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Novel microsatellite markers used to determine the population genetic structure of the endangered Roseate Tern, *Sterna dougallii*, in Northwest Atlantic and Western Australia

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Abstract

The Roseate Tern, *Sterna dougallii*, is an endangered species in the Northwest Atlantic, where it has undergone transient reductions in population size over the past 120 years. This population has been slow to regain former size and range, perhaps in part due to the female-biased sex ratio, which results in female–female pairs, reducing the average productivity of the colony. The larger populations of the Western Pacific and Indian Oceans are not endangered and there is no evidence of a biased sex ratio at breeding in Western Australia. We developed four novel microsatellite markers and adapted one other and these are the first used in the genus *Sterna*. We also determined the utility of these markers for 17 related species. Here we report the population genetic structure within and between two regions, the Northwest Atlantic and Western Australia. A significant finding is that the Northwestern Atlantic region has much lower allelic diversity than the Western Australia region, promoting the recommendation for increased protection of sites in this region in order to preserve remaining genetic diversity and new potential breeding habitats.

Introduction

The Roseate Tern, *Sterna dougallii*, is a cosmopolitan seabird, with large populations found in the western Pacific Ocean and throughout the Indian Ocean. Smaller populations are found in the Eastern and Northwestern Atlantic oceans and Caribbean Sea. The Northwest Atlantic population consists of about 4000 pairs, breeding on small islands from Nova Scotia, Canada to New York, USA. This population was listed as an Endangered Species in the US in 1987, based on several reductions in population size, and range constrictions beginning in the late 1800s and continuing throughout the last century (Nisbet 1980; Nisbet & Spendelow 1999). The population has been slow to recover, from the low estimated as 2000 pairs, to its original number estimated to have been 8500 pairs. Therefore it continues to be vulnerable to disturbances such as predators, and habitat loss or degradation. At the time of the study, there were only three colonies with more than 150 mating pairs in the entire Northwest Atlantic region. Long-term studies are in progress at several colonies in this region, some beginning more than 30 years ago (reviewed by Gochfeld et al. 1998), but nothing is known about the remaining genetic diversity or levels of gene flow between colonies.

In contrast to the North Atlantic population, tropical Roseate Tern populations are large. They have low breeding site fidelity, unpredictable breeding patterns, and seasonal mixing of distant regional populations. O'Neill et al. (2003) reported birds that were banded in Japan have been trapped on the Great Barrier Reef alongside Australian banded birds. It is also thought that birds from South Africa travel north into the Madagascar and Seychelles populations (Tree & Klages 2003). Western Australian populations are large; Surman et al. (2002) report more than 2500 summer breeding Roseate Terns at Houtman Abrolhos, an archipelago of small islands. In Eastern Australia populations are estimated to have between 1600 and 6500 breeding pairs and approximately 25,000 birds in the non-breeding season (Ross et al. 1995; O'Neill et al. 2003). These clusters are part of a much larger region known to have many similar populations of Roseate Terns. We expected that large colonies, linked by regular dispersal would show little genetic differentiation among colonies, and high genetic diversity within colonies.

Here we present four microsatellite loci developed in the Roseate Tern and optimize a fifth locus originally developed for Red-billed Gull, *Larus hovaehollandiae*, (Given et al. 2002). We used these loci to determine population genetic structure within and between populations in Northwest Atlantic and Western Australia. We also examined their utility in 17 other related species.

Methods

Sampling and study sites

Blood samples (~25 μl) were collected from individuals at four locations as described in Szczys et al. (2001). Samples from Bird Island, Massachusetts, USA ($N = 88$) were collected in 1997 and at Falkner Island, Connecticut, USA ($N = 336$) in 1998–1999 as part of studies of sex-ratio bias (Nisbet and Szczys 2000; Szczys et al. 2001; Szczys et al. In preparation). These two islands are approximately 150 km apart. Samples were collected from three colonies in the Houtman Abrolhos Archipelago ($N = 15$ total; Square, Pelsaert, and Wooded Islands) and on Lancelin Island, Western Australia ($N = 15$) in 1997. These collections were part of a study of sex ratio at breeding (Hatch & Szczys 2000). Lancelin Island and Houtman Abrolhos are located 110 km and 420 km north of Perth, respectively. Blood samples of 12 additional species of *Sterna* and 5 other genera were donated by many seabird researchers for use in testing the utility of these markers in related taxa (Table 3).

Microsatellite development and genotyping

DNA was extracted from red blood cells following a standard chloroform extraction and ethanol precipitation protocol modified from Muellenbach et al. (1989) as described in Szczys et al. (2001). Fragments ranging in size from 400 to 1000 bp were selected for cloning after a restriction digest with *DpnII*. These fragments were inserted into the Lambda Zap Express Vector Kit (Stratagene, La Jolla, CA) as described in Hughes and Moralez Deloach (1997). Clones were screened with each of the following ^{32}P-labeled oligos; $(TG)_{10}$, $(AAAC)_{10}$, $(AAAG)_{10}$, $(GATA)_{10}$, $(AAT)_{10}$, and $(AAC)_{10}$. We sequenced 166 positives. Primers were developed for six positives containing repeat motifs and appropriate flanking sequences. Amplification reactions (25 μl) contained 20–40 ng DNA, 2.5 μl 10× magnesium free buffer (Promega), 2.5 mM $MgCl_2$,125 μM each dNTPs, 5 pmol each primer, one of each pair with 5′ fluorescent tag, 1U Taq DNA polymerize (Promega). The amplification parameters were 2 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at annealing temperature (Table 1), 30 s at 72 °C and a final extension step of 2 min at 72 °C. Four primer pairs amplified fragments of expected size without spurious bands and were polymorphic in Roseate Terns. An additional five loci originally developed for the Red-billed gull (Given et al. 2002) were tested for amplification in the Roseate Tern. One of these was polymorphic and useful for analysis of population genetic structure.

Statistical analysis

Genotype data for the five loci were analyzed using Genescan 3.1.2 (PE Biosystems). Allele frequencies, expected and observed heterozygosity, allelic richness (R_S) Wright's F statistics (Weir & Cockerham 1984) were calculated using GENEPOP 3.1c (Raymond & Rousset 1995) and FSTAT

Table 1. Primer Sequence, GENBANK accession number, optimized annealing temperature, length of original clone, number of alleles and repeat motif of four microsatellite loci developed in the Roseate Tern

Locus (Acc. #)	Primer Seq. 5′-3′	Temp	Length (range)	No Alleles	Repeat Motif
Sdaat20 (AY597041)	F:CTGGCTATGCTGCAGACTGA R:GCATCAAGTGCTCGATACCA	58	196 (180–207)	7	$(AAT)_9T(AAT)_5AAA(AAT)_3$
Sdaat27 (AY597042)	F:TGAAAACAGATGAATCAAACCAA R:ATCTGGTCTCCCTCCAGCTT	55	250 (250–266)	6	$(A)_6CCAT(AAT)_6GAT(A)_6$
Sdaat46 (AY597043)	F:TTTGTTGACTCGTTTGAGTTCC R:CATGTTTGTGGGTAGACAGCTT	55	250 (243–250)	2	$(TTTTA)_2(T)_3ATCC(TAAA)_2(T)_4$ $AGGAT(A)_4(T)_3A(T)_4AATAAAT$ $(AAT)_2(AAATAAT)_2$ AATAAATATTAAT
Scaac20 (AY597044)	F:CTTCATAGTGCCCAATACATCAG R:TCACTTGTTTAGGCATTTGGTT	58	144 (131–150)	5	$(AAAAC)_6CAC\ (AAC)_2C$ $(A_7\ CC)_2$

(Goudet 2001). Wright's F statistics from both software packages were in agreement, and we report those from FSTAT.

Results

We screened approximately 250 million base pairs of the Roseate Tern genome and more than a million probe/clone combinations with six repeat motifs. This effort produced 166 positives, seven simple sequence repeats, and only four polymorphic loci. Primer sequences and characteristics of each locus are presented in Table 1. These microsatellite loci also proved to be polymorphic in many other species, even with the limited sampling of our preliminary survey (Table 3).

Two of the five loci were monomorphic in the Northwestern Atlantic, but polymorphic in Western Australia. For 4 loci Allelic Richness (R_S) was higher in western Australia than in the North Atlantic, ranging from 1.5 to 4 times higher (Table 2). At the fifth locus the value was similar in western Australia ($R_S = 3.0$) and North America ($R_S = 3.2$).

Population differentiation was marked at the global scale, but not at the local scale. Population differentiation between the Northwestern Atlantic and Western Australian populations was highly significant ($F_{ST} = 0.48$, $P < 0.05$). In contrast

Table 2. Number of individuals Sampled (N), number of alleles (n), alleles richness (R_S), heterozygosity (H_E), observed heterozygosity (H_O), the inbreeding coefficient (F_{IS}), and (F_{ST}) for each locus in the two Northwest Atlantic and four Western Australian Roseate Terns

Population	Parameter	Locus				
		Sdaat20	Sdaat27	Sdaat46	Sdaac20	RBG27
N. Atlantic	N	186	168	156	361	125
	n	4	1	1	4	4
	R_S	2.5	1.0	1.0	3.22	2.33
	H_E	0.39	0	0	0.57	0.46
	H_O	0.32	0	0	0.54	0.48
	F_{IS}	0.17	NA	NA	0.05	0.05
	F_{ST}	0.03	NA.	NA	0.03	0.02
W. Australia	N	27	28	28	11	28
	n	5	6	2	3	5
	R_S	3.8	4.2	2.0	3.0	4.1
	H_E	0.47	0.43	0.48	0.18	0.6
	H_O	0.26	0.43	0.32	0.18	0.63
	F_{IS}	0.47	0.01	0.37	0.05	0.01
	F_{ST}	0.04	0.04	0.08	−0.11	0.02

differentiation between colonies in the Northwest Atlantic population ($F_{ST} = 0.03$) was not significant. There was no evidence of inbreeding within these colonies ($F_{IS} = 0.05$). The large but ephemeral colonies of western Australian region show very low, non-significant, levels of differentiation ($F_{ST} = 0.003$). However there was a deficiency of heterozygotes detected within colonies ($F_{IS} = 0.19$) and two of the five, loci *Sdaat20* and *Sdaat27*, are in linkage disequilibrium ($P = 0.009$) in this region.

We characterized the utility of these markers in related species of birds (Table 3), Interpretable fragments were obtained for nearly all markers in all 17 species and, surprisingly, most were polymorphic despite extremely small sample sizes. Indeed, seven of the accessions with sample sizes of only 9 to 25, identified more alleles than were detected in the entire Northwest Atlantic collection of Roseate Terns.

Discussion

The scarcity of microsatellite loci in the genomic library is not entirely unexpected; avian taxa have the smallest known genome sizes of any vertebrates and have previously revealed fewer microsatellite loci than plants and mammals (Hughes & Moralez Deloach 1997; Primmer et al. 1997; Longmire et al. 1999). There also seems to be variation for the frequency of microsatellites among avian groups and it is likely that the family Laridae, the genus *Sterna* in particular, is especially lacking. Some of this variation among families is certainly a function of time and resources expended as hundreds of microsatellites have been identified in the agronomically important chicken (personal communication, H. Cheng, USDA East Lansing, MI, USA). However, other studies, comparable to ours have reaped substantially greater numbers of markers (Isaksson & Tegelstrom 2002; Korfanta et al. 2002; Thode et al. 2002). Only two studies have identified polymorphic microsatellite loci in Laridae (Given et al. 2002; Tirard et al. 2002).

Seabird capture and resighting data generally suggest high levels of natal philopatry in local populations (Coulson 2001). Roseate Terns in the Northwest Atlantic show strong site fidelity, as only 10% of recruits breed initially in a non-natal colony, and at least 97% of adults return to breed at the same colony year after year (Gochfeld et al. 1998). We found low levels of differentiation between populations within a region but high levels ($F_{ST} = 0.48$) between regions separated by oceans, consistent with phylogeographic studies of other

Table 3. Cross species amplification

Scientific name	Common name	Locus			
		Sdaat20	Sdaat27	Sdaat46	Sdaac20
Sterna maxima	Royal Tern	1(5)	2(5)	2(1)	2(5)
S. hirundinacea	South American Tern	5(6)	2(9)	3(10)	3(10)
S. superciliaris	Yellow-billed Tern	2(1)	1(2)	2(2)	1(1)
S. fascata	Sooty Tern	4(3)	2(4)	1(1)	1(2)
S. hirundo	Common Tern	6(25)	2(4)	2(3)	5(16)
S. antillarum	Least Tern	1(1)	2(7)	1(4)	1(8)
S. paradisaea	Arctic Tern	6(9)	3(7)	5(7)	–
S. bergii	Crested Tern	1(9)	1(10)	2(2)	2(4)
S. sumatrana	Black-naped Tern	3(5)	1(10)	1(3)	–
S. anaethetus	Bridled Tern	–	4(9)	–	1(1)
S. caspia	Caspian Tern	2(12)	1(16)	–	2(5)
S. forsteri	Forsters Tern	2(16)	2(10)	3(14)	5(10)
Thalasseus elegans	Elegant Tern	1(12)	3(15)	5(18)	2(6)
Chlidonias nigra	Black Tern	5(6)	6(10)	1(2)	1(1)
Rynchops niger	Black Skimmer	4(8)	2(18)	2(3)	3(12)
Anous stolidus	Brown Noddy	2(2)	2(6)	–	2(6)
Larus dorninucus	Kelp Gull	2(3)	2(2)	1(3)	2(4)

Number of alleles (number of individuals scored), – indicates that locus was not tested.

seabird species (Avise et al. 2000), which shows mitochondrial haplotype differentiation between rookeries in the Atlantic and Indo-Pacific Basins. We are unaware of other published studies of seabird regional differentiation between oceans analyzed with microsatellite loci rather than mitochondrial DNA. This very large F_{ST} value and the occurrence of private alleles at all five loci in the Western Australian population, and at three of the five loci in the North Atlantic population, supports the current classification of Atlantic Ocean Roseate Terns and Indian Ocean Roseate Terns as separate subspecies, *Sterna dougallii dougallii* and *Sterna dougallii gracilis*, respectively.

Reduced allelic richness and gene diversity in the Northwest Atlantic is consistent with expectations based on a small population size relative to the Indian Ocean populations compounded by repeated fluctuations in population size beginning in the late 1800s and continuing throughout the last century (Nisbet 1980; Nisbet & Spendelow, 1999). Bird Island, MA, with about 1800 mating pairs was one of only two large breeding colonies and at the time of the study, Faulkner Island, CT was the third largest with 150 pairs. The small size of the colonies coupled with the philopatric nature of seabirds (Coulson 2001) suggested that inbreeding and substantial isolation may be significant factors in the dynamics of this species in the Northwest Atlantic region. However, while a relatively small effective population size may have contributed to the overall reduction in genetic diversity in these colonies, genetic population structure and inbreeding effects were not evident. The comparatively high level of gene flow (low F_{ST}) between colonies in the Northwest Atlantic is qualitatively consistent with band resighting data. Roseate terns have been banded on Bird Island and Falkner Island for nearly 20 years and resighting data show that there is an interchange of individuals between colonies in this region (Spendelow et al. 1995; Lebreton et al. 2003).

Low levels of differentiation in Western Australia are consistent with expectations based on the large, transient nature of the populations and the potential for population mixing. Unexpectedly, we found high F_{IS}, and significant linkage disequilibrium at two loci, conditions not found in the Northwest Atlantic. The presence of null alleles may explain heterozygote deficiency, however, the combination of high F_{IS} with linkage disequilibrium found in only one region may indicate that colonies are mixtures. As colonies change locations between years, small groups from separate subpopulations colonize the new locations, and may thereby have created a Wahlund effect for our sample. This explanation is supported by the natural history data showing that colonies are ephemeral. For example, colonies were found at different locations on Pelsaert Island each year between 1998 and 2000 and a colony was found on Jon Jim Island in 1999, but not in 1998 or 2000 (Surman et al. 2002).

These data have conservation implications for the Northwest Atlantic. Continued protection of this locally endangered species should be provided to prevent erosion of the genetic diversity that is already low compared to Australian populations. This is particularly important given the significant genetic differentiation between this population and that in Australia. Protection of suitable breeding habitats that are not already colony sites is an essential component of this in order to promote the increase in population size through recolonization and further decrease loss of genetic diversity.

Recent colonizations have indeed been recorded. Ram Island, Massachusetts, USA is a new colony first reported in the late 1990s and is now the second largest colony in the region.

This study has produced the first useful microsatellite markers for the Roseate Tern. We anticipate their use in future studies of population structure, genetic diversity, mating systems, and kin groupings in terns and closely related species.

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