Genetic variation of four populations of the Little Blue Penguin, *Eudyptula minor*

Meredith A. M. Meredith and
Frank Y. T. Sin*

Department of Zoology, University of Canterbury,
Christchurch 1, New Zealand.

The biochemical differentiation of four geographic populations of the Little Blue Penguin, *Eudyptula minor* from Onawe Peninsula, Motunau Island, Maud Island (South Island of New Zealand) and Poor Knights Island (North Island) was determined. Ten isozymes, with a total of 24 loci were analysed. Seven of these loci were polymorphic. A south-north cline was detected in the allelic frequencies of all the polymorphic loci. Population specific loci were detected. The Yellow-eyed Penguin, *Megadyptes antipodes* offered a comparison between genera. In *Megadyptes* only two loci were polymorphic, and it had a lower mean heterozygosity than *E. minor*. Loci specific for this genus were detected. The two genera shared the same alleles at seventeen of twenty loci.

INTRODUCTION

There are a number of characteristics which distinguish the Little Blue Penguin, *Eudyptula minor*, from the other penguins. They are small, blue dorsally, and white ventrally, without any tufts or flashes. They are found only in Australasia. In New Zealand, *E. minor* is widely distributed along the coast and around the offshore islands. The coastal populations are presently subdivided into subspecies on the basis of external morphological characteristics proposed by Kinsky and Falla (1976). They give to White-flipped Penguin of the Canterbury coast the name *E.m. albosignata*; the North Island population *E.m. iredalei*; and the Cook Strait birds *E.m. variabilis*.

The present study attempts to determine the amount of genetic variation in these geographical populations of *E. minor* which are morphologically distinct according to (Kinsky and Falla, 1976).

MATERIALS AND METHODS

Study areas

The four *E. minor* study areas were (1) Onawe Peninsula (43°46'S, 172°55'E), situated approximately 30 km south-east of Christchurch in the

Akaroa Harbour; (2) Motunau Island (43°08'S, 173°10'E), about 1 km offshore and 64 km north of Christchurch; (3) Maud Island (41°01'S, 173°52'E) in the Marlborough Sounds; and (4) Poor Knights Islands (35°30'S, 174°45'E), approximately 24 km off the east coast of Northland (North Island). One population of Yellow-eyed Penguins (*Megadyptes antipodes*) was sampled from Otago Peninsula (45°54'S, 170°37'E).

The population, sample sizes and the time sampled are given in table 1.

Penguin handling and sexing

The penguins were caught at night on their breeding grounds and placed in a bag with a hole, which allowed a leg to protrude while blood was extracted. Only adult penguins were sampled.

The method described by O'Brien (1940) to distinguish the sexes by bill characteristics was used in this study. Difficulty in differentiating between juvenile males and adult females was sometimes experienced, in which case penguins were classified as juveniles. Those penguins found cohabiting were assumed to be adult male/female pairs, since two birds of the same sex seldom occupy the same burrow except in the moulting season (C. Challies, personal communication). Fledglings were easily identified by their smaller size and paler plumage.

* Author to whom correspondence should be addressed.

Table 1 Sampling data

Scientific name	Vernacular name	Sample size	Location	Month/Year
<i>Eudyptula minor albosignata</i>	White-flippered penguin	15	Onawe Peninsula	Dec 1981
		73	Onawe Peninsula	Feb 1982
		10	Onawe Peninsula	Apr 1982
		3	Onawe Peninsula	Jun 1982
		8	Onawe Peninsula	Aug 1982
		7	Onawe Peninsula	Oct 1982
		45	Motunau Island	Dec/Jan 1981/82
<i>E. minor iredalei</i>	Northern blue penguin	43	Poor Knights Is.	Nov 1981
<i>E. minor variabilis</i>	Cook Strait blue penguin	41	Maud Island	May 1982
<i>Megadyptes antipodes</i>	Yellow-eyed penguin	27	Otago Peninsula	Feb 1983

Blood sampling and preparation

Two 1 ml samples were drawn from either the anterior tibial (leg) or anterior digital (foot) vein of most individuals through 21 gauge hypodermic needles into sterile disposable syringes (Monoject, USA). The first samples were drawn into syringes containing 0.05 ml of 0.2 M disodium ethylene diamine tetra-acetate (EDTA); each 50 ml of EDTA stock containing 10 mg Thiomersal—a bacterial inhibitor. The other samples were drawn into syringes containing 0.05 ml of 0.2 M Heparin (Lithium salt). Once drawn, samples were carefully mixed with the anticoagulant and withdrawn into sterile 1.5 ml Eppendorf centrifuge tubes and stored on ice until centrifuged. As soon as possible, whole blood samples were centrifuged at 1500 rpm for 10 min. The plasma fraction was removed and frozen in Eppendorf tubes in liquid nitrogen held in a 20 l container with a 20 day static holding time. Red cells were washed three times in physiological saline (0.9 per cent NaCl) before freezing. The penguins from Onawe Peninsula were banded to allow blood to be resampled at differing times during the year. This was undertaken to test for seasonal variations in the proteins and enzymes.

Preparation of samples for electrophoresis

All samples were thawed slowly at 4°C. After use, they were immediately refrozen in liquid nitrogen. Enzymes were extracted from three blood fractions: plasma, erythrocytes and leukocytes. Every sample was mixed with a small quantity of Bromophenol Blue in buffer *H* (see table 2) before electrophoresis. The following processes were used to extract the proteins and enzymes from each blood fraction.

- (a) *Plasma fraction* The untreated plasma samples were used for electrophoretic analysis.
- (b) *Leukocyte fraction* Leukocyte samples were suspended in distilled water and leukocyte lysates were obtained by repeated freezing and thawing.
- (c) *Erythrocyte fractions* Erythrocyte samples were haemolysed using one of three methods, depending on the enzyme to be extracted.
 - (i) Packed red cells were suspended in an equal volume of distilled water, then frozen and thawed once.
 - (ii) As above, with 1 per cent (v/v) Triton X-100 added to the distilled water.
 - (iii) As in (i), with 1 per cent (v/v) Toluene added to the distilled water.

Erythrocyte lysates were centrifuged in an Eppendorf SP 1500 for 10 minutes immediately after lysis.

For the analysis of glucose-6-phosphate dehydrogenase (*G6PDH*), it was necessary to isolate this enzyme from the red blood cells which were lysed using one volume of red cells to 20 volumes of distilled water. The lysate was centrifuged at $18,400 \times g$ and the resulting supernatant discarded. The sediment was mixed with an equal volume of 0.05 M phosphate buffer (pH 6.5) containing 2 per cent (v/v) Triton X-100 and 2 per cent (v/v) 2-mercaptoethanol. This solution was left to stand for 20 minutes at room temperature, with occasional shaking. After 20 minutes, the solution was centrifuged at $18,400 \times g$ and the supernatant brought up to 60 per cent of saturation with ammonium sulphate (3.61 g in 10 ml). The resultant solution was centrifuged for 15 min and the insoluble component that formed on the surface of the solution removed. All three fractions (*i.e.*,

sediment, supernatant and the surface component) were tested for *G6PDH* activity. The region of highest activity was the insoluble component that formed on the surface of the supernatant. This insoluble component was redissolved in an equal volume of 0.05 M phosphate buffer containing 2 per cent (v/v) 2-mercaptoethanol and stored frozen until used.

A considerable amount of enzyme degradation was encountered. Red cell lysates that were released using Triton X-100 were used only once; toluene treated samples could be used twice and all other samples used four times before breakdown of enzymes was obvious.

The buffer systems used for electrophoresis and for the staining of the soluble proteins and enzymes are given in tables 2 and 3.

Ten isoenzymes were studied, 5 from group 1 (specific enzymes), and 5 from group 2 (non-specific enzymes) (Gillespie and Kojima, 1969) (table 4).

Blood collected in vessels containing EDTA as the anticoagulant was used for all enzymes except acid and alkaline phosphatases, which were analysed using blood extracted with Heparin.

Stain recipes

Detection of isozymes was essentially as described previously (Brewer, 1970; Harris and Hopkinson, 1976; Shaw and Prasad, 1970).

Table 2 Buffers used for electrophoresis and stains

A	0.1 M Tris/0.1 M Maleic acid/0.1 M Magnesium chloride—adjusted to pH 9.0 with 10 N NaOH
B	0.1 M Tris/0.1 M Glycine—pH 8.2
C	0.25 M Tris/0.1 M Citric acid—pH 8.6
D	0.9 M Tris/0.5 Boric Acid/0.02 M EDTA—pH 7.5
E	0.11 M Trisodium citrate/0.24 M NaH ₂ PO ₄ —pH 6.2
F	0.07 M Boric Acid adjusted to pH 9.3 with 10 N NaOH
G	0.1 M NaH ₂ PO ₄ adjusted to pH 7.0 with 1 M Na ₂ HPO ₄
H	0.25 M Tris adjusted to pH 7.0 with 1 N HCl.

Genetic interpretation

Enzyme followed by a number, e.g., *EST-1*, *EST-2*, indicating the bands anodal migration, where *EST-2* is the more anodal band. Allelic variants were designated by a superscript after the locus number, e.g., *EST-1^s* and *EST-1^f* where *s* indicates slow moving and *f*, fast moving bands.

The phenotypic frequencies of the populations were then tested against the Hardy-Weinberg equilibrium using the Chi-squared (χ^2) test of goodness of fit, with $1/2(n^2 - n)$ degrees of freedom, where *n* = number of alleles (Ferguson, 1980). The effective number of alleles per locus; the mean heterozygosity per locus (*H*); and the proportion of polymorphic loci were determined.

Table 3 Summary of methods for electrophoresis of 10 enzymes

Name	Gel concn. %; v/v	Electrode buffer	Gel buffer	pH	Comments
Plasma Cholinesterase	7.5	1:2 B	B	8.2	—
Red Cell Esterase	7.5	1:2 B	B	8.2	—
Carbonic Anhydrase	7.0	D	D	7.5	—
Acid Phosphatase	6.5	E	1:5 E	6.2	20 mg MgCl ₂ added to cathode buffer
Alkaline Phosphatase	5.0	F	1:5 F	9.3	20 mg MgCl ₂ added to cathode buffer
Glucose-6-phosphate Dehydrogenase	5.0	C	1:5 C + 2% 2 mercaptoethanol	8.6	5 mg MADP ⁺ added to gel mixture and 10 mg to cathode buffer
Malate Dehydrogenase	7.5	D	1:10 D	7.5	—
Glutamate Oxaloacetate Transaminase	5.0	A	1:8 A	9.0	—
Lactate Dehydrogenase	6.5	1:2 B	B	8.0	—
Glucose Phosphate Isomerase	6.5	D	D: 10 + 2% 2 mercaptoethanol	7.0	5 mg NADP ⁺ added to gel mixture and 10 mg to cathode buffer

Table 4 Enzymes and blood fractions used for this study

Enzyme E.C. no.	Enzyme or protein	Blood fraction
3.1.1.8	Cholinesterase (<i>EST</i>)	Plasma
3.1.1.1	Red cell esterase (<i>EST</i>)	Red blood cells
3.1.3.1	Acid Phosphatase (<i>AcP</i>)	Red blood cells
3.1.3.1	Alkaline Phosphatase (<i>ALP</i>)	Plasma
4.2.1.1	Carbonic Anhydrase (<i>CA</i>)	Red blood cells
1.1.1.27	Lactate Dehydrogenase (<i>LDH</i>)	Red blood cells
1.1.1.49	Glucose-6-phosphate Dehydrogenase (<i>G6PDH</i>)	Red blood cells
2.6.1.1	Glutamate oxaloacetate Transaminase (mitoch & soluble) <i>GOT_m</i> + <i>GOT_s</i>)	White blood cells
5.3.1.9	Glucose Phosphate Isomerase (<i>GPI</i>)	Red blood cells
1.1.1.37	Malate Dehydrogenase (<i>MDH</i>)	Red blood cells
	Malic Enzyme (<i>ME</i>)	Red blood cells

The normalised identify of genes (I) between populations was calculated using Nei's index and the mean genetic distance (D) between two populations was calculated (Nei, 1972).

RESULTS

Ten enzymes, with a total of 24 loci, were analysed. The allelic frequencies for all the loci are given in table 5.

Plasma *EST*

Three zones of plasma *EST* activity were detected in penguin plasma. All three zones had banding patterns characteristic of monomeric enzymes with two co-dominant alleles. Thus, they were assumed to be coded for by three distinct loci, designated *EST*-1, *EST*-2 and *EST*-3, respectively. The allelic variants were designated *EST*-1^s, *EST*-1^f, *EST*-2^s, *EST*-2^f, *EST*-3^s, *EST*-3^f. Over all *EST* loci, the slow allelic variants showed the highest frequencies. The *EST*-1 and *EST*-3 loci showed South to

Table 5 Allelic frequencies for the five penguin populations

Enzyme	Loci	Populations									
		s	f	s	f	s	f	s	f	s	f
<i>EST</i> (plasma)	1	0.965	0.035	0.967	0.033	0.915	0.085	0.884	0.116	1	0
	2	0.890	0.110	0.844	0.156	—	—	—	—	—	—
	3	0.888	0.112	0.801	0.199	0.744	0.256	0.701	0.299	0.963	0.037
<i>GOT</i>	1	1	0	1	0	1	0	1	0	1	0
<i>ALP</i>	1	1	0	1	0	1	0	1	0	1	0
	2	1	0	1	0	1	0	1	0	0	0
<i>MDH</i>	1	1	0	1	0	1	0	1	0	1	0
<i>CA</i>	1	0.027	0.973	0.044	0.956	0.085	0.915	0.140	0.860	0	0
	2	0	0	0	0	0	0	0	0	1	0
<i>LDH</i>	1	1	0	1	0	1	0	1	0	1	0
	2	1	0	1	0	1	0	0	0	0	0
	3	0	0	0	0	0	0	0	1	0	0
	4	0	0	0	0	0	0	0	0	1	0
<i>G6PDH</i>	1	0.658	0.342	0.622	0.378	0.488	0.512	0.418	0.582	0.722	0.278
<i>AcP</i>	1	0.014	0.986	0.033	0.967	0.049	0.951	0.058	0.942	1	0
	2	1	0	1	0	1	0	1	0	1	0
	3	1	0	1	0	1	0	1	0	1	0
<i>GPI</i>	1	0.893	0.107	0.884	0.116	0.914	0.086	0.953	0.047	1	0
<i>EST</i> (red cell)	1	1	0	1	0	1	0	1	0	1	0
	2	1	0	1	0	1	0	1	0	1	0
	3	1	0	1	0	1	0	1	0	1	0
	4	1	0	1	0	1	0	1	0	1	0
	5	1	0	1	0	1	0	1	0	1	0
	6	1	0	1	0	1	0	1	0	0	0

North variation in the allelic frequencies over the four *E. minor* populations. The frequency for the slow (*s*) alleles decreased as the populations moved from the southernmost Onawe Peninsula population to the northernmost Poor Knights Islands population.

EST-2 was undetected in the Poor Knights Island and Maud Island populations of Little Blue Penguins and in the Yellow-eyed Penguin samples. Other inconsistently resolved bands were not scored.

GOT

The form found in penguin red blood cells was probably the soluble (*GOTs*) isozyme as only the reticulocytes in the blood exhibit the mitochondrial (*GOTm*) isozymes. One protein band with common mobility and *GOT* activity was detected in the blood of all five penguin populations.

ALP

Two protein bands with *ALP* activity were detected in zymograms of the plasma of all populations of *E. minor*. These were considered to be the product of two distinct loci, designated *ALP-1* and *ALP-2*. *ALP-1*, but not *ALP-2*, was detected in the plasma of Yellow-eyed Penguins.

MDH

Red cells of penguins showed one band with MDH activity. This locus was monomorphic and the same allele was shared by all five penguin populations. Other inconsistently resolved bands were not scored.

CA

One zone of *CA* was detected in the red blood cells of all populations of *E. minor*. This was designated *CA-1* and had a banding pattern characteristic of a monomeric enzyme with two co-dominant alleles, designated *CA-1^s* and *CA-1^f*. The *CA-1^f* alleles were present in all *E. minor* populations in the highest frequencies. This locus, also, showed a south/north cline in the allelic frequencies with increasing frequency for the fast (*f*) allele from the southern to the northern populations.

One *CA* band was found in the blood of Yellow-eyed Penguin, with an electrophoretic mobility differing from either allelic form of all the *E. minor* populations.

LDH

Zymograms of plasma *LDH* showed a 5-banded pattern characteristic of a tetrameric enzyme with two co-dominant alleles, presumably the products of two distinct loci (*LDH-1* and *LDH-2*).

Variants of the *LDHh* loci were found in the Poor Knights (*LDH-3*) and the Yellow-eyed Penguin (*LDH-4*) populations.

G6PDH

One zone of enzymatic activity was seen in zymograms of penguin blood stained for *G6PDH*. This locus was polymorphic, showing a banding pattern characteristic of a dimeric enzyme with two co-dominant alleles, designated *G6PDH-1^s* and *G6PDH-1^f*.

The *G6PDH-1^s* allele was present in the highest frequencies in the Onawe Peninsula and Motunau Island populations whereas the *G6PDH-1^f* allele was present in greater frequencies in the Maud and Poor Knights Islands populations.

AcP

Three regions of *AcP* activity were observed in zymograms of all the penguin populations. These were designated *AcP-1*, *AcP-2* and *AcP-3*. *AcP-1* was a polymorphic locus, with two alleles. The allele *AcP-1^f* was present in the greatest numbers in all penguin populations. The *s* allele increased in frequency from the southernmost to the northernmost populations of *E. minor*. *AcP-2* and *AcP-3* were monomorphic.

GPI

One zone of *GPI* activity was seen in zymograms of all populations of the penguins. This polymorphic locus showed a banding pattern characteristic of a dimeric enzyme with two co-dominant alleles, designated *GPI-1^s* and *GPI-1^f* respectively, with *GPI-1^s* showing the highest allelic frequencies. The frequency of this slow allele increased from the southern to the northern populations.

EST

In the red cell fractions of the penguin blood, at least eight to nine protein bands showed *EST* activity but only six of these were consistently detected throughout the year. These were designated *EST-1*, *EST-2*, *EST-3*, *EST-4*, *EST-5*, and *EST-6*. *EST-6* was not detected in the blood from the Yellow-eyed Penguin populations.

All loci, except *EST-2* of the Onawe Peninsula population ($P < 0.001$), were in Hardy-Weinberg equilibrium (table 6). The *EST-2* locus had an excess of heterozygotes.

Table 7 summarises the genetic variation for the five penguin populations. Within the *E. minor* populations, the mean heterozygosity (H) value was highest in the Onawe Peninsula population (0.47), the Poor Knights Islands population had the lowest value (0.34). The mean heterozygosity of the Yellow-eyed Penguin population was 0.16.

The effective number of alleles for each polymorphic locus (table 8) also showed a north/south trend, with *EST-1*, *EST-3*, *CA-1*, *G6PDH-1* and *AcP-1* values all increasing and the *GPI-1* value decreasing from the Onawe Peninsula through the Motunau Island and the Maud Island populations to the Poor Knights Islands population of *E. minor*.

Nei's genetic identity and distance values, calculated from table 5, are given in table 9. The Onawe Peninsula and Motunau Island populations are the most similar, with an I value of 0.999. The pairwise comparisons between the Yellow-eyed Penguin population and each of the other four populations showed the least similarities, with a mean I value of 0.818. Maud Island compared to both Onawe Peninsula and Motunau Island populations were the second most similar, $I =$

0.977. The Poor Knights Islands population compared to the Maud Island population showed a genetic identity of 0.938 and compared to the Onawe Peninsula and Motunau Island populations showed genetic identities of 0.916 and 0.919 respectively.

DISCUSSION

An analysis of the allelic frequencies for the four geographic populations of *E. minor* revealed clinal variations in all the seven polymorphic loci. One of the most extreme cases is seen in the plasma *EST-3* locus. The Onawe Peninsula population shows an allelic frequency for the slow allele to be 0.888. The Poor Knights Island population, on the other hand, show the frequency of 0.701. The Motunau Island population has a value of 0.801, intermediate between the Onawe Peninsula and the Poor Knights Island values. In addition, the Maud Island frequency for the s allele (0.744) is intermediate between the Motunau Island frequency and the Poor Knights Island values.

The allele frequencies for the polymorphic loci of the Yellow-eyed Penguin are consistently closer to unity than those of *E. minor*. The mean number of alleles per locus for the four *E. minor* popula-

Table 6 Chi square values (χ^2) with levels of significance (in brackets) for the Hardy-Weinberg equilibrium test of genetic equilibrium at each locus

Loci	Populations				
	Onawe	Motunau	Maud	Poor Knights Is.	Yellowed-eyed P.
<i>EST 1</i>	0.36 (NS)	0.25 (NS)	0.48 (NS)	0.58 (NS)	0.55 (NS)
<i>EST 2</i>	6.07 ($p < 0.001$)***	1.04 (NS)	N/A	N/A	1.70 (NS)
<i>EST 3</i>	2.24 (NS)	0.45 (NS)	1.88 (NS)	0.26 (NS)	0.39 (NS)
<i>CA</i>	2.30 (NS)	1.81 (NS)	1.14 (NS)	0.04 (NS)	0.34 (NS)
<i>G6PDH</i>	1.49 (NS)	2.86 (NS)	0.58 (NS)	1.58 (NS)	3.00 (NS)
<i>AcP</i>	1.89 (NS)	1.11 (NS)	0.34 (NS)	0.34 (NS)	N/A
<i>GPI</i>	2.93 (NS)	0.01 (NS)	0.65 (NS)	0.95 (NS)	3.08 (NS)

NS = not significant—locus in Hardy-Weinberg equilibrium. $p < 0.001$ *** = significant—probability of 99.9% that locus is not in Hardy-Weinberg equilibrium.

Table 7 Summary of genetic variation

Population	No. of loci	Proportion of polymorphic loci	Mean no. of alleles/locus	Mean heterozygosity	
				Obs.	Exp.
Onawe Peninsula	21	0.33	1.08	0.47	0.51
Motunau Island	21	0.33	1.11	0.45	0.43
Maud Island	20	0.30	1.11	0.38	0.39
Poor Knights Island	20	0.30	1.12	0.34	0.37
YEP* Otago	18	0.11	1.04	0.16	0.17

* YEP = Yellow-eyed Penguin.

Table 8 Effective number of alleles per polymorphic locus

Enzyme	Loci	Onawe	Motunau	Maud	Poor Knights	Otago (YEP*)
<i>EST</i>	1	1.07	1.07	1.18	1.26	1
	2	1.24	1.36	—	—	—
	3	1.25	1.47	1.62	1.72	1.08
<i>CA</i>	1	1.07	1.09	1.18	1.32	1
<i>G6PDH</i>	1	1.82	1.89	2.00	1.95	1.67
<i>AcP</i>	1	1.03	1.07	1.10	1.12	1
<i>GPI</i>	1	1.24	1.26	1.19	1.10	1

* YEP = Yellow-eyed Penguin.

Table 9 Nei's Genetic identity and distance values for the five penguin populations

	Onawe	Motunau	Maud	Poor Knights Is.	Otago Yellow-eyed
Onawe		0.999	0.977	0.916	0.803
Motunau	0.001		0.978	0.919	0.813
Maud	0.022	0.023		0.938	0.828
Poor Knights Is.	0.087	0.084	0.063		0.828
Otago Yellow-eyed	0.213	0.206	0.189	0.188	

Genetic identity values in the upper triangle and genetic distance values in the lower triangle.

tions are very similar, but these values are all slightly higher than that of the Yellow-eyed Penguin.

The mean heterozygosity values of 0.041 for the four *E. minor* populations is similar to that calculated for other vertebrates. The mean heterozygosity from studies of birds has been calculated as 0.037, and mammals 0.039 (Avice and Aquadro, 1982). When the heterozygosity of the four populations are examined, it is clear that the southern populations (Onawe Peninsula and Motunau Island) have a much higher heterozygosity value (0.047 and 0.045) than the northern population (Poor Knights Islands), 0.034. Thus the southern populations are more polymorphic. This is consistent with the high intra-population variations detected in the southern populations by both the classical and numerical phenetic approaches (Meredith, 1984).

The mean heterozygosity (0.016) of the Yellow-eyed Penguin is lower than values reported for all other bird species and the *E. minor* populations. This may be due to the absence of panmixia in the taxon, or the presence of null alleles. It is probably not due to selection against heterozygotes because all the loci studied in this genus appear to be in genetic equilibrium.

How closely related are these four *E. minor* populations? From an analysis of Nei's genetic

identity values, it appears that the two southern populations from Motunau Island and Onawe Peninsula are the most similar. The population from Poor Knights Island is the most distant from these two southern populations and that from Maud Island is the less distant. This is consistent with the observation that population specific loci are found amongst the four penguin populations. Plasma *EST*-2 is found only in the Onawe and Motunau populations; and *LDH*-3 is only detected in the Poor Knights Island population. Thus, the genetic identity values also suggests a clinal pattern of divergence with the Onawe Peninsula and Motunau Island populations at one end of the cline and the Poor Knights population at the other. A comparison of Yellow-eyed Penguin with *E. minor* reveals a high degree of differentiation between the two genera. The Yellow-eyed Penguin has unique *CA* and *LDH* loci, and it lacks the red cell *EST*-6, *ALP*-2 and *LDH*-3 loci which are in all the *Eudyptula minor* populations.

Acknowledgements We thank Dr P. Harper for critical reading of the manuscript and Dr C. Challies for providing much help and advice on the penguins, the Wildlife Service for permits to handle the penguins and to visit Maud Island, and the Department of Lands and Survey for allowing our visit to Motunau Island. We are also grateful for help in the field provided by A. Meredith, M. Barth and P. Claman.

REFERENCES

- AVISE, J. C. AND AQUADRO, C. F. 1982. A summary of genetic distances in the vertebrates. In Hecht, M. K., Wallace, B. and Parnace, G. T. (eds.) *Evolutionary Biology*, 15, 151-180.
- BREWER, G. J. 1970. *An Introduction to Isozyme Techniques*. Academic Press, London.
- FERGUSON, A. 1980. *Biochemical Systematics and Evolution*. Blackie, Glasgow.
- GILLESPIE, J. H. AND KOJIMA, K. 1968. The degree of polymorphism in enzymes involved in energy production compared to that in nonspecific enzymes in two *Drosophila ananassae* populations. *Genetics*, 61, 582-585.
- HARRIS, H. AND HOPKINSON, D. A. 1976. *Handbook of Enzymes Electrophoresis in Human Genetics*. North-Holland, Amsterdam.
- KINKSY, F. C. AND FALLA, R. A. 1976. A subspecific revision of the Australasian Blue Penguin (*Eudyptula minor*) in the New Zealand area. *Rec. Nat. Mus. N.Z.*, 1(7), 105-126.
- MEREDITH, M. A. M. 1984. M.Sc. Thesis, University of Canterbury.
- NEI, M. 1972. Genetic distance between populations. *Amer. Nat.*, 106, 283-292.
- O'BRIEN, P. J. 1940. Some observations on the breeding habits and general characteristics of the White Flipped Penguin (*Eudyptula albosignata*). *Rec. Cant. Mus.*, 4, 311-324.
- REILLY, P. N. AND BALMFORD, P. 1975. A breeding study of little penguins (*Eudyptula minor*) in Australia. In Stonehouse, B. (ed.) *The Biology of Penguins*. MacMillan, London.
- SHAW, C. R. AND PRASAD, R. 1970. Starch gel electrophoresis of enzymes—a compilation of recipes. *Biochem. Genet.*, 4, 297-320.