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The subspecies of Antarctic Terns (*Sterna vittata*) wintering on the South African coast: evidence from morphology, genetics and stable isotopes

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Abstract. Five to seven subspecies of Antarctic Tern (*Sterna vittata*) are recognised, with at least three (*S. v. vittata*, *S. v. tristanensis* and *S. v. sanctipauli*) wintering in South Africa. Morphological characters used to define these subspecies are not perfectly reliable, but fidelity to nesting site suggests they could be genetically distinct. We used morphological data and DNA to investigate the validity of subspecies. We further used stable isotope analysis of feather samples collected from the non-breeding grounds in South Africa to attempt to ascertain the population of origin. Nuclear and mitochondrial DNA sequence data identified two major genetic clades: one mostly comprised individuals partially or completely matching the morphological description of *S. v. tristanensis*, the other included individuals from *S. v. vittata* and *S. v. sanctipauli*. Stable isotope values indicated that juveniles originated from at least three populations. Irrespective of their morphological and genetic characteristics, most immatures moulted in Antarctic waters, and adults moulted in various habitats. Their colony of origin could not therefore be inferred from stable isotope values from feathers. Results indicate that morphological groupings may reflect a north–south cline across the Indian Ocean. Adequate conservation strategies require rigorous reassessment of the currently accepted subspecies, including DNA analyses of samples from the breeding grounds, particularly on Amsterdam and St Paul Islands.

Additional keywords: carbon and nitrogen stable isotopes, feathers, intron sequences, microsatellites, mtDNA, population structure, Southern Ocean.

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Introduction

Concerns about seabird conservation are growing, as more than 47% of seabird populations are believed to be declining worldwide (Croxall *et al*. 2012). The implementation of adequate conservation measures requires biodiversity assessments based on accurate taxonomic identification and systematic relationships among and within species (Haig *et al*. 2006). Many species are taxonomically subdivided into multiple subspecies (Haig *et al*. 2006), including >30% of seabird species (Croxall *et al*. 2012; Gill and Donsker 2013). Despite the intense use of the subspecies concept, its validity is still under debate (reviewed in Haig *et al*. 2006), and it has been suggested that the concept can be treated as a testable hypothesis (Winker 2010). Various definitions for subspecies exist, including the reciprocal monophyly of multiple characters among a species' populations (Avise 2000), and the idea that 75% of a population must lie outside 99% of the range of other populations for a given character or set of characters (Patten and Unitt 2002). We propose that a multi-criterion approach is an appropriate way to evaluate subspecies, using information from completely independent datasets.

The Antarctic Tern *Sterna vittata* J. F. Gmelin, 1789 is a medium-sized tern (Higgins and Davies 1996) with an estimated global population of ~44 500 breeding pairs (Tree and Klages 2004). The species breeds on many islands in the Southern Ocean, from 68°S on the Antarctic Peninsula north to 37°S on the Tristan da Cunha Archipelago north of the Subtropical Convergence.

Breeding takes place mainly between mid-November and April, although with some geographical variation. Outside the breeding season, part of the population remains near the breeding grounds, whereas another moves north to the coasts of South Africa and Argentina (Higgins and Davies 1996; Tree and Klages 2004). Between five and seven subspecies are recognised on the basis of morphology, colouration and moult cycle, with the assumption that their breeding ranges are distinct (Fig. 1).

Previous studies based on morphology have postulated that many individuals could be identified to subspecies by experienced ornithologists, but that some are borderline in morphology or colour, making identification in non-breeding areas difficult (Tree and Klages 2004; Hockey *et al*. 2005). From April to October, at least three of the seven subspecies visit South Africa (Brooke *et al*. 1988; Tree and Klages 2004): *S. v. vittata*, *S. v. tristanensis* and *S. v. sanctipauli*. There is geographical segregation of their wintering quarters, with *S. v. tristanensis* recorded mainly on Dyer Island, on the southern coast of South Africa, whereas most birds identified on Bird Island on the south-eastern coast and on the western coast of South Africa are *S. v. vittata* (Tree and Klages 2004). As most of the breeding sites of this species are remote and access to them is difficult, and the birds are highly sensitive to disturbance at breeding sites (M. Favero and M. Connan, pers. obs.), we sampled birds from various breeding colonies when they were wintering in South Africa.

Stable isotopes are increasingly used to study the origins and migration patterns in avian ecology (e.g. Rubenstein and Hobson 2004) and rely on the fact that stable isotope values pass from prey to predator tissues in a predictable manner (Hobson and Clark 1992). Feathers are almost pure protein (keratin) and remain

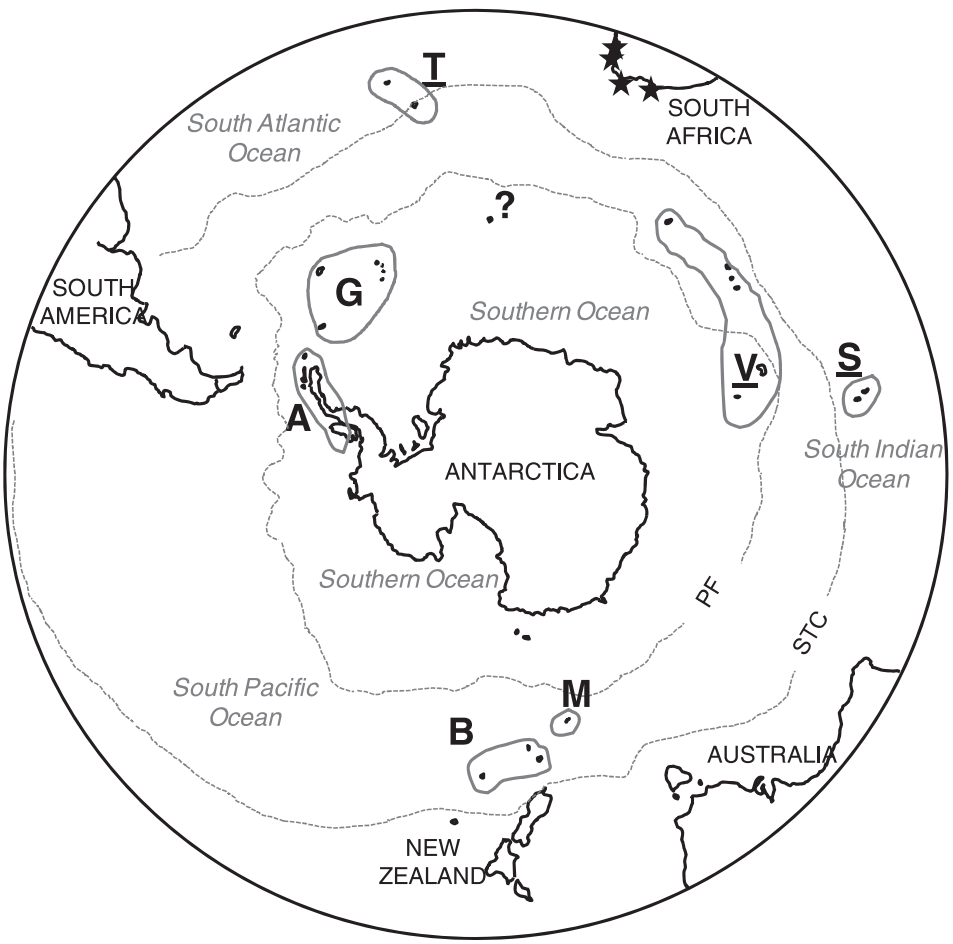


Fig. 1. Breeding localities of Antarctic Terns: A – *S. v. gaini*, Antarctic Peninsula and South Shetland Islands; G – *S. v. georgiae*, South Georgia, South Orkney and South Sandwich Islands; V – *S. v. vittata*, Heard, Kerguelen, Crozet, Prince Edward and Marion Islands; B – *S. v. bethunei*, Campbell, Antipodes, Bounty, Auckland, Snares and Stewart Islands; M – *S. v. macquariensis*, Macquarie Island (Falla 1937; Gochfeld and Burger 1996); T – *S. v. tristanensis*, Tristan da Cunha and Gough Islands; S – *S. v. sanctipauli*, Amsterdam and Saint Paul Islands (R. K. Brooke, unpubl. data; Tree and Klages 2004); ? – subspecific status unknown, Bouvet Island (adapted from Murphy 1938; Higgins and Davies 1996). Stars (★) show the four wintering sites in South Africa where birds were captured (from west to east): Cape Columbine, Mauritz Bay, Dyer Island and Bird Island. The dashed lines show the average positions of the oceanic fronts in the Southern Ocean: PF, Polar Front; STC, Subtropical Convergence (from Orsi *et al*. 1995; Moore *et al*. 1999). Subspecies visiting the South African coast are underlined.

isotopically inert once fully grown, so they can be used to infer the areas visited by birds (carbon isotopic value) as well as their trophic level (nitrogen isotopic value) when moulting (Mizutani *et al*. 1990; Hobson and Clark 1992). Sedentary populations of Antarctic Terns moult close to their breeding sites (Sadleir *et al*. 1986), whereas migratory populations may start their post-breeding moult while still on their breeding grounds (Weimerskirch *et al*. 1985; Higgins and Davies 1996). Latitudinal and inshore–offshore gradients in oceanic isotopic values exist in the Southern Ocean (e.g. Cherel and Hobson 2007; Quillfeldt *et al*. 2010) and there can be correspondence between the $\delta^{13}C$ values of predator tissues and the water masses where they feed (e.g. Cherel and Hobson 2007; Jaeger *et al*. 2010). Antarctic Tern breeding sites in the Atlantic and Indian Oceans are located in isotopically distinct water masses (Fig. 1; Fig. S1 in Supplementary Material, available online only); we thus predicted that birds in juvenile plumage should exhibit distinct feather stable isotope values depending on their natal colony.

Here, we combine morphometric, genetic and isotopic analyses to assess variation among the subspecies of Antarctic Tern that winter in South Africa. Our hypothesis is that, if these are valid subspecies, then genetics will confirm identifications based on adult morphology, and juveniles will exhibit different stable isotope values depending on their natal regions. In addition, as breeders may start their moult when finished breeding but still on their breeding grounds, stable isotopes were also measured in adult and immature feathers to investigate their moulting areas. Furthermore, stable isotopes would discriminate the Kerguelen Island populations of *S. v. vittata* from the Prince Edward and Crozet Island populations of *S. v. vittata*, as these islands occupy different positions in the Southern Ocean isoscape (Fig. S1). Finally, given the disparity in population numbers among breeding colonies (Tree and Klages 2004), a better understanding of meta-population dynamics within the species, and how these relate to the conservation status of the different subspecies, is necessary.

Materials and methods

Data collection

Birds were captured at four localities along the South African coast (Fig. 1) during the austral winter (June–September), from 2008 to 2011, using either mist-nets or whoosh-nets (Gosler 2004). Captured birds were ringed (banded) with a South African Bird Ringing Unit (SAFRING, Cape Town) stainless steel ring and many were also marked with a site-specific coloured Darvic leg-flag (flags made by A. J. Tree from Darvic strips, A. C. Hughes Ltd, Hampton, Hill, Middlesex, UK). Birds were assigned to three age-classes based on plumage colouration: juveniles (birds in their very first plumage), immatures (up to 2 years old), and adults (>2 years old) (Tree and Klages 1998, 2001). Birds arrived in South Africa in juvenile (first pennaceous) plumage and remained there for two or three months while their body and flight feathers were gradually replaced. This process was only completed after the birds' departure from their wintering grounds (A. J. Tree, pers. obs.). By the time the birds returned to the South African coast, from May in their second year, they were in full immature plumage with no traces of juvenile plumage. Total head-length (from back of skull to tip of bill), and length of exposed culmen and tarsometatarsus (tarsal length) were measured using dial callipers (±0.1 mm); and length of tail (from emergence from body to tip of central pair and outer pair of rectrices) and flattened, straightened wing were measured using a 30-cm ruler (±1 mm) (e.g. de Beer *et al*. 2001). Mass was measured using a 300-g Pesola spring balance (±1 g). When large numbers of birds were caught simultaneously, only total head-length, tarsal length and mass were measured to reduce stress by accelerating processing of birds. A cumulative moult-score (Prevost 1983) was also calculated to take into account the complex patterns of wing-moult that can occur in Antarctic Terns, with second series wing-moult found in second-year birds but seldom in adults (Hockey *et al*. 2005; A. J. Tree, unpubl. data). Additional qualitative information, such as colour of bill (black, blood red, red, coral red) or the shading of grey (light, medium or dark) on the underparts was recorded for some individuals. Birds were identified to subspecies whenever possible using morphology, colouration and moult-sequence (Table 1).

Blood was drawn from the brachial vein of 98 individuals, and immediately stored in a solution of 0.05 M sodium EDTA, 0.1 M Tris and 2% SDS for genetic analyses (Buffer, Bioline, Sydney, Australia). Scapular feathers were collected for stable isotopic analyses from 107 individuals and stored dry before analysis. Samples for isotopic investigation primarily targeted juveniles because they are known to grow their feathers in the natal colony, but samples from immatures and adults were also collected.

Genetic analyses

DNA was extracted with the DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands) following the protocol for nucleated blood. Of the 98 samples collected, 79 had the proper quality for further analyses.

To determine whether there is a genetic basis for the three subspecies, mitochondrial (mtDNA) sequence data, nuclear (nuDNA) sequence data and nuDNA microsatellites were used. The loci sequenced were the mtDNA NADH dehydrogenase subunit 2 (*ND2*) and the nuDNA signal recognition particle 54-kDa subunit (*SRP54*), which contains a single intron (Jarman *et al*. 2002; Table 2). Primers for 15 microsatellite loci that have previously cross-amplified in various species of Charadriiformes (Küpper *et al*. 2008) were tested on seven individuals identified as either *S. v. vittata* (3 individuals), *S. v. tristanensis* (2 individuals) or *S. v. sanctipauli* (2 individuals). Of all these loci, 10 amplified consistently, but only four (*Calex-01*, *K16*, *RBG13* and *RBG27*) showed variation in the tested samples and were used to genotype the remaining samples (Table S1). All four microsatellites had dinucleotide repeats.

Technical details about PCR reactions and sequencing are given in the Supplementary Material (available online only). Complete *ND2*, *SRP54* and microsatellite datasets and examples of *Calex-01* sequences are given in Tables S3–S6. A single example of each *ND2* haplotype and *SRP54* allele was submitted to GenBank (accession numbers KP241019–KP241035).

Subspecies-specific exact tests for Hardy–Weinberg equilibrium (Guo and Thompson 1992) were performed on the microsatellite data, with a forecast Markov chain length of 1000 and 1000 dememorisation steps. Bonferroni correction for multiple tests was applied. Tests for pairwise linkage disequilibrium that

Table 1. Measurements, colouration and moult-stages of the three subspecies of Antarctic Terns wintering on the coast of South Africa
Under Moult, 'Late' and 'Early' refer to later and earlier in the season

Subspecies	Breeding area	Measurements (all lengths, in mm)						Colouration			Moult
		Wing	Culmen	Total head-length	Tarsus	Tail	*N*	Culmen	Legs	Plumage	
Sterna vittata vittata J. F. Gmelin, 1789	Southern Indian Ocean: Heard, Kerguelen, Crozet, Prince Edward and Marion Islands	247–286	31.0–39.6	70.9–81.9	16.0–19.7	117–158	22	Black with partial blood-red tinge	Mixture of blackish and dull reddish	Underparts plumage dark	Late
Sterna vittata tristanensis R. C. Murphy, 1938	South Atlantic Ocean: Tristan da Cunha and Gough Island	246–296	33.0–40.0	75.8–83.8	17.6–22.0	144–186	46	Coral-red	Bright coral-red	Pale	Early
Sterna vittata sanctipauli Gould, 1865	Southern Indian Ocean: Amsterdam and St Paul Islands	242–277	31.5–40.0	70.4–81.1	17.0–19.4	131–188	51	Coral-red with slightly blackish tip	Almost coral-red	Pale	Late

Table 2. Details of loci, PCR primers and haplotype diversity of DNA sequences of Antarctic Terns
F, forward primer; R, reverse primer. Fluorescent dyes used for the microsatellites were: *Calex-01*, NED; *K16*, PET; *RBG13*, FAM; *RBG27*, VIC (Sigma-Aldrich, St Louis, USA). M13, a universal M13 sequence (TGTAAAACGACGGCCAGT), was attached to the 5′ end of the forward primers of all microsatellite loci (Schuelke 2000); PIG: a PIG-tail (GTTTCTT) was attached to the 5′ end of the *Calex-01* reverse primer (Brownstein *et al*. 1996)

Genome	Marker type	Locus name	Length	Primer sequences (5′–3′)	Number of Haplotypes/Alleles	Haplotype diversity	Reference
mtDNA	DNA sequence	*ND2*	466	F: AGGTCAGCTAATAAAGCTATC R: ATTTTTCGGACTTGTGTTTGG	9	0.467	This study
nuDNA	DNA sequence	*SRP54*	252	F: ATGGGTGAYATYGAAGGACTGATWGATAAAGTCAA R: TTCATGATGTTYTGGAATTGYTCATACATGTC	8	0.611	Jarman *et al.* 2002
nuDNA	Microsatellite	*Calex-01*	276–296	F: M13-CTTCTCCATTGTTGTCACCTCCAGT R: PIG-CTTGACTTGGCCTGAGGTTTAGGTT	9		Küpper *et al.* 2007
nuDNA	Microsatellite	*K16*	141–159	F: M13-TGCAATTTGTACAACCAGATTT R: GGGTTCCTGTTTGCAATGAA	4		Tirard *et al.* 2002
nuDNA	Microsatellite	*RBG13*	233–247	F: M13-CAGGAGGGAAAGCCCATATG R: GACAGGCAGGAAAGAATCTC	4		Given *et al.* 2002
nuDNA	Microsatellite	*RBG27*	159–167	F: M13-GGAATTTTCGTTGGCAGGAT R: GAAATCACAGTGAAAACGCC	4		Given *et al.* 2002

employ permutation tests of the expectation-maximisation (EM) algorithm (Slatkin and Excoffier 1996) were performed using 1000 permutations and two initial conditions for the EM algorithm. All calculations were performed using Arlequin version 3.5 (Excoffier and Lischer 2010).

The distinctness of the different subspecies was assessed using maximum likelihood phylogenetic trees (DNA sequence data), a Bayesian clustering method (microsatellite data), and pairwise estimates of genetic structure. For the first two types of analyses, individuals were assigned to seven groups based on presumed subspecies morphology: (1) *S. v. vittata* (abbreviated V); (2) *S. v. sanctipauli* (S); (3) *S. v. tristanensis* (T); (4) *S. v. sanctipauli* or *S. v. vittata* (SV); (5) *S. v. sanctipauli* or *S. v. tristanensis* (ST); (6) *S. v. tristanensis* or *S. v. vittata* (TV); and (7) subspecies not identified (N). Grouping of individuals assigned to a particular subspecies into the same cluster would support the subspecies taxonomy.

Phylogenies were constructed using the maximum-likelihood method in MEGA, with nodal support based on 1000 bootstrap replications (Felsenstein 1985). The Hasegawa-Kishino-Yano (Hasegawa *et al*. 1985) and Jukes–Cantor (Jukes and Cantor 1969) models were specified for *ND2* and *SRP54*, respectively, on the basis of the lowest Bayesian Information Criterion score by the MEGA nucleotide model selection tool. Sequences of the South American Tern (*Sterna hirundinacea*) and the Arctic Tern (*S. paradisaea*), two species with *ND2* sequences most similar to those of the Antarctic Tern (Bridge *et al*. 2005), were used to root the *ND2* tree, and a published sequence of the Antarctic Tern collected in Antarctica (Bridge *et al*. 2005; J. V. Remsen Jr, pers. comm.) was added to test distinctness from the South African samples (Palmer 2006). The *SRP54* tree was rooted arbitrarily using an ingroup individual as no suitable outgroup sequences were available. For the microsatellites, STRUCTURE version 2.3.4 (Pritchard *et al*. 2000) was used to determine the most likely number of distinct genetic clusters (K) to which individuals of Antarctic Tern could be assigned. An admixture model with subspecies assignment as location priors was used, and allele frequencies were set to be correlated among populations, with default settings for all advanced parameters. For each value of K (1–7, representing the subspecies groupings described), 20 replicates were run with a burn-in of 10^5 Markov Chain Monte Carlo generations, followed by 10^6 recorded generations. The program STRUCTURE HARVESTER (Earl and von Holdt 2012) was then used to determine which value of K had the highest probability, and to identify the greatest rate of change in the probability of the data between successive K values (Evanno *et al*. 2005). For the best-supported value of K, a bar-plot was created using CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) and DISTRUCT version 1.1 (Rosenberg 2004).

To test for significant genetic structure between individuals easily assigned to one of the three subspecies, we estimated pairwise Φ_{ST} (Excoffier *et al*. 2005) between subspecies in Arlequin version 3.5 (Excoffier and Lischer 2010) for the two DNA sequence datasets. For the microsatellite data, we estimated pairwise G''_{ST} (Meirmans and Hedrick 2011) in GenAlEx version 6.5.501 (Peakall and Smouse 2012). Φ_{ST} and G''_{ST} are summary statistics derived from FST (Wright 1965). Φ_{ST} is particularly suitable for identifying genetic structure on the basis of DNA sequence data because it can incorporate information on evolutionary relationships among haplotypes, while G''_{ST} is more suitable for microsatellites because it corrects for the high mutation rate of these loci (Meirmans and Hedrick 2011).

Stable isotope analyses

Feathers were cleaned in a 2 : 1 chloroform : methanol solution (Merck (Ptd) Ltd, Modderfontein, South Africa) placed in an ultrasonic bath for 2 min, rinsed in successive baths of methanol and deionised water, and then dried (at 50°C for 24 h). Each scapular feather was then cut into small pieces using stainless steel scissors. Relative isotope abundances of carbon ($^{13}C/^{12}C$, $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$) were determined for 0.5–0.6 mg subsamples of materials with either a Flash 2000 organic elemental analyser coupled to a Delta V Plus isotope ratio mass spectrometer (IRMS) via a Conflo IV gas control unit (Thermo Scientific, Bremen, Germany; Stable Light Isotope Unit, University of Cape Town, Cape Town, South Africa) or a Europa Scientific 20–20 IRMS linked to an ANCA SL Prep Unit (PDZ Europa Ltd, Crewe, UK; IsoEnvironmental cc, Department of Botany, Rhodes University,Grahamstown, South Africa). Results are presented in the usual δ notation relative to Vienna Peedee belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Replicate measurements of internal laboratory standards (Merck gel: $\delta^{13}C = -20.05‰$, $\delta^{15}N = +7.50‰$; seal $\delta^{13}C = -11.97‰$, $\delta^{15}N = +15.84‰$; valine $\delta^{13}C = -26.80‰$, $\delta^{15}N = +12.14‰$; casein $\delta^{13}C = -26.98‰$, $\delta^{15}N = +5.94‰$) indicated measurement errors <0.09‰ and <0.10‰ for stable-carbon and nitrogen isotope measurements, respectively.

Differences in stable isotopes between age-groups of Antarctic Terns were tested by non-parametric multivariate analysis of variance (PERMANOVA) using the Euclidean distance followed by a pairwise Hotelling's tests with Bonferroni correction (Hammer *et al*. 2001). Patterns in the isotopic values of Terns were investigated using a hierarchical clustering method (weighted pair-group average) on dissimilarities (Euclidean distance). One-way analysis of similarities (ANOSIM) was then used to test for significant differences between the identified groups, followed by pairwise ANOSIMs between all pairs of groups as a *post-hoc* test (Hammer *et al*. 2001).

Finally, physical measurements and moult-scores were compared between Terns from clades identified by genetics or from clusters identified by stable isotopes using either parametric or non-parametric tests after verification of normality (Shapiro–Wilk test) and homogeneity of variances (Fishers's test). All statistical tests were conducted using PAST version 1.85 (Hammer *et al.* 2001). The α level was set at 0.05 unless otherwise stated.

Results

A maximum of 145 birds sampled over 4 years was used in the combinations of morphological, genetic or stable isotope analyses (Table 3). Bird Island (86.1% of captures) and Dyer Island (9.3%) were the main capture localities, with smaller numbers from Cape Columbine (3.3%) and Mauritz Bay (1.3%). Juveniles were 35.2% of the birds caught, immatures 16.5% and adults 48.3% (Table 3).

Table 3. Numbers of Antarctic Terns examined in the present study

Numbers are number of individuals identified in the hand using morphology, colouration and moult stage; numbers in parentheses are: N_G, number of samples for genetic analyses, and N_S, numbers of samples for stable isotope analyses

Subspecies	Code	Adults	Immatures	Juveniles
S. v. vittata	V	19 (N_G = 12, N_S = 14)	1 (N_G = 1, N_S = 1)	1 (N_G = 0, N_S = 1)
S. v. tristanensis	T	13 (N_G = 12, N_S = 7)	2 (N_G = 0, N_S = 2)	4 (N_G = 2, N_S = 4)
S. v. sanctipauli	S	15 (N_G = 14, N_S = 2)	1 (N_G = 1, N_S = 1)	–
S. v. sanctipauli/*vittata*	SV	5 (N_G = 5, N_S = 1)	–	–
S. v. tristanensis/*vittata*	TV	1 (N_G = 1, N_S = 1)	–	–
S. v. sanctipauli/*tristanensis*	ST	16 (N_G = 14, N_S = 10)	1 (N_G = 1, N_S = 1)	–
Not identified	N	1 (N_G = 1, N_S = 1)	19 (N_G = 7, N_S = 16)	46 (N_G = 8, N_S = 45)
Total		70 (N_G = 59, N_S = 36)	24 (N_G = 10, N_S = 21)	51 (N_G = 10, N_S = 50)

Genetic analyses

Genetic data were generated for 79 Terns (Table 3). Aligned *ND2* sequences were 466 nucleotides in length and contained 11 variable sites, and a consensus segment of *SRP54* sequences that could be reliably scored was 252 nucleotides in length and contained seven variable sites. A single nucleotide insertion in the flanking sequence of *Calex-01* was found in 13 individuals (see examples in Table S5), resulting in intermediate allele sizes in these cases. Whenever this was identified, a single nucleotide was subtracted from the individual's *Calex-01* allele size. No evidence was found for scoring errors or null alleles.

Significant departures from Hardy–Weinberg equilibrium were found for locus *K16* ($P = 0.005$) in *S. v. tristanensis* and for loci *Calex-01* ($P = 0.021$), *K16* ($P = 0.010$) and *RBG13* ($P = 0.000$) in the group comprising Antarctic Terns that could not be assigned to any subspecies (Table S2). Following Bonferroni correction (corrected *P*-value 0.0023 for 22 tests, with loci being monomorphic in two cases), only the test for *RBG13* remained significant. Significant linkage disequilibrium was only found between loci *Calex-01* and *RBG27* ($P = 0.049$) in the group comprising individuals that were morphologically intermediate between *S. v. sanctipauli* and *S. v. tristanensis*. Given the inconsistencies of these results between subspecies, we considered the significant *P*-values to be artefacts of small sample sizes.

The maximum-likelihood tree constructed from *ND2* sequences recovered two major clades. One of these (Clade 2) comprised only 13 individuals, of which 11 were morphologically identified as *S. v. tristanensis* and two had morphological features of both *S. v. tristanensis* and *S. v. sanctipauli* (Fig. 2). Clade 1 comprised all remaining individuals, including the individual from Antarctica. The *SRP54* tree recovered three major clades, one mostly comprising individuals identified as *S. v. tristanensis* (Clade 2) whereas the others contained all subspecies (Clades 1a and 1b; Fig. 3). The composition of Clade 2 differed somewhat in the *SRP54* tree: first, individual T-4H38058 was not represented in this clade and, second, one of the alleles of T-4H38292, which was recovered in Clade 1 in the *ND2* tree, was recovered in Clade 2 in the *SRP54* tree. The highest likelihood in the STRUCTURE and STRUCTURE HARVESTER analyses was found for $K = 2$, and this was also the K with the highest value of Evanno's ΔK (Fig. S2). Most individuals in the seven morphologically based groups were assigned to the cluster represented in white in Fig. 4. There was strong support for assignment to the second cluster only for individuals that were identified as *S. v. tristanensis* and one individual morphologically intermediate between this subspecies and *S. v. sanctipauli* (in black in Fig. 4).

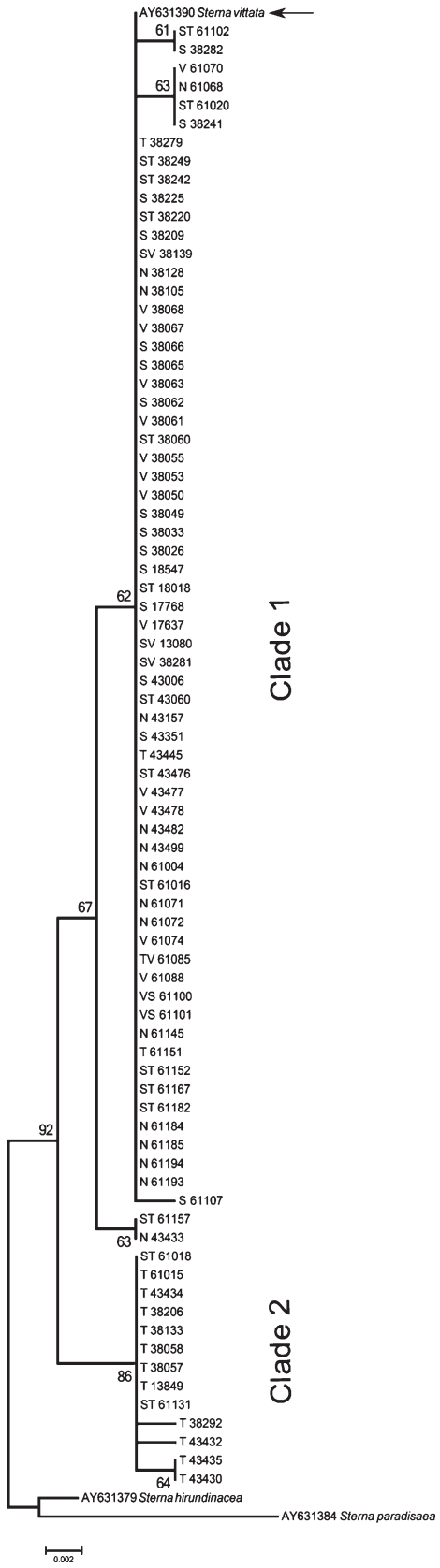
For all three genetic datasets of individuals that could be readily assigned to one of the three subspecies, significant genetic structure was found between *S. v. tristanensis* and *S. v. vittata* (*ND2*: $\Phi_{ST} = 0.41$; *SRP54*: $\Phi_{ST} = 0.29$; microsatellites: $G''_{ST} = 0.21$; $P < 0.01$ in all cases) and between *S. v. tristanensis* and *S. v. sanctipauli* (*ND2*: $\Phi_{ST} = 0.36$; *SRP54*: $\Phi_{ST} = 0.22$; microsatellites: $G''_{ST} = 0.22$; $P < 0.01$ in all cases), but not between *S. v. vittata* and *S. v. sanctipauli* (*ND2*: $\Phi_{ST} = 0.00$, $P = 0.56$; *SRP54*: $\Phi_{ST} = 0.01$, $P = 0.24$; microsatellites: $G''_{ST} = 0.00$, $P = 0.77$).

Interestingly, adults from Clade 2 in the *ND2* tree had significantly longer total head-length, culmen and tarsus than adults from Clade 1: total head-length, 80.9 ± 1.5 mm versus 75.7 ± 2.3 mm; culmen, 38.4 ± 0.8 mm versus 34.4 ± 1.9 mm; tarsus, 20.1 ± 0.8 versus 18.5 ± 0.8 mm (α after Bonferroni correction 0.017, *t*-test, $P < 0.001$ for all comparisons). Compared with *S. v. tristanensis* recovered in Clade 1, adults from Clade 2 again had significantly longer total head-lengths but not tarsal lengths ($P < 0.025$ significant after Bonferroni correction; total head-length: 80.9 ± 1.5 mm versus 77.5 ± 2.5 mm, $t = 3.11$, $P < 0.010$; tarsus: 20.1 ± 0.8 mm versus 19.4 ± 0.8 mm, $t = 1.48$, $P = 0.166$). Moult-scores in adults varied greatly among birds in both clades, ranging from 23 to 53 in Clade 2 and from 1 to 54 in Clade 1. However, birds from Clade 2 had significantly more newer feathers on average than birds from Clade 1 (average moult-scores: Clade 2, 39.5 ± 10.0 mm; Clade 1, 25.8 ± 10.5 mm; $t = 2.97$, $P < 0.005$).

Stable isotope analyses

Feather isotopic values of all Antarctic Terns encompassed huge ranges from –24.6‰ to –9.6‰ for $\delta^{13}C$ and from +7.8‰ to +16.6‰ for $\delta^{15}N$. Birds of different ages were segregated, with juveniles showing the highest $\delta^{13}C$ and $\delta^{15}N$ values and immatures the lowest $\delta^{13}C$ and $\delta^{15}N$ (PERMANOVA: $F_{2,104} = 53.85$, $P < 0.001$, pairwise comparisons all $P < 0.003$, immatures < adults < juveniles).

Carbon and nitrogen stable isotope values of juveniles, whose feathers were known to have grown in their colony of birth, ranged from –19.2 to –9.6‰ and from +10.0 to +16.6‰, respectively. The 50 juveniles were classified into three clusters (ANOSIM: $r = 0.967$, $P < 0.001$; Fig. 5*a*), and individuals from the three clusters were significantly different from each other



(pairwise ANOSIM, all $P < 0.003$). Three of the juveniles exhibiting morphological characteristics of *S. v. tristanensis* were in cluster 2, and the fourth juvenile identified as *S. v. tristanensis* was in cluster 3. The only juvenile *S. v. vittata* was in cluster 1.

When considering all 107 individuals, they were classified into six groups based on their carbon and nitrogen isotopic values (ANOSIM: $r = 0.971$, $P < 0.001$; Fig. 5*b*), and all groups were significantly different from each other (pairwise ANOSIM, all $P < 0.006$). However, no correspondence was found between the subspecies identified in the hand and the classification into a particular cluster (Fig. 5*b*). Cluster 1 ($\delta^{13}C < -22.2‰$) comprised only adults and immatures identified as *S. v. tristanensis*, *S. v. vittata*, or *S. v. tristanensis*/*sanctipauli*. Apart from one adult, cluster 4 was entirely juveniles (only one of which was identified as *S. v. vittata*), with $\delta^{13}C$ values ranging from –14.4‰ to –9.6‰ and $\delta^{15}N$ values from +13.4‰ to +16.4‰. Terns in clusters 2 and 3 exhibited intermediate values for both isotopes compared with those of clusters 1 and 4, and again comprised birds from all the identified subspecies. Cluster 5 mainly comprised adults (but included two immatures identified as *S. v. tristanensis*) with high $\delta^{15}N$ (> +15.2‰). Finally, cluster 6 consisted of three juveniles exhibiting intermediate values of $\delta^{13}C$ and high values of $\delta^{15}N$.

No statistical differences were found among adults of the different clusters on the basis of morphometrics (culmen: $H_{3,11} = 2.571$, $P = 0.277$; total head-length: ANOVA $F_{4,30} = 0.714$, $P = 0.552$; tarsus: ANOVA $F_{4,29} = 0.216$, $P = 0.885$) or moult-stage (ANOVA $F_{3,33} = 1.323$, $P = 0.285$).

Blood and feather samples were collected from 41 birds to conduct genetic and isotopic analyses on the same individuals. Seven of these samples were placed in Clade 2 by genetic analyses (*ND2* and *SRP54* trees), but fell into different clusters in the isotopic analysis (Fig. 6). Their $\delta^{13}C$ values ranged from –24.6 to –14.2‰ and $\delta^{15}N$ from +9.6 to +16.3‰ with two individuals being placed in cluster 5, four in cluster 3, and one in cluster 1. The remaining 34 specimens (all part of Clade 1 in *ND2* and *SRP54* trees) were classified as: 13 individuals in cluster 1, seven in each of clusters 3 and 4, five in cluster 2, and two in cluster 5.

Discussion

Across all levels of taxonomy, the development of robust genetic techniques over recent decades has challenged understandings of biology based solely on morphology. This can have critical implications for issues of conservation, as correctly identifying individual birds as belonging to reproductively isolated subspecies is clearly central to effective conservation efforts. Here we

Fig. 2. Maximum-likelihood tree constructed from *ND2* sequences of Antarctic Terns. Numbers without prefixes at some of the nodes are clade-support values from 1000 bootstrap replications (≥50%). The arrow indicates an individual Antarctic Tern from Antarctica (Bridge *et al.* 2005). Letters represent identified subspecies or morphologically intermediate forms, as per codes in Table 3, and following numbers are part of the ring-numbers that uniquely identify each individual Tern (ring numbers: 4H followed by five digits, e.g. 4H61085). GenBank accession numbers are shown for previously published sequences (AY631390, AY631379, AY631384). The scale bar represents the number of nucleotide substitutions per site.

Fig. 3. Maximum-likelihood tree constructed from *SPR54* sequences of Antarctic Terns. Numbers without prefixes at some nodes are clade-support values from 1000 bootstrap replications. Letters and subsequent numbers represent subspecies or morphologically intermediate forms and part of ring-numbers, as Fig. 2. Suffix numbers 1 or 2 after these codes represent allele phases. The tree was rooted *post hoc*, with an ingroup sample to maximise spaces between specimen codes.

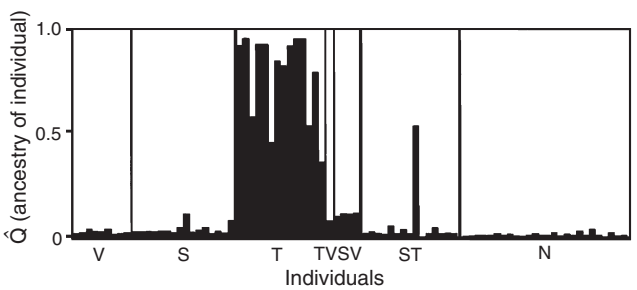


Fig. 4. STRUCTURE bar-plot depicting the assignment of Antarctic Terns to two clusters on the basis of microsatellite data. Each individual is represented by a vertical bar, with the relative probability that it originates from a particular cluster being indicated in white (cluster 1) or black (cluster 2). Individuals are also shown within one of the seven morphologically based groups as per codes in Table 3.

used the combination of morphology and DNA to test the validity of subspecies of Antarctic Terns, and stable isotopes to identify geographical areas where feathers had grown.

Genetic variability among putative subspecies

Three types of genetic markers were used to examine the genetic basis for subspecies of the Antarctic Tern. These data provided little support for the recognised subspecies, but rather recovered clades that could not be clearly distinguished on the basis of subspecies-specific morphological characters. On the basis of the *ND2* data, which allow a comparison of genetic distances between sequences generated in the present study and previously published sequences from other species (Bridge *et al*. 2005), there were two clades that were quite distinct. The most common haplotype of each clade differed from that of the other clade by

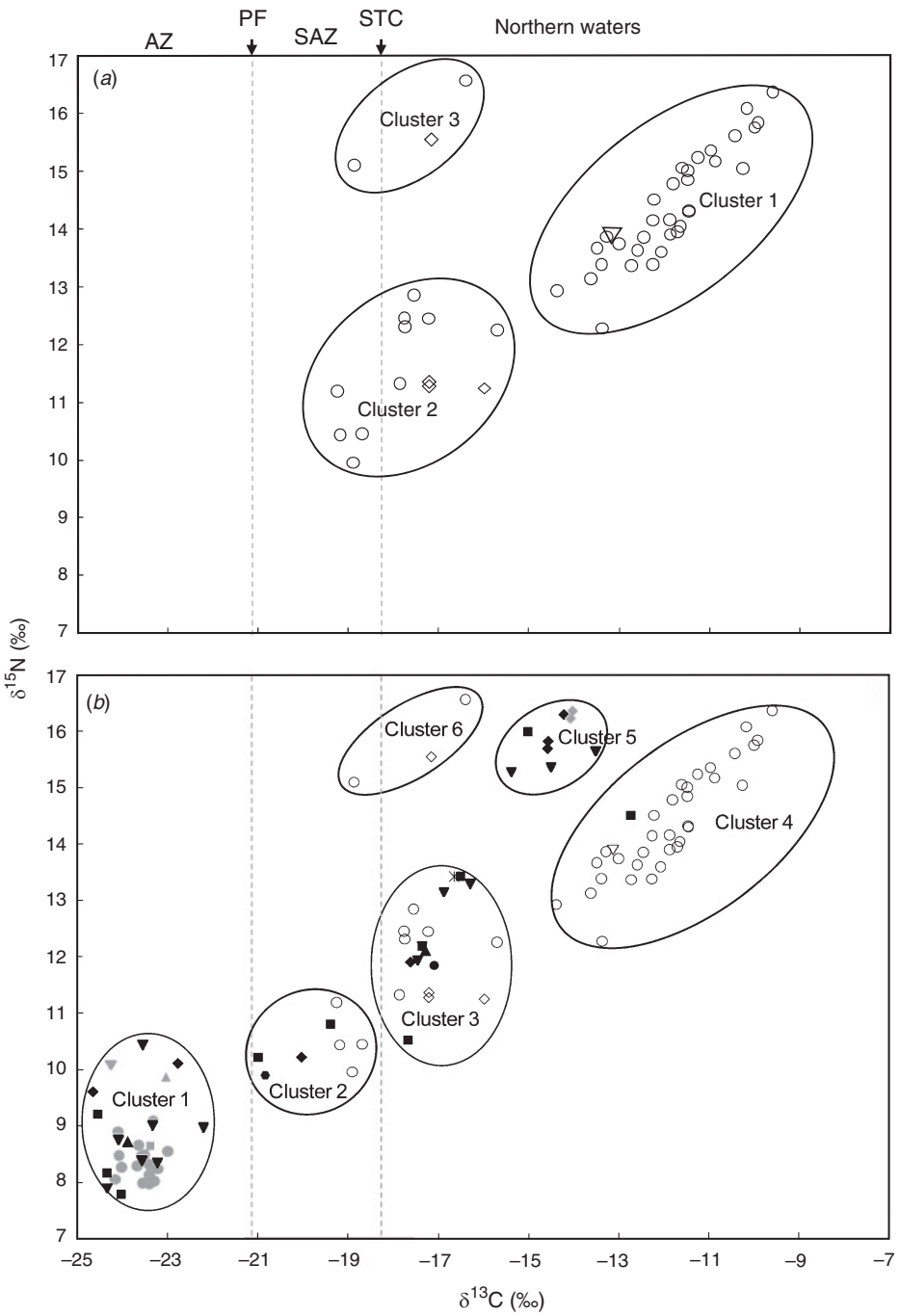


Fig. 5. Values of feather $\delta^{13}C$ and $\delta^{15}N$ for: (*a*) juvenile Antarctic Terns; and (*b*) all ages of Antarctic Terns combined. Subspecies identified based on morphological features are: *S. v. vittata* (inverted triangles, ▼); *S. v. tristanensis* (diamonds, ◆) *S. v. sanctipauli* (triangles, ▲); *S. v. sanctipauli*/*tristanensis* (squares, ■); *S. v. vittata*/*sanctipauli* (asterisks, *); *S. v. vittata*/*tristanensis* (hexagons, ⬣); and subspecies not identified (circles, ●). Solid black symbols, adult birds; grey symbols, immature birds; open symbols, juvenile birds. Water masses (AZ, Antarctic Zone; SAZ, Subantarctic Zone; northern waters, waters north of the Subtropical Convergence) and fronts (PF, polar front; STC, Subtropical Convergence) from Jaeger *et al*. (2010).

four nucleotide substitutions, whereas a previously published haplotype of the closely related South American Tern differed from both Antarctic Tern haplotypes by five substitutions. Interestingly, the corresponding portion of the *ND2* gene differed by only two substitutions between two other distinct species of tern, Elegant Tern (*Thalasseus elegans*) and Cabot's Tern

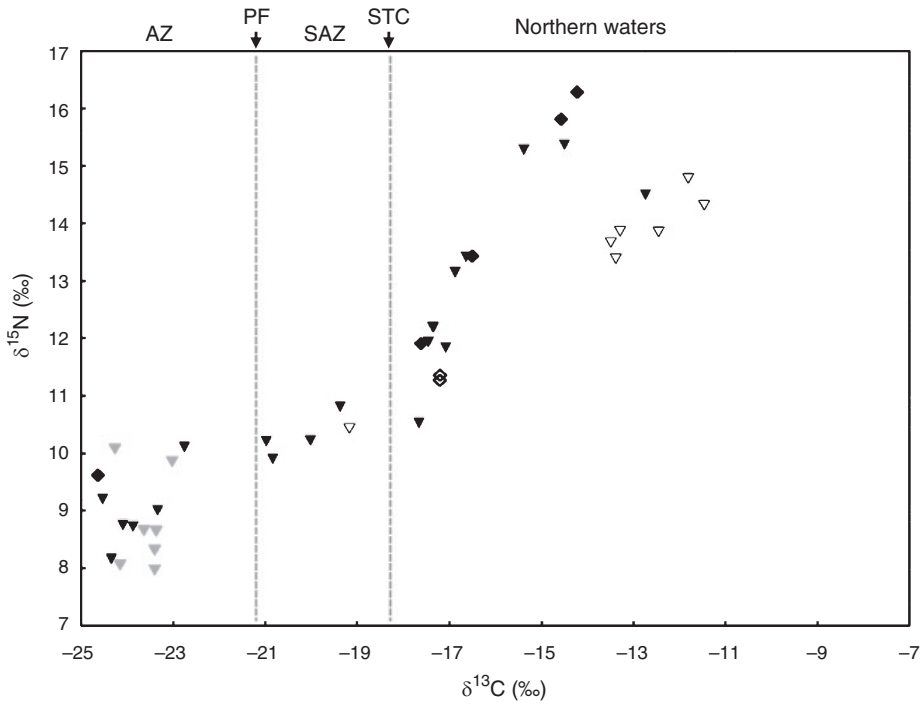


Fig. 6. Values of feather $\delta^{13}C$ and $\delta^{15}N$ for Antarctic Terns whose genetic profiles have been determined. Diamonds (♦) are individuals from genetic Clade 2, and inverted triangles (▽) are individuals from Clade 1 from *ND2* tree. Shading of symbols and water masses and fronts are as in Fig. 5.

(*Thalasseus eurygnathus*) (Bridge *et al*. 2005). This, and the finding that individuals of Clade 2 are morphologically distinct from those of Clade 1 on the basis of total head-length, and culmen and tarsal lengths, suggests that they clearly belong to different subspecies (see below). As 11 of 13 birds from Clade 2 were identified as *S. v. tristanensis* in the field, this suggests that Clade 2 represents the currently named subspecies *S. v. tristanensis.* This result was confirmed by (1) the *SRP54* data, in which Clade 2 also mostly comprised individuals identified as *S. v. tristanensis*; (2) tests for genetic structure, which were significant only between *S. v. tristanensis* and the other subspecies; and (3) STRUCTURE analyses, which identified two major clusters of which one almost exclusively comprised individuals identified as *S. v. tristanensis*.

A bird from Antarctica (Bridge *et al*. 2005), most likely a representative of *S. v. gaini* (a sedentary subspecies that is known to breed and winter only on the Antarctic Peninsula and South Shetland Islands and thus not to winter in South Africa), had the same *ND2* haplotype as most of the individuals sampled on the South African coast (Clade 1). While this suggests that Clade 1 may be widespread in the southern hemisphere and may not show any regional genetic differentiation, additional samples of known breeding origin and more loci are required to confirm this. Two main hypotheses could explain the lack of a correlation between genetic and morphological data. First, even though the different forms may currently be isolated during the breeding season, their divergence is so recent that this is not yet reflected in the fairly small number of genetic markers used in the present study (i.e. incomplete lineage sorting). Alternatively, the populations were separated in the past but the genetic signal was lost owing to secondary contact (Gay *et al*. 2007). This hypothesis is supported by the fact that some of the individuals identified as *S. v. tristanensis* could be assigned to the other cluster with approximately equal probability in the STRUCTURE plot (Fig. 4), and by the fact that the two *SRP54* alleles of individual S-4H38209 were in different clusters (Fig. 3). Friesen *et al*. (2007) proposed that isolation during the non-breeding season is a keystone in genetic differentiation among seabirds. Therefore, occasional gene flow could also be promoted by the fact that the subspecies share their wintering grounds; this would prevent the genetic divergence of the different subspecies and would complicate their morphological identification. In this case, the variation in morphology and colouration observed between subspecies may merely be the result of phenotypic plasticity rather than lack of gene flow.

Stable isotope values of feathers of juveniles and link to the natal colony

Juveniles were defined as first-year birds and were therefore just coming from their natal site where their feathers had grown. When considering their carbon and nitrogen isotopic values, they were classified into three clusters (Fig. 5*a*), indicating they likely originated from a minimum of three breeding regions rather than reflecting a metabolic effect of chick nutritional conditions. Indeed, Sears *et al*. (2009) did not find any effect of chick nutritional condition on the $\delta^{13}C$ values of feathers, whereas the decrease of 0.6‰ they observed in $\delta^{15}N$ between control and restricted chicks is very small compared with the wide range of $\delta^{15}N$ measured in our study among juvenile Antarctic Terns (+10.0‰ to +16.6‰). Juveniles from cluster 1 exhibited surpris-

ingly high $\delta^{13}C$ values (range: –14.4 to –9.6‰). Six of these individuals were identified as falling into genetic Clade 1 (*ND2* and *SRP54* trees), and one specimen exhibited morphological characteristics of *S. v. vittata*. The proximity of these enriched values to those of Kelp Gull (*Larus dominicanus*) chicks raised in the Kerguelen Islands (–12.8‰; Blévin *et al*. 2013) suggests that juveniles from cluster 1 may have originated from these islands. This would furthermore suggest that Clade 1 genetic samples would then represent *S. v. vittata*. Nevertheless, such high $\delta^{13}C$ values (–9.6‰) have not previously been observed in any bird species from the Kerguelen Islands (Y. Cherel, pers. comm.). Although there have been observations of Antarctic Terns feeding around these islands (Sagar 1991), no detailed dietary study has yet been published. The surprisingly high carbon values from birds assumed to be from the Kerguelen Islands could indicate that chicks were fed by adults that foraged mainly in Morbihan Bay, an enclosed embayment that does not have flushing to the same extent as exposed coastlines, and so would be naturally enriched (Cherel and Hobson 2007; P. Sagar *in litt*.). A Kerguelen origin is also supported by ringing recoveries of three Antarctic Terns ringed on the South African coast and recovered or resighted in the Kerguelen Islands (A. J. Tree, unpubl. data). Comparing the $\delta^{13}C$ values of juveniles from clusters 2 and 3 with the well-known $\delta^{13}C$ latitudinal gradients (Cherel and Hobson 2007; Jaeger *et al*. 2010; Fig. S1) suggests that these birds were raised near the Subtropical Convergence, probably in distinct water masses. Indeed, the difference in the $\delta^{15}N$ values of the two clusters could be explained by either the birds from cluster 3 having been fed on prey one trophic level higher than the juveniles from cluster 2, or more likely that juveniles from the two clusters were fed on prey from water masses with distinctly different $\delta^{15}N$ baselines. Although Amsterdam and St Paul Islands (Indian Ocean) and Gough Island (Atlantic Ocean) lie in different oceans, it would be difficult to discriminate between Antarctic Terns from these two island groups using stable isotopes, as these markers exhibit well-known latitudinal gradients but no clear longitudinal gradients have been found (Jaeger 2009). In accord with the stable isotope values, genetic analyses of three juveniles from cluster 2 classified them in Clade 2, which is otherwise largely *S. v. tristanensis*.

Stable isotope values of feathers of immatures and adults: can these stages be reliably assigned to their colony of origin?

More than 90% of the immature Antarctic Terns (1–2 years old), exhibited very low $\delta^{13}C$ values (< –23‰) and were classified in cluster 1 (Fig. 5*b*), with no correspondence between their isotopic values and their morphological identification to subspecies. Several species of petrel and fulmar wintering in high Antarctic waters exhibit similar very low $\delta^{13}C$ and $\delta^{15}N$ values (e.g. Hodum and Hobson 2000). This suggests that these immatures visited high latitude waters during their previous moult. The comparison of the $\delta^{15}N$ values of Antarctic Terns in cluster 1 with those of potential prey (e.g. Hodum and Hobson 2000; Quillfeldt *et al*. 2005) suggests that birds with values of ~+8.5‰ probably ate crustaceans (e.g. krill) whereas those with $\delta^{15}N$ values of ~+10‰ may have eaten a mix of crustaceans and fish when close to the Antarctic continent. This is in accordance with Antarctic Tern dietary studies conducted in the south where they were found to be opportunistic foragers with a preference for fish and Antarctic Krill (*Euphausia superba*; e.g. Ainley *et al*. 1992; Casaux *et al*. 2008). Two immatures caught at Dyer Island (South Africa) and identified as *S. v. tristanensis* were classified in isotopic cluster 5 with enriched $\delta^{13}C$ and $\delta^{15}N$ values indicating that these feathers had grown north of the Subtropical Convergence (Fig. S1). Altogether, these data suggest that immature Antarctic Terns are an unsuitable age class to sample if the stable isotope technique is intended to delineate their breeding origin. Nevertheless, stable isotopes have provided previously unknown information about where immatures forage before their first breeding attempts.

Adult birds (>2 years old) exhibited a wide range of values for carbon and nitrogen suggesting that moult took place in a variety of water masses (Fig. 5*b*). Most migratory Antarctic Terns start moulting on their breeding grounds immediately after completion of breeding and complete their moult away from their main wintering grounds (Weimerskirch *et al*. 1985; Higgins and Davies 1996), although some individuals, probably late breeders, wait until they reach their wintering grounds (A. J. Tree, pers. obs.). No correspondence could be identified between subspecies identified in the hand and their respective stable isotope values. Likewise, genetic and stable isotope analyses of the same birds placed specimens from genetic Clade 1 (*ND2* and *SRP54* trees) in five isotopic clusters. Adults from cluster 1 exhibited very low carbon and nitrogen values ($\delta^{13}C < –22.2‰$; $\delta^{15}N < +10.5‰$), which suggests that they moulted in high Antarctic waters. Two birds from cluster 1 exhibited morphological features of *S. v. tristanensis* and one of them was genetically identified from Clade 2 (unfortunately no sample was available for genetic analysis for the second bird). This suggests that adults originating from the northern islands of Tristan da Cunha and Gough Island may visit Antarctic waters.

When birds are caught in their wintering areas, nothing is known about their past breeding history and it is possible that failed breeders have different migratory behaviour from successful birds, heading to productive Antarctic waters or frontal systems early in the season before wintering on the coast of South Africa. Another possibility is that adults take a ‘gap’ year from breeding, as has been suggested for the Arctic Tern (Hatch 2002) and head to Antarctic waters. As for the juvenile birds, adults classified in isotopic clusters 2, 3 and 4 probably moulted at islands in the Subantarctic Zone,that is those islands close to the Subtropical Convergence and at the Kerguelen Islands. The enriched $\delta^{13}C$ and $\delta^{15}N$ values of adults from cluster 5 suggest that they moulted in very productive waters. Six of nine of these exhibited the morphological features characteristic of *S. v. tristanensis* and the two of those genetically analysed belong to Clade 2. This suggests that these adult birds might originate from the Gough Archipelago. However, the presence in this isotopic cluster of three adults with morphological characteristics of *S. v. vittata* (confirmed genetically for two of them that were recovered in Clade 1) remains unclear. Another hypothesis is that birds from cluster 5 might have moulted in their wintering areas in the waters of South Africa. Noticeably, birds from cluster 5 were caught at three locations in South Africa: Cape Columbine, Bird Island and Dyer Island; the latter is known to be a non-breeding site that is numerically dominated by *S. v. tristanensis* (Tree and

Klages 2004). Altogether, these data suggest that adult feathers are also inadequate for delineation of the origin of the birds, because they moult in a variety of geographical areas.

The subspecies of Antarctic Tern

The present study revealed that individuals in Clade 2 (Figs 2, 3) differed from the other Antarctic Terns examined. Morphological analyses showed that birds from Clade 2, likely *S. v. tristanensis*, were significantly larger than other Antarctic Tern populations examined, and that they started their moult-cycle earlier. Four nucleotide substitutions were found between the two *ND2* clades of Antarctic Terns. Stable isotope feather analyses suggest that immatures with features of *S. v. tristanensis* moult in different areas to immatures from other populations (although sample size was small). These differences support the interpretation of *S. v. tristanensis* as a subspecies of *S. vittata* if not a distinct species. Specific status has been afforded to populations of albatrosses (*Diomedea* spp.; Burg and Croxall 2004) and petrels (*Procellaria* spp.; Techow *et al*. 2009) at Tristan da Cunha and Gough Islands. Glacial cycles and associated ecological changes in the Southern Ocean are thought to be the main drivers of this evolution.

In the present study, no samples identified morphologically as belonging to *S. v. sanctipauli* were genetically distinct from *S. v. vittata*. Stable isotope analyses of the 13 adults identified as *S. v. sanctipauli* or *S. v. sanctipauli*/*tristanensis* in the field were classified in five different clusters (clusters 1 to 5; Fig. 5*b*). These results suggest that there may be a north–south cline in colour and size in the Indian Ocean populations, with the smallest and darkest birds in the south (Heard Island), and the largest and palest birds in the north (Amsterdam and St Paul Islands) challenging the existence of the two subspecies *S. v. vittata* and *S. v. sanctipauli*.

Conclusion

This study highlights the difficulties of identifying avian subspecies using morphology alone and the complexity of the Antarctic Tern subspecies complex, challenging the validity of the accepted subspecies. To implement adequate conservation strategies, we believe that a reassessment of the currently accepted subspecies is crucial. Comprehensive sampling should be conducted on birds in breeding plumage collected in their breeding areas to ascertain their origin. Specimens housed in museums would reduce the difficulty of accessing breeding birds in their natural habitat. This is critical to confirm or reject the current International Union for the Conservation of Nature evaluation of the species as least concern (BirdLife International 2013) and establish whether the vulnerability of these infraspecific units should be evaluated independently at a country level.

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References

Ainley, D. G., Ribic, C. A., and Fraser, W. R. (1992). Does prey preference affect habitat choice in Antarctic seabirds? *Marine Ecology Progress Series* **90**, 207–221. doi:10.3354/meps090207

Avise, J. C. (2000). 'Phylogeography.' (Harvard University Press: Boston, MA.)

BirdLife International (2013). Species factsheet: *Sterna vittata*. (BirdLife International: Cambridge, UK.) Available from http://www.birdlife.org [accessed 6 November 2013].

Blévin, P., Carravieri, A., Jaeger, A., Chastel, O., Bustamante, P., and Cherel, Y. (2013). Wide range of mercury contamination in chicks of Southern Ocean seabirds. *PLoS ONE* **8**, e54508. doi:10.1371/journal.pone.0054508

Bridge, E. S., Jones, A. W., and Baker, A. J. (2005). A phylogenetic framework for the terns (Sternini) inferred from mtDNA sequences: implications for taxonomy and plumage evolution. *Molecular Phylogenetics and Evolution* **35**, 459–469. doi:10.1016/j.ympev.2004.12.010

Brooke, R. K., Cooper, J., Hockey, P. A. R., Ryan, P. G., Sinclair, J. C., Suter, W., and Tree, A. J. (1988). Distribution, population size and conservation of the Antarctic Tern *Sterna vittata* in southern Africa. *Cormorant* **16**, 107–113.

Brownstein, M. J., Carpten, J. D., and Smith, J. R. (1996). Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: primer modifications that facilitate genotyping. *BioTechniques* **20**, 1004–1010.

Burg, T. M., and Croxall, J. P. (2004). Global population structure and taxonomy of the Wandering Albatross species complex. *Molecular Ecology* **13**, 2345–2355. doi:10.1111/j.1365-294X.2004.02232.x

Casaux, R., Baroni, A., Ramón, A., Favero, M., and Silva, P. (2008). Aspects of the foraging behaviour of the Antarctic Tern *Sterna vittata gaini* at Harmony Point, South Shetland Islands. *Polar Biology* **31**, 327–331. doi:10.1007/s00300-007-0362-3

Cherel, Y., and Hobson, K. A. (2007). Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Marine Ecology Progress Series* **329**, 281–287. doi:10.3354/meps329281

Croxall, J. P., Butchart, S. H. M., Lascelles, B., Stattersfield, A. J., Sullivan, B., Symes, A., and Taylor, P. (2012). Seabird conservation status, threats and priority actions: a global assessment. *Bird Conservation International* **22**, 1–34. doi:10.1017/S0959270912000020

de Beer, S. J., Lockwood, G. M., Raijmakers, J. H. F. A., Raijmakers, J. M. H., Scott, W. A., Oschadleus, H. D., and Underhill, L. G. (2001). 'SAFRING Bird Ringing Manual.' ADU Guide 5. (Avian Demography Unit, University of Cape Town: Cape Town.)

Earl, D. A., and von Holdt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359–361. doi:10.1007/s12686-011-9548-7

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**, 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x

Excoffier, L., and Lischer, H. E. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and

Windows. *Molecular Ecology Resources* **10**, 564–567. doi:10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Laval, G., and Schneider, S. (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**, 47–50.

Falla, R. A. (1937). 'B.A.N.Z. Antarctic Research Expedition 1929–1931, under the Command of Sir Douglas Mawson. Reports—Series B, Volume II. Birds.' (B.A.N.Z.A.R. Expedition Committee: Adelaide, SA.)

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791. doi:10.2307/2408678

Friesen, V. L., Burg, T. M., and McCoy, K. D. (2007). Mechanisms of population differentiation in seabirds. *Molecular Ecology* **16**, 1765–1785. doi:10.1111/j.1365-294X.2006.03197.x

Gay, L., Neubauer, G., Zagalska-Neubauer, M., Debain, C., Pons, J.-M., David, P., and Crochet, P.-A. (2007). Molecular and morphological patterns of introgression between two large white-headed gull species in a zone of recent secondary contact. *Molecular Ecology* **16**, 3215–3227. doi:10.1111/j.1365-294X.2007.03363.x

Gill, F., and Donsker, D. (2013). 'IOC World Bird List (v 3.4).' Available at http://www.worldbirdnames.org [accessed 28 November 2013].

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. *Molecular Ecology Notes* **2**, 416–418. doi:10.1046/j.1471-8286.2002.00261.x

Gochfeld, M., and Burger, J. (1996) Family Sternidae (Terns). In 'Handbook of the Birds of the World. Vol. 3: Hoatzin to Auks.' (Eds J. del Hoyo, A. Elliott and J. Sargatal.) pp. 624–667 (Lynx Edicions: Barcelona, Spain.)

Gosler, A. (2004) Birds in the hand. In 'Bird Ecology and Conservation: A Handbook of Techniques'. (Eds W. J. Sutherland, I. Newton and R. E. Green), pp. 85–118. (Oxford University Press Inc.: New York, NY.)

Guo, S., and Thompson, E. (1992). Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics* **48**, 361–372. doi:10.2307/2532296

Haig, S. M., Beever, E. A., Chambers, S. M., Draheim, H. M., Dugger, B. D., Dunham, S., Elliott-Smith, E., Fontaine, J. B., Kesler, D. C., Knaus, B. J., Lopes, I. F., Loschl, P., Mullins, T. D., and Sheffield, L. M. (2006). Taxonomic considerations in listing subspecies under the US Endangered Species Act. *Conservation Biology* **20**, 1584–1594. doi:10.1111/j.1523-1739.2006.00530.x

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST: palaeontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**(1), 9.

Hasegawa, M., Kishino, H., and Yano, T. (1985). Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**, 160–174. doi:10.1007/BF02101694

Hatch, J. J. (2002). Arctic Tern (*Sterna paradisaea*). In 'The Birds of North America'. (Eds A. Poole and F. Gill.) No. 707, pp. 1–39. (The Birds of North America Inc.: Philadelphia, PA.)

Higgins, P. J., and Davies, S. J. J. F. (Eds) (1996). 'Handbook of Australian, New Zealand and Antarctic Birds. Vol. 3: Snipe to Pigeons.' (Oxford University Press: Melbourne.)

Hobson, K. A., and Clark, R. G. (1992). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor* **94**, 189–197. doi:10.2307/1368808

Hockey, P. A. R., Dean, W. R. J., and Ryan, P. G. (Eds) (2005). 'Roberts Birds of Southern Africa.' 7th edn. (The Trustees of the John Voelcker Bird Book Fund: Cape Town.)

Hodum, P. J., and Hobson, K. A. (2000). Trophic relationships among Antarctic fulmarine petrels: insights into dietary overlap and chick provisioning strategies inferred from stable-isotope ($\delta^{15}N$ and $\delta^{13}C$) analyses. *Marine Ecology Progress Series* **198**, 273–281. doi:10.3354/meps198273

Jaeger, A. (2009). Etude isotopique des variations saisonnières et à long terme de l'écologie alimentaire des oiseaux marins de l'Océan Austral. Ph.D. Thesis, Université Pierre et Marie Curie, Paris.

Jaeger, A., Lecomte, V. J., Weimerskirch, H., Richard, P., and Cherel, Y. (2010). Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. *Rapid Communications in Mass Spectrometry* **24**, 3456–3460. doi:10.1002/rcm.4792

Jakobsson, M., and Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**, 1801–1806. doi:10.1093/bioinformatics/btm233

Jarman, S. N., Ward, R. D., and Elliott, N. G. (2002). Oligonucleotide primers for PCR amplification of coelomate introns. *Marine Biotechnology* **4**, 347–355. doi:10.1007/s10126-002-0029-6

Jukes, T. H., and Cantor, C. R. (1969). Evolution of protein molecules. In 'Mammalian Protein Metabolism'. (Ed. H. N. Munro.) pp. 21–132. (Academic Press: New York.)

Küpper, C., Horsburgh, G. J., Dawson, D. A., French-Constant, R., Székely, T., and Burke, T. (2007). Characterization of 36 polymorphic microsatellite loci in the Kentish Plover (*Charadrius alexandrinus*) including two sex-linked loci and their amplification in four other *Charadrius* species. *Molecular Ecology Notes* **7**, 35–39. doi:10.1111/j.1471-8286.2006.01517.x

Küpper, C., Burke, T., Székely, T., and Dawson, D. A. (2008). Enhanced cross-species utility of conserved microsatellite markers in shorebirds. *BMC Genomics* **9**, 502. doi:10.1186/1471-2164-9-502

Meirmans, P. G., and Hedrick, P. W. (2011). Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources* **11**, 5–18. doi:10.1111/j.1755-0998.2010.02927.x

Mizutani, H., Fukuda, M., Kabaya, Y., and Wada, E. (1990). Carbon isotope ratio of feathers reveals feeding behavior of cormorants. *Auk* **107**, 400–403. doi:10.2307/4087626

Moore, J. K., Abbott, M. R., and Richman, J. G. (1999). Location and dynamics of the Antarctic Polar Front from satellite sea surface temperature data. *Journal of Geophysical Research* **104**, 3059–3073. doi:10.1029/1998JC900032

Murphy, R. C. (1938). Birds collected during the Whitney South Sea expedition. XXXVII. On Pan-Antarctic terns. *American Museum Novitates* **977**, 1–17.

Orsi, A. H., Whitworth, T. III, and Nowlin, W. D. Jr (1995). On the meridional extent and fronts of the Antarctic Circumpolar Current. *Deep-sea Research. Part I, Oceanographic Research Papers* **42**, 641–673. doi:10.1016/0967-0637(95)00021-W

Palmer, J. (2006). Lack of mitochondrial DNA differentiation in four subspecies of Antarctic Tern, *Sterna vittata*. B. Sc. Hons Thesis, Rhodes University, Grahamstown, South Africa.

Patten, M. A., and Unitt, P. (2002). Diagnosability versus mean differences of Sage Sparrow subspecies. *Auk* **119**, 26–35. doi:10.1642/0004-8038(2002)119[0026:DVMDOS]2.0.CO;2

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics* **28**, 2537–2539. doi:10.1093/bioinformatics/bts460

Prevost, Y. (1983). The moult of the Osprey *Pandion haliaetus*. *Ardea* **71**, 199–209.

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Quillfeldt, P., McGill, R. A. R., and Furness, R. W. (2005). Diet and foraging areas of Southern Ocean seabirds and their prey inferred from stable isotopes: review and case study of Wilson's Storm-Petrel. *Marine Ecology Progress Series* **295**, 295–304. doi:10.3354/meps295295

Quillfeldt, P., Masello, J. F., McGill, R. A. R., Adams, M., and Furness, R. W. (2010). Moving polewards in winter: a recent change in the migratory

strategy of a pelagic seabird? *Frontiers in Zoology* **7**, 15–26. doi:10.1186/1742-9994-7-15

Rosenberg, N. A. (2004). Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**, 137–138. doi:10.1046/j.1471-8286.2003.00566.x

Rubenstein, D. R., and Hobson, K. A. (2004). From birds to butterflies: animal movement patterns and stable isotopes. *Trends in Ecology & Evolution* **19**, 256–263. doi:10.1016/j.tree.2004.03.017

Sadleir, R. M. F. S., Taylor, R. H., and Taylor, G. A. (1986). Breeding of Antarctic Terns. *Notornis* **33**, 264–265.

Sagar, P. M. (1991). Aspects of the breeding and feeding of Kerguelen and Antarctic Terns at the Kerguelen Islands. *Notornis* **38**, 191–198.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233–234. doi:10.1038/72708

Sears, J., Hatch, S. A., and O'Brien, D. M. (2009). Disentangling effects of growth and nutritional status on seabird stable isotope ratios. *Oecologia* **159**, 41–48. doi:10.1007/s00442-008-1199-3

Slatkin, M., and Excoffier, L. (1996). Testing for linkage disequilibrium in genotypic data using the EM algorithm. *Heredity* **76**, 377–383. doi:10.1038/hdy.1996.55

Techow, N. M. S. M., Ryan, P. G., and O'Ryan, C. (2009). Phylogeography and taxonomy of White-chinned and Spectacled Petrels. *Molecular Phylogenetics and Evolution* **52**, 25–33. doi:10.1016/j.ympev.2009.04.004

Tirard, C., Helfenstein, F., and Danchin, E. (2002). Polymorphic microsatellites in the Black-legged Kittiwake *Rissa tridactyla*. *Molecular Ecology Notes* **2**, 431–433. doi:10.1046/j.1471-8286.2002.00258.x

Tree, A. J., and Klages, N. T. W. (1998). Ageing techniques and age structure of a mid-winter roost of Antarctic Tern. *Safring News* **27**, 15–17.

Tree, A. J., and Klages, N. T. W. (2001). A reassessment of plumage characters in ageing Antarctic Terns. *Afring News* **30**, 28–29.

Tree, A. J., and Klages, N. T. W. (2004). Population size, distribution and origins of Antarctic Terns *Sterna vittata* wintering in South Africa. *Marine Ornithology* **32**, 55–61.

Weimerskirch, H., Jouventin, P., Mougin, J. L., Stahl, J. C., and van Beveren, M. (1985). Banding recoveries and the dispersal of seabirds breeding in French Austral and Antarctic territories. *Emu* **85**, 22–33. doi:10.1071/MU9850022

Winker, K. (2010). Subspecies represent geographically partitioned variation, a gold mine of evolutionary biology, and a challenge for conservation. *Ornithological Monographs* **67**, 6–23. doi:10.1525/om.2010.67.1.6

Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* **19**, 395–420. doi:10.2307/2406450