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Genetic analysis of wandering albatrosses killed in longline fisheries off the east coast of New Zealand

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ABSTRACT

1. Fisheries bycatch is an important global conservation issue. Each year many species, including seabirds, are killed accidentally in longline fisheries. Highly vagile marine species such as the wandering albatross species complex (*Diomedea* spp.), populations of which have declined dramatically due to fisheries-related mortality, traverse large areas of open ocean and as a result are at high risk of negative fisheries interactions.

2. For most wandering albatrosses found dead at-sea the provenance is unknown unless they are ringed, as their poor condition make species identification very difficult. However, molecular markers can be used as genetic tags to identify the population of origin of bycatch birds.

3. Five microsatellite markers and mitochondrial restriction fragment length polymorphism were used to genotype 41 individuals belonging to the wandering albatross species complex killed in longline fisheries in two locations off the east coast of the North and South Islands of New Zealand, an area known to be visited by at least three members of the wandering albatross groups.

4. Assignment tests indicate that the majority (22/30 of assigned individuals) of bycatch birds were *D. antipodensis antipodensis* from Antipodes Island, the closest breeding site. More than half of the North Island birds were assigned to *D. a. antipodensis* (8/14); the remaining samples were assigned to both the *D. a. gibsoni* from The Auckland Islands ($n = 3$) and *D. exulans* from the South Atlantic/Indian Ocean ($n = 3$). The South Island samples were almost exclusively assigned to *D. a. antipodensis* (14/16) of which 13 were males. In the North Island samples there were 14 males and 8 females.

5. This study has shown that molecular markers are a powerful tool to determine the species identity, population of origin and sex of bycatch birds to better understand the impact of fisheries-related mortality and conservation priorities for individual populations.

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KEY WORDS: longline fisheries; wandering albatross; genetic markers; conservation

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INTRODUCTION

Fisheries bycatch is an important conservation issue. Dolphins, turtles and albatrosses are only a few of the bycatch species accidentally killed in global fisheries (Brothers, 1991; Laurent *et al.*, 1998). Many of these marine species have complex life history patterns that make implementation of recovery plans difficult. For example, turtles and albatrosses are long-lived, have prolonged sexual immaturity, and migrate thousands of kilometres between foraging and breeding grounds. For albatrosses, the impact of fisheries bycatch is further complicated by low reproductive rates, life-long pair bonds between breeding pairs and obligate biparental care.

Longline fisheries are the single largest threat currently facing albatross populations worldwide and has led to all 21 species being listed as threatened or near-threatened (BirdLife International, 2004). Near one breeding site in the southern Indian Ocean (Prince Edward Islands; 46°60′S, 37°58′E), an estimated 3.5 million hooks per year are legally set for Patagonian toothfish (*Dissostichus eleginoides*; Ryan and Boix-Hinzen, 1998). On a global scale, an estimated 200 million hooks are set each year by the tuna (*Thunnus* species) fisheries alone. Despite increased efforts to obtain accurate estimates of the number of albatrosses killed, spatial and temporal variation in the fisheries, combined with illegal fishing and underreporting of bycatch, make it difficult to obtain accurate estimates (Tuck *et al.*, 2003). Klaer and Polacheck (1997) estimate 2450 albatrosses were killed annually between 1992 and 1994 in the Japanese longline fisheries off Australia. Reports from the New Zealand domestic tuna fisheries show 634 albatrosses killed between 1990 and 2001 (Murray *et al.*, 2002). Recent implementation of mitigation measures, such as thawed bait, tori lines, weighted lines, and night setting, has decreased bycatch (Brothers, 1991; Brothers *et al.*, 1999; Tuck *et al.*, 2003), but not all vessels use such deterrents and population declines continue.

Census data from many of the breeding colonies have shown drastic declines, including a 63% reduction in breeding birds at a colony of *Diomedea antipodensis* from 1973 to 1997 and declines in excess of 20% from 1960 to the 1990s at most *D. exulans* sites (Weimerskirch *et al.*, 1997; Croxall and Gales, 1998; Walker and Elliott, 1999; Nel *et al.*, 2003). Under current conditions, models of long-term population estimates predict declines up to 79% over the next three generations (70 years; BirdLife International, 2006). With as few as three to 17 breeding pairs on some islands (e.g. Campbell, Macquarie, and Amsterdam Islands; Figure 1) the conservation threat is apparent.

The conservation status of albatrosses is further complicated by the dynamic taxonomy of the group in which 14–24 species are described by various authors. There are currently five species/sub-species that form the wandering albatross species complex: *D. amsterdamensis* (<20 breeding pairs annually) endemic to Amsterdam Island in the Indian Ocean; *D. antipodensis antipodensis* (Antipodes Island 4600–5700 breeding pairs annually; Campbell Island six breeding pairs annually) and *D. a. gibsoni* (Auckland Islands 5800 breeding pairs annually) off the coast of New Zealand; *D. dabbenena* found on Tristan da Cunha (Gough Island 1500–2400 breeding pairs annually, Inaccessible Island three breeding pairs annually) in the Atlantic; and *D. exulans* (17 000 breeding pairs annually) in the South Atlantic and Indian Ocean (Figure 1; Table 1; Gales, 1998; Birdlife International, 2006). The current global estimate of the wandering albatross species complex for all four species is approximately 135 000 individuals including juvenile and non-breeding birds (Table 1). Weimerskirch *et al.* (1997) provide a non-conservative estimate of 122 000 'wandering albatrosses' killed in the Japanese longline fisheries in a 30 year period starting in the early 1960s. However, since the population sizes of individual species/sub-species vary by orders of magnitude the relative impacts of fisheries mortality on different populations will vary considerably. With large numbers of albatrosses being killed in longline fisheries it is critical to identify both the species and the provenance of these birds to fully understand the impact of longline mortality on individual species and populations.

Sex-specific foraging distributions mean that the sex ratio of birds killed as bycatch may be biased (Weimerskirch and Jouventin, 1987; Weimerskirch *et al.*, 1987, 1993; Bartle, 1990; Ryan and Boix-Hinzen, 1999; Mills and Ryan, 2005) which can have adverse demographic consequences (Weimerskirch *et al.*, 1987;

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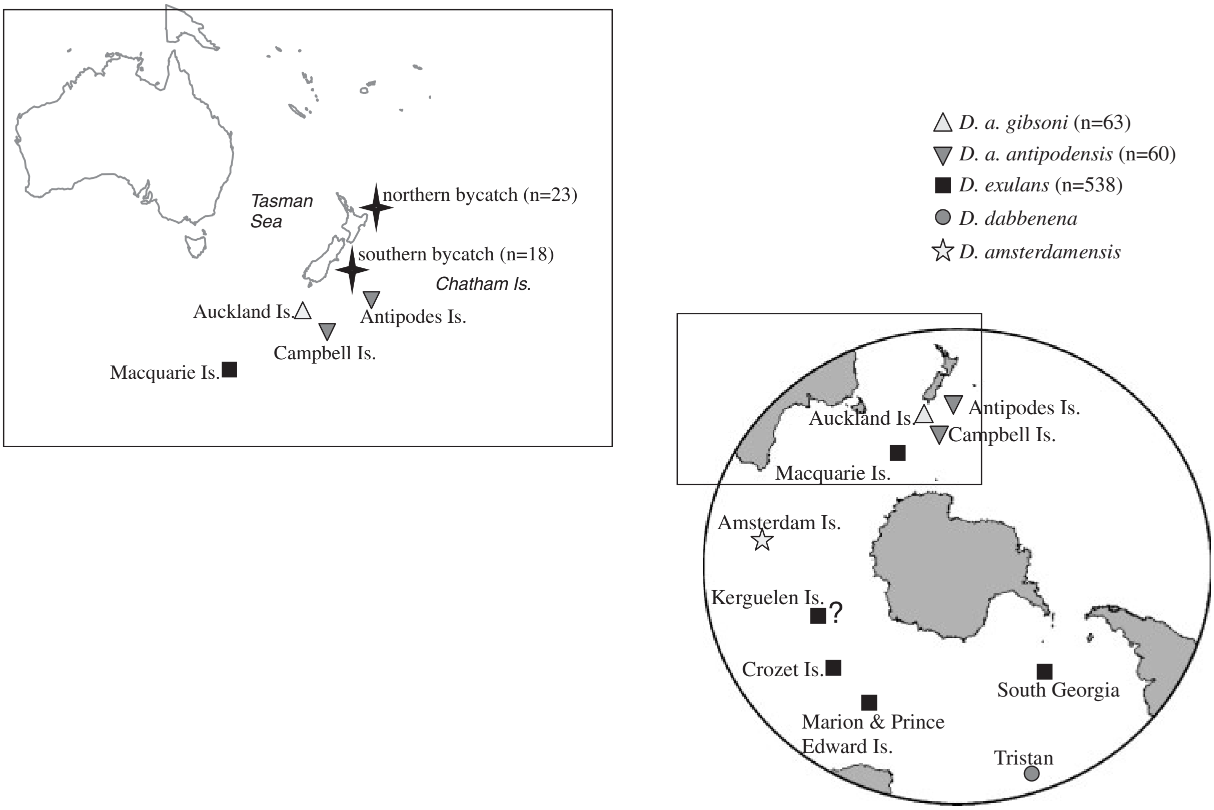

Figure 1. Distribution of breeding sites of wandering albatrosses in the Southern Ocean. Genetic analysis revealed the presence of five genetically distinct groups (Burg and Croxall, 2004; Alderman *et al*., 2005; Burg, unpublished). MtDNA from *D. amsterdamensis* ($n = 35$) confirmed that birds breeding on Amsterdam Island are genetically distinct from the four other groups (Burg, unpublished). Unsampled site (?) and sample sizes (n) for islands included in this study are also shown. Inset map shows bycatch sites in relation to breeding colonies around New Zealand and Australia.

Table 1. Global population status of the wandering albatross species complex. Population estimates provide the estimated total number of mature birds (Gales, 1998; BirdLife International, 2006). Breeding sites located in the same archipelago are counted as a single site. Geographic distribution of the sites is shown in Figure 1

Species	# breeding sites	Pop. estimates	Status	Pop. trends
D. amsterdamensis	1	80	Critically endangered	Decreasing
D. antipodensis	3[a]	37 000	Vulnerable[b]	Unknown
D. dabbenena	1	7000	Endangered	Decreasing
D. exulans	5	28 000	Vulnerable	Decreasing

[a] Includes a small number of individuals breeding on Campbell Island.
[b] May be re-classified as endangered or critically endangered pending additional data.

Gales *et al*., 1998; Ryan and Boix-Hinzen, 1999; Nel *et al*., 2003). For example, off the Crozet Islands the more southerly Patagonian toothfish fishery shows a strong male bias in bycatch birds, whereas the more northerly tuna longline fishery has a female bias (Weimerskirch *et al*., 1987; Ryan and Boix-Hinzen, 1999;

Weimerskirch and Wilson, 2000). This has been attributed to sex-specific differences in at-sea distribution during the breeding season. Such sex-biased mortality leads to a large number of unpaired birds (Weimerskirch and Jouventin, 1987; Jouventin *et al*., 1999; Nel *et al*., 2003) and interruptions to the breeding cycle due to mate loss can increase the rate of population decline as the loss of a mate can lead to an estimated loss in lifetime chick production of 15% as it takes an average of three years to re-mate following the loss of a partner (Jouventin *et al*., 1999).

Owing to the large distances travelled by albatrosses, birds caught in one area may breed on islands thousands of kilometres away. However, reliance on morphology or plumage characteristics for specific identification of 'wandering albatrosses' can be problematic as they show considerable overlap in both plumage scores and morphology (Gibson, 1967; Robertson and Warham, 1994; Cuthbert *et al*., 2003). In addition, bycatch birds are often badly damaged making accurate specific identification problematic (Murray *et al*., 2002). Molecular markers can be employed to identify the species being caught and therefore have the potential to identify the provenance of bycatch birds (Walsh and Edwards, 2005; Abbott *et al*., 2006). Microsatellite markers have been used successfully to determine mixture of fish stocks and for forensic conservation (Paetkau *et al*., 1999; Primmer *et al*., 1999; Hauser *et al*., 2006).

In order to use genetic markers to identify species and provenance of bycatch samples, one needs markers with high molecular resolution and the presence of both interspecific and intraspecific genetic structure. For the wandering albatross species complex, an extensive global population study revealed the presence of five genetically distinct groups: Amsterdam Island, Tristan, South Atlantic/Indian Ocean, Antipodes Island, and Auckland Island (Burg and Croxall, 2004; Alderman *et al*., 2005; Burg, unpublished).

In areas where the foraging ranges of multiple species overlap with fisheries it is particularly important to establish which populations are being impacted. In the waters east of New Zealand *D. exulans*, *D. a. antipodensis* and *D. a. gibsoni* (Figure 2, Nicholls *et al*., 2000, 2002) are known to overlap with longline fisheries.

The nearest breeding colonies of *D. a. antipodensis* and *D. a. gibsoni* are located 560–1825 km away (Figure 1). The closest *D. exulans* breeding colony is on Macquarie Island (55°30′S, 158°56′E), 1500 km to the south-west of New Zealand.

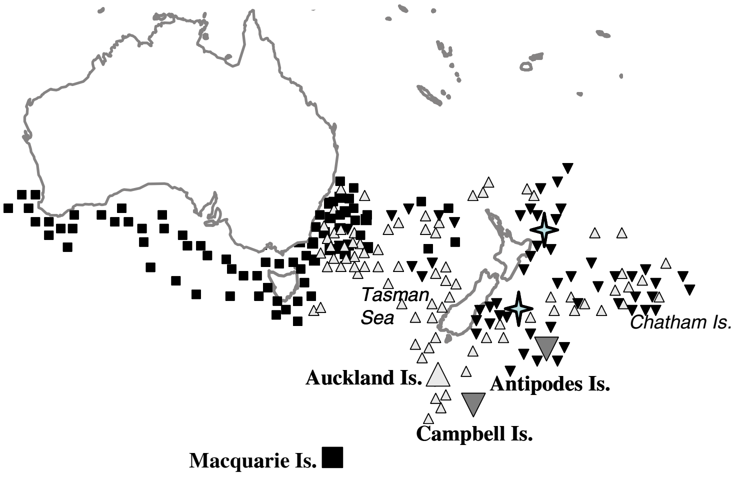

Figure 2. At-sea distribution of wandering albatrosses in Australia and New Zealand based on satellite telemetry data from Nicholls *et al*. (2000). Note that not all of the original data points are shown on the map. The western Tasman Sea is heavily utilized by *D. exulans* (black squares) and *D. a. gibsoni* (light grey triangles). Areas where bycatch samples were obtained overlap with the at-sea distribution of *D. a. antipodensis* (inverted, black triangles) and *D. a. gibsoni*.

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The main objectives of this study are to examine the feasibility of using genetic markers from birds killed in the New Zealand longline fishery to identify the sex and species, and hence the provenance, of members of the wandering albatross species complex killed in the longline fisheries off the east coast of New Zealand.

METHODS

Nomenclature

The nomenclature used by Brooke (2004), which is supported by genetic data (Burg and Croxall, 2004) and has been adopted by both the ACAP Taxonomy Working Group (Double, 2006) and BirdLife International (BirdLife International, 2000), has been adopted here. Furthermore, and to avoid confusion between *D. exulans* (wandering albatross) and the wandering albatross species complex, throughout the text any reference to wandering albatross refers to the complex, not the species *D. exulans*.

Bycatch samples

Tissue samples were collected by K. Walker (Department of Conservation, New Zealand) from members of the wandering albatross species complex caught by two Japanese longliners off the east coast of the North Island (37°S 179°E, $n = 23$; July 1997) and the South Island (46°S 175°E, $n = 18$; 22–27 April 1997) of New Zealand. Tissue samples were stored in salt saturated DMSO (Amos and Hoelzel, 1991) and DNA was extracted using a modified Chelex protocol (Burg and Croxall, 2004).

Known provenance samples

Genotypic data for *D. exulans* (Crozet, Marion and South Georgia), *D. a. antipodensis* (Antipodes and Campbell Islands), and *D. a. gibsoni* (Auckland Island) from Burg and Croxall (2004) were used to establish a baseline dataset to assess the accuracy of bycatch assignments. Significant differences between islands containing different species exist (pairwise F_{ST}: 0.57–0.86 mitochondrial DNA; and 0.07–0.13 microsatellites; Burg and Croxall, 2004).

Molecular markers

Bycatch samples were typed at five microsatellite loci (Dc5, De11, Dc16, De18 and De37) and the mitochondrial control region (details in Burg and Croxall, 2004). The set of microsatellite loci were chosen specifically for their allele frequency profiles to provide maximum resolution. All of the loci used were in Hardy–Weinberg equilibrium and were not linked. *AluI* digests of mtDNA (mtDNA RFLP) allowed for higher resolution between the two New Zealand *D. antipodensis* subspecies. Previous research by Burg and Croxall (2004) found 72.4% of *D. a. antipodensis* ($n = 58$) have an *AluI* restriction site compared with 17.5% of *D. a. gibsoni* ($n = 57$).

CHD primers (CHD1 2550F and CHD1 2718R, Fridolfsson and Ellegren, 1999) were used for molecular sexing of bycatch birds. Size differences between the W and Z chromosome fragment are smaller than those in other avian species and PCR products were resolved on acrylamide gels. PCR conditions and details of screening are described in Burg and Croxall (2006).

Assignment tests

A number of programs are available to determine the origin of unknown individuals (Manel *et al.*, 2005). However, a recent study has shown that different assignment methods have similar success (Hauser *et al.*,

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2006). WHICHRUN (version 4.1) was used in this study as it allows the inclusion of both haploid (mtDNA) and diploid (microsatellite) data to calculate likelihood estimates (Banks and Eichert, 2000). Individuals were assigned to one of three groups (*D. a. antipodensis*, *D. a. gibsoni* and *D. exulans*, representing Antipodes Island, Auckland Island or South Atlantic/Indian Ocean, respectively). WHICHRUN uses the log of the ratio of the population with the highest probability of assignment and the second most likely population to determine the LOD score allowing a critical assessment of individual allocation to each population (Banks and Eichert, 2000). Individuals with a LOD score >2 (corresponding to $P<0.01$) were assigned to the group with the highest likelihood probability.

RESULTS

Bycatch birds

Over 70% of the bycatch samples ($n = 14/23$ from North Island and 16/18 from South Island) were assigned to one of the three groups. Both sets of bycatch contained a majority (22/30 of assigned individuals) of *D. a. antipodensis*, although this was only significant for the southern bycatch samples ($\chi^2 = 21.53$, d.f. = 2, $P<0.001$). More than half of the North Island birds were assigned to *D. a. antipodensis* (8/14); the remaining samples were assigned to both the *D. a. gibsoni* ($n = 3$) and *D. exulans* ($n = 3$). The South Island samples were almost exclusively assigned to *D. a. antipodensis* (14/16) with 2 *D. a. gibsoni* ($n = 3$).

The overall sex ratio for both sets of bycatch combined was significantly different ($\chi^2=4.92$, d.f. = 1, $P=0.02$). The northern bycatch also comprised mostly males, but the sex ratio (14 males:8 females) was not significantly different from random ($\chi^2 = 0.86$, d.f. = 1, $P = 0.36$). However, samples off the South Island contained significantly more males than females ($\chi^2 = 8.86$, d.f. = 1, $P<0.01$) with all but one of the samples being male. Results of the assignments by sex show similar numbers of males and females within each of the groups for the northern bycatch (*D. a. gibsoni*: two females, one male; *D. a. antipodensis*: three females, five males; *D. exulans*: one female, two males). The lone female sampled in the southern bycatch was assigned to *D. a. antipodensis*.

Assignment of known provenance birds

In total 535 of 661 or 80.9% of known provenance individuals were assigned to species or subspecies at $P<0.01$ using microsatellite genotypes and mtDNA RFLP data. The majority of assigned birds (87.0% *D. exulans*, 88.0% *D. a. gibsoni*, and 89.6% *D. a. antipodensis*) from each of the groups were assigned correctly (Table 2). Lowering the stringency to $P<0.05$ increased the number of assignments by 94, but lowered the accuracy of the assignments (81.2 % *D. exulans*, 87.3% *D. a. gibsoni*, and 68.7% *D. a. antipodensis*).

Table 2. Allocation of known provenance and bycatch birds using five microsatellite loci and *AluI* mtDNA digest data. Individual assignments with a LOD score >2 are shown ($P<0.01$). Individuals that could not be assigned at $P<0.01$ are listed as unassigned. Known provenance individuals correctly assigned to breeding grounds are shaded and in bold

Origin	Assigned to:			
	D. a. gibsoni	*D. a. antipodensis*	*D. exulans*	Unassigned
D. a. gibsoni	**44**	6	0	13
D. a. antipodensis	2	**43**	3	12
D. exulans	24	33	**380**	101
South Island bycatch	2	14	0	2
North Island bycatch	3	8	3	9

DISCUSSION

The majority of the bycatch samples from the east coast of New Zealand were *D. a. antipodensis*, probably from the Antipodes Island, the closest breeding site and were predominantly male. The results from this study indicate that bycatch samples from members of the wandering albatross species complex can be correctly assigned to species/subspecies using a combination of mtDNA and microsatellite markers. Over 80% of the known provenance birds could be assigned using the suite of genetic markers described in this study and 87–90% of those were correctly assigned.

Both male and female sex biases have been reported in bycatch from longline fisheries (Weimerskirch and Jouventin, 1987; Bartle, 1990; Ryan and Boix-Hinzen, 1999). The wandering albatross bycatch from both fishing vessels showed a male bias, though a greater proportion of females were caught by the vessel fishing further north. This is consistent with the findings that females forage at more northerly latitudes than males (Weimerskirch and Jouventin, 1987; Weimerskirch and Wilson, 2000).

Differences in the composition of the two sets of bycatch can be explained by temporal and spatial variation in habitat usage by members of the wandering albatross species complex. The small number of bycatch *D. exulans* off the east coast of New Zealand corresponds with satellite telemetry data of their at-sea distribution: *D. exulans* are found in large numbers in the South Atlantic and Indian Ocean extending east to the west Tasman Sea (Figure 2) yet spend relatively little time in the South Pacific (Nicholls *et al*., 2000, 2002; BirdLife International, 2004). Murray *et al.* (2002) used morphological characters to analyse bycatch samples from New Zealand tuna fisheries and reported only two *D. exulans* over an 11 year period. However, the identification of three *D. exulans* in only 41 samples collected over a 2 month period suggest that they may be caught more frequently than indicated by analysis restricted to morphological characteristics.

Both *D. a. antipodensis* and *D. a. gibsoni* forage extensively off the east coast of New Zealand (Figure 2) and are found in greater numbers in New Zealand fisheries bycatch (Nicholls *et al*., 2000, 2002; Murray *et al*., 2002). *D. a. antipodensis* spend the majority of their time east of New Zealand near the Chatham Rise and off the west coast of South America (Nicholls *et al*., 2000, 2002; Hamilton *et al*., 2002; Walker and Elliott, 2002) with breeding birds foraging close to Antipodes Island and the Chatham Rise (Walker and Elliott, 2002).

The large number of *D. a. antipodensis* in the bycatch can further be explained by the timing of the fisheries: the samples from the South Island were obtained at the end of April, whereas the North Island samples were collected in July. The timing of the southern bycatch corresponds to the early-chick rearing period (Walker and Elliott, 1999, 2005) when breeding birds remain close to the breeding colony (Weimerskirch *et al*., 1993; Prince *et al*., 1998; Weimerskirch, 1998; Nicholls *et al*., 2002). Antipodes Island is the nearest breeding site to the southern bycatch site. The northern bycatch samples were collected three months later when breeding birds forage further away from breeding sites.

This study has shown that molecular markers are a powerful tool to determine species/subspecies composition of wandering albatrosses caught incidentally in longline fisheries. These techniques can now be employed on bycatch from other areas (e.g. Tasman Sea) to determine the species identity, population of origin and sex of bycatch birds to better understand the impact of longline mortality on individual populations.

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