DNA analysis of shore plovers

SCIENCE & RESEARCH INTERNAL REPORT 178

David Lambert, Hilary Miller, and Ian Anderson

Published by
Department of Conservation
P.O. Box 10-420
Wellington, New Zealand

Science & Research Internal Reports are written by DOC staff or contract scientists on matters which are on-going within the Department. They include reports on conferences, workshops, and study tours, and also work in progress. *Internal Reports* are not normally subject to peer review.
Publication was approved by the Manager, Science & Research Unit, Science Technology and Information Services, Department of Conservation, Wellington.
This report (DOC investigation no. 2505) was prepared by David Lambert and Hilary Miller (Molecular Ecology, Institute of Molecular BioSciences, Private Bag 11–222, Palmerston North), and Ian Anderson (Equine Blood Typing Unit, Massey University, Palmerston North, New Zealand).

ISSN 0114–2798
ISBN 0–478–21961–X

Cataloguing-in-Publication data

Lambert, David
DNA analysis of shore plovers / David Lambert, Hilary Miller, and Ian Anderson. Wellington, N.Z. : Dept. of Conservation, 2000.
1 v. ; 30 cm. (Science & Research internal report, 0114-2798 ; 178)
Includes bibliographical references.
ISBN 047821961X
1. Thinornis novaeseelandiae. 2. DNA fingerprinting. 3. Rare birds—New Zealand—Chatham Islands. I. Miller, Hilary II. Anderson, Ian III. Title. Series: Science and Research internal report ; 178.

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Abstract

Until recently the threatened shore plover (*Thinornis novaeseelandiae*) was thought to have survived as a single population on Rangatira Island in the Chatham Islands group, New Zealand. In 1999 a second population was discovered on the Western Reef, off main Chatham Island. Because the establishment of new self-sustaining populations is important in the conservation of an endangered species, it was considered important to determine whether the Western Reef population is part of the larger Rangatira Island population, or whether it represents a separate, genetically distinct population. It was also important to determine the sex ratios of both populations. Individuals from both Rangatira Island and Western reef were sexed using a DNA-based method. In addition, using a multilocus DNA profiling, the level of genetic divergence between these populations was estimated. Levels of bandsharing between the two populations were found to be lower than within each population. The level of population structure was investigated using Wright's F statistic and an F_{ST} value of 0.382 was recorded. Thus 38.2% of the minisatellite DNA variability detected in shore plovers was attributable to differentiation between the Rangatira Island and Western Reef populations. This result, combined with the presence of unique sets of restriction fragments in the DNA profiles of individuals from Western Reef, suggests that the two populations are genetically differentiated, with relatively low levels of migration between them. It is possible that the two populations are remnants of a series of highly structured, genetically divergent populations. Alternatively, it may be that the two populations have diverged relatively recently. A number of logically possible strategies for the continued management of these populations are discussed in the light of these findings.

Keywords: *Thinornis novaeseelandiae,* shore plover, threatened species, populations, multilocus DNA profiling, minisatellite DNA, Rangatira Island, Western Reef, Chatham Islands, New Zealand

1. Introduction

The shore plover *(Thinornis novaeseelandiae)* was once distributed throughout mainland New Zealand but is now confined to the islands in the Chatham Islands group. On Rangatira Island, the shore plover inhabits wave platforms and salt meadow. They are highly sedentary and adults rarely move away from their territories. The population is relatively stable, with numbers fluctuating between 37–45 breeding pairs and 100–130 individuals since regular censusing of the species began in 1981 (Kennedy, E. 1993: Shore plover recovery plan. Unpublished draft)*.

The shore plover was originally thought to have survived as a single population on Rangatira Island, but in 1999 a second population of 21 individuals was found on the Western Reef off main Chatham Island. Because of the risk of extinction faced by a species which has only two remnant populations, the Department of Conservation's Shore Plover Recovery Plan has focused on the establishment of new self-sustaining populations.

In this project we aim to estimate any genetic differences between the recently discovered shore plovers on the Western Reef and those from Rangatira Island. Specifically, the scope of this project (as detailed in original proposal) is to:

1. Develop a DNA-based test for sex in shore plovers.
2. Sex all blood samples provided to us by the Department of Conservation up to a maximum of 40 wild and/or captive individuals.
3. Extract DNA from the available samples, digest each of these appropriate restriction enzyme(s) and hybridise to a range of minisatellite DNA probes.
4. Digitise the resulting DNA profiles.
5. Quantify levels of minisatellite DNA variation in a sample of up to 40 wild/captive individuals. Specifically we will provide information on the mean number of bands per individuals for an appropriate enzyme/probe combination and the mean bandsharing between individuals.
6. Compare the results of minisatellite DNA studies of shore plovers with results from similar studies of other New Zealand avian species.
7. Provide an appropriate report to the Department of Conservation.

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* The latest recovery plan is not yet published, but is close to final approval. It is included in a document by Aikman, H.; Davis, A.; Miskelly, C.; Taylor, G. (Draft April 2000): 'Chatham Islands threatened birds; recovery and management plans'.
This document is a series of plans, of which the shore plover is listed as 'New Zealand Shore Plover Recovery Plan 2000–2010'.

2. Material and methods

2.1 SAMPLING

A total of 48 samples were collected from 15 male, 13 female and 20 juvenile shore plover on Rangatira Island. In addition four blood samples were collected from four males on the Western Reef (detailed in Table 1). Birds were caught by the noose-mat method using mealworms as a lure. Once caught, the condition of each bird was assessed and its weight and band number recorded. Blood was sampled from the brachial vein, using a 27 or 25 gauge hypodermic needle. Between 100 µL and 300 µL of blood was collected in 75 mm/1.2 mm ID heparanized capillary tubes (2–6 tubes). The content of each tube was then decanted (via a filtered blow tube) into an individually numbered nunc cryotube, and stored in liquid nitrogen (O'Connor 1999). Samples were collected in accordance with Department of Conservation shore plover recovery group approval.

2.2 SEXING METHODS

DNA extraction

A 4 µL sample of blood was diluted to 50 µL with distilled water in an Eppendorf tube and left at room temperature, for 5 mins to allow complete lysis of cells. After centrifugation the supernatant was removed and the pellet was resuspended in 40 µL of BioRad Instagene. The resulting solution was incubated at 56°C for 30 mins in water bath, vortexed for 10 seconds and then heated at 100°C for 8 mins. The vortexing and centrifugation was repeated again and 4 µL was used as template in PCR.

PCR and electrophoresis conditions

Amplifications were performed in 25 µL volumes, 67 mM Tris-HCl (pH 8.8), 6.7 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$ 10 mM 2-mercaptoethanol, each dNTP at 1 mM, each primer a 1 µM, and 0.5 units of *Taq* polymerase (Perkin-Elmer/ Roche). DNA template was added to this reaction. PCR primers with the following sequences were used: P2 5'–TCTGCATCGCTAAATCCTTT–3' and P3 5'–AGATATTCCGGATCTGATA–3' (Griffiths et al. 1996). Thermo-cycling (denaturation 94°C 45 sec; annealing 53°C 60 sec; extension 72°C 60 sec) for 40 cycles was performed on a Hybaid OmniGene thermal cycler. PCR products were digested with the restriction enzymes according to the manufacturers instructions. Amplified CHD sequences from both sexes were digested with the restriction enzyme *Hae* III. Amplified and digested products were size fractionated on agarose gels containing ethidium bromide (2% NuSieve/1% agarose in Tris acetic acid pH 8.0 buffer) and visualised under UV light. Negative controls were run in all experiments.

DNA methods for minisatellite DNA profiling

Genomic DNA was extracted from whole blood. Fifteen microlitres of blood was lysed by resuspension in 500 µL of lysis buffer (144 mM NH_4Cl; 10 mM NH_4HCO_3). The lysate was centrifuged at 16,000 × g for 10 min and the supernatant discarded. The resulting pellet was resuspended in 400 µL of SET buffer (0.1 M NaCl, 1 mM EDTA, 0.1 M Tris-HCl pH 8.0) to which sodium dodecyl sulphate (20 µL, 10% SDS) and proteinase K (10 µL, 20 mg/mL) were added, and incubated overnight at 55°C. DNA was then extracted and purified using standard phenol/chloroform methods and precipitation and resuspension of DNA was performed in accordance with Sambrook et al. 1989.

Approximately 20 µg of DNA was digested overnight with the restriction enzyme *Hae*III (10 units) in the presence of spermidine trihydrochloride and bovine serum albumin (BSA, 2 mg/mL) with the manufacturer's recommended buffer. The following morning a further 10 units of enzyme were added and incubation continued for a minimum of 1 hour. The concentration of the digested DNA was determined with a Hoefer TKO-100 DNA fluorometer. The digested DNA was then electrophoresed through 0.8% agarose gels in TBE buffer (134 mM Tris, 74.9 mM boric acid, 2.55 mM EDTA pH 8.8) for 48 hr at 55V. After electrophoresis was completed, gels were depurinated in 0.25 M HCl for 15 min, denaturated in 0.5 M NaOH, 1.5 M NaCl for 45 min, and then neutralised in 1.5 M NaCl, 0.5 M Tris-HCl pH 7.2, 0.5 M EDTA pH 7.2 twice for 15 min. DNA was transferred to Nylon membrane (Boehringer Mannhiem) by Southern blotting overnight in 6 × SSC (1 × SSC, 0.15 M NaCl, 0.015 M tri-sodium citrate). The membrane was subsequently washed briefly in 6 × SSC, air dried and baked at 80°C for 2 hours. Minisatellite probes pV47-2 and *per* were labeled with [α^{32}P]dCTP by random priming (GibcoBRL RTS RadPrime DNA Labelling System). Membranes were prehybridised in 0.25 M disodium hydrogen orthophosphate, 1 mM EDTA, 7% SDS at the hybridisation temperature for 2 hours before addition of the probe. Hybridisation temperatures used were 55°C (pV47-2) and 65° (*per*). Membranes were washed twice with 5 × SSC, 0.1% SDS for 30 min, then exposed to Fuji Medical X-ray film (RX) at –80°C for 1–7 days

2.3 DATA ANALYSIS

The molecular weight size ranges scored for each probe were as follows: >5 kb (pV47-2); >3.5 kb (*per*). Bands were considered to be identical if their centers were within a bin size of 1 mm for profiles produced with pV47-2, or 1.5 mm for profiles produced with *per*. The DNA profiles were digitised into Microsoft Excel by assigning 1 or 0 for the presence or absence of bands respectively. Using an Excel macro, the mean number of fragments per individual and mean bandsharing within and between the Rangatira Island and Western Reef populations was determined. Differentiation between the two populations was investigated by calculating Wright's index of population subdivision (F_{ST}), as described by Lynch (1991).

3. Results

3.1 DNA-BASED SEXING

All 52 blood samples collected from shore plover on Rangatira Island and Western Reef were sexed and the results are given in Table 1. All sexes of adult birds suggested from morphological data, were concordant the results of the DNA-based sex test. The sex ratio of adults was 19 males to 13 females. These values are not significantly different from a 1:1 ratio (χ^2=1.125; p>0.05). Juvenile individuals, unsexed prior to this testing were all successfully identified as male or female. A total of 20 individuals were assayed, revealing 13 males and 7 females. This ratio does not significantly differ from parity (χ^2=1.125; p>0.05). Despite these non-significant results, there is a bias towards males in both groups, and consequently additional sexing of individuals, as they become available, would seem advisable.

3.2 MINISATELLITE ANALYSES

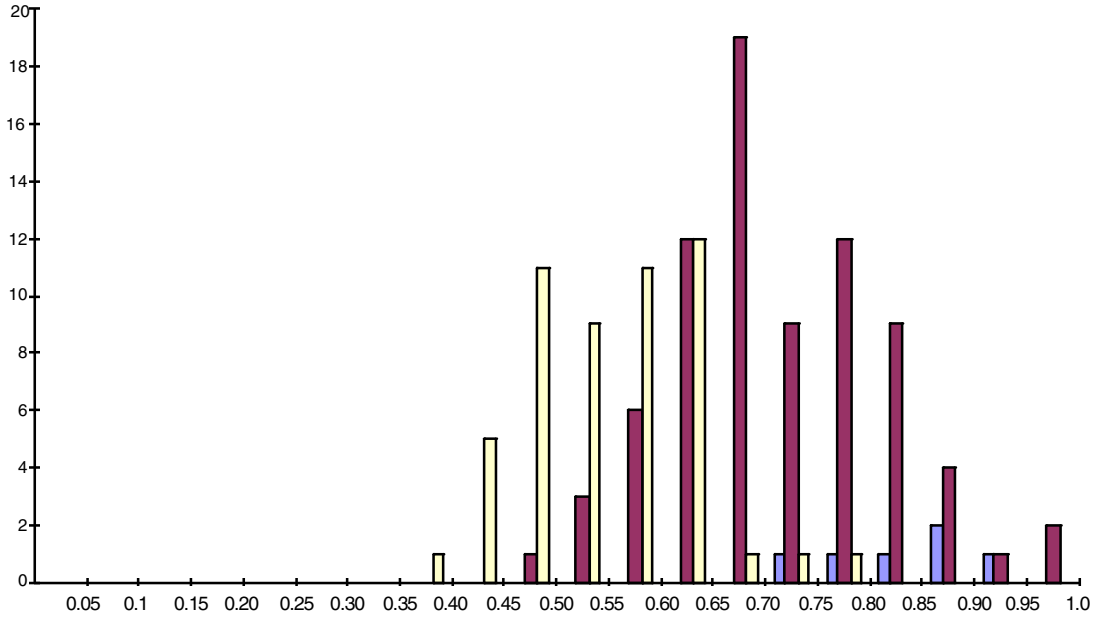
The four Western Reef samples were run alongside 14 randomly selected samples from Rangatira Island, on each of two gels. Each gel was probed with both pV47-2 and *per*. Examples of minisatellite profiles are shown in Fig. 1. The raw data, detailing the presence or absence of bands, and individual pairwise comparisons for each gel/probe combination is given in Appendix 1. These data are represented visually in Fig. 2 (A–D). Mean bandsharing was determined for individuals both within and between Rangatira Island and Western Reef populations (Table 2). An F_{ST} value of 0.382 was calculated, thus 38.2% of genetic variability detected is attributable to differentiation between the Rangatira Island and Western Reef populations. The level of minisatellite DNA variation recorded for shore plovers is compared to variation found in other New Zealand birds in Table 3.

Figure 1. Minisatellite DNA profiles of shore plovers from Western Reef and Rangatira Island. Hybridisation results for two gels probed with minisatellite DNA sequences *per* (A), and pV47-2 (B). Four samples from the Western Reef collection were directly compared by running on the same gel as individuals from Rangatira Island. The two open arrowheads indicate restriction fragments found only in Western Reef birds.

TABLE 1. SEX OF SHORE PLOVER SAMPLED FROM RANGATIRA ISLAND AND WESTERN REEF DURING FEBRUARY/ MARCH 1999. ENTRIES IN **BOLD** ARE THE INDIVIDUALS USED IN MINISATELLITE DNA ANALYSES.

TUBE NO.	BAND NO.	SEX (MORPHOLOGY)	SEX (DNA)	WEIGHT	LOCATION / TERRITORY
01	**28354**	**Male**	**Male**	**51g**	**Front Landing/ T6**
02	**28271**	**Male**	**Male**	**61g**	**Whalers Bay/ T10B**
03	57102	Juvenile	Male	57g	Whalers Bay/ T10B
04	**28361**	**Female**	**Female**	**58g**	**WB/ Island Point/ T11A**
05	**28244**	**Male**	**Male**	**63g**	**Thinornis Bay Nth/ T14**
06	**28203**	**Female**	**Female**	**60g**	**Thinornis Bay Mid/ Nth/ T15**
07	28292	Female	Female	59g	Thinornis Bay Mid/ Nth/ T15
08	**25550**	**Male**	**Male**	**68g**	**Thinornis Mid/ Nth/ T17**
09	28211	Female	Female	56g	Thinornis Bay Mid/ T17
10	**28208**	**Male**	**Male**	**63g**	**Thinornis Bay Mid/ T19**
11	**51864**	**Male**	**Male**	**59g**	**Thinornis Bay Mid/Sth/ T19A**
12	**51803**	**Female**	**Female**	**59g**	**Thinornis Bay Sth/ T20**
13	**57130**	**Male**	**Male**	**60g**	**Thinornis Bay Sth/ T20**
14	28228	Female	Female	56g	Thinornis Bay Sth/ T21A
15	**51808**	**Male**	**Male**	**63g**	**Thinornis Bay Sth/ T21A**
16	57103	Juvenile	Female	49g	Thinornis Bay Sth/ T21A
17	57113	Juvenile	Male	56g	Thinornis Bay Sth/ T21A
18	**28298**	**Male**	**Male**	**57g**	**Nogs Folly/ T22**
19	**51802**	**Female**	**Female**	**61g**	**Nogs Folly/ T22**
20	**28304**	**Female**	**Female**	**64g**	**Nogs Folly/ T22A**
21	51875	Female	Female	62g	East Clears/ T26A
22	**28303**	**Male**	**Male**	**63g**	**East Clears/ T26A**
23	**28269**	**Male**	**Male**	**60g**	**East Clears/ T26B**
24	**51846**	**Male**	**Male**	**59g**	**East Clears/ T26B**
25	**51847**	**Female**	**Female**	**61g**	**East Clears/ T26B**
26	57131	Male	Male	68g	East Clears/ T26B
27	**28368**	**Female**	**Female**	**62g**	**Clears/ Seal Gully/ T30A**
28	57106	Juvenile	Female	51g	WB/ Island Point/ T11A
29	**57132**	**Juvenile**	**Male**	**56g**	**WB/ Island Point/ T11A**
30	57111	Juvenile	Female	46g	Thinornis Bay Sth/ T20
31	57105	Juvenile	Male	48g	Thinornis bay Sth/ T20
32	57129	Juvenile	Male	48g	Front Landing/ T7A
33	57101	Juvenile	Male	52g	Front Landing/ T9
34	**57126**	**Juvenile**	**Male**	**55g**	**Front Landing/ T9**
35	**28224**	**Male**	**Male**	**60g**	**West Clears/ T40**
36	**57133**	**Juvenile**	**Male**	**53g**	**West Clears/ T37A**
37	57125	Juvenile	Male	49g	West Clears/ T37
38	**28279**	**female**	**Female**	**63g**	**West Clears/ 32C**
39	57134	Juvenile	Male	55g	West Clears/ 32C
40	**51804**	**Female**	**Female**	**62g**	**Clears/ T32**
41	57135	Juvenile	Female	55g	Clears/ T32
42	57107	Juvenile	Male	63g	Clears/ T32
43	57136	Juvenile	Male	48g	Clears/ T32
44	57127	Juvenile	Female	49g	Clears/ T30A
45	57114	Juvenile	Female	55g	Clears/ T36
46	**57137**	**Male**	**Male**	**61g**	**Clears/ T36**
47	**57138**	**Juvenile**	**Female**	**57g**	**Whalers Bay Wst/ T10**
48	57121	Juvenile	Male	53g	WB/ Island Point/ T11A
WR01	**C61801**	**Male**	**Male**	**66g**	**Western Reef**
WR02	**C61802**	**Male**	**Male**	**67g**	**Western Reef**
WR03	**C61803**	**Male**	**Male**	**64g**	**Western Reef**
WR04	**C61805**	**Male**	**Male**	**65g**	**Western Reef**

A.



B.

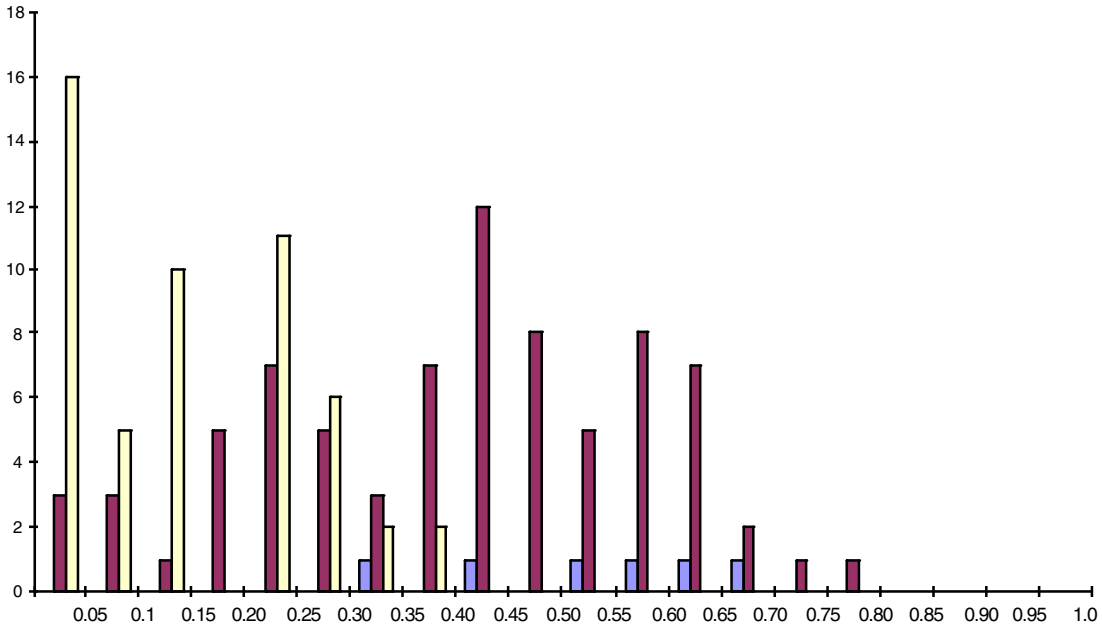


Figure 2A and B. Levels of minisatellite DNA bandsharing between individuals from Rangatira Island (dark bars), individuals from Western Reef (medium grey bars), and between individuals from Rangatira compared with the Western Reef (light bars). X axis=proportion of bands shared, Y axis=frequency. The gel/probe combinations are as follows: A.= SP1, pV47-2. B.= SP1, *per*.

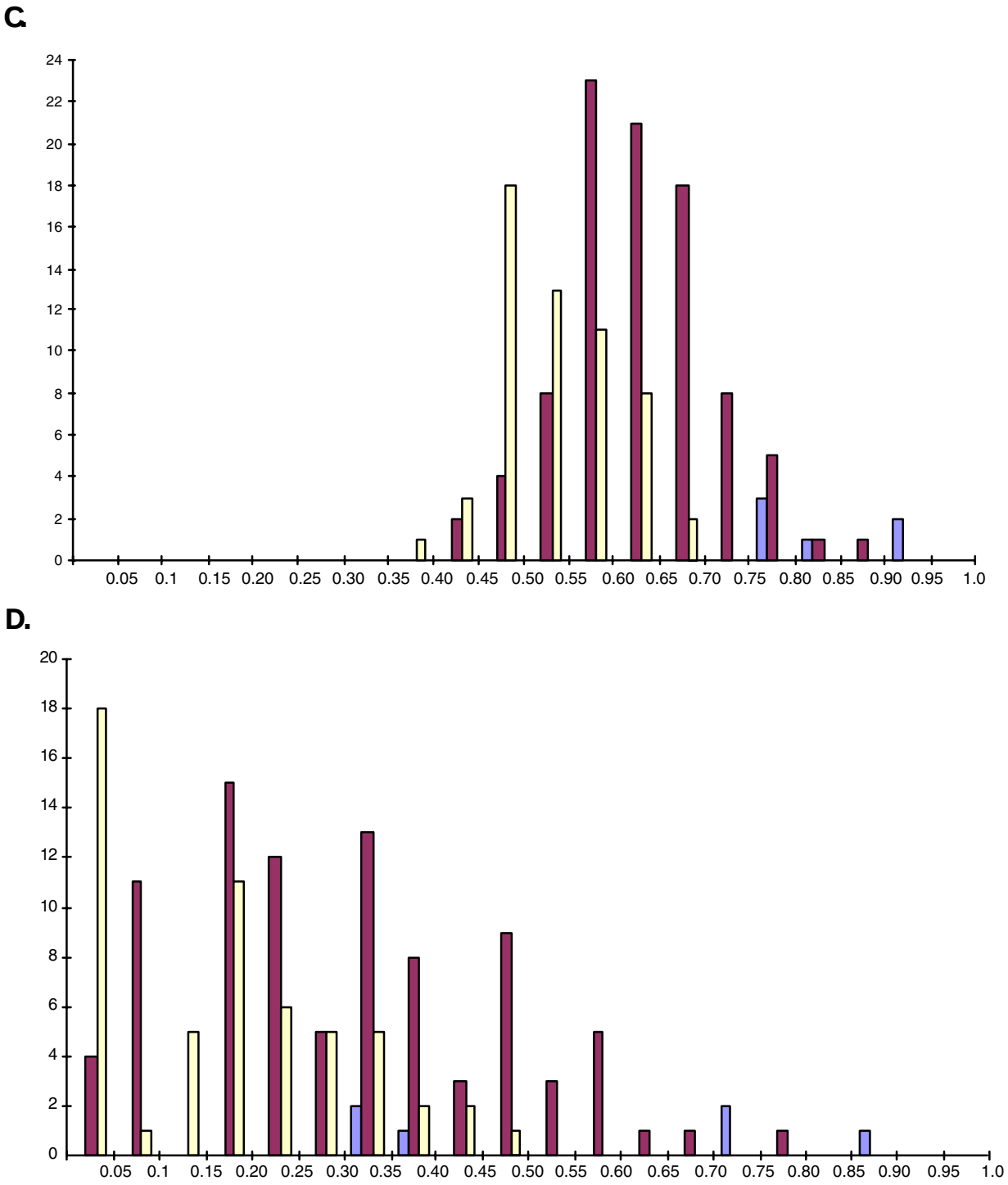


Figure 2C and D. Levels of minisatellite DNA bandsharing between individuals from Rangatira Island (dark bars), individuals from Western Reef (medium grey bars), and between individuals from Rangatira compared with the Western Reef (light bars). X axis=proportion of bands shared, Y axis=frequency. The gel/probe combinations are as follows: C.= SP2, pV47-2. D.= SP2, *per*.

TABLE 2. BANDSHARING (STANDARD ERROR) BETWEEN MINISATELLITE pV47-2 AND *per* DNA PROFILES OF SHORE PLOVER FROM RANGATIRA ISLAND POPULATION AND THE WESTERN REEF POPULATION.

GEL SP.1		RANGATIRA ISLAND	WESTERN REEF
Rangatira	pV47-2	(0.012)	(0.011)
	per	0.406 (0.021)	0.139 (0.016)
	33.15		
Western Reef	pV47-2		(0.030)
	per		0.525 (0.055)
	33.15		
GEL SP.2.		RANGATIRA ISLAND	WESTERN REEF
Rangatira	pV47-2	(0.009)	(0.010)
	per	0.297 (0.019)	0.164 (0.019)
	33.15		
Western Reef	pV47-2		0.830 (0.027)
	per		0.543 (0.098)
	33.15		

TABLE 3. NUMBER OF BANDS AND BANDSHARING INDICES FOR PAIRWISE COMPARISONS OF PRESUMPTIVE UNRELATED INDIVIDUALS BELONGING TO A NUMBER OF NEW ZEALAND AVIAN SPECIES. ALL SAMPLES WERE DIGESTED WITH *Hae*III RESTRICTION ENZYME.

SPECIES	NO. SAMPLED (COMBINATIONS)	MINISATELLITE PROBE USED	MEAN NO. OF BANDS SCORED	MEAN BANDSHARING INDEX (D)	REFERENCE
Shore plover (*Thinornis novaseelandiae*)					
Rangatira Island	13 (78)	pV47-2	12	0.714	This study
		per	10	0.406	
Western Reef	4 (6)	pV47-2	14	0.828	
		per	7.5	0.525	
Auckland Island teal (*Anas aucklandica aucklandica*)					
	16 (120)	pV47-2	15	0.71	Lambert & Robins
South Polar skua (*Catharacta maccormicki*)					
	33	pV47-2	26	0.28	Millar et al. 1994
Black robin (*Petroica traversi*)					
	15	pV47-2	3	0.84	Ardern & Lambert 1997; Ardern et al. 1997
South Island robin—Motuara Island (*Petroica australis australis)*					
	17	pV47-2	8	0.62	Ardern & Lambert 1994; Ardern et al. 1997
North Island robin (*Petroica australis longipes*)					
	15 (27)	pV47-2	35	0.37	Ardern & Lambert 1994; Ardern et al. 1997
Pukeko (*Porphyrio porphyrio melanotus*)					
	17	pV47-2	18	0.6	Lambert et al. 1994
	17	*per*	5	0.228	

4. Discussion

Mean bandsharing levels within each population of shore plover are relatively high when compared with other New Zealand bird species (Table 3). The low level of DNA variation seen in shore plover is also seen in other endemic Chatham Islands species including the black robin (Ardern & Lambert 1997, Ma & Lambert 1997) and the Chatham Island tomtit. The Chatham Island tomtit is not endangered but still shows extremely high levels of bandsharing, ranging from 0.79 ± 0.02 (SE) for the minisatellite probe 33.6, to 0.93 ± 0.07 (SE) for the Bkm probe (Ma & Lambert 1997). This suggests that this is a general feature of some avian species comprising the Chatham Island biota.

When the level of bandsharing between shore plover from the Rangatira and Western Reef populations was measured it was found to be lower than within each population. The extent of differentiation between the two populations was investigated by calculating Wright's index of population subdivision (F_{ST}), which is defined to be the fraction of total gene diversity that is attributable to population differentiation. The F_{ST} value is equal to one when the populations are fixed for different alleles (completely differentiated), and zero when there is no subdivision between populations (Lynch 1991). Wright (1978) suggested that values above 0.15 were characteristic of 'greatly differentiated' populations. In this study we recorded an F_{ST} value of 0.382 indicating that the Rangatira Island and western reef populations are greatly differentiated and that there is little movement of birds between the two locations.

The level of gene flow between populations can be estimated from the spatial distribution of genetic markers using several statistical approaches. The most common theoretical model of population structure which underlies these approaches is the 'island model'. This model assumes a species is divided into 'islands' of equal size, all of which are equally likely to exchange alleles. The 'stepping stone' model assumes only adjacent 'islands' exchange genes. Assuming alleles have neutral selective advantages in an island model, Nm, the absolute number of individuals exchanged between populations per generation can be estimated as:

$$Nm \cong (1 - F_{ST}) / (4 F_{ST})$$

In the case of shore plover, the F_{ST} of 0.382, suggests 0.404 migrants per generation between the Western Reef and Rangatira Island populations.

Although this approach is numerical it, nevertheless, represents a qualitative measure of gene flow. Theoretically an average exchange of one individual per generation ($Nm \cong 1$) is marginally sufficient to prevent dramatic genetic differentiation by genetic drift alone (Allendorf 1983). Hence the recorded value of 0.404 migrants per generation is well below the value necessary to prevent divergence due to genetic drift.

Additional evidence of a lack of migration of individuals between the two populations under investigation derives from the DNA profiles. Two sets of restriction fragments, one in the *per* profiles, and one in pV47-2 (Fig. 1) were found only in birds from the Western Reef. As these bands were not detected in

any of the 27 samples from Rangatira Island they may be useful markers for the Western Reef population, and hence could be used for the identification of unknown individuals. Assuming that the *per* and pV47-2 fragments, are diagnostic of Western Reef populations, none of the 27 birds from Rangatira were from Western Reef. In order to verify that these bands are present in all Western Reef birds, and not just the four birds sampled here (who may be closely related), further sampling will be required. There also appears to be a segregating locus of restriction fragments in the *per* minisatellite profiles of birds from Rangatira Island. These are not present, however, in any of the Western Reef birds (Fig. 1A, bands between 8.1–12.2 kb).

The level of panmixus in shore plover populations prior to the introduction of cats, Norway and ship rats, etc. to the Chatham Islands 160–200 years ago, cannot be precisely known. Certainly, given the observations that individuals of this species are sedentary and adults rarely move away from their territories, panmixia seems highly unlikely. Our results are consistent with the Western Reef and Rangatira populations representing remnants of a once highly structured series of populations that had minimal levels of migration between them, even in their past history. This current structure may have been remnant of an originally more diverse series of genetically divergent populations.

It is also possible that, given the high rate of mutation of minisatellite DNA, these populations diverged even since the introduction of pests. Although there is no direct evidence for founder events, and these could only be postulated, a population as small as the Western Reef one is almost certainly been affected by genetic drift. Whatever the time of origin of the observed divergence of populations, and whether they represent remnants of a once highly structured population, the issue of the conservation of these genetic diversity is of interest to the Department of Conservation. The trend towards lower bandsharing between populations compared with bandsharing within each population, coupled with a high F_{ST} value, and the presence of the above markers for the Western Reef population suggests that these populations of shore plovers are genetically distinct.

Several management strategies are logically possible:

1. The Western Reef and Rangatira populations are merged into one large one. That is, birds from Western Reef population are relocated to Rangatira Island. Since the shore plover recovery programme has focused on a reintroduction programme in New Zealand since the early 1990s, with the goal of establishing a second self-sustaining population; this option seems counter productive. Also, there are potential risks in having only one population as opposed to having two.
2. That 'scarce genes' should be 'salvaged' by reciprocal transfers of individuals between both populations. This approach would have the advantage of making available novel DNA sequences to each population. However, if continued, this would act to genetically homogenize the two populations, finally resulting in two populations with very similar genetic characteristics. Hence if individuals from one population were, at some later time, susceptible to, for example, an invading pathogen, then perhaps individuals from both populations would also have a similar characteristic. This would potentially increase

the population's susceptibility to a novel environmental challenge, e.g. the introduction of a new pathogen.

3. Both Western Reef and Rangatira populations are left as they are and monitored until additional information, e.g. sex ratios and levels of genetic variation in the Western Reef population, can be obtained. None of the genetic data collected as part of this study suggests that these populations warrant subspecies status in taxonomy.

5. Summary

(For a list of the numbered proposals to which this numbered summary refers, see section 1, the Introduction.)

1. & 2. All individuals supplied were sexed and these are detailed in Table 1.
3. DNA (from samples indicated in **bold**, Table 1) was extracted, digested and probed with minisatellite probes pV47-2 and *per.*
4. & 5. Resulting minisatellite profiles were digitised, and statistical details of these results are given in Table 2.
6. Levels of minsatellite variation detected are compared to that found in other New Zealand avian taxa (Table 3).
7. This report completes the original project, as requested.

6. References

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Appendix 1

PRESENCE/ABSENCE OF BANDS FOR EACH GEL AND PROBE

A. Gel SP1, pV47-2

Samples →	W1	W2	W3	W4	1	2	5	10	11	15	18	22	35	6	12	19	25
1	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0
2	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0
4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
5	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0
6	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	1	0	1	0	0	1	1	0	0
9	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	1	0	1	0	1	0	0	1	1	0	0	0	0
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0
13	0	0	0	0	1	1	1	1	1	1	0	1	0	1	1	1	0
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
17	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
18	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
19	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0
20	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0
22	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
23	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
24	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1	1	1
25	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0
26	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
27	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
28	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0
29	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
30	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1

B. Gel SP2, pV47-2

Samples →	W1	W2	W3	W4	8	13	23	24	46
1	0	0	0	0	0	0	1	0	0
2	0	0	0	0	1	1	0	0	0
3	0	0	0	0	0	0	0	0	1
4	1	1	1	1	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	1	0
7	0	0	0	0	0	0	1	0	1
8	0	0	0	0	0	0	1	0	0
9	0	0	0	0	0	0	0	0	0
10	0	0	0	0	1	1	1	0	1
11	1	1	1	1	0	0	0	0	0
12	0	0	0	0	0	0	1	0	0
13	0	0	0	0	1	0	0	0	0
14	0	0	0	0	0	0	0	1	1
15	1	1	0	1	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	1	0
20	1	1	1	1	1	1	1	1	1
21	1	1	1	1	0	1	0	0	1
22	0	0	0	0	0	1	0	0	1
23	1	1	1	1	1	1	1	1	1
24	0	1	1	0	0	0	0	0	0
25	0	0	0	0	0	1	0	0	1
26	1	1	1	1	1	1	1	1	1
27	0	1	1	1	1	1	1	1	1
28	0	1	1	1	0	0	0	0	1
29	1	1	1	1	1	0	0	1	1
30	1	1	1	1	1	1	1	1	1
31	0	0	0	1	0	0	0	0	0
32	0	0	0	0	0	0	1	0	1
33	1	1	1	1	0	0	0	1	0
34	0	0	0	0	1	1	1	0	0
35	1	1	0	1	0	0	0	1	1
36	0	0	0	0	0	0	0	0	0
37	0	1	1	1	0	1	1	1	1
38	0	0	0	0	1	0	0	0	0
39	1	0	1	0	0	0	0	0	0
40	0	0	0	1	1	0	1	0	1
41	1	1	1	1	1	1	1	1	1
42	0	1	1	1	0	0	0	0	0

C. Gel SP1 *per*

	Samples →																
	W1	W2	W3	W4	1	2	5	10	11	15	18	22	35	6	12	19	25
1	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
3	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0
4	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0
11	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	0	0
12	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	1	1
13	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
17	0	0	0	0	1	0	1	0	1	1	1	1	0	0	1	1	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
19	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
23	1	1	0	0	1	1	0	1	1	1	0	1	0	0	0	0	0
24	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	1	1
25	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
26	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1
27	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0
29	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	0
31	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
32	0	0	0	0	1	1	1	0	1	1	1	0	1	1	1	0	0
33	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
34	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	0
35	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
36	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0
38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
39	0	0	0	0	0	1	0	1	1	1	0	1	0	1	1	0	0
40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
41	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0
43	1	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0
44	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
45	0	0	0	0	1	1	1	1	0	1	0	1	0	1	1	0	0
46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

D. Gel SP2 *per*

	Samples →								
	W1	W2	W3	W4	8	13	23	24	4
1	0	0	0	0	0	1	1	1	
2	0	0	0	0	0	0	0	0	
3	0	0	0	0	1	1	0	0	
4	0	0	0	0	1	0	0	0	
5	1	0	0	0	0	0	0	0	
6	0	0	0	0	0	0	0	0	
7	0	0	0	0	1	1	1	0	
8	0	0	0	0	0	0	0	0	
9	0	1	0	1	0	0	0	0	
10	0	0	0	0	0	0	0	0	
11	1	1	1	1	1	0	1	1	
12	0	0	0	0	0	0	0	0	
13	0	0	0	0	0	0	1	0	
14	0	0	0	0	0	0	0	0	
15	0	0	0	0	0	0	0	0	
16	0	0	0	0	1	1	0	0	
17	0	0	1	1	0	0	1	1	
18	1	0	0	0	0	0	0	0	
19	0	1	1	1	0	0	0	0	
20	1	0	0	0	0	0	0	0	
21	0	1	0	0	1	1	0	0	
22	0	0	0	0	0	0	0	0	
23	0	0	0	0	0	0	1	0	
24	0	0	0	0	1	1	0	1	
25	0	0	0	0	0	0	0	0	
26	0	0	0	0	0	0	1	1	
27	0	0	0	0	0	0	0	0	
28	0	0	0	0	0	1	0	0	
29	0	0	0	0	0	0	1	0	
30	0	0	0	0	0	1	1	0	
31	0	0	0	0	1	0	0	0	
32	0	0	0	0	0	0	0	1	
33	0	0	0	0	0	0	0	0	
34	0	0	0	0	0	0	0	0	
35	0	1	1	0	1	1	1	1	
36	0	0	0	0	0	0	0	0	
37	0	1	1	1	0	1	0	0	
38	0	0	0	0	0	0	0	0	
39	1	1	0	0	0	0	1	0	
40	0	1	1	1	1	1	0	0	
41	0	0	0	0	0	0	0	0	
42	0	0	0	0	0	1	1	1	
43	0	0	0	0	1	0	0	0	
44	0	0	0	0	0	0	0	1	
45	0	1	0	0	0	0	0	0	
46	0	0	0	0	0	0	0	0	
47	1	1	1	1	0	0	0	1	
48	0	0	0	0	1	1	1	0	

Appendix 2

INDIVIDUAL PAIR-WISE COMPARISONS

(Band sharing coefficients for each pair of lanes)

A. Gel SP1, pV47-2

	W1	W2	W3	W4	1	2	5	10	11	15	18	22	35	6	12	19	25
W1		0.769	0.720	0.815	0.435	0.400	0.417	0.455	0.435	0.476	0.545	0.435	0.609	0.480	0.435	0.455	0.571
	W2		0.897	0.903	0.519	0.483	0.500	0.615	0.593	0.560	0.538	0.519	0.519	0.621	0.593	0.615	0.720
		W3		0.867	0.538	0.500	0.519	0.640	0.615	0.583	0.640	0.538	0.615	0.643	0.615	0.640	0.667
			W4		0.500	0.467	0.483	0.593	0.571	0.538	0.593	0.500	0.571	0.600	0.643	0.593	0.769
				1		0.846	0.960	0.870	0.833	0.909	0.609	0.750	0.583	0.615	0.667	0.696	0.636
					2		0.815	0.800	0.692	0.833	0.560	0.615	0.615	0.714	0.615	0.720	0.583
						5		0.833	0.800	0.870	0.583	0.800	0.560	0.667	0.640	0.667	0.609
							10		0.870	0.952	0.636	0.696	0.522	0.720	0.783	0.818	0.762
								11		0.818	0.696	0.750	0.583	0.769	0.833	0.783	0.727
									15		0.667	0.727	0.545	0.667	0.727	0.762	0.700
										18		0.696	0.696	0.640	0.783	0.727	0.667
											22		0.667	0.692	0.667	0.696	0.636
												35		0.692	0.500	0.609	0.545
													6		0.769	0.800	0.667
														12		0.870	0.818
															19		0.762
																25	

B. Gel SP2, pV47-2

	W1	W2	W3	W4	8	13	23	24	46	4	20	27	38	40	29	34	36	47
W1		0.800	0.759	0.774	0.462	0.462	0.357	0.615	0.500	0.414	0.462	0.467	0.571	0.467	0.519	0.538	0.519	0.563
	W2		0.909	0.914	0.467	0.533	0.438	0.667	0.611	0.485	0.467	0.588	0.500	0.529	0.581	0.533	0.452	0.611
		W3		0.824	0.483	0.552	0.452	0.621	0.571	0.500	0.483	0.606	0.581	0.545	0.600	0.552	0.533	0.686
			W4		0.516	0.516	0.485	0.645	0.649	0.529	0.452	0.629	0.485	0.514	0.563	0.516	0.438	0.595
				8		0.692	0.643	0.538	0.563	0.690	0.615	0.533	0.571	0.533	0.593	0.692	0.519	0.625
					13		0.643	0.538	0.688	0.621	0.615	0.667	0.571	0.533	0.667	0.615	0.667	0.813
						23		0.500	0.647	0.710	0.643	0.625	0.600	0.563	0.552	0.429	0.621	0.588
							24		0.625	0.552	0.615	0.600	0.571	0.533	0.667	0.615	0.444	0.500
								46		0.800	0.750	0.778	0.588	0.611	0.667	0.500	0.545	0.737
									4		0.897	0.727	0.710	0.667	0.600	0.621	0.667	0.686
										20		0.667	0.714	0.733	0.667	0.692	0.667	0.625
											27		0.563	0.588	0.645	0.600	0.581	0.667
												38		0.563	0.552	0.571	0.759	0.647
													40		0.774	0.800	0.645	0.556
														29		0.741	0.500	0.667
															34		0.593	0.563
																36		0.606
																	47	

C. Gel SP1, *per*

	W1	W2	W3	W4	1	2	5	10	11	15	18	22	35	6	12	19	25
W1	0.533	0.429	0.308	0.308	0.353	0.267	0.118	0.250	0.105	0.000	0.125	0.000	0.000	0.000	0.211	0.211	
	W2	0.588	0.625	0.250	0.300	0.222	0.100	0.316	0.091	0.000	0.105	0.000	0.000	0.000	0.273	0.273	
		W3	0.667	0.133	0.211	0.235	0.105	0.111	0.095	0.000	0.000	0.000	0.000	0.000	0.286	0.286	
			W4	0.143	0.222	0.250	0.222	0.235	0.100	0.000	0.000	0.000	0.111	0.105	0.400	0.100	
				1	0.444	0.625	0.444	0.588	0.500	0.400	0.471	0.429	0.444	0.421	0.300	0.100	
					2	0.600	0.455	0.571	0.500	0.211	0.286	0.111	0.273	0.435	0.250	0.250	
						5	0.400	0.632	0.545	0.588	0.316	0.250	0.300	0.476	0.545	0.182	
							10	0.571	0.750	0.421	0.571	0.444	0.636	0.609	0.250	0.083	
								11	0.696	0.556	0.500	0.353	0.381	0.545	0.435	0.174	
									15	0.571	0.609	0.400	0.500	0.640	0.231	0.154	
										18	0.444	0.533	0.421	0.700	0.381	0.095	
											22	0.353	0.476	0.545	0.261	0.000	
												35	0.778	0.421	0.200	0.000	
													6	0.609	0.250	0.000	
														12	0.320	0.160	
															19	0.308	
																25	

D. Gel SP2, *per*

	W1	W2	W3	W4	8	13	23	24	46	4	20	27	38	40	29	34	36	47
W1	0.375	0.308	0.308	0.105	0.000	0.211	0.235	0.111	0.200	0.211	0.211	0.000	0.000	0.000	0.000	0.111	0.222	
	W2	0.706	0.706	0.348	0.333	0.261	0.286	0.364	0.417	0.174	0.174	0.000	0.000	0.000	0.000	0.091	0.182	
		W3	0.857	0.300	0.286	0.300	0.444	0.316	0.476	0.200	0.200	0.000	0.000	0.000	0.000	0.000	0.105	
			W4	0.200	0.190	0.200	0.333	0.316	0.381	0.200	0.200	0.000	0.000	0.000	0.000	0.105	0.211	
				8	0.667	0.308	0.333	0.160	0.370	0.231	0.231	0.160	0.154	0.000	0.087	0.160	0.240	
					13	0.444	0.400	0.308	0.429	0.296	0.370	0.154	0.296	0.250	0.167	0.231	0.308	
						23	0.500	0.320	0.519	0.231	0.154	0.320	0.077	0.174	0.087	0.160	0.320	
							24	0.435	0.480	0.167	0.250	0.087	0.000	0.095	0.000	0.000	0.174	
								46	0.385	0.400	0.480	0.333	0.160	0.182	0.091	0.083	0.083	
									4	0.593	0.593	0.231	0.074	0.167	0.083	0.077	0.308	
										20	0.769	0.400	0.231	0.261	0.261	0.320	0.320	
											27	0.240	0.231	0.348	0.174	0.320	0.240	
												38	0.560	0.364	0.364	0.500	0.583	
													40	0.609	0.522	0.560	0.480	
														29	0.500	0.455	0.455	
															34	0.545	0.273	
																36	0.500	
																	47	