in small and potentially isolated wildlife populations.

Estimating changes to gene flow and genetic diversity through analysis of modern populations only may not provide a full picture of population dynamics, as static estimates of diversity in modern populations are determined by life history traits as well as historical demography (Romiguier et al. 2014; Díez-del-Molino et al. 2017). Additionally, inferences of historical gene flow from modern samples via coalescent-based methods can have reduced accuracy when changes in population connectivity rates have occurred over recent timescales (Samarasin et al. 2017), as is common for declining populations. Use of DNA derived from archival museum specimens, hereafter referred to as museum DNA, can subvert these issues by enabling direct comparisons of

historical and modern population parameters to evaluate temporal changes. Despite its great potential for such analyses, museum DNA is typically degraded and only present in low quantities (Burrell et al. 2015; McDonough et al. 2018), which can limit the extent of its use in population genomic studies requiring large numbers of loci. Targeted enrichment of museum DNA via synthetic probes can resolve this issue by enabling capture and amplification of genomic areas of interest only, which, combined with high throughput genomic sequencing, increases the potential of recovering adequate amounts of historical genetic material for analysis (Faircloth et al. 2012; Linck et al. 2017). Use of the same methodologies to capture variant sites in modern DNA can then facilitate direct comparison of genetic parameters between historical and modern populations (Crates et al. 2019). Such comparisons can provide a wealth of information on population-level changes to connectivity and genetic diversity across greater timescales, which has the potential to broaden the understanding of the temporal effects of human activities or other impacts on population dynamics (Schmid et al. 2018; Crates et al. 2019).

Museum DNA also represents an opportunity for enhancing the understanding of understudied wildlife populations, for which long-term demographic information can be incomplete or nonexistent. In North America, roseate terns (*Sterna dougallii*), a small, gull- like seabird, are divided into northwestern Atlantic and Caribbean breeding populations that appear to co- occur on a South American wintering ground (Hays et al. 1999, 2010). The endangered northwestern roseate tern population has experienced extensive population declines and range contractions since the early 20th century (Buckley and Buckley 1974; Nisbet et al. 2012; U.S. Fish and Wildlife Service 2020), and has accordingly been the subject of intensive research and conservation programs since the 1980s. By contrast, the demo- graphic history of the threatened Caribbean population is not well understood due to lower research effort in the region (Nisbet and Ratcliffe 2008; U.S. Fish and Wildlife Service 2010).

Genomic analysis of modern roseate terns has shown that the northwestern and Caribbean populations are genetically differentiated, with minimal contemporary gene flow between them (Byerly 2021). This finding is unusual, as the pattern for migratory seabirds typically reflects low rates of divergence and high connectivity among geographically segregated subpopulations within the same oceanic basins due to the great mobility potential of the guild (Friesen et al. 2007; Yannic et al. 2016; Booth Jones et al. 2017; Quillfeldt et al. 2017). Here, we use targeted sequencing of ultra-conserved elements (UCEs) to recover variant loci and evaluate roseate tern genetic diversity and population connectivity across both spatial and temporal scales. Given the known and inferred demographic changes of the two populations in the 20th century, evidence for differentiation among contemporary, but not historical, North American roseate tern populations

may be suggestive of disruptions to historical dispersal regimes along the Atlantic seaboard. Such changes to historical connectivity could have resulted in isolation of the northwestern population and subsequent loss of genetic diversity.

Methods

Footpad tissue was obtained from museum specimens collected from the Northwest (*n* = 20; hereafter H-NW for Historical NW) and Caribbean (*n* = 23; hereafter H-CAR for Historical CAR) regions between 1879– 1941 (see Supplementary Text S1 for a full description of specimen origins). We sampled tissue only from museum specimens collected during the breeding season (May–August) with the assumption that these individuals were presumably breeding in the sampling location. For museum samples, we extracted DNA and prepared genomic libraries in a dedicated PCR product-free laboratory. Museum DNA was extracted using a phenol–chloroform protocol modified from McDonough et al. (2018) with a negative control for every eight reactions. Following extraction, we quantified all extraction products using a Qubit Fluorometer 3.0 (Invitrogen, Carlsbad, CA, USA).

Past work on roseate terns has shown minimal genetic differentiation among subpopulations or colonies within the northwestern (Szczys et al. 2005; Dayton & Szczys 2021) and Caribbean (Byerly 2021) populations, and we therefore reasoned that modern samples collected from single sites within each population should provide adequate representation of genetic diversity across each population. Blood and tissue samples were collected from roseate tern chicks in the US Virgin Islands (*n* = 37; hereafter, C-CAR for Contemporary CAR) and Bird Island, MA, USA (*n* = 35; here- after C-NW for Contemporary NW) in 2017. Samples were stored in Queen's lysis buffer prior to extraction. We extracted whole genomic DNA from modern samples using Qiagen DNeasy Blood and Tissue kits (Qiagen Inc., Valencia, CA, USA) with a negative control for every 18 reactions and a modified 24-h digestion time for tissue samples.

For library preparation, we sheared DNA from mod- ern samples using a Biorupter with a target fragment size of <500 bp. DNA from museum specimens was al- ready fragmented and required no additional shearing. After extraction, shearing, and quantification, we pre- pared genomic libraries for museum and modern samples separately using the Blunt-end Single-tube (BEST) method (Carøe et al. 2018), with libraries ligated to bar- coded adapters, amplified for sequencing between 11– 14 (museum) and 6–9 (modern) PCR cycles, and purified. We then quantified concentration of multiplexed libraries using a Qubit Fluorometer and assessed mean fragment size using an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA).

For UCE enrichment, we combined multiplexed libraries into 500 ng (166 ng/library) pools for ancient samples and 750 ng (150 ng/library) pools for modern samples. We then enriched equimolar pools for 5060 UCE loci using the myBaits UCE Tetrapods 5Kv1 kit (Arbor Biosciences, Ann Arbor, MI, USA) following the recommended protocol. Samples were hybridized for 48 hours for museum specimens with 18 cycles of post-enrichment PCR and for 24 hours for modern specimens with 16 PCR cycles. We quantified fragment size distribution of enriched pools with a Bioanalyzer High Sensitivity dsDNA kit (Agilent Technologies, Santa Clara, CA, USA). Enriched pools were then combined at equimolar ratios and sequenced as paired-end 150 bp reads on a single lane of an Illumina HiSeq 2500.

Genotyping

Sequenced genomic reads were demultiplexed using Bcl2fastq 1.8.4 (Illumina). Following demultiplexing, we processed reads using the Phyluce bioinformatics pipeline (Faircloth 2016). First, we cleaned reads by re- moving adapter contamination and low-quality bases with Illumiprocessor (Faircloth 2016) and the trimming tool Trim Galore (https://github.com/FelixKrueger/TrimGalore) with default minimum retained contig size = 20. For *de novo* contig assembly, we chose a modern sample with an intermediary number of reads, which we reasoned, would maximize recovery of UCEs while minimizing the amount of duplicate contigs. To prepare our reference, we assembled the reference sample's Read 1 and 2 files *de novo* using the Trinity 2.0.6 assembler (Grabherr et al. 2011), mapped the output contigs to a FASTA file of the UCE probes using Phyluce, and converted the results to our final reference FASTA file. We aligned raw, clean reads from each sample to the reference using the "mem" algorithm in BWA 0.7.12 (Li and Durbin 2010) and cleaned the output using BWA and PicardTools 1.113 (http://picard.sorceforge.net) within the Phyluce wrapper. We rescaled quality scores for all samples using mapDamage 2.0 to correct for potential damage in museum samples (Ginolhac et al. 2011) and then called single nucleotide polymorphisms (SNPs) using HaplotypeCaller in GATK 4.1.8.1 (McKenna et al. 2010).

We used VCFtools to filter SNPs in the output VCF file by first removing indels and all loci below a Phred- scaled minimum genotype quality of 30. For population structure and gene flow analyses, we filtered samples by a minimum read depth of 3x, maximum read depth of 120x, and maf = 0.05, and removed SNPs with >30% missing data across all samples and samples with >90% missing data (hereafter, the "moderate" filter set). Because estimates of genetic diversity can be biased by missing data (Pompanon et al. 2005), which we expected would be higher for museum samples, we then removed SNPs with >20% missing data and samples with >75% missing data for

comparisons of F_{ST} and temporal genetic diversity (hereafter, the "strict" filter set). We also reperformed all population structure analyses using the strict filter set to investigate the effect of SNP filtering on output and validate results. To remove loci in linkage disequilibrium, we randomly restricted SNPs to 1 per UCE locus. For both filter sets, we then tested each sampling group separately for deviation from Hardy–Weinberg Equilibrium (HWE) using 1000 Monte Carlo replicates in the package "pegas" and removed loci out of HWE within any group from the entire dataset using a sequentially Bonferroni- corrected significance threshold of $\alpha = 0.05$. We note that this HWE filtering approach has the potential to artificially inflate population structure in some scenarios (Pearman et al. 2022), but do not feel that this was a potential source of bias in our study given our results and the minor number of loci we removed from the overall final SNP set.

Population genetics

For subsequent analyses, we treated each sampling group (H-NW, H-CAR, C-NW, C-CAR) as an individual population. To measure genetic diversity, we calculated observed and expected heterozygosity (respectively, H_o and H_e) and rarefied allelic richness (A_R) for each group in the R package diveRsity (Keenan et al. 2013). To test for differences in A_R between sample groups, we used a Friedman test with SNP locus as a blocking factor, and investigated output for post-hoc pairwise significance using a Wilcox test with Bonferroni-corrected *P*-values. For comparison of H_o and H_e between time periods, we used a two-way type III ANOVA with region and time as independent variables and SNP locus as a blocking factor, and investigated output for post-hoc pairwise significance using a Tukey test with *P*-values adjusted for multiple comparisons. We examined differentiation between populations with Weir and Cockerham's F_{ST} (Weir and Cockerham 1984) in diveRsity, using 10^4 permutations to calculate bias-corrected 95% confidence intervals (CIs). All analyses involving multiple comparisons were corrected with a sequential Bonferroni correction.

Population structure

We inferred population structuring or number of genetic clusters among our four groups using two methods. First, we used the Bayesian clustering pro- gram STRUCTURE v. 2.3.4. (Pritchard 2000) on a combined dataset of all four groups to evaluate differentiation on both spatial and temporal scales. We ran simulations for one to five possible clusters (*K*) for 10 iterations each with 10^4 MCM repetitions after a burn-in of 10^4 generations using the admixture model with correlated allele frequencies. Inclusion of location as prior information can be useful in STRUC- TURE when

signals of population structure are weak or low numbers of loci are used to make inferences about number of genetic clusters (Porras-Hurtado et al. 2013); however, we inferred that we would have high power to resolve structuring based on evidence for strong differentiation in modern samples (Byerly 2021), and used the "no prior" option. We used the online program STRUCTURE HARVESTER (Earl and von Holdt 2012) to infer the most likely value of *f..K* via the Evanno method and validated results with the mean log likelihood from each iteration of *K* (LnP(*K*); Pritchard 2000). We then aligned clusters, merged STRUCTURE runs by *K* value, and visualized output using the R package Pophelper 2.3.0 (Francis 2016).

In addition to STRUCTURE, we investigated population structuring with Discriminant Analysis of Principal Components (DAPC) in the R package "adegenet" (Jombart et al. 2010). DAPC is a multivariate analysis method that uses principal components (PCs) to infer genetic clustering and assign of individuals to groups by minimizing within-group variation (Jombart et al. 2010). Prior to implementing DAPC, we used the *K*-means clustering via the "find.clusters" function in adegenet to identify the most likely number of genetic clusters in our dataset without *a priori* population information, retaining all PCs, and selecting the number of clusters based on the lowest Bayesian information criterion (BIC) value. We visualized results both without and with *a priori* genetic clusters to describe the relationships among putative populations, and used *a priori* clusters ($K = 4$) to assign individuals to populations of origin. For DAPC analysis, retaining a suboptimal number of PCs can cause model under or overfitting (Jombart et al. 2010). For DAPC both with and without *a priori* grouping, we used cross-validation with 1000 permutations to identify the optimal number of PCs based on the lowest mean squared error rate, and used this as input for the discriminate analysis (DA).

Gene flow between regions was investigated for each time period using BayesAss 3.0.4 (Wilson and Rannala 2003) with modifications for SNP analysis (Mussmann et al. 2019). BayesAss estimates recent (less than three generations) unidirectional migration rate among populations as m_{ij}, with m as the proportion of population i resulting from gene flow from population j. We ran the program for 3×10^6 iterations, with a burn-in of 1×10^6 iterations and a sampling frequency of 2000. To evaluate convergence of MCMC values we performed the analysis five times using different starting seeds and compared the posterior mean parameter estimates for each run to check concordance. Delta values were adjusted with a goal of a 20–60% acceptance rate for each parameter (Wilson and Rannala 2003).

Results

After removing loci and samples during various steps of extraction, library preparation, sequencing, and filtering for quality and missing data (Supplementary Table S1), we were left with *n* = 76 samples (21 museum and 55 recent) and with 1522 SNPs for the moderate filter set and 729 SNPs for the strict filter set (Table 1, Supplementary Table S2). Over 50% (*n* = 22) museum samples were removed due to insufficient starting amounts of DNA, library preparation failure, or high rates of missing data post-sequencing. The median collection year of successful museum samples was 1920 from a range of 1883–1941 (Supplementary Table S1). Mean per individual read depth and % missing data varied by sample age (Table 1), with lower read depth and greater % missing data for museum versus contemporary samples.

Table 1 Comparison of summary statistics from population genetic analyses of roseate terns from 4 time periods and 2 sampling locations based on SNP markers and analyzed following moderate or strict SNP filtering parameters.

Population	*n*	Year	Depth	% miss.	A_R	H_o	H_e
H-CAR	10	1926	14.21x	0.52	**1.44** (0.02)	**0.15** (0.01)	**0.23** (0.01)
H-NW	11	1921	29.40x	0.27	**1.67** (0.01)	**0.20** (0.01)	**0.26** (0.01)
C-CAR	30	2017	68.56x	0.00	**1.90** (0.01)	**0.30** (0.01)	**0.29** (0.00)
C-NW	25	2017	72.05x	0.00	**1.85** (0.01)	**0.30** (0.01)	**0.30** (0.01)

Results presented with standard error in parentheses and with *n* = sample size, year = mean year of sample collection, % miss. = % of SNPs unable to be genotyped due to sequencing uncertainty, A_R = rarefied allelic richness, H_o = observed heterozygosity, and H_e = expected heterozygosity.

Table 2 F_{ST} with 95% CIs estimated from 10^4 bootstraps.

	H-CAR	H-NW	C-CAR
H-NW	0.04 (-0.05, 0.12)		
C-CAR	0.02 (-0.02, 0.08)	**0.02 (0.04, 0.09)**	
C-NW	**0.08 (0.03, 0.13)**	0.02 (-0.03, 0.05)	**0.06 (0.05, 0.06)**

Bolded values represent F_{ST} values with estimated 95% CIs that do not overlap 0.

Genetic diversity

We found significant differences in A_R among sampling periods and regions (χ^2 = 848.63, *df* = 3, *P* < 0.001; Table 1), with significant differences (*P* < 0.001) detected between all pairwise groupings except for C-CAR and C-NW (*P* = 0.15). We found significant differences for H_o by both time period and region (F_1 = 21.57, *P* < 0.001), with pairwise combinations significantly lower for both museum sample sets at adjusted *P* < 0.001, but with no difference between C-CAR and C-NW (*P* = 0.88). H_e also differed significantly by time period and region (F_1 = 19.12, *P* = < 0.001), again with pairwise combinations significantly lower for both museum sample sets (*P* < 0.001) and again with no difference between C-CAR and C-NW (*P* = 0.45). CIs for estimates of genetic differentiation using pairwise F_{ST} values overlapped 0 for H-CAR and H-NW, while C-CAR and C-NW were significantly differentiated (Table 2). However, values were similar be- tween the two time periods, and the difference between the modern and historical samples was likely driven by higher rates of uncertainty due to lower sample sizes and greater % of missing data for the latter, as indicated by wider CIs indicative of lower power (Table 2).

Population structure

STRUCTURE results were best supported at *K* = 2 (Supplementary Fig. S1), with higher *f..K* values at *K* = 2 also supported by lower LnP(*K*) at *K* = 1. STRUCTURE plots were representative of strong differentiation be- tween NW and CAR at all levels of *K* for both the historical and modern periods (Fig. 1), with no substantial differences in degree of admixture between time periods.

Identification of optimal number of clusters via *K*- means clustering was in agreement with STRUCTURE, with *K* = 2 identified as the most likely number of clusters among the 4 groups by the lowest BIC. For DAPC without *a priori* grouping, 40 PCs were retained for the moderate set, representing 64% of the conserved variance, and 40 PCs were retained for the moderate set, representing 76% of the conserved variance. For both types of prior groupings, 100% of samples clustered by region, but not time period (Fig. 2a). For DAPC with *a priori* grouping, 15 PCs were retained for both filter sets, representing 35% of conserved variance for the moderate set, and 40% for the strict. Plotted as *a priori* populations along the first two discriminate functions, samples showed differentiation by both region, and time period (Fig. 2b). Results from both STRUCTURE and DAPC were comparable when ran with the strict filter set, with both analyses showing strongest evidence for *K* = 2 and with little admixture between regions for either time period (Supplementary Figs. S2 and S3).

Bayesian estimates demonstrated low levels of gene flow (*m*) between regions for both the

historical and modern periods. 95% CIs overlapped or approached 0 for all estimates, suggesting that rates of migration between regions were similarly negligible for both the historical and modern periods (Fig. 3).

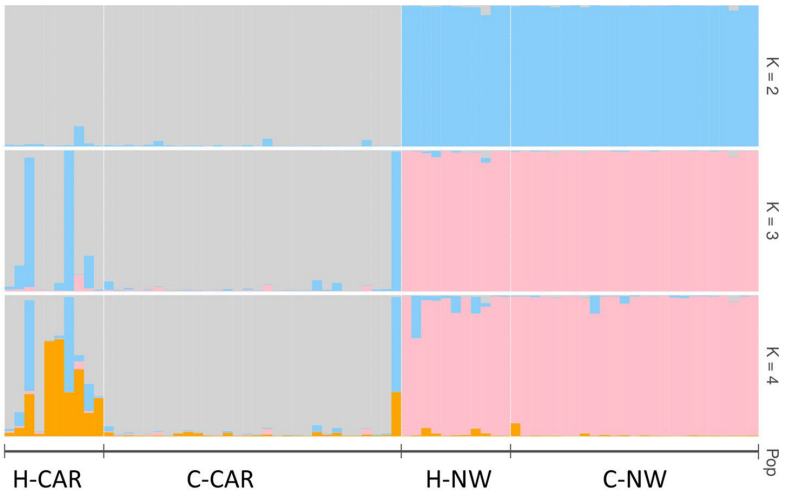

Fig. 1 Genetic clustering inferred by Bayesian structure analysis in STRUCTURE, with the *x*-axis representing sampling location and the
y-axis representing proportion of group membership, and with sampling location not included as prior information.

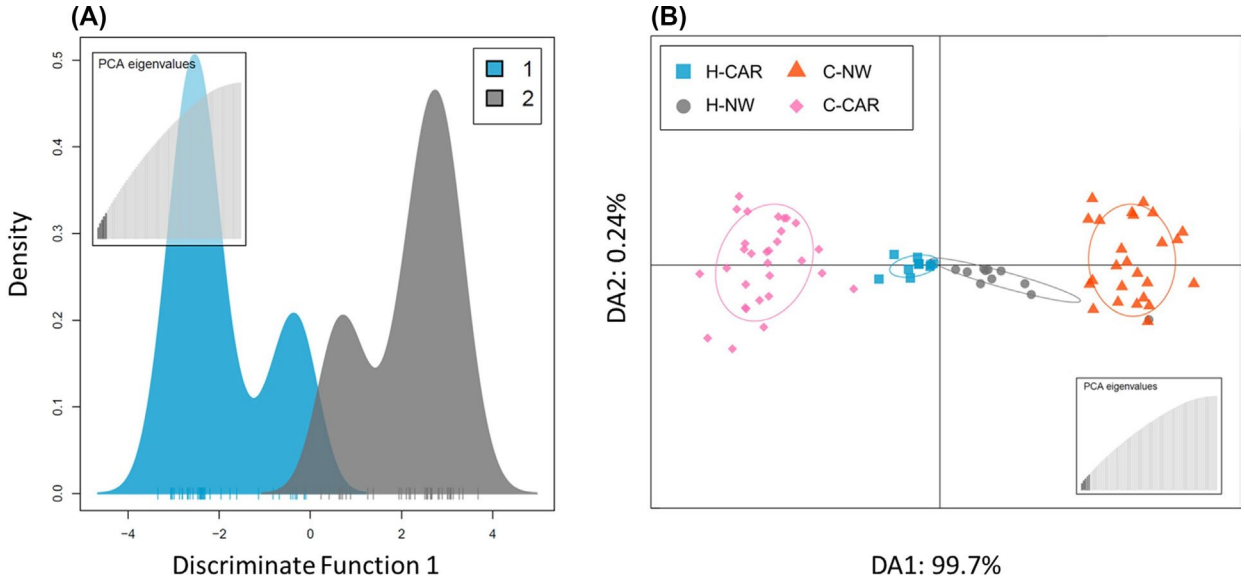

Fig. 2 DAPC of roseate tern SNP loci. Results are plotted on (**A**) One DA function with colors representing inferred clusters of *K* = 2 for groups 1 (H-NW, M-NW) and 2 (H-CAR, M-CAR); and (**B**) With *a priori* sampling locations specified, plotted on two discriminate axes of 25 PCs (51.9% cumulative variance from DA functions 1, 2, and 3), with shapes representing individual samples and colors representing sample locations within ellipses designating grouping.

Discussion

Using a genomic dataset spanning of 136 years, we found evidence for significant genetic differentiation of roseate terns breeding in the northwestern Atlantic and Caribbean for both the historical and modern periods. These results suggest that relatedness of roseate terns in the Western Atlantic was unlikely to have been higher prior to catastrophic declines of the northwestern Atlantic population in the early 20th century, and that past dispersal did not appear to have occurred at meaningful rates between these populations. Instead, our study provides support for long-term differentiation between these two populations. We did not find evidence that 20th century population declines led to genetic bottlenecking and loss of genetic diversity; however, these findings may have been biased by the higher rates of missing data in the museum samples. Our study highlights the value of museum DNA for addressing contemporary questions in conservation science and confirms the usefulness of DNA capture methods for sequencing large numbers of SNP loci from museum specimens.

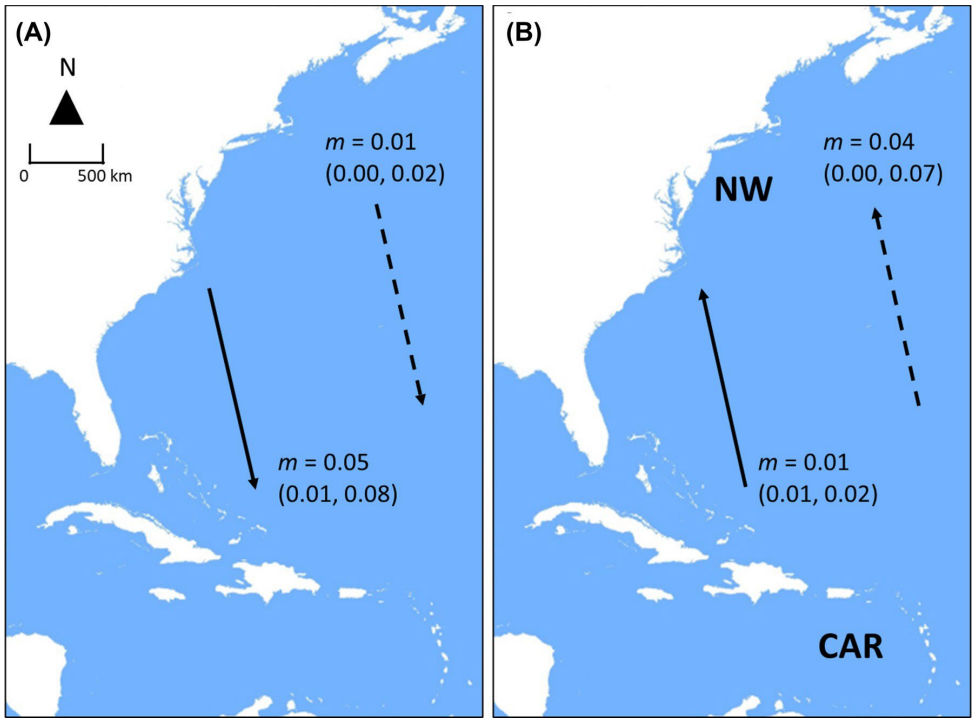

Fig. 3 Bayesian estimates of gene flow with 95% CIs derived from strict filtering parameters for roseate terns dispersing from (**A**) NW to CAR and (**B**) CAR to NW. The arrow signifies direction of dispersal between pairs of populations, with the solid arrow representing migration rates for historical samples and the dashed arrow representing migration rates within three generations of the sampling date for the modern samples.

Genetic diversity

Comparisons of genetic diversity between historical and modern time periods provided variable results. Unexpectedly, we found higher rarefied allelic richness and observed and expected heterozygosity for both mod- ern populations, with no differences between modern populations detected. As populations decline, rare alleles are lost due to the random force of genetic drift (Nei 1972). The NW population has experienced major population declines in the 20th century, declining from approximately 8500 breeding pairs in the 1930s to about 2600 pairs in the 1970s (Buckley and Buckley 1974). Long-term monitoring in the Virgin Islands has detected declines since the 1980s (Soanes et al. 2020), but it is probable that populations declined consider- ably there prior to the initiating of monitoring. These declines would lead us to expect loss, and not gain, of genetic diversity in the 21st century. However, our results are similar to those from a comparable study which used eight microsatellite loci to investigate temporal changes to the NW Atlantic population and found no difference in allelic richness and lower genetic diversity for modern vs historical (1870s) samples (Dayton and Szczys 2021).

It is possible that the greater differences in allelic rich- ness and heterozygosity between the two time periods in our study may be the product of allelic dropout in our historical specimens, as underestimated heterozygosity is a common outcome of genotyping error (Wandeler et al. 2007; Crates et al. 2019; Bresadola et al. 2020). In our study, false homozygotes were likely caused by both lower amplification of contigs with longer fragment size (Wandeler et al. 2003) and by lower cover- age and higher rates of missing data in the museum specimens (O'Leary et al. 2018), both of which can bias recovery of SNP loci. Potential for genotyping error was highest for the historical Caribbean population, which had greater rates of missing data and lower read depth, and this presumed error likely resulted in the greater differences in genetic diversity and allelic rich- ness between time periods for the Caribbean, whereas this source of error was reduced for the NW museum samples. This greater rate of missing data likely also ac- counts for the differences in genetic diversity between regions for the historical time periods, as we do not believe that the substantially lower genetic diversity in the Caribbean is reflective of actual biological differences in genetic diversity between the two regions. Higher rates of missing data have been associated with underestimated heterozygosity, and therefore genetic diversity, in museum specimens (Ewart et al. 2019). Our results are therefore comparable to those in other studies quantifying long-term temporal changes in population genetic parameters (Crates et al. 2019), and underscore the on- going challenge of controlling for genotyping error in museum genomics.

Population structure

For both population structuring analyses, populations clustered by region and not temporal period, and historical populations did not demonstrate greater levels of admixture. Although our population structuring analyses were also affected by greater rates of missing data in the museum specimens, missing data has been shown to have a minimal effect on estimates of genetic differentiation in species that exhibit high degrees of population structuring (Ewart et al. 2019). Additionally, the negative effects of missing data may be offset by the greater number of SNPs retained when missing data filters are relaxed, with more SNPs improving resolution of structure estimates (Hodel et al. 2017). Our findings of strong differentiation between the two regions, but not time periods, were similar for both the moderate and strict filter sets, highlighting the robustness of our results and leading us to believe that missing data did not greatly bias our population structuring analyses.

The relatively high F_{ST} and low estimates of migration between populations are also indicative of a lack of extensive gene flow for either time period. Although BayesAss can be biased when sample sizes are small or F_{ST} between populations is low (<0.1), inaccurate estimates tend to be biased upwards (Samarasin et al. 2017), and estimates of migration rates using Bayesian methodologies have been found to be largely consistent for SNP loci even for sample sizes of two individuals per population (McLaughlin and Winker 2020). Our findings of low migration rates between both historical and modern populations suggest that there was not substantial gene flow in either direction for either time period.

In other migratory seabird species such as Common terns (*Sterna hirundo*), asymmetrical dispersal has been demonstrated to result from migratory patterns, with birds more likely to disperse to regions passed through on migration routes (Szczys et al. 2017). As northwest- ern roseate terns are known to use the Caribbean as a stopover during migration (Auto structure), the finding that individuals do not appear to disperse is unusual, and may be indicative of potential assortative mating. Further research should focus on investigating potential ecological mechanisms for genetic differentiation, as well as modeling potential divergence dates between the two populations.

Conservation implications

The strong genetic differentiation between Caribbean and northwestern roseate tern populations has important implications for the persistence of the species in North America. Although the northwestern population has shown signs of recovery since the 1980s, the population is still small,

and individuals are heavily concentrated into just three primary colony sites (U.S. Fish and Wildlife Service 2020). In the Caribbean, subpopulations appear to be in active decline (Byerly et al. 2021, Soanes et al. 2020) and the minimal recovery efforts within the region are unlikely to reverse ongoing trends due to high rates of nest failure (Byerly et al. 2021). Even low levels of gene flow have been found to offset loss of genetic diversity resulting from declines in small, relatively isolated wildlife populations (Ramírez et al. 2013; Lonsinger et al. 2018). Evidence for the contemporary isolation of both populations of breeding roseate terns suggests that loss of genetic diversity is unlikely to be counteracted by influxes of new alleles from external populations. Although we did not find evidence for loss of genetic diversity, these results may be more indicative of both genotyping error and of the long generation time and lower reproductive rate of seabirds, which can stall erosion of heterozygosity. Given the small sizes and apparent isolation of both populations, future loss of genetic diversity is highly likely. Our findings highlight the need for ongoing conservation efforts in both breeding populations to preserve the remaining genetic diversity, and therefore evolutionary potential of roseate terns in the Western Atlantic.

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Supplementary data

Supplementary data available at *ICB* online.

Conflict of interest

We have no conflicts of interest to declare.

Data availability statement

Raw sequence data were deposited in the NCBI Sequence Read Archive (PRJNA847596). Genetic data for this paper are also available through USGS Science- Base at https://doi.org/10.5066/P9MTXDCH (Byerly et al. 2022). SNP VCF files, R code used for data analysis, and sample metadata are available at https://github.com/pabyerly/RoseateTern_PopulationGenomics_ICB.

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