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POPULATION GENETICS OF SOUTHERN SEABIRDS

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SUMMARY

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Results are provided of a population genetics study using the starch gel electrophoresis technique and concerning 22 species of seabirds from four localities of the Southern Ocean: Antarctic Continent (Adélie Land), two subantarctic islands (Crozet and Kerguelen Islands) and one subtropical island (Amsterdam Island). Concerning genetic differentiation, species classification is reviewed using data from electrophoresis: for example, populations of King Penguins *Aptenodytes patagonicus* from Kerguelen and Crozet Islands show a high level of isolation. For five species, electrophoretic data are compared with the preliminary results of a mitochondrial DNA study. At a higher taxonomic level, storm petrels Hydrobatidae are markedly distant from the other three families of Procellariiformes. Concerning heterozygosity, genetic variability is low in the Wandering Albatross *Diomedea exulans*, low on average for seabirds in general, and increases on average towards higher latitudes.

INTRODUCTION

Genetic studies have long been lacking for the class Aves, in comparison with the other vertebrates classes. They are now more numerous, with studies of protein electrophoretic variability within populations (Nevo *et al.* 1984, Barrowclough *et al.* 1985) as well as differentiation at diverse levels of taxonomic and evolutionary divergence (Avise 1983, Avise & Aquadro 1982, Barrowclough 1983). The DNA-DNA hybridization technique has also been employed for bird taxonomy (e.g. Sibley *et al.* 1988). Many recent avian genetic studies (for example Tegelström 1987, Van Wagner & Baker 1990, Zink 1991) are based on mitochondrial DNA (mtDNA), which is maternally inherited, so that one can follow maternal lineages since there is no recombination between generations (for a review, see Wilson *et al.* 1985).

Because genetic variations are calculated in a way which depends on the technique used, it seemed to us useful to take stock of more classic results that we obtained on a general survey of seabirds, and to compare them with the preliminary results of a mitochondrial DNA study. We used protein electrophoresis to obtain data on the genetics of seabirds of the three orders Sphenisciformes,

Charadriiformes and Procellariiformes, whose populations breed on four French localities of the southern hemisphere: Amsterdam, Crozet and Kerguelen Islands, and Adélie Land on the Antarctic Continent. Ornithological studies, conducted from permanent bases in these otherwise uninhabited regions, began about three decades ago.

These four localities constitute a transect from the high-latitude Antarctic (Adélie Land) to the Subtropical (Amsterdam Island), through Kerguelen Islands in a cold sub-Antarctic climate, and Crozet Islands in the temperate sub-Antarctic. Common to all four localities is their isolation from terrestrial habitats, and thus they serve as breeding grounds for species that are entirely dependent upon the ocean for their food. Even the Kerguelen Archipelago, with a total land area of 7000 km^2, has only one true terrestrial species, the Kerguelen Pintail *Anas eatoni*, whereas the Pointe Géologie Archipelago in Adélie Land is an oasis on the edge of the Antarctic Continent. The immense and diverse tracts of ocean around them explain why some of these places can shelter the world's richest seabird communities. On the Crozet Archipelago, one of us recently identified the 36th breeding species (Jouventin 1990). Although the two sites at extremes latitudes are less rich (nine breeding species on Amsterdam Island, and eight in Adélie Land), as a whole these islands constitute an exceptional observatory of vertebrate populations almost untouched by humans.

Protein electrophoresis has already been applied to studies of some of the species here: e.g. among the Procellariiformes, for a study of interrelationships (Harper 1978) and levels of differentiation (Barrowclough *et al.* 1981). A comparative biochemical study - with other localities and with up-to-date techniques, such as mtDNA study - is now required. The followings problems must be examined: (1) variability between populations and its possible link with the general biology of the populations; (2) genetic differentiation between the conspecific populations of Crozet and Kerguelen Islands; (3) genetic differentiation at lower taxonomic levels, between congeneric species (including twin species) and subspecies; (4) genetic differentiation at higher taxonomic levels (between genera and above). The biochemical work has been mainly conducted in laboratories at the bases of Crozet and Kerguelen Islands, showing that such studies are possible in the field.

MATERIALS AND METHODS

Protein electrophoresis

During the period 1981-1984, a total of 907 individuals has been analysed electrophoretically across all 24 taxa (28 populations) studied: 478 Sphenisciformes, 142 Charadriiformes, and 287 Procellariiformes. Appendix 1 gives the names of the species or subspecies studied, and the localities of the populations sampled. The study has relied mostly on blood samples. But some non-blood tissues have also been used (from birds taken for museum collections, dead chicks frozen during the winter in Adélie Land, and from accidental mortalities), including samples of liver, cardiac and skelatal muscle, and kidneys. Methods of blood sampling, treatment and conservation of extracts are described in Jouventin & Viot (1985).

Proteins were separated by horizontal 12% starch gel electrophoresis, except for alpha-amylase, separated on 6% acrylamid gel, and plasmatic esterases and proteins separated on vertical 6% acrylamid gels. Several buffer systems were tested on the proteins; electrophoretic conditions indicated for each in Appendix 2 are those that had the best resolving power. The enzymes and non-enzymatic proteins that were studied are given in Appendix 3.

Not all loci could be scored in all populations, due to the conditions of the study and to differences in the material studied. In particular, limited numbers of loci were studied in blood (plasma and haemolysate) samples. Interpretation of zymogram patterns follows Harris & Hopkinson (1976). Alleles (=electromorphs) at each locus that showed polymorphism are labelled alphabetically in order of decreasing mobility. The staining procedures of

Selander *et al.* (1971) and Harris & Hopkinson (1976) were used with minor modifications. These are described by Jouventin & Viot (1985), except for Adh and Idh stains from Selander *et al.*(1971), and Amy, Ak and Ck stains from Harris & Hopkinson (1976).

Appendix 1 gives for each population the total numbers of individuals and loci studied, and the numbers of individuals and loci for each type of material, blood or non-blood tissues, available for study.

Allelic frequencies are used to calculate the heterozygosity (H) and standard genetic distance (D) of Nei (1975). When several populations of a given species have been examined, mean heterozygosity of the species is calculated by weighing for the numbers of individuals and loci studied, as in Nevo *et al.* (1984).

Mitochondrial DNA study

DNA was extracted from frozen liver or feathers plucked from live birds (see Table 1), using the protocol established by Taberlet & Bouvet (1991) with minor modifications. A 307-base pair fragment of the cytochrome *b* mitochondrial gene was amplified via the Polymerase Chain Reaction (PCR) (Saiki *et al.* 1988), using primers cyt *b* L14841 and cyt *b* H15149 (Kocher *et al.* 1989), and directly sequenced (Sambrook *et al.* 1989) with the Sequenase 2.0 kit (United States Biochemical). The species studied (two Sphenisciformes, three Procellariiformes, and one Charadriiformes) are listed in Table 1; their taxonomic status is apparently well known, so that it will be possible to check the resolving power of this technique by comparing our results with electrophoretic, biometric, and fossil data.

TABLE 1

S=NUMBER OF PRELEVEMENTS FOR mtDNA STUDY IN THREE FRENCH SUB-ANTARCTIC LOCALITIES - A = SUCCESS AT THE AMPLIFICATION OF A PART OF THE MITOCHONDRIAL CYTOCHROME *b* GENE - S.O.= NUMBER OF SEQUENCES OBTAINED - L= LENGTH (BASE PAIR) OF THE mtDNA FRAGMENTS SEQUENCED

Species	*P.p*	*E.c.*	*P.d.*	*P.b.*	*H.c.*	*C.m*
S:						
Amsterdam	-	5	-	-	-	-
Crozet	6	6	-	-	-	3
Kerguelen	4*	6	3*	1*	1*	6
A:	2(4*)	4	3*	1*	1*	8
S.O:	2(4*)	-	3*	1*	1*	8
L:	157° 285°°	-	249	249	249	219

The abbreviations for the species are given in Appendix 1, except for *H.c.* =Blue Petrel *Halobaena caerulea.*
*: liver prelevements (the other ones are feather prelevements).

°: *P.p.C.* °°: *P.p.K.*

RESULTS

Allelic frequencies at the polymorphic loci in the various populations are listed in Appendix 2a (Sphenisciformes), 2b (Charadriiformes) and 2c (Procellariiformes). Appendices 1a-c give for each population its heterozygosity and the proportion of polymorphic loci. Nei's (1975) matrices of genetic similarity and distance between populations are given in Tables 2a-c for the three orders. The percentages of nucleotide sequence divergence are given in Table 3.

Genetic differentiation

Phenograms are shown in Figs 2 (UPGMA phenogram) and 3 (Neighbour-joining phenogram, from G.B. Nunn pers. comm.).

Differentiation between populations

For five species a comparison could be made between their respective populations at Crozet and Kerguelen Islands. Genetic distance is relatively high between the two populations of King Penguins *Aptenodytes patagonicus* (D=0.0781), but very low for Gentoo *Pygoscelis papua* (D=0.00045) and Rockhopper *Eudyptes chrysocome* (D=0.0007) Penguins; it is rather high for the Subantarctic Skua *Catharacta antarctica* (D=0.0294) and Lesser Sheathbill *Chionis minor* (D=0.0393; these populations belong to two different subspecies, see Appendix 1). A population of a different subspecies (*E. c. moseleyi*) of Rockhopper Penguin breeding on Amsterdam Island has been compared with the populations at Crozet and Kerguelen Islands (D=0.0060 and 0.0063, respectively). The mean genetic distance between conspecific populations is D=0.0257, and this has a s.d.=0.0303. With a standard deviation that is greater than the mean, large variations in genetic distance are demonstrated at this level. Comparing allelic frequencies, it must be noted in particular that the differentiation between the two populations of King Penguins is high for the Pgd locus (see Appendix 2a), indicating a low rate of gene flow between them. For the Subantarctic Skua there is likely to be a significant bias in our measure of the differentiation between Crozet and Kerguelen populations, because of the small sample size (loci studied in non-blood samples are derived from only one individual in each population).

Differentiation between taxa

At the next higher level of differentiation between congeneric species, we have distinguished the case of "twin" species (six pairs, see Table 4), between which the mean distance is 0.1220 (s.d.=0.1206): variations are still high, standard deviation being equal to the mean. We must point out the considerable distance between the Common *Pelecanoides urinatrix* and South Georgian *P. georgicus* Diving Petrels. Four pairs of non-twin congeneric species have also been examined (see Table 4): between the two albatross species, differentiation thus looks relatively low, despite a considerable morphological difference between them. The average of these four distances is D=0.1961 (s.d.=0.0844), which is higher than the mean distance between twin species. Variation between pairs is less at this level and the standard deviation is smaller than the mean. The average genetic distance for all pairs of congeneric species is 0.1516 (s.d.=0.1092). This is about six-fold greater than the average distance between conspecific populations. However, the highest genetic distance is observed between the closely related species of diving petrels, which are reliably distinguished morphologically only by the occurence of a bluish strip on the tarsus (Payne & Prince 1979).

Differentiation at higher taxonomic levels

At the genetic level, we shall treat the genera of penguins separately from the others. Considering firstly the other genera, distances were calculated between seven pairs; they are given in Table 5 and the mean distance is D=0.3758 (s.d.=0.1619). The mean distance between the sphenisciform genera (see Table 6) is D=0.8159 $\pm$ 0.3106. For the 10 distances between confamilial genera, the mean is

TABLE 2
GENETIC SIMILARITIES (I, ABOVE DIAGONAL) AND DISTANCES (D, BELOW DIAGONAL) CALCULATED ACCORDING TO NEI (1975) BETWEEN POPULATIONS OF TAXA.

(A) SPHENISCIFORMES

ID	*A.p.*	*A.p.c.*	*A.p.K.*	*P.a.*	*P.p.C.*	*P.p.K.*	*E.c.*	*E.c.c.C.*	*E.c.c.K.*	*E.c.m.*
A.p.		0.7990	0.7328	0.3989	0.3529	0.3566	0.4870	0.5261	0.5204	0.5210
Ap.c.	0.2244		0.9249	0.3671	0.3015	0.3031	0.4555	0.4656	0.4592	0.4673
A.p.K.	0.3109	0.0781		0.3855	0.2356	0.2384	0.3833	0.3886	0.3809	0.3909
P.a.	0.9190	1.0021	0.9532		0.7659	0.7662	0.5630	0.6205	0.6161	0.6102
P.p.C.	1.0416	1.1990	1.4456	0.2667		0.9996	0.5375	0.6176	0.6154	0.6043
P.p.K.	1.0311	1.1937	1.4338	0.2663	0.0004		0.5420	0.6225	0.6203	0.6091
E.c.	0.7195	0.7864	0.9589	0.5745	0.6208	0.6125		0.8625	0.8592	0.8481
E.c.c.C.	0.6423	0.7644	0.9452	0.4772	0.4819	0.4740	0.1479		0.9993	0.9940
E.c.c.K.	0.6532	0.7783	0.9652	0.4843	0.4855	0.4776	0.1518	0.0007		0.9937
E.c.m.	0.6520	0.7608	0.9393	0.4940	0.5037	0.4958	0.1648	0.0060	0.0063	

(B) CHARADRIIFORMES

ID	*C.m.*	*C.s.l.C.*	*C.s.l.K.*	*C.m.c.*	*C.m.m.*
C.m.		0.9862	0.9561	0.1415	0.1323
C.s.l.C.	0.0139		0.9718	0.1340	0.1247
C.s.l.K.	0.0449	0.02186		0.1120	0.1026
C.m.c.	1.9555	2.0099	2.1893		0.9614
C.m.m.	2.0227	2.0818	2.2769	0.0393	

(C) PROCELLARIIFORMES

ID	*D.e.*	*D.m.*	*P.p.*	*P.f.*	*T.a.*	*D.c.*	*P.n.*	*P d.*	*P.b.*	*P.s.*	*P.g.*	*P.u.*	*O.o.*
D.e.		0.9048	0.8210	0.8151	0.2651	0.3574	0.3641	0.4043	0.4226	0.4107	0.3963	0.3200	0.0518
D.m.	0.1001		0.9082	0.9070	0.3615	0.3975	0.3825	0.5132	0.5353	0.5240	0.4474	0.3734	0.1036
P.p.	0.1972	0.0962		0.9108	0.3652	0.3997	0.4159	0.5161	0.5384	0.5300	0.4498	0.3755	0.1041
P.f.	0.2044	0.0976	0.0935		0.4228	0.3758	0.3811	0.5542	0.5579	0.5491	0.4801	0.4490	0.1057
T.a.	1.3277	1.0176	1.0074	0.8609		0.7211	0.6604	0.5890	0.5640	0.5700	0.3625	0.3784	0.1730
D.c.	1.0288	0.9227	0.9171	0.9787	0.3270		0.8345	0.6224	0.6006	0.6064	0.4135	0.4318	0.1787
P.n.	1.0102	0.9610	0.8774	0.9648	0.4149	0.1810		0.6159	0.5908	0.5966	0.4673	0.4904	0.2287
P.d.	0.9055	0.6671	0.6615	0.5903	0.5293	0.4742	0.4847		0.9393	0.9254	0.4558	0.5754	0.2350
P.b.	0.8614	0.6250	0.6192	0.5836	0.5727	0.5099	0.5262	0.0627		0.8985	0.4766	0.5374	0.2215
P.s.	0.8899	0.6463	0.6349	0.5994	0.5621	0.5002	0.5165	0.0775	0.1070		0.4681	0.5283	0.2828
P.g.	0.9257	0.8043	0.7990	0.7338	1.0148	0.8831	0.7607	0.7858	0.7412	0.7591		0.6963	0.1060
P.u.	1.1393	0.9852	0.9796	0.8007	0.9719	0.8397	0.7126	0.5528	0.6211	0.6381	0.3620		0.2104
O.o.	2.9604	2.2674	2.2621	2.2472	1.7545	1.7218	1.4754	1.4482	1.5073	1.2630	2.2443	1.5587	

TABLE 3

RESULTS FROM mtDNA SEQUENCING. ABOVE DIAGONAL: VALUES OF THE SEQUENCE DIVERGENCE PERCENTAGE (p %) BETWEEN CONSPECIFIC AND CONFAMILIAL SPECIES PAIRS. BELOW DIAGONAL: ESTIMATES OF DIVERGENCE TIME (M.Y.) BETWEEN THE SAME PAIRS

	P. d.	*P. b.*	*H. c.*	*P. p. C*	*P. p. K*	*C. m. c.*	*C. m. m.*
P. d.	-	0.4 ± 0.4	7.23 ± 1.64				
P. b.	0.2 ± 0.2	-	7.63 ± 1.68				
H. c.	3.62 ± 0.82	3.82 ± 0.84	-				
P.p.C				-	5.10 ± 1.76		
P.p.K				2.55 ± 0.88	-		
C.m.c.						-	0.91 ± 0.64
C. m.m.						0.46 ± 0.32	-

p = number of substitutions observed/number of bases compared.

TABLE 4

NEI'S (1975) GENETIC DISTANCES AND ESTIMATES OF THE DIVERGENCE TIME BETWEEN SIX CONSPECIFIC POPULATIONS PAIRS AND TEN CONGENERIC SPECIES PAIRS, USING THE FORMULA t=26.3 x D (NEI 1975, GUTIERREZ *ET AL.* 1983)

Pairs	D	Divergence time (m.y.)
C.m.c./C.m.m.	0.0393	1.0335
C.s.l.C/C.s.l.K	0.0294	0.7732
P.p.C/P.p.K	0.0004	0.0105
E.c.m./E.c.c.	0.0062	0.1631
E.c.c.C/E.c.c.K	0.0007	0.0184
A.p.C/A.p.K	0.0781	0.2054
**C.s.l./C.m.*	0.0293	0.7706
**P.f./P.p.*	0.0935	2.4590
**P.d./P.b.*	0.0627	1.6490
**P.b./P.s.*	0.1070	2.814
**P.s./P.d.*	0.0775	2.0382
**P.u./P.g.*	0.3620	9.5206
***A.f./A.p.*	0.2677	7.0405
***P.p./P.a.*	0.2665	7.0089
***E.c./E.c.c.*	0.1499	3.9424
***D.e./D.m.*	0.1001	2.6326

*: twin species; **: non-twin species.

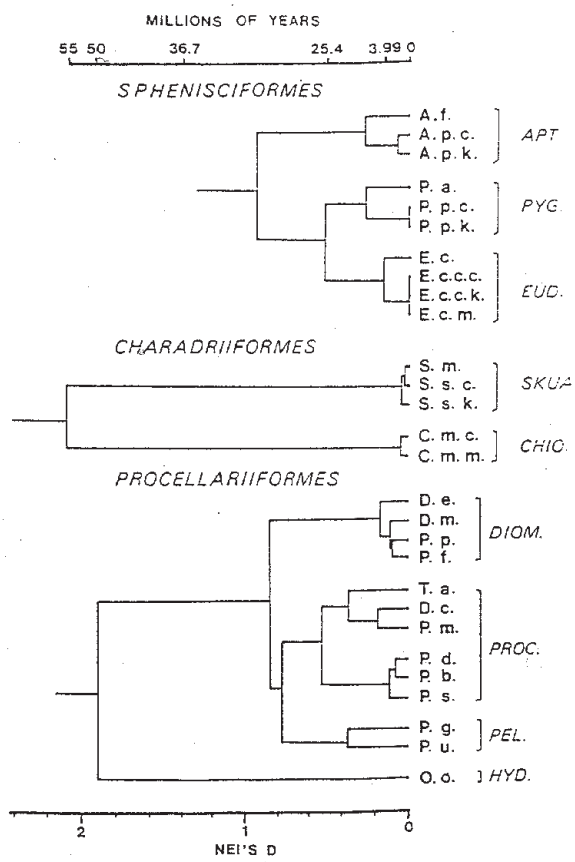

Figure 1

UPGMA phenogram based on Nei's D-values, for Sphenisciformes, Charadriiformes, Procellariiformes. See text for explanations of the dating scale.

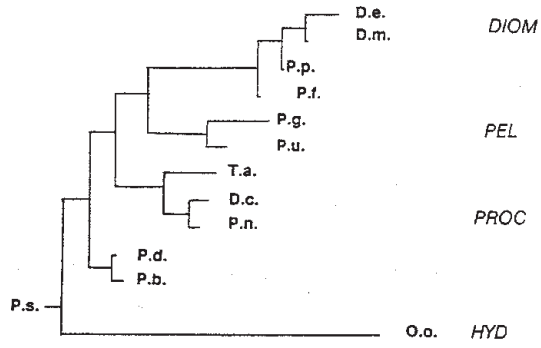

Figure 2

Neighbour-joining phenogram (unrooted tree) showing the distances between procellariiform species (from G. B. Nunn pers. comm.).

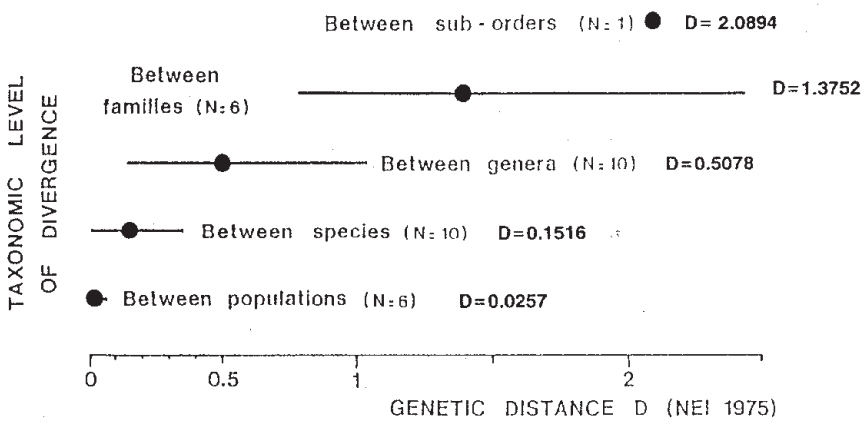

Figure 3

Mean Nei's D at different taxonomic levels calculated from values for the three orders.

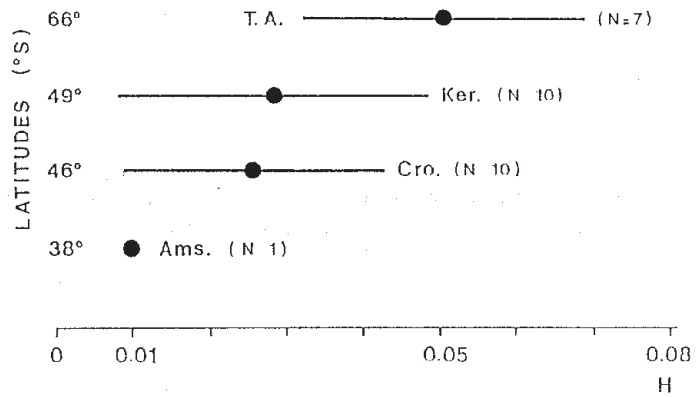

Figure 4

Mean heterozygosities (H) of the populations studied in the four localities: Amsterdam Island (AMS), Crozet Island (CRO), Kerguelen Islands (KER), and Adélie Land (A-L). Horizontal scale gives the latitude of the locality.

D=0.5078 (s.d.=0.2900). The variation between pairs appears somewhat less important at this level than at the level of congeneric species (mean to standard deviation ratio), although the mean distance is much higher between the three sphenisciform genera than between other confamilial genera.

Three interesting conclusions can be drawn from these results: *Aptenodytes* is well separated from the other two genera of penguins; *Daption* and *Pagodroma* are closer to one another than either one is to *Thalassoica*; the mean distance between these latter three genera (belonging to the sub-family Fulmarinae) and *Pachyptila* (sub-family Pachyptilinae) is not great (D=0.5195), relative to the distances between the three fulmarine genera (see Tables 2 and 5). The low genetic distance between the two genera of albatrosses is particularly surprising.

Comparisons between families concern the four families comprising the order Procellariiformes: Diomedeidae-Procellariidae (D=0.8399), Diomedeidae-Pelecanoididae (D=0.7735), Procellariidae-Pelecanoididae (D=0.7734), and Hydrobatidae with the other three (respectively D=2.4343, D=1.5284 and D=1.9015). The mean of these six distances is D=1.3752 (s.d.=0.6779). Their range is important; distances are rather homogenous between the first three families: Diomedeidae, Procellariidae and Pelecanoididae but much higher between these and Hydrobatidae (represented by only one species, Wilson's Storm Petrel *Oceanites oceanicus*). Thus the second and third families, Procellariidae and Pelecanoididae, are closest to one another, and both are close to Diomedeidae, whereas all three are very distant from Hydrobatidae.

TABLE 5

GENETIC NEI'S (1975) DISTANCES CALCULATED BETWEEN SEVEN PAIRS OF PROCELLARIIFORM GENERA. THE AVERAGE DISTANCE BETWEEN THESE GENERA IS 0.3758 ± 0.01619

	Phoebetria	*Thalassoica*	*Daption*	*Pagodroma*	*Pachyptila*
Diomedea	0.1489				
Thalassoica			0.3270	0.4149	0.5547
Daption				0.1810	0.4948
Pagodroma					0.5091

TABLE 6

GENETIC NEI'S (1975) DISTANCES CALCULATED BETWEEN THREE PAIRS OF SPHENISCIFORM GENERA. THE AVERAGE DISTANCE BETWEEN THESE GENERA IS 0.8159 ± 0.3106)

	Pygoscelis	*Eudyptes*
Aptenodytes	1.1355	0.7971
Eudyptes	0.5152	-

The highest taxonomic level examined here concerns the sub-orders Lari (genus *Catharacta*) and Charadrii (genus *Chionis*), and the distance between them is 2.0894.

Genetic distances and taxonomy molecular clock

On the whole, we can see that mean genetic distances increase with the level of taxonomic divergence (Fig. 4). This increase is not linear, but fits an exponential curve ($r=0.976$, 3 d.f., $p<0.01$).

The general increase in genetic distance with taxonomic level is predicted by theoretical considerations (see for example Nei 1975). Excepting the debate about neutralism, it has been confirmed in many instances (see reviews by Avise & Aquadro 1982, Avise 1983, Barrowclough 1983, Kessler & Avise 1985).

However, the genetic distances in our study show large variations (indicated by the high standard deviation relatively to the mean) at the lower levels of divergence, particularly conspecific populations and twin species. More or less considerable overlaps exist between the ranges of genetic distance at the different classes of taxonomic level.

In view of the increase of genetic distance with taxonomic status, it was considered worthwhile to calibrate a time scale against D, using the formula of Nei (1975): t (million of years before present) = k x D. The time scale shown in Fig. 2 and Table 4 uses the value $k=26.3$ calculated by Gutierrez *et al.* (1983) from fossil records of the taxa which they studied by electrophoresis. In fact, the fossil records are poor for birds in general, and particularly so for the taxa studied here: the reasons for this (fragile bones in general, and habit of breeding on quickly-eroding volcanic islands) have been discussed by Simpson (1975, 1976) and Bourne (1965). With $k=26.3$, we obtain for the mean time of divergence of populations ($D=0.0257 \pm 0.0303$) a value of $t=0.676$ million years; for congeneric species ($D=0.1516 \pm 0.1092$): $t=3.99$ m.y. (3.21 m.y. for twin species); for confamilial genera ($D=0.5078 \pm 0.2900$): $t=13.4$ m.y. (21.5 m.y. for sphenisciform genera); for families within the order Procellariiformes ($D=1.3752 \pm 0.6779$): $t=36.2$ m.y.; for suborders of Charadriiformes ($D=2.0894$): $t=55$ m.y.

These values are comparable to those from DNA studies (see for example Kessler & Avise 1984); they also show good agreement with what is known or conjectured from palaeontology. For 19 genera corresponding to 10 orders of birds, first fossil records go back, on average, to 9.1 m.y. (in Wyles *et al.* 1983), a value somewhat lower than our 13.4 m.y. However, if Sphenisciformes were excluded, our value would be 9.88 m.y. According to Simpson (1975), the sphenisciform genera probably originated from a Miocene (25-5.5 m.y.) stock, while according to Zusi (1975), the main split between them occurred in the Miocene or earlier. Our mean value for sphenisciform genera is 21.5 m.y., and 24.77 m.y. for the split between *Aptenodytes* and the other two genera, values which are not far from the hypotheses of these authors. The great adaptive branching of birds probably occurred in the Cretaceous (135-65 m.y.), and continued into the Paleocene (65-55 m.y.). Nineteen of the 27 present-day orders are known from the early Eocene or earlier (55-50 m.y.), and all orders and most families from the late Eocene (45-37 m.y.) (Simpson 1975, 1976, Fisher 1967). Our value of 55 m.y. for the two suborders of Charadriiformes is thus perhaps a slight overestimate. The same is true for the 36.2 m.y. calculated for the split between procellariiform families. According to Imber (1985), the origin of the family Procellariidae dates back to late Eocene (45-37 m.y.), and the split between *Pachyptila* and the sub-family Fulmarinae would appear to have taken place during the late Miocene. The value of t corresponding to our $D=0.5195$ for this split is 13.7 m.y. In respect of the ages of congeneric species, our mean value of t is 3.99 m.y., and for twin species it is 3.21 m.y., which would certainly appear to be overestimates. These high values are due to the considerable genetic distance ($D=0.362$) between the two twin diving petrels. However,

Mack *et al.* (1986) have obtained results of the same order: estimates of the age of their common ancestor between three *Parus* species are 1-2 m.y. to 2.5-4.5 m.y.

Thus, so far as is known, the estimates of age of divergence using our mean genetic distance values are not in considerable discrepancy with other data about evolution of these taxa. The values would appear however to be more or less overestimated, and a value of the coefficient k closer to 20 would probably be better fitted.

Within population variation

Estimates of heterozygosity (Appendix 1) range from 0.0 (in the Kerguelen Island populations of Subantarctic Skuas, Wandering Albatrosses *Diomedea exulans* and Blackbrowed Albatrosses *D. melanophris*) to 0.082 (Snow Petrel *Pagodroma nivea*). Average heterozygosities, calculated for all species (H=0.035), and for the species of each of the three orders (H=0.033, 0.031 and 0.036 respectively for Sphenisciformes, Charadriiformes and Procellariiformes) are lower than the average of 0.051 for 46 other species of birds (Nevo *et al.* 1984), most of which belong to the order Passeriformes. Proportions of polymorphic loci range from 0.00 (same populations or those with null heterozygosity levels), to 0.48 (Snow Petrel), with a mean of 0.17. This parameter is highly dependent on sample size. In fact, for populations whose sample sizes are not too small, polymorphic loci mostly show one predominant allele (frequency 0.95). That is, when sample size is small, very few variable loci will show polymorphism (the values of this parameter are in effect clearly lower when sample sizes are small). For this reason, in future references to the level of genetic variability within populations, only the value of heterozygosity will be considered.

On the whole, genetic variation among the Sphenisciformes is somewhat more homogenous, particularly if the Northern Rockhopper Penguin is excluded, in comparison with the other two orders. This can be explained at least a part by the fact that sample sizes are almost never small for the populations of Sphenisciformes, whereas they may be for the other two. The low level of variability observed in the Northern Rockhopper Penguin might be related to the fact that the population breeding on Amsterdam Island has been considerably disturbed by human activities for the last hundred years (Decante *et al.* 1987) and might have suffered more or less pronounced population bottlenecks. The zero variability in the Kerguelen Islands' population of the Subantarctic Skua, compared to the high variability on Crozet Island, is probably due to sampling error. For both *Diomedea* albatrosses also, it might be due to sampling; however, variability is rather low for all four species of albatrosses studied. In other species whose sample sizes are small, heterozygosities are not generally low, and there is no overall correlation between sample size and the level of genetic variation.

Mean heterozygosity at each study site

Mean population heterozygosities are H=0.051 (± 0.018) in Adélie Land, 0.028 (± 0.020) at Kerguelen Islands, and 0.026 (±0.017) at Crozet Islands. Variations in relation to latitude are given in Fig. 5. Amsterdam Island is also shown in this figure, although the value of H=0.010 for the Northern Rockhopper Penguin cannot be considered as reliably representative. The other three localities differ significantly from each other at $p<0.05$ (H=9.64, Kruskal-Wallis test).

Comparison with mtDNA study

Performing the amplification of a part of the cyt *b* gene was much less easy in Sphenisciformes; sequences were available for all species but the Rockhopper Penguin (see Table 1). They show a surprisingly high percentage of sequence divergence (*p*) between Crozet and Kerguelen Gentoo Penguins (in this species we also find a variation within the Kerguelen Archipelago), almost as high as the value observed between the Blue Petrel *Halobaena caerulea* and the Prions *Pachyptila* sp.. The

Antarctic Prion *P. desolata* and the Slenderbilled Prion *P. belcheri* seem very close to each other, as do the two populations of Lesser Sheathbills. The values of sequence divergence percentages are given in Table 3. If we consider a 2% per million year evolution rate of substitutions for mitochondrial DNA (see reviews by Wilson *et al.* 1985, Avise *et al.* 1987), the estimates of divergence time (Table 3) do not seem in disagreement with the mean dates calculated from protein electrophoresis (Table 4). We have not caculated any divergence date for higher taxonomic levels, because the relation between p and divergence time becomes curvilinear when $p > 0.15\text{-}0.16$ and ends reaching a plateau (Brown *et al.* 1982).

DISCUSSION

Numerous logistical difficulties, in field work and shipping of the frozen samples, together with our desire not to disturb the local fauna, have dictated the types of tissues collected and the irregularity of the sample sizes. The large proportion of blood samples (for which fewer loci can be analysed) and the small number of individuals representing some of the populations, has to some extent limited the interpretative power of our analysis. The implications of the more significant results are examined below, leading to a general discussion of variation within and among populations.

Within-species variation

King Penguin

The most remarkable and least expected result of the comparison between conspecific populations concerns the King Penguin. The Crozet and Kerguelen Islands populations show differences in allelic frequencies that imply a high level of isolation between them. This result led us to a morphological comparison of the two populations. For flipper as well as bill length, the Kerguelen Island population exhibits measurements much smaller than those on Crozet Islands (Viot 1987). This corroboration was the more surprising considering that Stonehouse (1975) and Watson (1975) regard the species as homogenous. By contrast, Prevost & Mougin (1971), Barrat (1976) and Jouanin & Mougin (1979), distinguish two subspecies, the first comprising birds in the region around the lower end of South America, and the second comprising birds in all other (and more numerous) breeding places, including Crozet and Kerguelen Islands.

Gentoo Penguin

Whereas the Kerguelen Island population has much smaller flippers and culmens than that on Crozet Island (Viot 1987), the genetic differentiation on the loci studied between the two populations is extremely low. Because of the limited number of loci identified by electrophoresis, it cannot be ruled out that a "diagnostic" locus such as that found for the King Penguin may have been missed, despite a general similarity in the patterns of morphological variation for the two species.

Mitochondrial DNA sequencing gives a value of p in agreement with the existence of two distinct species (see Kessler & Avise 1985) at Crozet and Kerguelen Islands. These surprising results, which contradict previous ones, can be explained in part by the small sample size in our study and by the length of the DNA fragment sequenced (only 157 bp, whereas the whole cyt *b* gene is about 1100 bp long - see Anderson *et al.* 1981, Irwin *et al.* 1991).

Rockhopper Penguin

As was expected, no significant genetic differences have been found between the populations of Crozet and Kerguelen Islands. In our study, the Northern Rockhopper Penguin of Amsterdam Island revealed very little differentiation from the other two islands, in respect of its ecology, ethology and in morphology. It is to be noted that genetic variation within the Amsterdam Island population is about four times lower than in the other two populations. Although the limited sample size must cast some doubt on this figure, it may well be that the

slaughters to which the population on Amsterdam Island was submitted at the end of the 19th Century caused a drastic reduction in its level of polymorphism (Jouventin *et al.* 1984, Decante *et al.* 1987). Similar circumstances have been cited to explain the lack of genetic variability among Northern Elephant Seals *Mirounga angustirosris* on the Californian coast (Nei *et al.* 1983, Bonnell & Selander 1974).

Variation in *Catharacta* skuas

The zero genetic variability in the Kerguelen Islands population of the Subantarctic Skua, in comparison with the rather high variability of the Crozet Island population may be attributed to sampling error, as discussed above. The low genetic differentiation observed between the two skua species is perhaps related to hybridization, existing in particular on the South Orkney Islands and the Antarctic Peninsula (Hemmings 1984, Parmelee 1988).

Variation in sheathbills

Results for the Lesser Sheathbill surprisingly involve both genetic variability within populations and differentiation between them. The populations of Crozet and Kerguelen Islands are more differentiated than are the two species of skuas. This may be related to the poor flying ability of these essentially terrestrial birds (only true, however, for *C. minor*, not *C. alba*), which are unable to venture far from their breeding islands. Morphological differences are well-marked between the populations of Crozet and Kerguelen Islands (Weimerskirch *et al.* 1989). However, ecological causes could equally contribute, as is probably the case on Kerguelen Island which has three times the level of heterozygosity than does the Sheathbill population at Crozet Island. A higher diversity of food resources on Kerguelen Island is thought to be an important factor here (P. Jouventin unpubl. data).

The estimates of divergence time calculated from both electrophoresis and mtDNA studies are not very different from each other (Tables 3 and 4), especially if the dates obtained from the former technique have been overestimated. The value of p (Table 3) seems consistent with the existence of two subspecies (see Kessler & Avise 1985, Tegelström 1987).

Variation in albatrosses

The two twin species of sooty albatrosses *Phoebetria* show a genetic differentiation surprisingly close to that between the genus *Phoebetria* and the Blackbrowed Albatross. The latter species appears to be no closer to the Wandering Albatross than to the genus *Phoebetria*.

The relatively low intergeneric genetic differentiation could be related to the rather low within-population variability (on average) on the same loci. The rather high interspecific differentiation observed in *Phoebetria* is accompanied by a not very low level of heterozygosity. Further studies of other albatross species will help to establish whether or not low heterozygosity levels are common to this group.

Intergeneric differentiation in penguins

In contrast to the opinion of Zusi (1975) and in agreement with systematics, the genus *Aptenodytes* appeared much differentiated from the other two studied genera, *Pygoscelis* and *Eudyptes*. Intergeneric distances observed in this order are relatively high, in common with the findings of many authors.

Genetic differentiation among petrels

Procellariidae

The Antarctic Petrel *Thalassoica antarctica* is generally considered to be closely related to the Pintado Petrel *Daption capense* and the Snow Petrel (Mayr & Amadon 1951, Meredith 1985, unpubl. data), which are closer still to one another. Our results are consistent with the previous study of Barrowclough *et al.* (1981). Among the prions,

Antarctic and Slenderbilled Prions are very close to one another, more than either is to Salvin's Prion *P. salvini*, in agreement with most authors (e.g. Bretagnolle *et al.* 1990) and with our mtDNA study, in spite of a loss of resolving power induced by the observation of only one substitution, so that divergence time cannot be accurately estimated (0.2 ± 0.2 m.y.).

Concerning the Blue Petrel *Halobaena caerulea* and the pair Antarctic - Slenderbilled Prions, the mean value of p (0.0743) is compatible with the existence of distinct species; the mean divergence time (about 3.7 m.y.) is lower than the mean values obtained from electrophoresis between the genus *Pachyptila* and the Fulmarinae (13.66 m.y.), and between confamilial genera (13.4 m.y.), suggesting that *Halobaena* and *Pachyptila* are closely related, in agreement with previous data (Barrowclough *et al.* 1981, Bretagnolle 1990). A comparison with paleontology, where the first fossil records of prions are from the early Pliocene (Olson 1985), does not show any considerable discrepancy with our mtDNA data.

Pelecanoididae

Interspecific differentiation is very high between the two studied diving petrels; their morphologies can be explained by selective constraints induced by their identical, and very peculiar, living habits.

Differentiation among Procellariiformes

According to our results, there are four distinct groups corresponding to the four presently recognized families, which are shown (Fig. 2 and 3) in both UPGMA and Neighbour-joining trees (from G.B. Nunn pers. comm.). Procellariidae, which include two groups, prions and Fulmarinae, appear closest to one another, and Diomedeidae join them at a slightly higher level, whereas storm petrels Hydrobatidae are very distinct from the other three families and constitute the "outgroup". Pelecanoididae are found between Diomedeidae and Procellariidae. The UPGMA phenogram considers ultrametric distances (two sister taxa have equally diverged from a common ancestor) and assumes the existence of a molecular clock, even if genetic distances may not increase linearly with time (Swofford & Olsen 1990). The Neighbour-joining method does not take into account the existence of this molecular clock, and it considers the distances between nodes are additive. If the data are not ideal, which is the case here, Nei's (1975) distances depending on heterozygosity, there will be no optimal tree (reviewed by Swofford & Olsen 1990). However, there are a few differences between the phenograms showed in this paper; they concern the position of Diomedeidae and the relation between *Phoebetria* and *Diomedea*.

Commonly expressed opinions have been, and are still in part, very different about the evolution of Procellariiformes. For a long time, only three families were recognized, Hydrobatidae being a sub-family of Procellariidae (Mayr & Amadon 1951). However, Alexander *et al.* (1965) considered that four families should be recognized, but stated that Pelecanoididae are well separated from the other three, whereas according to Cracraft (1981) the Diomedeidae form a sub-order, the other being composed of families Hydrobatidae and Procellariidae (including *Pelecanoides*). Sibley *et al.* (1988), who used DNA-DNA hybridization techniques, put albatrosses, petrels, shearwaters and storm petrels together into the same order (Ciconiiformes) and the same family (Procellariidae); they distinguished three sub-families: Hydrobatinae (storm petrels), Procellariinae (petrels, diving petrels and shearwaters), and Diomedeinae (albatrosses). Our results are in agreement with Harper (1978), who considers that the Hydrobatidae diverged very early. We also have similar results to the genetic distance data obtained by Barrowclough *et al.* (1981), firstly for the differentiation among Fulmarinae as noted above, and secondly for the very high genetic distances between Hydrobatidae and the other families. However, a greater number of species must be studied if we are to have a clear view of this

order, particularly of the complicated Procellariidae family.

Patterns of variability

Average across-species levels of heterozygosity

The average heterozygosity level for the species studied here is H=0.035. It is lower than the mean H=0.051 for 46 bird species in the review by Nevo *et al.* (1984). However, the latter value concerns mostly the order Passeriformes. The 40 species of Passeriformes give a value of H=0.052 whereas the six remaining species of other orders have a value of H=0.043. According to the results of Nevo *et al.* (1984), the different vertebrate classes show significantly different average levels of heterozygosity. This seems to be a controversial conclusion because the class Aves had previously been considered to have the lowest average of polymorphism, after the study of Kitto & Wilson (1966), whereas nowadays the lowest rank is given to Mammalia with H=0.041 (N=184 species, Nevo *et al.* 1984). The number of bird species studied is still rather low (Nevo *et al.* 1984 studied 46 species with sufficient numbers of individuals and loci). But it may be that the class Aves is not homogenously polymorphic, especially as concerns the order Passeriformes compared to other orders (see the particular characteristics of the order Passeriformes in Bock & Farrand 1980).

The three orders studied were not significantly different from each other (by Kruskal-Wallis test, H=0.07, $p>>0.05$). The differences in average heterozygosity between the three localities: Adélie Land, Kerguelen and Crozet Islands, showing an increase towards the higher latitudes, is comparable to the variations of mean polymorphism for bird species in the review by Nevo *et al.* (1984). They demonstrated that three broad life zones, Tropical (H=0.027 $\pm$ 0.028, N=2), Temperate (H=0.044 $\pm$ 0.019, N=24) and Arctic (H=0.050 $\pm$ 0.034, N=5), were significantly different at $p<0.05$. However, the numbers of populations studied are often very low in both cases, and, moreover genetic variability can be very different between animal groups (Nevo *et al.* 1984).

Patterns of variability

Even without taking into account the populations studied with too few loci and/or individuals, there is no clear correlation of the patterns of variation in our heterozygosity estimates with biological characteristics. Similarly, Nevo *et al.* (1984), in their review concerning genetic variation, found few correlations with biological features among the 968 species (551 vertebrate species), subjected to electrophoretic study. The most significant correlation was with the spatio-temporal heterogeneity of the environment, giving the interesting result that demography does not seem to be an important parameter. It is likely that such factors as, for instance, past fluctuations of population size could play an important role. Population "bottlenecks" in fact induce more or less important reductions of genetic polymorphism (Nei *et al.* 1978), whereas such variations more often cannot be precisely known (Barrowclough 1983). Moreover it is clear that studies of different populations of the same species can give rather different values of genetic polymorphism, as is the case here, for which explanations are available, but which mean that very often a population cannot be taken as representative of a whole species. We noted above however, in the cases of the Lesser Sheathbill and the Northern Rockhopper Penguin, some biological and historical factors that could explain their low levels of variability.

Snow Petrel

In other genetic variation studies concerning bird species, the most important factor determining higher levels of polymorphism appeared to be hybridization between differentiated populations (Barrowclough 1980, Corbin 1981). This is a very plausible explanation for the rather high genetic polymorphism in the Snow Petrel (Jouventin & Viot 1985). In Adélie Land this species shows a considerable morphological variability, of a level

unique among seabirds. It has been shown that within this population some birds are twice as large as others (Guillotin & Jouventin 1981). We proposed that this region constitutes a secondary hybridization zone. Following the last ice-age, two subspecies of very different sizes, of which "pure" populations exist in other breeding places of the species, are thought to have established colonies in such ice-free areas as Adélie Land, thus creating a hybrid population.

Perspectives

Our results from mtDNA sequencing do not seem in disagreement with electrophoretic data, except for the Gentoo Penguin. However, our samples and the DNA fragments which have been sequenced remain too small to allow of firm conclusions. Sequencing the whole cytochrome *b* gene from a greater number of individuals will complete information; if too few substitutions, inducing a loss of resolving power, are still observed, it would be possible to sequence the D-loop region of mtDNA, which evolves as much as ten times faster than the rest of the mitochondrial genome (Wilson *et al.* 1985).

Mitochondrial DNA analysis seems to be a less polyvalent technique than protein electrophoresis, especially if the sequence divergence percentages are too low (prions), or are too high (see Brown *et al.* 1982) as it is the case between families or orders.

DNA extraction from feather pulp appears as an elegant and non-invasive method; but it seems to provide too little amounts of this molecule in Sphenisciformes (because of a very high rate of keratin in their feathers), so that amplification using PCR can almost never be performed. Taking blood samples from these species would be a more suitable technique.

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APPENDIX 1

(A) SPHENISCIFORMES (N=6 SPECIES) H=0.033±0.011

Species	Location	Abbreviation	H	P	N	L	NB/NT	LB/NT
Emperor Penguin								
Aptenodytes forsteri	A-L	*A.f*	0.034	0.32	28	22	-28	-/22
King Penguin								
A. patagonicus	CRO	*A. p.C.*	0.027)	0.26	173	22	166/7	13/22
			) 0.025					
A.patagonicus	KER	*A.p.K.*	0.018)	0.29	50	13	50/-	13/-
Adélie Penguin								
Pygoscelis adeliae	A-L	*P.a*	0.046	0.28	15	22	-/15	-/22
Gentoo Penguin								
P. papua	CRO	*P.p.C.*	0.030)	0.23	32	13	32/-	13/-
			) 0.034					
P. papua	KER	*P.p.K.*	0.040)	0.31	24	13	24/-	13/-
Macaroni Penguin								
Eudyptes chrysolophus	CRO	*E.c.*	0.018	0.23	54	22	54/-	13/22
Rockhopper Penguin								
E. c. chrysocome	CRO	*E.c.c.C.*	0.044)	0.23	79	13	79/-	13/-
E. c. chrysocome	KER	*E.c.c.K.*	0.053)	0.23	16	13	16/-	13/-
			) 0.043					
E. c. moseleyi	AMS	*E.c.m.*	0.010	0.08	7	13	7/-	13/-

(B) CHARADRIIFORMES (N=3 SPECIES) H=0.031±0.019

Species	Location	Abbreviation	H	P	N	L	NB/NT	LB/NT
South Polar Skua								
Catharacta maccormicki	A-L	*C.m.*	0.038	0.17	13	27	-/13	-/27
Subantarctic Skua								
C. antarctica lonnbergi	CRO	*C.s.l.C.*	0.056)	0.11	9	27	9/1	10/27
			) 0.046					
C. a. lonnbergi	KER	*C.s.l.K.*	0.000)	0.00	2	27	2/1	10/27
Lesser Sheathbill								
Chionis minor minor	KER	*C.m.m.*	0.023)	0.07	12	29	6/6	10/27
			) 0.010					
C. m. crozettensis	CRO	*C.m.c.*	0.008)	0.03	106	29	102/6	10/27

(C) PROCELLARIIFORMES (N=13 SPECIES) H=0.036±0.025

Species	Location	Abbreviation	H	P	N	L	NB/NT	LB/NT
Wandering Albatross								
Diomedea exulans	CRO	*D.e.*	0.000	0.00	106	22	106/1	11/22
Blackbrowed Albatross								
D. melanophris	KER	*D.m.*	0.000	0.00	1	21	-/1	-/21
Lightmantled Sooty Albatross								
Phoebetria palpebrata	CRO	*P.p.*	0.018	0.09	4	23	4/1	12/22
Sooty Albatross								
P. fusca	CRO	*P.f.*	0.018	0.08	14	12	14/-	12/-
Antarctic Petrel								
Thalassoica antarctica	A-L	*T.a.*	0.038	0.08	1	26	-/1	-/26
Pintado Petrel								
Daption capense	A-L	*D.c.*	0.051	0.19	6	26	-/6	-/26
Snow Petrel								
Pagodroma nivea	A-L	*P.n.*	0.082	0.48	31	27	21/10	11/27
Antarctic Prion								
Pachyptila desolata	KER	*P.d.*	0.056	0.18	7	22	5/7	11/22
Slenderbilled Prion								
Pachyptila belcheri	KER	*P.b.*	0.024	0.05	1	21	-/1	-/21
Salvin's Prion								
Pachyptila salvini	CRO	*P.s.*	0.042	0.10	2	21	-/2	-/21
South Georgian Diving Petrel								
Pelecanoides georgicus	KER	*P.g.*	0.044	0.10	2	20	-/2	-/20
Common Diving Petrel								
Pelecanoides urinatrix	KER	*P.u.*	0.026	0.05	1	19	-/1	-/19
Wilson's Storm Petrel								
Oceanites oceanicus	A-L	*O.o.*	0.068	0.25	5	20	-/5	-/20

TAXA STUDIED (ALL: 22 SPECIES H=0.035±0.020)

Localities of the populations sampled (A-L= Adélie Land, Ker= Kerguelen Island, Cro= Crozet Islands, AMS= Amsterdam Island); abbreviations used later; genetic heterozygosity (H) for each population and mean heterozygosity across each order and for all populations; proportion of polymorphic loci (P, most common allele μ 0.99); total sample size (N); total number of loci studied (L); sample size for blood/non-blood tissues (NB/NT); number of loci studied in blood/non-blood tissues (LB/LT).

APPENDIX 2: (A) SPHENISCIFORMES

Locus	*A.f.*	*A.p.C.*	*A.p.K.*	*P.a.*	*P.p.C.*	*P.p.K.*	*E.c.*	*E.c.c.C.*	*E.c.c.K.*	*E.c.m.*	B*
Aat-1	d (0.97) e (0.03)	d (0.979) e (0.018) a (0.003)	d	c (0.96) b (0.04)	f	f p(0.95) e (0.05)	b (0.98) a (0.01) e (0.01)	b	b	b	1; 5
Aat-2	c (0.98) a (0.02)	c	-	d (0.93) b (0.07)	-	-	d	-	-	-	2; 5
Acp-2	a (0.96) b (0.04)	a	-	a	-	-	a	-	-	-	2
Ak	a	a	-	a	-	-	a	-	-	-	5
Alb	b	b	b	a	a	a	a	a	a	a	6
Amy	a	b	b	c	e	e	d	e	e	e	7
Ck	b	c	-	a	-	-	a	-	-	-	5
Est-1	b	b (0.964) c (0.020) a (0.016)	b (0.97) c (0.02) a (0.01)	c	c	c (0.98) b (0.02)	d (0.92) e (0.06) g (0.02)	f (0.72) i (0.25) h (0.03)	f (0.75) i (0.25)	f	6
Est-3	a (0.64) b (0.36)	b	b	a	a	a	a	a	a	a (0.93) b (0.07)	4
Glo	b	b (0.996) c (0.004)	b (0.93) c (0.07)	b	b	b	b	b (0.98) a (0.02)	b (0.90) a (0.10)	b	4; 5
Gpd-2	c	c (0.92) a (0.08)	-	b	-	-	d	-	-	-	5
Ldh-1	b	b (0.997) c (0.003)	b	b	a	a	c	c	c	c	5
Ldh-2	a	a	a (0.99) b (0.01)	a	a (0.98) b (0.02)	a	a	a	a	a	5
Mdh-1	a	c	c	b	b (0.98) c (0.02)	b	b	b (0.99) c (0.01)	b	b	2
Mpi	a (0.98) b (0.02)	a	-	c (0.91) b (0.09)	-	-	a	-	-	-	5
Pgd-1	d (0.98) a (0.02)	d (0.982) e (0.015) a (0.003)	c (0.97) e (0.03)	d (0.92) b (0.08)	d (0.81) b (0.19)	d (0.79) b (0.17)	d (0.97) a (0.03)	d (0.99) e (0.01) a (0.04)	d	d	3; 5
Pgd-2	b	b	-	a	-	-	b	-	-	-	8
Pgi 5	d (0.98)	d (0.922)	d (0.97)	d (0.94)	e		e (0.98)	d (0.97)	d (0.96)	d (0.93)	d
	a (0.02)	a (0.050) b (0.025) e (0.003)	b (0.02) a (0.01)	c (0.06)			f (0.02)	e (0.03)	b (0.04)	b (0.07)	
Pgm-1	b	b	-	c (0.75) a (0.25)	-	-	a	-	-	-	5
Sod-1	a	a	a	b	b	b	a (0.95) c (0.05)	a	a	a	4
Sod-2	c	d (0.93) c (0.07)	-	a	-	-	b	-	-	-	2

Locus Mdh-2 appeared monomorphic and fixed for the same alleles in all populations.

* buffer systems - (gel/electrode pH):
(1)TC 6.0/5.4 described in Jouventin & Viot (1985)
(2) TC 6.7/6.3; (3) TM 6.9/6.9; (4) TM 7.4/7.4,; (5) TC 8.0/8.0; (6) LiOH 8.3/8.1; (7) TG 8.5/8.1; (8) TcB 8.7/8.2; (9) TEB 8.6/8.6 described in Selander *et al.* (1971)

APPENDIX 2: (B) CHARADRIIFORMES.

Locus	*C.m.*	*C.s.l.C.*	*C.s.l.K.*	*C.m.c.*	*C.m.m.*	B*
Aat-1	a	a	a	b	b	1
Acp-1	a	a	a	b	b	2
Acp-2	yb	b	b	a	a	2
Adh-1	a	a	a	b	b	4
Adh-2	b	b	b	a	a	4
Alb	a	a	a	b	b	6
Amy	a	a	a	b	b	7
Eo-1	b	b	b	a (0.86) c (0.14)	a	6
Est-8	-	-	-	a	b	6
Gpd-1	a (0.73) b (0.27)	a (0.50) b (0.50)	b	a	a	5
Gpd-2	a	a	a	b	b	5
Icd-1	b	b	b	a	a	2
Icd-2	a (0.79) c (0.21)	a (0.50) c (0.50)	c	b	b	2
Ldh-2	b	b	b	a	a	4
Mdh-1	b	b	b	a	a	1
Mdh-2	b	b	b	b	b (0.75) a (0.25)	1
Me-1	b	b	b	a	a	5
Me-2	c (0.96) b (0.04)	c (0.50) b (0.50)	c	a	a	5
Mpi	a	a	a	b	b	5
Np	a	a	a	b	b	9
Pgd-1	c (0.92) b (0.08)	c	c	a	a	2
Pgi	a (0.96) c (0.04)	a	a	b	b	5
Pgm-1	b	b	b	a	a	5
PiP	-	-	-	a	a (0.83) b (0.17)	6
Sdh	b	b	b	a	a	5
Sod-1	b	b	b	a	a	4
Sod-2	a	a	a	b	b	4

Aat-2 and Ldh-1 appeared monomorphic and fixed for the same alleles in all populations.

APPENDIX 2: (C) PROCELLARIIFORMES

Locus	*D.e.*	*D.m.*	*P.p.*	*P.f.*	*T.a.*	*P.n.*	*D.c.*	*P.d.*	*P.b.*	*P.s.*	*P.g.*	*P.u.*	*O.o.*	B*
Aat-1	d	d	d	d	d (0.50) a (0.50)	d	d	d	d	d	f	e	b (0.90) c (0.10)	5
Aat-2	a	b	b	-	b	b	b	b	b	b	b	b	b	5
Acp-1	-	-	-	-	a	a	a	-	-	-	-	-	-	1
Acp-2	b	b	b	-	b	b (0.95) d (0.05)	b	b	b	b	c	a	a	2
Acp-3	a	-	a	-	-	a	-	-	-	-	-	-	-	1
Alb	d	d	d (0.88) c (0.12)	d	g	g (0.58) f (0.42)	g	e	e	e	b	b	a	6
Est-2	a	a	c (0.88) b (0.12)	c	f	d (0.90) f (0.10)	d (0.50) e (0.50)	-	-	-	-	-	-	6
Est-4	g	g	g	-	e	f	f (0.75) e (0.25)	b (0.64) c (0.36)	b	c	a (0.50) d (0.50)	d	c	6
Est-5	d	d	d	-	a	b	c	-	-	-	b	b	e	6
Est-6	-	-	-	-	-	-	-	c	a	b	-	-	-	6
Est-7	-	-	-	b	a	-	-	-	-	-	-	-	-	6
Glo	b	b	b	b	b	b (0.95) c (0.05)	b	a (0.57) b (0.43)	a (0.50) b (0.50)	a (0.50) b (0.50)	b	-	d (0.90) e (0.10)	4
Gpd-2	b	b	b	-	a	c (0.90) a (0.10)	a (0.92) c (0.08)	c	c	c	d	e	c	5
Ldh-1	d	d	d	d	b	b (0.95) c (0.05)	b	b (0.93) a (0.07)	b	b	d	d	c	8
Ldh-2	a	a	a	a	d	d	d	b	b	b	b	b	c	8
Mdh-1	b	b	b	b	b	b (0.98) a (0.02)	b	b	b	b	b	b	b	1
Mdh-2	b	b	b	b	b	b	b	b	b	b	b	b	a	1
Me-2	e	e	f	-	c	c (0.90) b (0.10)	g (0.92) d (0.08)	d (0.92) a (0.08)	d	d	j	i	b	1; 4
Mpi	c	c	c	-	c	c (0.90) d (0.10)	c	c	c	c	c	c	a(0.90) b(0.10)	5
Np	-	-	-	-	a	b	-	-	-	-	-	-	-	8
Pgd-1	f	c	c	c	c	b (0.92) h (0.05) a (0.03)	b	c	c	c	d	d (0.50) g (0.50)	e	2

Pgd-2	-	-	-		b (0.90) b c (0.05) a (0.05)	c	c	c	-	-	-	1	
Pgi	d	d	d	d (0.88) e b (0.12)	e (0.95) e c (0.05)	d	d		d (0.75) d (0.75) d b (0.25) a (0.25)		e (0.50) 5 f (0.50)		
Pgm-1	c	c	c	-	b	b	b	b	b	c	b	b (0.80) 5 a (0.20)	
Pi-L	-	-	-	-	a (0.50) b	b	-	-	-	-	-	-	6
Pi-M	-	-	-	-	a	b	a	a	a	-	-	-	6
Sdh	d	d	d	-	c	d (0.85) d	d	d	d a (0.10)	d	d	b	5
Sod-1	a	a	a	a	b	e (0.05) b	b	b	b	b	b	c	1
Sod-2	a	a	a	-	b	b	b (0.92) b a (0.08)	-	-	b	b	c	2

APPENDIX 2. ALLELIC FREQUENCIES FOR VARIABLE LOCI. FIGURES IN PARENTHESIS ARE FREQUENCIES FOR ALLELES (ALPHABETICALLY LABELED) WHEN A PARTICULAR ALLELE IS NOT FIXED AT THAT LOCUS IN THE POPULATION.

APPENDIX 3

LIST OF THE ENZYMES AND NON-ENZYMATIC PROTEINS STUDIED, WITH THEIR CORRESPONDING NUMBERS AND ACRONYMS USED IN THE TEXT AND OTHER TABLES

Enzymes and proteins	Number	Acronym
Aspartate amino transferase	2.6.1.1	Aat-1, 2
Acid phosphatase	3.1.3.2	Acp-1, 2, 3
Alcohol dehydrogenase	1.1.1.1	Adh-1, 2
Adenylate kinase	2.7.4.3	Ak
Albumin	-	Alb
Amylase	3.2.1.1	Amy
Creatine kinase	2.7.3.2	Ck
Esterase	3.1.1.1	Est-1*
		Est-2*
		Est-3**
		Est-4***
		Est-5***
		Est-6***
		Est-7****
		Est-8****
Glyoxalase	4.4.1.5	Glo
Á-glycerophosphate dehydrogenase	1.1.1.8	Gpd-1, 2
Isocitrate dehydrogenase	1.1.1.42	Icd-1, 2
Lactate dehydrogenase	1.1.1.27	Ldh-1, 2
Malate dehydrogenase	1.1.1.37	Mdh-1, 2
Malic enzyme	1.1.1.40	Me-1, 2
Mannose-6-phosphate isomerase	5.3.1.8	Mpi
Nucleoside phosphorylase	2.4.2.1	Np
Phosphogluconate dehydrogenase	1.1.1.44	Pgd-1, 2
Phosphoglucoisomerase	5.3.1.9	Pgi
Phosphoglucomutase	2.7.5.1	Pgm-1, 2
Protein in liver	-	Pil
Protein in skeletal muscle	-	tPim
Plasmatic protein	-	Pip
Sorbitol dehydrogenase	1.1.1.14	Sdh
Superoxyde dismutase	1.1.1.5.1.1.	Sod-1, 2

(*: plasma and non-blood tissues; **: haemolysate and non-blood tissues; ***: non-blood tissues; ****: haemolysate).