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Variable Connectivity Among Black Skimmer (*Rynchops niger*) Populations in North and South America: A Population Genetics Investigation

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Abstract.—The Black Skimmer (*Rynchops niger*) is a charismatic representative of coastal-nesting waterbird communities in North America and freshwater riverine and coastal systems in South America. Skimmers are at high risk of decline due to threats including habitat loss, human activity, and climate change throughout the annual cycle. To understand range-wide population connectivity, eight microsatellite loci were used to reveal genetic differentiation between North American Pacific and Atlantic Coasts, including the Gulf of Mexico, and very strong differentiation between the North American sites and a site in Peru, South America. A reduced dataset, incorporating previously published data from South America, documented strong inter-continental differentiation and moderate differentiation among sites within South America in a pattern inconsistent with current subspecies designations, and suggesting a more complex pattern of dispersal. We recommend combined use of telemetry and genomic tools to assist in future delineation of skimmer subspecies ranges and non-breeding habitat use in South America. Because genetic diversity was relatively low in North America and higher in South America, genetics could determine whether skimmers in South America represent the ancestral population now expanding along the Atlantic and Pacific Coasts of North America. Detailed knowledge of seasonal movement and non-breeding habitat use that can be obtained by telemetry and population-wide molecular analyses will be essential to informing effective management across the Americas. *Received 30 Aug 2023, accepted 5 May 2024.*

Key words.—Black Skimmer, dispersal, latitudinal distribution, microsatellites, population genetic structure, *Rynchops niger*, subspecies

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Loss of genetic diversity is a central concern in conservation and population biology as high genetic diversity allows populations to tolerate a wide range of environmental regimes (Frankham *et al.* 2002). Conversely, populations with low genetic diversity may suffer from inbreeding and reduced fitness, lack the potential to adapt to long-term changes to the environment, and experience higher risk of extinction (Reed *et al.* 2003; Jangjoo *et al.* 2016). Dispersal of individuals among populations (gene flow) acts to maintain genetic variability and thus, positively impacts long-term population or species survival as even a small amount of gene flow is

sufficient to prevent genetic differentiation among populations (Slatkin 1985; Clobert *et al.* 2012). Dispersal capability strongly influences individual fitness, which can then impact the social and genetic structures of populations and their long-term viability (Greenwood 1980; Driscoll *et al.* 2014). Because dispersal has such an important ecological and genetic role, knowledge of where and when species move is critical for managing and conserving biodiversity and maintenance of global population connectivity (Swift and Hannon 2010; Clobert *et al.* 2012). Unfortunately, dispersal data are generally depauperate for many species as it is

particularly challenging to differentiate dispersal from other demographic processes such as mortality and emigration.

One method used to explore population connectivity is to measure population structure and describe patterns of structure across the range of a species (Szczyś et al. 2017; Byerly et al. 2023). Genetic differentiation can be explained by the occurrence of physical barriers limiting dispersal, and thus gene flow, among populations. However, in highly vagile species such as birds, non-physical barriers and behaviors such as non-breeding distribution and breeding site fidelity can play an equally important role in promoting genetic differentiation (reviewed by Friesen et al. 2007; Milot et al. 2008; Szczyś et al. 2017; Lombal et al. 2020).

The Black Skimmer (*Rynchops niger*) is a charismatic representative of both coastal nesting waterbird communities in North America and freshwater riverine and coastal systems in South America that provides an important link between terrestrial habitats and aquatic resources (Erwin et al. 2006). Waterbird species such as the Black Skimmer are at high risk of decline at regional levels and globally influenced by a combination of threats including habitat loss, degradation of remaining habitat (pollution), and the synergistic effects of climate change and continued land use alteration (Erwin et al. 2006). Further, modeled scenarios of climatic change on various waterbird populations predict continuous population declines until the end of the twenty-first century (Rehfishch et al. 2004; Breiner et al. 2022) highlighting a critical need to document factors influencing population connectivity.

Black Skimmers exhibit an unusually large latitudinal component to their distribution which provides a unique opportunity to examine population connectivity at both the regional and hemispheric scale. The Black Skimmer ranges along both coastlines of North and South America and within inland freshwater river systems in South America. Skimmers are currently classified into three subspecies (*R.n. niger*, *R.n. cinerascens*, and *R.n. intercedens*) identified by morphology, and by

apparently distinct breeding ranges (reviewed by Vieira et al. 2018). *R.n. niger* is smaller than the South American subspecies and has an entirely white underwing. Subtle differences are noted among subspecies in South America: *R.n. cinerascens* has dark gray underwing coverts with a definitive black margin on the anterior edge of the underwing surface. *R.n. intercedens* is similar in size to *R.n. cinerascens* but has pale gray to white underwing coverts and the dark line along the anterior margin of the underwing surface is broken, irregular, or much narrower than in *R.n. niger* (Wetmore 1944). The global population size of the Black Skimmer is unknown as there are no available estimates for *R.n. cinerascens*, although a general estimate for the global population ranges between 124,600 to 207,900 individuals (Vieira et al. 2018; Gochfeld et al. 2020). The *R.n. niger* range includes coastal North America south to the Caribbean and Central America to Panama. *R.n. cinerascens* occurs along the coasts of eastern Colombia south and southwest to the Pacific Coasts of Ecuador, Peru, and Chile, and from southwest Panama west to Trinidad and the northern coast of Brazil. *R.n. intercedens* occurs along the large rivers of eastern Brazil from Maranhão to eastern Mato Grosso, eastern Paraguay, Uruguay and in northeast Argentina to Bahía Blanca. Although breeding distributions along the Brazil-Paraguay border and in the southern Brazilian Amazon basin, reproductive timing could effectively maintain breeding isolation for the South American subspecies as *R.n. cinerascens* begins breeding in late May- June while *R.n. intercedens* breeds from October to March (Blake 1977; Vieira et al. 2018; Gochfeld et al. 2020).

The Black Skimmer has a life history strategy that includes migration and residency during the nonbreeding season and a variable but usually high degree of site fidelity during the breeding season (Gochfeld 1978; Gazzaniga 1996; Snipes and Sanders 2012) which complicates understanding of population connectivity for the Black Skimmer throughout its range. Populations in Florida, California, and about one-third of the populations in Texas do not migrate but instead make shorter local movements away from

the breeding colonies to coastal wintering locations (Gazzaniga 1996; Gochfeld *et al.* 2020). The more northern Atlantic populations (north of latitude 37°N) are obligatory migrants with all individuals moving southward by the end of October annually (USGS Bird Banding Lab).

The overarching goal of this study is to use genetic metrics to understand population connectivity for the Black Skimmer. At a hemispheric scale, there have been no reported sightings of *R.n. niger* in South America nor those of *R.n. cinerascens* and *R. n. intercedens* in North America (Gillespie 1931; Burger and Gochfeld 1990; Sprandel *et al.* 1999; Snipes and Sanders 2012). Hence, we expect to detect distinct differences in population structure when comparing North and South American populations. More regionally, there are no reports of *R.n. niger* populations from the Pacific Coast documented on the Atlantic Coast and vice versa (USGS Bird Banding Laboratory data). For some populations in Texas, the Black Skimmer has been documented to migrate overland between the Gulf of Mexico and the Pacific Coast (Newstead unpub data), therefore the Texas populations could act as a connection between Pacific and Atlantic populations and thus share genetic profiles from both oceanic basins. Across South America, Davenport *et al.* (2016) suggested a diverse, near continental migration strategy, although Mariano-Jelicich and Madrid (2014) reported non-panmictic populations of the Black Skimmer suggesting there is some separation among breeding populations in South America.

For this study, we sampled individuals at one breeding site on the North American Pacific Coast, one breeding site on the North American Atlantic Coast, three sites on the Gulf of Mexico (hereafter called Texas), and one breeding site inland in South America at Manu National Park, Peru (hereafter called Manu). Further, we examined four of the five genetic markers used in Mariano-Jelicich and Madrid (2014) to make direct comparisons with the only previous genetic study of Black Skimmers. Our primary objective was to document genetic

diversity and genetic differentiation among skimmer populations representing the three subspecies and spanning the large latitudinal distribution.

METHODS

Sample Collection

Feather or blood samples from 91 adult skimmers were sampled from three different breeding locations: San Diego Bay NWR San Diego, California, U.S.A. ($n = 16$; 2019), Lido Beach, Long Island, New York, U.S.A. ($n = 30$; 2018 and 2019), and Manu National Park, Peru ($n = 18$; 2017 and 2018). Adults from Texas were sampled in the month immediately pre- or post-breeding at roosts near South Padre Island ($n = 12$), Corpus Christi ($n = 11$), and Galveston, Texas, U.S.A. ($n = 4$). Because of their proximity (< 400 km) and because they were part of a tracking study, these samples were considered one sampled site representing the Texas Gulf Coast in 2017 (Fig. 1 and Table 1). All blood samples were preserved in alcohol or on PTA filter paper. Feather samples were stored individually in paper envelopes until DNA extraction.

DNA Extraction and Microsatellite Testing

DNA was extracted from liquid blood samples, dried blood samples on PTA filter paper (Smith and Burgoyne 2004), or feather samples using DNeasy (QIAGEN), following the blood or tissue protocols. Concentration of DNA was quantified using a NanoDrop and samples were diluted using QIAGEN AE buffer if DNA concentration was over 50 ng/mL.

Twenty-five microsatellite loci were tested for eight individuals to optimize amplification and assess polymorphism levels for overall suitability for this study. These individuals were subsequently used as standards. Microsatellite amplifications were performed in 10 μ L volumes with primer concentrations of 5 μ M using 2X GoTaq PCR Master Mix (Promega) that included 400 μ M each dNTP, 3 mM MgCl₂, and 5 units *Taq* DNA Polymerase. Cycling parameters were: 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 50-60°C for 30 sec, 72°C for 1 min, and a final 60°C extension for 30 min. PCR products were diluted in formamide loading dye and genotypes were resolved on a 4300 DNA Analyzer using IRDye size standard (LiCOR) and alleles identified by SAGA G2 software (LiCOR).

Five loci were known to be polymorphic in Black Skimmer populations across South America (Mariano-Jelicich and Madrid 2014; Supplemental Table 1: K16, RBG18, RBG27, RBG28, and Sdaat27). Four additional loci were determined to be polymorphic by our testing: K6, MsSh7, MsSh8, and MsSh 9 (Supplemental Table 1). Sixteen additional microsatellite loci were tested, and either did not amplify, resulted in non-specific amplification, or were monomorphic and thus these 16 loci were not used for this study: RBG13, RBG29, Lasa2,

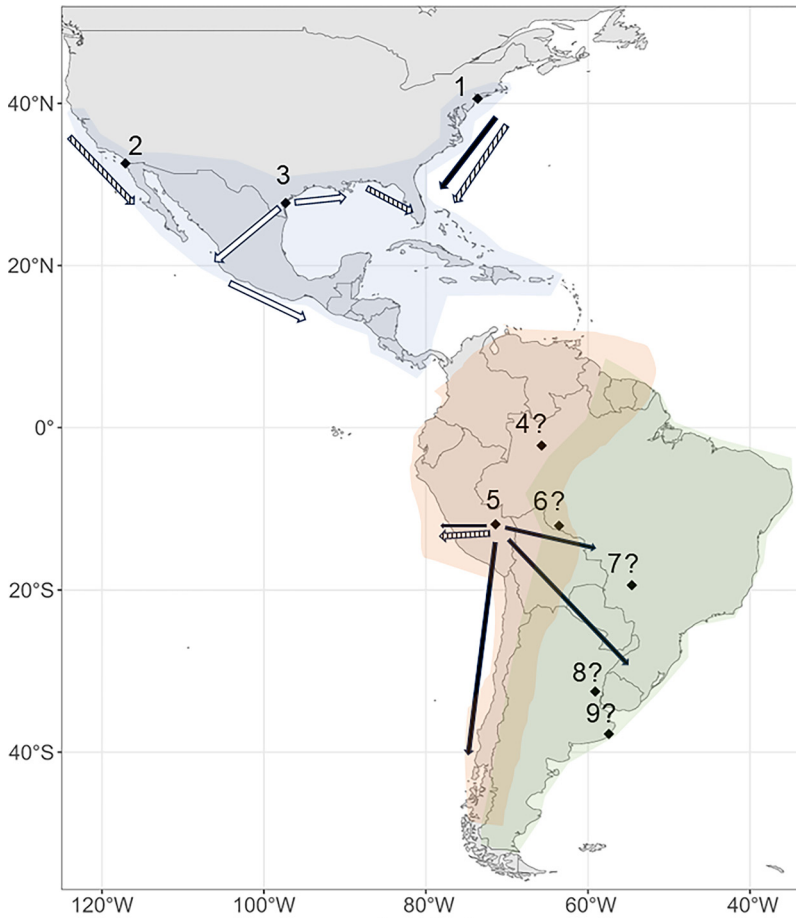


Figure 1. Black Skimmer sample locations in North and South America (black diamonds) with range distribution for *Rynchops niger niger* (blue), *R.n. cinerascens* (orange), *R.n. intercedens* (green) based on Vieira et al. (2018). There is some overlap between *R.n. cinerascens* and *R.n. intercedens* distributions outside of the breeding season as both subspecies can be found on both coasts of the South American continent. Black arrows indicate confirmed movements via telemetry (Davenport et al. 2016). Barred arrows suggest movements inferred from mark-recapture data. White arrows indicate proposed migratory direction. Question marks (?) indicate unknown dispersal and migratory movements. Numbers indicate sample locations and are listed below. North American sample locations included breeding birds from Lido Beach, New York (1); Galveston, Texas; Corpus Christi, Texas (3); and San Diego, California (2). In South America, we sampled at Manu National Park, Peru (5) and incorporated data from Brazil- Mamiraua (4), Sao Francisco Guapore (6), and Pantanal (7); and from Argentina- Entre Rios (8) and Mar Chiquita (non-breeding 9). (Supplemental Table 2; Mariano-Jelicich and Madrid 2014).

Sdaat20, Sdaac20, MsSh18, MsSh20, MsSh23, C1, C2, C5, C17, C22, C35, C39, and C40 (Supplemental Table 1). Because locus K6 amplified inconsistently in all sampled populations and was likely to harbor a very high frequency of null alleles (Mariano-Jelicich and Madrid 2014), this locus was omitted from further analysis.

To combine the dataset from this study and the Mariano-Jelicich and Madrid (2014) study, allele frequencies were calculated for each of the five loci and the most common alleles were used to calibrate the two datasets, resulting in conservative estimates of genetic structure. The decision process was straightforward for four of the five loci and we provide a full accounting in Supplementary Table 3. Because allele calibration for

Sdaat27 was not straightforward, it was omitted from the comparative analysis so that dataset became a four-locus analysis rather than five-locus.

Statistical Analysis

Genetic diversity was quantified in GenAlEx 6.5 (Smouse and Peakall 2012) by allele frequency, number of alleles, and observed and expected heterozygosities, and assessed for deviations from Hardy-Weinberg Equilibrium (HWE). Allelic richness was estimated by FSTAT (Goudet 1995). Microsatellite loci were evaluated for the presence of null alleles by 10,000 bootstrap replicates and the ENA method in FreeNa (Chapuis and Estoup 2007). The conservative B-Y

Table 1. Indices of genetic diversity for Black Skimmer for the 8-locus and 4-locus datasets. Number of individuals genotyped (N), number of alleles (Na), observed heterozygosity (H_O), and expected heterozygosity (H_E), Smouse and Peakall (2012). Allelic Richness (A_R) standardized to $n = 10$ (8-locus), $n = 7$ (4-locus, nine-site), and $n = 25$ (4-locus, 3 cluster). Goudet (1995). South American samples include those previously reported by Mariano-Jelicich and Madrid (2014).

	Site	N	A_R	Na	H_O	H_E
8-locus	New York	28.1	3.02	3.63	0.53	0.47
	Texas	25.8	3.46	4.38	0.43	0.45
	California	14.0	2.43	2.50	0.40	0.42
	Manu, Peru	17.6	3.29	3.50	0.46	0.52
4-locus	New York	27.5	2.84	3.75	0.53	0.50
	Texas	25.5	3.20	4.25	0.37	0.43
	California	14.3	2.42	2.50	0.30	0.43
	Manu, Peru	18.0	3.33	3.75	0.56	0.58
	Mamiraua	7.5	3.93	4.50	0.71	0.62
	Guaporé	9.0	3.62	4.50	0.58	0.62
	Pantanal	10.0	4.19	4.25	0.53	0.60
	Entre Rios	9.5	3.88	4.00	0.58	0.55
	Mar Chiquita*	19.0	4.47	5.00	0.58	0.62
	4-locus [^]	North American Cluster	67.3	3.59	4.75	0.42
Peru Cluster		18.0	3.75	3.75	0.56	0.58
Brazil Cluster		26.5	5.50	5.75	0.60	0.64
Argentina Cluster*		28.5	5.31	6.00	0.58	0.61

*Mar Chiquita is a non-breeding site.

[^]Diversity indices for the four clusters identified by the STRUCTURE analysis and depicted in Figure 2B.

correction of critical P-value was used for all analyses of loci (Narum 2006). MicroChecker estimated genotyping errors and null allele frequencies for quality control of microsatellite amplifications (Van Oosterhout *et al.* 2004).

Population structure was described using genotypes for eight microsatellite loci obtained for this study from three North American sites and one site in Manu National Park, Peru. We used FreeNa (Chapuis and Estoup 2007) to investigate the influence of null alleles on population structure as measured by F_{ST} . We quantified F_{ST} and Jost's D_{ST} (for differentiation using the effective number of alleles to correct for allele frequency biases; Meirmans and Hedrick 2011) in GenAlix 6.5 (Smouse and Peakall 2012) with 999 permutations. The admixture model with correlated allele frequencies (Falush *et al.* 2003; Falush *et al.* 2007) was employed in STRUCTURE to identify population differentiation among sites. Analysis was conducted with an initial alpha of 0.25 to control for uneven sampling (*i.e.*, 1/K; Wang 2017) using sampling location as prior information. For each value of $K = 1$ to 5, STRUCTURE executed 25,000 burn-in steps followed by 250,000 MCMC iterations for 15 replications. Genetic clusters were visualized across all possible values via Structure Harvester (Earl and von-Holt 2012) and CLUMPAK (Kopelman *et al.* 2015) in STRUCTURE SELECTOR (Li and Liu 2018) but only the best fit K-value histograms are reported here. The number of clusters was determined following the methods of Pritchard *et al.* (2000), Evanno *et al.* (2005), and Puechmaile (2016). To assess the potential impact of uneven sampling among sites (Meirmans 2019), a

random subsampling of 16 individuals from each of New York, Texas, and Manu and all 16 California samples was used to repeat the cluster analysis in STRUCTURE under the conditions outlined above.

Four loci were used to quantify genetic diversity indices (above) and population structure (F_{ST}) in GenAlix 6.5 (999 permutations) comparing nine sites across North America (three) and South America (six, including five breeding and one non-breeding site published by Mariano-Jelicich and Madrid, 2014). STRUCTURE analysis included 148 individuals for the reduced-locus dataset (K16, RBG 18, 27, and 28; Supplementary Table 1) and proceeded as detailed above for the 8-locus dataset.

RESULTS

Microsatellite Diversity

A total of 91 Black Skimmers sampled from three sites in North America and one site in South America (Fig. 1) were genotyped for eight previously published microsatellite loci (Table 1 and Supplementary Table 1). Populations conformed to HWE and three loci (RBG 18, MsSh9, and AAT 27) were estimated to harbor a null allele at low to moderate frequency in one or two

Table 2. Population differentiation (F_{ST}) estimates derived from the 8-locus dataset. Uncorrected F_{ST} (below diagonal) and F_{ST} corrected (above diagonal). The 95% confidence intervals from 10,000 bootstraps in parentheses implemented in FreeNa (Chapuis and Estoup 2007).

	New York	Texas	California	Manu
New York		0.024 (0.010–0.044)	0.055 (0.000–0.111)	0.254 (0.079–0.438)
Texas	0.023 (0.007–0.036)		0.057 (0.017–0.102)	0.237 (0.065–0.437)
California	0.063 (0.000–0.11)	0.052 (0.016–0.099)		0.251 (0.076–0.446)
Manu	0.253 (0.078–0.436)	0.239 (0.068–0.436)	0.252 (0.086–0.441)	

populations. These three loci did not influence population structure estimates (below), so they were retained for full analysis.

Using eight microsatellite loci, we documented higher levels of genetic diversity in New York and Texas ($A_R > 3.00$; $H_O > 0.43$) compared to California ($A_R = 2.43$; $H_O = 0.40$). Diversity in Manu was comparable to New York and Texas ($A_R = 3.29$; $H_O = 0.46$). Further, compared to individual North American populations ($n = 3$), individual South American populations ($n = 6$) had considerably higher genetic diversity (allelic richness, number of alleles, observed and expected heterozygosity; Table 1) at four microsatellite loci genotyped for this study and compared with data from Mariano-Jelicich and Madrid (2014).

Population Genetic Structure

The global fixation index values were not influenced by the possibility of null alleles as indicated by the comparison of F_{ST} based on uncorrected allele frequencies and from adjusted allele frequencies where differences in F_{ST} ranged from 0.001–0.008. (FreeNa, 8-locus dataset; Table 2). Genetic differentiation was identified among the three North American sites (F_{ST} ranged 0.02–0.05) and strong differentiation was noted between each of them and Manu in South America (F_{ST} ranged 0.08–0.09; 8-locus dataset; Table 2). That pattern of strong inter-continental differentiation was retained in pairwise estimates of F_{ST} and $Dest$ among all nine sites (4-loci, Table 3).

When site sample sizes for the 8-locus dataset were randomly standardized to $n = 16$, STRUCTURE clustering results were robust to both inclusion/exclusion of prior

population information where North American sites cluster together separately from the South American site (Fig. 2A). We used Puechmaille's unbiased estimators (MedMeaK and MedMedK, 2016) to avoid splitting clusters when populations may have been unevenly sampled or may exhibit hierarchical structuring. The most likely number of clusters was $K = 3$ where New York and Texas formed one cluster separately from California and Peru (Fig. 2A).

The four-locus dataset for nine sites, clustered North American sites separately from South American sites (Fig. 2B; $K = 2$, Mean $\ln P(K) = -1316.2$ and $\Delta K = 65.1$, Evanno et al. 2005), and identified $K = 4$ as most likely where genetic clusters aligned by country in South America (Puechmaille, 2016 MedMeaK and MedMedK, Fig. 2B). The differentiation detected here should be considered conservative estimates because of the calibration of the two datasets (see DNA Extraction and Microsatellite Testing above and Supplementary Table 3).

DISCUSSION

This study extends the knowledge of genetic diversity and population connectivity of the Black Skimmer by building upon the only previous genetic investigation, which was limited to the South American distribution. It also highlights the importance of including molecular techniques to provide a more comprehensive understanding of population connectivity. We have now documented genetic diversity across the species' range from 40°N to -37°S latitude and report strong genetic differentiation between skimmers in North and South America. Black Skimmer populations within

Table 3. Pairwise population differentiation estimates among nine sites or four genetic clusters for Black Skimmer using the reduced 4-locus microsatellite dataset. F_{ST} below the diagonal and D_{ST} above the diagonal based on 999 permutations as implemented in GenA1Ex 6.5, (Smouse and Peakall, 2012). Bold font indicates significant difference where $P \leq 0.05$. The four genetic clusters were identified by STRUCTURE analysis (See Figure 2B).

Site	New York	Texas	California	Manu	Mar Chiquita	Entre Rios	Guaporé	Pantanal	Mamiraua
New York		0.021	0.058	0.181	0.196	0.324	0.303	0.241	0.422
Texas	0.021		0.023	0.165	0.185	0.308	0.313	0.227	0.445
California	0.046	0.032		0.156	0.167	0.295	0.322	0.226	0.430
Manu	0.080	0.084	0.085		0.021	0.089	0.019	0.035	0.091
Mar Chiquita	0.080	0.087	0.085	0.021		0.002	0.068	-0.003	0.110
Entre Rios	0.138	0.149	0.148	0.051	0.022		0.118	0.007	0.175
Guaporé	0.118	0.135	0.143	0.029	0.041	0.064			0.011
Pantanal	0.103	0.110	0.115	0.033	0.021	0.031	0.044	0.047	0.043
Mamiraua	0.151	0.176	0.175	0.050	0.053	0.080	0.035	0.043	
Genetic Cluster	Site	Subspecies		North America		Peru	Argentina	Brazil	
North America	New York	<i>niger</i>				0.163	0.222	0.304	
	Texas	<i>niger</i>							
	California	<i>niger</i>							
Peru	Manu	<i>cinerascens</i>		0.075			0.044	0.033	
Argentina	Mar Chiquita	non-breeding		0.091		0.026			
	Entre Rios	<i>intercedens</i>						0.054	
Brazil	Guaporé	<i>cinerascens</i>		0.111		0.023	0.025		
	Pantanal	<i>intercedens</i>							
	Mamiraua	<i>cinerascens</i>							

both North America and South America exhibit detectable differences that represent non-panmictic populations (Fig. 2 and Table 3).

We documented greater genetic diversity (allelic richness and heterozygosity) at sites on the Atlantic and Gulf Coasts compared to the Pacific Coast within North America. This may reflect the larger migratory population in the east compared to the smaller and resident population in southern California. Given the northward range expansions on both Pacific and Atlantic Coasts of North America, and the greater genetic diversity documented in South America, it is possible that South America represents the ancestral population while North American populations represent a historical range expansion.

Genetic diversity in Manu was comparable to Atlantic Coastal sites in North America (8-locus dataset), however when compared to all North American sites ($n = 3$; 68 individuals), all South American sites ($n = 6$; 73 individuals) harbored considerably higher genetic diversity (Table 1) in the four-locus dataset. Higher genetic diversity in South America is not

consistent with the reports of similar overall population sizes: 93,000 in North America by Gochfeld *et al.* (2020) and 25–100,000 in South America (Blanco *et al.* 2008) and could be a sign that skimmer populations are larger than estimated, or may reflect that North American populations are a result of range expansion from the core ancestral population in South America (Excoffier *et al.* 2009). This interpretation should be considered with caution because of the small genetic dataset, potentially outdated population size estimates, and lack of detailed surveys within South America.

We have documented higher diversity in South America compared to North America in both the 8-locus and 4-locus datasets, consistent with previously reported low genetic diversity for this species in South America (Table 1; $N_A = 2.5-5$, $A_R = 2.43-4.47$. $H_E = 0.42-0.62$). Faria *et al.* (2007) tested 10–12 individuals for seven loci and reported $N_A = 2-5$. Mariano-Jelicich and Madrid (2014) reported indices for five loci: $N_A = 4.0-5.2$, $A_R = 3.51-4.08$, $H_E = 0.60-0.68$. Although we caution against direct comparison, in continental-scale genetic diversity assessments for terns that utilized microsatellites all reported

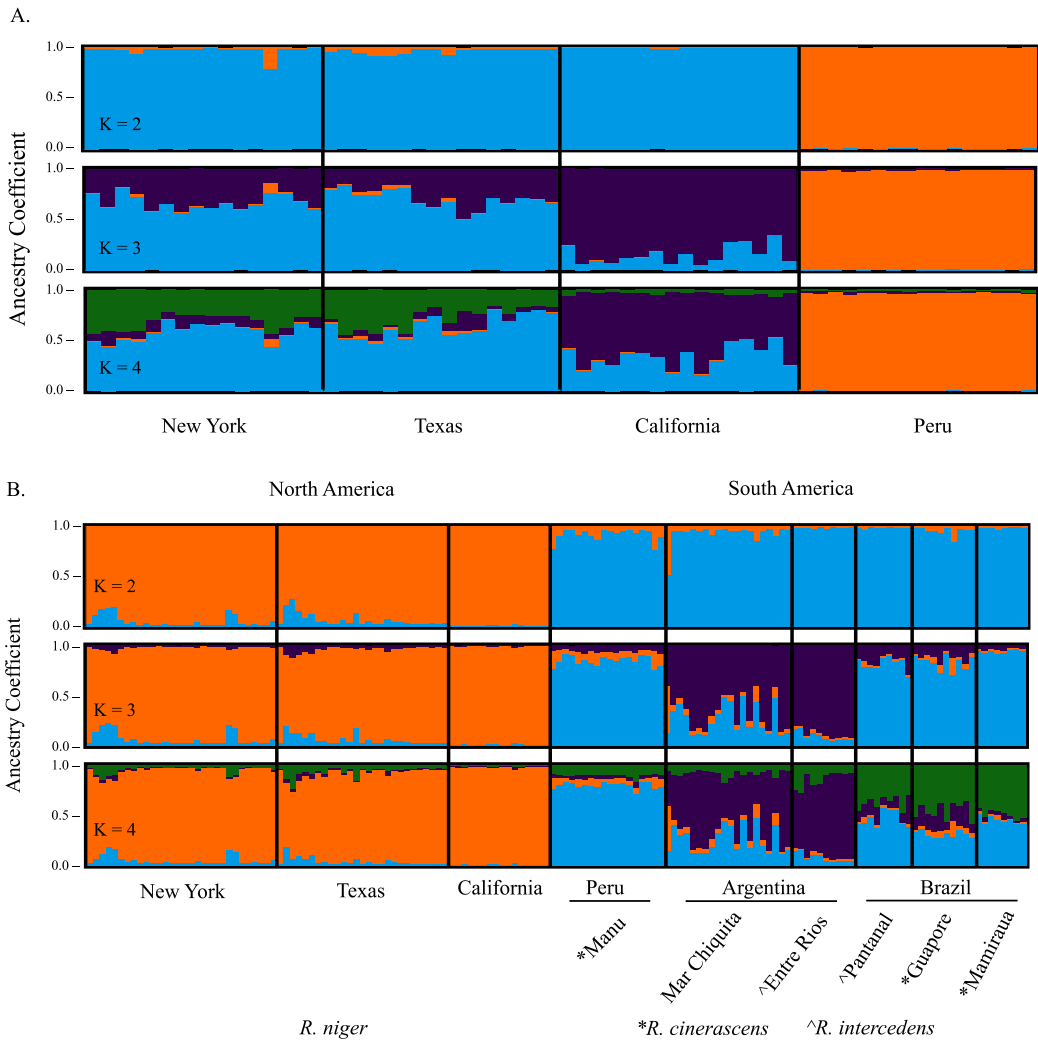


Figure 2. (A) Histogram of ancestry coefficient for 91 Black Skimmer (*Rynchops niger niger*) for the 8-locus dataset with randomly evened sample size ($n = 16$) using sample site *a priori* and reporting tests for $K = 2-4$. $K = 3$ is the most likely number of clusters by MedMeaK and MedMedK (Puechmaile, 2016). (B) Histogram of ancestry coefficient for 148 Black Skimmer for the 4-locus dataset, with *a priori* identity by individual site with unequal sample sizes reporting tests for $K = 2-4$. $K = 4$ is the most likely number of clusters (MedMeaK and MedMedK, Puechmaile, 2016) while ΔK (Prichard et al. 2000) identified $K = 2$. Although $K = 2$ is biologically relevant, this metric represents an underestimate of true K due to the uneven sampling of populations (Puechmaile, 2016).

generally higher N_a , A_R and H_E values: Black Tern (Shephard et al. 2023; 8 loci; $N_a = 7.4-7.5$; $A_R = 7.2-7.9$; $H_E = 0.71-0.80$); Common Tern (Arnold et al. 2022; 6 loci $N_a = 5.4-6.4$; $H_E = 0.63-0.70$); and Black-fronted Tern (Schesselman and Robertson 2017; 345 individuals, 18 loci $N_a = 4-23$, $H_E = 0.445-0.861$). In contrast, two studies of the endangered Roseate Tern reported low diversity $A_R = 2.52-4.52$ and $H_E = 0.38-0.50$ (Dayton and Szczys 2021; Byerly et al. 2023).

In their survey of molecular markers for terns, Faria et al. (2007) reported low frequency of occurrence of microsatellite loci in the Black Skimmer genome and our data support that claim. For this study, we tested a total of 25 highly polymorphic microsatellite loci: nine had been tested by Faria et al. (2007) and five of those were subsequently implemented in the only population genetic study of Black Skimmer (Mariano-Jelicich and Madrid 2014). Therefore, we tested an

additional 15 loci that were developed after 2014 and were reported to be polymorphic in terns (Janowski *et al.* 2016; Schlesselmann and Robertson 2017). These loci were initially attractive because they proved polymorphic and informative in endangered Roseate Tern populations (Dayton and Szczys 2021) where microsatellites were difficult to identify *de novo* and genetic diversity was depauperate (Lashko 2004; Szczys *et al.* 2005). However, only three of 15 newly tested loci were polymorphic—providing support for the assertion that the abundance of microsatellite loci may be low in skimmers (Faria *et al.* 2007).

Genetic differentiation between the *niger* subspecies and both South American subspecies was pronounced (F_{ST} ranged from 0.08–0.25; Fig. 2B, Table 2, and Table 3). These results, combined with morphological data support the subspecies classification separating *R.n. niger* from the two South American subspecies. However, the subspecies classification within South America was not consistent with the measured genetic differentiation despite the subtle morphological differences. Based on the reduced dataset of four loci, the three sites within the *cinerascens* range (Manu, Peru; Guapore, Brazil; and Mamiraua, Brazil) formed two clusters that separated the Peru site from the Brazil sites. From the *intercedens* range, Entre Rios, Argentina clustered with the non-breeding location in Argentina, Mar Chiquita but the Pantanal, Brazil site clustered with the other sites in Brazil from the *cinerascens* range. This pattern is not precisely the cluster pattern reported by Mariano-Jelicich and Madrid (2014) using five microsatellite loci where Guapore, Brazil clustered with Entre Rios, Argentina. However, in both that study and this one, genetic clusters did not align with subspecies classifications (Viera *et al.* 2018), suggesting that subspecies designations for Black Skimmer require more detailed genetic elucidation. The genetic clusters identified here support the observations that the large river systems including their tributaries may serve as movement corridors for waterbirds

transiting throughout the Amazon Basin (Davenport *et al.* 2016).

Within North America, genetic data support capture-recapture data indicating that Atlantic Coast (Gillespie 1931; Sprandel *et al.* 1999; Snipes and Sanders 2012) and at least some Texas populations (Newstead unpub. data) are seasonally migratory. Seasonal migration to shared non-breeding sites use can facilitate gene flow by increasing opportunities for interaction with spatially distributed individuals or breeding sites (Friesen *et al.* 2007; Quillfeldt *et al.* 2017). For example, female winter-site fidelity was greater than breeding-site fidelity and initiation of pair formation there, drove differentiation among breeding populations of snow geese through male dispersal (Shorey *et al.* 2011). Similarly, common eider breeding population structure was linked to sex-specific patterns of geneflow associated with interaction at the wintering site (Sonsthagan *et al.* 2011). Our study did not investigate sex-specific gene-flow, however we report evidence of genetic similarity (Fig. 2A) among migratory breeding populations that share wintering sites and genetic differentiation among those breeding populations that do not, i.e., the resident California population (Collins and Garrett 1996; Gazzaniga 1996; Gochfeld *et al.* 2020). Some individuals from the Texas populations have been documented to migrate south and west, overland, to Mexico (Newstead unpub. data) where they could potentially interact with the Pacific Coastal populations in Mexico and California. But if this occurs, it may not occur very often, as the genetic signature of gene flow is weak in our dataset (Fig. 2A, Table 2 and Table 3).

Genetic differentiation among sites in North America is the result of competing forces of natal philopatry, breeding site fidelity, local adaptation, and dispersal. Although skimmers appear to be less philopatric than the co-occurring tern species on North American beaches (Viera *et al.* 2018), regular juvenile recruitment to their natal population could account for the differentiation we documented (F_{ST} values, Table 2). Northward range expansion on

both Pacific and Atlantic Coasts highlights strong dispersal tendencies and the capability to form new breeding colonies. For example, the Black Skimmer was not breeding in California prior to the 1950s and its range continues to expand with documented breeding in south San Francisco Bay, California by the late 1990s (Layne *et al.* 1996). Along the Atlantic Coast, there has been an increase in breeding numbers in Massachusetts since 2014 (Mostello *et al.* 2019). Combined with the opportunity for northern juveniles to prospect at southern colonies during migration on the Atlantic Coast (Snipes and Sanders 2012), the gene flow detected between New York and Texas could be shaped by the shared use of non-breeding sites in the Gulf of Mexico and the Yucatan.

The resident skimmer population in Florida was not sampled for this study but offers an interesting opportunity to tease out the relative contributions of winter residency and over-lapping winter distributions to gene flow in skimmers. Studies of niche-tracking across the annual cycle suggest that variability of climate and environmental tolerance across the breeding range can drive the degree of migratory connectivity and divergence among breeding sites (Bay *et al.* 2021; Ruegg *et al.* 2021). The California skimmer population is both resident and apparently isolated from other sampled populations outside the breeding season, conversely skimmers in Florida are residential and overlap the winter distribution of migrating Atlantic Coast birds. If the opportunity to interact with individuals from spatially distributed breeding populations during the non-breeding season is an important driver of geneflow, we predict that although the Florida population is resident (sedentary), it would cluster with the Atlantic Coast population.

This inference of gene flow within continents, breeding success in dynamic riverine environments, and observed northward range-shift in the northeast USA and California (Layne *et al.* 1996) suggests an ability in this species to shift habitat and range as individual breeding sites are impacted by

rising sea level, flooding, and human disturbances. However, this study has also bolstered evidence from previous studies suggesting a low level of genetic variation in Black Skimmer which could limit the ability of skimmer populations to adapt to the rapidly changing conditions forecasted in this century (Rehfishch *et al.* 2004; IPCC 2023), and is in contrast to results from similar species (e.g., Black Tern, Shephard *et al.* 2023). In Europe, Whiskered Terns (*Chlidonias hybrida*) share interesting life history characteristics with Black Skimmers, including rapid northward range expansion, dependence on dynamic freshwater environments for breeding and foraging, and relatively low site fidelity that promotes dispersal (Ledwoń *et al.* 2013). Dayton *et al.* (2017) reported variable genetic diversity estimates similar to the skimmer diversity documented here (N_a ranged 3.5–7.17, H_E ranged 0.47–0.67), where the highest values were associated with core populations versus low diversity in the leading-edge populations. That study also identified two genetic clusters that corresponded to discrete migratory flyways and wintering distributions.

Our data support the mark-recapture, telemetry, and genetic studies suggesting some mixing of populations during the non-breeding season (Mariano-Jelicich and Madrid 2014; Davenport *et al.* 2016; Vieira *et al.* 2018), but an expanded genetic investigation, both in geographic range, sample size, and genome coverage will be required to elucidate the fine details of population connectivity in this complex region. In parallel, a precise understanding of habitat use that includes both breeding and wintering habitat will identify threats faced across the annual cycle and is critical for this declining species. Our results suggest that effective conservation measures can consider North American and South American populations separately, but within continents, management will require multi-state and in South America, multi-national collaborations that leverage the newest technologies for tracking skimmers throughout the annual cycle. A concurrent sampling scheme to support genomic study would be required to fill

significant gaps in knowledge that impact our ability to support long-term persistence under changing climate, food, and human-wildlife interactions.

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