RESEARCH NOTE

The origins of white-chinned petrels killed by long-line fisheries off South Africa and New Zealand

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Abstract

The white-chinned petrel (Procellaria aequinoctialis) is the seabird species most frequently killed by fisheries in the Southern Ocean and is listed by the International Union for the Conservation of Nature and Natural Resources as globally vulnerable. It breeds around the sub-Antarctic, but genetic data identified two subspecies: P. a. aequinoctialis from islands in the Atlantic and Indian Oceans and P. a. steadi from the New Zealand sub-Antarctic islands. We identify the region of origin of birds killed by two long-line fisheries based on differences in the mitochondrial gene cytochrome b. All 113 birds killed off South Africa had the haplotype of P. a. aequinoctialis, whereas all the 60 birds from New Zealand had P. a. steadi haplotypes. The two subspecies of white-chinned petrels thus appear to disperse to different regions irrespective of their age, which accords with the tracking data of adult birds. Our finding has significant implications for managing the bycatch of this species by regional fisheries.

Caught on longlines or colliding with trawl cables, many seabirds are killed at sea (Croxall et al. 2012). As a result, most albatrosses and several large petrels are listed as threatened (ACAP 2009; BirdLife International 2016). The white-chinned petrel (Procellaria aequinoctialis) is the seabird species killed most frequently in Southern Ocean fisheries, with peak estimates of more than 50,000 birds killed annually (Ryan et al. 2012). It is confined to the Southern Ocean, with a circumpolar breeding distribution at sub-Antarctic islands (Fig. 1). Accurate population estimates are complicated by their burrow-nesting behaviour (Brooke 2004), but there is evidence that populations are decreasing (Phillips et al. 2006; Barbraud et al. 2009) and the species is listed as vulnerable, with the main threat being accidental mortality on fishing gear (BirdLife International 2016). Mitigation measures have been developed (Robertson et al. 2006) and national action plans implemented to reduce bycatch (Croxall et al. 2012). However, in order to assess the impact of fisheries on different island populations, we need to know where birds killed by specific fisheries breed, because differences in dispersal and foraging areas among island populations may affect their susceptibility to fishery bycatch (Berrow et al. 2000; Catard et al. 2000). Genetic markers can be useful to identify regions or colonies most at risk (e.g., Abbott et al. 2006).

White-chinned petrels traditionally have been regarded as monotypic with few, if any, morphological differences among populations. Techow et al. (2009) recognized two subspecies based on partial cytochrome b sequences: the nominate race from islands in the Atlantic and Indian Ocean sectors of the Southern Ocean, and P. a. steadi from the New Zealand sub-Antarctic islands (Fig. 1). Reasonably accurate population estimates are available for all breeding colonies of the nominate race (Ryan et al. 2012), but estimates of P. a. steadi are more crude. Best estimates

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suggest that they are much less abundant, comprising perhaps 20–25% of the nominate population (Table 1). Given this uneven distribution of breeding numbers, it is important to identify the colonies of origin for birds killed in different regional fisheries.

Tracking and banding studies have found no evidence of movement of white-chinned petrels between colonies (Marchant & Higgins 1990; Hockey et al. 2005; Phillips et al. 2006; Péron et al. 2010) but relatively few individuals and colonies have been studied. The year-round movements of adults from four colonies have been tracked with light-logging geolocators. During the non-breeding season—the austral winter, from May to September—adults from South Georgia mainly occur off the east coast of South America (although some venture around Cape Horn to the west coast [Phillips et al. 2006]), whereas those from the Prince Edward Islands and Kerguelen occur off southern Africa (Péron et al. 2010; PGR unpubl. data) and those from New Zealand’s Antipodes Islands visit the west coast of South America (D. Thompson, pers. comm.). However, these studies have only tracked adult birds. Genetic markers allow insights into the movements of all age classes, which is important given that non-breeders comprise more than half of the total white-chinned petrel population (Barbraud et al. 2008). In this study, we use the sequence differences in cytochrome b to assess whether the two subspecies of white-chinned petrels disperse to different foraging areas when not breeding.

**Materials and methods**

Blood and muscle tissue samples were collected from white-chinned petrels at their breeding islands and from birds killed by fishing vessels. Birds from breeding islands are summarized in Table 1, with full details in Techow et al. (2009). Samples also were collected from birds of unknown provenance killed away from breeding colonies: 113 caught on longlines off South Africa in winter (May–October; 111 on tuna [Thunnus spp.] longlines and two on hake [Merluccius spp.] longlines), and 60 caught on New Zealand ling (Genypterus blacodes) longlines off southern and south-eastern New Zealand in early summer (November–December). Birds were sexed based on examination of gonads, and birds killed off South Africa were aged as juveniles (age 0–1 year), immature (maximum ca. 5 years old) or adult (> 5 years) based on a combination of gonad condition, moult and bill structure (Marchant & Higgins 1990). Birds killed by long-line vessels were kept frozen until tissue samples could be collected and stored in 96% ethanol. Genomic DNA was extracted using an overnight Proteinase K digestion in extraction buffer (Fermentas; 10 mM Tris-HCl [pH 7.5], 10 mM MgCl2, 0.1 mg/ml BSA), followed by a phenol-chloroform extraction (Ausubel et al. 1989; Sambrook et al. 1989).

**Cytochrome b sequencing and data analysis**

A subset of 25 bycatch birds, selected at random, was sequenced for the cytochrome b gene; 11 from South Africa and 14 from New Zealand. PCR conditions are described by Techow et al. (2009). Bands were excised from the agarose gel with a sterile blade and purified with the Promega

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**Abbreviations in this article**

PCR: polymerase chain reaction  
SNP: single nucleotide polymorphism

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**Table 1** Population estimates for white-chinned petrel breeding colonies (Brooke 2004; Barbraud et al. 2008; ACAP 2009; Barbraud et al. 2009; Martin et al. 2009; Ryan et al. 2012; BirdLife International 2016) and the number from each population used in this study. Numbers in parentheses are adult birds caught off breeding islands during the breeding season. See Techow et al. (2009) for additional details.

| Colony          | Colony size (pairs) | Number sampled |
|-----------------|---------------------|----------------|
| Falklands/Malvinas | 70                  | 0              |
| South Georgia   | 681 000             | 15             |
| Prince Edward Islands | 36 000             | 19             |
| Crozet Islands  | 23 600              | 11             |
| Kerguelen Islands | 234 000             | 0              |
| Auckland Islands | 100 000             | 16 (9)         |
| Campbell Island | 10 000              | 0              |
| Antipodes       | 100 000             | 22 (6)         |

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Restriction enzyme analysis of a SNP

The remaining 148 white-chinned petrels of unknown origin (102 from South Africa and 46 from New Zealand) were subjected to a restriction enzyme digest after amplifying the same fragment of the cytochrome b gene. Three fixed mutational differences were found between the two subspecies of white-chinned petrel (\(n=83\) birds; Techow et al. 2009), allowing the two populations to be segregated with the restriction enzyme Csp6l (supplied by Fermentas). Cytochrome \(b\) genes from the New Zealand population \(P. a. steadi\) have two Csp6l sites resulting in three fragments of 311, 173 and 114 bp, whereas \(P. a. aequinoctialis\) from islands in the Atlantic and Indian Ocean have only one Csp6l site resulting in two fragments of 484 and 114 bp. PCR amplification of cytochrome \(b\) was as described by Techow et al. (2009). PCR products were electrophoresed on 2% agarose gels. Size of products was determined according to a DNA ladder (Promega 100 bp DNA ladder). Digest conditions were as follows: five units of Csp6l, 1 \(\times\) digestion buffer B, 5 \(\mu\)l of PCR product in a total volume of 14.5 \(\mu\)l. Digests were incubated at 37°C for two hours and inactivated by incubating at 65°C for 20 min. Products were electrophoresed on 3% agarose. Each agarose gel contained a control sample of known origin.

Discussion

Most procellariiformes are philopatric to their natal and breeding sites (Warham 1990), which is conducive to the
evolution of population differentiation among colonies over time. Among albatrosses, species that feed primarily in shelf waters are more strongly structured genetically than are those with oceanic foraging ranges (Abbott & Double 2003; Burg & Croxall 2001, 2004; Friesen et al. 2007). Breeding white-chinned petrels range widely, foraging in both oceanic and coastal shelf waters (Weimerskirch et al. 1999; Catard et al. 2000; Delord et al. 2010) but mainly disperse to shelf waters when not breeding (Phillips et al. 2006; Péron et al. 2010). As a result, we might expect them to demonstrate some genetic structure. Cytochrome b analysis has found two distinct regional populations, one from New Zealand and another in the Atlantic and Indian Oceans (Techow et al. 2009). This conclusion was supported by the larger samples of birds included in our study, which identified a few additional haplotypes to those reported by Techow et al. (2009).

Tracking studies using geolocators show that adult white-chinned petrels from South Georgia, the Prince Edward Islands, Kerguelen and the Antipodes Islands disperse to largely discrete non-breeding areas (Phillips et al. 2006; Péron et al. 2010; D. Thompson, pers. comm.; PGR unpubl. data). Sample sizes in these studies are modest, but the limited individual variation detected suggests that adults from different breeding islands tend to have different non-breeding sites, at least at a regional level, even within subspecies. Nominate adults from South Georgia spend the non-breeding season off South America (Phillips et al. 2006), whereas those from the Prince Edward Islands and Kerguelen spend the winter off southern Africa (Péron et al. 2010; PGR unpubl. data). However, little is known about the dispersal patterns of juvenile and immature white-chinned petrels. Only three birds banded as chicks have been reported away from their breeding islands: all were from the Crozets and were recognized by restriction enzymes, the potential ambiguity in SNP results caused by one P. a. steadi haplotype could be resolved with mismatch primers (FitzSimmons et al. 1997). However, the level of discrimination offered by cytochrome b is too coarse to segregate between specific island populations. Other tools that could be used to attempt to segregate populations include morphometric differences or biogeochemical markers such as stable isotope ratios (Gómez-Díaz & González-Solís 2007). Such additional markers are needed to segregate between nominate white-chinned petrels from the large South Georgia population and those from breeding colonies in the southern Indian Ocean.

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