CONSERVATION
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CONSERVATION ADVISORY SCIENCE NOTES

No. 41

GENETIC VARIATION AND SYSTEMATICS OF THE NEW ZEALAND DOTTEREL

(Short Answers in Conservation Science)

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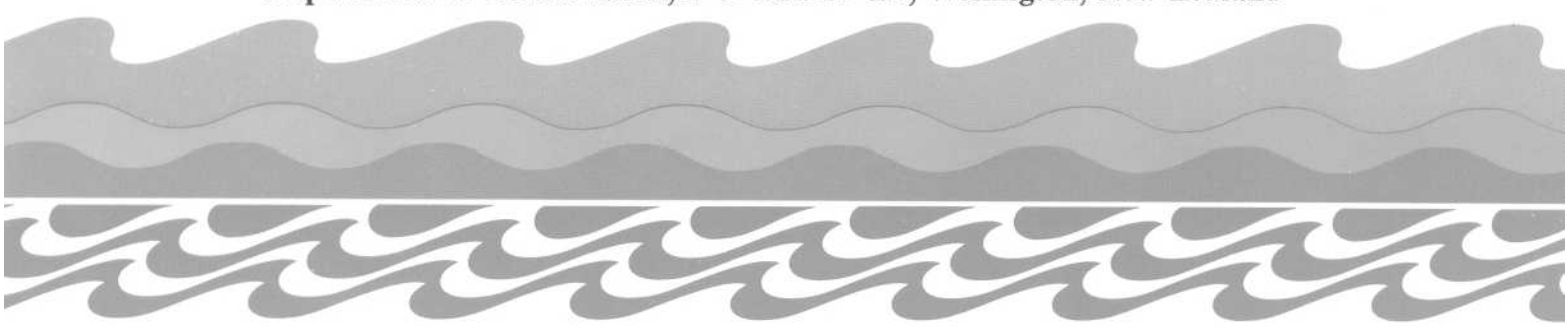

ISSN 1171-9834

Reference to material in this report should be cited thus:

Herbert, J.M., Dowding, J.E. and Daugherty C.H., 1993.
Genetic variation and systematics of the New Zealand dotterel.
Conservation Advisory Science Notes No. 41, Department of Conservation,
Wellington. 14p.

Commissioned by: Threatened Species Unit
Location: NZMS

Genetic Variation and

Systematics of the New Zealand

Dotterel

TO: NEW ZEALAND DEPARTMENT OF CONSERVATION
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DATE: 16 June 1993

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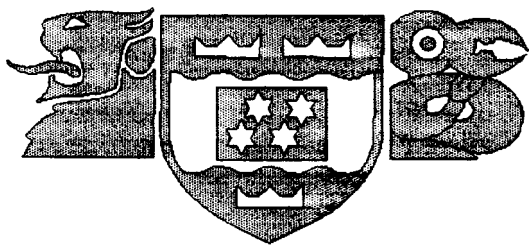

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SUMMARY

The New Zealand Dotterel (*Charadrius obscurus* Gmelin) is composed of two populations: a small southern population that breeds at Stewart Island and a large northern population that breeds in the North Island. The Stewart Island population shows some behavioural and morphological differences compared to the North Island birds. This study was conducted to examine the level of genetic differentiation that occurs between these two populations.

All populations examined using the technique of allozyme electrophoresis were found to have very low levels of genetic differentiation. This result was consistent with the low level of genetic differentiation found among other conspecific populations of shorebirds.

The low level of genetic differentiation found so far does not support taxonomic subdivision of the New Zealand Dotterel, but our findings do not mean that genetic differences do not exist between northern and southern populations. More sensitive genetic analysis may yet provide evidence to support subspecific recognition of the populations within the species.

INTRODUCTION

New Zealand Dotterels (*Charadrius obscuncs* Gmelin) were formerly widespread throughout New Zealand. Breeding populations were reported from subalpine and from coastal habitats (Buller, 1888, Oliver, 1930). Today, New Zealand Dotterels consist of two populations: (1) a northern population consisting of birds that breed on suitable coastline of the North Island, mostly north of 38°S, and (2) a southern population of birds that breed only on Stewart Island (Turbott, 1990). There is no evidence that these populations ever meet to interbreed (Dowding and Murphy, 1993).

Other evidence suggests that these populations may be taxonomically distinct. The Stewart Island population shows differences in breeding behaviour and morphology compared to their northern counterparts. For example, they tend to nest only on the open subalpine tops (Dowding and Murphy, 1993), whereas North Island birds nest on beaches at sea level (Turbott, 1990). Stewart Island birds tend to overwinter in flocks that stay together, a phenomenon that does not occur in the North Island. The Stewart Island birds also tend to be significantly heavier and larger in some measurements and have noticeably darker plumage than the North Island birds (Dowding and Murphy, 1993).

The current classification of New Zealand Dotterel is given in Turbott (1990):

Family	*Charadriidae* (Dotterels, Plovers, Lapwings)
Subfamily	*Charadriinae*
Genus	*Charadrius* Linnaeus
Species	*obscurus* Gmelin (New Zealand Dotterel (= Tuturiwhatu))

The Banded Dotterel, compared as an outgroup in this study is classified as a separate species: *Charadrius bicinctus bicinctus*.

Of particular concern the Stewart Island population of the New Zealand Dotterel has declined rapidly over the last few decades. Dowding and Murphy (1993) noted that "if most (or all) of the New Zealand Dotterels seen in the South Island are from Stewart Island, the loss of that population would greatly reduce the species range. With the physical and behavioural differences we see in birds of the southern population, there would also be a loss of diversity, some of which may well be genetically based".

Allozyme electrophoresis has been widely used to examine geographic variation and the taxonomic status of many avian species (Baker *et al.*, 1985, Baker and Strauch, 1988, Barrowclough and Johnson, 1988, Triggs *et al.*, 1989, Stangel, 1990, Zink, 1990). Generally, most avian congeneric species show low levels of genetic differentiation compared to other vertebrates, perhaps due to slower rates of protein evolution within these animals, or some other reason (Prager and Wilson, 1975, Avise *et al.*, 1980). However, electrophoresis has been particularly useful for the discrimination of congeneric shorebird species, since taxonomic differences between these birds are normally relatively great compared to other congeneric avian species (Baker *et al*, 1985, Baker and Strauch, 1988).

On the other hand, intraspecific variation is often particularly low among shorebirds (Baker and Strauch, 1988). Therefore, some caution has to be exercised when evaluating intraspecific taxonomic differentiation.

The low levels of genetic variation found among avian species, despite often considerable morphological variation, caused Zink (1990) to comment, "I see little value in continuing protein Eectrophoretic studies of geographical variation in temperate-breeding birds, unless pilot studies indicate otherwise." This paper reports the results of a pilot study which is intended to give the baseline data to determine whether allozyme electrophoresis is useful for evaluating genetic and taxonomic differences between the northern and southern populations of the New Zealand Dotterel.

METHODS

I. Collection:

A sample of blood was collected from 28 individuals of New Zealand Dotterel from three locations and two Banded Dotterels (outgroup) between November 1992 and March 1993. These samples are representative of the known populations of New Zealand Dotterel (Figure 1, Appendix 1).

Blood was immediately centrifuged in order to separate the red blood cells from the plasma. Red cell components were diluted 1:1 with distilled water, in order to lose the red cells. All samples were immediately snap frozen and stored at $-80^{\circ}C$ in the ultracold freezer at Victoria University.

II. Electrophoretic Techniques:

Genetic data were obtained using standard techniques of starch gel electrophoresis as

described in Triggs *et al.*, (1989) and Daugherty *et al.* (1990). Since birds show low levels of genetic diversity as compared to other vertebrates, we attempted to maximise the number of loci examined: all combinations of five gel/electrode buffer systems and 26 different protein (mainly enzyme) stains were examined for electrophoretic activity and resolution. Individuals which showed the same mobility on a gel stained for a particular enzyme were considered to be encoded by the same Mendelian allele, whereas those showing differences in mobility were assumed to be encoded by a different allele. Not all amino acid substitutions alter the mobility of the resulting protein, with no more than perhaps 30% of any genetic variation being detected at any given structural locus (Lewontin, 1974, King and Wilson, 1975). Therefore, estimates of genetic divergence can be assumed to be substantial underestimates.

III. Allozyme nomenclature and analysis:

The following nomenclature, based on the recommendations of Murphy and Crabtree (1985), has been used when labelling genetic loci and alleles: (1) loci have been indicated in italics; (2) multiple loci have been labelled sequentially beginning from the most anodal, and (3) alleles have been indicated by a lower case letter written within parentheses. Therefore, *Ada-1(b)* refers to the b allele at the most anodal locus encoding the enzyme Adenosine deaminase.

The BIOSYS-1 programme (Swofford and Selander, 1981) was used to compute (1) gene and genotype frequencies, Hardy-Weinberg analyses, and average unbiased heterozygosity per locus (H; Nei, 1978) for each population; (2) F-statistics to analyze population structure within and between all populations (Wright, 1978); (3) unbiased estimates of genetic distance (D; Nei, 1978) between pairwise combinations of populations, and (4) a phenetic clustering algorithm (WPGMA; Sneath and Sokal, 1973) over all populations.

RESULTS:

A total of 28 blood loci were examined (Table 1). Only three loci showed polymorphism, mostly between the Banded Dotterels and the New Zealand Dotterels (Figures 2-4). At only one locus was any variation observed within the New Zealand Dotterel group (Figure 4); a single New Zealand Dotterel was heterozygous at the *Lap-1* locus (Table 2).

None of the New Zealand Dotterel populations could be distinguished from one another using the loci detected in this study. All populations were in Hardy-Weinberg equilibrium. Mean unbiased measurements of heterozygosity were low, ranging from 0.000 to 0.004. Genetic structuring within the New Zealand Dotterel populations was low ($F_{ST}=0.034$), however, structuring between the New Zealand and Banded Dotterels was high ($F_{ST}=0.890$). Genetic distance (D, Nei, 1978) equalled 0.000 within the New Zealand Dotterel group, but ranged between 0.091 and 0.094 when New Zealand and Banded Dotterels were compared. Cluster analysis of the populations revealed that all New Zealand Dotterel populations clustered separately from the Banded Dotterels. No subclusters were observed between the New Zealand Dotterel populations (Figure 5).

Table 1: Loci examined using allozyme electrophoresis:

Locus	Enzyme involved	Reference	E. C. Number
Ada-1	Adenosine deaminase	1	3.5.4.4
Ak-1	Adenylate kinase	6	2.7.4.3
Ck-2	Creatine kinase	1	2.7.3.2
Gdh-1	Glutamate dehydrogenase	1	1.4.1.3
Got-1	Aspartate aminotransferase	1	2.6.1.1
Gp-2	General protein	1	-------
Gp-3			
G6p-1	Glucose-6-phosphate dehydrogenase	3	1.1.1.49
Gpi-1	Glucose-phosphate isomerase	1, 3	5.3.1.9
Hb-1	Haemoglobin	1	-------
Hb-2			
Hb-3			
Idh-1	Isocitrate dehydrogenase	3	1.1.1.42
Lap-1	Leucine aminopeptidase	3	3.4.13
Ldh-1	Lactate dehydrogenase	1	1.1.1.27
Ldh-2			
Mdh-1	Malate dehydrogenase	3	1.1.1.37
Me-1	Malic enzyme	3	1.1.1.40
Me-2			
Mpi-1	Mannose-phosphate isomerase	2	5.3.1.8
Np-1	Purine nucleoside phosphorylase	3	2.4.2.1
Pep-1	Peptidase	1, 4	3.4.11
Pep-2			
6pg-1	6-phosphogluconate dehydrogenase	3	1.1.1.44
Pgm-1	Phosphoglucomutase	5	2.7.5.1
Pgm-2			
Sod-1	Superoxide dismutase	2	1.15.1.1
Xdh-1	Xanthine dehydrogenase	1	--------

1. Richardson *et al.*, 1986, modified for starch; 2. Cheliak and Pitel, 1984; 3. Allendorf *et al* 1977; 4. Conkle *et al.*, 1982; 5. Stuber and Goodman, 1983; 6. Vallejos, 1983.

Table 2: Allozyme frequencies for variable loci

Locus *(allele)*	Population (N)			
	North Auckland	Coromandel	Stewart Island	Banded Dotterel
Ada-1	(6)	(9)	(8)	(2)
(a)	1.00	1.00	1.00	----
(b)	----	----	----	1.00
Lap-1	(10)	(9)	(9)	(2)
(a)	0.95	1.00	1.00	----
(b)	0.05	----	----	1.00
Mpi-1	(10)	(9)	(9)	(2)
(a)	----	----	----	0.75
(b)	1.00	1.00	1.00	0.25

DISCUSSION:

The present taxonomy of the New Zealand Dotterel places all populations examined in this study within the same species (Turbott, 1990). No subspecies of New Zealand Dotterel have been reported. Dowding and Murphy (1993), however, described morphological and behavioural differences that are suggestive of taxonomic disparity between the northern and the Stewart Island populations of New Zealand Dotterel. Nevertheless, this study did not reveal genetic divergence between any populations. However, moderate levels of genetic divergence, relative to other avian studies, were revealed between the New Zealand and Banded Dotterels (Nei's D=0.091-0.094).

Most studies of birds have shown, in general, low levels of genetic divergence among avian compared to non-avian vertebrates of similar taxonomic rank (Avise *et al.*, 1980, Baker *et al.*, 1985). Reported values of genetic divergence for shorebirds range from Nei's D (1978) = 0.000-0.031 among local populations, 0.000-0.028 among subspecies, and 0.000-0.319 among species (Baker and Strauch, 1988). F_{ST} values reported for shorebirds range from 0.019 to 0.054 (Stangel, 1990). Intraspecific variation within the family *Charadriidae,* as measured by mean heterozygosities (H unbiased, Nei, 1978), range from 0.000 $\pm$ 0.000 to 0.021 $\pm$ 0.011 (Baker and Strauch, 1988). The values of D, F_{ST} and H reported in this study fall within the ranges reported for those values in previous avian studies, as shown above.

The maximum values of genetic divergence reported for congeneric avian species are small compared to the maximum values reported for non-avian vertebrates (Nei's similarity is normally less than 0.85 (D > 0.16) among mammalian species, Thome, 1982: F_{ST} values normally exceed 0.5 among small mammals and amphibians, Barrowclough and Johnson, 1988). There is also the difficulty that genetic divergence of shorebirds overlaps at the population, subspecies, and species levels of taxonomy. The minimum genetic distances for each of these taxonomic levels falls at Nei's (1978) D = 0.000 (Baker and Strauch, 1988). This problem of low genetic differentiation among avian species where allozyme data has been compared has on occasions occurred even where morphological differentiation has been great. Braun and Robbins (1986) noted that "Extreme protein similarity cannot by itself be taken as strong evidence for conspecificity of bird populations". Yang and Patton (1981) also argued that large differences in diversity and feeding behaviour among Galapagos finches were achieved without significant genetic change, at least as indexed by allozyme differentiation. It appears, therefore, that among birds major shifts in morphology are not necessarily reflected in structural gene changes as measured by electrophoresis.

Therefore, we are not able to directly assess the conspecificity the New Zealand Dotterel populations from the data revealed in this study. Either (1) divergence between the populations is recent, or (2) the rate of protein evolution within these birds is too slow to reveal differences between the populations, or (3) allozyme electrophoresis has failed to reveal real taxonomic differences that occur between some or all of their populations.

Conclusions and recommendations.

Analysis of 28 blood loci show no differences between the New Zealand Dotterel populations. Either the observed morphological variation is environmentally induced, or the genes surveyed by protein electrophoresis do not reflect the patterns of variation underlying the morphological variation (Zink, 1990). Future studies, if any, will require more sensitive techniques of analysis to reveal genetic differences that may exist between these populations.

The draft recovery plan for the New Zealand Dotterel states that allowing the southern population to become extinct is not considered a justifiable option. "The recovery option chosen should aim to preserve the maximum diversity possible within the species, whether the origin of that diversity is known or not" (Dowding, 1992). Given that the New Zealand Dotterels are concentrated into only two breeding populations, and that the Stewart Island population shows ecological and morphological differences to the northern population (Dowding and Murphy, 1993), we recommend that both populations should be managed separately so that maximum diversity in this species is maintained.

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ACKNOWLEDGEMENTS:

Primary support for this research was provided by a grant from the New Zealand Department of Conservation. Other assistance was provided by a New Zealand Lotteries Board grant, and by the School of Biological Sciences, Victoria University of Wellington. We thank Simon Chamberlin and Elaine Murphy for help in collecting samples.

APPENDIX 1:

Collection dates and localities of New Zealand Dotterel and Banded Dotterel blood samples:

Sample Number	Date	Locality
912	09-11-92	Matarangi Spit, Coromandel
913	09-11-92	Matarangi Spit, Coromandel
914	09-11-92	Matarangi Spit, Coromandel
915	09-11-92	Matarangi Spit, Coromandel
814	10-11-92	Matarangi Spit, Coromandel
916	10-11-92	Gray's Beach, Coromandel
917	10-11-92	Gray's Beach, Coromandel
918	10-11-92	Wharekaho, Coromandel
919	10-11-92	Wharekaho, Coromandel
921	11-11-92	Waipu Spit, North Auckland
922	11-11-92	Waipu Spit, North Auckland
923	11-11-92	Waipu Spit, North Auckland
924	11-11-92	Waipu Spit, North Auckland
058	11-11-92	Waipu Spit, North Auckland
068	11-11-92	Waipu Spit, North Auckland
925	12-11-92	Omaha, North Auckland
390	12-11-92	Omaha, North Auckland
513	12-11-92	Omaha, North Auckland
052	12-11-92	Omaha, North Auckland
579	23-03-93	Mason Bay, Stewart Island
950	23-03-93	Mason Bay, Stewart Island
001	24-03-93	Mason Bay, Stewart Island
506	24-03-93	Mason Bay, Stewart Island
048	24-03-93	Mason Bay, Stewart Island
944	25-03-93	Mason Bay, Stewart Island
002	25-03-93	Mason Bay, Stewart Island
003	25-03-93	Mason Bay, Stewart Island
004	25-03-93	Mason Bay, Stewart Island
BD I.	23-03-93	Mason Bay, Stewart Island
BD2	23-03-93	Mason Bay, Stewart Island

Figure 1: Locations from which Dotterel blood samples were collected.

(Sample sizes in parentheses)

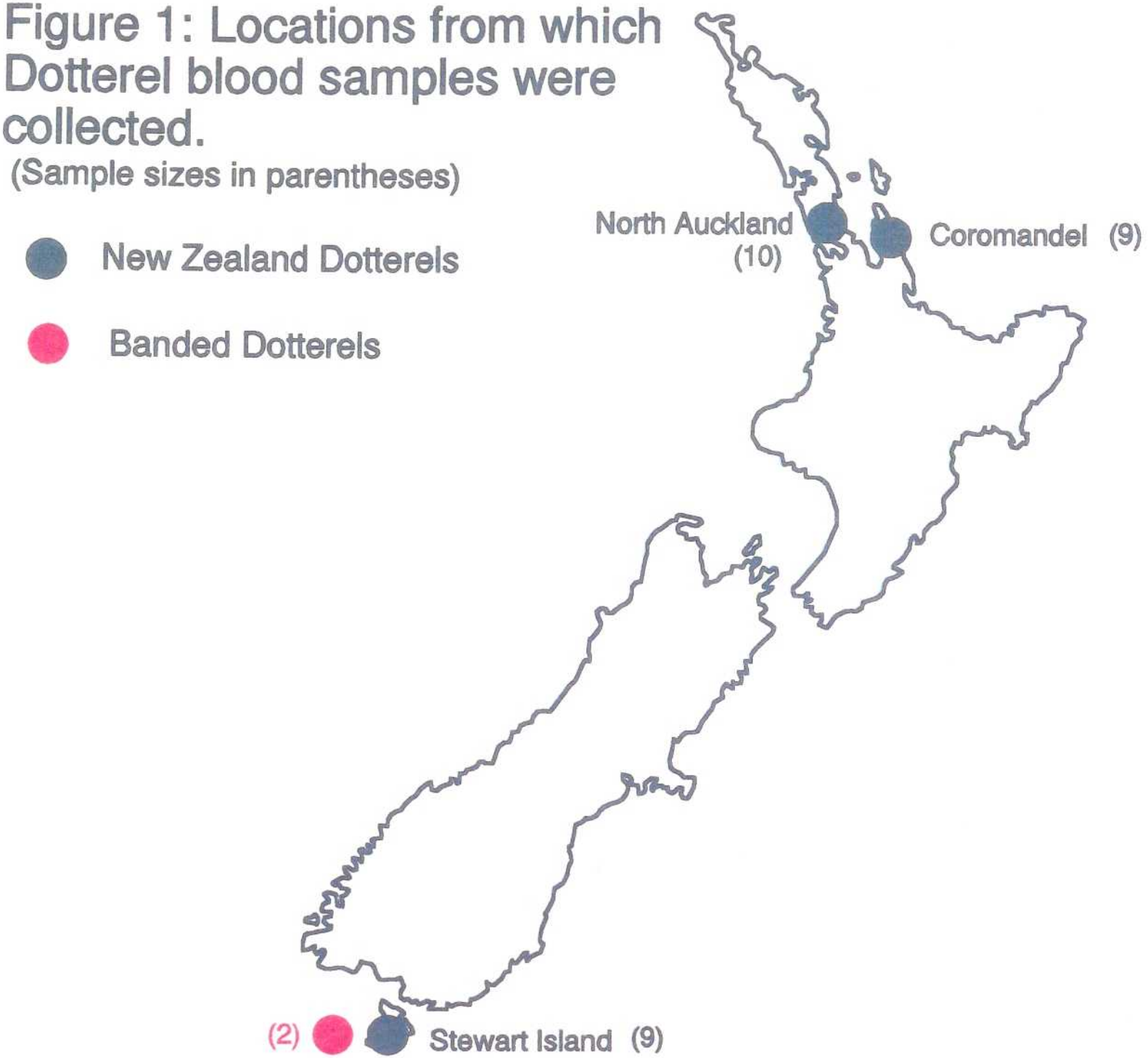

Figure 2: Allozyme frequencies at locus *Ada-1*.

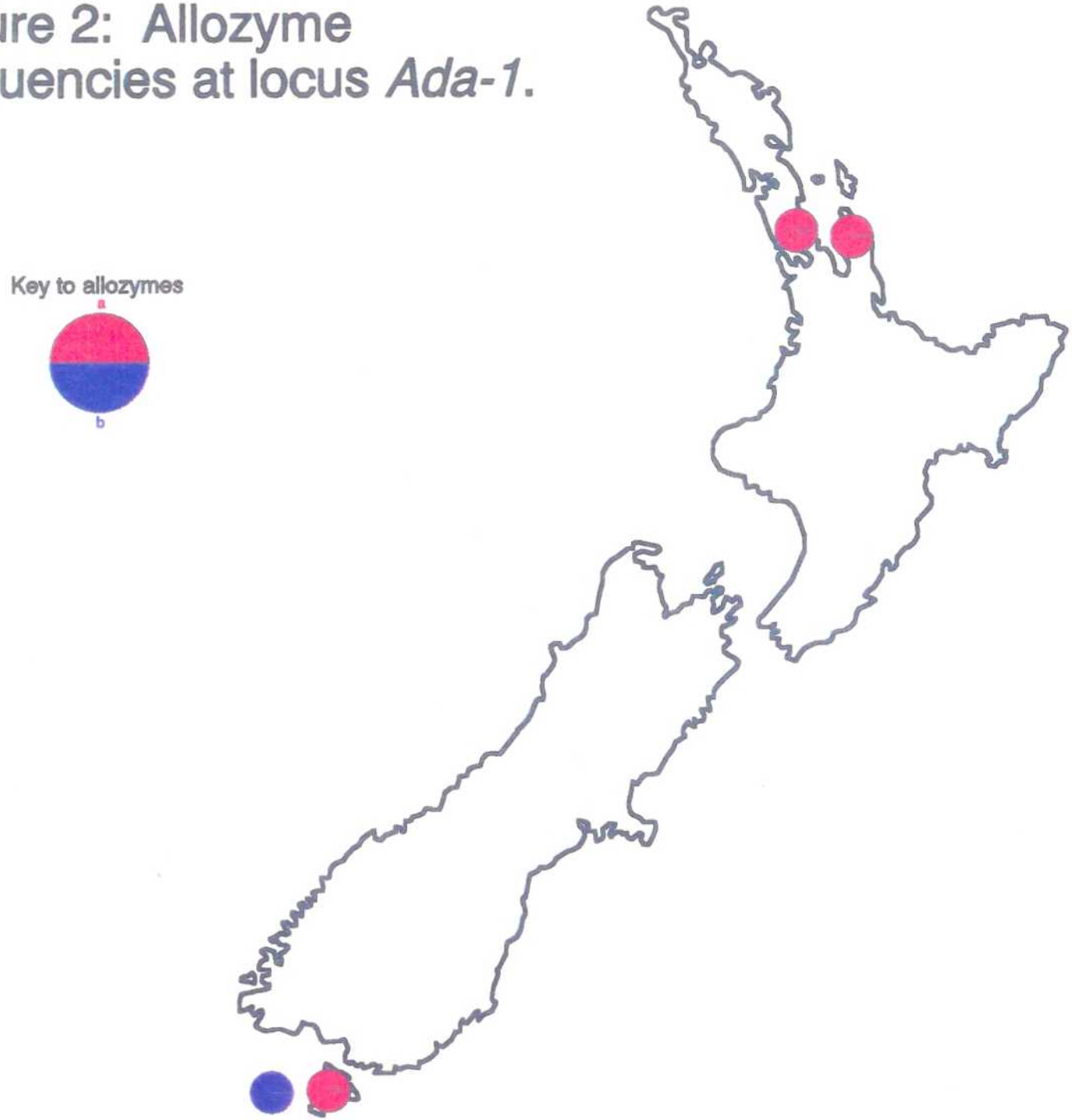

Figure 3: Allozyme frequencies at locus *Lap-1*.

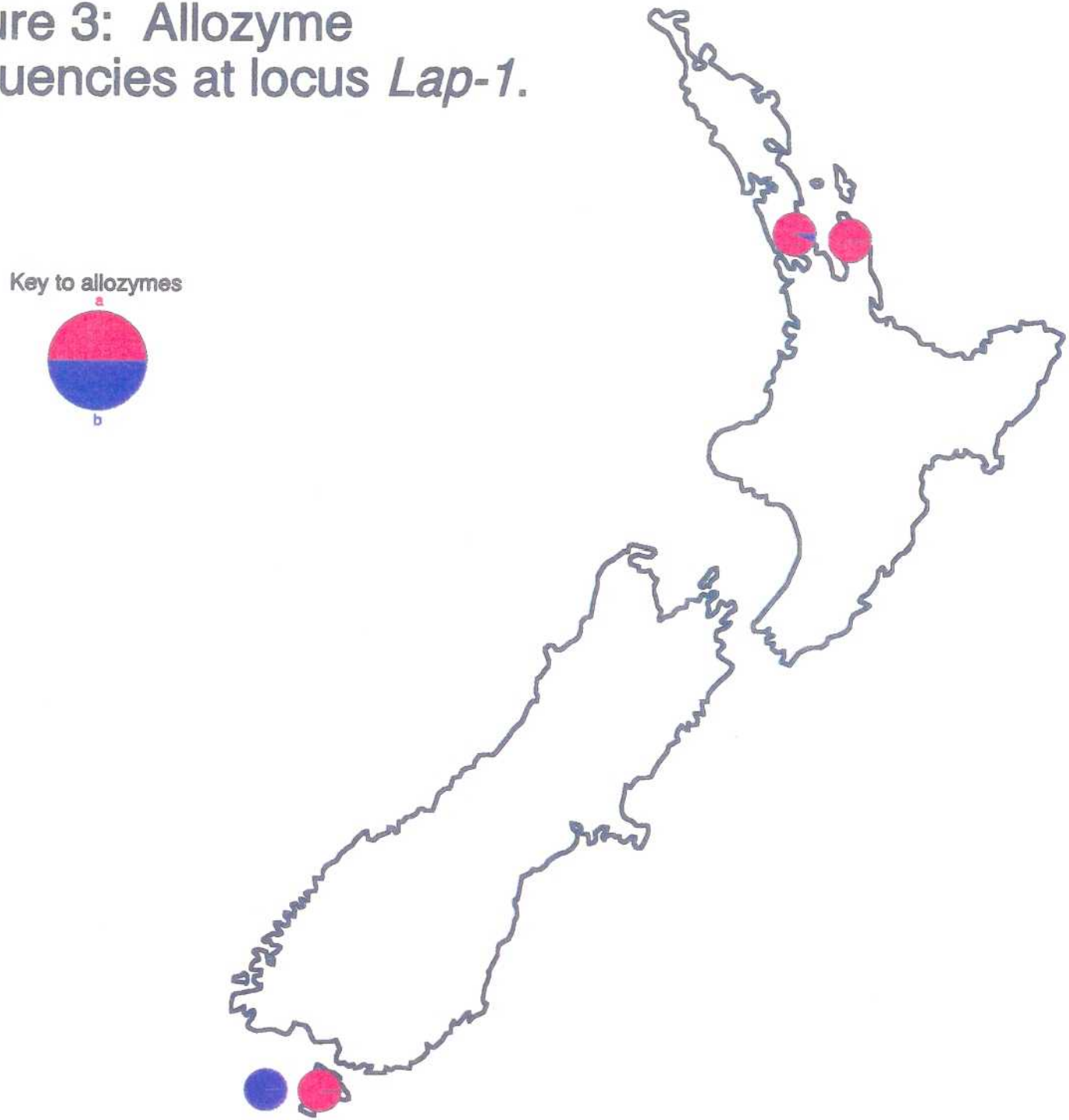

Figure 4: Allozyme frequencies at locus *Mpi-1*.

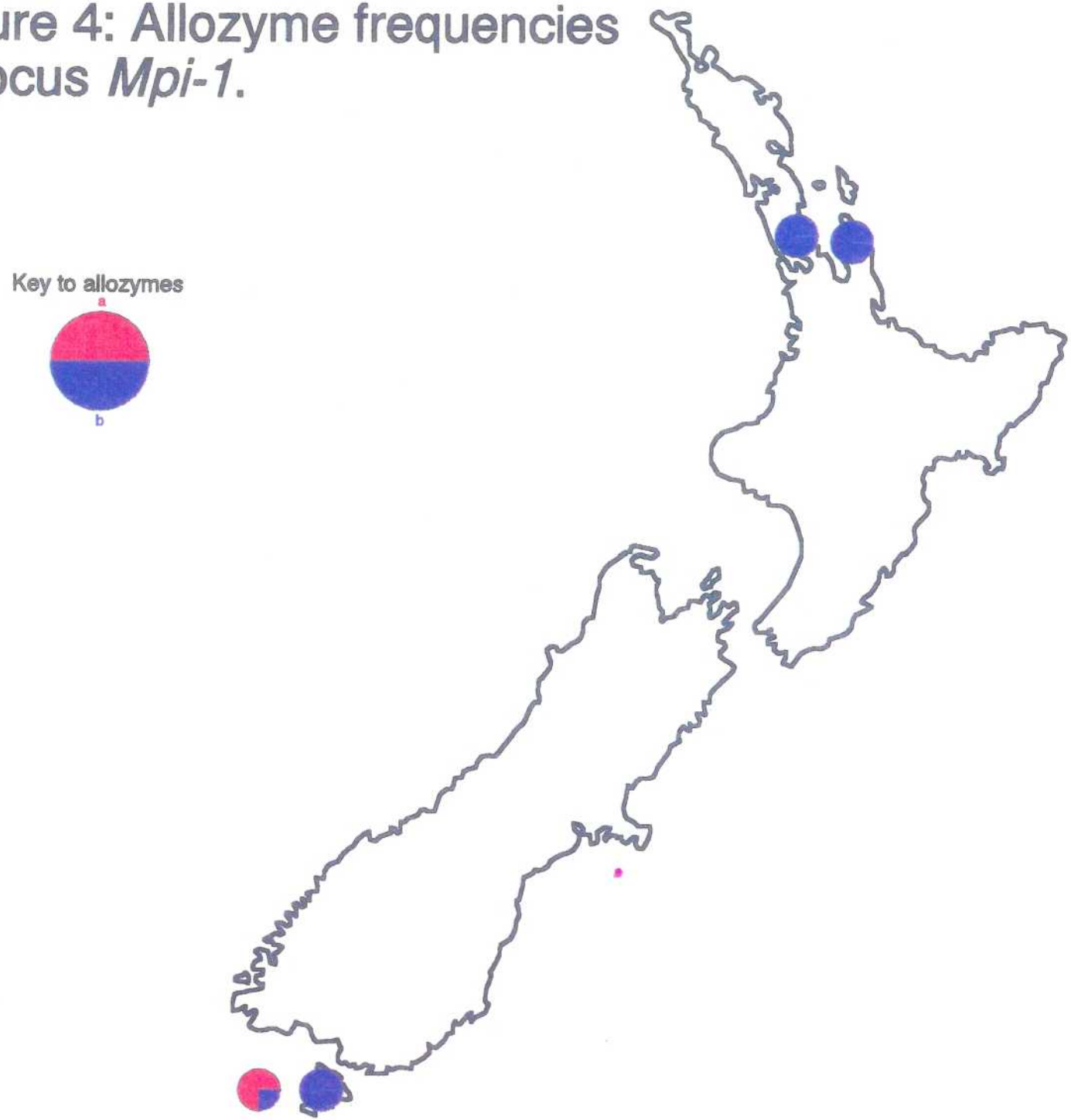

Figure 5: Genetic relationships of the New Zealand Dotterel using Nei's D (1978) and the WPGMA algorithm (Sneath and Sokal 1973).