Molecular Genetic (RAPD) Analysis of Leach's Storm Petrels (*Oceanodroma leucorhoa*) From Three Breeding Islands in Atlantic Canada

by

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Abstract

Leach's storm petrel (*Oceanodroma leucorhoa* Vieillot 1817) is one of the most populous seabirds breeding in maritime Atlantic Canada. Leach's storm petrel breed colonially on offshore islands, and exhibit strong site tenacity, returning to the same nest burrow each season, often with the same mate. I used random amplified polymorphic DNA (RAPD) to estimate population differentiation between three colonies, Bon Portage I., N.S., Big White I., N.S. and Gull I., Nf. Three oligonucleotide primers were used to identify 23 polymorphic loci within the genomic DNA of 96 individual petrels. Analysis of molecular variance (AMOVA) indicate small but statistically significant variance among groups (3.47%, $p < 0.001$, Nova Scotia and Newfoundland) and among colonies within groups (2.4%, $p = 0.038$, three nesting islands). Similarity and percent match estimates of genetic distance failed to recover colonial groupings. The observed structure is likely a result of high contemporary gene flow or a historical association following the most recent glaciation.

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Introduction

Leach's Storm Petrel, *Oceanodroma leucorhoa* Vieillot 1817, is the smallest breeding seabird in the North Atlantic (19-22cm long). This colonial bird is common offshore in Atlantic Canada during the summer breeding season but is seldom seen inshore. It breeds throughout the Northern Hemisphere; the primary western Atlantic breeding range extends from Maine to Newfoundland where petrel numbers have been estimated in the millions (Cairns *et al.* 1989). Main eastern Atlantic colonies include the Faeroes; Westmann I., Iceland; Lofoten I., Norway; and St. Kilda, Flannan I., North Rona, and Sula Sgeir in Scotland (Harrison 1983b).

Leach's Storm Petrels dig their nest burrows on offshore islands which are devoid of mammalian predators. They are nocturnally active at the breeding islands, returning to sea before first light or remaining in the burrow during the day. Leach's petrels share incubating and rearing duties, lay a single white egg each season, and seldom relay if this egg fails. These long lived seabirds (>36 years, Huntington *et al.* 1996) exhibit high site and mate fidelity; adults return to the same colony, often with the same mate and to the same burrow each season. Burrows are easy to monitor for activity by observing disturbance of a stick lattice placed across the burrow entrance, and the petrels' lack of physical defenses make them easy to handle. These features have contributed greatly to the documentation of the breeding habits of Leach's storm petrels (Gross 1935, Ainslie and Atkinson 1937, Wilbur 1969).

Many of these same features have impeded research into their population ecology. Individual Leach's petrels are indistinguishable without prior banding or colour marking.

At sea their small size makes them difficult to spot, and identification of marked individuals is nearly impossible. They are too small for all but the lightest, and therefore expensive, electronic devices. Their enormous numbers, exclusive nocturnal activity at the colony and burrow nesting behaviour would incur massive effort and cost in order to mark sufficient numbers for a large scale study. Such effort must be maintained for a number of years and at numerous colonies in order to identify patterns of dispersal and migration. Fortunately, recent advances in molecular genetics have introduced novel ways of investigating population ecology allowing researchers to address population questions directly through genetic analysis. To date, there has been only one published report of DNA analysis of Leach's storm petrel in which Mauck *et al.* (1995) used DNA fingerprinting to investigate monogamy.

The recent American Ornithologist Union publication on Leach's Storm Petrels (Huntington *et al.* 1996) identifies some priorities for future research, which include "(improved knowledge of)...wintering grounds of individuals and populations or fidelity to wintering grounds", the differences between large central colonies and peripheral colonies, and if there are "consistent genetic differences between colonies". Molecular genetic techniques can be used to infer relatedness based on differences in genetic variability at all levels of population structure, from identifying individuals to generating phylogenies and may be the only practical way of approaching such questions in pelagic seabirds.

I used the PCR, RAPD (polymerase chain reaction, randomly amplified polymorphic DNA) technique to investigate Leach's petrels from three breeding colonies.

This technique uses short, arbitrary oligonucleotide primers to target unspecified regions of the genomic DNA. The PCR produces DNA fragments which are visualized using electrophoresis, resulting in banding patterns which are analysed as phenotypic data. The use of RAPD analyses in population ecology has proven effective for a range of taxa since the introduction of the technique by Williams *et al.* in 1990 (e.g. Castiglione *et al.* 1993, Tibayrenc *et al.* 1993, Haig *et al.* 1994, Patwary *et al.* 1994). RAPD analyses can be conducted without prior knowledge of the target genome, which makes them relatively inexpensive and much less time consuming than most other techniques. RAPD also requires minimal amounts of DNA, which allows for non-destructive sampling. These attributes make the RAPD technique appropriate for an introductory analysis of the genetic divergence between western Atlantic petrel colonies.

Methods and Materials

Collection Sites

Samples were collected during the breeding season from two Nova Scotian islands in 1995, and one Newfoundland island in 1996 (Table 1, Fig. 1).

Table 1. Leach's Petrel colonies sampled in the 1995 and 1996 breeding seasons.

Island	Location	Lat/Long	No. Samples Collected	Estimated Breeding Pairs
Bon Portage	Shelburne Co.N.S.	43° 28' N, 65° 45' W	N= 36	54 000[1]
Big White	Halifax Co., N.S.	45° 54' N, 62° 08' W	N= 34	30 000[2] *
Gull Island	Witless Bay, Nf.	47° 15.8'N, 52° 46.3'W	N= 34	530 000[3]

1. MacKinnon 1988., 2. M. O'Brien pers. comm., 3. Cairns *et al.* 1989.
*no census, rough estimate based largely on comparison to Bon Portage census.

Blood Collection:

Petrels were captured in 50 mm mesh mist nets at night or removed from their nest burrows by hand during the day. The brachial vein was exposed using an alcohol tipped swab and pierced with a 30 gauge needle. Welling blood was collected in 100 µl haematocrit tubes and immediately placed in 500 µl lysis buffer (1.25 % w/v sodium dodecal sulfate, 0.3 M Tris-HCl pH 8, 0.1 M EDTA pH 8, 5 % w/v sucrose, Jowett 1986). Blood flow usually stopped prior to collection of a full 100 µl, while on occasion slight pressure with a sterile swab was necessary to stop the flow. Mist net captured birds were banded and held individually in cotton bags for at least 20 min prior to release to ensure clotting and to prevent wing strain. Birds caught in burrows were banded and returned to their burrow immediately following blood collection. Lysed blood was refrigerated as soon as possible and held at 4 ^{o}C until the DNA was extracted.

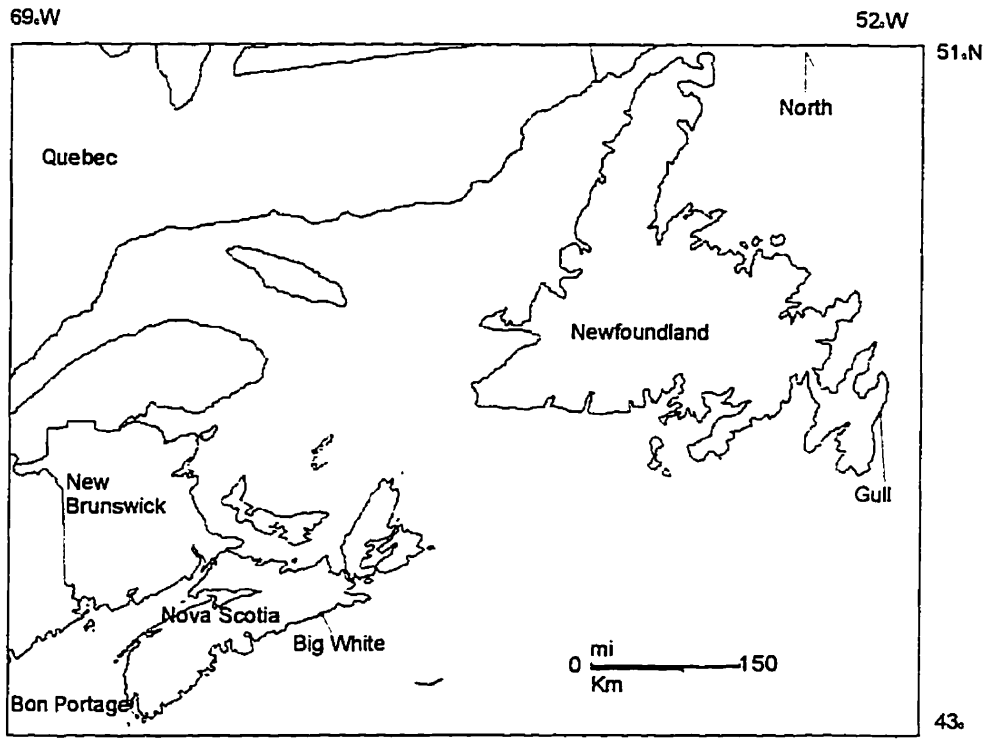

Figure 1. Study islands where petrel blood samples were collected during the 1995 (N.S.) and 1996 (Nf.) breeding seasons.

DNA Extraction

DNA extraction followed a modified protocol from Jowlett (1986). Samples were incubated for at least 30 min at 60 °C, agitated with 350 µl Tris-equlibrated phenol (pH 8, 0.1 % 8-Hydroxyquinoline, Maniatis *et al.* 1982) and 100 µl $CHCl_3$ then centrifuged (13 600 rpm) for 20 min in a Fisher Scientific Microcentifuge model 235C. The supernatant was removed to a sterile tube, agitated with 500 µl of 24:1 $CHCl_3$-isoamyl alcohol, and spun for 30 s. The supernatant was then removed to a sterile tube, agitated with 500 µl $CHCl_3$, spun 30 s and the supernatant removed again, mixed gently with 500 µl isopropyl alcohol, incubated at least one hour at -20 °C then centrifuged for 20 min. The

isopropanol was discarded and the pellet washed gently with 50 µl of 70 % ethanol. The pellet was left to air dry by covering the tube with parafilm, punching holes in the film with a sterile needle and placing the tube in a 35 ^{o}C incubator.

Samples collected in 1995 were extracted at least two months after collection and produced an average of 200 µg DNA per sample. Samples from 1996 were extracted within two weeks of collection, formed a gelatinous pellet, yielded less than 20 µg DNA per sample and produced inconsistent banding under PCR. The PCR results were corrected by re-treating the 1996 samples with 500 µl lysis buffer, incubating at 35 ^{o}C for 48 hours and then proceeding with the preceding phenol-chloroform protocol.

Yields

Pelleted DNA was resuspended in 50 µl TE (10 mM Tris-HCL pH 8, 1 mM EDTA pH 8) and visualized on 1 % w/v agarose gels by running 2 µl suspended DNA, 3 µl load-stop dye (0.05 % Bromophenol blue, 40 % w/v sucrose, 100 mM EDTA, Maniatis *et al.*1982) and 5 µl ddH_2O (10 µl total) at 115 volts for 0.1 hours, then 80 v for approximately 1.0 h. The DNA concentration was estimated based on the band intensity compared to the intensity of the λ HindIII molecular weight marker. Gels were made using electrophoresis grade agarose, 0.5X TBE (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA pH 8, Sambrook *et al.* 1989) and between 0.002-0.004 µg / ml ethidium bromide (EtBr). Stock DNA solutions were diluted to approximately 400 ρg DNA / µl with ddH_2O. The use of the term 'DNA' during reactions refers to stock dilutions.

Polymerase Chain Reaction

DNA was amplified using the PCR RAPD technique (Williams *et al.* 1990). Optimized reactions included 1.2 ng DNA, 1.25 µl 10X buffer (800 mM Tris-HCl pH 9, 200 mM $(NH_4)_2SO_4$, 35 mM MgCl, 1.5 mg / ml BSA), 80 µM each dNTP, 41 ng primer (University of British Columbia, Nucleic Acid - Protien Service Unit, Dr. J.B. Hobbs, Director), 0.5 units Appligene Taq polymerase and ddH_2O to a final volume of 12.5 µl. Reactions were covered with light mineral oil to prevent evaporation and placed in an MJ Research Inc. PTC-100 thermocycler for an initial 2 min at 93 ^{o}C, then cycled 40 times through 1 min at 93 ^{o}C, 1 min at 35 ^{o}C, and 2 min at 72 ^{o}C, then 5 min at 72 ^{o}C and indefinite hold at 4 ^{o}C. Reactions were stopped by adding 3 µl load-stop dye.

Amplification products were run on 1.7 % or 1.4 % agarose gels for 0.1 h at 115 v then 3 h at 90 v with Promega pGEM molecular weight marker. Gels were viewed on a Spectroline Transilluminator TR302 UV light table and photographed with Polaroid 667 or 665 film. Gels were remelted and reused until the point when the amount of dispersed DNA caused the background fluorescence to be too bright for high quality photographs.

Ninety-one UBC decamer primers were scanned using DNA from a single individual to identify primers which produced bands with petrel DNA. Twenty primers which produced the clearest banding patterns were scanned using DNA from two individuals from each Nova Scotia colony to identify primers which produced consistent banding patterns. Data collection proceeded using four primers which met this criterion.

Samples were run in duplicate during each PCR, and each reaction was run twice to ensure repeatability. Banding patterns were scored if they were consistent across all

four gel lanes per individual and were not present in the control lanes. Occasional bands in the control lanes were ignored if they did not correspond to the pattern observed in the experimental lanes. If control bands and sample bands coincided, the entire PCR was rerun.

Statistical Analysis

RAPD bands were scored as present (1) or absent (0) for each individual and for each primer (Appendix). RAPDPLOT (Black, 1995) was used to construct two types of genetic similarity matrices. One is based on the Nei and Li (1985) similarity index:

$$S = 2NAB / (NA + NB)$$

where NAB is the number of bands individuals A and B share, NA and NB are the number of bands in each individual. The other based on a percent match:

$$M = NAB / NT$$

where NAB is the number of matches (both present and absent matches), and NT is the total number of loci (Black 1995). These matrices were consequently used as input to the NEIGHBOR program of PHYLIP 3.5c (PHYLogeny Inference Package, Felsenstein, 1993) to calculate phylogenies (distance measures) based on the Saitou and Nei (1987) neighbor joining method, from which dendrograms were produced. The PHYLIP, CONSENSE program was used to construct a single consensus tree for each of the two similarity measures.

Analysis of molecular variance (AMOVA) was performed using the WINAMOVA program of Excoffier *et al.* (1992) based on Euclidian distance. The input matrix of

Euclidean distances was formed using a Qbasic program (T. Wilson, unpublished program). Wright's F statistics for population subdivision were also calculated using WINAMOVA. Wright's F statistics are fixation coeffiecients which indicate the relationship between the variance components of a population. They are expressed at three levels of complexity in relation to individuals, subpopulations and total population, indicated by the subscripts I, S, and T respectively. F_{ST} or fixation index, is the most commonly used F statistic and reflects the reduction of heterozygosity in a subpopulation relative to the total. The value can range from 0 (totally random mating, subpopulations are in Hardy-Weinburg equilibrium) to 1 (subpopulations are completely differentiated). F_{IS} measures the inbreeding of individuals relative to the subpopulation and F_{IT} measures the degree of inbreeding of an individual relative to the total population. F_{IS} is expected to be near zero in natural populations (indicating random mating) and if F_{IS} is 0 then $F_{IT} = F_{ST}$ (Slatkin 1985). Random mating is often assumed for natural populations, as a result most studies report only F_{ST} values.

Number of migrants per generation (Nm) was calculated using Wright's island model equation (from Hartl and Clark 1989):

$$F_{ST} = 1 / 4\,Nm + 1$$

where $F_{ST} = F_t$ = the probability that two alleles chosen at random within the same population in generation *t* are identical by descent, N is the number of individuals and m is the migration rate.

Results

Of the 91 primers scanned, 68 (75 %) produced potentially scorable banding patterns. Secondary scans of twenty primers resulted in four primers being chosen for data collection: 389, 370, 376 and 333 (Table 2). Because samples collected from Newfoundland in 1996 did not produce consistently scorable patterns with primer 333, this primer was eliminated from subsequent analysis. Consistent banding was not obtained for two samples (GL20 and BW01), these were also removed from the analysis.

Thirty-two loci were identified (Table 2) and scored for band presence (1) or absence (0) for 96 individual petrels. Nine alleles were present in all individuals, and ten alleles were present in frequencies too low to calculate error based on a normal approximation to binomial distribution.

Seventy-six haplotypes were identified, 67 of which were unique and 9 of which were represented by two or more individuals (Table 3).

Table 3. Petrels representing non-unique haplotypes from Bon Portage (BP), Big White (BW) and Gull (GL) Islands. Haplotype number designations were assigned sequentially as each type was discovered.

Haplotype	Samples	Haplotype	Samples	Haplotype	Samples
	BW49		BW63		BP09
	BW54	52	GL07	67	GL21
44	BW57		BW40		BP16
	BW58	54	GL12	70	BP28
	BW67		GL28		BW02
	GL11		BW37	72	BW42
	BW41		BW39		BW46
48	GL10	66	BW51		BP30
	GL19		BW52	74	BP33
	GL23		GL15		

Table 2. Band frequency for Leach's storm petrels at 32 loci, generated with three RAPD primers. Error (95% confidence) was calculated for neither homozygous alleles (p = 1, indicated with '-') nor alleles whose frequencies did not meet a normal approximation to binomial distribution (*). Fragment lengths are reported in base pairs (bp).

Primer Sequence	Fragment length (bp)	Frequency (p)	Error
376	1100	0.938	0.048
CAGGACATCG	1000	1.0	-
	800	1.0	-
	670	0.938	0.048
	640	0.792	0.081
	520	0.969	*
	460	0.958	*
	350	0.990	*
	330	0.344	0.095
	290	0.115	0.064
	270	0.969	*
	240	0.990	*
389	900	1.0	-
CGCCCGCAGT	800	1.0	-
	600	0.979	*
	560	1.0	-
	400	0.896	0.061
	380	1.0	-
	360	0.333	0.094
	300	1.0	-
	275	0.885	0.064
370	1150	0.615	0.083
TCAGCCAGCG	1000	0.781	0.083
	860	0.948	0.044
	750	0.969	*
	600	0.958	*
	520	0.969	*
	430	0.958	*
	300	0.927	0.052
	220	1.0	-
	150	1.0	-

The two genetic distance measures produced similar dendrograms. Consensus trees for similarity (Fig. 3) and percent match (Fig. 4) recovered neither populations (3 colonies) nor groups (2 regional groups, Nova Scotia and Newfoundland). Analysis of molecular variance shows 94.1 % ($p < 0.001$) of the observed variation occurs between individuals, while a low but significant fraction (3.5 %, $p = 0.038$) is accounted for among groups and among populations (2.4 %, $p < 0.001$, Table 4).

Table 4. Variance components of RAPD allele frequencies for Leach's storm petrels from two groups (Nova Scotia and Newfoundland) and three populations (Bon Portage I., Big White I., and Gull I.) calculated using AMOVA (Excoffoier *et al.* 1992).

Variance Component	Degrees Freedom	Variance	Percent of Variance	F Statistics	P (more extreme random value)
Among groups	1	0.066	3.5 %	$F_{ST} = 0.035$	< 0.001
Among populations / within groups	1	0.046	2.4 %	$F_{IT} = 0.059$	0.038
Within populations	93	1.79	94.1 %	$F_{IS} = 0.025$	< 0.001

Number of migrants per generation (Nm) was calculated as 6.9.

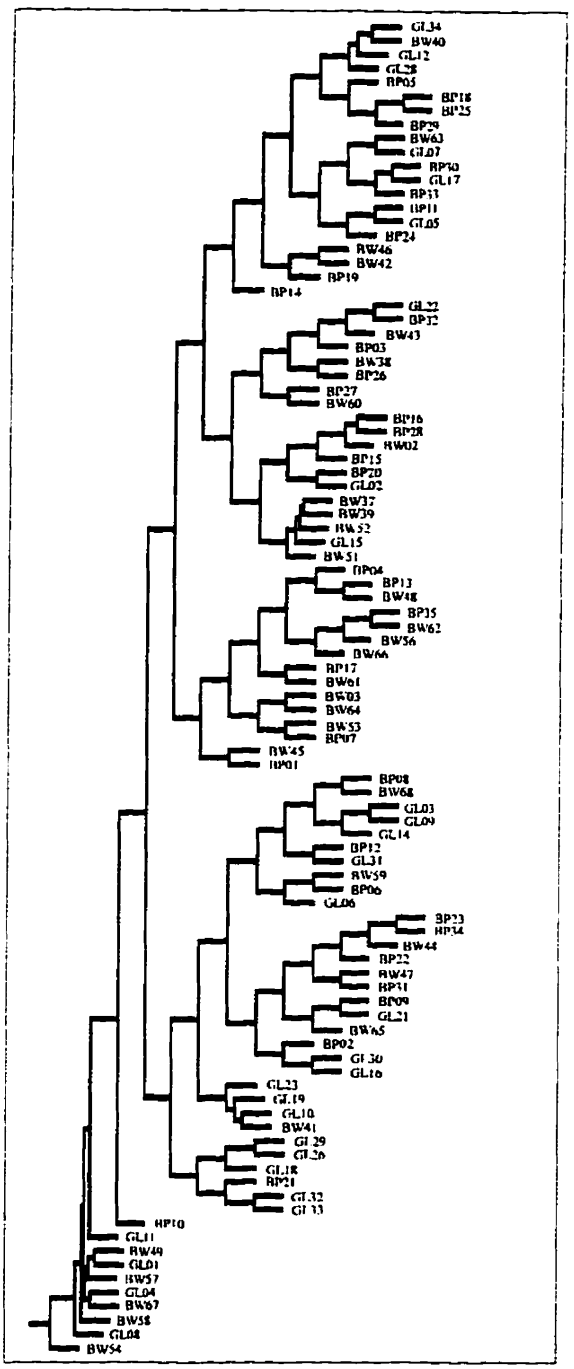

Figure 2. Consensus dendrogram of 96 Leach's storm petrels from 3 breeding islands (BP# = Bon Portage I., BW# = Big White I., GL# = Gull I.) based on Nei and Li (1985) similarity index S = 2NA / (NA + NB) where NAB is the number of bands individuals A and B share, NA and NB are the number of total bands per individual, respectively. This tree is a consensus of 100 Neighbor joining trees generated using the NEIGHBOR program in PHYLIP (Felsenstein 1993) using random input order.

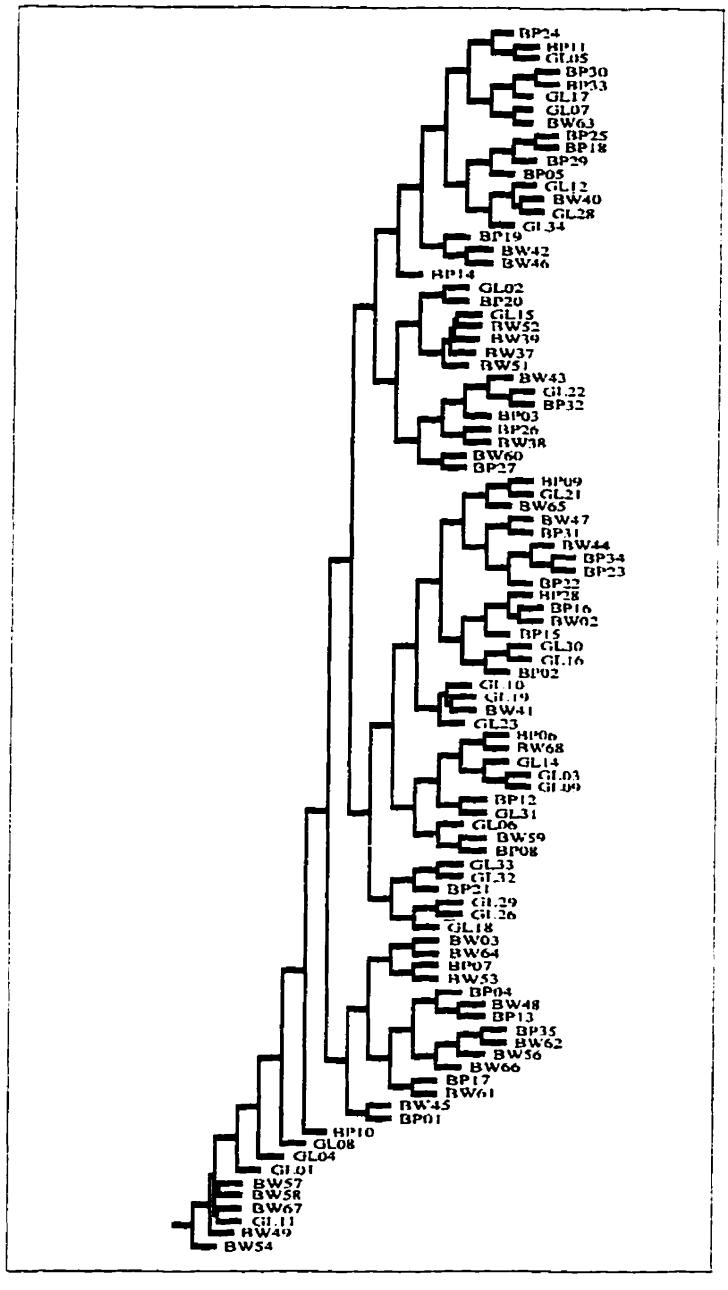

Figure 3. Consensus dendrogram of 96 Leach's storm petrels from 3 breeding islands (BP# = Bon Portage I., BW# = Big White I., GL# = Gull I.) based on the fraction of matches M = NAB / NT where NAB is the number of total matches in individuals A and B (both bands present and bands absent) and NT is the total number of loci scored (from Black 1995). This tree is a consensus of 51 Neighbor joining trees generated using the NEIGHBOR program in PHYLIP (Felsenstein 1993) using random input order.

Discussion

RAPD analysis has been proven to be an inexpensive but effective method of genetic analysis, capable of identifying numerous polymorphisms in a small amount of time. Initial concerns over reliability and repeatability have been addressed (e.g. Hadrys *et al.* 1992, Munthali *et al.* 1992, Micheli *et al.* 1994), and RAPD patterns have been shown to be repeatable between laboratories (Penner *et al.* 1993). The problems of dominance and the inability to differentiate between +/+ homozygotes and +/- heterozygotes have been discussed by Lynch and Milligan (1994) and Clark and Lanigan (1993) propose a method for estimating nucleotide divergence with RAPDs by correcting for dominance. Multivariate statistical methods have proven applicable to RAPD data sets, (e.g. Felsenstein 1993, PHYLIP; Rohlf 1993, NTSYS-PC; Excoffier *et al.* 1992, AMOVA) while others have been designed specifically for RAPD analysis (e.g. Black 1995, RAPDPLOT).

The major remaining concerns with RAPD analyses involve the inherent unknown binding sites of the primers, the risks of scoring comigrating non-homologous bands as alleles (Reiseburg 1996), and the concern whether shared band-absence can be used as a measure of similarity. For intraspecific comparisons, as in this study, the risk of incorrectly scoring co-migrating, non-allelic bands as alleles is minimal. This risk increases as the taxa in question become more genetically distant. Shared band absence was addressed by comparing one distance metric which scores 0 as a similarity, and one metric which does not.

The number of primers screened and number of polymorphisms scored varies greatly between studies (Table 5). I used three primers to analyse the three petrel colonies and scored 32 loci. This number of primers may seem low but RAPD loci segregate independently (> 95 %, Williams *et al.* 1990), allowing each band to be scored as a single locus.

Table 5. Some recent population studies using RAPD, the numbers of primers used and the numbers of polymorphic loci scored for analysis.

Study	Species Studied	Individuals sampled	Primers scanned	Primers Scored	Polymorphic Primers	Polymorphic loci
Ballinger-Crabtree *et al.* 1992	Mosquito	120	40	3	3	16
Haig *et al.* 1994	Woodpecker	101	34	14	13	13
Patwary *et al* 1994	Scallop	24	222	40	10	21
Gibbs *et al.* 1994	Rat Snake	43	20	4	4	25
Gibbs *et al.* 1994	Rattlesnake	18	16	4	4	9
This Study	Storm Petrel	96	91	3	3	23

The two methods of generating similarity indices are the percent match method and the Nei and Li(1987) method (Black 1995). Neither method clustered petrels according to colony or region (Figures 3 and 4) indicating a generally heterogeneous population. The two analyses differ in that the percent match method considers shared band absence as a similarity while the Nei and Li method does not. The assumption that band absence is allelic may not be valid due to the number of dissimilar events which can lead to absence. Black (1995) recommends using the Nei and Li method for comparing different species and the percent match method for intraspecific comparisons, presumably

on the assumption that shared absence is most likely to be allelic within closely related groups. The differences in the similarity indices can be seen in the following example: Consider three individuals scored for three loci as A: 001, B: 001, and C:101. The percent match method considers individuals A and B to be identical with three shared loci while A and B each share two loci with C. The Nei and Li method does not recognize the zeroes as a similarity, so A, B and C are all equally similar, sharing only the third locus. Thus, despite the fact that A and B have the same haplotype, they are not more closely related to each other than either is to C. This accounts for the minor differences between the two dendrograms.

Analysis of molecular variance of genomic petrel DNA indicated small but significant statistical evidence of population structuring, both between colonies and populations (Table 4). The majority of variation within most natural populations is attributable to individuals, and was measured at 94.1 % in this study. The remaining variation is attributed to differences between the three colonies (2.4 %) and differences between the two groups (3.5 % between Nova Scotia and Newfoundland). The fact that significant differences between colonies and groups can be identified using AMOVA indicates there are measurable genetic differences, despite the fact that phylogenetic cluster analyses were unable to recover the colonial aggregations. This may be the result of incomplete lineage sorting, due to recent colonization of a large number of founders (Friesen, pers. comm.). Analyses of more rapidly evolving loci, such as microsatellite or mitochondrial DNA analyses may serve to resolve these differences.

Barriers to genetic exchange are primarily physical, temporal or behavioural. There are no obvious temporal or physical barriers to exchange between Atlantic petrel colonies yet behavioural barriers may exist. Philopatry, the behaviour of returning to the natal colony to breed, is a behaviour which promotes genetic differentiation (Ovenden 1991, Birt-Friesen *et al.* 1992) which many seabirds and most procellariiforms exhibit (Warham 1990 and 1996). Fisher (1976) suggested that philopatry may explain the existence of subcolonies within a main colony of a large procellariid, the Laysian albatross. Such subcolonies (areas of synchronized laying and fledging) are apparent on Bon Portage Island (pers. obs.) and presumably in other petrel colonies. Huntington *et al.* (1996) report recaptures of only 1.1% (87 of 7674) of Leach's petrel chicks banded over a 41 year period on Kent Island, New Brunswick. This low number of returns may indicate that such subcolonies within storm petrel colonies are be a result of factors other than inbreeding.

However, 42 of the 87 chicks recaptured on Kent Island were captured near their natal burrow (Huntington, pers. comm.). While a low percentage of breeders return to the natal colony, those that do seem to nest their natal burrow. Friesen *et al.* (1996a) suggest that the preferential recruitment of native over foreign young at murre ledges "will promote formation of family groups, whereas even high emigration rates will not disrupt substructuring if recruits tend to settle in new areas". As long as returning and breeding native petrels proportionally outnumber emigrants, there exists the possibility of kin groups, hence kin selection, within petrel colonies.

Factors which inhibit genetic mixing will act to enhance population differentiation. Conversely, factors which promote dispersal, hence genetic mixing, will act against population differentiation. However, even among species with high gene flow between populations some structuring is expected merely because mating is more likely to occur between individuals from local colonies than from distant colonies (Rockwell and Barrowclough 1987). The fixation index (F_{ST} = 0.035) of the three colonies is low, suggesting a high gene flow, hence, high mean number of migrants per generation (Nm = 6.9) although this value represents an evolutionary average and not an actual number of migrants.

One migrant per generation (Nm >1) is enough to prevent fixation through genetic drift (Wright 1931). However, a high Nm does does not necessarily indicate a high degree of contemporary gene flow. Populations reach genetic equilibria at a rate determined primarily by population size (N), mutation rate (m) and population age in generations (t) (Slatkin 1985a, Friesen *et al.* 1996b) such that many seabirds (which tend to be long lived, have correspondingly long generation times and support large populations) will have been unable to reach an equilibria since the last Pleistocene ice age began to recede circa 15 000 years ago (eg. Randi *et al.* 1989, Austin *et al.* 1994, Friesen *et al.* 1996b). The receding ice will have opened up new nest habitat while water levels rose, drowning the (then) current nest islands. Sequential founder events and large scale founder events likely occurred as birds left the 'sinking' islands. This situation is essentially one of panmixia and according to current theory, there has been insufficient time for the subsequent island populations to come to genetic equilibria. Thus, present levels of genetic differentiation

are likely caused by, but cannot be ascribed to, either contemporary gene flow or historical association.

A study of wider scope which includes samples from European and Pacific colonies is of interest. Geographic variation among Leach's petrels is more pronounced in Pacific birds (Harrison 1983b) with a cline towards dark rumped individuals in more southerly waters and the taxonomy of subspecies has been controversial (Ainley 1980, 1983, Power and Ainley 1986). Also of interest are the Leach's storm petrels of Guadeloupe Island, which are split temporally into a summer nesting population and a winter nesting population.

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Appendix 1. Raw data in format ready for use as input for RAPDPLOT (Black 1995). The data are presented in 3 tables, each provided with its own header information. The actual input file is a continous list of samples with a single header.

Number of individuals: 96
TITLE: Petrels from Bon Portage (BP), Big White (BW) ; and Gull I. (GL)
:PRIMER ORDER 376(12 loci), 389(9 loci), 370(8 loci)
Number of fragments: 32
(4(9(1X,A7)/),4(1X,A7))
3761100 3761000 376-800 376-670 376-640 376-520 376-460 376-350 376-330
376-290 376-270 376-240 389-900 389-800 389-600 389-560 389-400 389-380
389-360 389-300 389-275 3701150 3701000 370-860 370-750 370-600 370-520
370-430 370-360 370-300 370-220 370-150
(A9,1X,31A1)

BP01	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1
BP02	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1
BP03	1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BP04	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1
BP05	1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BP06	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
BP07	1 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 0 1 1 1
BP08	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1 0 1 1
BP09	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1
BP10	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 0 1 1 1 1 1 1 1 1
BP11	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
BP12	1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1
BP13	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
BP14	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1
BP15	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 1
BP16	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BP17	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1
BP18	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BP19	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BP20	0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BP21	1 1 1 0 1 1 1 1 0 0 1 1 1 1 1 1 0 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BP22	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 0 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1
BP23	1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1 0 1 0 1 1 0 0 1 1 1 0 1 1 1 1
BP24	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1
BP25	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BP26	0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1
BP27	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 0 0 1 1 1 1 1 1 1 1
BP28	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BP29	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BP30	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1
BP31	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
BP32	1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1
BP33	1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1
BP34	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 0 1 1 0 0 1 1 1 1 1 1 1
BP35	1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1

Appendix 1. Cont.

Number of individuals: 96
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:PRIMER ORDER 376(12 loci), 389(9 loci), 370(8 loci)
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389-360 389-300 389-275 3701150 3701000 370-860 370-750 370-600 370-520
370-430 370-360 370-300 370-220 370-150
(A9,1X,31A1)

BW37 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW38 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW39 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1
BW40 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BW41 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
BW42 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
BW43 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
BW44 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 0 1 0 1 1 0 0 1 1 1 1 1 1 1 1 1
BW45 1 1 1 1 0 1 0 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1 1 1
BW46 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
BW47 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BW48 1 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1
BW49 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1
BW51 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW52 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW53 1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1
BW54 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW56 1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BW57 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1
BW58 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1 1
BW59 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 0 1 1 1 1 1 1 1 1 1 1
BW60 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1
BW61 1 1 1 1 0 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1
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BW66 1 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
BW67 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
BW68 1 1 1 1 0 0 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1 1
BW02 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
BW03 1 1 1 1 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1

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389-360 389-300 389-275 3701150 3701000 370-860 370-750 370-600 370-520
370-430 370-360 370-300 370-220 370-150
(A9,1X,31A1)

GL01	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 0 1 1 1 1
GL02	0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
GL03	0 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1
GL04	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 0 1 0 1 1 1 1 1 1 1 1
GL05	0 1 1 1 1 0 0 1 0 0 0 1 1 1 1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1
GL06	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1
GL07	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1
GL08	1 1 1 1 1 1 1 1 0 0 0 0 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
GL09	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1
GL10	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
GL11	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
GL12	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
GL14	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1
GL15	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 1 1 1 1 1 1
GL16	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1
GL17	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 0 1 1 1 1 1
GL18	1 1 1 0 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1
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GL21	1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 1
GL22	1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1
GL23	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 1 1 1
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GL28	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
GL29	1 1 1 0 1 1 1 1 0 0 0 1 1 1 1 1 1 1 0 1 1 1 1 1 0 1 1 1 1 1 1 1
GL30	1 1 1 1 1 1 1 1 0 0 1 1 1 1 1 1 1 1 0 1 1 1 0 1 1 1 1 1 1 0 1 1
GL31	1 1 1 1 1 1 1 1 0 0 1 1 1 1 0 1 1 1 0 1 0 1 1 1 1 1 1 1 1 1 1 1
GL32	1 1 1 0 1 1 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 0 1 1 1 1
GL33	1 1 1 0 1 0 1 1 0 0 1 1 1 1 1 1 1 1 1 0 1 1 0 1 1 1 1 0 1 1 0 1 1
GL34	1 1 1 1 1 1 1 1 0 0 1 1 1 1 0 1 1 1 1 1 1 1 1 1 1 0 1 1 1 1 1 1