The Auk 111(1):70–79, 1994

POPULATION-GENETIC STRUCTURE OF A PHILOPATRIC, COLONIALLY NESTING SEABIRD, THE SHORT-TAILED SHEARWATER (*PUFFINUS TENUIROSTRIS*)

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ABSTRACT.—Short-tailed Shearwaters (*Puffinus tenuirostris*) are a numerous, colonially nesting seabird that is strongly philopatric. We applied restriction-enzyme analysis of mitochondrial DNA (mtDNA) to 335 individuals from 11 colonies across southeastern Australia to assess population-genetic structure and the amount of genetic variability in this species. Eleven 6/5.33-base and four 4-base restriction enzymes revealed 25 and 48 mtDNA haplotypes in two overlapping surveys of 215 individuals from seven colonies and 231 individuals from eight colonies, respectively. A low mean sequence diversity among individuals (0.247%) and lack of spatial structuring of mtDNA haplotypes suggest a lack of population-genetic structure and a reduced ancestral population size during glaciation, followed by a population and range expansion. Intracolony mtDNA diversities in three recently established colonies and in one colony that has experienced a recent bottleneck were comparable to mtDNA diversities within larger and older colonies. This suggests that, despite strict philopatry in those colonies, colony founding and recovery from population reduction occurs via immigration of a large number of individuals. *Received 27 April 1993, accepted 11 November 1993.*

DIRECT (genetic) and indirect (observational) estimates of the type of genetic-population structure within a species often can produce conflicting results (Moum et al. 1991, Avise et al. 1992, Birt-Friesen et al. 1992). Evidence from band returns suggests that many bird species exhibit strong natal philopatry to a particular site or region. Based on this indirect evidence, Quinn and White (1987) suggested that genetic differentiation among populations of philopatric species may be substantial as a consequence of limited gene flow. However, indirect methods are limited in terms of the number of colonies (space) and the number of years (time) from which data can be collected. These methods, therefore, can often fail to detect rare, but evolutionarily important, events such as large-scale demographic changes and episodic or continuous, low-level dispersal that can affect the genetic structuring of populations (Slatkin 1987, Avise et al. 1992).

Analysis of mitochondrial DNA (mtDNA) has proved to be an informative and useful method for examining population-genetic structure in a variety of organisms, including birds (Ball et al. 1988, Ovenden et al. 1991, Avise et al. 1992, Birt-Friesen et al. 1992). Evolution of mtDNA is more rapid than for nuclear DNA (Brown et al. 1979) and the nucleotide sequence of mt DNA is highly polymorphic among individuals (Avise et al. 1987). Maternal inheritance and haploid transmission result in an effective population size for the mitochondrial genome about one-quarter of that for nuclear genes. Mitochondrial genes, therefore, are more sensitive to the effects of random genetic drift, founder events and bottlenecks than are nuclear genes (Birky et al. 1983). Direct estimates of the type of genetic structure of populations, based on the geographic distribution of extant mtDNA haplotypes that retain a phylogenetic record of population connectedness and historical demographic changes, therefore, reflect past as well as contemporary gene flow.

The Short-tailed Shearwater (*Puffinus tenuirostris*), or muttonbird, is a colonially nesting seabird breeding on islands and headlands along the southern coastline of Australia. Population size is estimated to be 23 million breeding birds with numbers concentrated around Tasmania and the islands of Bass Strait (Skira et al. 1985). Although Short-tailed Shearwaters are trans-equatorial migrants, long-term banding studies suggest strong philopatry, with only limited natal dispersal to adjacent colonies (Wooller et al. 1990). For example, of 23 banded adults found in a survey of 4,573 birds breeding on Little Green Island in Bass Strait during 1988, 17 had

been banded on Little Green Island as adults or nestlings, 3 as nestlings on Great Dog Island, and 3 as surface adults on Fisher Island (Skira pers. comm.). The latter two colonies are less than 5 km from Little Green Island. In addition, approximately 73,000 individuals were banded at various colonies in Victoria, New South Wales (NSW), South Australia and elsewhere in Tasmania over 18 years, but none have been recovered in a number of large surveys on Little Green Island or in annual surveys on Fisher Island (Serventy and Curry 1984).

Populations of the Short-tailed Shearwater have experienced considerable flux within the last 15,000 years. Most current breeding sites would have been unsuitable breeding habitat during the last ice age, when the sea level was at least 100 m lower than it is today (Milliman and Emery 1968); thus, these sites must have been colonized within the last several thousand years. Prior to European settlement (ca. 1800), Aborigines in Tasmania harvested eggs and chicks during the breeding season for food (Ryan 1981). Subsequently, shearwaters have formed the basis of a muttonbird industry with up to one million chicks taken each year for their meat, feathers and oil (Skira et al. 1985). In the last 100 years many new colonies have been established both within and outside the historical range of the species, representing both a major range, and possible population, expansion (Davies 1959, Harris and Bode 1981). Individual colonies have become extinct, fluctuated in size, and suffered severe bottleneck events (Gilham 1962, Harris and Bode 1981).

We applied restriction-enzyme analysis of mtDNA to examine the population-genetic structure of Short-tailed Shearwaters, representing 11 distinct nesting colonies, throughout southeastern Australia. We included one colony that had been subjected to a recent population bottleneck (Gabo Island) and several recently established colonies (Cape Direction, Cape Deslacs and Montague Island). The remaining seven colonies were all known or presumed to have predated European settlement. Philopatry may be acting to maintain genetic differentiation between breeding colonies in this species, as has been shown in colonially nesting Fairy Prions (*Pachyptila turtur*) occupying a similar breeding range (Ovenden et al. 1991). If individual colonies represent distinct intraspecific assemblages of mtDNA haplotypes, colony-specific mtDNA markers could be used to identify the natal origins of captured birds. This could provide valuable information on the relationship between birds during migration and foraging, on wintering grounds, and during the breeding season. In addition, the natal origins of founding females of the recently established colonies could be determined. However, although breeding colonies may be genetically isolated through contemporary philopatry, patterns of mtDNA differentiation may be complicated by the unstable demographic history of this species.

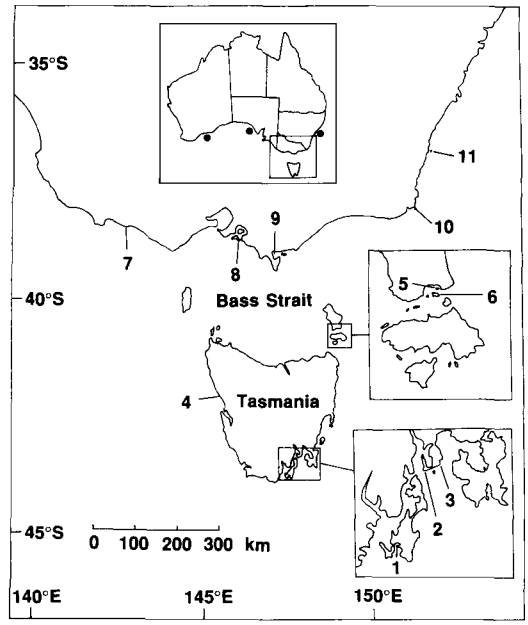

Fig. 1. Locations of breeding colonies of Short-tailed Shearwaters (approximate size in parentheses): (1) Whalebone Point (3,000 burrows; Naarding 1980); (2) Cape Direction (20,000 burrows; Naarding 1980); (3) Cape Deslacs (20,000 burrows; Naarding 1980); (4) Trial Harbour (15,000 burrows; Naarding 1980); (5) Little Green Island (150,000 burrows; Naarding 1980); (6) Great Dog Island (375,000 pairs; Skira et al. 1985); (6) Port Fairy (52,100 burrows; Harris and Norman 1981); (8) Cape Woolamai (356,000 burrows; Harris and Bode 1981); (9) Doughboy Island (2,000 burrows; Harris and Norman 1981); (10) Gabo Island (30,000–40,000 pairs; Reilly 1977); (11) Montague Island (15,000 pairs; Lane 1979). Dots on map inset indicate positions of colonies at extremes of breeding range.

Methods

We collected 335 shearwater chicks during the southern summer breeding seasons from 1989 to 1992 from 11 colonies in southeastern Australia (Fig. 1),

TABLE 1. Numbers of 6/5.33-base restriction enzyme haplotypes scored from each of seven Short-tailed Shearwater colonies. Each haplotype composed of morph designations for restriction enzymes *Afl* II, *Apa* LI, *Ava* I, *Ban* I, *Bcl* I, *Bgl* II, *Bst* XI, *Eco* RV, *Hae* II, *Hin* dIII and *Pvu* II, respectively.

		Locality (*n*)							
	Haplotype	1 Whale-bone Point (29)	3 Cape Deslacs (29)	2 Cape Direc-tion (30)	4 Trial Harbour (30)	5 Little Green Island (34)	6 Great Dog Island (39)	11 Mon-tague Island (22)	Total
a	AAAAAAAAAAA	14	19	17	17	23	25	13	128
b	AAAAAAAABAA	6	5	6	5	4	7	4	37
c	AAAAAAAACAA	2	1	2	4	1	2	1	13
d	AABAAAAABAA	1	0	2	0	2	2	2	9
e	AAAAAABAAAA	1	0	2	0	0	0	0	3
f	AAAAABAABAA	0	0	0	1	0	1	0	2
g	AAAACAAAAAA	0	1	0	0	1	0	0	2
h	AAAAAAAADAA	2	0	0	0	0	0	0	2
i	AABAAABAEAA	1	0	0	0	0	0	0	1
j	AAAADAAABAA	1	0	0	0	0	0	0	1
k	AAAAAADAAAA	1	0	0	0	0	0	0	1
l	AAAAAAAAFAA	0	1	0	0	0	0	0	1
m	AAAAAABABAA	0	1	0	0	0	0	0	1
n	AAAAAACAAAA	0	1	0	0	0	0	0	1
o	AABABAABBAA	0	0	1	0	0	0	0	1
p	AAABAAAAAAA	0	0	0	1	0	0	0	1
q	ABAAAAAAAAA	0	0	0	1	0	0	0	1
r	BABAAAAABAA	0	0	0	1	0	0	0	1
s	AAAAABAACAA	0	0	0	0	1	0	0	1
t	AABABAAABAA	0	0	0	0	1	0	0	1
u	BAAAAAAAAAA	0	0	0	0	1	0	0	1
v	AABAAACABAA	0	0	0	0	0	1	0	1
w	AAAAABAAAAA	0	0	0	0	0	1	0	1
x	AAADAAAAAAA	0	0	0	0	0	0	1	1
y	AABCAAAABAA	0	0	0	0	0	0	1	1

comprising: 6 colonies in Tasmania at Whalebone Point, Bruny Island (43°28'S, 147°14'E, n = 29), Cape Deslacs (42°59'S, 147°33'E, n = 30), Cape Direction (43°06'S, 147°25'E, n = 30), Trial Harbour (41°55'S, 145°09'E, n = 30), Little Green Island (40°13'S, 148°15'E, n = 35) and Great Dog Island (40°15'S, 148°14'E, n = 39); 4 colonies in Victoria at Port Fairy (38°24'S, 142°15'E, n = 30), Cape Woolamai, Phillip Island (38°34'S, 145°22'E, n = 30), Doughboy Island (38°46'S, 146°17'E, n = 30) and Gabo Island (37°34'S, 149°55'E, n = 30); and Montague Island (36°15'S, 150°13'E, n = 22) in NSW. Liver tissue was dissected from dead birds and stored in liquid nitrogen.

We extracted mtDNA from thawed liver samples by a modification (Brasher et al. 1992) of the method of Chapman and Powers (unpubl. report). The mtDNA of 215 individuals collected from Whalebone Point, Cape Deslacs, Cape Direction, Trial Harbour, Little Green Island, Great Dog Island and Montague Island was digested with eight 6-base restriction enzymes (*Afl* II, *Apa* LI, *Bcl* I, *Bgl* II, *Bst* XI, *Eco* RV, *Hin* dIII, *Pvu* II) and three 5.33-base restriction enzymes (*Ava* I, *Ban* I, *Hae* II). For sample sizes see Table 1. Subsequently, with the availability of samples from more colonies and based on the results of the 6/5.33-base enzyme survey, the mtDNA of 231 individuals from Whalebone Point, Trial Harbour, Great Dog Island, Port Fairy, Cape Woolamai, Doughboy Island, Gabo Island and Montague Island were analyzed with four 4-base restriction enzymes (*Hha* I, *Hinf* I, *Msp* I, *Taq* I). All of the birds from Whalebone Point, Trial Harbour, Great Dog Island and Montague Island analyzed with 4-base restriction enzymes were included in the 6/5.33-base enzyme survey. Samples were digested with up to a 10-fold excess of enzyme, but otherwise according to the supplier's (New England Biolabs, USA) instructions. Restriction fragments produced by 6/5.33-base and 4-base restriction enzymes were radioactively labelled with ^{35}S-deoxycytosine triphosphate (Ovenden et al. 1989) and separated by molecular weight through 1.4% agarose gels (Ovenden et al. 1989) and 5% polyacrylamide gels (Smolenski et al. 1993), respectively.

The 6/5.33-base and 4-base restriction-enzyme data were analyzed separately. Unique fragment patterns identified with each enzyme were assigned an uppercase letter, the most common fragment pattern, or morph, for each enzyme being designated "A." For

the 6/5.33-base restriction-fragment profiles, the loss or gain of a restriction site was determined by the loss or gain of appropriately sized fragments. It was difficult to determine 4-base restriction-site gains and/or losses between individuals due to the large number of sites. Subsequent analyses of the 4-base data, therefore, were based on restriction-fragment presence and absence. Each individual in the 6/5.33-base and 4-base enzyme surveys was assigned a composite 11- or 4-letter code or haplotype, respectively, corresponding to the fragment patterns produced from that individual for each restriction enzyme. For the 6/5.33-base enzyme data, nucleotide sequence divergence was calculated between pairs of mitochondrial genomes, with standard errors, using the maximum-likelihood method of Nei and Tajima (1983). An estimate of sequence divergence between individuals was calculated from the presence or absence of 4-base restriction-fragment data using equation 20 in Nei and Li (1979). Net nucleotide sequence divergences and standard errors between pairs of populations were estimated by the method of Nei and Jin (1989). A Student's *t*-test, with Bonferroni correction for the large number of pairwise comparisons (Rice 1989), was used to determine whether the net divergence estimates were significantly different from zero.

A chi-square test was used to compare observed and expected haplotype frequencies to detect possible population subdivision among colonies. The significance of the chi-square value was tested using a Monte-Carlo method (Roff and Bentzen 1989), as expected class sizes were often less than five. An estimate of genetic variability due to geographic subdivision was gained using G_{ST} analysis (Takahata and Palumbi 1985). We used equations 17 and 19 of Takahata and Palumbi (1985) to estimate the intrademe (I) and interdeme (J) identity probability using restriction-site (6/5.33-base enzyme data) or restriction-fragment (4-base enzyme data) presence/absence data from each population. The significance of the G_{ST} value was evaluated by bootstrapping (Palumbi and Wilson 1990), where the true G_{ST} value is compared to 1,000 random G_{ST} values obtained from random rearrangements of the raw data. If the true G_{ST} value was greater than 95% of the random G_{ST} values, we concluded that population subdivision was present. Wagner-parsimony analysis of the 6/5.33-base restriction-enzyme site data was performed using the tree-bisection-reconnection branch-swapping algorithm of version 3.1 of the computer program PAUP (supplied by D. L. Swofford, Illinois Natural History Survey, Champaign).

Results

6/5.33-base enzyme survey.—The 11 restriction enzymes identified 63 restriction sites among 215 shearwater mitochondrial genomes, with 44 to 52 restriction sites assayed per mtDNA clone. For four enzymes an approximately 200-base-pair insert in two individuals compared to all other mtDNA genomes was observed. These individuals were omitted from subsequent analyses. The size ($\bar{x} \pm$ SE) of the shearwater genome was estimated to be 20,768 $\pm$ 62 base pairs from the sum of all fragments for a single individual representing each enzyme morph on all gels.

Each restriction enzyme yielded one to six morphs ([1] *Hin* dIII and *Pvu* II; [2] *Afl* II, *Apa* LI, *Ava* I, *Bgl* II and *Eco* RV; [4] *Ban* I, *Bcl* I and *Bst* XI; [6] *Hae* II), which in combination identified 25 haplotypes, differing by 1 to 10 restriction-site gains or losses. There were 40 monomorphic restriction sites (present in all haplotypes) and 23 polymorphic sites (occurring in some but not all haplotypes). The three most common haplotypes (a, b and c) occurred in all seven colonies and collectively accounted for 84% of all individuals surveyed (Table 1). The remaining 22 haplotypes were represented by only one to nine individuals and, with one exception (haplotype d), were shared between two colonies (haplotypes e, f and g) or exclusive to single colonies (haplotypes h-y). Parsimony analysis of the substitutional relationships among the 25 haplotypes produced 675 most-parsimonious networks of length 31 steps. A 50% majority-rule consensus network, of length 34 steps, revealed two groups centered on common haplotypes a and d, separated by five mutational steps (Fig. 2). Two of these changes involved a single *Bgl* II restriction site (i.e. a reversal) and a third (*Ava* I site 2) exhibited considerable homoplasy in the group centered on haplotype d. Most haplotypes within each group were separated by only two or three restriction-site changes.

The mean mtDNA sequence diversity among the 213 shearwater genomes was 0.247 $\pm$ 0.100%, with a range of 0 to 1.850%. No variation in the intrapopulational sequence diversities was detected between the seven shearwater colonies (Table 2). The net interpopulational nucleotide sequence divergence between birds from pairs of colonies was zero ($n = 17$) or not significantly different from zero ($P > 0.2$; $n = 4$) in all 21 possible pairwise comparisons. Haplotype frequencies for individuals sampled from the seven colonies were also not significantly different ($X^2 = 41.24$, $P = 0.49 \pm 0.032$). The amount of genetic variation among colonies due to geographic subdivision (G_{ST}) was 0.284. This value

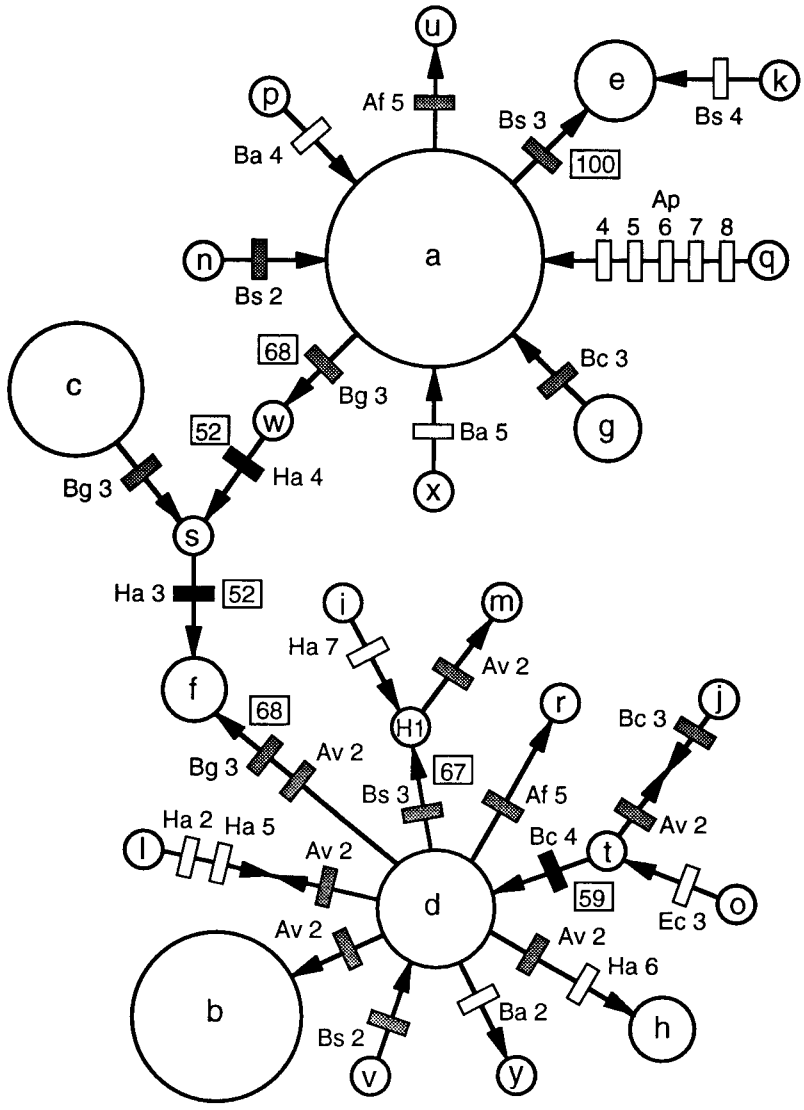

Fig. 2. Unrooted consensus network describing relationships among 25 Short-tailed Shearwater mtDNA haplotypes. Values in boxes alongside branches represent percentage of times that group above that branch (assuming haplotype d represents bottom of network) occurred in 675 parsimonious 31-step trees. Haplotype designations as in Table 1. Haplotype H1 represents a hypothetical haplotype that was not observed. Labelled bars and arrows on lines joining haplotypes indicate direction of loss of restriction sites. Each restriction site is denoted by first two letters of enzyme and site number for that enzyme (e.g. Bg 3 = *Bgl* I site 3). Open and solid bars represent site changes that were autapomorphic with respect to haplotypes and clades, respectively. Shaded bars represent convergent synapomorphic site changes. Size of each circle is proportional to frequency of that haplotype.

does not demonstrate significant population differentiation as it is greater than only 46% of the 1,000 bootstrapped G_{ST} values (range 0.264 to 0.323).

4-base enzyme survey.—The four restriction-enzymes produced 117 restriction fragments from the 231 shearwater mitochondrial genomes. Almost twice as many fragments (83–86) were identified per mtDNA clone, compared to the 6/5.33-base enzyme survey.

We identified 48 composite haplotypes, consisting of 6 to 15 morphs per restriction enzyme ([6] *Hinf* I; [8] *Taq* I; [11] *Msp* I; [15] *Hha* I). Three haplotypes (1, 2 and 3) were common to all eight

TABLE 2. Percent intrapopulational mtDNA nucleotide sequence diversity ($\bar{x} \pm$ SE) for 11 Short-tailed Shearwater colonies.

Locality	6/5.33-base enzyme	4-base enzyme
1 Whale Point	0.3286 ± 0.1396	0.2038 ± 0.1104
4 Trial Harbour	0.2710 ± 0.1342	0.2304 ± 0.0980
2 Cape Direction	0.2565 ± 0.1312	—
3 Cape Deslacs	0.2142 ± 0.1132	—
6 Great Dog Island	0.2205 ± 0.1215	0.2639 ± 0.1004
5 Little Green Island	0.2142 ± 0.1026	—
7 Port Fairy	—	0.2455 ± 0.1000
8 Cape Woolamai	—	0.2573 ± 0.0943
9 Doughboy Island	—	0.2952 ± 0.1078
10 Gabo Island	—	0.2145 ± 0.0948
11 Montague Island	0.2581 ± 0.1300	0.2462 ± 0.1081

colonies sampled and represented 39% of all individuals (Table 3). Three haplotypes (4, 5 and 6) were found in seven colonies. The remaining 42 haplotypes were represented by eight or fewer individuals, and occurred in fewer than five of the eight colonies. Thirty haplotypes were exclusive to a single colony.

The mean mtDNA sequence diversity among the 231 shearwater genomes was 0.247 ± 0.060%, with a range of 0 to 0.890%. Mean intrapopulational diversities were similar both among colonies and with estimates derived from the 6/5.33-base enzyme survey (Table 2). Pairwise comparisons of all eight colonies revealed 27 net interpopulational mtDNA divergence estimates that were either zero ($n = 13$) or not significantly different from zero ($n = 14$). The one remaining comparison between the Doughboy Island and Trial Harbour colonies yielded a significantly large net interpopulational mtDNA divergence of 0.0158 ± 0.0048% ($0.002 > P > 0.001$). A chi-square test of homogeneity of morphs for all haplotypes occurring more than once was nonsignificant ($X^2 = 130.65$, $P = 0.195 \pm 0.025$). The G_{ST} value for the eight populations was 0.1922, being greater than only 42% of the 1,000 bootstrapped G_{ST} values (range 0.172 to 0.209).

DISCUSSION

The phylogeographic structure of a species comprises two, partly interdependent components: (1) the magnitude of mtDNA sequence diversity; and (2) the degree of spatial structuring of mtDNA haplotypes (Avise 1989). Short-tailed Shearwaters across southeastern Australia exhibited a low estimated mean sequence diversity among pairs of mtDNA genomes and no geographic structuring among mtDNA haplotypes, with the most common haplotypes present in colonies throughout the surveyed range. The phylogenetic network (Fig. 2) revealed two closely related groups of mtDNA haplotypes that were geographically widespread. Together, these aspects of shearwater mtDNA variability demonstrate a lack of detectable phylogenetic population structure in the Short-tailed Shearwater.

This result is, in part, unexpected for a number of reasons. First, the colonies included in this study encompass the majority (in terms of numbers of birds) of the species' breeding range; individual colonies were separated by distances of up to 860 km. Second, no long-distance natal or breeding dispersal has been recorded in this species despite large-scale and long-term banding and recovery surveys. Over a 40-year period, only 23 successful natal dispersals have been recorded in surveys of tens of thousands of burrows; each dispersal event involved a distance of less than 5 km between natal and breeding colony (Skira pers. comm., Serventy and Curry 1984, Wooller et al. 1990). Avise et al. (1992) have discussed the problems raised by such contrast between band-return studies and the geographic distributions of mtDNA. They suggested that "an understanding of population structure requires the integration of both evolutionary (genetic) and contemporary (direct observational) perspectives." Third, strict philopatry has been demonstrated by restriction-enzyme analysis of mtDNA in at least one colony of the Fairy Prion, a colonially nesting seabird occupying a breeding range similar to that of the Short-tailed Shearwater (Ovenden et al. 1991).

The significantly large net interpopulational sequence divergence between the Doughboy Island and Trial Harbour colonies suggests lim-

TABLE 3. Numbers of 4-base restriction enzyme haplotypes scored from each of eight Short-tailed Shearwater colonies. Each haplotype composed of morph designations for restriction enzymes *Hha* I, *Msp* I, *Hinf* I and *Taq* I, respectively.

	Locality (*n*)								
Haplotype	1 Whale-bone Point (29)	4 Trial Harbour (30)	6 Great Dog Island (30)	7 Port Fairy (30)	8 Cape Woolamai (30)	9 Dough-Boy Island (30)	10 Gabo Island (30)	11 Montague Island (22)	Total
1 AAAA	11	7	3	5	10	2	5	3	46
2 ABAA	3	3	5	4	1	3	5	2	26
3 CAAA	4	3	2	2	1	2	3	1	18
4 BAAA	0	4	4	3	4	2	6	5	28
5 BBAA	1	2	2	5	2	1	3	0	16
6 DAAA	3	2	1	2	2	0	1	2	13
7 CBAA	3	0	1	0	0	2	0	2	8
8 CDAA	1	1	0	0	2	1	0	1	6
9 DBAA	0	2	0	0	1	2	1	0	6
10 EAAA	1	0	0	2	0	2	0	0	5
11 FAAA	0	0	3	0	0	1	0	1	5
12 CAAB	0	1	2	1	0	0	1	0	5
13 FBAA	0	0	0	1	0	3	1	0	5
14 CCAA	0	0	0	1	0	1	1	2	5
15 EBAA	0	1	1	0	0	0	1	0	3
16 AAAC	0	0	1	0	1	0	0	0	2
17 EAAB	0	0	1	0	0	1	0	0	2
18 CFAA	0	0	0	0	1	1	0	0	2
19 IAAA	1	0	0	0	0	0	0	0	1
20 BGAA	1	0	0	0	0	0	0	0	1
21 AADA	0	1	0	0	0	0	0	0	1
22 ABCA	0	1	0	0	0	0	0	0	1
23 GAAA	0	1	0	0	0	0	0	0	1
24 BAAE	0	1	0	0	0	0	0	0	1
25 BCAA	0	0	1	0	0	0	0	0	1
26 HDAD	0	0	1	0	0	0	0	0	1
27 JBBA	0	0	1	0	0	0	0	0	1
28 BBAF	0	0	1	0	0	0	0	0	1
29 BIAA	0	0	0	1	0	0	0	0	1
30 KAAA	0	0	0	1	0	0	0	0	1
31 BEAA	0	0	0	1	0	0	0	0	1
32 CAAC	0	0	0	1	0	0	0	0	1
33 CCAG	0	0	0	0	1	0	0	0	1
34 CAAH	0	0	0	0	1	0	0	0	1
35 DDAA	0	0	0	0	1	0	0	0	1
36 LAAC	0	0	0	0	1	0	0	0	1
37 AHAA	0	0	0	0	1	0	0	0	1
38 AJAA	0	0	0	0	0	1	0	0	1
39 AEAA	0	0	0	0	0	1	0	0	1
40 DKAA	0	0	0	0	0	1	0	0	1
41 ECEA	0	0	0	0	0	1	0	0	1
42 EAFA	0	0	0	0	0	1	0	0	1
43 MCBA	0	0	0	0	0	1	0	0	1
44 DAAB	0	0	0	0	0	0	1	0	1
45 AAAB	0	0	0	0	0	0	1	0	1
46 NAAA	0	0	0	0	0	0	0	1	1
47 ACAA	0	0	0	0	0	0	0	1	1
48 OAAA	0	0	0	0	0	0	0	1	1

ited gene flow between them. This result is difficult to understand since: (1) the Doughboy Island colony is situated near the center of the species' range; (2) neither colony displays any apparently unique characters in terms of its size, age or history compared to any other colony; and (3) neither colony appears to be genetically isolated from the three more geographically

distant colonies at Montague Island, Gabo Island and Whalebone Point.

The limited geographic structuring among mtDNA haplotypes suggests contemporary gene flow and/or recent evolutionary connections among populations (Slatkin and Maddison 1989). Indeed, the geographically widespread occurrence of both the common and rare haplotypes, observed in more than one individual, indicates an extensive and recent intercolony exchange.

Birky et al. (1983) and Slatkin (1987) have shown that an exchange of as few as one or two females per generation would be sufficient to counteract genetic drift in the mitochondrial genome. Assuming genetic equilibrium within populations, the effective gene flow, or number of females exchanged per generation (N_em), among colonies can be estimated as

$$N_em = (1/G_{ST} - 1)/2 \quad (1)$$

(Takahata and Palumbi 1985). The G_{ST} values calculated from the 6/5.33-base and 4-base enzyme data give N_em estimates of 1.4 and 2.1, respectively. This suggests a low level of gene flow, which would be sufficient to maintain the observed genetic homogeneity of mtDNA haplotypes. However, due to recent fluctuations in overall population and individual colony size and colony number (see below), it is unlikely that Short-tailed Shearwater populations have reached genetic equilibrium. It is not possible, therefore, to distinguish between contemporary gene flow or historical associations of colonies using this method (Slatkin and Maddison 1989). Banding studies suggest strong philopatry, although a small number of natal dispersals to geographically very close colonies have been recorded (Wooller et al. 1990). However, no long-distance dispersal has been reported. Based on this evidence it is unlikely that a low level of intercolony movement by females is responsible for the observed homogeneity of mtDNA haplotypes.

Gene flow among colonies, however, may involve rare, large-scale movements of individuals (Slatkin 1985, Avise et al. 1992). Circumstantial evidence of this type of gene flow is available, given the low intrinsic population growth through reproduction (Wooller et al. 1988). The Montague Island colony and others on the NSW coast were founded in the last 35 years (Lane 1979) and have grown rapidly. Similarly, colonies at Cape Direction and Cape Deslacs appeared early this century (Bryden 1966). The Gabo Island colony suffered a severe population bottleneck in 1959 (Gillham 1962) from which it has rapidly recovered to a size of 30,000 to 40,000 pairs (Reilly 1977). The genetic data support this hypothesis. The intrapopulational sequence diversities (Table 2) in the recently established colonies at Cape Direction, Cape Deslacs and Montague Island, and in the Gabo Island colony following the bottleneck event are similar to the genetic diversity for the population overall. This suggests large founder populations and population recovery via a substantial, and probably opportunistic, immigration of birds (Slatkin and Maddison 1989). Birt-Friesen et al. (1992) have suggested a similar scenario for philopatric, colonially nesting Thick-billed Murres (*Uria lomvia*). In contrast, a recently formed Fairy Prion colony was found to be devoid of mtDNA restriction-site variation, suggesting a very small founder population and a lack of large-scale gene flow (Ovenden et al. 1991).

Regardless of current levels of gene flow, the limited mtDNA differentiation among colonies may have occurred through recent evolutionary interconnections. Most current breeding colonies must have originated in the last 10,000 years and may have been derived from a restricted breeding population during the Pleistocene glaciation.

The mean nucleotide sequence diversity among pairs of shearwater mtDNA genomes is consistent with the common pattern of low intraspecific mtDNA variability found in birds (Ball et al. 1988, Moum et al. 1991, Birt-Friesen et al. 1992). A low intraspecific mtDNA sequence diversity suggests a reduced long-term effective population size (N_e) relative to the current breeding population size, as a consequence of a restricted number of female ancestors that have contributed to the current gene pool (Avise et al. 1988). The last glacial period played a major role in influencing mtDNA diversity in avian species through population bottlenecks and founder events during subsequent population expansion from a restricted number of breeding refugia (Tegelström et al. 1990, Moum et al. 1991, Ovenden et al. 1991, Birt-Friesen et al. 1992).

The low mtDNA sequence diversity in shearwaters indicates similar fluctuations in population size, probably involving one or more population bottlenecks during glaciation and a recent expansion to current population levels. Shearwaters may have experienced a very re-

cent population expansion given that a number of new colonies have been formed both inside and outside the historical breeding range (Bryden 1966, Harris and Bode 1981).

The pattern of population-genetic structure in the Short-tailed Shearwater indicates that shearwater colonies have had extensive and evolutionarily recent genetic contact. These results do not refute observational data indicating strong contemporary philopatry, nor do they imply present-day panmixia. Instead the mtDNA data suggest a scenario involving a population bottleneck during the last glaciation, and subsequent population and range expansion that has continued until present times. In combination with observational data, the genetic data also suggest gene flow occurring via rare, large-scale dispersal events. In contrast to the sympatric species, Fairy Prion, where very small founding populations are implicated, establishment of Short-tailed Shearwater colonies and recovery from population bottlenecks appear to involve an immigration of large numbers of individuals.

ACKNOWLEDGMENTS

We thank the following for logistical support: the Department of Parks, Wildlife and Heritage, Tasmania; Department of Conservation and Environment, Victoria; and the NSW National Parks and Wildlife Service. Matt Edmunds, Irynej Skira, Tim Lamb, Greg Austin, Peter Fullagar and Mike Crowley assisted with collection of material. Eldredge Birmingham, Vicki Birt-Friesen and Bill Montevecchi provided helpful comments that improved the manuscript. The project was funded by Pasminco Metals-EZ and a grant from the Victorian Institute of Marine Science.

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