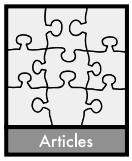

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Population structure in the South American tern *Sterna hirundinacea* in the South Atlantic: two populations with distinct breeding phenologies

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The South American tern *Sterna hirundinacea* is a migratory species for which dispersal, site fidelity and migratory routes are largely unknown. Here, we used five microsatellite loci and 799 bp partial mitochondrial DNA sequences (Cytochrome *b* and ND2) to investigate the genetic structure of South American terns from the South Atlantic Ocean (Brazilian and Patagonian colonies). Brazilian and Patagonian colonies have two distinct breeding phenologies (austral winter and austral summer, respectively) and are under the influence of different oceanographic features (e.g. Brazil and Falklands/Malvinas ocean currents, respectively), that may promote genetic isolation between populations. Results show that the Atlantic populations are not completely panmictic, nevertheless, contrary to our expectations, low levels of genetic structure were detected between Brazilian and Patagonian colonies. Such low differentiation (despite temporal isolation of the colonies) could be explained by demographic history of these populations coupled with ongoing levels of gene flow. Interestingly, estimations of gene flow through Maximum likelihood and Bayesian approaches has indicated asymmetrical long term and contemporary gene flow from Brazilian to Patagonian colonies, approaching a source–sink metapopulation dynamic. Genetic analysis of other South American tern populations (especially those from the Pacific coast and Falklands–Malvinas Islands) and other seabird species showing similar geographical distribution (e.g. royal tern *Thalasseus maximus*), are fundamental in gaining a better understanding of the main processes involved in the diversification of seabirds in the southern hemisphere.

The genetic structure and speciation of many animal groups has traditionally been explained by the occurrence of physical barriers disrupting dispersal among populations and/or species. However, in seabirds non-physical barriers and behavioural processes such as non-breeding distribution and phylopatry, also play an important role in promoting genetic differentiation (reviewed by Friesen et al. 2007a). More recently, isolation by time has also been described as a key mechanism preventing gene flow in seabirds (Friesen et al. 2007b, Overeem et al. 2008).

Isolation by time (IBT), or temporal isolation, is defined as the disruption of gene flow among populations due to heritable reproductive times (i.e. breeding phenology or allochrony) (Hendry and Day 2005). Population structure can develop in populations that are composed of a mixture of individuals with distinct breeding phenologies within a particular season and/or location (Hendry and Day 2005). The effect of seasonal breeding variation in genetic structure has been investigated in band-rumped storm petrels *Oceanodroma castro* (Monteiro and Furness 1998, Friesen et al. 2007b, Smith and Friesen 2007) where temporally segregated populations using the same colony site were genetically differentiated (Friesen et al. 2007b). Furthermore, breeding phenology is directly influenced by environmental conditions and limited gene flow between reproductive seasons, where those populations experiencing distinct selective environments may adapt to these specific habitats (Hendry and Day 2005).

The South American tern *Sterna hirundinacea* is a migratory seabird which is restricted to South America and breeds in the Atlantic Ocean from southern Brazil, through to Argentina (including the Falklands–Malvinas Islands) and along the Pacific coast from southern Peru to Chile (Harrison 1983, Gochfeld and Burger 1996). This species constitutes a valuable model to study the role of temporal isolation on genetic structure, as Atlantic populations show two distinct breeding phenologies: austral winter for colonies from the Brazilian coast (April–September, Branco 2003, Campos et al. 2004) and austral summer in the Argentinean Patagonia (November–January, Scolaro et al. 1996). Because reproduction is dependent on environmental quality (especially food availability, Schreiber and Burger

2002), differences in breeding timing between these populations may be influenced by locally distinct oceanographic features. Brazilian colonies are under the influence of the Brazil current that runs south along the coast of Brazil and is characterized by warm, saline and relatively oligotrophic waters. The Patagonian colonies on the other hand are influenced by the Falklands/Malvinas current that carries cool and nutrient-rich waters from the sub-Antarctic region (Bisbal 1995). As a consequence, local adaptation and genetic isolation of these populations could occur.

To our knowledge this is the first study addressing genetic variation and population structure of the migratory South American tern. Five colonies from the Brazilian coast and one from Punta Loma in Argentinean Patagonia were analyzed using both nuclear (five microsatellite loci) and mitochondrial markers (799 bp of partial Cyt b and ND2 genes), this in order to gather information about the spatial structure of this species in the South Atlantic. We tested the hypothesis that a significantly different genetic structure has developed between the Brazilian and Patagonian colonies via the influence of distinct oceanographic features promoting local adaptation and consequently two distinct breeding phenologies. This assessment of the genetic structure in the South American tern may help us to understand the role of breeding phenologies and oceanographic features on the diversification of other southern hemisphere seabird species. Furthermore, if populations of this tern are genetically structured then the data might be used in the future to identify the origin of South American terns in non-breeding areas, and help in further studies of their migratory movements.

Material and methods

Sampling and DNA extraction

Blood samples were collected from 159 South American terns chicks *Sterna hirundinacea* at six breeding sites (Fig. 1); five from along the Brazilian coast (Escalvada Island (ES, n = 36; 20°42′S, 40°24′W), Itaçucê Island (IT, n = 22; 23°50′S, 45°26′W), Apara Island (AP, n = 36; 23°49′S, 45°32′W), Laje de Santos (LS, n = 15; 24°19′S, 46°11′W) and Cardos Island (IC, n = 34; 27°48′S, 48°34′W) and one in the Argentinean Patagonia region Punta Loma, Chubut (AR, n = 16; 42°49′S, 64°28′W). The chicks were captured by hand and were banded with metal rings to avoid re-sampling the same individual. In order to avoid the possibility of sampling siblings, when several nests were situated closer to each other, only one chick was sampled. Blood samples were kept in absolute ethanol at 4°C and total DNA was isolated by standard phenol-chloroform extraction (Sambrook et al. 1989).

MtDNA sequencing

The most suitable mtDNA region for vertebrate population genetic and phylogeographic studies is usually the control region (Avise 2004). However, previous attempts to obtain these sequences failed and we have reported the occurrence of nuclear copies in this region in terns (Faria et al. 2007). As a result, additional mtDNA gene sequences were required to conduct such studies and partial sequences of the Cytochrome *b* (Cyt *b*) and ND2 genes were selected. PCR amplifications were performed in a 10µl volume using a MJ Research thermocycler (PTC-100 and PTC-200). Cycling conditions consisted of an initial denaturation step (95°C for 5 min), followed by 35 cycles of 95°C for 40 s, an annealing step (58 for Cyt b and 60°C for ND2) for 40 s and 72°C for 40 s and a final elongation step (72°C for 10 min). Cyt b partial sequences (318bp) were amplified using the light strand primer L15008 (AACTTCGGATCT CTACTAGG) and heavy strand primer H15326 (GAATA AGTTGGTGATGACTG) (Desjardin and Morais 1990), while ND2 sequences (472 bp) were amplified using MetL primer (AAGCTATCGGGCCCATACCCG) and ND2H primer (GATGAGAAGGCTAGGATTTTKCG) (Sorenson et al. 1999).

PCR products were purified using SAP/Exonuclease enzymes (Werle et al. 1994), with both forward and reverse strands being directly sequenced using the Big Dye Terminator Sequencing Kit following the standard protocol. The products were analysed using an automated sequencer (ABI 3100 and 3700) with the sequences being visualized via the software Sequence Navigator. Forward and reverse sequences were aligned by eye and double checked at the mutation points.

Microsatellite screening

To date, there have been no microsatellites loci isolated from South American terns, with previous attempts to generate species-specific primers (through the construction of genomic libraries) being unsuccessful (Faria et al. 2007). As a result, we conducted cross-species amplification tests using 14 microsatellite primers published for two species of gulls (Given et al. 2002, Tirard et al. 2002) and four isolated from the roseate tern (Szczys et al. 2005). Six of these loci (K6, K16, RBG13, RBG18, RBG29 and Sdaat20) were polymorphic and were subsequently used. Microsatellite amplifications were performed in a Perkin Elmer thermocycler using a modified primer with a fluorescent tail (TET, HEX or 6-FAM) attached to the 5′ end of the oligonucleotide primer. The protocol used was the Qiagen Multiplex PCR Kit, following the manufacturer's instructions. PCR products were visualized and sized on an automated DNA sequencer using TAMRA 350 size standard and the software GENESCAN 2.0 (ABI) and GENOTYPER 1.1 (ABI).

Molecular data analysis

MtDNA

ARLEQUIN 3.1 (Schneider et al. 2000) was used to estimate the amount of genetic variation within colonies through haplotype diversity (H) and nucleotide diversity (π) (Nei 1987). The program DNASP 4.1 (Rozas et al. 2003) was used to calculate parameters for the demographic history of the populations through mismatch distributions: unimodal curves are expected in populations that have undergone a rapid population expansion (Rogers and Harpending 1992, Harpending 1994). To test for deviations between the observed and expected mismatches we used a parametric bootstrap test, the sum of square

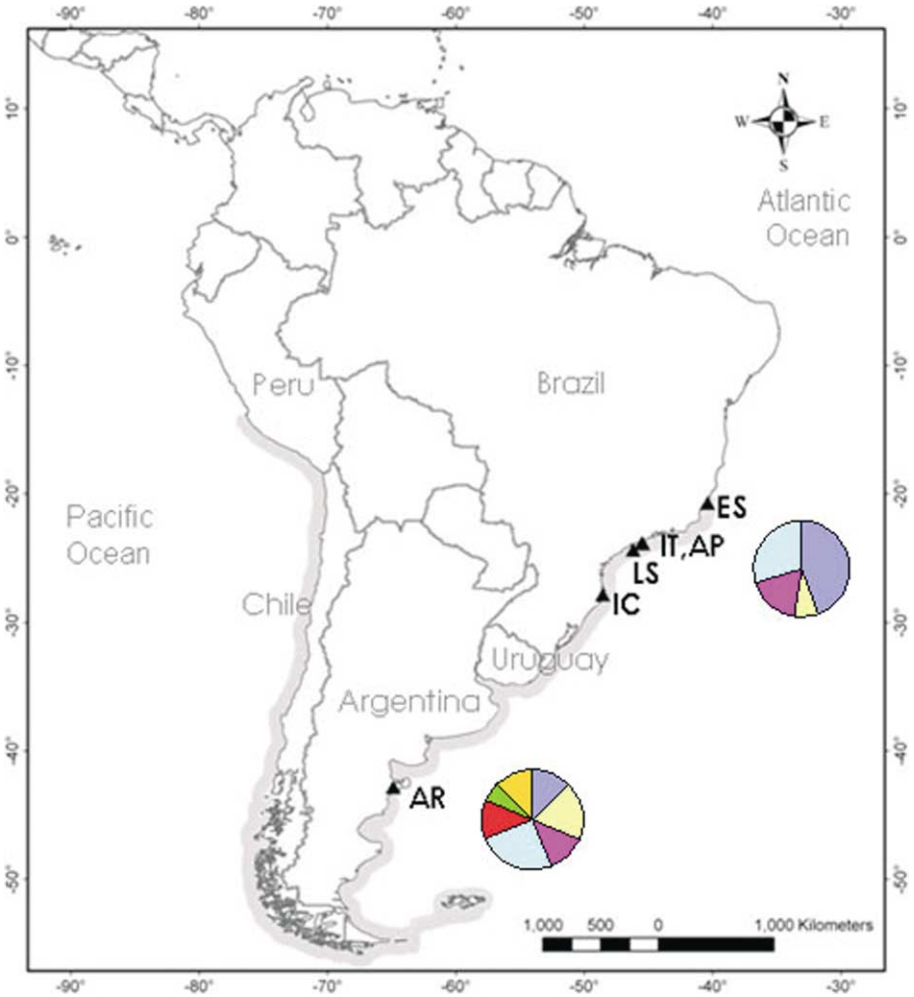

Figure 1. South American tern sampling sites. LS = Laje de Santos, AP = Apara Island, IC = Cardos Island, IT = Itaçucê Island, ES = Escalvada Island and AR = Punta Loma, Argentina. IT and AP appear as a single point on the map because these colonies are situated approximately 0.2 km from each other. Highlighted in light grey is the breeding range of the species (modified from Harrison 1983). Pie charts show mtDNA haplotype frequencies in the two main regions.

deviations (SSD) (Schneider and Excoffier 1999). We also applied two neutrality tests (Fu's Fs tests, Fu 1997; Tajima's D, Tajima 1989) in order to detect whether the population had expanded, decreased or has been stable in the past. Median joining networks were estimated using NETWORK 4.1.1.1 (Bandelt et al. 1999, <http://www.fluxus-engineering.com/>).

Microsatellites

Observed (Ho) and expected (He) heterozygosity under Hardy–Weinberg equilibrium and allele richness were calculated using ARLEQUIN. Each locus and population was tested for departures from Hardy–Weinberg proportions through a Markov chain simulation with 100 000 steps. The occurrence of linkage disequilibrium between each pair of loci was also tested with a similar approach using 10 000 steps.

Assessment of genetic differentiation and gene flow among colonies

Genetic divergence among populations was examined by F-statistics (Wright 1951) and hierarchical analyses of molecular variance (AMOVA, Excoffier et al. 1992). Significance was estimated through 10 000 permutations tests as implemented in ARLEQUIN. A coalescent theory approach implemented in the program MIGRATE 3.0.3 (Beerli and Felsenstein 1999) was used to estimate unequal migration rates between populations using mtDNA. The default settings in MIGRATE were used except for the number of trees sampled for the short and long chains, these being increased to 500 and 5000 respectively. Four-chain heating was used with temperatures of 1, 1.2, 1.5 and 3 in order to improve the sampling of trees. The program was run ten times with a different random number of seeds. Initial estimates were generated from Fst values for the first run and subsequent runs were performed using the estimates from previous runs, this in order to determine if the 95% confidence intervals were overlapping.

For microsatellites, we estimated recent migration rates using BAYESASS 1.3 (Wilson and Rannala 2003) which apply a Bayesian approach to multi-locus genotypes. The program was run with 5 000 000 MCMC iterations with the first 1 000 000 iterations discarded as burn-in and a sampling frequency of 2000. In addition, 95% confidence

intervals (CI) were calculated and these values were compared to mean migration rates and CI for data generated when there was insufficient information for estimating migration. Finally, in order to verify the consistency of our data, ten runs with different seed numbers and/or different delta settings were performed to verify the convergence between runs.

The program GENECLASS 2.0 (Piry et al. 2004) was used to assign individuals to their natal colonies, using the method from Rannala and Mountain (1997). A Bayesian-likelihood approach was applied to infer the number of populations without prior information of the sampling location as implemented in the program BAPS 3.2 (Corander et al. 2003, Corander and Marttinen 2005). Ten independent runs were performed in order to check the consistency of the results. The resulting mixture clustering was used for an admixture analysis using 50 interactions, 100 reference individuals from each population and 20 interactions for the reference individuals. The best clustering partition was determined by the highest value of likelihood.

Results

Mitochondrial DNA

Seven haplotypes were obtained from the combination of the Cyt b (318 bp) and of the ND2 data (481bp) (Table 1) (GenBank accession no. EU572709–572716). The Cyt b and ND2 sequences had only three variable sites each, all of which were transitions at the third codon position and showed no amino-acid replacements. Four haplotypes were shared among all the colonies (SH_1, SH_2, SH_3 and SH_4) and three were exclusively found in AR (SH_5, SH_6 and SH_7) (Table 1). Nucleotide diversity (π) ranged from 0.0009 in AP and ES to 0.0015 in AR, while haplotype diversity (H) ranged from 0.6383 in ES to 0.8667 in AR (Table 1). The distribution of the haplotypes in the two main regions (Brazil and Patagonia) is shown in Fig. 1.

The evidence indicating that the sequences amplified in this study are authentic mitochondrial DNA is that: 1) single PCR fragments were always detected on agarose gels, 2) clear sequences were always generated with no ambiguities present, and 3) there were no stop codons, deletions, insertions or frame-shifts.

The median joining network obtained for 799 bp of mtDNA (Fig. 2) revealed a shape where all haplotypes diverged by a single mutation, this pattern is often described as a characteristic of a population expansion. All Tajima's D neutrality tests were positive and non-significant ($p > 0.31$), except for a negative value obtained for AR colony (Table 2). Fu's F values were negative for all populations, except for a positive value for IT and were significant only for AR (Table 2). Fu's statistic is thought to be more sensitive in indicating range expansions than Tajima's D (Fu 1997). Mismatch distributions had a unimodal pattern for all populations (data not shown), however SSD tests were significant only for AP and IC colonies ($p = 0.04$), suggesting support for a sudden expansion model for these populations (Table 2).

Microsatellites

The number of alleles per locus ranged from two for K6 to eight for Sdaat20. The levels of observed heterozygosity ranged from 0.11 to 0.91 (Table 3). With the exception of locus Sdaat20, the Patagonian population had the highest values of heterozygosity. In addition, exclusive alleles were found within this population for loci R20, K6 and Sdaat20. Significant departure from Hardy–Weinberg equilibrium ($p > 0.05$) was found in locus K6 for almost all populations, consequently this locus was excluded from further analysis. Linkage disequilibrium ($p < 0.05$) was found between some pairs of loci in LS, IT, ES and IC. However, none of these showed significant values for the same combination, providing no evidence for physical linkage among loci.

Population differentiation and gene flow

Significant pairwise Fst values were obtained for mtDNA when AR was compared against the AP, ES and IC colonies (Table 4). AMOVA was performed by comparing one group formed by all Brazilian colonies with a second group

Table 1. Variable sites, absolute haplotype frequencies, nucleotide diversity (π) and haplotype diversity (H) in the mtDNA genes (Cytochrome *b* and ND2) and the geographical distribution of haplotypes amongst South American sampling sites. Standard deviation for π and H in brackets.

Haplotype	Cyt b	ND2	Colonies					
	222 679 164	134 647 264	LS (n = 15)	AP (n = 34)	IT (n = 22)	ES (n = 33)	IC (n = 31)	AR (n = 16)
SH_1	T G A	G C C	5	17	6	17	15	2
SH_2	. . G	. . .	2	1	4	1	3	3
SH_3	. . .	. . T	4	6	6	5	3	2
SH_4	. A .	. . .	4	10	6	10	10	4
SH_5	C A .	. . .	–	–	–	–	–	2
SH_6	. . .	A . .	–	–	–	–	–	1
SH_7	. . .	. T .	–	–	–	–	–	2
π			0.0014 (+/−0.0011)	0.0009 (+/−0.0008)	0.0014 (+/−0.0011)	0.0009 (+/−0.0008)	0.0010 (+/−0.0008)	0.0017 (+/−0.0013)
H			0.7810 (+/−0.0532)	0.6506 (+/−0.0521)	0.7792 (+/−0.0322)	0.6383 (+/−0.0559)	0.6645 (+/−0.0561)	0.8917 (+/−0.0439)

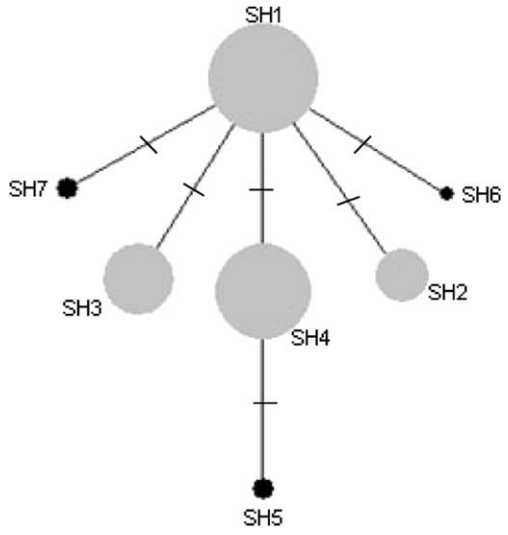

Figure 2. Median spanning network of South American tern mtDNA sequences (799 bp of partial Cytochrome *b* and ND2). The diameter of each circle is scaled to the haplotype frequency and the number of substitutions among haplotypes is indicated by the number of crosshatches between them. Black circles represent haplotypes exclusively from Argentina.

formed by the Patagonian colony (AR). The results revealed that 99.47% of the variation was partitioned within populations and the among-group component of genetic variation was not significant (4.28%, $p = 0.16$).

Microsatellite results were very similar to the mtDNA results with significant pairwise Fst values being obtained when the Patagonian colony was compared to the IT, ES and IC colonies (Table 4). AMOVA also detected significant genetic variation between the two groups (6.34%, $p = 0.0003$). Results from BAPS corroborates these results, as the best clustering partition (highest likelihood value of -2164.5) in 10 independent runs was two populations, one with all the colonies from Brazil and the other comprising of the Patagonian population (Fig. 3). Correct assignment rates using GeneClass were low in the colonies from the Brazilian coast, ranging from 40% in IC to 61.11% in AP, compared to the Patagonian colony, where 87.5% of the sampled individuals were correctly assigned (Table 5).

When analysing migration rates between the six colonies using BayesAss and Migrate, inconsistancies were encountered in the convergence among different runs for microsatellites and mtDNA markers. For each run, a different Brazilian population was identified as the source of migrants to the remaining Brazilian colonies (unpubl.). As the method implemented in BayesAss assumes low levels of migration ($<1/3$ of the total population per generation), and estimates of migration rates using the program Arlequin were extremely high (unpubl.), all the Brazilian colonies were pooled into a single population. Using this approach convergence was obtained in both nuclear and mtDNA data-sets. Migrate identified asymmetrical gene flow, with high migration rates from Brazilian colonies into Patagonia (mean = 130.67; CI = 54.17–218.75) but not in the opposite direction (mean = 0; CI = 0–0.0006). Similar results were obtained using BayesAss, which also identified unidirectional gene flow from Brazilian to Patagonian colonies (mean = 0.2084; CI = 0.0721–0.3264) and low levels from Patagonia into Brazilian colonies (mean = 0.0032; CI = 0–0.0112). In the latter, the values of gene flow from Brazilian to Patagonia are significantly higher than values expected in cases of insufficient signal in the data (non-informative CI: 0.0078–0.325).

Discussion

In this study, genetic homogeneity was found among South American tern breeding colonies from Brazil. Low, but significant, genetic differentiation was detected between the Patagonian and some Brazilian colonies (ES, AP, IT and IC) providing evidence that the Atlantic populations of these terns are not completely panmictic. These findings are supported by two different types of molecular markers (799 bp of mtDNA and five microsatellites), which despite their dissimilar inheritance models and evolutionary rates, provided concordant results. Three exclusive mtDNA haplotypes (Table 1) and three exclusive microsatellites alleles (loci R29, K6 and Sdaat20; Table 4) were found in the Patagonian population. Further support for this differentiation between regions was provided by the three exclusive mtDNA haplotypes (Table 1) and three exclusive microsatellites alleles (loci R29, K6 and Sdaat20; Table 4) found only in the Patagonian population. Furthermore, results from a Bayesian-likelihood method implemented in BAPS support the existence of two populations, one formed by Brazilian colonies and another in Patagonia (Fig. 3). On the other hand, estimations of recent and long term migration rates using BayesAss and Migrate, respectively, suggested significant unidirectional gene flow from Brazilian colonies to Patagonia.

Contrary to our hypothesis, genetic differentiation between Brazilian and Patagonian colonies was low, indicating that despite distinctive breeding phenologies, gene flow is high enough to prevent genetic isolation. Temporal isolation (IBT) has been described as an important force preventing gene flow among seabird populations (Friesen et al. 2007b). In band-rumped storm petrels *Oceanodroma castro* sympatric seasonal populations from two of the archipelagos studied (the Azores and Cape Verde) have ceased to exchange genes, providing the first example of sympatric speciation by allochrony in a tetrapod (Monteiro and Furness 1998, Friesen et al. 2007b). In penguins, sea surface temperatures seem to be

Table 2. Neutrality tests (Tajima's D and Fu's Fs) and SSD tests for mismatch distributions. *$p < 0.05$.

	LS	AP	IT	ES	IC	AR
Tajima's D	0.5257	0.1623	1.0054	0.0687	0.1924	−0.5799
Fu's Fs	−0.1543	−0.0660	0.4116	−0.1669	−0.0664	−3.2999*
SSD	0.02189	0.0243*	0.0192	0.02507	0.02416*	0.03913

Table 3. Allelic variation (A), expected heterozygosity (H_e) and observed heterozygosity (H_o) at microsatellite loci. *p <0.05.

Nesting site	RBG13	RBG18	RBG29	K6	K16	Sdaat20
LS (n =15)						
A	2	3	3	2	3	4
He	0.3711	0.3011	0.5701	0.5080	0.4666	0.7425
Ho	0.4667	0.3333	0.6000	0.3333	0.6000	0.7333
AP (n =36)						
A	3	3	3	2	6	6
He	0.1819	0.4338	0.5090	0.5090*	0.5066	0.7312
Ho	0.1944	0.3611	0.4444	0.2500*	0.2500	0.7222
IT (n =22)						
A	2	3	3	3	4	4
He	0.0888	0.5317	0.4640	0.5317*	0.5317	0.7146
Ho	0.0909	0.5909	0.4090	0.1818*	0.6363	0.7272
ES (n =36)						
A	2	3	3	2	3	5
He	0.0547	0.4291	0.4831	0.5203*	0.2957	0.7288
Ho	0.0555	0.4444	0.4722	0.1111*	0.3333	0.6944
IC (n =34)						
A	2	3	3	2	5	4
He	0.0294	0.2410	0.4543	0.4543*	0.5272	0.7335
Ho	0.0294	0.2647	0.3823	0.2647*	0.5882	0.8235
AR (n =16)						
A	3	4	4	3	6	8
He	0.4667	0.5726	0.6593	0.5181*	0.7903	0.4207
Ho	0.4667	0.6250	0.5000	0.5625*	0.7500	0.4000

primarily related to differences in breeding phenology which seems to have an effect on the diversification among populations of the little penguin *Eudyptula minor* (Overeem et al. 2008) and speciation of the rockhopper penguin *Eudyptes chrysocome* (Jouventin et al. 2006). Furthermore, rockhopper penguins are separated by the sub-tropical convergence, which also separates the Brazilian and Patagonian populations of the South American tern. The degree of influence a distinct environment has in promoting habitat specialization will depend primarily on how dependent species are to this particular marine environment. Distinct oceanographic environments have also played a role in the diversification of shearwaters of the genus *Calonectris* (Gómez-Díaz et al. 2006).

Patterns of genetic structure detected by molecular markers may not reflect the current levels of gene flow, particularly if the populations are not in genetic equilibrium (Beaumont 2004). Several seabirds have meta-population dynamics showing that they have experienced recent bottlenecks, range expansion, colonization, fluctuations in population size and/or a change in the number of colonies (e.g. short-tailed shearwaters, Austin et al. 1994; shy albatrosses, Abbott and Double 2003; common murres, Morris-Pocock et al. 2008). Therefore, it is unlikely that they have reached genetic equilibrium, and the low genetic differentiation might reflect populations that have not had time to diverge significantly as against the consequence of high levels of current dispersal. Consequently, the genetic structure of many seabirds seem to be a result of demographic histories influenced by long-term climate oscillations, glaciations events (Friesen et al. 1996, Congdon et al. 2000, Liebers et al. 2001) and sea level changes during the Pleistocene (Peck and Congdon 2004). Even though there was no ice covering the habitats in the tropics, it is possible that glacial maxima during Pleistocene has promoted changes in sea temperature, sea level, currents and other physiochemical characteristics that may have affected the population history of tropical birds (Peck and Congdon 2004).

The absence of strong genetic structure in the South American tern may be explained by either 1) the absence of current gene flow among recently separated populations which did not have sufficient time to attain genetic equilibrium, or 2) contemporary gene flow among populations which were historically isolated. The evidence shown in the present study suggest that South American tern populations might not be in genetic equilibrium, especially the Patagonian population: the Tajima's D value was negative for the Patagonian colony, negative values of Fu's F were obtained for all colonies and were significant for the Patagonian colony (Table 2), mismatch distributions had a unimodal curve (unpubl.) and the median spanning network had a star-like structure with haplotypes diverging by single

Table 4. Pairwise Fst for microsatellites (above diagonal) and mtDNA (below diagonal). *p <0.05.

	LS	AP	IT	ES	IC	AR
LS	–	−0.0027	0.0020	−0.0005	0.0027	0.0105
AP	−0.0138	–	−0.0027	0.0021	0.0027	0.0077
IT	−0.0550	0.0224	–	0.0069	0.0050	0.0092*
ES	−0.0058	0.0312	0.0312	–	−0.00367	0.0184*
IC	−0.0096	−0.0222	0.0206	−0.0252	–	0.0184*
AR	−0.0038	0.0872*	−0.0059	0.0938*	0.0680*	–

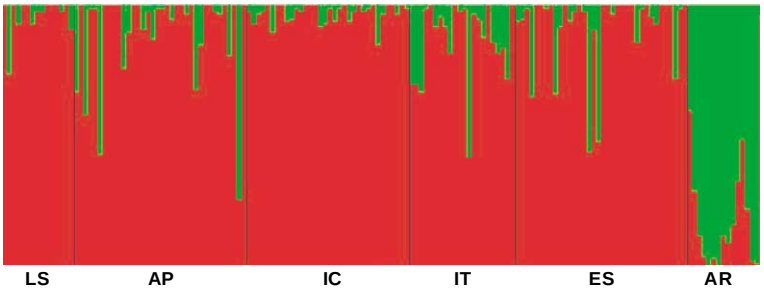

Figure 3. Admixture analysis results from BAPS using five microsatellite loci for the South American tern samples. Each vertical bar represents an individual divided into the two inferred clusters. LS = Laje de Santos, AP = Apara Island, IC = Cardos Island, IT = Itaçucê Island, ES = Escalvada Island and AR = Punta Loma, Argentina.

mutations suggesting a recent population expansion (Fig. 2). Nevertheless, as we obtained significant contemporary dispersal rates from the Brazilian to Patagonian colonies (using microsatellites markers), the first explanation evoking absence of current gene flow as a reason to the pattern of structure observed can be discarded.

The higher genetic diversity, haplotype diversity, and allelic richness in the Patagonian population in both marker types (despite a smaller sample size and exclusive haplotypes) suggest that this population could be acting as a source from which the other populations might have originated and expanded afterwards. Inversely, the migration rates obtained using BayesAss and Migrate suggested the opposite scenario, where the Brazilian colonies are the source of migrants to Patagonia.

This source–sink dynamic has been observed in other seabird species such as the Magellanic penguin *Spheniscus magellanicus* (Bouzat et al. 2009), and it is likely to be a common mechanism in several species of terns, as these birds frequently change breeding sites and colonize new areas when the habitat become unsuitable. Possible scenarios to explain higher diversity in the Patagonian population include: 1) migration from different source populations (e.g from the Falkland Islands), 2) erosion of genetic diversity in the source population (Brazilian colonies) due to recent bottlenecks, and 3) a larger effective population size. Trustworthy population censuses of South American tern colonies are still lacking, especially because of the frequent changes of breeding sites displayed by these birds. This behaviour requires simultaneous censuses in different breeding locations in order to correctly estimate the total population sizes of this species. The census of Brazilian colonies has reported the occurrence of no more than 5000 breeding pairs along the coast (Branco 2004a). In Punta Tombo, Argentina, 67 500 pairs were reported in 1877 (Durnford 1878), however this number has decreased to "tens of thousands of pairs" in 1969 (Gochfeld and Burger 1996). Furthermore, from 1996 to 2003 breeding did not occur at this site (Yorio 2005). Additional population censuses in six other Argentinean localities in 1995 reported 7242 pairs (Yorio 2005).

Table 5. Assignment tests results provided by GeneClass. Populations from which specimens were sampled are shown in rows, and the number of individuals that were assigned to that respective population with 95% probability is shown in columns.

		Assigned colony					
		LS	AP	IT	ES	IC	AR
Sampled colony	LS	8	3	1	2	1	0
	AP	3	22	1	4	6	0
	IT	4	2	11	3	2	0
	ES	6	7	3	15	4	1
	IC	6	4	3	8	14	0
	AR	–	–	–	2	–	14

Gene flow among spatially isolated colonies of South American terns might occur through contact in non-breeding grounds. These sites constitute an opportunity for contact between birds from distant colonies, where they can potentially switch future reproduction sites following encounters with individuals from different areas. If each colony has a population-specific non-breeding site, the chance of encounters among individuals from distant colonies (and potential switching) will be much lower, if not absent. In a recent review Friesen et al. (2007a) found a strong correlation between genetic structure and the occurrence of multiple population-specific non-breeding areas in migratory species.

The migratory routes and population composition of nonbreeding grounds of the South American tern are not fully known, especially because direct dispersal data (e.g. banding returns) are scarce. Analysis of the abundance of South American terns in non-breeding grounds in Rio Grande do Sul State, Brazil (31°S) and Uruguay shows peaks during July to September (when reproduction is occurring along the coast of Brazil) and an absence of individuals from November to March (coincident with the breeding season in Patagonia), this suggests that these wintering areas are visited mainly by birds from Patagonia (Bugoni and Vooren 2005, Alfaro and Clara 2007). However, due to the occurrence of few birds in breeding plumage during the austral autumn in the over-wintering area in southern Brazil (31°S) and the proximity of this site (around 450 km) to the southern distributed colonies of South American tern in Brazil (in Santa Catarina State, 26–27°S), it is feasible that individuals from different populations may co-exist at these grounds occasionally (Bugoni and Vooren 2005).

Seasonal migratory movements from the Patagonian populations seem to be related to the displacement of anchovies *Engraulis anchoita* (Rodriguez et al. 2005), a

major prey item in the South American tern diet (65% in Buenos Ayres Province, Argentina; Favero et al. 2000), as during the winter, spawning concentrations of anchovy travel northwards reaching southern Brazil (Bakun and Parrish 1991). As productivity is higher in the Patagonian region (due to the effect of the Falklands/Malvinas Current carrying nutrient-rich waters from the sub-Antarctic region, Bisbal 1995), we can speculate that in years where food availability is low along the Brazilian coast, some individuals might migrate southwards following better food provision (Bisbal 1995). This would establish individuals in new breeding sites in the Patagonia region, promoting unidirectional gene flow from Brazil to Patagonia as suggested by the migration estimates in this study.

The South American tern is a poorly studied species, where population census, basic ecological knowledge, migratory routes, historical demographic data and even direct dispersal data (e.g. banding returns) are still unknown. In order to adequately understand the diversification of South American tern populations in the South Atlantic and identify the principal processes that have played a role in its evolutionary history, it is essential to gather ecological, genetic and demographic information for the whole distribution range of this species. The molecular analysis of additional colonies from Patagonia, the Falkland–Malvinas Islands and the Pacific Ocean with a broader range of molecular markers than those used in the present study, is needed to corroborate the results reported here and promote a better understanding of the processes underlying such patterns.

Conservation implications

The South American tern is not considered globally threatened, however its total population size is inadequately documented (Gochfeld and Burger 1996) and population declines have already been reported (BirdLife Int. 2007). In Brazil they are classified as near-threatened in São Paulo state (SMA 2008) as the colonies are small (less than 800 individuals, Campos et al. 2004), are situated on off-shore islands (maximum distance from the continent is 35 km) and the birds frequently desert colonies/islands when disturbed (Branco 2004b, Campos et al. 2004). In general, the main threats are from egg collecting, disturbance caused by intruders at the colonies and the predation of chicks/eggs by kelp gulls (*Larus dominicanus*) (Branco 2004b, Campos et al. 2004), this species now thought to be expanding due to increasing food availability from fishing vessels discards and urban landfills (Bertellotti et al. 2001).

Based on the molecular analysis presented here, we suggest that populations from the coast of Brazil and Patagonia should be managed separately until further information about the connectivity between Patagonian, Falkland Islands and Pacific coast colonies is obtained. In addition, it is essential to ascertain the stopover and wintering areas of this species, to guarantee effective conservation of its populations.

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References

Abbot, C. L. and Double, M. C. 2003. Genetic structure, conservation genetics and evidence of speciation by range expansion in shy and white-capped albatrosses. – Mol. Ecol. 12: 2953–2962.

Alfaro, M. and Clara, M. 2007. Assemblage of shorebirds and seabirds on Rocha Lagoon sandbar, Uruguay. – Neotrop. Ornitol. 18: 421–432.

Austin, J. J., White, R. W. G. and Ovenden, J. R. 1994. Population genetic structure of a philopatric colonially nesting seabird, the short-tailed shearwater *Puffinus tenuitrostris*. – Auk 111: 70–79.

Avise, J. C. 2004. Molecular markers, natural history and evolution. – Synauer Associates.

Bakun, A. and Parrish, R. H. 1991. Comparative studies of coastal pelagic fish reproductive habitats: the anchovy *Engraulis anchoita* of the southwestern Atlantic. – J. Mar. Sci. 48: 343–361.

Bandelt, H. J., Forster, P. and Rohl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. – Mol. Biol. Evol. 16: 37–48.

Beaumont, M. A. 2004. Recent developments in genetic data analysis: what can they tell us about human demographic history? – Heredity 92: 365–379.

Beerli, P. and Felsenstein, J. 1999. Maximum likelihood estimation of migration rates and population numbers of two populations using a coalescent approach. – Genetics 152: 763–773.

Bertellotti, M., Yorio, P., Blanco, G. and Giaccardi, M. 2001. Use of tips by nesting kelp gulls at a growing colony in Patagonia. – J. Field Ornithol. 72: 338–348.

Birdlife international. 2007. Species factsheet: *Sterna hirundinacea*. – <http://www.birdlife.org>.

Bisbal, G.A. 1995. The southeast South American shelf large marine ecosystem. – Mar. Policy 19: 21–38.

Bouzat, J. L., Walker, B. G. and Boersma, P. D. 2009. Regional genetic structure in the Magellanic penguin *Spheniscus magellanicus* suggests metapopulation dynamics. – Auk 126: 326–334.

Branco, J. O. 2003. Reprodução de *Sterna hirundinacea* lesson e *S. eurygnatha* Saunders (Aves, Laridae), no litoral de Santa Catarina, Brasil. – Rev. Brasil. Zool. 20: 655–659.

Branco, J. O. 2004a. Aves marinhas insulares brasileiras: bioecologia e conservação. – Editora da Univali, Itajaí.

Branco, J. O. 2004b. Aves marinhas das ilhas de Santa Catarina. – In: Branco, J. O. (ed.), Aves marinhas insulares brasileiras: bioecologia e conservação. Editora da Univali, Itajaí, pp. 15–36.

Bugoni, L and Vooren, C. M. 2005. Distribution and abundance of six tern species in southern Brazil. – Waterbirds 28: 110–119.

Campos, F. P., Paludo, D., Faria, P. J. and Martuscelli, P. 2004. Aves insulares marinhas, residentes e migratórias do litoral do Estado de São Paulo. – In: Branco, J. O. (ed.), Aves marinhas insulares brasileiras: bioecologia e conservação. Editora da Univali, Itajaí, pp. 57–82.

Congdon, B. C., Piatt, J. F., Martim, K. and Friesen, V. L. 2000. Mechanisms of population differentiation in marbled murrelets: historical versus contemporary processes. – Evolution 54: 974–986.

Corander, J. and Marttinen, B. 2005. BAPS: Bayesian analysis of population structure. Manual ver. 3.2. – Dept of Mathematics, Univ of Helsinki.

Corander, J., Waldmann, P. and Sillanpää, M. J. 2003. Bayesian analysis of genetic differentiation between populations. – Genetics 163: 367–374.

Desjardin, P. and Morais, R. 1990. Sequence and gene organization of the chicken mitochondrial genome: a novel gene order in higher vertebrates. – J. Mol. Biol. 212: 599–634.

Durnford, H. 1878. Notes on the birds of central Patagonia. – Ibis 2: 389–406.

Excoffier, L., Smouse, P. E. and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. – Genetics 131: 479–491.

Faria, P. J., Baus, E., Morgante, J. S. and Bruford, M. W. 2007. Challenges and prospects of population genetic studies in terns (Sternidae, Charadriiformes, Aves). – Gen. Mol. Biol. 30: 681–689.

Favero, M., Bó, M. S., Silva, M. P. and García-Mata, C. 2000. Food and feeding biology of the South American tern during nonbreeding season. – Waterbirds 23: 125–129.

Friesen, V. L., Montevecchi, W. A., Baker, A. J., Barret, R. T. and Davidson, W. S. 1996. Population differentiation and evolution in the common guillemot *Uria aalge*. – Mol. Ecol. 5: 793–805.

Friesen, V. L., Burg, T. M. and McCoy, K. D. 2007a. Mechanisms of population differentiation in seabirds. – Mol. Ecol. 16: 1765–1785.

Friesen, V. L., Smith, A. L., Gomez-Diaz, E., Bolton, M., Furness, R. W., Gonzalez-Solis, J. and Monteiro, L. R. 2007b. Sympatric speciation by allochrony in a seabird. – Proc. Natl Acad. Sci. USA 104: 18589–18594.

Fu, Y. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. – Genetics 147: 915–925.

Given, A. D., Mills, J. A. and Baker, A. J. 2002. Isolation of polymorphic microsatellite loci from the red-billed gull *Larus novaehollandiae scopulinus* and amplification in related species. – Mol. Ecol. Notes 2: 416–418.

Gochfeld, M. and Burger, J. 1996. Family Sternidae (terns). – In: Del Hoyo, J., Elliot, A. and Sargatal, J (eds), Handbook of the birds of the world. Vol. 3. Lynx Edition, pp. 572–623.

Gómez-Díaz, E., González-Solís, J., Peinado, M. A. and Page, R. D. M. 2006. Phylogeography of the *Calonectris* shearwaters using molecular and morphometric data. – Mol. Phylogenet. Evol. 41: 322–332.

Harpending, R. C. 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. – Hum. Biol. 66: 561–600.

Harrison, P. 1983. Seabirds: an identification guide. – Houghton Mifflin Company, Boston, EUA.

Hendry, A. P. and Day, T. 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. – Mol. Ecol. 14: 901–916.

Jouventin, P., Cuthbert, R. J. and Ottvall, R. 2006. Genetic isolation and divergence in sexual traits: evidence for the northern rockhopper penguin *Eudyptes moseleyi* being a sibling species. – Mol. Ecol. 15: 3413–3423.

Liebers, D., Helbig, A. J. and Knijff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans–fuscus* group (Aves: Charadriiformes). – Mol. Ecol. 10: 2447–2462.

Monteiro, L. R. and Furness, R. W. 1998. Speciation through temporal segregation of Madeiran storm petrel *Oceanodroma castro* populations in the Azores? – Phil. Trans. R. Soc. Lond. B 353: 945–953.

Morris-Pocock, J. A., Taylor, S. A., Birt, T. P., Damus, M., Piatt, J. F., Warheit, K. I. and Friesen, V. L. 2008. Population genetic structure in Atlantic and Pacific Ocean common murres *Uria aalge*: natural replicate tests of post-Pleistocene evolution. – Mol. Ecol. 17: 4859–4873.

Nei, M. 1987. Molecular evolutionary genetics. – Columbia Univ. Press.

Overeem, R. L., Peucker, A. J., Austin, C. M., Dann, P. and Burridge, C. P. 2008. Contrasting genetic structuring between colonies of the World's smallest penguin, *Eudyptula minor* (Aves: Spheniscidae). – Conserv. Genet. 9: 893–905.

Peck, D. R. and Congdon, B. C. 2004. Reconciling historical processes and population structure in the sooty tern *Sterna fuscata*. – J. Avian Biol. 35: 327–335.

Piry, S., Alapetite, A., Cornuet, J. M., Paetkau, D., Baudouin, L. and Estoup, A. 2004. GeneClass2: A software for genetic assignment and first-generation migrant detection. – J. Hered. 95: 536–539.

Rannala, B. and Mountain, J. L. 1997. Detecting immigration by using multilocus genotypes. – Proc. Natl Acad. Sci. USA 94: 9197–9201.

Rodríguez, M. P. S., Favero, M., Berón, M. P., Mariano-Jelicich, R. and Mauco, L. 2005. Ecología y conservación de aves marinas que utilizan el litoral bonaerense como área de invernada. – Hornero 20: 111–130.

Rogers, A. R. and Harpending, H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. – Mol. Biol. Evol. 9: 552–569.

Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X. and Rozas, R. 2003. DNASP, DNA polymorphism analyses by the coalescent and other methods. – Bioinformatics 19: 2496–2497.

Sambrook, J., Fritsch, E. and Maniatis, T. 1989. Molecular cloning: a laboratory manual. –Cold Spring Harbor Press.

Schneider, S. and Excoffier, L. 1999. Estimation of demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. – Genetics 152: 1079–1089.

Schneider, S., Roessli, D. and Excoffier, L. 2000. Arlequin version 2.000: a software for population genetics data analysis. – Genetics and Biometry Lab, Univ. of Geneva, Switzerland.

Schreiber, E. A. and Burger, J. 2002. Biology of marine birds. – CRC Press.

Scolaro, J. A., Laurenti, S. and Gallelli, H. 1996. The nesting and breeding biology of the South American tern in northern Patagonia. – J. Field Ornithol. 67: 17–24.

SMA. 2008. Fauna ameaçada no Estado de São Paulo. – Secretaria do Meio Ambiente. São Paulo.

Smith, A. L. and Friesen, V. L. 2007. Differentiation of sympatric populations of the band-rumped storm-petrel in the Galapagos Islands: an examination of genetics, morphology, and vocalizations. – Mol. Ecol. 16: 1593–1603.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T. and Mindell, D. P. 1999. Primers for a PCR based approach to mitochondrial genome sequencing in birds and other vertebrates. – Mol. Phylogenet. Evol. 12: 105–114.

Szczys, P., Hughes, C. R. and Kesseli, R. V. 2005. Novel microsatellite markers used to determine the population genetic structure of the endangered roseate tern, *Sterna dougallii*, in northwest Atlantic and western Australia. – Conserv. Genet. 6: 461–466.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. – Genetics 158: 1147–1155.

Tirard, C., Helfenstein, F. and Danchin, E. 2002. Polymorphic microsatellites in the black-legged kittiwake *Rissa tridactyla*. – Mol. Ecol. Notes 2: 431–433.

Werle, E., Schneider, C., Renner, M., Volker, M. and Fiehn, W. 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. – Nucleic Acids Res. 22: 4354–4355.

Wilson, G. A. and Rannala, B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. – Genetics 163: 1177–1191.

Wright, S. 1951. The genetic structure of populations. – Ann. Eugen. 15: 323–354.

Yorio, P. 2005. Estado poblacional y de conservación de gaviotines y escúas que se reproducen en el litoral marítimo argentino. – Hornero 20: 75–93.