A COMPARATIVE STUDY OF PLASMA PROTEINS FROM TWENTY-NINE SPECIES OF ALBATROSSES AND PETRELS (Order PROCELLARIIFORMES).

by

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Submitted for the degree
of Doctor of Philosophy in Zoology
at Victoria University of Wellington
NEW ZEALAND, 1978

ABSTRACT

The plasma proteins of 29 species of albatrosses and petrels were electrophoretically separated in acrylamide gels to clarify relationships at the species-group to family-group levels. Little in the resulting data from 472 birds seriously contests the present classification of the Procellariiformes; much of the biochemical evidence supports, confirms, and clarifies the proposals of conventional taxonomic methodology. The biochemical data give fresh insights into the interrelationships of procellariiform taxa, and highlight intriguing new problems.

Sex, season, age, and other sources of non-genetic protein variation are insignificant for taxonomic purposes. Proteins of comparable value include the transferrins, some α and β globulins, albumins, prealbumins, and non-specific esterases. Genetic variations in the mobility of these proteins are useful at the genus-group level and below. Other proteins are monomorphic at genus and family level, and three are monomorphic in both number and mobility throughout the Procellariiformes; these are useful reference points for calibrating samples on different gels. One conspicuous α protein is absent in the Hydrobatidae but present in all other families; the implications of this are discussed.

Polymorphic proteins at the population or species level were not detected; this conspicuous phylogenetic conservatism is discussed with regard to its possible evolutionary significance.

Following a summary of the protein data, three categories of defined probability statements, based on the biochemical and other evidence, allow speculative comment on the evolutionary relationships and history of the taxa within the Procellariiformes. The value of further biochemical research into the marine birds is emphasised.

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"... before long we shall have a subject called protein taxonomy ..."

Francis Crick, 1958.

"The value of the chemical approach to taxonomy is that it brings additional and independent evidence to a taxonomic discussion."

Holger Erdtman, 1968.

"Both chemical and morphological properties of the phenotype are the outcome of biosynthetic processes governed by the genome, and so far as it can be said to be the taxonomist's aim to classify genotypes, he is likely to find as reliable guides in the one as in the other."

J. Heslop-Harrison, 1968.

"Thus we are made aware, once again, of how useful protein studies can be when used in conjunction with systematic data based on traditional phenetic approaches."

Linda Maxson, 1977

INTRODUCTION

The fact that the Procellariiformes are an ancient group of marine birds is evidenced by their fossil record, structural isolation, behaviour and distribution. They probably originated along the shores of a fragmenting Gondwanaland in the late Cretaceous, when a fauna of aquatic birds of distinctly modern appearance existed. These included loons, grebes, penguins, and cormorants, in which affinities with the ocean birds have long been recognised (Brodkorb 1971, Murphy 1936). Avian fossil evidence is consistent with an average interordinal divergence time of 80-90 million years (Fisher 1967, Brodkorb 1971, Prager et al. 1974, Cracraft 1973, Wilson et al. 1977). Procellariiform fossils are not common; two Lower Tertiary albatrosses have been tentatively identified: *Gigantornis eaglesomei*, an immense creature twice the size of the largest living albatross with some possible skeletal relationships with the cormorants (Pelecaniformes), comes from the Middle Eocene of Nigeria (Andrews 1916); *Manu antiquus*, from New Zealand's South Island Upper Oligocene (Marples 1946), was probably similar in size to the wandering albatross (*Diomedea exulans*). Smaller Miocene albatrosses are known from California and South Australia. *Diomedea thyridata*, from Victoria, Australia, was described from an incomplete fossil bill with lateral nostrils and nasal sulci identical to that of extant *Diomedea* species (Wilkinson 1969). The living group of *Puffinus* shearwaters (family Procellariidae) was represented during the Oligocene (Lambrecht 1933), and no less than nine Miocene species are known from France, Maryland, and California (Brodkorb 1963). The fulmarine petrels (family Procellariidae)

are found in the Lower Patagonian (Lower Miocene) formations of Argentina and the Burdigalian formation near Bordeaux, France. The storm petrels (family Hydrobatidae) date back to the Upper Miocene of California (Brodkorb 1971).

From a practical viewpoint, the Procellariiformes are "storm" birds in more ways than one. Although abundantly distributed over all oceans, they are not easy birds to study. In addition to the difficulties of reaching their breeding grounds, many petrels disappear on migrations over vast tracts of ocean, so that for most of their life cycle they are not amenable to scientific study in the sense that land birds with defined habitats are. Consequently, knowledge from all levels of field research into the biology of these birds is fair at best and non-existent at worst. For some species such as the Laysan albatross (*Diomedea immutabilis*), the northern fulmar (*Fulmarus glacialis*), or the short-tailed shearwater (*Puffinus tenuirostris*) the volume of information is impressive, thanks to the thorough and long-term work of James Fisher (1952), Dom Serventy (1967) and Harvey Fisher (1971). For other taxa, such as the prions (*Pachyptila* spp.), even skilled ornithologists cannot always correctly identify museum skins, let alone distinguish the living birds at sea (Harper 1972). As a final insult, the whereabouts of some petrels' nesting grounds is unknown; Pacific storm petrels afford but one example (Crossin 1974). Hutton's shearwater (*Puffinus huttoni*) is another example of this sort of problem, solved only recently when Harrow (1965) found burrows tunnelled into snow tussocks 1524 m (5000 ft) up the Seaward Kaikoura mountains of New Zealand's South Island - a unique nesting habitat for New Zealand petrels, and thus not the place one would normally look for them.

Yet such discoveries are not astonishing to those who recognise the long ancestry of the Procellariiformes. Their phyletic history spans perhaps 80-90 million years, a period which has seen the evolution of a group of highly successful and specialised oceanic birds with few if any present-day (even ancestral?) competitors in other avian orders. However, the very success of the Procellariiformes has resulted in competition from within their own ranks. There is much information to suggest that it has been, and remains, a relentless and compelling selective pressure (e.g., Murphy 1936; and see below).

In order to meet this challenge, the evolutionary stratagem of the Procellariiformes has been (1) to benefit from the selective advantages afforded by differential body size; (2) to diversify prey-catching behaviour; and (3) to fully exploit the space/time continuum. The facts bespeak the outcome. The petrels exhibit a range in size unparalleled in other avian taxa. They obtain their food prey by "dipping, pattering, hydroplaning, surface seizing and filtering, and pursuit plunging and diving" (Ashmole 1971). Spectacular transequatorial and circumpolar migrations in search of food supplies are a feature of many procellariiform genera. Eggs are laid consistently within a week, year after year, by a particular species, and burrows can be used throughout the year by different genera of comparable size. Two species of petrel will occasionally use a single burrow with a branched interior to raise their young (see, for example, Oliver 1955). Albatrosses sit overtly on their tussock nest mounds, while small petrels return secretively to their nests tunnelled into the mud bases of the albatross's handiwork.

These exemplary adaptations to minimise intraordinal competition, fostered over the millenia, compound the puzzles for seabird taxonomists and all those requiring a stable nomenclature from which to work. Not only does the taxonomist have a patchy framework of information to contend with, but speciation in the Procellariiformes is often bewildering in its complexity. The enigmatic gadfly petrels (*Pterodroma* spp.) afford an excellent example of this phenomenon. Students of the oceanic birds can appreciate Professor A.J. Cain's pithy remark: "All that the really competent taxonomist needs to know is everything about his subject and about the groups he works on".

Because Sibley & Ahlquist (1972) have recently presented a cogent review of the taxonomic history of the petrels, I need not enlarge here on the same topic. They comment that although the albatrosses and petrels are widely accepted as being a natural monophyletic group of marine birds, the relationships between the original groups have been the subject of conflicting and diverse opinion. An unfortunate series of inconsistent new classifications devised by the quixotic Gregory Mathews between 1933 and 1948 produced taxonomic chaos in an already difficult group (Serventy 1950).

I have no wish to rekindle a controversy best left dead; the matter is fully discussed by many of its international participants - see Alexander et al. (1965). These 15 authors of an open letter to the Editor of "Ibis" stressed the need for a restabilised nomenclature, and set about producing guidelines based on reasoned opinion, concluding with a classification which has, understandably, met with wide approval; I follow it throughout this thesis. Alexander et al. (1965) emphasise that

"... few of the major changes in the classification of the petrels proposed in recent years have been based upon new research or involve important reassessments of relationships. Most have been based upon a subjective reassessment of existing information."

One new line of inquiry, as a rapidly growing number of workers are discovering, is to probe taxonomic problems on all levels with electrophoretic techniques. The results have been both encouraging and exciting. For instance, although two populations of a Florida species of sea-cucumber, *Thyonella gemmata*, were found by Manwell & Baker (1963) to differ very slightly in morphological and behavioural characteristics, an absence of gene flow between the 'stout' and 'thin' populations was clearly evident in their completely different haemoglobin and esterase patterns. The starch-gel electrophoresis showed the 'populations' to be reproductively isolated, and thus more properly regarded as sibling species.

In his review of the biochemical evidence of relationships in amphibians and reptiles, Dessauer (1974) notes that whereas the paleontological approach is so useful in establishing evolutionary events within certain other groups, it is often of little use in the above heterotherms owing to a lack of critical fossils. This fact, together with the conservative skeletal structure and external morphology of many reptiles and amphibians, has created daunting taxonomic problems; Blair (1962, cit. Dessauer 1974) writes "The classification of anuran amphibians from the level of suborder to species formation from purely morphological criteria may be best described as chaotic." Dessauer (1974) compiles many examples to show how study of the electrophoretic patterns of proteins is providing a promising

approach to many of these problems. The successes of biochemical techniques in the taxonomy and population genetics of fish are discussed at length by O'Rourke (in Wright 1974; see also Manwell et al. 1963).

The grouping of the orang-utan, chimpanzee, gorilla, and man into a single family, the Pongidae, by Tashian (1968) using evidence from esterases in their erythrocytes is one of numerous examples from the Mammalia. Johnson (1974), in his informative and entertaining review of mammalian taxonomy, notes: "Because good criteria are already available, taxonomists in mammalogy have been less sanguine to accept radically new techniques. By the same token, a trial of new methods is probably best evaluated in situations where a maximum background of information is already available. Relative to molecular taxonomy, it may be stated that this newest of taxonomic data fits well with traditional evaluation, as it should."

Sibley et al. (in Wright 1974) have given a thorough and comprehensive review of the application of biochemical and immunological techniques to problems of avian systematics. They give many examples of where these techniques have unearthed errors and clarified relationships in numerous avian taxa. Electrophoresis of the egg-white of a little known Costa Rican bird, *Zeledonia coronata*, "showed it to be a member of the New World nine-primaried oscine assemblage, and not related to the thrushes. Subsequent study of specimens and a re-evaluation of previous anatomical papers indicated that *Zeledonia* is most closely allied to the wood warblers (Parulidae)." Since 1960, Sibley and his associates have studied 1450 species of birds representing all the orders recognised by Wetmore (1960) - a major and pioneering effort which has highlighted and elucidated

many taxonomic problems (see, for example, Sibley 1970, Sibley & Ahlquist 1972).

The petrels first came under scrutiny in 1960, when Sibley included four shearwaters, a fulmar, and a *Bulweria* petrel among a comparison of 359 species of non-passerine birds using paper electrophoresis. His interordinal comparisons led him to remark: "The egg-white profiles show general resemblances to some Pelecaniformes, some Ciconiiformes, some Charadriiformes and some Anseriformes. Nevertheless the profiles of the Procellariidae are clearly different from all of these and a choice is not possible."

Twelve years later Sibley & Ahlquist (with the help of 350 assistants) expanded the endeavour to include 32 species from the four procellariiform families. Their primary objective was the relationships at the family level or higher, not the intra- or interspecific and generic ones. Their methods using starch gels were extremely comprehensive, but were limited by a lack of resolution in the separated proteins; the comparative value of their results suffers accordingly. They conclude:

> "The Procellariiformes are a monophyletic group of birds. This is demonstrated by the egg white protein evidence and is supported by a large array of previous evidence from a variety of sources. Because of the uniformity of the starch-gel patterns it is not possible to speculate upon relationships within the order. The egg white pattern of *Pelecanoides* is indistinguishable from those of some petrels. From this evidence and from that of previous studies we conclude that *Pelecanoides* is more closely allied to the Procellariiformes than to any other group. The Procellariiformes appear to be allied

to the Sphenisciformes, and they may be related to some or all of the following groups: Charadriiformes, *Gavia, Fregata, Phaethon, Pelecanus*."

Brown & Fisher (1966) were the first to probe procellariiform serum proteins and the investigation of procellariiform interspecific relationships (2 albatrosses, 2 shearwaters, and a gadfly petrel - 5 species, $\underline{n}$ = 31). Using paper electrophoresis, they found that "The genera were well marked, and congeneric species exhibited the greatest similarities. The families Diomedeidae and Procellariidae did not show marked separation". Paper electrophoresis has even greater limitations than starch gels as regards resolution and subsequent interpretation of protein fractions; newer techniques are considerably more helpful.

Shaughnessy (1970) examined a 97-bird sample of two sibling species of giant petrel (*Macronectes*) at Macquarie Island, and compared their serum proteins. Using starch-gel as his electrophoretic medium, he found that the mobilities of transferrin, albumin, and a haem-binding protein were identical in *M. giganteus* and *M. halli*. He therefore concluded that the two species are genetically very similar. *Macronectes* was the only petrel genus among six Antarctic birds investigated by Murrish & McMahon (1975) using 7% acrylamide gel discs and a tris/glycine buffer (cf. Materials and Methods).

An interesting approach of doubtful taxonomic significance is Jacob's (1976) analysis of the uropygial gland wax of seven species of Procellariiformes representing the four families. My conclusion from Jacob's data is that the albatrosses (*Diomedea* spp.) are more closely related to flamingoes (*Phoenicopterus* spp.) than to other petrels - the chemical approach to taxonomy is not

without its pitfalls.

Boulter & Thurman (1968) have laid down constructive guidelines which bear reiteration: "To be useful, chemical characters must be shown to be 'good' taxonomic characters, i.e., they should not vary within the samples being considered, should not be susceptible to environment modification and should show consistency, that is correlate with existing classifications constructed using other characters. For the methods to be feasible, they must be within the reach of the average taxonomist from a technical standpoint."

Advantages and disadvantages of electrophoretic data, as compared with more conventional systematic criteria such as morphology, are reviewed by Avise (1975). Advantages are as follows.

(1) Objectivity: "The enumeration of alleles and their frequencies are objective determinations, based solely on the mobility of bands in gels. Subjectivity may occasionally enter into some morphological data (body form) or behavioural data" (cf. Mathews 1933, 1948).

(2) Most of the loci routinely examined show little or no age or sex specific variation: "This observation may be of particular value in cases where there is little life history information about an organism" (this statement is true for many of the Procellariiformes).

(3) Amount of genetic information: plasma contains information of a large number of loci, whereas some sub-species of procellariids (e.g., the *Pachyptila* prions) are distinguished solely by 0.5 mm differences in bill width.

(4) "Electrophoretic techniques yield very precise data on genetic contents of organisms - only amino acid or nucleotide sequencing (at present, very laborious techniques) promise to give greater precision." Such information is especially pertinent to genera such as *Pterodroma* (see below).
(5) For most loci, common function strongly implies common origin (useful for comparative relationships; see below).

Avise continues with the "several real and theoretical disadvantages of the electrophoretic approach to systematics": (1) the technique is applicable to living animals only; (2) the chance of identity in band mobility (Sibley (1970) considers this very remote); (3) some practice and training is required in scoring differences in protein band mobilities; (4) some protein differences may remain undetected (see my 'minor' protein classification, below); (5) since the proteins subjected to electrophoresis are water-soluble, an unknown amount of bias could be present; and (6), electrophoretic data do not include information on the number of amino acid differences between proteins - "Two alleles may be separated by one or many mutational steps" (Avise 1975).

Despite these limitations, however, the volume of papers now appearing in the literature is clear testimony of the value and potential of electrophoretic and immunological techniques (e.g., Wright 1974). In recent years several theories involving micro-complement fixation and electrophoretic data have been used to compute the rates of evolution of species and their times of divergence (see Wilson et al. 1977). These proposals and analyses are indeed most exciting and thought-provoking, but I agree with Johnson (1974) that they need "more seasoning and correlated data" before being accepted as fact.

This thesis, therefore, follows the pioneering work of both Sibley and his associates and Brown & Fisher's (1966) investigation of the Procellariiformes.

Its scope is limited in five ways.

(1) I took up the task having no background in biochemistry whatsoever. My only qualification was 19 years of studying the Procellariiformes in the field, coupled with a growing dissatisfaction with the paucity of knowledge of the inter-relationships within and between the procellariiform genera. What is related to what, and how closely? How good are the species characters of the prions (genus *Pachyptila*), and are the *Procellaria* petrels really large shearwaters? These and a host of other questions needed a fresh appraisal and new answers.

(2) No petrel was killed for the purpose of tissue analysis or for any other reason. If one must kill a creature in order to study it I would rather not do so. The removal of penguins' heads - "One Emperor and fourteen Adelie penguins were exsanguinated by decapitation ..." (Allison & Feeney 1968) - in order to obtain large quantities of blood is unwarranted in my opinion. Fortunately, all petrels nesting around New Zealand are legally protected, and some species, such as the endemic *Procellaria* petrels, are so rare that I doubt whether permission to take specimens would have been approved even if I had requested it.

(3) This inquiry has been restricted to relationships <u>within</u> the Procellariiformes. For inter-ordinal relationships of non-passerine birds see Sibley & Ahlquist (1972).

(4) This thesis study was distinctly constrained as regards availability of finance for obtaining some chemicals and hardware.

(5) From the first field trip to the final thesis presentation, a 3-year time limit was strictly adhered to.

Despite these limitations it seemed to me entirely appropriate that the elucidation of genetic relationships within the extant species of albatrosses and petrels should be attempted in New Zealand, which has the world's largest and most diverse assemblage of Procellariiformes either resident or regularly visiting its waters. Moreover, none of New Zealand's petrels had hitherto been subjected to plasma protein analyses. In December 1974 I began the work, very much unaware of its possible outcome.

It became increasingly obvious during the study that the results might lead into speculation regarding the phylogenetic history of the Procellariiformes, and that some of the findings would be more equivocal than others. In recognition of this problem I have first summarised the plasma protein data (see page 102), and second I have adopted three categories of opinion in which to place additional statements whose validity I regard as 'Highly Probable', 'Probable', and 'Possible' - see Discussion for definitions and comment. (The reader wishing to avoid my speculative remarks need not look beyond the protein summary.) Only in this way can I hope to satisfy both conservative and liberal points of view. My speculations are drawn from many sources, both in the literature and from conversations with colleagues over many years. I have drawn upon the philosophy of Robert Cushman Murphy in his masterful treatise on the oceanic birds of South America (Murphy 1936),

and on discussions with Robert A. Falla over the past 12 years. To this background I have added my own information resulting from 2½ years of ornithological reasearch in the southern oceans aboard U.S.N.S. Eltanin, and data obtained from visits to the breeding grounds of many species of petrel (see below).

Because of the time factor and logistic problems involved in a thesis study of this nature, the Annotated Checklist of the Birds of New Zealand (OSNZ 1970) was perused, and a list of petrels was compiled having regard to the following criteria:

(1) The desirability of studying at least one species from all four families (Diomedeidae, Procellariidae, Hydrobatidae, and Pelecanoididae), and as many genera as possible;

(2) The accessibility of the candidate species' breeding grounds, in terms of both cost and time;

(3) a purely subjective view of a species' interest in the context of the project.

Once this list of 15-20 petrel species was completed, another inventory was prepared of questions inviting solution:

(1) would there be any age, sex, or seasonal variation in the plasma proteins?

(2) In the context of (1), what would be an adequate sample size - 1, 10, or 100?

(3) Would any intraspecific variation make interspecific comparisons difficult or untenable?

(4) What effect would sample collection techniques and storage have on plasma samples?

To these anticipated practicalities were added unforeseen obstacles and benefits. The record-setting trend in the 1974-77 weather was both unseasonable and atrocious. Gales and floods bent field-trip timetables into something unrecognisable, and

petrel colonies usually easy to get to became difficult if not hexed.

The parade of storms, however, brought the unexpected benefit of vagrant petrels driven ashore and retrieved alive. This broadened the scope of the inquiry in a way otherwise impossible: examples include an Antarctic fulmar (*Fulmarus glacialoides*) retrieved at Petone after a southerly gale; a white-chinned petrel (*Procellaria aequinoctialis*) which found itself fogbound in a Wellington garden; and a lost wandering albatross (*Diomedea exulans*) which hid under some coastal trees until discovered by an adventurous domestic cat.

The field work involved visits to 31 petrel colonies, 13 on islands and 3 at mainland locations (Table 1). Of the four procellariiform families, 29 species representing 11 genera were sampled (over 31% of the world total of 96 species). Sex, age, and seasonal variation in plasma proteins was tested using 24 mated pairs (6 species), 39 fledglings and 84 adult birds (4 species), and by visiting 20 colonies more than once. Nineteen populations (7 species) were monitored for intraspecific variation, and population samples where $\underline{n}$ <10 were bled from 11 species to give a measure of intrapopulational variation. In all 472 birds were sampled, and their plasma proteins were subjected to vertical slab polyacrylamide gel electrophoresis (hereafter referred to as PAGE) run in a continuous 0.05 M tris/ 0.05 M glycine buffer system at pH 8.4. The electrophoresis apparatus is a modified version of previously described hardware (see Akroyd 1967, Reid & Bieleski 1968, Billington 1969). Techniques such as disc electrophoresis (Davis & Ornstein 1961) and two-dimensional electrophoresis (Poulik & Smithies 1958,

Wein 1969, Margolis & Kenrick 1969, Kaltschmidt & Wittman 1970) were attempted also, but these failed to yield the consistently good results of the simpler PAGE system. Its advantages are:

(1) small volumes of up to 16 samples can be run under identical conditions in a single gel;

(2) the method yields highly reproducible results;

(3) the apparatus is simple to construct and easily cooled;

(4) running time is usually only 3 h, and the protein fractions can often be examined within 30 min after this period (depending on the type of stain employed).

Its disadvantages are:

(1) the toxicity of acrylamide monomer (see below);

(2) the non-linearity of some protein bands, especially when the gel is heavily loaded with samples. This 'bowing' effect is particularly apparent with the more mobile proteins, and unless precautions are taken can give rise to misleading comparisons of scan profiles from different gels (see below).

Immunoelectrophoresis (IEP) was employed also in order to confirm the results of the PAGE technique, and to provide additional data on the affinities within the procellariiform taxa (see page 99).

TABLE 1. PROCELLARIIFORM BIRDS SAMPLED FOR THIS STUDY

Scientific name	Vernacular name	n	Location	Date
FAMILY DIOMEDEIDAE	ALBATROSSES			
Diomedea exulans	wandering albatross	1	Waikanae Beach	Feb. 77
epomophora sanfordi	northern royal albatross	4	Taiaroa Head	Jan. 77
melanophris	black-browed mollymawk	1	collected at sea	Jan. 77
Phoebetria palpebrata	light-mantled sooty albatross	2	Petone beach	Jun. 76, Jul. 77
FAMILY PROCELLARIIDAE	FULMARINE PETRELS: PRIONS: GADFLY PETRELS: SHEARWATERS			
Macronectes halli	northern giant petrel	1	Paekakariki beach	Dec. 76
Fulmarus glacialoides	Antarctic fulmar	1	Petone beach	Sep. 75
Daption capense	Cape pigeon	4	Petone; at sea	Sep. 75, 76, Nov. 76
Procellaria aequinoctialis	white-chinned petrel	1	Wellington	Apr. 76
parkinsoni	black petrel	22	Great Barrier I.	Mar. & Dec. 75, Dec. 76
westlandica	Westland black petrel	52	West Coast	May 75, Sep. 76
Pachyptila turtur	fairy prion	89	Poor Knights, Motunau, & Stewart Is	Oct. 75, Jan., Sep., Oct. & Nov. 76
crassirostris	fulmar prion	5	Bounty Is	Nov. 76
desolata	Antarctic prion	5	Auckland Is	Jan. 77
vittata	broad-billed prion	16	North Is (Stewart)	Sep. & Oct. 76
Pterodroma macroptera	grey-faced petrel	17	Whangmata, Tiri-Tiri, & Korapuki Is	Jun. & Aug. 75, Dec. 76
lessoni	white-headed petrel	3	Auckland Is, Petone beach	Dec. 76, May 77, Sep. 77
inexpectata	mottled petrel	1	The Snares	Jan. 77
pycrofti	Pycroft's petrel	1	Poor Knights Is	Oct. 75
cooki	Cook's petrel	2	Great Barrier I.	Mar. 75
Puffinus bulleri	Buller's shearwater	59	Poor Knights Is	Oct. 75, May, Dec. 76
carneipes	flesh-footed shearwater	10	Karewa, Korapuki Is	Jan, Dec. 76
griseus	sooty shearwater	28	Korapuki, Titi (Marlb.), & Stewart Is	Feb., Oct., Dec. 76
gavia	fluttering shearwater	29	Poor Knights, Great Barrier, & Korapuki Is	Oct., Dec. 75, May, Dec. 76
huttoni	Hutton's shearwater	10	Seaward Kaikoura Mountains	Feb. 76
assimilis	allied shearwater	49	Poor Knights Is	Jul., Oct. 75, May, Nov. 76
FAMILY PELECANOIDIDAE	DIVING PETRELS			
Pelecanoides urinatrix	northern diving petrel	38	Whangmata, Poor Knights, & North Is (Stewart)	Jul. 75, May, Nov. 76 Sep., Oct. 76
u. exsul	subantarctic diving petrel	1	Auckland Is	Dec. 76
FAMILY HYDROBATIDAE	STORM PETRELS			
Fregetta tropica	black-bellied storm petrel	1	collected at sea	Jan. 77
Garrodia nereis	grey-backed storm petrel	1	Auckland Is	Jan. 77
Pelagodroma marina	white-faced storm petrel	18	Poor Knights & North (Stewart) Is	Oct. 75, Oct. 76
FAMILIES 4; Genera 11; species 29		472		

MATERIALS AND METHODS

Sampling Techniques

Blood plasma was used in all the electrophoretic separations discussed in this study. Of the 472 birds sampled, 458 were captured at night on their breeding grounds and 14 were retrieved alive from windward coasts after gales had driven then ashore. The species list (Table 1) follows nomenclature given in the Annotated Checklist of the Birds of New Zealand (OSNZ 1970).

To prevent the bird from biting either itself or the operator a rubber band was used to secure the beak. Birds were marked with stainless steel leg bands obtainable from the Wildlife Service, Department of Internal Affairs, Wellington. Banding prevents sampling errors and enables re-analysis at a later date to detect age, seasonal, and any indeterminate variation in the plasma constituents. All birds were released immediately after banding.

Blood samples were drawn from either the brachial vein (wing) or the infratarsal vein (leg). For storm petrels this procedure was impracticable, and cardiac puncture proved a useful alternative (Utter et al 1971). One ml of whole blood was taken from most species, and this generally proved a sufficient sample; 2 ml was sometimes drawn from larger procellariids. The blood was drawn through a 27-gauge needle into a 1 ml sterile syringe containing 0.05 ml of 0.2M disodium ethylenediamine tetraacetate (EDTA) anticoagulant. (Each 50 ml of EDTA stock solution was treated with 10 mg of Thiomersal (BDH), a bacterial inhibitor.) The syringes were immediately centrifuged in batches of two or four at c.125 rad/s for 10 min.

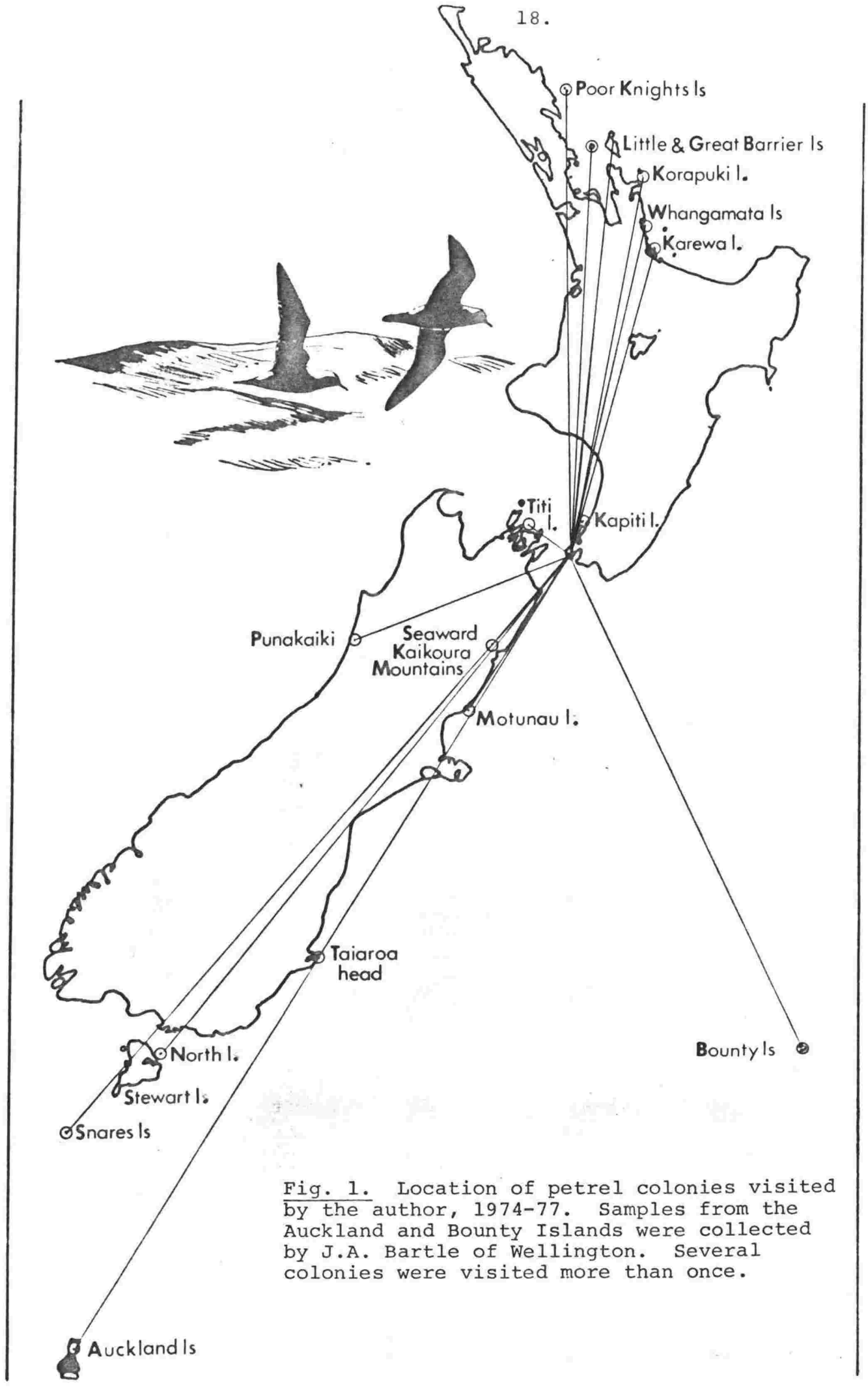


Fig. 1. Location of petrel colonies visited by the author, 1974-77. Samples from the Auckland and Bounty Islands were collected by J.A. Bartle of Wellington. Several colonies were visited more than once.

The portable centrifuge, powered by a 6-V, 11-Ah motorcycle battery, was designed for this purpose by the author in consultation with staff of the university mechanical workshop.

The plasma supernatant was drawn into a sterile 1-ml syringe, which was sealed and then placed into either a 0.5 litre "Thermos" flask packed with ice or a 10 litre container of liquid nitrogen. Ice was adequate for approximately 8 h, when the samples were being dispatched daily to the nearest freezer; liquid nitrogen had a maximum field working life of 14 days, sufficient for prolonged expeditions to outlying islands. One ml of whole blood yields 0.3 - 0.4 ml of plasma in the syringe. Some erythrocytic material was collected to provide haemoglobin markers. All samples were kept frozen solid at $-15^{o}C$ in a large freezer until required for analysis in the laboratory.

Preparation of Stock Solutions

Analytical grade chemicals and freshly distilled water must be used in the preparation of gels. Acrylamide is a cumulative neurotoxin and must be handled accordingly (Reid & Jones 1973).

To prepare stock acrylamide monomer solution containing 15% acrylamide (Eastman Kodak Co., Rochester N.Y.) and 0.3% NN bisacrylamide (Eastman) weigh 18.75 g acrylamide into a 200 ml beaker and carefully add 50 ml water. Allow to dissolve. Weigh 0.37 g bisacrylamide on to metal foil and rinse into beaker. Stir until dissolved, then add water to 125 ml. Since the shelf life of the acrylamide mixture is about 1 month at room temperature, it is advisable to make up small volumes only (Brownstone 1973).

To make 0.1 M tris/0.1 M glycine stock buffer solution, add 12.1 g tris ("Sigma 7-9"; Sigma Chemical Co., St. Louis, Missouri, U.S.A.) and 7.5 g glycine to 100 ml of water, stir until all solids are dissolved, and make up to 1 litre with water. Store at $5^{o}C$. The nominal pH, 8.4, tends to increase slightly if the shelf life of the buffer exceeds 2-3 months.

Preparation of Samples for Electrophoresis

Sample protein concentration can be determined by either the Biuret or the Lowry method. To quickly test whether a protein sample is more dense than the buffer, and hence will maintain close contact with the gel surface, take a 5 µl aliquot of raw sample up into a micropipette and gently release it into a test tube containing the running buffer (stock buffer diluted 1:1 with water). By holding the tube up to the light, the downward (or upward) path of the plasma can be traced readily by eye. All procellariiform plasmas tested could be applied raw to the gel.

Immediately before electrophoresis, plasma samples should be allowed to thaw slowly until liquid - about 20 min. in the main section of a refrigerator. Samples can be applied raw to the gel or, if necessary, diluted 3:1 with 5% sucrose solution. (These diluted samples should be discarded after three uses).

Preparation of Acrylamide Gel

To a conical flask containing 10 ml of the 0.1 M tris/ glycine buffer solution, add 10 ml stock acrylamide monomer. De-gas the gel mixture until all bubbling ceases. When ready for gel casting, quickly add 0.2 ml freshly prepared 10% w/v ammonium persulphate and 0.2 ml of 10% N, N, N^{1}, N^{1}-tetra-methylethylene diamine (TEMED) in ethanol to initiate chemical polymerisation.

Casting the Gel

The simple electrophoresis apparatus, constructed by perspex (Plexiglas), is shown in Fig. 2. It consists of two buffer compartments each supplied with a platinum electrode which terminates in a banana plug. Between the offset upper and lower buffer compartments is the glass cell containing the gel. The circuit is completed by a paper wick and a power pack which delivers 10 mA at a potential difference of at least 300 V DC.

The cell consists of a 150 x 90 x 1.8 mm glass plate at each end of which 15 x 90 x 1.8 mm glass strips are cemented with epoxy resin. A second glass plate of the same dimensions is clamped with large spring clips on to the Vaseline-smeared glass strips to form a shallow, rectangular box with open top and bottom. The bottom of the cell is sealed by firmly applying a strip of plasticine along its length.

After adding the catalyst and initiator, gel mixture is quickly pipetted into the glass cell to within 1 cm of the top, using a long-tipped 10 ml pipette equipped with an adjustable filler (' 8 ' in Fig. 2). Care should be taken not to introduce air bubbles into the gel, or to spill any down the outside of the cell. Gels prepared with ammonium persulphate are sensitised such that contact with atmospheric oxygen will prevent polymerisation (Brownstone 1973; P.C. Harper, pers. obs.). As well as increasing the risk of skin contact, liquid allowed to flow down the outside of the cell will disrupt the plasticine seal and cause leakage.


CONSTANT
CURRENT
AnalaR
AnalaR
Glycine
VASELINE

1 the simple electrophoresis apparatus
2 the glass cell
3 the wick made of Whatman No. 3 chromatography paper
4 plasticene seal
5 5 ml glass syringe for water-layering on gel before polymerisation
6 de-gassing flask
7 power unit
8 auto-pipette used for filling the electrophoresis with the toxic unpolymerised gel mixture
9 5 µl pipette for applying samples to gel
10 the 1.7 mm diameter rubber tubing used for separating protein samples on the gel

Fig. 2. The basic laboratory chemicals and harware used for the acrylamide gel electrophoresis (see text). The gell illustrated is being pre-run for 45 min at 100 V DC and 8 mA to remove the initiators. The protein samples will then be applied between the rubber spacers just visible in the glass cell.

Distilled water is carefully layered on top of the gel solution using a glass (not plastic) syringe fitted with a recurved 18-gauge needle: the layering must be done slowly, with particular care not to disturb the gel mixture. Polymerisation is evident from the clearly visible and stable sharp interface between polymerised gel and covering layer. At room temperature the gel should set within 20 min - if it does not, check the quantity and purity of both the ammonium persulphate and acrylamide monomer solutions.

When polymerisation is complete the plasticine is carefully peeled away from the cell, which is then placed vertically in the electrophoresis apparatus as shown in Fig. 2. The liquid on top of the gel is carefully poured off to waste, and the gel surface is rinsed and covered with the 0.05 M running buffer. Small lengths of 1.7 mm diameter 'Esco-rubber' (translucent, soft silicone rubber tubing; bore 1.0 mm; wall thickness 0.4 mm) are then pressed gently down on top of the gel with two dissecting needles, to make 8 mm wide wells for the samples. The tubing must make unbroken contact with the gel surface, to ensure that no liquid can pass under it, yet the gel surface must not be distorted when the tubing is positioned. Both upper and lower buffer compartments are filled, and a 120 x 160 mm wick of Whatman No. 3 chromatography paper, folded in half and wrapped in a polythene sleeve, is introduced into the top buffer compartment to connect it with the running buffer above the gel. The platinum electrode of the lower compartment is connected to the anode (+) of the power supply, and the top compartment to the cathode (-). The gel is pre-run at 100 V DC and 8 mA in a refrigerator or cold room for at least 45 min, to clear the chemical initiators from the gel.

Calibration of Gels; Application of Samples; Running Conditions

Bovine albumin fraction V is one of several standards suitable for protein mobility calibration. Its migration rate through gels is identical to that of a common procellariiform prealbumin and this fact was taken into account when formulating the reference petrel plasma (*Procellaria westlandica*; see below).

Following the pre-running of the gel the system is switched off, the wick is withdrawn into the upper buffer compartment, and the desired aliquots (usually 5 µl) of sample are pipetted into each well on the gel surface. A felt-tipped pen is used to mark the orientation of the glass cell and number each sample well. Five µl of 0.02% bromophenol blue in tris/glycine buffer in 5% sucrose is placed at each end of the sample wells as a visible marker of electrophoretic progress. After reconnecting the wick, electrophoresis is begun at a constant 10 mA and a potential difference of about 180 V. After 30 min when the proteins have migrated into the gel, the tubing spacers are carefully removed and electrophoresis is allowed to continue for a total of exactly 3 h. A rise in voltage during the run is probably due to increasing conductivity of the tris/glycine buffer (Brownstone 1973). This can be minimised by replacing (or recycling) the buffer once or twice during the run. Heating of the gel can be reduced by completing the run at 3-4^{o}C in a refrigerator or cold room; running temperatures do not exceed 4^{o}C under these conditions.

Fig. 3. The Westland Black Petrel (*Procellaria westlandica*) on its breeding grounds near Punakaiki, New Zealand. The plasma complement of this petrel was used as a 22-banded reference for all calibration gels (see text).

Pooling of Plasma

After individual variation in the plasma samples had been assessed by PAGE, the plasma was carefully thawed and drawn into a large sterile syringe. The pooled plasma was then passed through a sterile Swinnex-13 millipore filter and into one or more sterile 2 ml 'Vacutainers' (Becton Dickinson). Electrophoresis of these plasmas proved invaluable for discerning populational and specific variations which may not be readily apparent from a single individual's plasma. Pooled plasmas were also required for immunological studies.

Derivation of Standard Species' Patterns

All plasma samples were run many times on many gels (see below). This exhaustive process was considered mandatory to obtain an accurate electrophoresis pattern for each petrel species. Each species's protein 'fingerprint' was compiled through continual monitoring and cross-referencing with other species' patterns. Each of the 20 or so protein bands were individually checked in this manner, and alsc against a reference plasma, the 22-protein-banded plasma of *Procellaria westlandica*. Aliquots from this species were run on all calibration gels as an internal multiple reference marker, because: (1) the mobility of its prealbumin Rmu 100 is identical to that of bovine albumin fraction V (No. A-4503: Sigma); (2) it breeds on the mainland in a place accessible by car, hence problems of blood preservation are minimal; (3) a large quantity of plasma was obtained (n = 52 birds); (4) the species is a typical procellariid, in that nearly all the protein bands found in other petrels are present.

Removal, Staining, and Preservation of Gels

On completion of electrophoresis, the glass cell is removed from the apparatus and gently prised apart. The gel is notched, to identify its orientation, and carefully placed in a staining tank.

Many staining and precipitation techniques are documented (see Maurer 1971). Those described below have proved the most suitable for this study.

Total Protein

Coomassie Brilliant Blue R250, a very sensitive total protein stain, is recommended. The gel is stained overnight in a 0.25% solution of Coomassie Blue in methanol:glacial acetic acid:water (5:1:5). Destaining in the solvent mixture takes 6h, with gentle shaking and frequent changes of the destaining solution. The protein fractions ultimately appear as blue bands on a clear background. At this stage the gel will have shrunk appreciably; this can be remedied by placing it in 5% acetic acid. About 30h later it will have expanded by 11.6% of its original size, which greatly improves the apparent band separation and so renders the gel more suitable for analysis.

Before any study involving its mutilation, the gel should be photographed. I used a Zeiss Contarex equipped with an F2/50 mm Planar lens and two synchronised electronic flash units. Colour photographs are distinctly preferable to monochrome prints (Kodachrome 25 ASA and Ektachrome 64 ASA transparencies were used in this study; colour negative film is a useful alternative, in that prints enlarged to actual or greater size can readily be obtained. Once photographed, the protein bands can be measured from the origin with vernier

calipers. The data are laid out on good-quality tracing paper or plastic, so that an accurate, to-scale drawing of the gel is available for scrutiny, along with the photograph.

Gels may be preserved whole in 5-7% acetic acid within a sealed container - a plastic lunch box is useful - stored in a cool, dark place. Alternatively, when densitometric scanning is desired the gel can be sectioned (a microtome blade is excellent for this purpose). I used the 1:1 and 3:1 scales on a Joyce-Loebl Chromoscan. The first graph can be compared directly with the drawing; the second is useful for detecting any subtle or obscure protein band patterns.

Transferrins, Albumins, and Globulins

'Rivanol' (2-ethyl-6, 9-diamino acridine lactate) is a useful chemical for the sequential precipitation of proteins (Schultze & Heremans 1966). Dilute 0.05 ml raw plasma 1:1 with 0.05 M tris/glycine buffer, to increase its alkalinity, and add 0.3 ml 0.003 M 'Rivanol'. Agitate, allow to stand at room temperature for 1 h, then centrifuge at c.1000 rad/s for 30 min. The supernatant can now be either subjected to electrophoresis, or mixed with a further 0.3 ml of Rivanol and re-centrifuged. The first centrifugation precipitates the prealbumins, some albumin, lipoprotein, and macroglobulin, and several minor proteins. The second precipitates most of the remaining proteins, leaving the gamma globulin IgG, transferrins, haemopexins, and α globulins of taxonomic interest, and frequently sufficient albumin to reveal its identity as a single or polymorphic system. (Albumins can also be identified by precipitating the globulins from the plasma in half-saturated ammonium sulphate).

Aliquots of Rivanol-treated plasma are applied to the gel as described above. Haemopexins can be identified with the benzidine reaction (see below). Transferrins, IgG, and other proteins present will stain in Coomassie Blue.

Esterase Activity

The napthylacetate method was used in this study to detect non-specific esterases. Dissolve 10 mg α-napthylacetate in 0.5 ml acetone, add to 10 ml sodium phosphate buffer (0.12 M, pH 6.0), and shake. To this solution add 10 mg Fast Blue BB; shake and filter. Immediately pour into a flat Petri dish, carefully add the freshly electrophoresed gel, and incubate for 10 min at 37°C. The coloured reaction products become visible within 15 min.

Stained bands can be sketched and photographed before the gel is rinsed with distilled water and counterstained in Coomassie Blue. Esterases can thus be located precisely in relation to the other proteins and markers present. Further photography and documentation can be done as necessary.

Haem-Containing Proteins

Proteins of this diverse and heterogeneous group can be stained using the benzidine reaction (Uriel 1964), to which red cell haemoglobin responds particularly strongly. (Warning: benzidine hydrochloride is carcinogenic). Dissolve 64 g trihydrated sodium acetate in 400 ml of 7% acetic acid. Saturate with disodium EDTA (about 1 g), filter, saturate with benzidine hydrochloride (about 1 g), and filter again. Store at room temperature. To one part red cell precipitate add three parts haemolysate reagent (Helena Labs, Beaumont, Texas) and centrifuge at c.200 rad/s for 30 min. Dilute the supernatant

containing haemoglobin 3:1 with 5% sucrose solution and apply 1 µl aliquots to the gel. The red colour remains visible during and after electrophoresis. Pour 30 ml prepared stain into a large Petri dish containing the gel and add 1 ml of 3% (v/v) aqueous hydrogen peroxide. The blue bands which quickly appear are unstable to light, and should be recorded promptly.

Plasma containing the haemproducts from lysed RBCs is occasionally encountered, especially from small birds or birds which have been allowed to struggle during blood sampling. When stained, this haemoglobin may mask the bands of plasma protein of similar electrophoretic mobility.

Immunoelectrophoresis

The principle of immunoelectrophoresis (IEP) is the electrophoretic separation of plasma proteins in an agar-gel medium followed by the diffusion of precipitating antibodies into the same gel at right angles to the direction of electrophoresis. The precipitating antibodies unite with their corresponding antigens to produce arcs of specific precipitate in the gel (Grabar & Burtin 1964, Clausen 1972, Crowle 1973, Brewer et al. 1974).

The advantages of IEP are as follows:

(1) Only 1 µl quantities of sample are required.

(2) The precipitation reaction is specific and very sensitive, hence it may be used for complex mixtures (such as total plasma) in which some proteins may have identical electrophoretic mobilities and/or be present in very small quantities < 0.1%.

(3) Electrophoresis in agarose gels composed of 98-99% liquid closely approaches electrophoresis in a liquid medium.

Liquid/solid interface effects, as present in polyacryamide gels, are minimised in 1% agar substrates.

(4) Colour reactions are the same as for the PAGE system.

(5) Precipitation arcs are highly reproducible.

The disadvantages of the system are as follows:

(1) Initial capital and running costs are high.

(2) The main reagent, the immune plasma, is a biological product which is difficult to standardise. Several immune plasmas from different animals may be required to detect the maximum number of constituents in the protein mixture.

(3) The method is essentially qualitative, not quantitative.

Antibodies to procellariiform plasma were generated in laboratory-reared guinea pigs obtained from Tasman Vaccine Laboratories, Upper Hutt. Preparation of antigens followed directions kindly supplied by Dr Kevin Moriarty (pers. comm.) and the preparative techniques given by Schreiner & Pesce (1973). Immunoelectrophoresis for this study uses the Millipore IEP System (Millipore Corp., Bedford, Mass.). A full and detailed description of this technique, which employs immuno-agaroslides and a sodium barbital ($NaC_8H_{11}N_2O_3$) buffer, is given in Millipore Catalogue AR311.

Preparation of Antigens and Antisera

Antigenic material consists of total plasma in saline emulsified with Freund's adjuvant. Two animals are used to raise antibodies. The second injection is administered 4 weeks after the first, and a booster injection is given 3 weeks later. The animals are bled 14 days after the booster injection.

To prepare the first injection, 0.2 ml of pooled plasma was drawn into a syringe containing 0.8 ml of physiological (0.85%) saline. This solution was passed into 1.2 ml of Freund's complete adjuvant (Difco) contained in a 5 ml reuseable glass syringe which had been autoclaved to sterility. An 18 gauge needle with a Luer Lok cap fitted at either end was secured to the syringe, and the Freund's adjuvant and the protein solution were thoroughly emulsified by passing them between two syringes. The completeness of emulsification was tested by allowing a drop of the mixture to fall into cold water; the antigenic material formed a spherical drop if emulsification was complete, otherwise it rapidly dispersed.

Once emulsification was complete, the antigenic material was divided equally and injected intramuscularly into the two guinea pigs. The injection was made in the upper thigh near the lymph node where antibodies are generated. The second injection was similar to the first except that Freund's incomplete adjuvant was used and the antigenic plasma was increased to 0.25 ml, it is administered 4 weeks after the first injection. An identical booster injection was given 3 weeks later and the first bleed of 10 ml whole blood by cardiac puncture is taken 14 days later. In all, 8 weeks elapsed from time of first inoculation to first bleed. Reinoculations were regularly administered to maintain a high antibody titre, and standard 'ring' tests were used to monitor precipitation products. The guinea pig antisera were treated exactly as the avian plasmas described above.

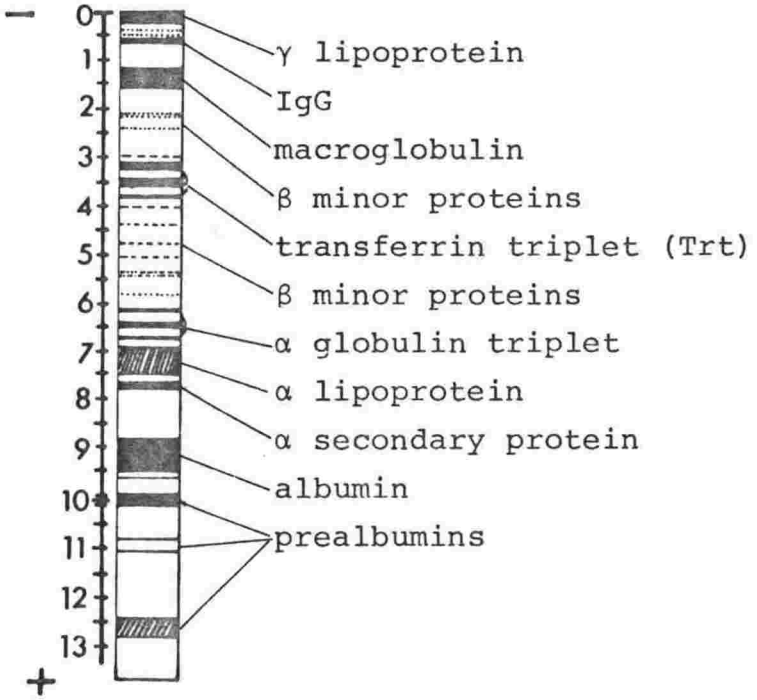
For this study, antibodies were raised against the following procellariid blood plasmas:

Genus *Procellaria*	n = 52 (3 species)
Procellaria westlandica	n = 37
Genus *Puffinus*	n = 185 (6 species)
Puffinus bulleri	n = 30
Pterodroma macroptera	n = 5
Pachyptila turtur	n = 29
Pelecanoides urinatrix	n = 10

Of the above seven antisera, five produced good globulin precipitations but a weak response to albumins and prealbumins. For comparative purposes they were not entirely satisfactory, and were thus rejected. The other two antisera were excellent, however. Because the large sample from *Puffinus* shearwaters, contained such a variety of plasma, it was selected as the medium by which precipitation complexes were formed for all other species of Procellariiformes for which I had sufficient plasma.

Differences at the species-, genus-, and family-group levels were investigated; the results are discussed on page 99. Relationships within the genus *Pachyptila* were investigated with the antisera developed against *Pachyptila turtur* plasma antigens.

Fig. 4. KEY TO PROTEIN BANDS IN THE REFERENCE PLASMA OF *Procellaria westlandica*



The identification of the illustrated protein bands was obtained by analogy with data from several sources and the purification techniques described in the Methods section.

From petrel and penguin data: Brown & Fisher (1966), Allison & Feeney (1968), Shaughnessy (1970), Sibley & Ahlquist (1972), Murrish & McMahon (1975).

Other avian taxa and mammals, including man: Sibley (1960), Uriel (1964), Schultze & Heremans (1966), Campbell (1970), Manwell & Baker (1970), Sibley (1970), Maurer (1971), Clausen (1972), Frelinger (1972), Wright (1974). The IgG band was kindly identified for me by Dr K. Moriarty, Immunologist, Massey University.

Definitions

(1) petrel: any member of the order Procellariiformes.

(2) protein complement: all proteins from total plasma stained by Coomassie Blue R250.
Divisible into three classes, as follows:

(a) primary proteins: proteins of taxonomic interest - transferrins, α globulins, albumins, some prealbumins, β globulins, and esterases.

(b) secondary proteins: proteins found in all blood plasmas examined, each having the same mobility in all plasmas. Valuable reference markers. Examples include prealbumin 126, macroglobulin 15, and α globulin 76.

(c) minor proteins: proteins of varying usefulness. Usually present in trace amounts; obscured by primary or secondary proteins, or change rapidly during storage, thawing, or preparation of plasma for electrophoresis. Examples include several β proteins and the lipoprotein Rmu 70-80.

(3) protein denaturation: any observable change, usually with time, in the electrophoretic appearance or mobility of a protein.

(4) Rmu: "relative mobility units", given by the equation:

$$\text{Rmu} = \frac{\text{distance of protein band from origin}}{\text{distance of bovine albumin (V) from origin}} \times 10^{-1}$$

(5) Trt: "transferrin triplet", the three-banded electrophoretic phenotype of procellariiform transferrins. Under electrophoretic analysis the bands behave as a single unit.

(6) measurements and statistical calculations: the Welch statistic

$$tw = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(S.E._1)^2 + (S.E._2)^2}}$$

is used for comparisons between species.

(7) NS: not significant at the 98% level of confidence.

RESULTS

This section is divided into two parts. The first deals with non-genetic plasma protein variation, the second with genetic variation.

(1) Non-Genetic Variation

(a) Sex Variation

Where a pair of birds was found together in a burrow with egg or chick, both adults were caught, sampled, and banded. From a total of 24 such pairs (6 species) the separated proteins gave no indication of sexual differences. Each pair was rated independently, since apart from three *Puffinus bulleri* females carrying shelled eggs in their oviducts I was not able to positively distinguish the sexes on morphological characters. As a general rule males tend to be slightly larger than their mates, but there are sufficient exceptions to this rule to prevent its use as a reliable criterion.

(b) Age Variation

TABLE 2. AGE CATEGORIES OF PROCELLARIIFORMES SAMPLED FOR PLASMA PROTEINS

Species	Adults (n)	Fledglings (n)	Chicks (n)	Totals
Pachyptila turtur	8	10	-	18
Procellaria parkinsoni	21	-	1	22
Puffinus bulleri	42	17	-	59
Pelecanoides urinatrix	13	12	-	25
TOTALS	84	39	1	124

A 39-fledgling sample taken from the same colonies as 84 adult procellariids (Table 2) yielded insignificant differences between the age groups. One large *Procellaria parkinsoni* chick (Band No. K 4451) still clad in mesoptyle down was sampled on February 1975 at the Mount Hobson colony, Great Barrier Island. Although at the time my gel preparative techniques were in their infancy, a clear difference in the albumin and prealbumin mobilities was detectable; both proteins travelled faster than those of the adults. The maturation of protein phenotypes into the adult configuration may occur during the fledgling period prior to the departure of the bird from its nest. More data are needed.

(c) Seasonal Variation

Of the 31 petrel colonies visited during 1974-77, 20 were visited more than once (Table 1). Some return visits were made after only a matter of weeks (for example, North Island in Foveaux Strait, and the Poor Knights off North Auckland's east coast); others were made several months (Westland, Great Barrier and Poor Knights Islands) or a year later (Great Barrier and Poor Knights). The reason for this - and the resulting 315 birds (8 species) being bled - was to ascertain whether plasma proteins are susceptible to seasonal variation which would impinge on the value for comparative purposes of plasma samples taken at different times of the year. All results were negative. Several birds were recaptured once, and one *Puffinus assimilis* was sampled at its nest on three separate visits to the Poor Knights Islands.

(d) Denaturation of Plasma Proteins During Collection and Storage

No differences were found between samples preserved in ice or liquid nitrogen prior to their delivery to the laboratory. This was tested simply by dividing samples between ice and liquid nitrogen and comparing the results.

Because the plasma samples held in 1 ml syringes were small and preserved with Thiomersal (Sigma), a small quantity of material could be thawed as and when required. Repeated thawing and refreezing of a single sample caused proteins to denature, especially those lacking a preservative. Bacterial contamination flourished under these circumstances. Denaturation requires more sample to be run on the gel, and causes a streaked and blurred appearance of the separated proteins, as shown by Sibley (1970).

One particularly sensitive protein is an α lipoprotein which migrates behind the procellariiform albumins. With prolonged storage it quickly loses its staining intensity, and finally disappears. This can be useful, because (as the Rivanol treatment demonstrates) several useful α globulins which are frequently obscured by lipoprotein are unmasked.

If properly cared for, plasma lasts over 12 months in storage. A 0.05 ml plasma sample from *Procellaria parkinsoni* (No. K 4401) was thawed and refrozen no less than 18 times during a 20-month period before being discarded as useless. A 2 ml plasma sample containing no preservative in a sealed, sterile 'Vacutainer' was inadvertantly left out at room temperature for a week before being relocated. Careful comparison with its frozen counterpart revealed no apparent changes to any proteins of taxonomic interest. The antibacterial and fungistatic properties of transferrin are well known (Schade & Caroline 1944, cit. Frelinger 1972).

(2) Genetic Variation

Key to Presentation of Genera

(1) The nomenclature used in the following pages is essentially that of the Annotated Checklist of the Birds of New Zealand (OSNZ 1970). Some amendments are incorporated; for example, the 'subspecies' of *Macronectes giganteus* have been shown to be full species (Bourne & Warham 1966).

Nomenclatural histories of the procellariiform birds considered here are fully reviewed by Condon (1975).

(2) The Synopses introducing the genera in the pages that follow are deliberately neither complete nor exhaustive. Rather, they provide information of special relevance to this thesis, the threads of which are brought together in the Discussion (page 107). Data not referenced come from my own observations, made either during my 2½ year ornithological research programme in subantarctic and Antarctic oceans - Eltanin Cruises 16, 20, 21, 22, 23, 26, 27, and 28 (in part) - or in New Zealand (Harper 1972, 1973a, b, 1976, in press, in prep., and unpubl. data).

(3) Measurements and other relevant data were obtained from museum skins in the following institutions:

(a) New Zealand

Auckland Institute and Museum, Auckland
National Museum of New Zealand, Wellington
Canterbury Museum, Christchurch
Otago Museum, Dunedin.

(b) Australia

National Museum of Victoria, Melbourne.

(c) U.S.A.

Smithsonian Institution, Washington, D.C.
American Museum (Nat. Hist.), New York.

(d) England

British Museum (Natural History), London.

Fig. 5.

Fig. 5. A wandering albatross (*Diomedea exulans*) leaving its nest on the Antipodes Islands.

Family Diomedeidae : albatrosses and mollymawks

Phoebetria fusca Hilsenburg, 1822 - sooty albatross

P. palpebrata Forster, 1785 - light-mantled sooty albatross

Diomedea about 12 species, 2 species of 'great' albatrosses, 10 species of smaller albatrosses and mollymawks

Synopsis

Although the great size, beauty, and majestic flight of the albatrosses are legendary, these birds have remained a little known group until quite recently (Sorenson 1950, Fisher 1967-76, Tickell 1964-8, Rice & Kenyon 1962; Harris 1975). The polytypic genus *Diomedea* has 12 recognised species, some of which have several doubtful subspecies (*D. exulans dabbenena* and *D. e. chionoptera*, for example); other 'subspecies' appear to warrant full specific status within the superspecies concept (e.g., the *cauta* group of larger mollymawks). Comparative studies of the elaborate courtship displays and hoarse conversations between albatross partners such as those of Fisher (1972) and Johnson et al. (1975), are a first step only recently undertaken, although albatrosses must surely rank as the easiest procellariiform birds to observe and study (Murphy 1936).

Despite their long, powerful beaks, unique among petrels in that the wide culminicorn divides the nostrils to either side of the bill, albatrosses are unusually docile for such sizeable surface-nesting birds. Such fearlessness renders albatrosses particularly vulnerable to mammalian predators - as many as 20 rats (*Rattus exulans*) have been reported feeding

at night on a single incubating Laysan albatross (*D. immutabilis*) at Kure Atoll in the Hawaiian Islands (Kepler 1967, cit. Fleming 1969). Crawling up the birds' backs, the rats open a wound which can be "enlarged to 5 or 7 inches diameter, often exposing the thoracic cavity, ribs or lungs." A more grisly example of what happens when a highly successful mammalian predator meets an order of birds which has clearly evolved in places far removed from such selection pressures is difficult to imagine.

The climatic zonal distribution patterns of the Diomedeidae are particularly evident in the Southern Hemisphere, and can be illustrated with representative species or races of the three natural groups of living albatrosses. Southern examples which have a circumpolar distribution and which feed well south of the Antarctic Convergence in summer include the wandering albatross (*D. exulans*), black-browed mollymawk (*D. melanophris*), grey-headed mollymawk (*D. chryostoma*), and light-mantled sooty albatross (*P. palpebrata*). The royal albatross (*D. epomophora*) and several of its smaller congeners (the *cauta* group and Buller's mollymawk, *D. bulleri*) are typical of Australasian subtropical waters; their opposite numbers in the South Atlantic Indian Ocean are the yellow-nosed mollymawk (*D. chlororhynchos*) and the sooty albatross (*P. fusca*).

A number of morphological and behavioural distinctions between the two long-tailed, slender-winged species of *Phoebetria* and the well speciated genus *Diomedea* point to the primitive character of *Phoebetria*. (The osteological characters of the bill separating the two genera are described by Wilkinson (1969).)

Phoebetria	*Diomedea*
(1) Bill black in all age groups.	Apart from *D. nigripes*, bill totally dark in immatures only; in adults, pale or conspicuously coloured.
(2) Fleshy sulcus divides mandibular latericorns.	Sulcus rudimentary, absent, or modified.
(3) Plumage dark in all age groups.	Plumage dark in young birds only.
(4) Tail long, cuneate.	Tail short, rounded.
(5) Solitary nesters; use nest only once.	Gregarious; frequently refurbish a single nest for each season's nest activities.
(6) Aerial courtship displays featured.	Courtship displays on ground or sea (close visual contact).

In being surface-nesters like the fulmarine petrels, the albatrosses have been able to fully exploit coloration to an extent unparalleled among the smaller nocturnal members of the Procellariiformes. The striking head, bill, gape, and mouth patterns and colours strongly suggest the possession of good colour vision by larger procellariiform birds, but conclusive data are completely lacking in this regard; indeed, the subject appears to have been largely overlooked.

Fig. 6. Family Diomedeidae : albatrosses

Species	n (birds)	n (gels)	n (runs)	Source	Date
Phoebetria palpebrata	2	20	20	Petone beach	Jun. 76 Jul. 77
Diomedea exulans	1	8	8	Waikanae beach	Jan. 77
D. epomophora sanfordi	4	10	14	Taiaroa Head (2♂; 2♀)	Jan. 77
D. melanophris	1	5	5	Collected at sea	Jan. 77
	8	43	47		

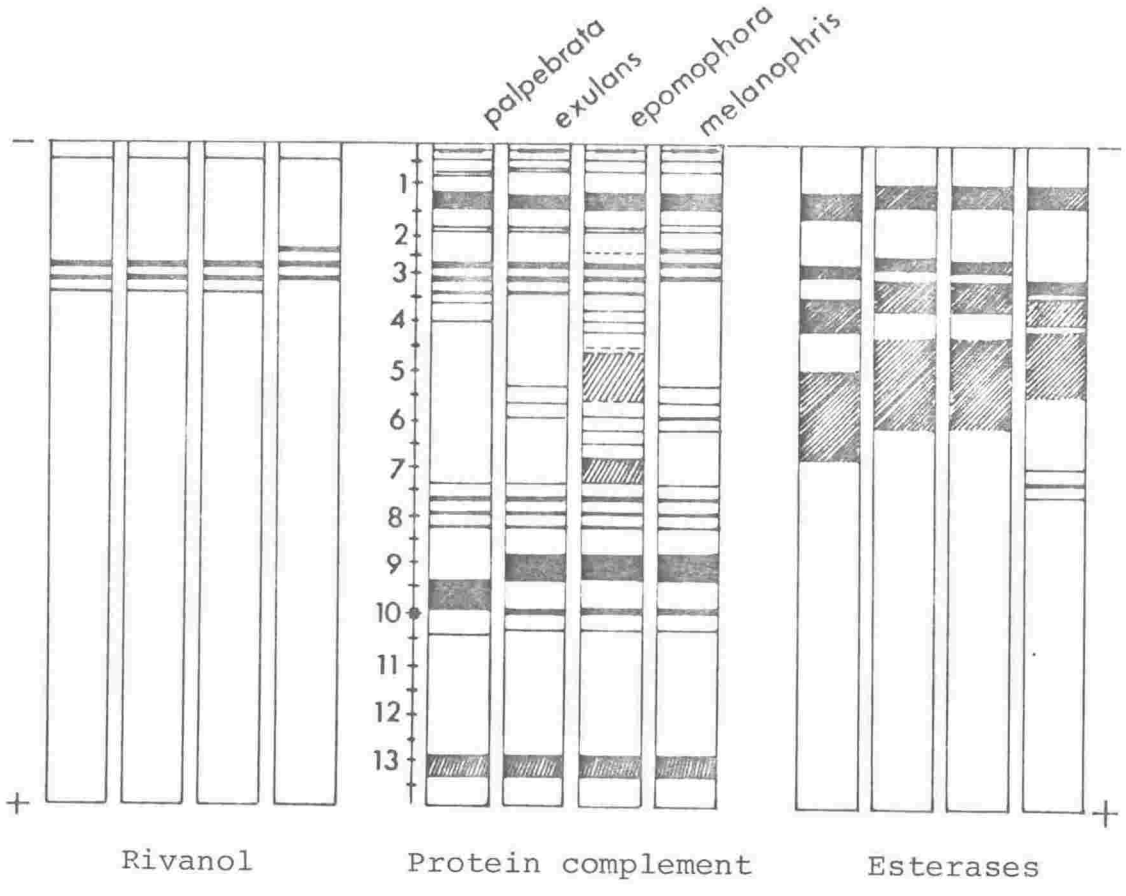


Fig. 6 is a composite drawing (to scale) of the banding patterns of plasma proteins from eight albatrosses representing both *Phoebetria* and *Diomedea*. Some of the minor proteins have been omitted from *palpebrata*, *exulans*, and *melanophris* because they could not be adequately defined from the amount of available plasma.

Note the following:

(1) In *melanophris*, the lower mobility of the Trt at Rmu 25-32. The Trt's of the other species are identical in both mobility and staining behaviour, the upper two bands staining more densely than the third; whether *melanophris* conforms to this pattern is not clear from the data. This type of staining pattern is typical of the shearwaters (*Puffinus*) and other procellariids (see below).

(2) The greater mobility of the *palpebrata* albumin at Rmu 94-99.

(3) There is a good correlation of mobility in the following protein bands:

   (a) The two minor proteins running ahead of the densely staining procellariiform macroglobulin (Rmu 12-15) at Rmu 18. They appear to be characteristic of the Diomedeidae.

   (b) The α globulin triplet (Rmu 77-83) was conspicuous in all eight albatrosses analysed, after the lipoprotein (shown as a broad band in *epomophora* at Rmu 69-74) had been precipitated with Rivanol. The albatross triplet appears to be complementary to the *Procellaria* triplet of slower mobility at Rmu 61-67 (see page 54).

   (c) The gamma globulins show some resemblance to those of *Procellaria aequinoctialis*.

(d) The reference prealbumin protein Rmu 100 is identical in mobility in *Phoebetria*, *Diomedea*, and five genera of the Procellariidae (*Procellaria*, *Macronectes*, *Fulmarus*, *Daption*, *Pachyptila*, and *Pterodroma*; see below).

(4) Overall configurations of the albatross esterase patterns are similar in all but *melanophris*. Those of the royal and wandering albatrosses are identical, whereas the esterases of *palpebrata*, although similar in staining appearance, are slightly more mobile. *D. melanophris* esterases are distinctive in that bands 2, 3, and 4 from the origin are very like those of *Procellaria*, except that band 2 (Rmu 32) is not as sharp. The three high-mobility *melanophris* bands not found in the other albatross plasmas are also found in diving petrels (Pelecanoididae) and storm petrels (Hydrobatidae).

Four species of albatross, representing the genera *Phoebetria* and *Diomedea*, are closely related. Monomorphic protein bands include (1) the reference prealbumin Rmu 100, (2) the α globulin triplet, and (3) the two minor protein bands Rmu 12-15. Significant differences in mobility are apparent in the albumin of *Phoebetria* and the transferrin triplet in *D. melanophris*. The esterase patterns are generally very similar; the three bands of high mobility in *melanophris* are peculiar to it. Further information might validate the genus *Thalassarche* prescribed for the southern mollymawks, and reveal affinities with the fulmarine petrels, Pelecanoididae, and Hydrobatidae. The albatrosses have a close affinity with the Procellariidae, and with genus *Procellaria* in particular.

Fig. 7.

Fig. 7. *Procellaria westlandica* outside its burrow at Punakaiki. This particular bird was caught and sampled for its blood proteins in May 1975 and September of the following year: no seasonal variation in the plasma proteins was detected.

Family Procellariidae : fulmars, petrels, prions, and shearwaters (I)

Genus *Procellaria* - four Southern Hemisphere species:

P. aequinoctialis Linnaeus, 1759 - white-chinned petrel, shoemaker, Cape hen.

P. westlandica Falla, 1946 - Westland black petrel

P. parkinsoni Gray, 1862 - black petrel, Parkinson's petrel

P. cinerea Gmelin, 1789 - grey petrel, pediunker

Synopsis

The little-known Southern Hemisphere genus *Procellaria* (Linnaeus) comprises four large, burrow-nesting petrels which are usually considered to be related to the cosmopolitan shearwater genus *Puffinus* (Brisson). In four species the plumage is wholly dark brown or black (see Fig. 7), whereas the grey petrel *Procellaria cinerea* is prevailingly greyish-brown above with dark underwing coverts and chiefly white underbody.

In addition to the large size of its species, the genus is characterised by a long, stout bill, which is either pale in colour or suffused with dark pigment on the terminal hook, the culminicorn, and much of the nostril plate. Its morphology suggests an adaptation to the seizing, holding, and biting of slippery prey. Oceanic species of squid captured from the sea's surface at night appear to be the principal natural diet, (P.C. Harper, pers. obs.) although *Procellaria* petrels have recently become persistent followers of large trawlers, aggressively fending off all but the albatrosses (*Diomedea* spp.) in pursuit of fresh fish thrown overboard (J.A. Bartle, pers. comm.). If forthcoming regularly and in quantity this new food supply may affect population numbers of several scavenging

species of petrels, for such food is readily accessible when rough seas might otherwise prevent petrels from feeding. The notable increase in the northern fulmar populations is believed to be attributable in part to the availability of such food supplies (Fisher 1952).

Despite their conspicuous nostrils (see Fig. 7) being elevated from the culminicorn, with the twin nasal tubes facing forward and divided by a septum - as in the surface-feeding genus *Pterodroma* and the fulmarine petrels - the *Procellaria* petrels are surprisingly good divers for such large birds, plunging beneath the sea's surface for bait and living prey (Murphy 1936; J.M. Moreland, pers. comm.; pers. obs.). This is in contradiction to the smaller shearwaters (*Puffinus* spp.), whose diving ability and remarkable structural modification for diving are well documented; their nostrils are much compressed, and open directed upward (Kuroda 1954).

The four species of *Procellaria* have evolved isolating mechanisms which exemplify the competitive strategy towards obtaining similar prey adopted by bird species of comparable size. The species *aequinoctialis* and *cinerea* occupy the sub-antarctic zone of surface water on a circumpolar basis, and in some instances use the same island to breed (the Kerguelen, Antipodes, and Campbell Islands are examples). However, whereas the larger, dark, white-chinned petrel nests in summer, its smaller grey-and-white counterpart, the grey petrel, breeds in winter. Eggs of the white-chinned petrel are laid in mid November, fledged young departing the burrow in May; grey petrel eggs appear in April-May, and the young depart in November-December. Nest sites are similar for both species - crooked burrows are dug deep into the upper parts of steep, damp slopes on headlands and hillsides. A feature of the nest site of

aequinoctialis is water, which either trickles through the tunnel or forms a pool at its entrance (Murphy 1936, Falla 1937). The nest itself is often a shaped mound or platform raised above the floor of the nest chamber and constructed either of mud or plant material (Rankin 1951: text, and plates 74 and 75). As nest builders, *Procellaria* petrels are comparable to the albatrosses (Falla 1937); no shearwater to my knowledge builds a nest so assiduously.

In addition to constrasting breeding regimes and plumage characters, their cries are distinctive. The white-chinned petrel makes a series of penetrating tapping sounds by rapidly opening and closing the bill, just as when two stones are firmly and rhythmically struck together; hence its vernacular names 'shoemaker' and 'cobbler'. The grey petrel's call is a speeded-up version of the same sound, creating a distinctive and strident whirring call, apparently produced by the throat instead of the beak (J. Warham, pers. comm.).

Their counterparts in subtropical waters are New Zealand's endemic black petrels, *Procellaria parkinsoni* and *P. westlandica*, which are identical in plumage and bill characters. Until the turn of the century, *parkinsoni* was a widespread breeding species of New Zealand's mountain ranges in both the North and South Islands (Reischek 1885, Oliver 1955; R.A. Falla, pers. comm.), whereas *westlandica* is known only from a small area on the west coast of the South Island (Falla 1946). The black petrel is about 18% smaller than the Westland petrel, except that its wing length is proportionally greater. This adaptation has undoubtedly arisen from the black petrel's migratory trait, which takes it across the equator to the rich, 30^{o}C waters of the west coast of middle America (Jehl 1974). *P. parkinsoni* is very similar in wing length to the more heavily built *P. cinerea*

(P = NS; see Table 3).

While its smaller, montane, summer-breeding relative is absent from New Zealand, the sedentary *P. westlandica* breeds during the winter in the wet, low-altitude broadleaf forests of Westland. Eggs are laid in May, and fledglings depart in late November - a schedule remarkably similar to that of its southern counterpart *cinerea*. In a subtropical environment, the preference for mountain-tops and seasonal timing of the breeding seasons ensure the wet and misty habitat required by the *Procellaria* petrels while nesting. The tapping noise of *parkinsoni* is strongly reminiscent of *aequinoctialis*; *westlandica* has a more varied vocal repertoire which includes a shearwater-like braying and howling.

TABLE 3. DIMENSIONS (mm) OF THE FOUR SPECIES OF *PROCELLARIA* PETRELS.

Species	n	Bill length	Wing	Tail	Tarsus	Toe
aequinoctialis	12	51.1±0.6	387.4±3.9) NS	125.7±1.3	68.1±1.1) NS	87.7±1.0) NS
westlandica	18	49.9±0.4	391.2±2.5)	132.4±1.2	62.2±0.5)	84.4±0.7)
cinerea	9	47.1±0.7	358.3±1.9) NS	115.2±1.4	64.0±0.9	77.5±0.6
parkinsoni	8	41.2±0.3	357.0±3.7)	106.1±1.7	54.9±0.8	70.8±1.0

Fig. 8. Family Procellariidae : Genus *Procellaria*.

Species	n (birds)	n (gels)	n (runs)	Source	Date
Procellaria westlandica	52	37	122	Westland	May 75, Sept. 76
P. parkinsoni	22	24	54	Great Barrier I.	Mar. 75, Jan., Dec. 76
P. aequinoctialis	1	11	11	Wellington	Apr. 76
	75	72	187		

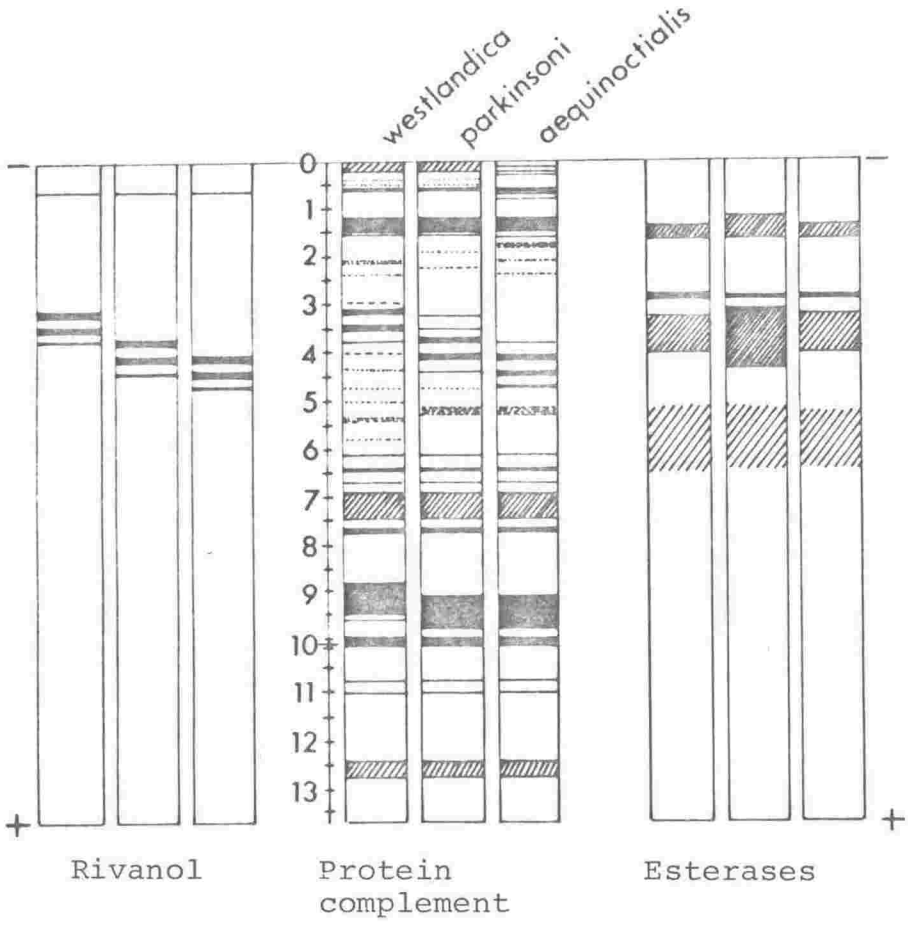


Fig. 8 is a composite drawing (to scale) of the banding patterns of plasma proteins from 75 *Procellaria* petrels representing three of the four species of the genus. Three major areas of distinction are:

(1) The differing mobilities of the Trt. Rmu's from the central transferrin band through *westlandica*, *parkinsoni*, and *aequinoctialis* are 35, 41 and 44 respectively. No individual variation in this pattern was found. Note that the Trt mobility of the latter two species is closer than the former two.

(2) *P. westlandica* albumin is significantly slower in mobility (92 Rmu : $\underline{n}$ = 52) as compared to that of its congeners (95 Rmu : $\underline{n}$ = 23).

(3) *P. aequinoctialis* gamma globulins (Rmu 0-10) are unique; those of *parkinsoni* and *westlandica* are identical. This fact would implicate a species difference rather than an individual one.

Areas of significant similarity are:

(1) The prealbumins (Rmu 100, 110);

(2) The α globulins (Rmu 61, 64, 67 and 77); and

(3) The esterase patterns.

Prealbumins at the Rmu 110 locus are unique to the *Procellaria* petrels. The distinctive esterase band at Rmu 29 shown as a clearly defined sharp band has an identical mobility to that of the Pelecanoididae - cf. the gadfly petrels (*Pterodroma* spp.), shearwaters (*Puffinus* spp.), and some fulmarine petrels illustrated below. Although their mobilities differ, the esterases of albatrosses (*Diomedea* spp.) have a similar pattern to those of *Procellaria* (see page 46).

Minor proteins such as those at Rmu 17-25 and Rmu 43-57 appear to show consistent differences, but being present in only trace amounts they are difficult to quantify accurately. Staining with benzidine suggests that some have haem-binding properties; whether this reflects a genetic difference or merely a transitory enrichment from dietary iron is not known.

The black petrels of the genus *Procellaria* are related, and are correctly placed within a single genus. The Westland petrel is more closely related to the black petrel than to the white-chinned petrel. The genus is allied to the Diomedeidae, the shearwaters (*Puffinus*), and the diving petrels (Pelecanoididae). It is considered to be one of the oldest living groups of petrels (see below).

Family Procellariidae : fulmars, petrels, prions, and shearwaters (II)

The fulmarine petrels - seven species in five genera; one Northern Hemisphere species, the remainder Southern Hemisphere:

Macronectes (two species) - giant petrels

Fulmarus (two species) - northern and southern fulmar

Thalassoica antarctica (Gmelin, 1789) - Antarctic petrel

Daption capense (Linnaeus, 1758) - Cape pigeon

Pagodroma nivea (Forster, 1777) - snow petrel

Synopsis

Fulmarine petrels comprise seven species of medium-sized to large birds belonging to five highly distinct genera. Their lineage is a long one, fossils dating back 22 million years to the lower Miocene of Argentina. All except the northern fulmar, *Fulmarus glacialis* (a pre-Pleistocene migrant from the south?), have a circumpolar breeding distribution and a winter range usually confined to the Southern Hemisphere. The Antarctic and snow petrels are the most restricted in range, frequenting the cold waters surrounding Antarctica and rarely venturing across the Antarctic Convergence. The southern fulmar and Cape pigeon (two subspecies: *capense* - high latitudes; *australis* - centred at The Snares) both nest on Antarctica and disperse more widely. This is especially true of the genus *Daption*, which is conspicuously abundant in southern oceans during the non-breeding season. Giant petrels (*Macronectes*) comprise two ecologically distinct species: *giganteus*, of Antarctic waters and the cooler subantarctic, a gregarious nester; and *halli*, its more northern congener, which is a more solitary species resident on many

subantarctic islands (Bourne & Warham 1966). Both forms occur in Marion, the Crozets, Macquarie, and Kerguelen(?) Islands (Watson 1975).

The albatrosses and southern fulmars show a number of interesting similarities and differences. Both groups nest in the open, a trait regarded by some workers as the more primitive one (Murphy 1936, Kuroda 1954). In retaining the old nesting habit and body size, the fulmars have had to retain and perfect their defence strategies towards aerial predators. To this end, they are capable of squirting an oily secretion from their throats at any intruder venturing within 1-2 m of an occupied nest. Should the victim be a bird, it will immediately retire to set about energetically cleansing itself of the discharge, even if this means immersing the feathers in mud, sieving snow through them (Brown 1966), or rubbing them against vegetation or rock. This is an effective deterrent for a group of petrels which nest exclusively above ground. Albatrosses, by virtue of their great size, assure themselves of competitive strength and relative freedom from the attention of predators.

The giant petrel builds a large, crater-like nest of plant and gravel debris situated often in open, flat areas. This habit, together with a trait of obtaining much of its food ashore, renders the giant petrel one of the best walkers of all petrels. This is in sharp contrast to its smaller kin, the high-latitude *Fulmarus*, *Thalassoica*, and *Daption*, all of which construct nests on cliff ledges using stones and gravel and are notably poor walkers on flat ground. Like Murphy (1936), I

have discovered *Daption* and *Fulmarus* to be "quite incapable of standing on straight tarsi or of springing into flight from a level surface".

By having a highly synchronised laying period as early in the summer as the snow cover and food supplies will allow, the large Antarctic fulmars are able to complete both their breeding season and post-nuptial moult before food supplies dwindle in early winter. In contrast, smaller birds such as the prion *Pachyptila desolata* and the storm petrels *Fregetta tropica* and *Oceanites oceanicus* must wait for the spring melt to free their burrows and crevices of snowdrifts, and must delay their post-nuptial moult until they reach their winter feeding grounds (Beck 1970). It is thus obvious that greater body size offers an important selective advantage for petrels nesting in the Antarctic, an interesting fact in that summer food abundance mitigates competition for food between procellariid species (see below).

Fulmarine petrels are noted for their aggressive and carnivorous behaviour. A disabled bird will be quickly set upon by its own kind, and disputes over territory are fierce and sometimes wounding. Despite having a wing all but amputated by gunshot, snow petrels will furiously fight any assailant that attempts to retrieve them from the water (P.C. Harper, pers. obs.) At the Crozets, Falla (1937) reported three giant petrels "sitting patiently round a fledgling albatross on its nest". Giant petrels will also beat small petrels into the water using their wings (Buller, cit. Oliver 1955; and pers. obs.).

Fig. 9. Family Procellariidae : fulmarine petrels

Species	n (birds)	n (gels)	n (runs)	Source	Date
Macronectes halli	1	11	11	Paekakariki beach	Dec. 76
Fulmarus glacialoides	1	9	9	Petone beach	Sept. 75
Daption capense	4	11	15	Petone beach	Sept. 75, 76,77 (1♂; 3♀s)
	6	31	35		

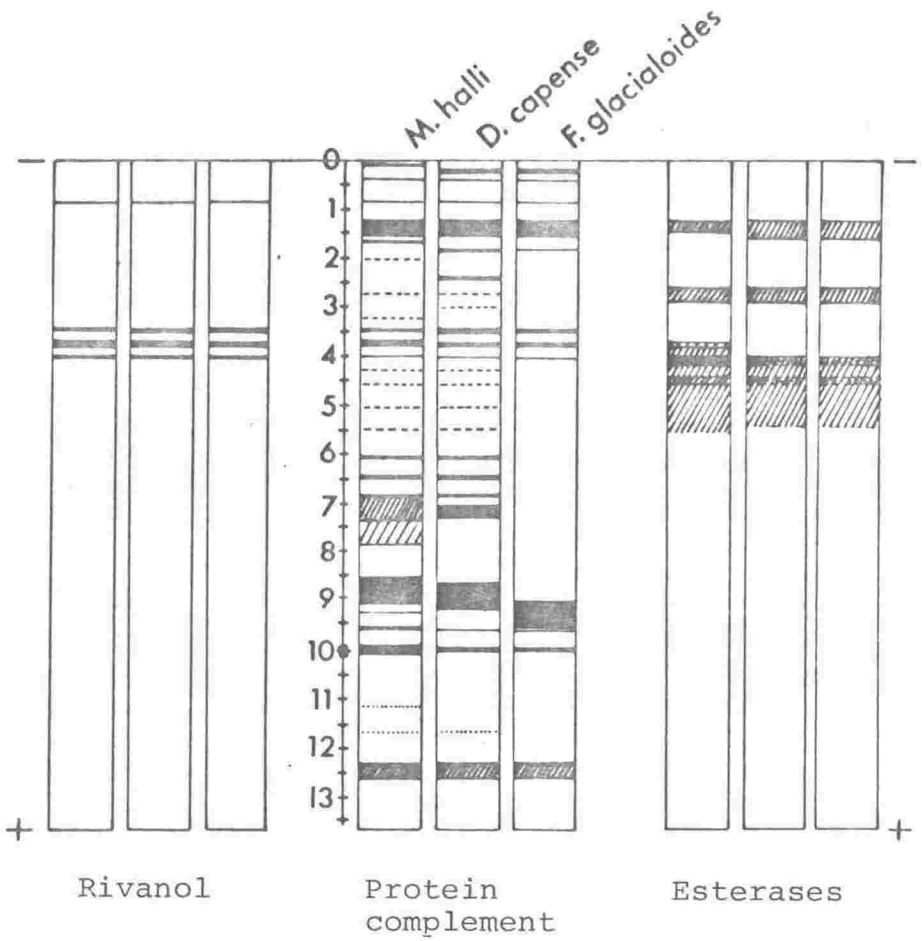


Fig. 9 is a composite drawing (to scale) of the banding patterns of plasma proteins from six fulmarine petrels representing three of the five genera. The data are to be regarded as only tentative, since the sample size is not large and the amount of plasma obtained from the single specimen of *Fulmarus glacialoides* was small; hence, some protein bands were not sufficiently quantified to be shown in the protein complement of this species. Sufficient quantities of plasma (> 2 ml) were obtained from *Macronectes halli* and *Daption capense*.

Note the following:

(1) The identical mobilities of the Trt at Rmu 35-40. In *Macronectes* the central band of the Trt is the strongest-staining, followed by the top band and the lower band (Rmu 40), which stains weakly. The upper two bands of *Daption* (Rmu 35 and 37) are approximately equal in staining intensity ($\underline{n}$ = 3 birds). This finding appears to be paralleled in *Fulmarus glacialoides* (n = 1), and concurs with the situation found in the genus *Puffinus* (see page 83).

(2) The α globulin triplets (Rmu 60-68) in *Macronectes* and *Daption* are of similar mobility, as are four bands of minor proteins spanning Rmu 42-55.

(3) The gamma globulins (Rmu 0-5) of *Daption* and *Fulmarus* are identical in mobility, and very different from those of *Macronectes*. The γ globulins share similarities with the white-faced storm petrel, *Pelagodroma marina* (Hydrobatidae). The strong *Daption* band at Rmu 71 is also very similar to that of storm petrels.

(4) There are several clear distinctions between *Macronectes* and *Daption* in the minor proteins spanning Rmu 16-33 and

the prealbumins. The remarkable configuration of the *Macronectes* esterases is of special interest, since it differs from that of the other fulmarine genera. The esterase patterns show similarities with those of the Hydrobatidae and Pelecanoididae.

(5) The position of the albumin of *Fulmarus* is tentative; a greater mobility than for either *Macronectes* or *Daption* is indicated (11 gels), but more data are required.

The fulmarine genera *Macronectes*, *Fulmarus*, and *Daption* are closely related. Several protein differences between the genera support the hypothesis of Alexander et al. (1965) that the fulmarine petrels are old, highly differentiated stock showing relationships with other primitive procellariiform birds such as the diving petrels (Pelecanoididae) and the southern storm petrels (Hydrobatidae); see below.

The uniform mobility of the transferrin triplet throughout the three phenotypically distinct fulmarine genera is both unusual and intriguing; a long-standing genetic equilibrium maintained under strong environmental selection pressures appears likely.

Fig. 10.

Fig. 10. A fairy prion (*Pachyptila turtur*) tending its chick at the Poor Knights, November 1976.

Family Procellariidae : fulmars, petrels, prions, and shearwaters (III)

Genus *Pachyptila* - prions; six Southern Hemisphere species:

P. turtur Kuhl, 1820 - fairy prion

P. crassirostris Mathews, 1912 - fulmar prion

P. belcheri Mathews, 1912 - thin-billed prion

P. desolata Gmelin, 1789 - Antarctic prion

P. salvini Mathews, 1912 - Salvin's prion

P. vittata Forster, 1777 - broad-billed prion

Synopsis

The six species of prion have the dubious distinction of being the most difficult of all the Procellariiformes to identify, either at sea or as museum skins. Names galore have been appended to them, at taxonomic levels ranging from geographical race to subfamily. In addition, misunderstanding of the differences between adult and immature bill morphology - a crucial criterion in separating the species - have resulted in a continuing bewilderment regarding *Pachyptila* among many ornithologists. Attempts by Murphy (1936), Falla (1940), and Fleming (1941) to remedy Mathews's chaotic treatment of the petrels (and this genus in particular) with names "so copiously and irresponsibly conjured out of nothing" (Murphy 1936) have clarified the nomenclatural problem considerably. Some 30 years later, Harper (1972) presented a guide to the identification and distribution of the two southern species, *desolata* and *belcheri*, and a full revision of *Pachyptila* is soon to be published (Harper, in press).

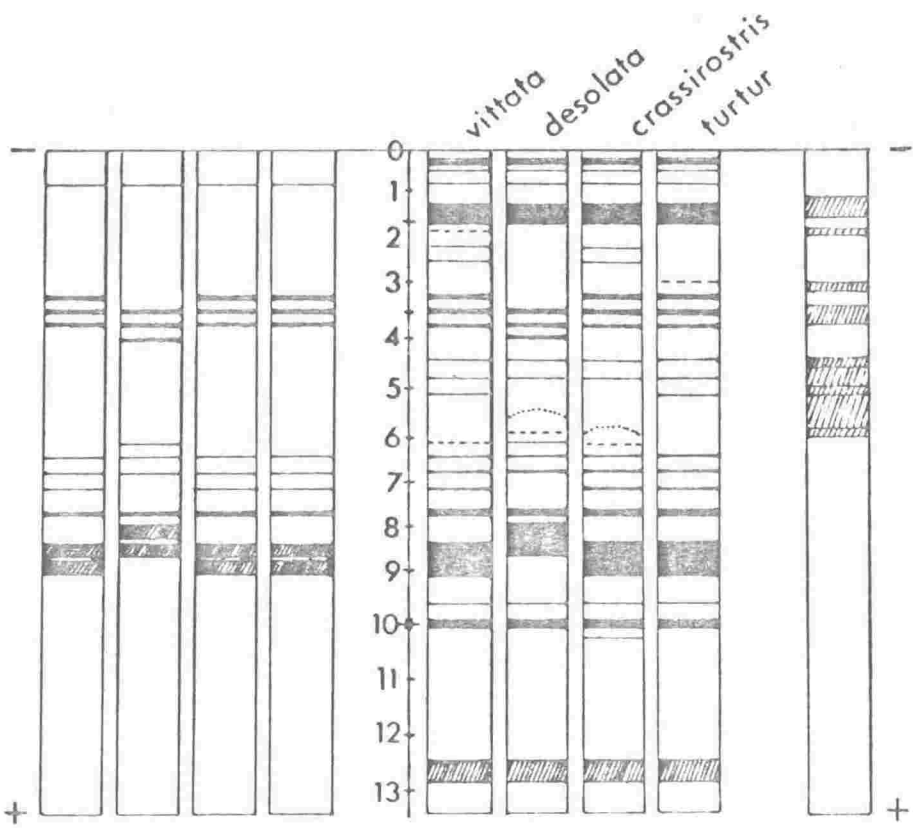
Confusion of the prions results from the species all being of similar colour, pattern, and body size. Facial pattern and the configuration of black pigment on the rectrices are useful identification criteria, but bill structure, in *Pachyptila* a superb example of adaptive responsiveness to selection pressures, is the key characteristic. The maxilla of the larger-billed species (*desolata, salvini, vittata*) is fringed with comb-like lamellae which strain food from seawater taken into the mouth; the smaller-billed *crassirostris, turtur,* and *belcheri* possess transverse maxillary rugi. The latter use their beaks for seizing, holding, and biting crustaceans and squid. Food is obtained by "hydroplaning" or "contact dipping" (Ashmole 1971) at the sea's surface. As with the fulmarine and gadfly petrels, the soft plumage and light body of *Pachyptila* (cf. diving shearwaters, *Puffinus* spp.) favour food gathering at or near the ocean's surface rather than below it.

Prions have a circumpolar distribution patterned on the differing zones of surface water. *P. desolata* breeds chiefly on high-latitude Antarctic isles, and on a few islands in subantarctic waters (Watson et al. 1971). Only on Kerguelen, positioned near the Antarctic/subantarctic water interface (Antarctic Convergence), do two species cohabit in substantial numbers (*desolata* and *belcheri*). A dominant species, *salvini*, occupies all nest zones from high tide level to near the summit of l'île de l'Est and l'île aux Cochons (Despin et al. 1972, Derenne & Mougin 1976) in numbers measured in millions; other *Pachyptila* (*turtur* and *desolata*) are tenuous breeders there. Islets off the Falklands are replete with *belcheri*; only at Beauchêne Island do a few fairy and fulmar(?) prions manage to raise chicks (Strange 1968; P.C. Harper, unpubl. data). This breeding pattern also occurs at Macquarie, Heard, and the Auckland and Chatham Islands.

Clearly the disruptive patterning and highly cryptic coloration of *Pachyptila* (and the blue petrel, *Halobaena caerulea*) appear to have evolved to minimise detection by a diurnal predator, most likely the Tertiary equivalent of the southern skua, *Catharacta lonnbergi*. The consummate, almost diabolical skill with which the skuas perceive and dispatch small petrels makes the predator - prey association appear a very long-standing one. The fact that prions do not nest in winter may, in part, be attributable to predation pressures. By swamping the appetite of their predators in summer ("arithmetic mimicry"), only to desert the breeding grounds during winter so as to deprive potential predators of prey, certainly appears to be an effective ploy for keeping the numbers of predators at a low level. On breeding grounds removed from intensive skua predation (chiefly those within subtropical waters), both *Pachyptila vittata* and *P. turtur* appear to be evolving away from a summer reproductive schedule (Harper 1976 and in prep.). Fleming (1941) has shown that the beak of the larger-billed prion species passes through an embryonic neotenous stage of appearing in structure like that of the smaller-billed members of the genus. Hence, at a latter stage of development, the bill of immature *belcheri* looks like that of adult *turtur*; of immature *desolata* like adult *belcheri*; and of immature *salvini* like adult *desolata*. The speciation of *Pachyptila* is thus elegant in design, but it has certainly proved to be a trap for ornithologists, and possibly for the birds themselves, since visitations of one species on another's breeding grounds have been reported (e.g., Falla 1937, Tickell 1960). Information on the breeding biology of *Pachyptila* can be found in Murphy (1936), Falla (1937), Oliver (1955), Tickell (1962), Richdale (1965), and Harper (1972, 1976, and in prep.).

Fig. 11. Family Procellariidae : Genus *Pachyptila*

Species	n (birds)	n (gels)	n (runs)	Source		Date
Pachyptila turtur	89	31	199	Poor Knights Is	n=50	Oct. 75, Nov. 76
				Motunau I.	n=20	Jan. 76
				Stewart I.	n=19	Sept., Oct. 76
P. crassirostris	5	8	13	Bounty Is		Nov. 76
P. desolata	5	10	18	Auckland Is		Jan. 77
P. vittata	16	10	27	Stewart I.		Sept., Oct. 76
	115	59	257			



Rivanol Protein complement Esterases

Fig. 11 is a composite drawing (to scale) of the banding patterns of plasma proteins from 115 prions representing four of the six species comprising the genus *Pachyptila*.

Note the following:

(1) In *desolata* the Trt shift to a greater mobility, and the opposite move for the albumins and the α globulin triplet. The result is a complement pattern distinctly different from those of the remaining *Pachyptila*.

(2) The important similarities in:
   (a) the double-banded albumin (Rivanol);
   (b) the esterase pattern;
   (c) the gamma globulins

(3) The importance in genetic terms of the differences in the minor proteins is not clear; some may be significant:
   (a) Rmu 44-51, *vittata* and *turtur*;
   (b) the 'domed' protein of *desolata* and *crassirostris*;
   (c) the consistent trace protein (Rmu 31) of *turtur* and protein Rmu 21-24 of *vittata* and *crassirostris*;
   (d) the prealbumin 103 of *crassirostris*.

The three populations of *Pachyptila turtur* (Poor Knights, n = 50; Motunau, n = 20; Stewart Island area, n = 19) proved to have remarkably similar protein complements and esterase patterns. Slight individual variation was detected in the esterase staining intensities, but this was not held to be significant. Indeed, the stability of the genes producing esterase activity shows valuable potential for comparisons above the species-group level, especially where more mobile loci such as the transferrin triplet and the albumins illustrate dissimilarities (cf. *P. desolata* and *Procellaria*, page 55).

The four species of prions considered here have many protein similarities, and are clearly closely related. The double albumins, which appear to be a generic character, are unique at this taxonomic level (cf. *Puffinus assimilis* and the Hydrobatidae). The esterase pattern of *Pachyptila* shows some similarities with those of the fulmar group. In differing from its congeners in the mobility of the transferrins, the globulin triplet, and the albumins, *desolata* is unusual. A much older phylogeny for *Pachyptila* than that proposed by Fleming (1941) appears likely (see Discussion, below).

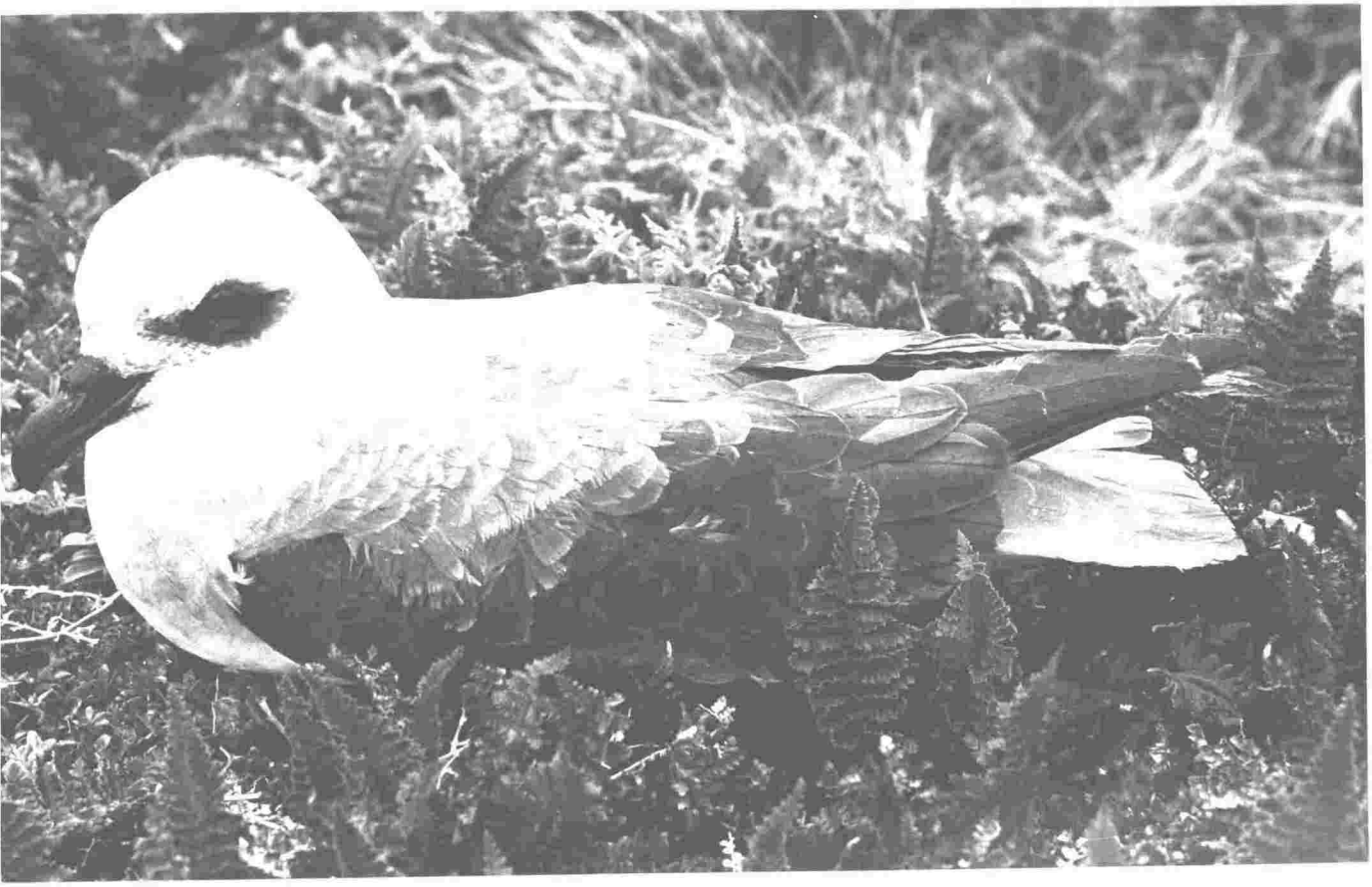


Fig. 12.

Fig. 12. A white-headed petrel (*Pterodroma lessoni*) near its burrow at the Antipodes Islands. The plasma proteins of this species are remarkably similar to those of the grey-faced petrel (*Pterodroma macroptera*).

Family Procellariidae : fulmars, petrels, prions, and shearwaters (IV)

Genus *Pterodroma* (also *Halobaena*) - the gadfly petrels; c. 25 species, most in temperate or tropical waters of the Southern Hemisphere; a few in the North Pacific and North Atlantic Oceans.

Synopsis

The gadfly petrels of the genus *Pterodroma* (Greek: *pteron* = wing, *dromos* = running; hence, 'wing-runner') are so named for the swift, dashing flight characteristic of these widely distributed birds. Although no fossils have been found to date, their lineage, distribution, structure, plumage patterns, and habits clearly mark the gadfly petrels as originating from ancient stock, probably at least as old as that of the present-day *Puffinus* shearwaters, which date back to the Oligocene (Lambrecht 1933).

Throughout this polytypic genus, the structural conformity to a single pattern is remarkable. The bill is predominantly short, deep, and well hooked, and shows little tumescent development of the latericorns (cf. *Pachyptila*; see above); it is consistently black, and the nostrils are separated by a weak septum and enclosed in short tubes (cf. fulmarine petrels) facing directly forward over the culminicorn (cf. shearwaters). In a detailed comparative osteological analysis of *Pterodroma* and *Puffinus*, Kuroda (1955) concluded that "*Pterodroma* makes a group with the fulmars and storm petrels and combines the characteristics of both ... it shares a few partial shearwater characters in, for example, the sternum, humerus, and skull (lacrymal ossicle)."

In most *Pterodroma* species the plumage is greyish-black above and white below, with typically mottled white feathering giving a scaled appearance to the forehead. Dark eye patches are prevalent (a protective device minimising reflection into the eyes?); the wings are long for the size of the birds; tails are typically cuneate in larger species, grading to almost square or gently rounded (*Halobaena*); and the contour plumage is characteristically long and soft. Such features are mirrored in vernacular names such as soft-plumaged petrel (*P. mollis*) and great-winged petrel (*P. macroptera*).

All these peculiarities, together with their comparatively low body weight yet rather bulky appearance, mark the *Pterodroma* petrels as a specialised group adapted to obtaining their food from the ocean at night when hosts of planktonic animals raft near the surface. It is the bioluminescent cuttlefish which attracts the attention of gadfly petrels (by smell, movement or other visual cues?); the short, powerful beak and hovering flight allow the birds to capture their prey with speed and accuracy. Large gadfly petrels such as *P. lessoni* and *P. macroptera* can deal effectively with squid 40 cm in length - as long as the birds themselves (pers. obs.). The specialised character of both bill morphology and feeding behaviour suggest the *Pterodroma* petrels to be highly stenophagous, hence their proclivity for extensive migrations.

Pterodroma petrels exhibit a comprehensive range of plumage colour and pattern. The surface-nesting species *neglecta* and *arminjoniana* are dichromatic to the point where the dark and pale phases "are linked by a series of intermediates representing nearly every possible blend. In addition, asymmetrical and partially albinistic styles crop out in a significant proportion of all populations" (Murphy & Pennoyer 1952: text,

and fig. 3). The Kerguelen petrel, *Pterodroma brevirostris*, is an interesting example of an apparently hitherto dichromatic species adopting a solely dark phase, in that the basal portions of the feathers are white; its skull is likewise uniquely ornate and fenestrated to accommodate and protect the large eyes (Harper 1972). Two species of identical morphology, the Atlantic and white-headed petrels (*Pterodroma incerta* and *lessoni* respectively) offer an example of a brown, dark-headed species inhabiting the South Atlantic (breeding Tristan da Cunha group) and a grey, white-headed petrel occupying subantarctic waters of the remaining southern oceans (breeding Auckland, Antipodes, Macquarie, Kerguelen and Crozets: Oliver 1955; Warham 1967; Barre 1976). These two sibling species were initially considered by Murphy (1936) to be merely a colour variation of a single species, a view later rescinded on account of the birds' differing water zone preferences and contrasting undertail covert colouration (Murphy and Pennoyer 1952). Recently Jouanin and Mougin (in press, cit Barre 1976) have sought to place *incerta*, *macroptera*, *magentae*, *solandri* and *lessoni* in a 'superspecies' group under *lessoni* (Garnot 1826).

Perhaps the most striking phenomenon associated with the genus *Pterodroma* is their conspicuous underwing patterns which are particularly prevalent amongst the medium to small-sized migratory members of the genus. Whereas body colour can vary phenotypically almost in random fashion with non-migratory species such as *neglecta* and *arminjoniana*, the underwing pattern is quite the opposite, with every indication that selective pressure maintaining it is very strong. The significance of this, in conjunction with behaviour patterns and protein polymorphism will be dealt with in the Discussion (see page 107).

Fig. 13. Family Procellariidae : Genus *Pterodroma*

Species	(birds)	n (gels)	(runs)	Source	Date
Pterodroma macroptera	17	18	21	Whangamata Is n = 3 Tiri-Tiri I. n = 2 Korapuki I. n =12	June 75 Aug. 75 Dec. 76
P. lessoni	3	11	11	Auckland Is Petone beach	Dec. 76 May 77 Sept. 77
P. inexpectata	1	13	13	Snares Is	Jan. 77
P. pycrofti	1	10	10	Poor Knights Is	Oct. 75
P. cooki	2	10	10	Great Barrier I.	Mar. 75
	24	62	65		

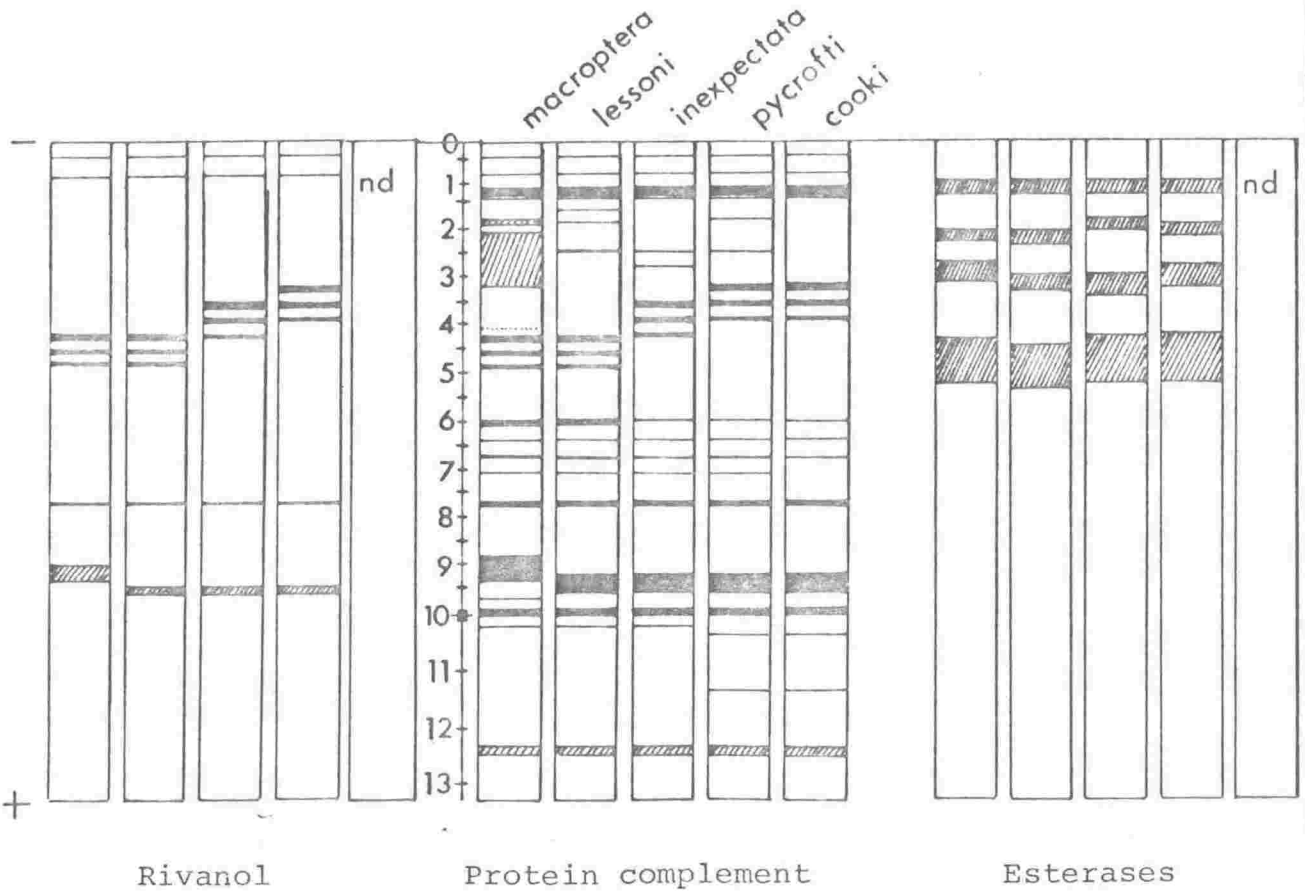


Fig. 13 is a composite drawing (to scale) of the banding patterns of plasma proteins from 24 gadfly petrels representing five of the c. 25 species comprising the genus *Pterodroma*. Two large, one medium-sized and two small species were examined. Significant results are as follows:

(1) In the protein complements, note the conspicuous step-like decrease in the Trt mobility from the largest to smallest members of the genus.

(2) The strong α globulin Rmu 60 is peculiar to both *macroptera* and *lessoni*: in the remaining species it is notably less distinct.

(3) *P. macroptera*'s albumin (Rmu 88-93) has a slower mobility than that of the four other species (Rmu 94-96).

(4) Note the prealbumins Rmu 102 and 113 peculiar to *pycrofti* and *cooki*.

(5) The minor proteins between Rmu 15-30 show specific differences; the validity of these is not known. The diffuse *macroptera* band at Rmu 21-32 may conceal additional trace proteins.

(6) Note the strong similarities in the esterase patterns (no data from *cooki* for esterases and Rivanol analyses). Species' patterns differ, however, (particularly bands Rmu 20 and 30) to support clear genetic dissimilarity.

The three populations of *macroptera* (all within 120 km of each other) proved to have identical protein complements and very similar esterase patterns. Sampled birds were adults incubating eggs or feeding young: seasonal variation in the protein complement and esterase patterns was not evident.

A breeding male *lessoni* from the Auckland Is incubating an egg in December, and 2 non-breeding females taken from Petone beach in winter and spring had identical protein patterns.

The *Pterodroma* petrels show striking transferrin mobility changes and differences in prealbumins and α globulins. The 2 largest species, *macroptera* and *lessoni* are closely related with identical transferrin mobilities, and a conspicuous α globulin band (Rmu 60) unique to them. Another subgroup, (the subgenus *Cookilaria*: see Fleming 1941) represented here by *pycrofti* and *cooki*, also have identical Trt mobilities, α globulins, albumins and a unique prealbumin pattern. The esterase patterns show distinctions at the species level, but are very similar as generic characters. They stain similarly to the shearwaters *Puffinus* sp. In examining recent fossil material from St. Helena Island, (South Atlantic) Olson (1975) presents skeletal evidence for a tropical species group of *P. rupinarum* (St. Helena; recently extinct); *becki*, *rostrata*, and *atercima*. "These four species constitute a very distinct group within the genus *Pterodroma*." (Olson, 1975). As noted above, Jouanin and Mougin (in press, cit. Barre 1976) are seeking to place the larger *Pterodroma* petrels within a *lessoni* superspecies group. The plasma protein data support the hypothesis that the gadfly petrels are a heterogenous assemblage of highly differentiated birds with an ancestry probably equal to that of the shearwaters.

Fig. 14.

Fig. 14. The Buller's shearwater (*Puffinus bulleri*) about to enter its nesting crevice. This large shearwater breeds only on the Poor Knights Islands, and migrates to the north Pacific for the austral winter. This and several other migratory New Zealand shearwaters return south to their breeding colonies in September. Fifty-nine *bulleri* were sampled for their plasma protein information.

Family Procellariidae : fulmars, petrels, prions, and shearwaters (V)

Genus *Puffinus* - shearwaters. About 12 species with numerous subspecies; distributed over all oceans; most in the Southern Hemisphere.

Synopsis

Shearwaters rank among the better known medium-sized petrels because some species such as the trans-equatorial migrants, the sooty (*P. griseus*) and short-tailed shearwaters (*P. tenuirostris*) are among the world's most abundant birds (*tenuirostris* = 150 million: Peterson 1948), and their fledglings are taken in great numbers as food both in Tasmania and southern New Zealand. It is not surprising, therefore, that their biologies are familiar to local "mutton birders" and have been the objects of scientific study: Richdale (1963), Marshall & Serventy (1956), and Serventy (1967).

The shearwater dynasty is ancient, dating back to the Oligocene (Lambrecht 1933), and has resulted in a heterogeneous group of slender-billed procellariids with a world-wide distribution. Northern trans-equatorial migrations are undertaken annually by six austral species; the Buller's shearwater, *P. bulleri*, the flesh-footed shearwater, *P. carneipes*, and its close ally the pink-footed shearwater, *P. creatopus*; the greater shearwater *P. gravis*, and the well known *P. tenuirostris* and *griseus* mentioned above. Several of the New Zealand species migrate together in a vast mixed concourse of birds. Such flocks of petrels are able to search for food resources more effectively than individuals, and to use such

resources more efficiently when they are found. Migrations into the Southern Hemisphere include those of the Mediterranean shearwater *Calonectris diomedea* and the manx shearwater *P. puffinus*. Other species such as the fluttering and Hutton's shearwaters (*P. gavia* and *P. huttoni*) fly the shorter distance from New Zealand to south-east Australian waters. All the above shearwaters breed in summer whereas the little shearwater *P. assimilis* is a sedentary, winter breeder. The extraordinary spread of its 17 subspecies across the world's oceans clearly shows its migration patterns of the past. The distribution of eight subspecies or species (according to your preference) of the manx shearwater *Puffinus* spp. found from Great Britain to New Zealand is another example of a relic-type distribution. Such behavioural traits clearly support the fossil evidence for the shearwaters temporal longevity.

Two types of feeding behaviour and adaptive morphology are illustrated by shearwaters. The larger, subtropical species, *Calonectris diomedea*, *P. bulleri* and the wedge-tailed shearwater, *P. pacificus*, are noted for their long, strong bills, comparatively broad wings, long wedge-shaped tails, soft plumage as the *Pterodroma* petrels, together with their contact-dipping feeding habits (King 1974). The other shearwaters are highly specialised in every adaptive character towards a more aquatic life style (Kuroda 1954); these heavy-bodied, streamlined shearwaters can wing-row themselves underwater with remarkable agility after their prey of shoaling fish. The two distinctly different feeding techniques can readily be observed by watching a mixed flock of feeding *bulleri* and *gavia* shearwaters in the Hauraki Gulf of New Zealand. The Buller's shearwater, unique to the Poor Knights Islands, (Falla 1924) is a large, broad-winged bird with a long neck and wedge-shaped tail.

It is conspicuously patterned mouse-grey and black above with a dark grey cap (see Fig. 14). Although the slightly larger greater shearwater *P. gravis* of the subtropical Atlantic is very similarly marked, it is a diving species and consequently, (like *carneipes*), has a much shorter tail (105.7 - 116 mean $\underline{\Omega}$ 111 mm; 12 ♂, 11 ♀, as compared to 114 - 131.8 mean 125.3 mm $\underline{n}$ = 24: Murphy 1936). Flocks of *bulleri* pursue shoaling fish by encircling them, whereupon the birds will alight quickly on the sea's surface, and with their wings held high, snatch their fleeting prey with a sudden darting movement of their long neck. The same feeding behaviour by *pacificus* has been described by King (1974). The long, laterally compressed bluish-grey bill of *bulleri*, which terminates in a slender yet powerfully decurved hook both impales the prey and prevents it sliding from the beak. The bill's hook, in conjunction with the sharp, flinty claws, is also distinctly functional in enabling the birds to climb trees when leaving their breeding grounds.

While the *bulleri* are so engaged at the sea's surface, fluttering shearwaters sweep low overhead before plunging in a low-angled dive clean beneath the water in pursuit of the fish below. Alternatively, these smaller shearwaters will quietly move forwards over the sea's surface with their heads submerged searching visually below for prey. Once detected, the birds paddle rapidly forwards so as to raise the body at a high angle before disappearing below. *P. gavia* can remain out of sight for at least 15 sec before reappearing sometimes 6 m from the submersion point. In the calm, sunlit waters of Wellington harbour on 30 June 1973 I saw *gavia* quickly disappear from view 3-5 m below the surface within 7 sec. Such a feat would seem impossible for a Buller's shearwater, yet the slightly larger, all dark, flesh-footed shearwater *carneipes* can accomplish this

with ease (Falla 1934). *P. carneipes*, is however, heavier in body weight and shorter tailed (Harper, unpubl. data 1963-69). Morphologically, *carneipes* forms an intermediate form between surface and submarine feeding shearwaters (Kuroda 1954).

Migratory shearwaters are also famous for their highly synchronised breeding cycles (e.g. Murphy 1936; Serventy 1967). At the Poor Knights Islands on 19 January 1973, I recorded the first hatchings of *bulleri* eggs for that year; a date precisely the same as that obtained by Wilson (1959) 30 years earlier. Egg-laying data for *carneipes*, *griseus*, and *tenuirostris* are equally impressive: the last week in November in all cases (the night of 25 November as vowed by some local "mutton birders" is too exact).

Fig. 15(a). Family Procellariidae : Genus *Puffinus*

Species	(birds)	n (gels)	(runs)	Source		Date
Puffinus bulleri	59	24	81	Poor Knights Is	n = 25	Oct. 75
					n = 14	Mar. 76
					n = 20	Dec. 76
P. carneipes	10	6	14	Karewa I.	n = 2	Jan. 76
				Korapuki I.	n = 8	Dec. 76
P. griseus	28	21	40	Titi I. (Marlborough)	n = 11	Feb. 76
				Stewart I.	n = 14	Oct. 76
				Korapuki I.	n = 3	Dec. 76
P. gavia	29	23	47	Poor Knights Is	n = 4	Oct. 75
						May. 76
				Saddle I. (G.B.I.)	n = 6	Dec. 75
				Korapuki I.	n = 19	Dec. 76
P. huttoni	10	23	48	Kaikoura Mountains		Feb. 76
P. assimilis	49	29	63	Poor Knights Is	n = 17	July 75
					n = 13	Oct. 75
					n = 19	Nov. 76
	185	126	293			

Fig. 15(b). Family Procellariidae : Genus *Puffinus*

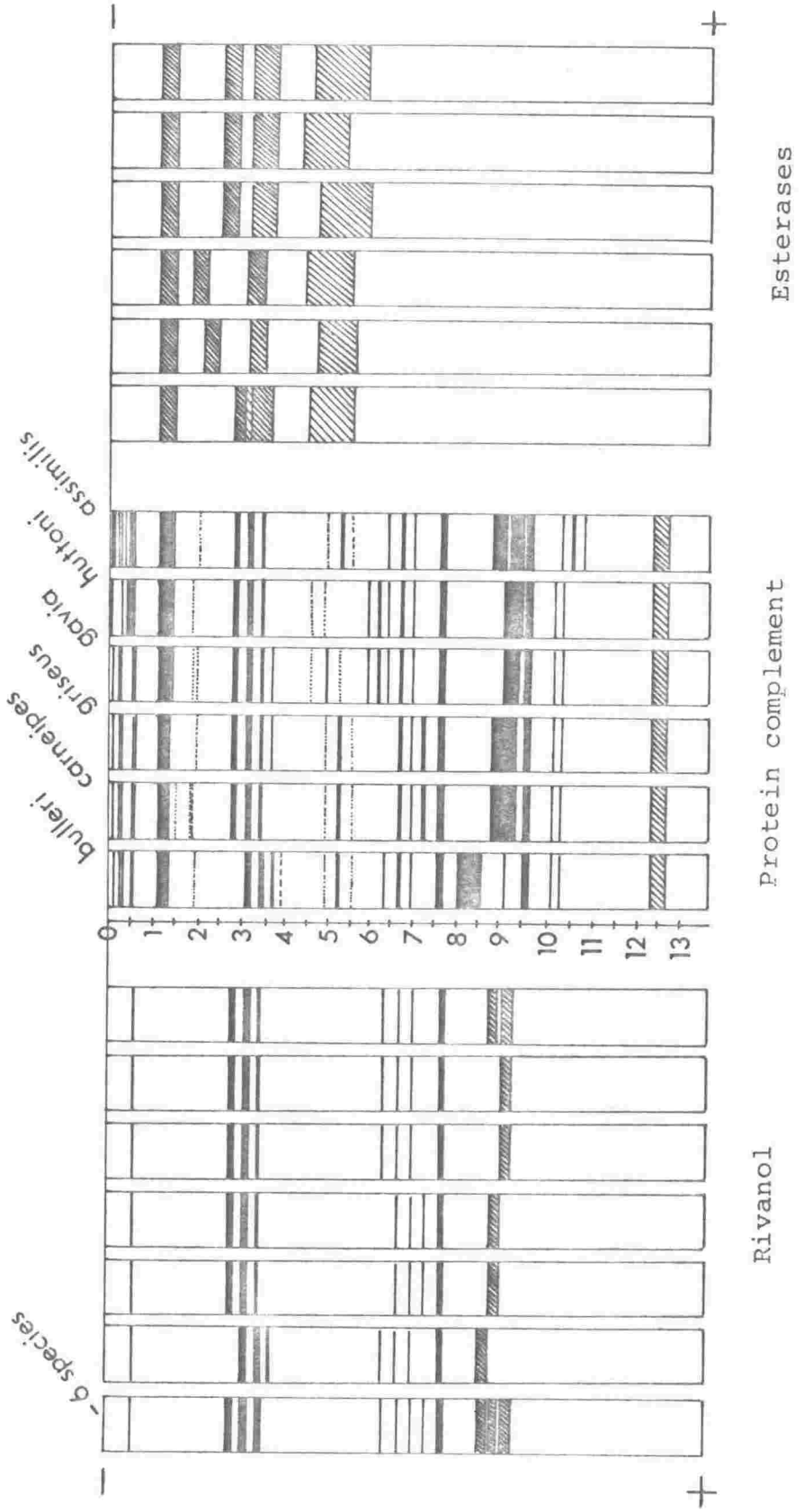


Fig. 15 is a composite drawing (to scale) of the banding patterns of plasma proteins from 185 shearwaters from six of the 12 species of *Puffinus* group. Good quantities of plasma were obtained from all species representing the genus in New Zealand.

Note the following:

(1) Apart from *bulleri*, five species have identical Trt mobilities. In all species the top two transferrin bands stain more strongly than the third; this was consistently the case in all birds taken from differing age groups and time of year (n = 185). A similar finding occurs in the Diomedeidae, the genus *Procellaria* and some fulmarine petrels.

(2) The differing albumin mobilities. Adult *assimilis* (n = 49) are remarkable in having two albumins at Rmu 89 and 93 respectively. The samples obtained from *carneipes*, *griseus*, *gavia* and *huttoni* were all from adult individuals and a twin albumin was not detected in this sample(n = 87). By pooling the adult bird plasma (n = six species) three albumin bands were defined after Rivanol treatment (see Fig.15b: far left). The data suggest that the *bulleri* albumin and the slower *assimilis* albumin are different in mobility and mutually exclusive.

(3) A divergence in the α globulin triplet is clearly apparent in Fig. 15. Rivanol reveals the triplet to be similar in *bulleri*, *gavia*, *huttoni* and *assimilis*; in *carneipes* and *griseus* it is of faster mobility (Rmu 66-75).

(4) The prealbumins (Rmu 101-109) of *assimilis* are unique to that species. Those of all the other shearwaters were twin-banded at Rmu 101-103.

(5) Minor proteins show similarities and differences. Those at Rmu 49-55 are similar in appearance and mobility in *bulleri*, *carneipes*, *griseus*, and *assimilis*; *gavia* and *huttoni* appear to have the same proteins at a slower loci (Rmu 46-54); the faster band (Rmu 54) was not positively confirmed in *huttoni* and hence is not shown

Some individual variation was found in the esterases of *bulleri*, resulting in faint minor banding both above and below the major and consistent second and third bands shown in Fig. 15. These were not age or sex specific. Fledgling esterase patterns were identical to those of the adults; they tended, however, to be slightly paler in staining intensity.

It is clear from the *Puffinus* data that a sharp distinction can be drawn between this genus and *Procellaria*. They differ greatly in the configuration of the protein complement constituents and in their esterase patterns. Despite recognition of the genus *Procellaria* as a discrete entity in a recent multi-authored taxonomic review (Alexander et al. 1965) some ornithologists still foster the notion that the *Procellaria* petrels are merely large shearwaters. My data oppose this.

The genus *Puffinus* contains a heterogeneous group of aerial and diving shearwaters which clearly are related but which exhibit several peculiar features. Of the diving species, the monomorphic nature of the transferrins is in sharp contrast with the gadfly group *Pterodroma*. The differing albumin, Trt, and α globulin mobilities mark *bulleri* as a distinctive species undoubtedly related but not closely so to the other shearwaters. *Puffinus assimilis* is unusual for its prealbumins, 2 albumin bands, its immunoglobulins, and high density lipoproteins. Because of this, more rigorous analyses will most likely show *assimilis* to be sufficiently distinct from other shearwaters to be accorded full generic status.

Family Pelecanoididae : diving petrels

Genus *Pelecanoides* - 4 species restricted to the Southern Hemisphere.

P. urinatrix Gmelin 1789 - common diving petrel

P. georgicus Murphy & Harper 1916 - South Georgian diving petrel

P. garnoti Lesson 1828 - potoyunco

P. magellani Mathews 1912 - Magellanic diving petrel

Synopsis

Diving petrels form a close-unit group of small, plump-bodied, black and white petrels sufficiently distinct from other procellariids for most taxonomists to accord them full family status. They are considered to be the most primitive of the procellariiformes although their fossils are unknown prior to the Pleistocene (Brodkorb 1963). Murphy (1936) has presented evidence for a South American origin for the Pelecanoididae. He writes that all the species "... are indigenous within a relatively short distance of the southern extremity of South America. It would seem as though the agencies which have been determining the course of evolution within the family have been most active in the region referred to, and that the point of original dispersal may not have been far from Cape Horn. In this locality we find today *Pelecanoides magellani*, the most strongly marked and distinctive of all diving petrels, and in this sense, the most advanced member of the genus." More distinct and remote races of *P. urinatrix* "share with one another strong superficial resemblances of color and pattern, small size, etc., which are probably not due to close relationship, nor to convergence, but rather to the retention among

these peripheral forms of primitive, non-adaptive characters. In any group of organisms, we should look to the point of origin for advanced present-day types rather than for primitive present-day types, and upon this criterion, southern South America fulfils the requirements." (Murphy 1936). This view that derived-specialised forms are more closely tied to their places of origin while primitive-unspecialised forms move freely into new places, is an opinion championed by Darlington (1970), and one not without its adversaries. Hennig (1966) and Brundin (1972) maintain the opposite is true: relatively primitive species are generally less apt to disperse than their relatively advanced relatives. In a succinct comment on the primitive species concept, Horton (1973) emphasises that "The place of origin is the area where the ancestral species occurs, not the area where the derived species occur", and that paleoecological parameters are important: "If environments similar to the ancestral environment still occur in the original centre of dispersal, then primitive species are likely to still occur there. If such environments now occur only in distant peripheral areas then we will find primitive species only in those areas." It is my belief that a South American origin for the Pelecanoididae is distinctly possible, but not necessarily for the reasons suggested by Murphy or Darlington.

Because diving petrels spend more of their life under water than in the air above it, their structural and behavioural modifications favour the denser medium. Their nostrils, divided by a strong septum, point upwards like those of the diving shearwaters, and a "curious lateral flange" appears to protect the nasal orifices from excess water pressure. "In the trunk skeleton, the sternum and rib-basket have become extended further caudad than other petrels of other groups, producing an

excellent structural adaptation for plunging and subsurface progression and resembling to a remarkable degree the condition in the auklets." The resemblance of the Fuegian diving petrel *P. magellani* to the Northern Hemisphere dovekie *Alle alle* is "one of the best examples of convergent evolution known among vertebrates." (Murphy 1936). The wings and tail are short, the former being rather broad but attenuating remarkably when the wing is partially folded. This provides a very efficient rowing organ for a bird underwater. The rapid, direct and whirring flight low above the sea's surface is characteristic of diving petrels. Such flights usually by small groups of feeding birds are generally short-lived, the birds abruptly disappearing beneath the water only to burst from the sea some distance away and continue in their active pursuit of food (Harper and Kinsky 1974). Their comparatively heavy body weight limits buoyancy and enables the birds to dive beneath the surface from a low angle.

The structural adaptations to living underwater are accentuated remarkably when diving petrels moult. Their quills are usually lost completely so that for a time each year the birds are entirely aquatic. "The stomach of the naturally 'crippled' temporarily penguin-like Diving petrels, which have lost all their flight feathers, prove to be as well filled with crustaceans or small fishes as those of their flighted contemporaries. Thus, so far as feeding is concerned, they might just as well be flightless birds. The only indispensable use of the full grown primaries would seem to be to bear the Potoyuncos to and from the nesting burrows on the islands." (Murphy 1936).

Fig. 16. Family Pelecanoididae : Genus *Pelecanoides*

Species	(birds)	n (gels)	(runs)	Source	Date
Pelecanoides					
u. urinatrix	38	23	79	Whangamata Is n = 2	June 75
				Poor Knights Is n = 19	July 75
u. chathamensis				Stewart I. n = 11	Sept, Oct. 76
				Poor Knights Is n = 6	Dec. 76
u. exsul	1	4	4	Auckland Is n = 1	Dec. 76
	39	27	83		

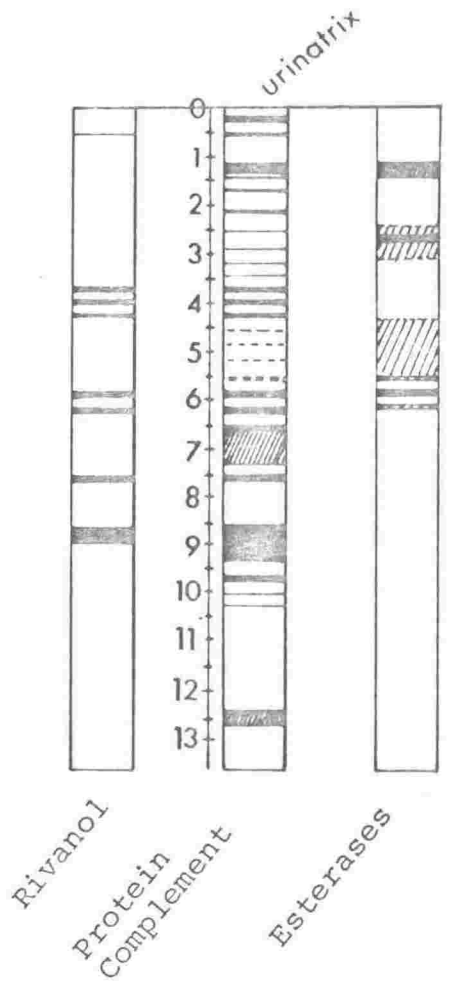


Fig. 16 is a composite drawing (to scale) of the banding patterns of plasma proteins from 39 diving petrels. Five populations of *Pelecanoides urinatrix* were sampled: the Poor Knights and Whangamata (n = 27) populations represent the nominate subspecies *urinatrix*; the population at North Island (NW sector off Stewart Island) appears to be composed of both *urinatrix* and *chathamensis* races such that no clear distinction was possible, and one bird of the race *exsul* was collected from the subantarctic Auckland Islands by Mr J.A. Bartle in December 1976.

Note the following:

(1) The Trt was between Rmu 37-43 in all cases (n = 39). Like the Diomedeidae and other procellariids, the top 2 bands were the densest staining. The high mobility of the Trt is similar to *Procellaria parkinsoni* and *aequinoctialis*; the 2 largest *Pterodroma* petrels, *macroptera* and *lessoni*, and the fulmarine *Macronectes*, *Fulmarus* and *Daption* petrels.

(2) The twin-banded prealbumins at Rmu 100-103 are identical to 5 species of shearwater *Puffinus* sp. Those of *Procellaria* are significantly faster in mobility at 108-110 Rmu.

(3) The 2 conspicuous bands at Rmu 17 and 21 appear similar to those of *Daption* but are of slower mobility; the albatrosses have 2 comparable bands closer together in a similar position; other procellariids are intermediate.

(4) The α globulins of *Pelecanoides* are enigmatic. They are very strong in staining; the 2 at Rmu 55 and 65 vary in density, possibly on a subspecific basis: more data are required.

(5) The esterase patterns of *Pelecanoides* show strong similarities with the Hydrobatidae, particularly *Pelagodroma marina*, the fulmarine petrels, and *Diomedea melanophris*. All these taxa are typically of the Southern Hemisphere.

Pelecanoides urinatrix is clearly a petrel with close affinities with the Procellariidae and Hydrobatidae (see also Fig. 18 for immunoelectrophoretic data). Sibley and Ahlquist's (1972) information from starch-gels clearly show the patterns of *garnoti* and *georgicus* have differing transferrin mobilities, suggesting a situation paralleling *Procellaria*, in which each species has a differing Trt phenotype.

Storm petrels (Family Hydrobatidae)

"Here within Night's dominion in the midst of a no less funereally garbed throng of flitting forms, seeming to speak most earnestly in a subhuman, unknown tongue, which is answered by their encaverned mates in purring tones and pleading wails, the mind may readily picture a most animated gathering of the black elves of old, hurrying to and fro for the accomplishment of some important mission, ere dreaded Day begins ....
But with the coming of the dawn, calm, damp and chill, this strange vision of the night has faded as a dream."

A.H. Norton, 1891.

Family Hydrobatidae : storm petrels

8 genera - at least 15 species; distributed over all oceans.

Synopsis

The Hydrobatidae are the smallest procellariids. A 450 g albatross egg weighs the same as 20 adult storm petrels (data from Crossin 1974 and Westerskov 1974). Storm petrels form two natural groups; those genera with long legs including the southern *Oceanites*, *Garrodia*, *Fregetta* and *Pelagodroma*, and short-legged genera embracing many tropical and Northern Hemisphere birds. The genus *Loomelania* has the "leaping" flight behaviour of the northern group and the long legs of the southern: its intermediate morphology and tropical distributional status is summarised by Murphy (1936). The family trait common to all hydrobatids is a slender black beak with the nostril tubes united into a conspicuous tube facing forwards and upwards over the culminicorn.

Although found in all oceans and zones, the primitive storm petrels are the least known procellariiform group (Crossin 1974). Their erratic flight behaviour, nocturnal nesting habits and nest sitings, make these secretive birds daunting objects for a study. Nests can be concealed in small fissures in large slabs of rock: one can see but seldom touch. Grass or soil tunnels some 15 cm long are used by low latitude breeding birds; the entrance is no bigger than a mouse hole. Storm petrels are flexible in their nest-building proclivities: some are industrious, gathering "good handfuls" of grass, feathers or stony material; others use none whatsoever - a slight depression in the ground suffices.

Moulting penguin feathers are light, heat-retentive building materials (Falla 1937). A propensity for ejecting streams of proventricular oil via their throat at an intruder is a trait shared by the ledge-nesting fulmarine petrels. Some data suggest males incubate more often than their partners which, tend, against the general rule in Procellariiformes, to be larger in body size (Murphy 1936). Young Leach's petrels lie full length in the nest chamber, their bill tips touching the ground and their feet extending forwards beneath the body. Only their rapid breathing signifies life (Norton 1891).

Flight behaviour varies in such a manner that it is a valuable identification aid. *Oceanodroma* storm petrels described as having a curious spring-like flight followed by shearwater-like glides only interupted for frequent settlings in the water during which the wing-tips are held high while the birds forage. The long slow wingbeats are totally unlike the butterfly-like aerobatics of the austral group *Oceanites* (Beck & Brown 1971). The widespread southern *Fregetta* and *Garrodia nereis* have a characteristic side-to-side swinging aerial motion, with the wings held just above the horizontal while the bird proceeds over the sea's surface in a series of skips (Beck & Brown 1971).

Courtship displays are conspicuous among hydrobatids and involve high speed aerial chases accompanied by loud chirping. This pursuit behaviour is also the feature of the gadfly petrels which engage in the behaviour for several hours after darkness has fallen. Both these groups lack the precision and grace of the courtship flights of the sooty albatrosses.

Fig. 17. Family Hydrobatidae : storm petrels

Species	n (birds)	n (gels)	n (runs)	Source	Date
Fregetta tropica	1	6	6	collected at sea	Jan. 77
Garrodia nereis	1	6	6	Auckland Is	Jan. 77
Pelagodroma marina	18	16	31	Poor Knights Is n = 9 North I (Stewart) n = 9	Oct. 75 Oct. 76
	20	28	43		

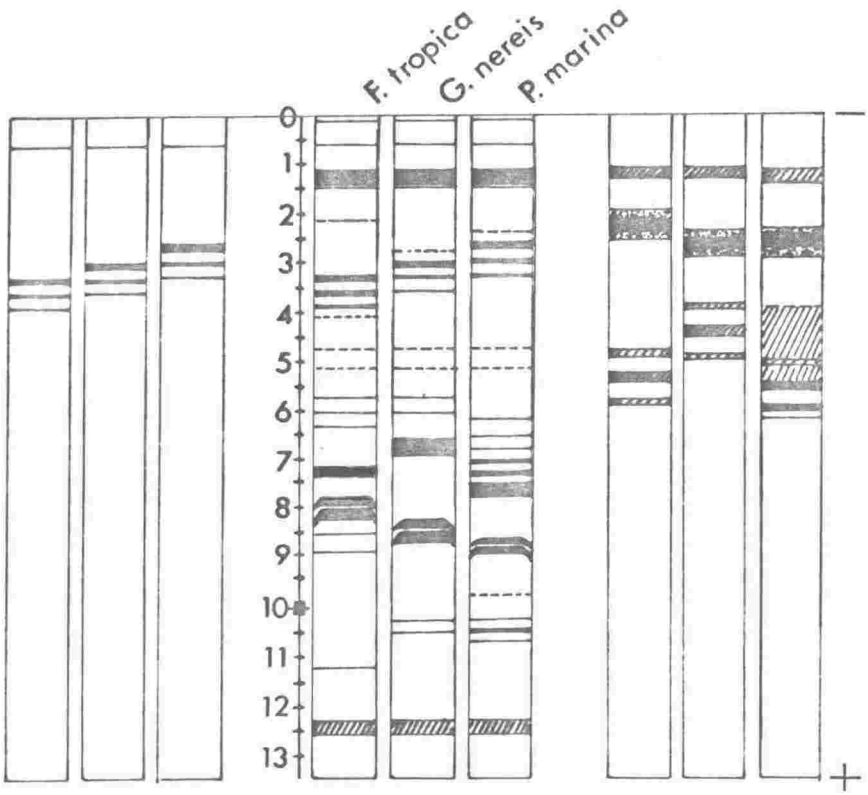


Fig. 17 is a composite drawing (to scale) of the banding patterns of plasma proteins from 20 storm petrels representing three Southern Hemisphere genera.

Note the following:

(1) The hydrobatids are unique in entirely lacking the reference prealbumin protein band found in all other procellariiform families (see preceding data).

(2) The conspicuous double-banded albumin and its differing mobility among the genera. *Puffinus assimilis* (family Procellariidae) is the only other petrel known to me that retains double banded albumin beyond the fledgling stage.

(3) The transferrin triplet varies in mobility and appears to have the top two bands of denser staining than the third; a condition prevalent in other procellariiform taxa.

(4) Many other mobility differences are present in the protein complement - particularly in the prealbumins. The dense-staining macroglobulin Rmu 12-14, the IgG, and the fast prealbumin (Rmu 125) have identical mobilities to the other procellariiform taxa analysed.

(5) Note the similarities and differences of the esterase patterns. The patterns are distinctly reminiscent of the Pelecanoididae and the fulmarine petrels.

The 3 genera of hydrobatids examined are clearly related; the protein data confirm the correct placing of the species within 3 genera as proposed by Alexander et al. (1965). Alliances of these 3 distinct and probably very old genera of hydrobatids with the primitive fulmarine petrels and shearwaters are clearly implied. The double albumin is regarded as a neotenous condition.

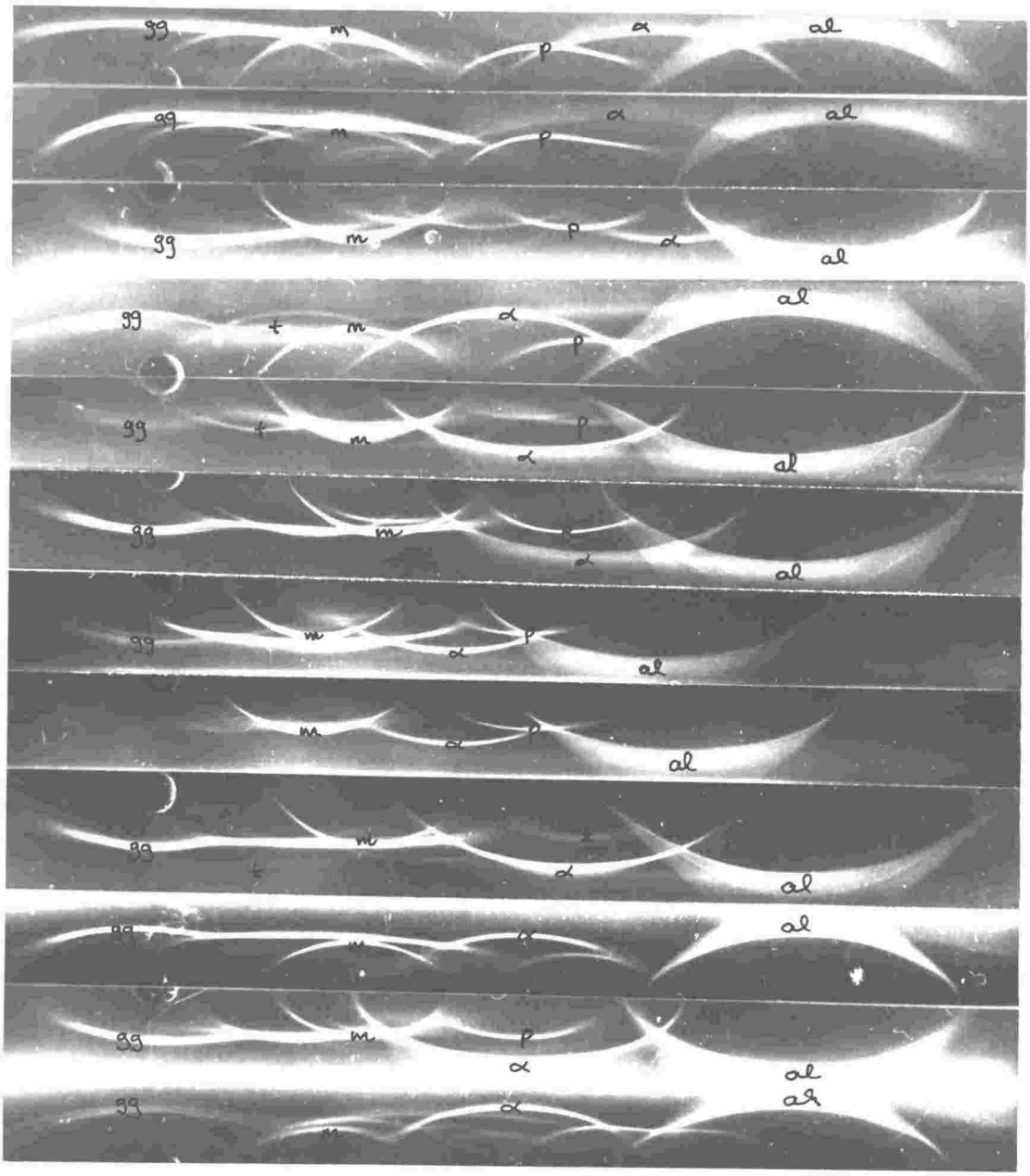

gg
m
p
α
al

Fig. 18.

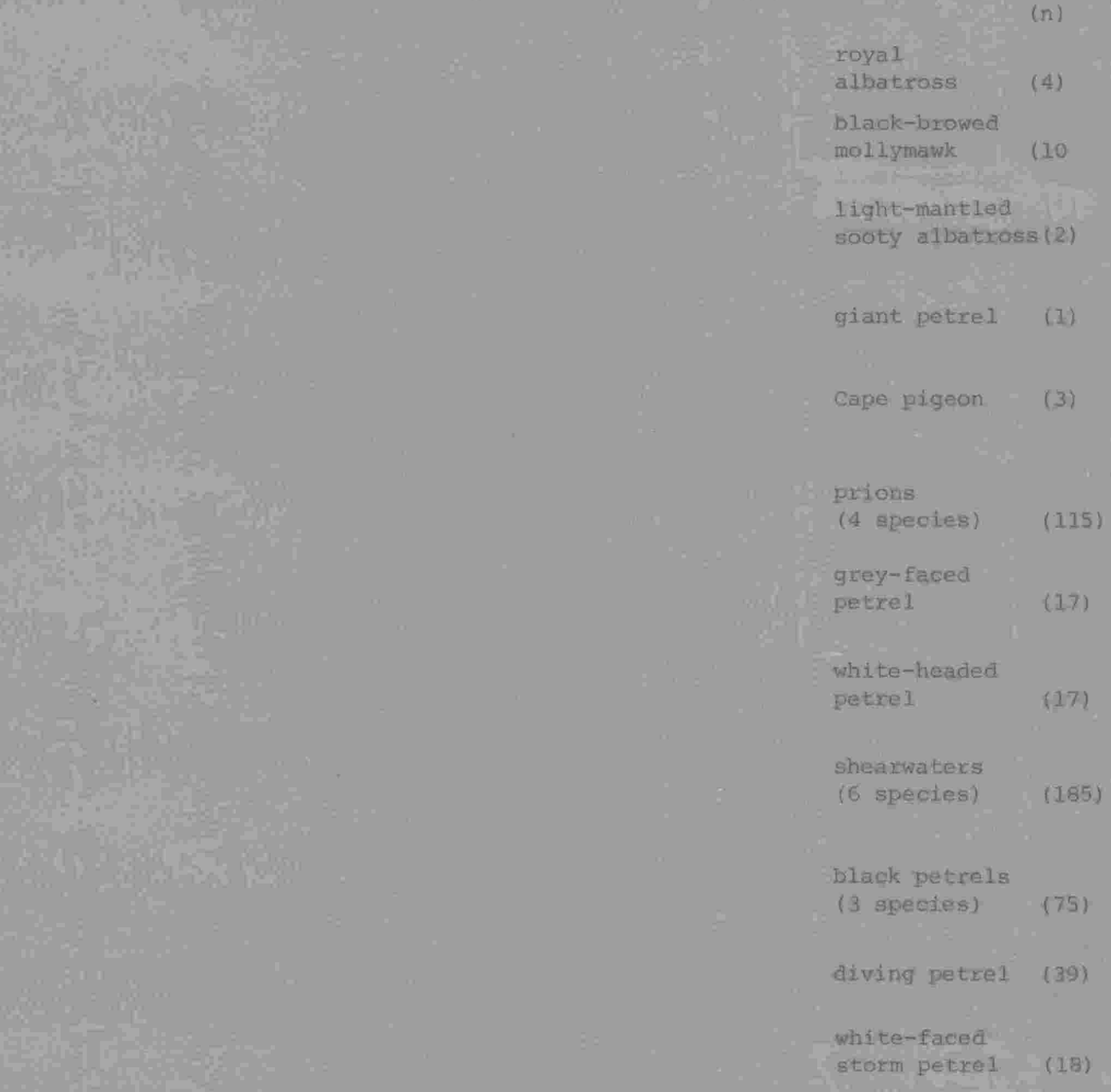

(n)
royal albatross (4)
black-browed mollymawk (10
light-mantled sooty albatross (2)
giant petrel (1)
Cape pigeon (3)
prions (4 species) (115)
grey-faced petrel (17)
white-headed petrel (17)
shearwaters (6 species) (185)
black petrels (3 species) (75)
diving petrel (39)
white-faced storm petrel (18)
KEY
al = albumin
α = α globulin triplet
p = post-albumin
m = macroglobin
t = transferrin triplet (Trt)
gg = immunoglobulins
(n) = number of birds in pooled IEP samples

Fig. 18. A composite photograph summarising the immunoelectrophoresis results.

This figure illustrates the precipitation products resulting from a cross reaction between the antisera developed against 6 species of shearwater (genus *Puffinus*) and antigenic plasma from other procellariiform taxa.

One µl of pooled plasma from the Procellariiformes illustrated was electrophoresed at 100 VDC at 9 mA/agar plate for 35 min; direction of electrophoresis left to right; diffusion at room temperature for 48 h; stain Amido Black. Fig. 18 represents an enlarged composite example from 40 IEP plates. Some arcs are identified, many are not. The macroglobulin arc is a useful internal reference: the *Pterodroma* petrel plasmas have not migrated the full distance; this is an experimental artifact. Note the good cross-reactivity of all taxa to the shearwater antisera. The Hydrobatidae had notably less precipitation products to the antisera than other taxa analysed. The 2 components of the storm petrel albumins were clearly discrete on some plates. The albumin arcs of *Puffinus* and *Pachyptila* are also shown to be heterogenous, a fact confirmed by PAGE analyses. Some transferrin arcs are just visible in the photograph.

These data support the findings of the acrylamide gel electrophoresis, and provide proof that the procellariiformes are indeed monophyletic and closely related, despite remarkable phenetic dissimilarities between the various taxa. Further research employing IEP and microcomplement fixation techniques will undoubtedly be rewarding.

Fig. 19.

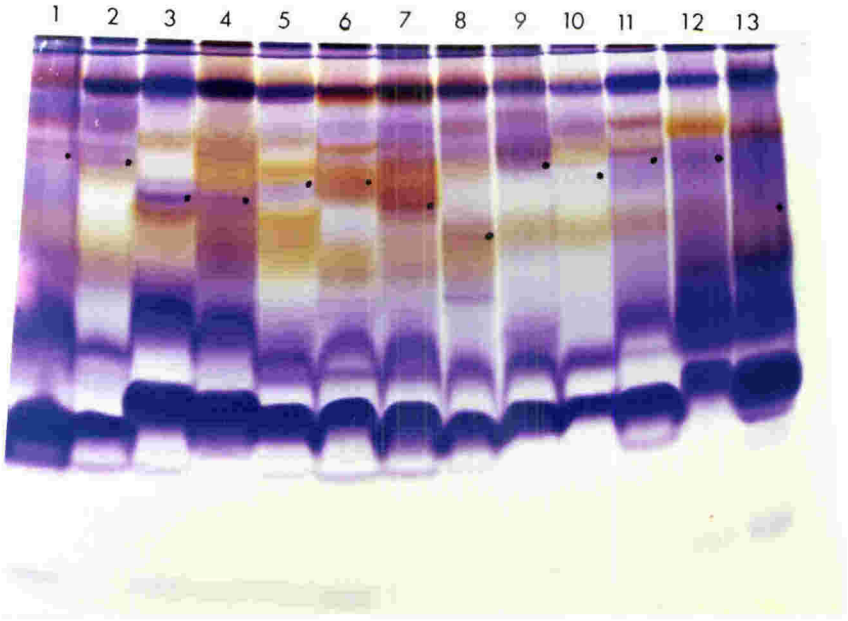


Fig. 19. A colour photograph of a typical gel (No. 146) illustrating the electrophoresed plasma proteins of 12 species of Procellariiformes.

Esterase activity is represented by the orange-coloured bands; the gel has been counterstained in Coomassie blue to visualise and calibrate the other proteins present. Direction of electrophoresis is from top to bottom.

From left to right, the samples are:

1. *Diomedea exulans*
2. *D. epomophora*
3. *Macronectes halli*
4. *Daption capense*
5. *Pachyptila turtur*
6. *Procellaria westlandica*
7. *P. parkinsoni*
8. *Pterodroma macroptera*
9. *Puffinus carneipes*
10. *P. bulleri*
11. *P. assimilis*
12. *Pelagodroma marina*
13. *Pelecanoides urinatrix*

The black dot indicates the central band of the transferrin triplet: note the differing mobilities characteristic of each species. The dense band, three-quarters of the way down the gel is albumin. Macroglobulin (Rmu 12-15) is represented by a heavily stained band near the origin; the band nearest the bottom of the photograph is the prealbumin (Rmu 125-135): both these proteins are monomorphic for all procellariiform taxa (see text). Note the conspicuous α globulin band of *P. macroptera* (8) unique to *macroptera* and *lessoni*; the diffuse, sometimes curved protein lying behind the albumin bands is α lipoprotein: note how it obscures the α globulin triplet in several of the protein complements illustrated; Rivanol removes it (see text). The 3-banded prealbumins, (Rmu 101-109) of especial interest in *P. assimilis* (11), are just visible in the photograph: the hydrobatid *Pelagodroma marina* has an identical set (see text).

(3) Summary of Plasma Protein Data

1. There is little in the information gained from the electrophoresis of plasma proteins from 29 species of albatrosses and petrels that contests the present classification of the order Procellariiformes as proposed by Alexander et al. (1965); indeed, there is much to support, confirm, and clarify such proposals.
2. Plasma will keep for 12 months if sterile procedures are maintained during its collection, and the plasma remains deep frozen at -15°C prior to its use. Samples can be chilled in liquid nitrogen before being returned to a freezer. If facilities are available, long-term storage under liquid nitrogen would be preferable. Denaturation, usually as a consequence of bacterial contamination, follows repeated freezing and thawing of small samples. Macroglobulin, albumin, and transferrin are very resistant to denaturation; α lipoprotein is very susceptible to it.
3. Seasonal variation in procellariiform plasma proteins does not occur (data: n = 315 birds; 3 families, 6 genera, 8 species).
4. No sex differences were detected (data: n = 24 mated pairs; 4 families, 6 species).
5. Age variation in plasma proteins of birds beyond the post-fledgling stage is not evident (data: n = 84 adults, 39 fledglings; 2 families, 8 species).
6. No significant variance in either the number or the mobility of plasma proteins was detected at the population level (data: n = 299 birds; 3 families, 5 genera, 7 species, 19 populations.

7. A conspicuous phylogenetic conservatism among plasma protein bands are monomorphic in both number and relative mobility throughout the Procellariiformes. They are IgG (Rmu 7-10); macroglobulin (Rmu 12-15); and the 'fast' prealbumin at Rmu 125-135. For comparative purposes these are valuable marker proteins.

8. The protein complement contains several protein groups of taxonomic and comparative usefulness. These include the transferrin triplet (Trt); albumins, prealbumins, and the α globulin triplet; and some minor β proteins and γ globulins. Non-specific esterases are a valuable adjunct to the protein complement.

9. Three transferrin bands are represented in all taxa examined. They migrate through PAGE gels as a unit, and appear to be controlled by a single co-dominant allele of an autosomal gene; in IEP they produce a single precipitation arc. Because two bands of slower mobility stain more densely than the third, problems of band identification during comparisons of taxa are avoided. The procellariiform Trt phenotype appears to be an ordinal character, presumably 80-90 million years old (see Prager et al. 1974).

10. The Trt phenotype, although numerically monomorphic, varies in mobility between species, but among 19 populations of 7 species examined no change was found in the species' specific Trt mobility. The transferrin triplet is therefore a valuable aid for distinguishing groups of taxa. Contexts in which it presents useful possibilities for further investigation include:

    (1) for distinguishing the 'mollymawks' of the earlier-recognised genus *Thalassarche* from other albatrosses (*Diomedea* and *Phoebetria*);

(2) for distinguishing aerial shearwaters (e.g. *Puffinus bulleri*) from the more aquatic, diving *Puffinus* species;

(3) for separating the species within genus *Procellaria*;

(4) for following possible evolutionary lines in the heterogeneous genus *Pterodroma* (see also Sibley & Ahlquist 1972: fig. 5);

(5) for distinguishing *desolata* from other species of genus *Pachyptila*;

(6) for separating the diving petrel species (Pelecanoididae) from each other (see Sibley & Ahlquist 1972: fig. 5 & 6);

(7) for distinguishing the highly differentiated Southern Hemisphere storm petrel genera *Fregetta*, *Garrodia*, and *Pelagodroma* from each other, and possibly from Northern Hemisphere taxa.

11. Genera which are closely related and which have identical Trt mobilities are the fulmarines *Macronectes*, *Fulmarus*, and *Daption*, and the albatrosses *Phoebetria* and *Diomedea*. Closely related species with identical Trt mobilities include the five diving shearwaters of the genus *Puffinus* and the subtropical species of *Pachyptila*. These facts would appear to rule out genetic drift as a plausible explanation for the present mobility differences of the Trt at various taxonomic levels throughout the Procellariiformes. Indeed, selection pressures appear to have favoured the retention of the Trt as a discrete unit for a very long time; mobility differences are held to be highly significant, because they may vary between species but not between populations of a species. (This, of course, does not necessarily imply that selection is operating specifically on the transferrin locus, but rather

on the organism as a whole, which must adapt to meet a new challenge or fail to perpetuate itself.

12. A single or double albumin phenotype is present in the Procellariiformes. Those with a double albumin phenotype include three genera of hydrobatids, all species of *Pachyptila*, and *Puffinus assimilis*. All remaining taxa studied have a single albumin. (Data: n = 470 birds; 4 families, 11 genera, 29 species).

13. Significant correlations of prealbumin band mobility were found in several taxa. For example, the reference pre-albumin marker protein (Rmu 100 *Procellaria westlandica* ≡ bovine albumin fraction V) is present in all procellariiform families except the Hydrobatidae. Within the Procellariidae (genus *Puffinus*) and Pelecanoididae (*Pelecanoides urinatrix*) it migrates at the slightly slower rate of Rmu 96. Other prealbumins are monomorphic at the generic level (e.g. the *Procellaria* petrels twin phenotypes at Rmu 108 and 110), and hence are good generic characters; a homologous prealbumin occurs at Rmu 101 and 103 in five species of *Puffinus* shearwaters and *Pelecanoides urinatrix*. The conservative nature of these prealbumin phenotypes suggests a common ancestry for these petrels. The prealbumins of the smaller *Pterodroma* species, *cooki* and *pycrofti*, also provide support for a reinterpretation of subgenus *Cookilaria* (Fleming 1941); those of the prions (*Pachyptila*) and the fulmarine petrels show a common ancestry, in agreement with the view that these taxa are closely related.

The prealbumin triple-band phenotype of *Puffinus assimilis* is totally unlike that of other shearwaters. It is, instead identical to that of the storm petrel *Pelagodroma marina*, and

this is intriguing. Still more provocative is the twin-banded phenotype of another hydrobatid genus, *Garrodia*; it is identical to that of five *Puffinus* shearwaters, one of them an aerial species and the others highly adapted divers. A late Cretaceous - early Tertiary ancestor common to three procellariiform families - represented by the present-day genera *Procellaria* and *Puffinus* (Procellariidae), *Pelecanoides* (Pelecanoididae), and *Pelagodroma* and *Garrodia* (Hydrobatidae) - would be distinctly fascinating. My data clearly support such an alliance.

DISCUSSION

I fully agree with Throckmorton (1968) and Sibley (1970) that no single line of inquiry into phylogenetic taxonomy can produce absolute answers; it would be pretentious and short-sighted to think that it could. Electrophoretic data, like all previous data, must be viewed in this light.

Accordingly I have analysed and summarised my plasma protein data with due regard for previously proposed alliances and for a spectrum of other interdisciplinary information known to me. This amalgamation of data has been a pleasant exercise because the information fits together very well - as, indeed, it should. Nonetheless, in order to separate fact and analytical inductive reasoning from intuitive speculation, I have recognised Sibley's (1970) categories of opinion in formulating statements whose conclusions are HIGHLY PROBABLE, PROBABLE, or POSSIBLE. These are defined below.

HIGHLY PROBABLE

"Our understanding of a few problems is now advanced so that we can consider them solved" (Sibley 1970).

1. The plasma protein data fully support the theory that the albatrosses and petrels (order Procellariiformes) are monophyletic in origin. Heavy intraordinal competition and predation pressures have been largely responsible for determining the evolutionary strategies of the Procellariiformes.
2. The four families currently recognised (Diomedeidae, Procellariidae, Pelecanoididae, Hydrobatidae) are all phylogenetically old, and are now clearly differentiated from each other.

3. The Hydrobatidae diverged from other procellariiform stock very early (pre-Miocene: Brodkorb 1971), and are now only distantly related to existing taxa.
4. The Pelecanoididae are more closely related to the Procellariidae than to any other existing taxon.
5. The genus *Procellaria* is allied to the albatross family Diomedeidae.
6. The genus *Procellaria* comprises a small group of southern petrels genetically distinct from the shearwaters of the genus *Puffinus*.
7. The genus *Procellaria* and the Mediterranean shearwater genus *Calonectris* are related (see below).
8. *Puffinus assimilis* does not belong in the genus *Puffinus*.
9. The storm petrels *Fregetta tropica*, *Garrodia nereis*, and *Pelagodroma marina* are correctly place in separate genera.

Most of the above are apparent from the previously summarised data; No. 5 merits further consideration.

Several lines of information indicate an early Tertiary alliance between the black *Procellaria* petrels (Procellariidae) and the albatrosses (Diomedeidae). This kinship may either have been linked by a pre-Miocene group of large, dark-plumaged, surface-nesting petrels (see below or be a direct line of descent from the *Procellaria* ancestor(s). (The modern-day albatross genus *Diomedea* was fully differentiated by the Upper Miocene: Wilkinson 1969). My reasons for formulating this hypothesis are as follows:

(a) the plasma protein data are remarkably similar in several respects, and fully consistent with the hypothesis;
(b) osteological data compiled by Kuroda (1954) support the close alliance;

(c) albatrosses considered to be representative of the most primitive stock are all dark-plumaged (both species of *Phoebetria* and *Diomedea nigripes*; and the immatures of both *D. exulans* and *D. albatrus* (a retained neotenous character?): Murphy 1936 , Kuroda 1954, Fisher 1972).

(d) both taxa appear to have evolved in the Southern Hemisphere where they are presently abundant, and fully differentiated into geographical isolates of the high-latitude subtropical and subantarctic life zones;

(e) the white face mask, which is retained as a neotenous character in chicks and fledglings of the Diomedeidae, remains in *Procellaria aequinoctialis* as a variable (sometimes absent) white chin patch, or as an outcropping of white feathering above and behind the eyes in *conspiculata* - the so-called spectacled Atlantic race of *aequinoctialis* (the conspicuous semicircle of white feathering immediately behind the eyes in adult *Phoebetria* may well be a functional adaptation of the same theme);

(f) *Procellaria* petrels are unique among burrowing procellariids for their bill-clapping calls and sky-pointing behaviour, which is both characteristic of and highly developed in the Diomedeidae;

(g) the unusual hunched attitude and waddling gait of some albatrosses (e.g., *D. nigripes*: see Fisher 1972, fig. 3) is remarkably similar to the posture adopted by *Procellaria* petrels;

(h) the extraordinary diligence with which *Procellaria* petrels build their substantial underground nests is peculiar to them among the Procellariidae, and matched only in the nest-building behaviour and abilities of the southern Diomedeidae.

PROBABLE

Reasonable certainty exists for the relationships and comments given below; conclusive evidence remains to be presented, and in many instances additional details remain to be worked out.

1. The genus *Procellaria* represents one of the most primitive petrel groups in existence (*P. arvenensis* from the European Oligocene is believed by Kuroda (1954) to have affinities with *P. cinerea*, being "probably all-dark with some aquatic adaptation"). The plasma protein data show that the three species examined are closely related, despite their being isolated from each other by morphological, behavioural, geographical, and seasonal mechanisms to an extent unparalleled among species of other petrel genera. This complete separation probably evolved from a near absence or paucity of food, and/or competition, or other strong selective pressures, including prolonged isolation (P.C. Harper, in prep.).

   The peculiar transequatorial migration of *P. parkinsoni* to the west coast of middle America probably pre-dates the closure of the Panama Portal some 5-7 million years ago (see Cracraft 1973), before which the petrel flew into the Atlantic. The close alliance of *Procellaria* with the primitive Mediterranean shearwater genus *Calonectris* is intriguing for both evolutionary and zoogeographical reasons, and presumably precedes the evolution of the highly adapted aquatic shearwaters (see below; also Kuroda 1954).

2. The southern fulmarine petrels are highly differentiated early procellarian stock (Alexander et al. 1965). They initially escaped competition from more advanced petrels by remaining in high latitudes and shifting into the polar

niche about the periphery of Antarctica. The fulmars remarkable adaptation to their frigid environment is illustrated by their strikingly different plumage and morphological characters, together with the constancy of their plasma protein mobilities. The phenotypic variations may be due to competitive and predation strategies; the contrasting biochemical uniformity is perhaps due to convergent evolution in the face of a very hostile polar environment.

3. The gadfly petrel group (*Bulweria, Pterodroma,* and *Halobaena*) is at least as old phylogenetically as the fulmars and the shearwaters.
4. The genera *Pachyptila* and *Halobaena* are only very distantly related; the prions evolved from a small fulmarine ancestor, whereas the *Pterodroma* group gave rise to the blue petrel. Convergence between the two taxa was the result of predation pressures, as exemplified by the activities of the present-day skuas (Stercorariidae).
5. The allied shearwater (*Puffinus assimilis*) is a modern-day representative of a primitive shearwater stock conceivably pre-dating its nominal congeners.
6. An alliance exists between the allied shearwater, the southern storm petrels (Hydrobatidae), and the diving petrels (Pelecanoididae). This statement was derived from plasma protein data; Kuroda's osteological analyses led him to remark "The Diving Petrels share some skeletal and anatomical characters with Hydrobatidae and Procellariidae".

POSSIBLE

The following statements are designed to stimulate discussion, controversy, and further work.

1. The North Pacific albatrosses are more closely related to the southern great albatrosses (*Diomedea exulans* and *D. epomophora*) than to the smaller sooty albatrosses and mollymawks. Fossils show that albatrosses were in the North Pacific in the Miocene (Wilkinson 1969), and these were probably derived from the greater albatrosses, possibly like those of *Manu antiquus*, an Oligocene albatross equal in size to the present-day *Diomedea exulans*. In bill shape the North Pacific albatrosses show striking resemblance not the smaller, southern mollymawks but to *D. exulans*, particularly in the very broad base to the culminicorn (see Fisher 1971: fig. 29). The dark brow and eye patch, the curiously patterned underwing (Fisher 1972: fig. 1), and overall size suggest convergent evolution of *D. immutabilis* with the southern hemisphere *D. melanophris*. Andersen (1895, cit. Murphy 1936) suggested that one of these two species has been derived from the other. In view of the differing bill adaptations, this direct relationship is, in my opinion, highly unlikely. The southern mollymawks are little changed from their Miocene ancestors (e.g., *Diomedea thyridata*: Wilkinson 1969). They have remained within their ancestral southern homeland since the mid Tertiary, and are sufficiently distinct from other *Diomedea* and the genus *Phoebetria* to warrant their return to the genus *Thalassarche*.

2. An extinct group of large petrels within the size parameters of the *Procellaria* petrels and the smaller species of the Diomedeidae existed in the Southern Hemisphere during the early Tertiary. They were surface-nesters, and had dark plumage and a white face similar to that of a fledgling *D. exulans*; their bills were black or pale, similar to

that of *P. aequinoctialis*. Their ancestors included a phenotype akin to the race *conspicillata* of *P. aequinoctialis*; among their descendants is the dark-plumaged, black-footed albatross *D. nigripes* (see Fisher 1972). The genus *Phoebetria*, a primitive offshoot of these hypothetical birds, has retained several of their morphological and behavioural characteristics. The genera *Macronectes* and *Procellaria* have evolved towards filling their ecological niche.

3. The phylogenetic history of the shearwaters is little known, complex, and intriguing. My data and speculations on their evolution were derived independently of Kuroda's (1954) masterful treatise, in which he based his findings on the shearwaters' osteology and life habits. Having read his publication, I find very little on which we disagree.

   1. The genera *Procellaria* and *Calonectris* were split from their common ancestor before the Oligocene. This ancestor lived in the Atlantic close to the origin of the Procellariiformes. *P. arvenensis* from the European Oligocene, which has affinities with *P. cinerea* (Kuroda 1954), provides fossil evidence for this view. The migration of *Puffinus borealis* from the Mediterranean down the west coast of Africa, and the flight of *Procellaria parkinsoni* to central America, are related phenomena consistent with this view.
   2. *Puffinus carneipes* and its close ally *P. creatopus* were derived from *Calonectris* (they each have a number of adaptive characteristics in common; their bill morphology and colour are strikingly similar). *P. creatopus* gave rise to *P. carneipes* (?); their ancestor passed westward through the Panama Portal from the Atlantic.

4. *Puffinus gravis* is an old species. (A Miocene shearwater, *P. conradi*, apparently lived until the Pleistocene and is believed by Kuroda (1954) to be the ancestor of *P. gravis*. Its humerus was flattened, indicating an aquatic adaptation.). The Pacific counterpart of *P. gravis* is *P. bulleri*, an endemic subtropical New Zealand species with aerial adaptations; both species have conspicuous upperparts (the opposite of the subtropical *Pterodroma*, which has conspicuous underwings; see below).

5. The highly adapted aquatic species *P. carneipes*, *P. griseus*, *P. gavia*, and *P. huttoni* are closely related, and are correctly placed within the single genus *Puffinus*.

6. The *Pterodroma* group of gadfly petrels are southern in origin, having been derived from *Procellaria* - fulmarine stock through an ancestral line of larger dimensions but structurally akin to the present-day *Bulweria* (the free lachrymal and perforated antorbital wall is still retained as a neotenous condition in young and fledgling *Pterodroma*: P.C. Harper, unpubl. data).

The subtropical *Pterodroma* petrels offer a striking example of evolutionary release in the absence of serious predation pressures. Whereas the high-latitude procellariids (e.g., *Pterodroma macroptera* and *Halobaena caerulea*) have morphological and behavioural adaptations to render them as inconspicuous as possible, subtropical gadfly petrels show the opposite trend, with their striking underwing patterns, aerial courtship displays, and proclivity towards nesting in the open. The progressive evolution from an all-dark underwing (a subantarctic phenotype where high predation pressures exist) to a conspicuously barred, almost white one is illustrated in Fig. 20. The plasma

Fig. 20.

Fig. 20. The underwing patterns of *Pterodroma* petrels. All dark underwings a feature of species nesting in cooler zones of high predation pressures; strikingly patterned wings are typical of subtropical species breeding in areas of low predation (see text).

proteins from two large-sized subantarctic, one intermediate, and two small-sized *Pterodroma* petrels corroborate the apparent shift in phenotypic emphasis (see page 74). Selection pressures for the underwing pattern appear to be high, in that very little individual variation occurs (P.C. Harper, unpubl. data); this is in marked contrast to the highly variable body pigmentation exemplified in the dichromatic subtropical *Pterodroma neglecta* (Murphy & Pennoyer 1952). The 'white window' wing signal is clearly a successful recipe for visual contact - it has evolved apparently independently in three different taxa: the southern fulmarine petrels (*Daption, Fulmarus, Thalassoica*); the subtropical *Pterodroma* petrels (*P. solandri, P. neglecta, P. arminjoniana*); and the skuas (Stercorariidae). The consequences of a conspicuous petrel attempting to spread southward into zones of high predation pressure can readily be seen in the southern islets of New Zealand, where *Pterodroma inexpectata* is slaughtered in enormous numbers on its breeding grounds by the southern skua, *Catharacta lonnbergi* (e.g., Stead 1932).

7. Inasmuch as Cenozoic plate tectonics have greatly altered the southern ocean environment, I give below some speculative thoughts on how these geological processes may have affected the phylogenetic evolution of the southern albatrosses and petrels. Four fundamental parameters are:
   1. the effect of Antarctica's ice on the southern oceans;
   2. the evolution of the circumpolar water current and the west-wind drift;
   3. the evolution of the Antarctic Convergence; and
   4. the distribution of islands and land masses.

I propose that the environmental trigger which initiated the mid-Tertiary radiation of the penguin and petrel faunas (see Murphy 1936) was the major global cooling (see below) which began at the Eocene Oligocene boundary (38 million years B.P.) together with the development of the circumpolar Antarctic current during the late Oligocene (30-25 m.y. B.P.).

Before the major global cooling which began about 38 million years ago, southern ocean Paleocene and Eocene surface temperatures were considerably higher than they have been at any time since (Devereux 1967). South of New Zealand and about the South Tasman Rise the sea temperatures were a warm 18-20^{o}C (Shackleton & Kennett 1975). Primary productivity was probably low and ocean currents sluggish (Andrews et al. 1975). The Antarctic Convergence was non-existent, since Antarctic ice was restricted to higher altitudes on the continent; in the Ross Sea area palynological data show evidence of a shrub or tree cover of low diversity "with *Nothofagus* and Podocarpaceae dominant, and Proteaceae and Myrtaceae among the minor elements" (Kemp 1975). Fossil data show that Eocene penguins already had characters basic to the Sphenisciformes, and were divisible into several genera. One of these, *Palaeeudyptes*, was distributed from Australia (Christie's Beach, near Adelaide, South Australia) to New Zealand (Burnside, near Dunedin; at least two species) and Seymour Island (off the northeastern tip of the Antarctic Peninsula). Simpson (1975) does not regard the late Eocene penguins as being primitive in a structural sense, and believes they had been "evolving for many tens of millions of years" prior to this time.

He is further convinced that the Sphenisciformes "were almost certainly derived from aerial flying birds, and their ancestors probably resembled and possibly belonged to the order Procellariiformes". I have no data to refute this view.

"At or near the beginning of the Oligocene, a dramatic decrease in temperature apparently reduced the surface temperature at the Antarctic coast close to freezing. This would have produced glaciers at sea level if precipitation was adequate and would have led to extensive sea-ice production and hence represents a critical stage in the development in Antarctic glacial history" (Kennett et al. 1975). As cold water moved northward from the continent (see Kennett et al., fig. 9) deep water circulation began, causing deep sea-floor erosion and marked changes in marine faunas. Procellariiform birds were probably adapting to the cooling climate, diversifying into newly created ecological niches, and some were evolving migration routes north through the South Atlantic to spend the austral winter in the warm 'Middle Seas' which at various times during the Tertiary separated the Americas and the African continent from Europe.

The circum-Antarctic current - which today transports more than 200 x 10^6 m^3/s of water, and which is the only current to mix the waters of all oceans (Gordon 1967, 1973) - originated some time after the rifting between the Australian continent and East Antarctica was well under way. According to Kennett et al. (1975), this was because the continental structure of the South Tasman Rise was sufficient constriction to prevent an active, deep-water

current circulation from moving between Australia and Antarctica until the late Oligocene, by which time the Drake Passage had been open for some time (Sproll & Dietz 1969). Fluctuations in sedimentation rates associated with the increased biogenic productivity of the Antarctic Convergence zone suggest that this Antarctic/subantarctic interface did not become a prominent boundary until the late Miocene, by which time the circumpolar Antarctic Current and (presumably) the west-wind drift were well established.

No-one who has studied southern oceanic birds can have failed to recognise the profound effect that the circumpolar west-wind drift has on their marine environment. For a graphic overview of the dynamic power of this climatic system one has only to peruse the mosaic satellite photographs of the Southern Hemisphere (Satellite Data Services Branch, National Climatic Center, Washington, D.C.) and count the cyclonic disturbances circling Antarctica at any given time of the year. Alternatively one can observe at first hand the sudden influx of petrels about a ship in the southern oceans as the wind rises and the barometer tumbles. As the storm sweeps on, frequently bearing Procellariiformes of several species before it, the wind oscillates then drops, and the wind-ploughed sea moderates. The flush of birds vanishes with the wind, leaving the usual scavengers hunting behind the ship's stern for galley scraps.

Just as dramatic in its effects is one of the most important boundary zones in the world's oceans, the Antarctic Convergence. Its position is often marked by steep temperature and salinity gradients (Mackintosh 1946) and

marked changes in productivity values and the standing crop of plankton (El-Sayed 1970). A ship passing through the convergence is frequently beset by a pervading sea-mist which finally unveils to reveal to the ornithologist a remarkable change in procellariiform bird species, as subantarctic forms are replaced by less varied but more abundant Antarctic ones (pers. obs. in 13 crossings of the Antarctic Convergence, 1965-67). Although there are local environmental factors which can modify a convergence zone, such as to render it unstable or obscure, the compelling effect of the Antarctic Convergence (and to a less extent the Subtropical Convergence) is already expressed in the speciation of procellariiform genera, different species occupying the distinctive life zones to the north and south of it.

A more southern barrier to petrels is the ice front, in that it prevents the birds from feeding and obtaining shelter during a gale. Icebergs, however, in addition to providing a convenient roosting place (I have counted 2105 Antarctic petrels (*Thalassoica antarctica*) sitting on a single berg some 700 m in length), provide marine birds with a highly effective shelter, and enable them to continue feeding in their lee while a gale rages to windward. The importance of these refuges, particularly for subantarctic petrels which might otherwise be stressed by wind-chill in a summer gale in Antarctic waters, should not be overlooked.

The attraction of the ice front to Procellariiformes during the austral summer is probably the vast swarms of krill revealed at the front as it melts southward. Krill is found hundreds of kilometres south of the spring

position of the ice edge, and "cannot have travelled there with the open water, for there is no general southward drift of the surface layer, and the krill's power of locomotion would scarcely allow for one mile a day even if it moved continuously in one direction. It must live under the ice, waiting, as it were, to be uncovered" (Mackintosh 1970). If this intriguing hypothesis is correct, it may assist in explaining why many subantarctic Procellariiformes cross the Subantarctic Convergence during the summer to feed (Harper 1973). Moreover, with a retreat of the ice beginning in early October, Antarctic-breeding petrels and penguins would have the selective advantage of nesting close to their food supplies; hence their distinctive phenetic and physiological adaptations to breeding and living in a polar environment. That they have achieved these adaptations, probably over several million years, is reflected in the recognition of several distinctive fulmarine petrel genera each of which contains a single species unique to Antarctica and its high-latitude island outliers.

I doubt that we shall ever fully appreciate the profound effects that Cenozoic plate tectonics and the associated environmental changes have had on the marine birds of the southern oceans. Fossil data from southern Australia, New Zealand, Patagonia, California, and Europe clearly show that Procellariiformes were widespread and probably at their peak during the Miocene (Brodkorb 1960, 1963, 1971, Murphy 1936, Wilkinson 1969). It is highly probable that all living procellariiform families were extant at that time, and contained at least as many genera as they do at present - perhaps more, since conspicuous gaps in size

(such as between the Diomedeidae and Procellariidae) create disruptions in an otherwise remarkable continuum unmatched in other avian taxa. (Such disruptions may, however, be the result of some taxa evolving faster than others.) Simpson (1952) and Rensch (1960) present data suggesting the average duration of an animal species's survival to be from 500 000 to 5 million years, a hypothesis supported by recent molecular studies (Nei 1975). I am convinced that many of the surviving petrel species, having long since adjusted to a stable marine environment, and by virtue of their migration patterns together with their other behaviour and ecology, are among the most ancient of bird species existing today.

8. Geneticists would probably regard the plasma protein data from the albatrosses and petrels as rather dull. The proteins differ in mobility but rarely in number, and gene frequencies of 1.0 are not very exciting. For the taxonomist, however, this state of affairs is almost heavenly, for he has at his disposal an entire order of birds uncluttered by transient variation or a large number of polymorphic loci. So conspicuous is the allelic constancy that an explanation for it must be sought. The phylogenetic history of the Procellariiformes probably began 80-90 million years ago, when the petrels were evolving in the Southern Hemisphere along the shores of a fragmenting Gondwanaland (Simpson 1975, Brodkorb 1971, Sibley & Ahlquist 1972, Fisher 1967, Prager et al. 1972, Harper 1975). Murphy's (1936) intuitive hypothesis that the primitive diving petrels (Pelecanoididae) originated near Cape Horn (see page 87) may well provide a plausible centre of origin for the petrel-penguin ancestral stock

also. I can think of no better place or candidate for the morphological and ecological adaptations required than those demonstrated by the Pelecanoididae. (Falla (1974) is of similar opinion).

Thus, beginning probably as small to medium-sized birds exploiting inshore habitats similar to those today occupied by diving petrels and cormorants, the petrel group was perhaps one of the first and certainly one of the most successful replacements for the nekton-feeding marine reptiles which died out during the late Cretaceous, and which left a large number of ecological niches unoccupied behind them. A lack of terrestrial predation, perhaps a dearth of competition, and the wider niche breadth of these early days must have eased the difficulty of selective pressures for the petrel ancestors. Selective tolerance of bold new mutations such as adaptive novelties of a relatively imprecise and/or imperfect function (Frazzetta 1975) could have become established. Assuming *Gigantornis eaglesomei* to be in fact a petrel, the enormous size of this mid-Eocene albatross may well have been one of these evolutionary novelties.

As the petrels differentiated and spread across the southern oceans and northward through the Atlantic into the Tertiary 'Middle Seas', they occupied a continuum of marine niches. During this expansion stage of procellariiform evolution, their genetic variability was probably extensive, necessary, and highly adaptive. Indeed, their polymorphic nuances at this time probably equalled those of some terrestrial avian taxa existing today (Sibley et al. 1974). If the ends are any indication of the means, the present-day phenetic diversity of the Procellariiformes is a clear reflection of

this ancestral heyday of genetic variability and successful adaptation.

Following the mid-Tertiary radiation of the Procellariiformes came a gradual loss of evolutionary novelty leading into a phase of specialisation - a pattern described for numerous other organisms (Simpson 1953, Rensch 1960, Frazzetta 1975, Mayr 1970). Competition and other selective agencies led to the extinction of many (?) petrels unable to tune their genome precisely to the marine environment. In terms of proteins, the hitherto high degree of genetic variation was lost, and many once polymorphic alleles became fixed. Sarich (1977); see also Wilson et al. 1977) discusses the concept of proteins evolving at grossly dissimilar rates, such that: "Early in the process of genetic differentiation between two diverging lineages, the bulk of any measured (genetic) distance will be contributed by the rapidly evolving loci (albumin, transferrin, haemoglobin, and one or more esterases). This contribution will be essentially complete by 5-6 M yr of separation (that is, all the rapidly evolving proteins will have different electrophoretic mobilities by that time), and any further accumulation of genetic distance will be seen only in the slowly evolving loci." In other words, a well known zoological trend on the phyletic macroscale (see Rensch 1960, Mayr 1970) appears to have its analogue at the molecular level. My plasma protein data strongly support such a view, for there are several major groups of proteins which are apparently monomorphic throughout the Procellariiformes. Sibley et al. (1974) comment that 60% of birds for which adequate data exist (mostly terrestrial taxa) are polymorphic at their transferrin locus; apart from mobility changes, the

petrels are monomorphic without exception. Such data support what is intuitively obvious to those familiar with the petrels; namely, that they are an ancient and very primitive group. They are highly adapted to their marine environment, to the severe pressures of competition from their own kin, and to heavy selection from aerial predators. They give the impression of having been so adapted for millions of years.

ACKNOWLEDGMENTS

It is a pleasure to record here my sincere appreciation of all the willing and generous people who assisted me during the preparation of this thesis. Special thanks must go to my enthusiastic group of friends, several of whom accompanied me up and down New Zealand in pursuit of petrels over the past 3 years: Simon Towle, Geoffrey Arnold, Sandy Bartle, Ian Granville, Kay Fountain, Paul Kennedy, Mary Livingston, John Warham, Ricardo Palma, Ross McQueen, Harry Patterson, and the team of Titirangi Bay. Their comradeship, sometimes in times of danger and difficulty, is something I shall cherish always.

Other helpers kindly took the time and effort to find and/or bring to my attention live petrels cast ashore after storms: Shane Cotter and his family, of Petone, Doug Sinclair of Waikanae; Ken Miers of Paekakariki; F.C. Kinsky and Sandy Bartle, both from Wellington, and Max Falconer of Lower Hutt.

The following skippers provided our parties with launch transport and services sometimes far beyond the call of duty: my special thanks to Fred Cotterill (Matira) of Tutukaka, Ces Harrison (Anne Maree) of Whitianga, and Peter Tait of Halfmoon Bay, Stewart Island.

I thank the following government personnel for their co-operation and help: Dr G.R. Williams and Brian D. Bell (Wildlife Service, Department of Internal Affairs, Wellington) for permission to take the blood samples used in this study; Dick Veitch (Wildlife Service, Auckland) for his generous help in providing transport to the Whangamata Islands, June 1975; Alan Wright (Wildlife Officer, Dunedin) for his help during my visit to the Taiaroa Head albatross colony, January 1977.

The Lands and Survey Department were extremely kind and helpful to this programme. My association with the Hauraki Maritime Park Board through its Chairman (J.D. O'Brien and George MacMillan) and their staff has been a long and friendly one; my thanks for their permission to visit several islands under their jurisdicton, and for their unstinting support. Mr Kerry Johnson (Ranger, Marlborough Sounds Maritime Park) very kindly provided me with transport to Titi, Chetwode, and Trio Islands. Mr Harry Patterson of Titirangi Bay made me welcome and raised a local band of helpers to catch shearwaters on Titi Island; the success of the trip was in large part due to their efforts. Mr Bob McMinn and Bert and Ngaire Youngman (Stewart Island Maritime Park) provided our parties with outstanding support; on behalf of all the party members, I thank them for their comradeship and efforts to ensure the success of our field trips to the islets off Stewart Island.

Wulf and Beryl Young (Forest Service, Great Barrier Island) went to great pains to make us welcome at the Barrier in every way possible; two nicer people one could not meet.

Financial assistance for this research was met jointly by the Internal Research Grants Committee (travel), the Zoology Department (Professor J.A.F. Garrick), and the Botany Department (Professor J.K. Heyes) of Victoria University of Wellington. Throughout the 3-year period of this project I received financial support as a New Zealand post-graduate scholar from the University Grants Committee. To all these generous avenues of funds I owe a special debt of gratitude.

Although this thesis was originated and followed through by me alone, I have bounced numerous ideas about with several colleagues and friends, on whom I could always rely for constructive critisism and encouragement. Sir Robert Falla was

a continual source of scholarly advice and cerebral discussion, and the bearer of numerous relevant papers. Sandy Bartle and Fred Kinsky argued for hours with me on matters great and small, as we have been doing for years; long may we argue still. To these three dear friends I offer my grateful appreciation and thanks.

To my supervisor, Professor Ken Heyes of the Botany Department, VUW, I offer my sincere gratitude for everything he has done for me over the past 3 years. I think he still definitely prefers tomato plants and flowing sap to the strange seafowl which often appeared in his laboratory and office unannounced. Through his guidance and unflagging support, this thesis grew in concept and flowered in reality; I can never hope to repay my debt to him.

For valuable theoretical advice and practical problem solving during the duration of this thesis, I thank Messrs Mike Arapoff, Les Thorne, the technicians of the Electronics Workshop (Physics Department) and Professor J.F. Duncan (Chemistry Department).

The manuscript of this thesis was read in its entirety by Professors Heyes and Garrick and Sir Robert Falla. I thank them for their expert criticisms and reasoned advice.

Mrs Olga Vincent and Mrs Kathryn Hastings provided me with friendly, patient, and quick typing services: my sincere thanks to them. The final draft was typed by Mrs Olga Vincent.

In closing, I must thank all the Botany and Zoology staff and students who dropped in to look, query and discuss the strange happenings in Professor Heyes' laboratory while I held territory there. It has been a delight meeting them.

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