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Behavioural and molecular evidence for specific status of light and dark morphs of the Herald Petrel *Pterodroma heraldica*

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The Herald Petrel *Pterodroma heraldica* has an extensive subtropical breeding range across the width of the Pacific Ocean. It occurs in dark and light morphs, and, at most breeding colonies, the latter is more numerous. However, on Henderson Island, one of the Pitcairn Islands of the Central Pacific, the dark morph is more numerous. During a field study at Henderson Island, we found evidence of reproductive isolation between light and dark Herald Petrels. The morphs bred and courted assortatively. They also tended to breed in different parts of the island (dark birds nearer the coast) and at slightly different seasons (dark birds mostly in the austral winter, light birds more evenly throughout the year) and uttered different calls. Together, these data strongly suggested that the morphs are distinct species. This conclusion was supported by sequence data from a 307-base pair region of the cytochrome *b* gene of the mitochondrial genome. When birds of both morphs from Henderson and light morphs from Ducie Atoll (345 km west of Henderson) were sampled, five haplotypes were restricted to light Herald Petrels; they did not occur in dark birds. In contrast, three haplotypes were restricted to dark Herald Petrels on Henderson Island. Thus, the haplotypes of the two morphs were mutually exclusive, further indicating reproductive isolation between them. We propose that the white-bellied form should retain the name Herald Petrel *P. heraldica*, while the dark-bellied form should be granted specific status as the Henderson Petrel *Pterodroma atrata* (Mathews 1912). The name *atrata* derives from a description written by Solander on Cook's first voyage. Molecular data from Round Island Petrels *Pterodroma arminjoniana* confirmed the close relationship between this form and *heraldica*. However, *arminjoniana* and *atrata* did not share sequences for the cytochrome *b* region studied.

When Murphy (1949) described a new *Pterodroma* gadfly petrel, he gave it the specific epithet *ultima* in the belief that it would be the last species from the genus to be described. This proved premature, for Barau's Petrel *Pterodroma baraui* was described subsequently (Jouanin 1963). However, the fact remains that, in common with other seabird groups, the majority of the approximately 28 (Warham 1990) gadfly petrels were known scientifically by the end of the last century. Nevertheless, the classification of the gadfly petrels has continued to provoke debate (Mathews 1934, Imber 1985, Warham 1990) since the boundaries between species appear difficult to draw, even when evidence from different characters is applied.

Evidence marshalled by Imber (1985) in his extensive study of the taxonomy of the group emphasized skeletal features, intestinal twisting, underwing pattern, calls and feather lice. Bourne (1983) highlighted how data on the timing of breeding may bear on taxonomic decisions, while Bretagnolle (1990, 1995) showed how calls can play a part in elucidating taxonomic problems.

Partly because gadfly petrels generally nest on the remotest oceanic islands and because the breeding season is characteristically protracted, the basic breeding biology of some species has not been described. This is of especial relevance where species exist in more than one morph and the breeding relationships between birds belonging to different morphs, a point of obvious taxonomic significance, remain unknown. Furthermore, the molecular techniques that have proved so useful in other taxonomic areas have as yet been little used on *Pterodroma* species (Nunn 1994, Paterson *et al.* 1995).

The study we report here on the polymorphic Herald Petrel *Pterodroma arminjoniana* (*sensu* Warham 1990) combines behavioural data from a field study with mitochondrial DNA (mtDNA) sequence data. Both strands of data suggest the need for taxonomic revision.

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The historical picture

Pterodroma arminjoniana breeds in the (sub)tropical Atlantic, Indian and Pacific Oceans. The taxonomic picture, both past and present, relates to this widespread distribution. *Pterodroma arminjoniana* was described by Giglioli and Salvadori (1869) on the basis of a type specimen collected in 1868 near the South Atlantic breeding station of Trindade (=South Trinidad) Island. This specimen was white-bellied; however, the species is polymorphic. There are also a wholly dark phase and forms intermediate between it and the brown-and-white pattern of the type. Various synonyms, now out of use, have been ascribed to these other forms (*trinitatis, sandaliata, wilsoni* and *chionophara*; Hellmayr & Conover 1948, Murphy & Pennoyer 1952). As far as we know, the Atlantic birds currently nest only at Trindade and the neighbouring rocks of Martin Vaz (Murphy & Pennoyer 1952, Harrison 1987).

In 1948, another population of *arminjoniana* was discovered breeding on Round Island off Mauritius in the Indian Ocean (Vinson *in* Gardner *et al.* 1985). According to Murphy and Pennoyer (1952, p. 38), "the Indian Ocean specimens are quite indistinguishable from the South Atlantic examples but are definitely larger than birds in our extensive South Pacific series". No scientific names have been ascribed exclusively to the Round Island petrels, which, as is true on Trindade, occur in dark, light and intermediate forms.

From the Pacific, *Aestrelata* (=*Pterodroma*) *heraldica* was described in 1888 from two specimens obtained at Chesterfield Island, northwest of New Caledonia. Both the type and the paratype are white-bellied (Salvin 1888, M. de L. B. & G. R., pers. obs. of the two specimens held in the British Museum). However, *P. heraldica* in its extensive South Pacific breeding range—islands off northeastern Australia east to Easter Island (Warham 1990)—occurs in both light (i.e. white-bellied) and dark (i.e. dark brown-bellied) forms. The birds from Easter Island have been differentiated as *Pterodroma heraldica paschae*, primarily on the basis of differences in undertail-coverts (Lönnberg 1921). All three full-grown birds discussed by Lönnberg were light.

To our knowledge, the only name that may attach to the dark form in the Pacific is *Procellaria atrata*. This name was given to a bird collected on 21 March 1769 during Cook's first voyage at 25°21'S, 129°W and described by Solander. A translation of Solander's description is given in Appendix 1, which indeed seems a valid description of the dark morph of the Herald Petrel. However, Solander did not publish his description. Only a century and a half later was the description reproduced by Mathews (1912–1913) from Solander's notebooks, with Mathews uncertain as to *atrata*'s identity. It fell to W.R.P. Bourne (*in* Lysaght 1959) to suggest that *atrata* might be the dark morph of *heraldica*.

Despite the size differences between *arminjoniana* and *heraldica* (the former is the larger) Murphy and Pennoyer (1952) appreciated the close relationship between the two and treated *heraldica* as the Pacific representative of Atlantic *arminjoniana*. They wrote (on p. 38) "the two forms have precisely the same proportions and plumage pattern". In the next major review of the genus *Pterodroma*, Imber (1985) gave specific status to both *arminjoniana* and *heraldica* because of their geographic separation and because they host different *Halipeurus* feather lice. He placed the two forms in the subgenus *Hallstroma* that also included the species *alba*, which is exclusively white-bellied, and *neglecta*, which is polymorphic like *arminjoniana* and *heraldica*. The subgenus also included the more distantly related species *phaeopygia, externa* and *baraui*. Warham (1990), giving no reason for adjusting Imber's classification, restored *heraldica* to subspecific status.

Background to present study

Reporting on *heraldica* in the American Museum's collection, Murphy and Pennoyer (1952) noted striking differences in the morph proportions at different breeding stations, with light birds usually more numerous than dark birds. In particular, there were differences between the birds collected at the Central Pacific islands of the Pitcairn group in 1922 by the Whitney South Sea Expedition (see also Bourne & David 1983, 1985). The low atolls of Ducie and Oeno held predominantly light birds (42:2 and 22:0 [light:dark], respectively), while the situation was reversed (5:53) on the raised limestone island of Henderson.

During fieldwork of the 1991–1992 Sir Peter Scott Commemorative Expedition to the Pitcairn Islands, we had an opportunity to explore whether, 70 years later, the interisland differences had been maintained. In so doing, we found strong evidence for assortative mating among the morphs. Backed by further behavioural and molecular evidence, the case for treating the former *heraldica* as two species grew. As this case strengthened, we expanded the study to the wider *heraldica/arminjoniana* complex.

METHODS

Study area, field methods and analysis

Fieldwork was undertaken from January 1991 to March 1992. The greater part of this period was spent on Henderson Island (24°22'S, 128°20'W), where most work was done. However, short visits of approximately 5 days were made to the atolls of Oeno (235 km to the west) and Ducie (345 km to the east). Oeno was visited four times and Ducie thrice in the study period. Precise dates are given by Brooke (1995).

On the 37 km^2 of Henderson Island, nests of Herald Petrels were found dispersed across the plateau about 25 m a.s.l. As nests were encountered opportunistically, usually during incubation, they were marked and then revisited about once a week. The position of each nest in relation to a grid of paths of measured length was noted.

Breeding adults on Henderson were measured using standard methods (Brooke 1990) and, wherever possible, sexed

by cloacal examination (Serventy 1956). Eggs were measured to 0.1 mm using Vernier calipers.

Because nests were located after the eggs were laid, the timing of laying was, where possible, determined retrospectively by subtracting the incubation period of around 50 days (Gill *et al.* 1970, Warham 1990) from the hatching date. Where the egg was lost before hatching, the laying date could sometimes be assigned only to 1 of 2 months. In the analysis below (Table 2), such cases (10 of 36) were scored as two "half-layings," one in each of the 2 months involved.

On the three Pitcairn Islands, Herald Petrels were easily classified as light or dark since no intermediate birds were found.

Courting birds frequently called in pairs in flight. This activity occurred through the daylight hours, reached a peak around dusk and then ceased shortly after dark, about 2000h. Daytime observations were made on the phase of birds calling in flight in pairs, identified because they flew in unison in close proximity. By restricting the observations to calling birds, we could be certain that the aerial observations did not include any Kermadec Petrels *Pterodroma neglecta*, which also nest on the islands (Brooke 1995) and look, but do not sound, similar.

Systematic observations were made on Henderson Island on pairs of calling, courting birds in all months from July onwards. Since flying pairs might call in more than one location on the same day or in a similar location on different days, observations were considered independent if they referred to pairs separated by more than 200 m (same day) or to pairs separated by more than 2 weeks (same location). Although it was usually very evident which two birds were associating in a courting pair, there were a few occasions, involving several birds flying together, when subjective judgement was exercised.

Tape recordings of Herald Petrels were obtained on Henderson Island between August 1991 and February 1992 using a Realistic Minisette-20 cassette recorder and a Realistic 33-1073A microphone. The birds were not prompted to call by playback; they were all calling naturally, either from the ground or in the air. In both circumstances the commonest call was a *kyek-kyek-kyek* . . . cry, sustained for a few seconds. Most calling birds belonged to a pair; therefore, a careful watch of beak movement was made to ensure that each recording referred to the call of a single bird which was assigned to a morph. The gender of the calling birds was not determined, but, given the variety of dates and circumstances when recordings were made, there was no reason to suspect that calls obtained from light and dark morph birds differed significantly with respect to the sex ratio of the birds recorded. Although other types of call besides *kyek* were heard (Holyoak & Thibault 1984), they were not analysed.

In the laboratory analysis of *kyek* calls, aerial and ground vocalizations were considered together, for they sounded similar in the field. No correction for possible Doppler shifts was made since recordings of flying birds were typically

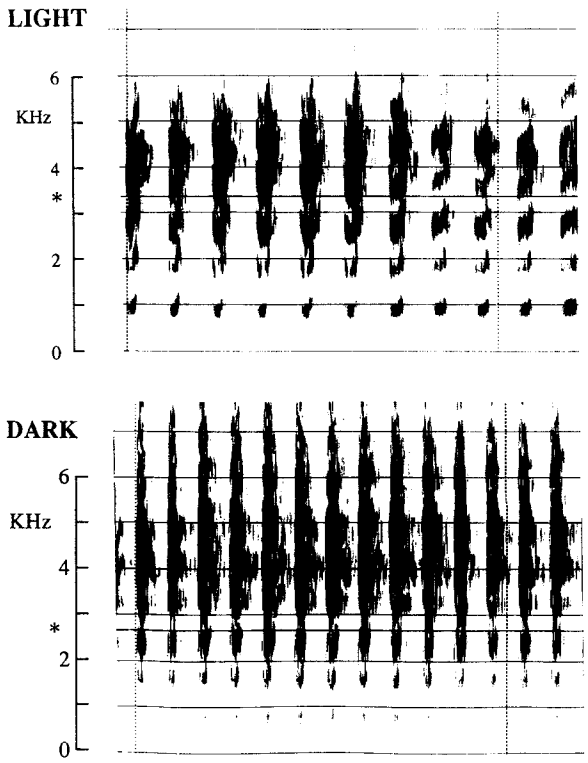

Figure 1. Sonagrams of the calls of light and dark morph Herald Petrels on Henderson Island. The distance between vertical dotted lines represents 1 s, a time which spans nine *kyeks* given by the light bird and 12 given by the dark bird (see Methods). Asterisks are placed alongside horizontal lines which show the highest frequency of the call's third harmonic, the frequency measure used in this study.

made as a bird approached the microphone, passed close and then retreated. After discarding imperfect recordings (e.g. where there was evidence of weak batteries), the remaining calls were analysed using a Kay DSP Sona-Graph (model 5500- 1). Using the Sona-Graph's time and frequency cursors, two measures of call structure were made. The first was the time to complete 10 *kyeks*, measuring from the first *kyek* that began at least half a second after the start of the call. This constraint was introduced to remove any effects associated with the call's start. The second measurement was the highest frequency of the third harmonic of the first *kyek* to the right of the time cursor. These measures are illustrated in Figure 1.

To compare the structure of *heraldica* and *arminjoniana*, we measured skins held by the American Museum of Natural History, New York. In 1992, we measured the entire holding of *heraldica* from the Pitcairn Islands of Oeno, Ducie and Henderson (all specimens were collected during the Whitney Expedition) and the entire holding of *arminjoniana*.

Pterodroma heraldica blood samples were obtained from dark and light birds on Henderson but only from light birds on Ducie since no dark birds nested there. The Henderson birds sampled were mostly adults (30 of 34), whereas those on Ducie were mostly chicks (15 of 16). Twenty *arminjon-*

Table 1. *Mean (±s.d.) measurements and egg sizes (both mm) of dark and light morph Herald Petrels breeding on Henderson Island. Samples sizes: 25 for dark and 19 for light measurements; 20 for dark and 10 for light eggs. Significant differences (t-test) are indicated by a single asterisk (P < 0.05)*

	Dark morph		Light morph
Wing	280.8 ± 5.54		279.8 ± 5.50
Culmen	27.3 ± 0.88		27.4 ± 0.80
Tarsus	35.2 ± 1.04		35.1 ± 1.05
Tail	114.3 ± 3.74		114.3 ± 3.98
Egg			
Length (L)	58.7 ± 2.93		58.0 ± 2.37
Breadth (B)	41.7 ± 1.42	*	40.2 ± 1.83
Volume index ($L \times B^2/10^3$)	102.2 ± 10.1	*	93.9 ± 10.2

iana samples were obtained from birds of dark, light and intermediate phases on Round Island. Blood samples were also obtained from *P. alba* on Christmas Island, South Pacific ($n = 6$). In all cases, 100-µl samples were taken from the brachial vein and stored in 1 cm^3 of absolute ethanol at ambient temperature until we returned to the laboratory. They then remained refrigerated at 5°C until analysis.

Laboratory analysis

PCR amplification

DNA was extracted following the procedure of Burke and Bruford (1987). For each individual, 0.5 µg of the genomic DNA provided templates in a polymerase chain reaction (PCR) (Saiki *et al.* 1985, 1988) using Kocher *et al.*'s (1989) mtDNA cytochrome *b* primers (Light: 5'-AAAAAGCTTCCA-TCCAACATCTCAGCATGATGAAA-3' and Heavy: 5'-AA-ACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3'). The 3' bases of the Light and Heavy primers are located at positions 14990 and 15298, respectively, of the complete chicken mitochondrial genome (Desjardins & Morais 1990), which is used in this paper as a reference sequence.

The PCR reaction was carried out in a total volume of 20 µl, using the mix of Jeffreys *et al.* (1988), with 1.0 µM of each primer and 2 units of "Thermostable" *Taq* polymerase (Advanced Biotechnologies). After mixing, the sample was overlaid with 40 µl sterile paraffin oil and microfuged.

Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler using the following conditions: denaturation, 94°C, 1 min; annealing, 45°C, 1 min; extension, 72°C, 2 min. This was cycled 35 times. A 45°C annealing temperature increased the PCR product yield without any apparent decrease in the specificity of priming or the quality of the sequences obtained. A PCR product was still obtained at an annealing temperature of 55°C; the upper bound was not investigated. After amplification, the reaction was extracted with 200 µl chloroform to remove the oil, and the 20-µl reaction volume was removed and added to 5 µl of 5× loading buffer in a separate tube. The samples were loaded onto a 1.5% agarose (SeaKem) gel containing 0.5 µg per cm^3 ethidium bromide. The amplified 376-base pair product was made visible using a UV hand-wand. The PCR product was run to the end of the gel and electro-eluted onto boiled dialysis membrane. The PCR product was spun off the membrane (tethered in the cap of a 1.5-cm^3 microfuge tube) by a 5-min microcentrofugation, giving an approximately 100-µl volume. Precipitation of the PCR product was by addition of 400 µl 4M ammonium ethanoate and 400 µl propan-2-ol, followed by a 30-min microfugation at 14,000 rpm. The pellet was washed in 80% ethanol and dried. The DNA was resuspended in 30 µl ultrapure water for dideoxy-terminator sequencing (Sanger *et al.* 1977).

Sequencing

The concentration of the template was checked, by eye, by running 1.0 µl on an agarose gel. We used 1.0–6.0 µl for direct double-stranded sequencing with T7 polymerase (Pharmacia), using a USB (Amersham) sequencing kit and following the manufacturer's instructions except the labelling mix was diluted 1:20.

Two separate annealing reactions were prepared in microfuge tubes for each template, each using 1.5 µl of one of the 10 µM primers used in the original amplification. After mixing, the samples were boiled for 3 min and snap-cooled in dry-ice. Then [α^{35}S]-dATP (Amersham) was incorporated as a label. The termination reactions were carried out in a 60-well (5.5 cm × 8 cm) Terasaki microtest plate (Nunc) held on a heating block at 46°C.

Six percent polyacrylamide sequencing gels were run on a BRL S2 sequencing kit, prepared and run following the manufacturer's instructions. The gels were pre-warmed for 30 min, and before loading, the samples were heated in a Terasaki plate on a heating block at 85°C for 3 min to denature the DNA. The gels were run as standard for 2.5 h.

The sequences were read directly from the film after obtaining the optimal exposure. As the templates were sequenced using both Light and Heavy primers, approximately 60% of the sequence could be read from both strands. This helped to improve the accuracy of base-calling. For more than half of the sequencing reactions, a second gel was run for 4.5 h. These long-run gels aided the calling of bases distant from the sequencing primer.

Sequence data from this study have been deposited with GenBank.

RESULTS

From the field

The Herald Petrels breeding on Henderson Island did not differ significantly in size according to the colour morph (Table 1). In fact, the most obvious plumage difference between

Table 2. *Recorded laying months of dark and light morph Herald Petrels on Henderson Island, early 1991 through early 1992*

Morph	Number of nests													Total nests
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	
Dark	—	1	1	3	11	5.5	1.5	—	—	1	—	—	—	24
Light	0.5	1.5	3	—	1	2	—	—	—	1	1	1	1	12

morphs, belly colour, was not even skin deep. The superficially dark feathers of dark-bellied birds were brown only along the distal third of their length. The remainder of the feathers was white, as in light birds.

Although morphological differences were slight, the morphs maintained total breeding separation on Henderson Island. In 19 pairs where it proved possible to catch both members, there were ten dark–dark pairs and nine light–light pairs (and no mixed pairs). The likelihood of 19 male and 19 female birds of these morph proportions pairing at random without generating mixed pairs is extremely low ($P < 0.0001$).

The pattern of isolation was maintained in calling pairs apparently courting in the air. Of 86 such pairs, 63 were dark–dark, 21 light–light and two mixed, a distribution significantly different from random ($\chi^2_2 = 76$, $P < 0.0001$).

Egg length did not differ between morphs, but dark birds laid significantly broader and therefore larger eggs (Table 1). To increase the sample size, we included nest sites where we caught one but not both members of the pair. We then assumed, using the above evidence for reproductive isolation, that any egg incubated by a light bird was laid by a light bird—and similarly for dark birds.

Making a similar assumption, we determined the nest site location of 30 pairs. Nine dark pairs were found less than 1 km from Henderson's coast and nine farther inland. All 12 light pairs nested more than 1 km from the coast ($\chi^2_1 = 6.35$, $P < 0.05$).

Herald Petrels nested throughout the year (Table 2). Whereas the laying dates of light birds were apparently uniformly distributed, there was a peak of laying by dark birds in June and July. Comparing the proportion of all recorded pairs laying in June and July, there is a significant difference between dark and light pairs ($\chi^2_1 = 4.37$, $P < 0.05$). However, the overall pattern of a peak of laying in the austral winter appears to be repeated elsewhere in the Pacific (Holyoak & Thibault 1984).

Breeding success was low (Brooke 1995), and therefore it was not possible to make any detailed study of the inheritance of plumage. However, three dark pairs studied did rear fledglings, all three of which were also dark.

Example sonagrams of the calls of dark and light birds are shown in Figure 1. The calls of the two morphs differed in both parameters measured. The mean (±s.d.) time to complete 10 *kyeks* was 1.055 ± 0.118 s ($n = 14$) for light birds but only 0.836 ± 0.068 s ($n = 12$) for dark birds, a significantly shorter time (Mann-Whitney $U = 7$, $P < 0.001$). In fact, the ranges of times, 0.884–1.311 s for light birds and 0.728–0.967 s for dark birds, showed limited overlap, and therefore the rate at which *kyeks* were repeated is a potentially powerful means of morph discrimination. There were also frequency differences between the morphs. The mean (±s.d.) frequency of light birds was 3167 ± 163 Hz ($n = 12$), significantly higher ($t_{21} = 2.41$, $P < 0.05$) than the frequency of dark birds [2982 ± 197 Hz ($n = 11$)]. However, the overlap in frequency was considerable, and frequency may be a less reliable indication of morph than is repetition rate.

Other islands in the Pitcairn group

Only light Herald Petrels were seen at Oeno in 1991–1992. Birds were normally seen flying over the island or offshore. During only one visit (31 August–5 September 1991) were birds (about five individuals) recorded on the ground, but none was established as breeding. If the species still breeds at all on the island, it is in small numbers (see Brooke 1995).

Table 3. *Measurements of AMNH skins of* Pterodroma heraldica *(all Pitcairn Islands) and* P. arminjoniana *(18 Trindade, 2 Round Island), all morphs combined*

	P. heraldica			*P. arminjoniana*				
	Mean	s.d.	*n*	Mean	s.d.	*n*	*t*	*P*
Wing	276.3	6.42	119	283.8	9.49	20	4.48	<0.001
Culmen	27.0	0.84	112	29.3	1.21	19	11.33	<0.001
Tarsus	34.1	1.07	119	36.8	1.29	19	9.53	<0.001
Tail	111.8	6.35	119	121.9	4.93	20	6.64	<0.001

Table 4. *Length and breadth (±s.d.) of eggs laid by* Pterodroma heraldica *on Henderson Island (data re-analysed from Table 1) and by* P. arminjoniana *on Round Island (from Gardner* et al. *1985)*

	Length	Breadth	*n*
P. heraldica egg	58.5 ± 2.74 mm	41.2 ± 1.70 mm	30
P. arminjoniana egg	61.5 ± 2.91 mm	44.8 ± 1.83 mm	46
t-value	4.46	8.50	
P	<0.001	<0.001	

Only light Herald Petrels were seen during three landings on Ducie totalling 14 days in March, July and October 1991. The breeding population is of Order 5: 10,000–100,000 pairs (Brooke 1995), and there was ample opportunity to see dark birds had they been present. It therefore seems that dark Herald Petrels have ceased to breed on the atoll since 1922 (Murphy & Pennoyer 1952).

Comparison of *heraldica* with *arminjoniana*

The two forms, *heraldica* and *arminjoniana*, differ in three respects addressed by our study, in structure, morph-dependent mating behaviour and mtDNA haplotype frequency.

Although Murphy and Pennoyer's (1952) study indicated size differences between *heraldica* and *arminjoniana*, their study did not provide statistical support. The series of skins we measured showed clearly that *heraldica* is smaller than *arminjoniana* (Table 3). Moreover, the eggs laid by *heraldica* on Henderson Island were smaller than those laid by *arminjoniana* on Round Island (Table 4).

Whereas light and dark Herald Petrels breeding on Henderson Island show no intermediates and mate assortatively (data above), the situation is different in both respects for *arminjoniana* breeding on Round Island. First, there are birds classified as intermediate (Gill *et al.* 1970). The proportions classified as dark, intermediate and light seem to have varied little over 20 years (Gardner *et al.* 1985). Second, Round Island birds apparently pair without respect to phase (Table 5). There are no comparable data from the other breeding station of *arminjoniana*, Trindade Island.

From the laboratory

The 307-base-pair sequence of cytochrome *b*, spanned by the primers, of the first Herald Petrel examined is shown in Figure 2. This proved to be the most frequent haplotype, but, altogether, 13 different haplotypes were found and assigned the letters A–M (Fig. 3, Table 6) on the basis of the order of discovery during sequencing. For the 76 *Pterodroma* samples sequenced, there were 12 nucleotide positions where there was a base substitution (Fig. 3) when compared with the *heraldica* reference sequence (haplotype A). Two variable positions were at the first codon position and ten at the third position. Two sequences, haplotypes D and G, representing one and two individuals, respectively, showed a first position change resulting in an amino acid replacement; these are also given in full (Fig. 2). The amino acid replacements are both Val ↔ Ile but result from two different codon changes: haplotype D, codon position 15244–15246 gtt ↔ att and haplotype G, codon position 15091–15093 gtc ↔ atc.

Because there is an evolutionary bias toward transitions (pyrimidine ↔ pyrimidine {cytosine ↔ thymine} and purine ↔ purine {adenine ↔ guanine} substitutions) in mitochondrial DNA (Brown *et al.* 1982), the observed ratio of transitions to transversions (purine ↔ pyrimidine substitutions) is time dependent. For recently diverged taxa, over 90% of base substitutions are transitions, and the basal transition : transversion ratio may be as high as 20:1 in avian mtDNA (Edwards & Wilson 1990). For the 12 variable positions found among the 13 *Pterodroma* haplotypes, all observed substitutions were transitions (Fig. 3).

Table 5. *Pairings of* Pterodroma arminjoniana *on Round Island. Data from 19 pairs recorded by Bullock* et al. *(1983)*

	Dark × dark	Dark × intermed.	Dark × light	Intermed. × intermed.	Light × intermed.	Light × light	Total
Observed	9	7	2	1	—	—	19
Expected	9.6	6.4	1.4	1.1	0.5	0.1	

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Position                14991
Haplotype A                 cttt gga tcc ctc cta ggc atc tgt cta ata acc caa att cta acc ggc ctc
Haplotype G                 .... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Haplotype D                 .... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Haplotype A                  Phe Gly Ser Leu Leu Gly Ile Cys Leu Met Thr Gln Ile Leu Thr Gly Leu
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .

Position                15043
Haplotype A                 cta cta gcc atg cac tac aca gct gac aca acc tta gcc ttc tca tcc gtc
Haplotype G                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... a..
Haplotype D                 ... ... ... ... ... ... ... ... ... ... ... ... ... ..t ... ... ...

Haplotype A                 Leu Leu Ala Met His Tyr Thr Ala Asp Thr Thr Leu Ala Phe Ser Ser Val
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .  Ile
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .

Position                15094
Haplotype A                 gcc cac acc tgt cga aac gta caa tac ggt tga cta atc cga aac cta cat
Haplotype G                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Haplotype D                 ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ... ... ...

Haplotype A                 Ala His Thr Cys Arg Asn Val Gln Tyr Gly Trp Leu Ile Arg Asn Leu His
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .

Position                15145
Haplotype A                 gca aac gga gcc tca ttt ttc ttc att tgc atc tac ctg cac atc gga cga
Haplotype G                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Haplotype D                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Haplotype A                 Ala Asn Gly Ala Ser Phe Phe Phe Ile Cys Ile Tyr Leu His Ile Gly Arg
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .

Position                15196
Haplotype A                 gga ttc tac tac ggc tcc tac ctg tac aaa gag aca tga aat aca gga gtt
Haplotype G                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Haplotype D                 ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ... a..

Haplotype A                 Gly Phe Tyr Tyr Gly Ser Tyr Leu Tyr Lys Glu Thr Trp Asn Thr Gly Val
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .  Ile

Position                15247
Haplotype A                 ata ctt cta ctt acc ctc ata gca act gcc ttc gta ggg tac gtc ctg ccc
Haplotype G                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
Haplotype D                 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

Haplotype A                 Met Leu Leu Leu Thr Leu Met Ala Thr Ala Phe Val Gly Tyr Val Leu Pro
Haplotype G                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
Haplotype D                   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
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Figure 2. The 307-base pair Light strand sequence amplified with Kocher *et al.*'s (1989) cytochrome *b* primers for three Herald Petrels (haplotypes A, D and G, see text). Haplotype A is used as a reference. The numbers refer to the position of the first base on the complete chicken mtDNA (Desjardins & Morais 1990). The amino acid sequences are given below the DNA sequences.

Phylogenetic analysis of molecular data

A phylogenetic parsimony network constructed using the 13 haplotype sequences has a length of 14 steps (Fig. 4). A small number of base changes separate all the haplotypes. In this network, the maximum distance between haplotypes (E or G to I or M) is six steps, while all haplotypes are three or fewer steps from haplotype L. Thus the network supports the idea that the haplotypes may have evolved from a common ancestor relatively recently. It also shows the clustering of the various haplotypes into distinct clades and the branch lengths between them. Three clades can be defined: (B, C and D), (A, E and G) and (F, I and M). Figure 4 is not intended to show the absolute evolutionary relationships between the clades (although it may do so). The base changes are not sufficient to indicate the phylogenetic relationships between the *Pterodroma* species sequenced.

The distribution of the haplotypes indicates their taxonomic significance (Table 6). Five haplotypes (A, E, F, G and H) occurred within the light Herald Petrels and not within the dark birds, while three haplotypes (B, C and D) occurred solely within the distinct dark Herald Petrel clade recorded on Henderson Island (Fig. 4, Table 6). The haplotypes of

→

Figure 3. Distribution of 12 variable cytochrome *b* bases over the 76 individual *Pterodroma* sequenced and their assignment to haplotypes A–M (right-hand column). Note that dark "Herald" Petrels from Henderson Island are termed *atrata* (see Discussion). The position refers to that on the complete chicken mtDNA (Desjardins & Morais 1990).

INDIVIDUAL	Position	14997	15015	15030	15081	15091	15123	15189	15237	15244	15246	15285	15288	HAPLOTYPE
P. heraldica - Hendsn11 - Light		A	T	T	C	G	T	C	T	G	T	G	C	A
P. heraldica - Hendsn12 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn16 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn23 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn24 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn25 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30A - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30E - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30F - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30G - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30H - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30I - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30J - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30L - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Hendsn30M - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie41 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie42 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie44 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie45 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie46 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie50 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie52 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie53 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie54 - Light		.	.	.	.	.	.	.	.	.	.	.	.	A
P. heraldica - Ducie43 - Light		.	.	.	T	.	.	T	C	.	.	.	T	F
P. heraldica - Ducie47 - Light		.	.	.	T	.	.	T	C	.	.	.	T	F
P. heraldica - Ducie49 - Light		.	.	.	T	.	.	T	C	.	.	.	T	F
P. heraldica - Ducie48 - Light		.	.	.	.	A	.	.	.	.	.	.	.	G
P. heraldica - Ducie51 - Light		.	.	.	.	A	.	.	.	.	.	.	.	G
P. heraldica - Hendsn30N - Light		.	.	.	.	.	.	.	.	.	.	A	.	E
P. heraldica - Ducie55 - Light		.	.	.	.	.	.	.	.	.	.	A	.	E
P. heraldica - Ducie56 - Light		G	.	.	T	.	.	.	C	.	.	.	.	H
P. alba - ChristmasIsland01		.	.	.	.	.	.	.	.	.	.	.	.	A
P. alba - ChristmasIsland02		.	.	.	.	.	.	.	.	.	.	.	.	A
P. alba - ChristmasIsland03		.	.	.	.	.	.	.	.	.	.	.	.	A
P. alba - ChristmasIsland04		.	.	.	.	.	.	.	.	.	.	.	.	A
P. alba - ChristmasIsland05		.	.	.	.	.	.	.	.	.	.	.	.	A
P. alba - ChristmasIsland06		.	.	.	.	.	.	.	.	.	.	.	.	A
P. atrata - Hendsn13 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn14 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn15 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn18 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn20 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn21 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn22 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn26 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn27 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn28 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn29 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn30B - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn30C - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn30D - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn30K - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	.	.	B
P. atrata - Hendsn17 - Dark (heraldica)		.	.	.	T	.	C	.	C	.	.	A	.	C
P. atrata - Hendsn19 - Dark (heraldica)		.	.	.	T	.	C	.	C	A	.	.	.	D
P. arminjoniana - Roundl278 - Unknown		.	.	.	.	.	.	.	.	.	.	.	.	A
P. arminjoniana - Roundl226 - Dark		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl295 - Unknown		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl564 - Dark		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl566 - Unknown		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl569 - Medium		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl570 - Dark		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl571 - Dark		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl582 - Light		G	.	.	T	.	.	.	C	.	.	.	.	H
P. arminjoniana - Roundl237 - Light		G	.	.	T	.	.	.	C	.	.	A	.	J
P. arminjoniana - Roundl276 - Unknown		.	.	.	T	.	.	.	C	.	.	.	.	L
P. arminjoniana - Roundl238 - Medium		.	.	.	T	.	.	.	C	.	C	.	.	K
P. arminjoniana - Roundl229 - Dark		.	.	C	T	.	.	T	C	.	.	.	T	I
P. arminjoniana - Roundl561 - DarkIMed		.	C	.	T	.	.	T	C	.	.	.	T	M
P. arminjoniana - Roundl572 - Light		.	C	.	T	.	.	T	C	.	.	.	T	M
P. arminjoniana - Roundl573 - Dark		.	C	.	T	.	.	T	C	.	.	.	T	M
P. arminjoniana - Roundl574 - Unknown		.	C	.	T	.	.	T	C	.	.	.	T	M
P. arminjoniana - Roundl231 - Medium		.	.	.	T	.	.	T	C	.	.	.	T	F
P. arminjoniana - Roundl568 - Dark		.	.	.	T	.	.	T	C	.	.	.	T	F
P. arminjoniana - Roundl577 - Dark		.	.	.	T	.	.	T	C	.	.	.	T	F

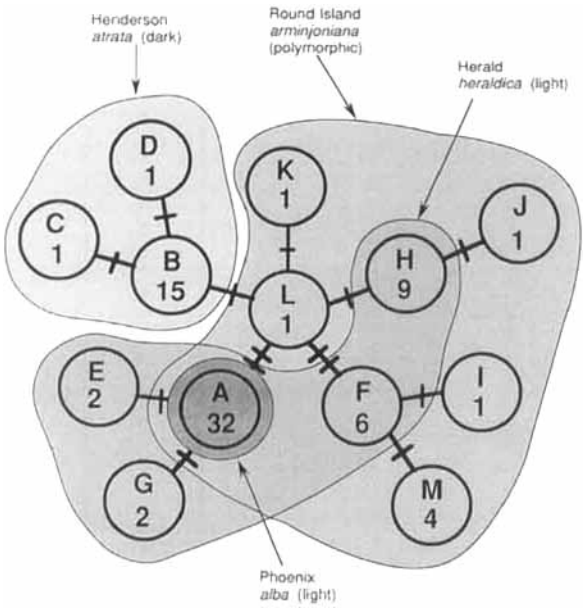

Figure 4. Unrooted phylogenetic network constructed by maximum parsimony using one representative of each *Pterodroma* haplotype. Slashes diagonal to the line indicate the number of inferred base changes between the haplotypes. Within the circles, the letter refers to the haplotype designation (Fig. 3 and text) and the digit refers to the number of individuals with that haplotype (Table 6). The four shaded areas, indicated by arrows, enclose haplotypes discovered in light Herald Petrels *P. heraldica*, in dark Henderson Petrels *P. atrata* (see Discussion), in Phoenix Petrels *P. alba* and in Round Island Petrels *P. arminjoniana*.

these two groups are mutually exclusive, indicating reproductive isolation between them, and the chance of the two common haplotypes, A and B, being represented 25:0 and 0:15 in pale and dark Herald Petrels, respectively, is low ($\chi^2_1 = 35.8$, $P < 0.0001$).

The light Herald Petrels of Ducie seem more polymorphic than those on Henderson. Among 16 Ducie birds sampled, five haplotypes were found (Fig. 3), while Henderson birds were mostly haplotype A (16 of 17), the single exception being a bird of haplotype E in the same clade (Fig. 4). Furthermore, Ducie was the only location where the haplotypes F and H, shared with *arminjoniana*, were found among Pacific birds.

Haplotypes A, F and H are common to *arminjoniana* and light *heraldica*, although haplotype A, predominant in the Pacific (25 of 33), was rarer (1 of 20) in *arminjoniana* ($\chi^2_1 = 22.2$, $P < 0.0001$). The modest sample sizes could account for the absence of two uncommon light Herald Petrel haplotypes, E and G (both 2 of 33), from the 20 *arminjoniana* sampled. Conversely, five *arminjoniana* haplotypes (I [1 of 20], J [1 of 20], K [1 of 20], L [1 of 20] and M [4 of 20], a total of 8 of 20) were not found amongst the *heraldica* sampled. Altogether, eight haplotypes were found in the 20 *arminjoniana* sampled. These results indicate incomplete reproductive isolation between light *heraldica* and *arminjoni-*

Table 6. *Distribution of* Pterodroma *haplotypes A–M with respect to species, island and colour morph. Note that dark "Herald" Petrels from Henderson Island are designated* P. atrata *(see Discussion)*

Haplotype	*n*	Species				Island				Colour phase				
		heraldica	*atrata*	*alba*	*arminjoniana*	Henderson	Ducie	Round	Christmas	Light	Medium	Dark/ medium	Dark	Unknown
A	32	25	—	6	1	16	9	1	6	31	—	—	—	1
B	15	—	15	—	—	15	—	—	—	—	—	—	15	—
C	1	—	1	—	—	1	—	—	—	—	—	—	1	—
D	1	—	1	—	—	1	—	—	—	—	—	—	1	—
E	2	2	—	—	—	1	1	—	—	2	—	—	—	—
F	6	3	—	—	3	—	3	3	—	3	1	—	2	—
G	2	2	—	—	—	—	2	—	—	2	—	—	—	—
H	9	1	—	—	8	—	1	8	—	2	1	—	4	2
I	1	—	—	—	1	—	—	1	—	—	—	—	1	—
J	1	—	—	—	1	—	—	1	—	1	—	—	—	—
K	1	—	—	—	1	—	—	1	—	—	1	—	—	—
L	1	—	—	—	1	—	—	1	—	—	—	—	—	1
M	4	—	—	—	4	—	—	4	—	1	—	1	1	1
Total	76	33	17	6	20	34	16	20	6	42	3	1	25	5

ana, or polymorphism which pre-dates the species' divergence (Avise *et al.* 1987).

DISCUSSION

On Henderson Island, there was evidence of reproductive isolation between light and dark Herald Petrels. The morphs bred and courted assortatively. They also bred in different parts of the island at slightly different seasons and uttered different calls. Together, these data strongly suggest that the morphs are distinct species.

Because most courting activity occurred during daylight, the plumage difference between the morphs could itself act as a species recognition signal. This signal would be reinforced by the vocal differences. Thus plumage differences could be more significant in these petrels than in other, nocturnal, burrowing species (Bretagnolle 1993). That these signals are effective is confirmed by the molecular data.

The difference in laying periods raises the possibility the morphs utilize different feeding zones. Possibly the dark morph feeds to the south of Henderson and breeds in the austral winter when its favoured feeding zone moves northward closer to the island. Meanwhile, the light morph breeds throughout the year while feeding in warmer waters to the north of Henderson.

As a molecular marker, mtDNA has the advantage over nuclear genes of a faster rate of evolution (Brown *et al.* 1979), which makes it more useful in resolving population questions. Mitochondrial cytochrome *b* codes for a functional protein of the electron transport and oxidative phosphorylation system contained in the inner mitochondrial membrane (Hatefi 1985, Howell & Gilbert 1988). As such, it is a relatively conserved gene, which, despite certain drawbacks (Meyer 1994), has become widely used for phylogenetic reconstructions. However, studies yielding sequence data from many individuals of a taxon (e.g. Finnerty & Block 1992, Quinn 1992, this study) have led to an increasing appreciation of the level of intraspecific DNA polymorphism. Obviously, caution is needed when describing new species solely on the basis of absolute DNA sequence divergence, particularly from a single individual.

Our cytochrome *b* data from many individuals support the behavioural case for a taxonomic split of the Herald Petrels of the Pacific. The approximately 1% difference (Figs 3 and 4) between the mtDNA haplotypes of light and dark Herald Petrels is less than the 6–10% difference observed between species within other petrel genera where the partial cytochrome *b* gene has been sequenced (*Puffinus*: Wink *et al.* 1993, *Oceanodroma*: Dawson 1992). Nevertheless, the difference is comparable with that observed between species within other genera and between sibling bird species. Such species may show mtDNA differences between 0.5% and 10% when studied by restriction fragment analysis (Avise & Zink 1988, Tegelström & Gelter 1990).

The utility of cytochrome *b* as a taxonomic marker in this study would be weakened if there were any connection between cytochrome *b* haplotype and plumage. The evidence of this study argues against such a connection. Haplotypes F and H (shared between *heraldica* and *arminjoniana*) and haplotype M (*arminjoniana* only) consist of light, medium (or dark/medium) and dark birds. Excluding three individuals with haplotypes D and G, 73 of the 76 *Pterodroma* individuals (96%) sequenced in this study, representing all recorded colour phases, shared the same amino acid sequence for this part of the cytochrome *b* protein. Consequently, we do not think this objection valid since there is no evidence, or known mechanism, to suggest the cytochrome *b* sequence determines the *Pterodroma* colour phase.

Quinn and White (1987) and Quinn (1992) reported on the transposition of part of the mtDNA into the nuclear genome of the Lesser Snow Goose *Anser caerulescens caerulescens*. This transposed region, part of which Quinn (1992) amplified from blood-derived DNA samples by PCR, may contain part or all of the cytochrome *b* region amplified in this study. However, there is no evidence from the sequences obtained here to suggest that a similar transposition has occurred in the *Pterodroma* lineage. No double banding was observed on the autoradiographs, indicating differentiated nuclear and mitochondrial copies, and the sequences show no sign of being released from functional constraints. Thus, no stop codons or frame shift mutations were found. Furthermore, only a few first and no second codon position base substitutions were detected.

In sum, we are confident that the behavioural and molecular data indicate that the dark and light Herald Petrels of the the Pitcairn Islands are good species and should be treated accordingly. On the question of nomenclature for the two species, formerly subsumed under Herald Petrel *Pterodroma heraldica*, it is evident that the white-bellied form should continue to be known as the Herald Petrel *P. heraldica* on grounds of priority. Because there is a prior name of *atrata* for the dark-bellied form, we propose

Pterodroma atrata (Mathews 1912) Henderson Petrel

The vernacular name of Henderson Petrel is appropriate since the island is apparently the species' stronghold (Murphy & Pennoyer 1952). It is also appropriate because Solander collected the bird he described as *atrata* about 100 km southwest of Henderson Island.

To the best of our knowledge, specimens collected by Solander on Cook's first voyage have not survived. The type of *atrata* is therefore lost. Since collecting was not permitted during our study, we designate as the neotype one of the specimens obtained during the Whitney Expedition's visit to Henderson Island in 1922.

Neotype

American Museum of Natural History (AMNH), New York, no. 191641, collected at Henderson Island on 8 April 1922.

Sex: female. Wing 279 mm; tail 111.4 mm; culmen 26.2 mm; tarsus 34.7 mm. A description of the neotype and of variation among specimens of *P. atrata* held in AMNH is given in Appendix 2.

Relationship between *Pterodroma heraldica* and *P. alba*

The close relationship between the Herald Petrel and the Phoenix Petrel *P. alba* of the Central Pacific has been recognized already (Imber 1985, Warham 1990). Our molecular data support this affinity. The six *alba* sequenced, from Christmas Island (Kiritimati), about 4000 km from the Pitcairn Islands, all have haplotype A, the commonest *heraldica* sequence. This may indicate recent divergence, a contention supported by the similarity in plumage between *alba* and *heraldica* (Murphy & Pennoyer 1952). Indeed, there may not be reproductive isolation between them. However, it would not be unique for unquestioned, morphologically divergent, congeneric species to have exactly the same cytochrome *b* sequence (G.R., unpubl. data).

Brooke (1995) reported that a Phoenix Petrel population, perhaps involving several thousand pairs, disappeared from Ducie between the Whitney Expedition of 1922 and the present study 70 years later. This loss, of course, could have been caused by adverse factors acting on the population. However, the present data raise another possibility, that the loss has been due to hybridization between *alba* and the larger *heraldica* population on Ducie. To assess this possibility morphologically would have required the collection of a very large skin series, a course that we were not permitted to follow. To assess the possibility genetically would require the use of a marker more specific than cytochrome *b*.

Relationship between *P. heraldica*, *P. atrata*, *P. arminjoniana* and *P. neglecta*

Because of the distribution of feather lice species on different host petrels, because of differences in intestinal twisting (transitional intestines in *heraldica* and *alba*, more twisted intestines in *arminjoniana* and *neglecta*) and because of geographic distribution (*neglecta* in the Pacific and *arminjoniana* in the Atlantic and Indian Oceans), Imber (1985) suggested that *heraldica* branched first from the lineage leading to *neglecta* and to *arminjoniana*. For these reasons he separated *heraldica* and *arminjoniana* as distinct species.

Imber's separation divides smaller *heraldica* from the larger *neglecta* of the Pacific (Murphy & Pennoyer 1952) and *arminjoniana* of the Indian and Atlantic Oceans (Tables 3 and 4). The latter two species, both polymorphic, share another feature—mating at random with respect to plumage (Merton 1970, M. de L. Brooke & G. Rowe, unpubl. obs. in the Pitcairn Islands for *neglecta*; Table 5 for *arminjoniana*).

Within the Pacific, the differentiation of dark-bellied *atrata* and white-bellied *heraldica* and *alba* occurred. The latter pair may exploit warmer, equatorial feeding zones to the north of Henderson Island.

The molecular data confirm the close relationship between *heraldica* and *arminjoniana*. They are not more divergent from each other than is *heraldica* from the newly proposed species, *atrata*. If *heraldica* and *arminjoniana* are both closely related to *neglecta* within the subgenus *Hallstroma*, we would expect *neglecta* haplotypes to be similar to those given in Figure 3.

In summary, within the Pacific the ancestral petrel gave rise to the light-bellied *heraldica* and the similar-sized, dark-bellied *atrata*. At about the same time, this ancestor gave rise to the larger, polymorphic *neglecta* in the Pacific and the larger, polymorphic *arminjoniana* within the Atlantic and Indian Oceans. This hypothetical sequence is at least not incompatible with the phylogenetic network (Fig. 4). It is a sequence which makes no comment on the precise relationship between the *arminjoniana* of Round Island and those of the Atlantic, since we have no molecular data from the latter.

A point to be investigated in the future is the relationship between the *heraldica* and *atrata* of the Pitcairn Islands and the petrels of the Marquesas, 2000 km north of the Pitcairns, that are currently classified as *P. (arminjoniana) heraldica*. These birds are cliff-nesting like some *neglecta* populations (Holyoak & Thibault 1984), and they also appear to court without regard to morph, suggesting they do not mate assortatively (V. Bretagnolle, pers. obs.). We suspect the taxonomy of this entire group of petrels may remain labile as further behavioural and molecular studies reveal a complex and possibly evolving situation.

If mtDNA differentiation occurs at about 2% per million years (Martin & Palumbi 1993), the molecular divergence revealed in Figures 3 and 4 and the differentiation discussed in the previous paragraphs probably would have occurred within two million years. Such a timescale is compatible with the absence of any known pre-Pleistocene *Pterodroma* fossils (Warham 1990).

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APPENDIX 1

A translation by M.D. Reeve of Mathews's (1912–1913) reproduction of Solander's Latin description of the bird *Procellaria atrata*. Underlined words in parentheses indicate uncertainties in the translation. Punctuation, layout and use of italics follow Mathews.

atrata *Procellaria* black(ish), underneath paler, with tail rounded, feet (legs) white and longish: palm black with base white

It lives in the southern Ocean (commonly known as the pacific sea) at lat. 25°21′S, long. 129°W (March 21, 1769)

The whole bird is blackish, but underneath somewhat paler or a dirty dark colour, because the feathers are blackish only at the tip

Tail wedgeshaped-round, somewhat longer than the feet

Bill black

Upper jaw curved, furrowed with a double groove from the tube of the nostrils to the sinus

Tube of nostrils convex, barely extended beyond a quarter of the bill, with two compartments (slots)

Partition not reaching the orifice

Apertures oval

Lower jaw straight, barely curved, marked on both sides from base to hump

Ribbon cutaceous, narrow, broadened in front, truncated

Eyes black

Feet white

Palm black, behind the first joint (i.e. the one nearest the base) white

Claws black Hind sessile

Length from tip of bill to end of tail	13½ in
Length between tips of spread wings	37 in
Weight	9 oz

APPENDIX 2

By Mary LeCroy

Description of neotype of *Pterodroma atrata* (AMNH specimen no. 191641)

A grey gadfly petrel which is sooty grey below, with the bases of the feathers white for about two-thirds of their length. The base of the throat feathers is white and the tips are sooty grey, varying in width, and giving a mottled appearance to the throat. The upperparts are more blackish, with grey feather bases. The bird has greyish tips on the feathers of the forehead, lores and cheeks, with a spot in front of and above the eye where the tips are more whitish. There are no white patches at the base of the bill. The legs and proximal one-third of the feet are pale pink (cf. white in Appendix 1); the distal two-thirds of the feet are black.

Variation among specimens of *P. atrata* held at AMNH

Variation occurs mainly in the head. The forehead, lores and cheeks vary from feathers that are concolorous with the underparts, but have a darker centre that gives them a scalloped appearance, to feathers with extensive whitish tipping in these areas, giving a very mottled appearance. There is sometimes a band of white below the eye and a spot of black just in front of the eye.