Notornis, 2008, Vol. 55: 209-211
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SHORT NOTE

DNA sexing of the critically endangered New Zealand storm petrel (*Oceanites maorianus*, or *Pealeornis maoriana*)

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On 25 Jan 2003 a small black and white storm petrel that did not match the description of any extant storm petrel was sighted off Whitianga, northern North I, New Zealand (Saville *et al*. 2003). Its plumage pattern and morphology, however, were very similar to the specimens that Oliver (1955) described under the name *Oceanites maorianus*, and called the New Zealand storm petrel, a species presumed extinct for over 150 years. Subsequent regular observations (Flood 2003; Gaskin & Baird 2005) and the examination of 4 birds captured in the 2005/2006 austral summer have resulted in the view that the New Zealand storm petrel had been overlooked since its discovery in 1827 (Saville *et al*. 2003; Flood 2003; Gaskin & Baird 2005). This conclusion has resulted in the species being upgraded from Extinct to Critically Endangered (BirdLife International 2006), although still being regarded as data deficient. Here we report on the molecular sexing of the recently-captured 4 specimens, which was done to assist with the interpretation of plumage and morphometric characteristics, because sexual differences in morphometrics are common in other storm-petrels (Brooke 2004, Marchant & Higgins 1990).

The 4 individuals of *O. maorianus* examined in this study were captured in the Hauraki Gulf, northern New Zealand, during the 2005/2006 austral summer. The 1st bird was caught by chance on 4 Nov 2005, when it flew into the cabin of a fishing boat anchored in Waimaomao Bay, Little Barrier I (36°10′10″S; 175°05′46″E) at *c*.2145 h (G. Murman, *pers comm*.). Three similar birds were captured at *c*.35°57′53″S; 175°58′53″E during 2 trips (5-6 Jan 2006; 8-10 Jan 2006) in the outer Hauraki Gulf, New Zealand that were undertaken with the objective of catching and examining more individuals. For molecular analyses, 5 contour feathers were collected from the bird captured on 4 Nov 2005 and blood samples (≤ 250 μL, preserved in lysis buffer; Seutin *et al*. 1991) were collected by wing venipuncture (Ardern *et al*. 1994) from each of the 3 individuals caught in Jan 2006. Whole genomic DNA was extracted from the feathers (2 feathers extracted) using a chelex extraction method (Walsh *et al*. 1991) and from the blood samples using a standard phenol/chloroform extraction (Sambrook & Russell 2000).

The molecular sex of the 4 individuals was determined using 2 independent molecular tests of sex to reduce the possibility of errors in sexing (Robertson & Gemmell 2006). Molecular sexing was done using polymerase chain reaction (PCR) primers for the chromo-helicase-DNA-binding gene (primer pair P2 & P8: Griffiths *et al*. 1998; primer pair 2550 & 2718: Fridolfsson & Ellegren 1999), which detect males as a single fragment (ZZ) and females as 2 fragments, each corresponding to one of the sex chromosomes (ZW). PCR amplifications were done in 25 μL reaction volumes: *c*.50 ng of template DNA, 1.0 pmol of each primer, 200 μM each of dATP, dGTP, dTTP, and dCTP, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$ and 0.5 unit of *Taq* polymerase (Bioline USA, Inc, Randolph MA 02368-4800). The thermal cycling parameters were an initial 2 min denaturation at 94°C, followed by 30 cycles at 94°C/15 s, 48°C/25 s, and 72°C/45 s. PCR products for 2550/2718 were resolved on a 1% agarose gel (TBE: 134 mM Tris, 74.9 mM boric acid, 2.55 mM EDTA pH 8.8) or, for P2/P8, using 5% native polyacrylamide gel electrophoresis (Sambrook & Russell 2000).

Received 17 Jun 2006; accepted 31 Mar 2007
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All 4 captured birds were identified as males by the molecular sexing protocol. The absence of females in the sample could have resulted from the small sample size, which may be resolvable by further captures, or because the sexes differ in ease of capture (for example, females may avoid feeding around boats; Ryan & Boix-Hinzen 1999). Alternatively, the observed predominance of males could reflect a real skew in the representation of the sexes in the Hauraki Gulf in the austral summer. Skewed sex ratios encountered in populations of storm petrels outside the breeding season have been attributed to differences in the timing of migration (Huber 1971), while sex biases in other seabirds such as the snowy wandering albatross *Diomedea exulans* (Weimerskirch & Jouventin 1987; Weimerskirch *et al*. 1997), the grey petrel *Procellaria cinerea* (Bartle 1990), or behavioural differences between the sexes in general (Ryan & Boix-Hinzen 1999), including the critically endangered Magenta petrel *Pterodroma magentae* (Imber *et al*. 2005) have been attributed to different foraging ranges of males and females.

Our molecular sexing of the 4 captured individuals as males demonstrated that the variation in plumage and morphometric characteristics apparent in these individuals was not related to their sex, as it commonly is in other storm petrels (Brooke 2004; Marchant & Higgins 1990). Examination of photographs taken of the birds captured in Jan 2006, indicates that the birds were at least 18 months old, and probably 2-4 years of age (Rob Thomas, *pers. comm.*), based on wear of the plumage and moult status of the primary feathers. Pre-breeders of the European storm-petrel, *Hydrobates pelagicus*, do not return to the colony until they reach 2 years of age and do not commence breeding until they are 4 years old (Warham 1990; Brooke 2004; Okill & Bolton 2005). We therefore conclude that if the New Zealand storm petrel breeds between Oct and Mar, the birds captured in Jan 2006 were old enough to be visiting a colony, but may not have been breeding. While this suggests that the New Zealand storm petrel has bred recently, its breeding site is still unknown.

It has been suggested that the New Zealand storm petrel is represented now by a small population that breeds in the Hauraki Gulf, possibly on the Mokohinau Is (Gaskin & Baird 2005), which are <30 km from where the 4 birds were captured. If that assumption is correct, then, based on when the birds are present in the Hauraki Gulf and breeding seasons of other storm petrels (Marchant & Higgins 1990; Brooke 2004), any local breeders should have been at the late incubation stage or have been rearing chicks in early Jan 2006.

The presence of pre-breeding birds in the Hauraki Gulf is consistent with the species breeding there. Non-breeding Procellariiformes, many of pre-breeding age, frequent breeding colonies (Warham 1990; Brooke 2004; Imber *et al*. 2005). For example, 67% of black-bellied storm-petrels *Fregetta tropica* captured at the breeding colony at the end of the hatching period were non-breeders (Hahn 1998). However, it is just as likely that the birds captured were pre-breeders using a male-specific foraging range which is not necessarily near the breeding colony, either during the breeding season itself or even in the non-breeding season. Clearly, we are no closer to locating a breeding colony of the New Zealand storm petrel, which would be a major step in the conservation of this obviously very rare species. Until we know where the species breeds, little can be done to protect it and location of a colony is a conservation priority.

ACKNOWLEDGEMENTS

Permits for this work were provided by the New Zealand Department of Conservation (No. 0442 & No. AK-15117-FAU), and the New Zealand Department of Conservation Animal Ethics Committee (No. AEC 108). We thank Karen Baird, Rohan Clarke, John Ewen, Richard Griffiths, Halema Jamieson, Chris Gaskin, Geordie Murman, and Brett Rathe for their involvement with the captures of the 4 specimens and the taking of samples for DNA analysis. Rob Thomas, Renata Jorge, and Mark Bolton provided information and help on ageing the captured birds, Fiona Robertson assisted in the laboratory. We also thank 2 anonymous referees for helpful comments. This research was supported financially by the New Zealand Department of Conservation (Halema Jamieson) and University of Canterbury funding to BR.

LITERATURE CITED

Ardern, S.L.; McLean, I.G.; Anderson, S.; Maloney, R.; Lambert, D.M. 1994. The effects of blood sampling on the behaviour and survival of the endangered Chatham Island black robin *Petroica traversi*. *Conservation biology 8*: 857-862.

Bartle, J.A. 1990. Sexual segregation of foraging zones in procellariiform birds: Implications of accidental capture on commercial fishery long-lines of grey petrels (*Procellaria cinerea*). *Notornis 37*: 146-150.

Brooke, M. de L. 2004. *Albatrosses and petrels across the world*. Oxford, Oxford University Press.

Fridolfsson, A.K.; Ellegren, H. 1999. A simple and universal method for molecular sexing of non-ratite birds. *Journal of avian biology 30*: 116–121.

Griffiths, R.; Double, M.C.; Orr, K.; Dawson, R.J.B. 1998. A DNA test to sex most birds. *Molecular ecology 7*: 1071–1075.

Hahn, S. 1998. Brutphänologie und morphometrie des schwarzbauchmeerläufers (*Fregetta tropica*) auf King George Island, Antarktis. *Journal of ornithology 139*: 149-156.

Huber, L.N. 1971. Notes on the migration of the Wilson's storm-petrel *Oceanites oceanicus* near Eniwetok Atoll Western Pacific Ocean. *Notornis 18*: 38-42.

Imber, M.J.; Taylor, G.A.; Tennyson, A.J.D.; Aikman, H.A.; Scofield, R.P.; Ballantyne, J.; Crockett, D.E. 2005. Non-

breeding behaviour of Magenta petrels *Pterodroma magentae* at Chatham Island, New Zealand. *Ibis 147*: 758-763.

Marchant, S.; Higgins, P.J. (Co-ordinators) 1990. *Handbook of Australian, New Zealand and Antarctic Birds. Vol 1. Ratites to ducks.* Oxford University Press, Oxford.

Okill, J.D.; Bolton, M. 2005. Ages of storm petrels *Hydrobates pelagicus* prospecting potential breeding colonies. *Ringing & migration 22*: 205-208.

Robertson, B.C.; Millar, C.D.; Minot, E.O.; Merton, D.V.; Lambert, D.M. 2000. Sexing the critically endangered kakapo *Strigops habroptilus*. *Emu 100*: 336–339.

Robertson, B.C.; Gemmell, N.J. 2006. PCR-based sexing in conservation biology: Wrong answers from an accurate methodology? *Conservation genetics 7*: 267-271.

Ryan, P.G.; Boix-Hinzen, C. 1999. Consistent male-biased seabird mortality in the Patagonian toothfish longline fishery. *Auk 116*: 851-854.

Sambrook, J.; Russell, D. 2000. *Molecular cloning: a laboratory manual. 3rd ed*. New York, Cold Spring Harbor Laboratory Press.

Seutin, G.; White, B.N.; Boag, P.T. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian journal of zoology 69*: 82-90.

Warham, J. 1990. *The petrels: their ecology and breeding systems*. London, Academic Press.

Weimerskirch, H.; Jouventin, P. 1987. Population dynamics of of the wandering albatross, *Diomedea exulans*, of the Crozet Islands: Cause and consequences of the population decline. *Oikos 49*: 315-322.

Weimerskirch, H.; Cherel, Y.; Cuenot-Chaillet, F.; Ridoux, V. 1997. Alternative foraging strategies and resource allocation by male and female wandering albatrosses. *Ecology 78*: 2051-2063.

Keywords New Zealand storm petrel; *Pealeornis maoriana*; *Oceanites maorianus*; molecular sexing; conservation