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Booth Jones, K.A., Nicoll, M.A.C., Raisin, C. et al. (2017) Widespread gene flow between oceans in a pelagic seabird species complex. Molecular Ecology, 26 (20). pp. 5716-5728. ISSN: 0962-1083

https://doi.org/10.1111/mec.14330

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Article type : Original Article

Title: Widespread gene flow between oceans in a pelagic seabird species complex

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Key words: dispersal, gene flow, hybridisation, petrel, Pterodroma, tracking.

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Running title: Gene flow in a seabird species complex

Abstract

Global-scale gene flow is an important concern in conservation biology as it has the potential to either increase or decrease genetic diversity in species and populations. Although many studies focus on the gene flow between different populations of a single species, the potential for gene flow and introgression between species is understudied, particularly in seabirds. The only well studied example of a mixed-species, hybridising population of petrels exists on Round Island, in the Indian Ocean. Previous research assumed that Round Island represents a point of secondary contact between Atlantic

(Pterodroma arminjoniana) and Pacific species (P. neglecta and P. heraldica). This study uses microsatellite genotyping and tracking data to address the possibility of between-species hybridisation occurring outside the Indian Ocean. Dispersal and gene flow spanning three oceans was demonstrated between the species in this complex. Analysis of migration rates estimated using BAYESASS revealed unidirectional movement of petrels from the Atlantic and Pacific into the Indian Ocean. Conversely, STRUCTURE analysis revealed gene-flow between species of the Atlantic and Pacific Oceans, with potential three-way hybrids occurring outside the Indian Ocean. Additionally, geolocation tracking of Round Island petrels revealed two individuals travelling to the Atlantic and Pacific. These results suggest that inter-specific hybrids in Pterodroma petrels are more common than was previously assumed. This study is the first of its kind to investigate gene flow between populations of closely related Procellariform species on a global scale, demonstrating the need for consideration of widespread migration and hybridisation in the conservation of threatened seabirds.

Introduction

Gene flow can be considered as a constraining or a creative force in evolution (Slatkin 1987). When occurring between closely related or recently diverged species, gene flow may dilute the gene pool of rare species (Gottelli et al. 1994; Muñoz-Fuentes et al. 2007; Rhymer & Simberloff 1996), oppose natural selection and the evolution of new species (Slatkin 1987) or reduce current biodiversity through reverse speciation (Seehausen 2006). Conversely, genetically isolated populations may represent unique sources of biodiversity, therefore maintaining the possibility of gene flow between discrete units can boost the genetic fitness of small populations affected by inbreeding depression (Grant et al. 2003). Moreover, population connectivity has been shown to be important to the persistence of threatened populations (Bicknell et al. 2012; Blower et al. 2012).

In marine ecosystems physical barriers to gene flow are few. Marine species that could potentially form panmictic populations due to their incredible mobility show behavioural constraints to gene flow, for example in whales (Baker et al. 1994), sharks (Blower et al. 2012; Portnoy et al. 2010) and tuna (Grewe et al. 2015). In particular, seabirds provide an interesting model in which to examine evolutionary genetics and the relationship between dispersal and gene flow, due to the dichotomy between their huge potential for dispersal coupled with their reluctance to disperse, driven by a strong instinct for philopatry (Friesen et al. 2007; Steeves et al. 2005). This preference for returning to their natal island to breed can lead to island populations of seabirds becoming distinct (Abbott & Double 2003; Austin et al. 1994; Avise et al. 2000; Burg & Croxall 2001; Welch et al. 2012). Natal philopatry does not always result in population differentiation between islands (Ando et al. 2011; Dearborn et al. 2003; Gómez-Díaz et al. 2009; Milot et al. 2008; Morris-Pocock et al. 2010), but there are many other potential barriers to gene flow in seabirds, such as non-breeding distribution (Burg & Croxall 2001; Morris-Pocock et al. 2010) and adaptation to local ocean regimes that may also play an important role in dividing seabird populations (reviewed in Friesen 2015; Gómez-Díaz et al. 2009; Schluter 2009).

Although many studies focus on the gene flow and genetic structure within single seabird species at different island colonies, the potential for gene flow between species at different spatial scales remains poorly understood (Friesen 2015). Introgression between bird species is fairly common in nature (Grant & Grant 1992; Mallet 2005; McCarthy 2006), but is usually prevented by physical (e.g. distance, mountain ranges, oceans) or biological barriers. Biological barriers to interspecific gene flow have been documented in a wide range of bird species; for example, hybrid phenotypes may be unsuitable to changing environmental conditions e.g. in Darwin's finches (Grant & Grant 1992), hybrids may suffer reduced reproductive fitness in one or both sexes e.g. in Flycatchers (Gelter et al. 1992; Svedin et al. 2008) and Spotted eagles (Helbig et al. 2005), or have a reduced adult survival rate e.g. in Larus gulls (Neubauer et al. 2014). Gene flow between distinct populations of a single seabird species is often restricted (Friesen 2015), therefore introgression of genes from one seabird species to another may be considered very unlikely. However, examples of naturally occurring seabird hybrids exist (murres, Friesen et al.1993; boobies, Taylor et al. 2010, 2012; giant petrels, Hunter 1982, Brown et al. 2015).

On Round Island, off the coast of Mauritius in the south-western Indian Ocean there is an unusual colony of Pterodroma petrels. The population includes three species (Trindade petrel, P. arminjoniana, Kermadec petrel, P. neglecta and Herald petrel, P. heraldica), known to extensively hybridise here (Brown et al. 2011; Brown et al. 2010), and is the only confirmed colony of these species in the Indian Ocean. The only other breeding location of the Trindade petrel is in the South Atlantic at the Trindade and Martim Vaz archipelago (Brooke 2004). Unlike the population in the Indian Ocean, in their Atlantic range Trindade petrels have no confirmed contact with Kermadec or Herald petrels, although the possible presence of Kermadec petrels in the Atlantic Ocean has been debated and largely dismissed due to insufficient evidence (Imber 2004, 2005; Imber 2008; Tove 2005). In contrast, in the Pacific Ocean Kermadec and Herald petrels share a similar range, and several breeding locations (BirdLife International 2016b; Brooke 2004). Despite the overlapping Pacific range of Kermadec and Herald petrels, they are not known to hybridise in the Pacific. The petrel population on Round Island therefore represents a particularly interesting 'natural experiment' in which to study the role of dispersal, gene flow and the breakdown of barriers between species that are both formerly allopatrically and sympatrically separated. Given the threatened conservation status of many wide-ranging seabirds (Croxall et al. 2012) the potential for inter-ocean gene flow has important implications for their ability to colonise new areas and survive under different environmental conditions.

Here microsatellite genotyping data, supplemented by geolocation tracking data, are used to distinguish between two potential models of gene flow involving the Round Island Pterodroma population. There is likely to be a range of possibilities that describe gene flow in petrels to and from

Round Island, however the models tested in this study represent two extreme cases. In the past, it has been presumed that Round Island is a point of secondary contact between Atlantic and Pacific species (Brown et al. 2011; Brown et al. 2010). In this scenario, referred to here as the '*secondary contact model'*, individuals disperse from the Atlantic and Pacific to Round Island in the Indian Ocean, and therefore co-occurrence of Atlantic and Pacific species and their hybrids should only arise on Round Island. However, given the huge dispersal potential of Pterodroma petrels, it is possible that introgression and gene flow between species may occur at a global scale. In this scenario, hereafter referred to as the '*widespread gene flow model'*, Atlantic and Pacific species and their hybrids may co-occur on islands other than Round Island, outside the Indian Ocean. Given the historical evidence that Pterodroma petrels disperse between oceans, we investigate the possibility of introgression of Trindade genotypes into the Pacific Ocean, and Pacific genotypes into the Atlantic. By genotyping island populations across the Atlantic, Indian and Pacific Oceans we aim to investigate the evidence for our two basic models of gene-flow.

Methods

Monitoring and tracking of Round Island Petrels

Since 1994, petrels have been routinely ringed on Round Island Nature Reserve (19.85° south; 57.78° east, Figure 1B). Between 2009 and 2012, 116 of the 330 petrels fitted with geolocators were recorded on migration, and two of these were recorded making inter-ocean trips. For details on ringing and geolocation tagging, see Nicoll et al. (2016) and the Supporting Information.

Sample Collection and DNA extraction

Blood samples were collected from Round Island Pterodroma (hereafter referred to as 'Round Island petrels'. Images of Round Island petrel plumage morphs can be seen in Figure S1), Trindade petrels from the Trindade Islands and Kermadec petrels from the Kermadec Islands (Table 1, Figure

1B). Blood samples were unavailable for other Pacific populations of Kermadec petrel and for Herald and Murphy's petrels due to the inaccessible nature of many of the islands in the Pacific range of tropical Pterodroma. To represent the Pacific ranges of the study species footpad tissue was sampled from the American Museum of Natural History's collection (Figure S2). In addition to Herald and Kermadec petrels, Brown et al. (2011) proposed that there could be additional Pterodroma species reaching Round Island from the Pacific. To investigate this, samples from Phoenix (P. alba) petrels were also collected, since there have been sightings of petrels with Phoenix petrel-like plumage (having a uniform brown underwing, Figure S2C) at Round Island. Additionally, samples from two island populations of Murphy's petrel (P. ultima), another tropical Pacific gadfly petrel (phenotypically less similar than the other species), were included as an out-group for genotyping (Table 1, Figure 1B). The Supporting Information provides details of sample collection and storage methods, in addition to details of the DNA extraction methods, which used standard procedures.

Microsatellite marker development and genotyping

Two di- and tetra-nucleotide repeat enriched genomic libraries were created from blood samples from one P. arminjoniana from the Trindade Islands and one P. heraldica from the Gambier Islands. From the libraries, 11 validated microsatellite loci (five from P. arminjoniana and six from P. heraldica) were chosen that amplified in the three focal species: P. arminjoniana, P. neglecta and P. heraldica. The genetic diversity of these markers was compared between the three study species to look for evidence of ascertainment bias caused by the selection of the markers that were isolated from only two of the three study species (Ellegren et al. 1995). In the final marker set, the following additional loci that also showed cross species utility in our study species were also included; TG03-002, TG13-009, TG13-017 (Dawson et al. 2010), Tgu06 (Slate et al. 2007) and Calex-01 (Küpper et al. 2007), giving a total of 16 loci. DNA was amplified using QIAGEN Multiplex PCR kits with HotStar Taq DNA polymerase and a touchdown PCR cycle. Estimates of null allele frequency, genotyping error rates and tests of Hardy-Weinberg equilibrium were carried out for each locus within each population. Further details of the library development method, testing of new microsatellite loci, PCR conditions and marker selection can be found in the Supporting Information.

Calculation of genetic diversity

Genetic diversity was measured by calculating the number of alleles per locus, observed and expected heterozygosity (H_o and H_e), and allelic richness for each species (Trindade petrel, Herald petrel, Kermadec petrel, Murphy's petrel and Phoenix petrel) in its native range, using the 12 markers in the final set (out of 16 potential markers, see Results and Table S1). The number of alleles per locus, H_o and H_e were calculated in CERVUS v.3.0.7 (Kalinowski et al. 2007), and allelic richness (Ar) was calculated in FSTAT v.2.9.3 (Goudet et al. 2002). Since three of the 12 markers used were developed from Trindade petrel (*P. arminjoniana*) DNA, and five were developed from Herald petrel (*P. heraldica*) DNA, evidence for ascertainment bias (Ellegren et al. 1995) was tested by comparing observed heterozygosity and allelic richness in these eight markers between the different species. This test was performed using ANOVA in the programming environment R v.3.1.2. (R Development Core Team 2013).

Estimation of genetic differentiation

F_{ST} calculations were performed using FSTAT v.2.9.3.2 (Goudet 2001) to describe genetic differentiation between island populations. Additionally, F_{ST} was calculated to quantify the genetic difference between the historical Kermadec petrel samples from the Kermadec Islands and the contemporary samples of the same species and location, to investigate whether there was evidence of genetic drift between the historical and contemporary samples. Population structure across all samples was estimated using the clustering software STRUCTURE v.2.3.4. (Falush et al. 2003; Pritchard et al. 2000). Preliminary analyses using STRUCTURE (Supporting Information) showed that the most likely number of clusters lay between three and five, therefore the number of specified clusters was narrowed to K= 3–5 based on this evidence. Fifteen independent models were run for each number of specified clusters, with 10^6 MCMC iterations, and a burn-in period of 10^5. All STRUCTURE analyses were run without any prior population information, and assuming admixture. The 10 models with the highest mean Ln likelihood for each value of K were chosen out of the total 15 using the online service STRUCTURE HARVESTER v.0.6.94 (Earl 2012). These models were then re-entered into STRUCTURE HARVESTER to identify the most likely value of K using the mean Ln (K) probabilities for each value of K (Evanno et al. 2005). The output for K= 3–5 from STRUCTURE HARVESTER was then summarised using the software CLUMPP (Jakobsson & Rosenberg 2007) and visualised using the software DISTRUCT v.1.1 (Rosenberg 2004), as shown in Figure S5. This analysis was separately carried out with the ANCESTDIST option turned on (ANCESTDIST = 1), to examine the effect of credibility intervals on hybrid identification (Supporting Information). Separate STRUCTURE analyses were also performed to compare clustering between Round Island and Trindade Island (Figure S6), and Herald petrel populations and Phoenix petrel populations (Figure S7). Details on the settings and outcomes of these can be found in the Supporting Information.

Hybrid classification

Petrels were assigned to one of nine possible pure or admixed 'classes' based on their estimated membership (Q) to clusters identified using STRUCTURE analysis (Figure 1). These classes were: 'Trindade type' (PureT), 'Kermadec type' (PureK), 'Herald-Phoenix type' (PureHP), 'Murphy's type' (PureM), Trindade and Kermadec hybrid (TxK), Trindade and Herald-Phoenix hybrid (TxHP), Herald-Phoenix and Kermadec hybrid (HPxK), Murphy's and Herald-Phoenix hybrid (MxHP) and Trindade, Herald-Phoenix and Kermadec hybrid (TxHPxK). Classes were distinguished using cluster membership thresholds for belonging solely to a particular species group or to a hybrid of two or more groups (Table S2), based on Vähä and Primmer (2006) and Marie et al. (2011). For example, individuals were identified as belonging to a single cluster if their estimated membership (Q) was $>=0.9$ for one cluster and < 0.1 for all other clusters. Two-way hybrids were identified if $Q >= 0.1$ for two clusters, and $Q < 0.1$for the remaining two clusters. Three-way hybrids were identified if $Q >= 0.1$ for three clusters, and $Q < 0.1$for the remaining cluster.

Estimation of migration

Gene flow was estimated using the software BAYESASS v.3.0.3. (Rannala 2012; Wilson & Rannala 2003). Based on preliminary STRUCTURE results, Murphy's petrel was not included in analyses of gene flow, as these populations were genetically distinct (Figure 1). Mixing parameters were adjusted to 0.15, 0.40 and 0.60 for migration rate, allele frequency and inbreeding coefficients, respectively, in preliminary runs to ensure that the acceptance rate fell between 20% and 60% and that adequate parameter space was sampled (Beerli 2009; Beerli & Felsenstein 2001). Following Davy et al. (2015), ten separate analyses were run using different random starting seeds. Each run had 2.5×10^7 iterations and a 1.5×10^7 burn-in, and the default sampling interval of 2000 iterations. The optimal run of the ten was identified using Bayesian deviance calculated using an R-script developed in Meirmans (2014), and the mixing parameters and starting seed for this run were used in a final, longer run with 10^8 iterations and a burn-in of 10^7. To investigate the role of Round Island as a possible stepping stone for introgression or point of secondary contact between populations of the Atlantic and Pacific Oceans, the analysis was also run using the same parameters as above but without the Round Island samples, as seen in Davy et al. (2015).

Results

The microsatellite loci used in the analyses were autosomal in all of the five petrel species tested (Trindade, Kermadec, Herald, Phoenix and Murphy's petrel) based on the presence of heterozygotes in known females and males for each species. Analysis of the suite of autosomal microsatellite markers separately within each species revealed that four out of the 16 loci had a high estimated null allele frequency (>0.2) in three or more of the five species, and these were therefore discarded from further analysis (Parm20, Parm34, Phel30 and TG13-017). Of the remaining 12 markers, eight (Parm34, Parm29, Parm22, Parm20, Calex01, TG13-017, Phel12, Phel30) deviated from the Hardy-Weinberg equilibrium (P-value <0.05) for three or more of the five species (Table 2). STRUCTURE analysis was conducted both with and without eight markers (Parm34, Parm29, Parm22, Parm20, Calex01, TG13-017, Phel12, Phel30, see Figure 1A and S4) that due to the Wahlund effect deviated from the Hardy-Weinberg equilibrium (P-value <0.05) for three or more of the five species (Table 2). Deviation from the Hardy-Weinberg equilibrium can indicate migration and genetic structure between groups, which were of interest to this study, or linked selection. Out of 396 paired calculations of linkage disequilibrium between all loci in all six groups (five species and the mixed species population at Round Island), seven pairs of loci displayed linkage disequilibrium (LD, Table S4), five in the Round Island population, and two in Herald petrels. Since few pairs of loci displayed LD and no LD was detected in the other species groups (Trindade, Kermadec, Phoenix and Murphy's petrels), these loci were left in the dataset. Strong LD can lead to an overestimate of clustering in STRUCTURE analyses (Kaeuffer et al. 2007). However, STRUCTURE analyses were performed using different numbers of markers and the results were consistent between runs (Figure 1A, S4 and S5), reflecting a genetic structuring that was consistent with the species included in the analysis, with the exception of Herald and Phoenix petrels which were clustered together. Data from samples that failed to amplify for at least 75% (eight) of the remaining 12 markers were removed from further analyses, leaving a total sample size of 885 individuals (Table 1). Average allelic dropout rate per-genotype was 0.02 (S.E. ±0.01) and the occurrence of false alleles in the dataset was estimated to be negligible (Table S5). Per-genotype allelic dropout was higher for museum samples (mean = 0.15, S.E. ±0.07) than blood samples (mean = 0.02, S.E. ±0.01). There was no significant effect of sample type (blood or museum) on the observed heterozygosity and allelic richness across the marker set (one-way ANOVA, H_O : $F_{1, 11} = 0.09$, $P = 0.77$; Ar: $F_{1, 11} = 1.09$, $P = 0.32$).

The observed heterozygosity and allelic richness in the eight markers developed using Pterodroma sample DNA did not differ significantly between the different species (H_O between species: $F_{4, 28} = 0.47$, $P = 0.76$; Ar between species: $F_{4, 28} = 0.38$, $P = 0.82$), although genetic diversity did differ between loci (H_O: $F_{7, 28} = 11.48$, $P < 0.0001$; Ar: $F_{7, 28} = 17.97$, $P < 0.0001$). This comparison of genetic diversity revealed there was no evidence of ascertainment bias (Ellegren et al. 1995) in the species-specific markers between the different species we sampled.

Analysis of genetic structure between the island populations of petrels using F_{ST} suggested that most were significantly differentiated, with only seven out of 105 island population pairs being non-significantly different (Table S6). Of these seven comparisons, the majority (five) were between island populations of the same species. However, Phoenix petrel samples collected from the Pitcairn Islands were not significantly differentiated from the two Pitcairn Island-populations of Herald petrels from Ducie Atoll ($F_{ST} = -0.01$, $P = 0.56$), and from Oeno Island ($F_{ST} = 0.01$, $P = 0.24$). Comparison between the museum sampled Kermadec petrels from the Kermadec Islands (1920s) and those collected recently (Brown et al. 2011) demonstrated there was no significant genetic differentiation between these temporally separated populations ($F_{ST} = 0.004$, $P = 0.10$).

The most likely number of genetic clusters in the dataset using STRUCTURE was found to be four, and these appeared to describe Trindade-type petrels, Kermadec-type petrels, Herald- or Phoenix-type petrels and Murphy's-type petrels (Figure 1A). Herald and Phoenix petrels across their ranges were both assigned to the same cluster. Estimated membership (Q) to each of these clusters was used to assign individuals to either a 'pure' species, based on the four possible clusters, or a hybrid of two or more species groups. As might be expected from the STRUCTURE clusters that largely adhered to species distinctions, Trindade-type individuals were characteristic of the Atlantic Ocean, whereas Kermadec-type, Herald/Phoenix-type and Murphy's-type were characteristic of the Pacific. The proportion of individuals in each class for island populations is shown in

Figure 1B, and numbers of individuals calculated to be in each class per island population can be found in Table S3. Round Island was the most admixed population, with 43.2% of individuals classified as hybrids. Admixture between clusters was not unique to Round Island however; of the other islands sampled 31.0% of Kermadec petrels from Rapa Island, 20.8% of Kermadec petrels from the Kermadec Islands and 22.2% of Trindade petrels from the Trindade Islands were classified as hybrids. In these groups, 24.1%, 18.9% and 22.2 % of each population, respectively, appeared to be hybridised with a cluster originating from a different ocean to that of the island. Two individuals sampled from the Kermadec petrel population of the Kermadec Islands were classified as belonging to the Trindade-type species cluster, and one individual from the sampled Trindade petrels of the Atlantic Ocean was classified as a Kermadec-type. Of the 885 petrels genotyped globally, 48 had a three-way split assignment between the Trindade, Kermadec and Herald-Phoenix cluster, of which 39 originated from Round Island.

Estimates of migration rates between island populations of Trindade petrels, Kermadec petrels, Herald petrels, Phoenix petrels and the mixed Round Island population are described fully in Tables S7 and S8 in the Supporting Information. Significant migration rates are illustrated by Figure 2. No significant migration was detected by the BAYESASS analysis from Round Island to other islands, in either the Atlantic or Pacific Oceans, whereas there was significant movement from Trindade (0.183 ± 0.036 of Round Island individuals per generation), Herald (Marquesas Islands, 0.014 ± 0.01) and Kermadec (Kermadec Islands, 0.048 ± 0.01) to Round Island. Significant migration rates were calculated from Herald petrel populations on the Marquesas Islands and Ducie Atoll to Phoenix petrel populations at a number of different islands (Herald (Marquesas Islands) to Phoenix (Pitcairn Islands) 0.132 ± 0.071; Herald (Ducie Atoll) to Phoenix (Marquesas Islands) 0.04 ± 0.04, Phoenix (Christmas Island) 0.076 ± 0.052, Phoenix (Phoenix Islands) 0.048 ± 0.046 and Phoenix (Pitcairn Islands) 0.063 ± 0.051). However, no reciprocal gene flow was observed from the sampled Phoenix petrel populations into the Herald petrel populations. Kermadec petrels were not observed to exchange individuals with other petrel species in the Pacific. When Round Island was removed from the analysis, no significant migration was seen between the Atlantic and Pacific populations.

Only two individuals (~1% of petrels successfully tracked with geolocators) left the Indian Ocean (Figure 3). In February 2010 petrel 5H41524 left Round Island and travelled eastwards into the Pacific Ocean. Here it travelled close to the nearest colony of known Kermadec petrels on Lord Howe Island and also close to Raine Island (Figure 3), where Herald petrels are known to breed. The petrel was recovered with the geolocator on Round Island on 12th November 2012. Unfortunately no genotyping information was available for the 5H41524, although phenotypically this bird resembled a Kermadec petrel as it was comparatively large and pale in plumage, with characteristic pale primary shafts. In contrast, petrel 5H41919 departed Round Island on 2nd October 2012 and remained within the Indian Ocean until it passed around the southerly tip of Africa and travelled into the Atlantic Ocean, close to the Trindade and Martim Vaz archipelago, the Atlantic breeding site of the Trindade petrel. The petrel was subsequently recaptured on Round Island on 1st June 2013 (Figure 3). Petrel 5H41919 was assigned predominantly to the Kermadec cluster (61.9%) but also to the Trindade cluster (28.7%), and had a low assignment to the Herald/Phoenix cluster (7.7%) and Murphy's cluster (1.7%).

Discussion

This study presents the first evidence of inter-oceanic gene flow between tropical Procellariform seabirds. Analysis of microsatellite genotyping data using STRUCTURE found that four clusters best represented the population structure between the five species sampled across three oceans. The population of petrels on Round Island was shown to consist mainly of individuals belonging to the Trindade-type cluster, however levels of admixture between Kermadec and Herald-type clusters were higher on Round Island than in the other island populations sampled (Figure 1). Notably, admixture between clusters was also seen outside the Indian Ocean, in the Trindade petrel population of the Atlantic Ocean and Kermadec, Herald and Phoenix petrel populations of the Pacific, providing strong evidence for dispersal and gene flow outside the Indian Ocean. However, no significant per-generation migration rates were detected from the Atlantic Ocean to the Pacific, or reciprocally (Figure 2), a result that persisted when Round Island was removed from the analysis. The contrasting secondary contact and widespread gene flow models presented in this study are extreme scenarios, and it is plausible that in reality the admixed Round Island population may facilitate migration and gene flow between the Atlantic and Pacific Oceans. Therefore there are a wide range of more specific gene flow models that might best represent gene flow between the tropical Pterodroma populations included in this study, and more detailed phylogenetic research is needed to clarify links between the species.

Historically speaking, the population of petrels on Round Island consists entirely of immigrants from outside the Indian Ocean, and it was clearly seen in the STRUCTURE analysis (Figure 1A) that the Round Island population appeared to be more admixed (43.2%) than Atlantic and Pacific populations (Figure 1B). This may relate to the Hubbs' principle, or the "desperation hypothesis" (Hubbs 1955), whereby hybrids are a result of a deficiency in conspecific mating options for rarer species in a population of related species (McCracken & Wilson 2011; Randler 2002, 2006). However, some petrels in the Atlantic Trindade population were classified as having either Kermadec-type or Herald/Phoenix-type hybrid genotypes (17.8% and 4.4% of the sample, respectively), and one individual was classified as a pure Kermadec-type migrant (Figure 1B). Similarly, Atlantic (Trindade-type) hybrid genotypes were found in Pacific Kermadec petrel populations (Ducie Atoll 4%; Juan Fernández Islands 17.9%; Kermadec Islands 18.9; Rapa Iti 24.1%), Herald petrel populations (Ducie Atoll 7.1%; Marquesas Islands 13.0%; Oeno Island 4.8%) and Phoenix petrel populations (Christmas Island 6.6%; Marquesas Islands 3.4%; Phoenix Islands 6.3%; and the Pitcairn Islands 11.1%, Figure 1B). This evidence of population mixing corroborates the findings of Brown et al. (2011), who found that one sampled Ducie Island Herald petrel possessed a Trindade-type mitochondrial cytochrome b haplotype, in addition to some Ducie Island Herald petrels sharing haplotypes with Kermadec petrels from the Kermadec Islands.

The 90% credibility intervals calculated during STRUCTURE analysis suggested that putative hybrids must be assigned with caution in this study. Intervals ranged from zero to one for assignments to potential clusters for all hybrids, casting doubt on their true hybrid status. However, investigations using a simulated hybrid population showed that at a 90% credibility interval, STRUCTURE's ANCESTDIST option would reject 88.4% of individuals correctly classified as hybrids using the criteria used in this study (Supporting Information). Therefore although hybrids may not be as common as identified here, STRUCTURE analysis supports the stepping-stone variant of the widespread gene flow model. Hybrid genotypes were not exclusively found on Round Island, but the Round Island population had the highest proportion and is therefore likely acting as an intermediate population for gene flow between the Atlantic and Pacific populations.

These findings are apparently contradictory to the lack of migration from the Indian Ocean to the Pacific or Atlantic Oceans, or directly between the Pacific and Atlantic Oceans as found in the BAYESASS analysis. Estimates of per generation migration rate suggested that there was a high level of gene flow from the Trindade petrel population of the Atlantic into the Round Island population (18.3 ± 4%), and likewise migration from the closest sampled Herald petrel population (Marquesas Islands, 1.4 ± 1%) and the closest sampled Kermadec petrel population (Kermadec Islands, 4.8 ± 1%) to Round Island (Figure 2). However, no reciprocal gene flow was observed.

BAYESASS has been shown to overestimate migration rates in certain circumstances, for example if population connectivity has reduced in recent generations (Samarasin et al. 2016), or if genetic divergence between populations is low ($F_{ST} \leq 0.05$, Faubet et al. 2007). Genetic divergence between Round Island petrels and the Trindade petrels of the Atlantic was estimated to be below this threshold ($F_{ST} = 0.02$, $P < 0.01$), and therefore the calculated migration rate of 18.3 ± 4% migrants per generation may be an overestimate. However, dismissing the calculated migration rate between Round Island and the Trindade Islands based on F_{ST} must be viewed with caution, as maximum F_{ST} can be influenced by allele frequency (Jakobsson et al. 2013) and locus polymorphism (O'Reilly et al. 2004). Additionally, due to the difficulty of gaining samples from the remote islands of the Pacific and Atlantic Oceans, the number of genotyped individuals from each of these island populations was very small, on average 26.7 (S.E. ± 1.7). Therefore, sample sizes for other populations are unlikely to include enough potential migrants to be detectable at a per-generation rate if the migration rates are very low (Meirmans 2014). Finally, BAYESASS analysis assumes that populations are separated by only a few generations, usually taken as fewer than five (Chiucchi & Gibbs 2010; Faubet et al. 2007; Wilson & Rannala 2003). However, when F_{ST} was calculated between contemporary and historical Kermadec petrel samples from the Kermadec Islands, there was no significant difference in genetic variation between the two temporally separated groups, and it was assumed that this lack of difference would be the same for all populations. The collection of modern samples would be useful to further studies; but the islands where these petrels are found are very remote and infrequently visited by researchers.

Although there was no evidence of ascertainment bias (Ellegren et al. 1995) , it would have been advantageous to have a larger microsatellite marker set of homologous markers with complete primer specificity to all of our five study species. This would have increased the power of the analyses to detect genetic structure between Herald and Phoenix petrels particularly (Figure S7), and may have enabled the detection of Phoenix petrel genotypes on Round Island. However, it may be that these two species are not well resolved, as no detailed phylogenetic studies have been conducted on Pacific Herald petrels and Phoenix petrels.

Although the number of tracked petrels from Round Island was limited (N = 116) two individuals (~2%) were tracked coming close to other colonies in the Pacific and Atlantic Oceans, demonstrating the potential connectivity of their isolated populations (Figure 3). It is interesting that these two petrels were tracked travelling counter to estimated migration directions. Most surprisingly, the petrel travelling to the Atlantic Ocean from Round Island appeared more genotypically similar to Kermadec petrels of the Pacific Ocean than Trindade petrels (61.9% assignment to the Kermadec-type cluster in STRUCTURE analysis The possibility of Kermadec petrels visiting and potentially breeding in the Atlantic Ocean has been contested in the past (Imber 2004, 2005; Imber 2008; Tove 2005), but both the genotyping and tracking data presented here adds evidence to support their presence in the Atlantic.

Of course, the strong philopatry of Procellariform seabirds means that not all visits to other breeding colonies will result in a switching of breeding locations. Both petrels tracked outside the Indian Ocean subsequently returned to Round Island, although both were initially caught and ringed as adults on Round Island, so their natal colonies are unknown. Unfortunately, since geolocators must be recovered for data to be obtained, any birds tracked with geolocators that permanently change colony would not return data. However, during routine seabird monitoring on Round Island in April 2006, a ringed, pale-plumaged petrel was discovered with a young chick (Tatayah 2010). The petrel was originally captured as a Herald petrel on Raine Island in 1984, where it bred with the same partner until 1987 (Figure 3; King & Reimer 1991). This individual was subsequently recorded as present on Round Island with an egg in October 2008 and again in May 2012, at a minimum age of 28 years old. However, the occurrence of ringing records providing evidence of colony-switching is highly unusual, as the islands inhabited by Pterodroma petrels are isolated and rarely visited by researchers.

To date the only well-studied instance of introgression between species of Pterodroma petrel is from Round Island (Brown et al. 2011; Brown et al. 2010), although mitochondrial and phenotypic study of a single museum specimen collected during the Whitney South Seas Expedition east of the Antipodes Islands is posited as a hybrid between a White-headed petrel (Pterodroma lessonii) and

Soft-plumaged petrel (P. mollis) (Tennyson et al. 2013). Indeed there are few published examples of two-way hybridisation between other Procellariform species, and these are based on small sample sizes or single individuals (Brown et al. 2015; Holdaway et al. 2001; McCarthy 2006; Moore et al. 2001; Moore et al. 1997; Tennyson et al. 2013). Naturally occurring three-way (compound) hybrids are rarely documented in birds, although anecdotally described in ducks (Harrison & Harrison 1965) and hummingbirds (McCarthy 2006). Avian three-way hybrids are more commonly reported in captive-bred birds such as pheasants, falcons, and cage birds (McCarthy 2006). Here, not only are inter-species hybrids widespread between the populations of tropical Pterodroma, but possible three-way hybrids are occurring on Round Island (8.1%) and outside the Indian Ocean (Kermadec petrels: Juan Fernández Islands 7.1%, Rapa Iti 6.9%; Herald petrels: Ducie Atoll 3.6%; Phoenix petrels: Pitcairn Islands 5.6%, Marquesas Islands 3.4%, Christmas Island 3.3%; Trindade petrels: 2.2%, Figure 1B). This finding is therefore currently unique.

Our results provide evidence of gene flow between three oceans in Pterodroma petrels, supporting widespread dispersal and gene flow between these closely related species. Within this complex of different species and island populations, Round Island is clearly an important zone of secondary contact between species originating in the Atlantic and Pacific Oceans. However, inter-ocean migrants and hybrids are not unique to Round Island. The wide-ranging behaviour of tropical Pterodroma may help them to disperse and colonise new or extirpated islands, and the potential to thrive in new environments bodes well for their future. This study highlights how little is known about gene flow and dispersal between populations of closely related, wide-ranging species. Consideration of migration and hybridisation with other species and colonies may be particularly relevant to the assessment of the conservation status and management of some seabirds. For example, the 'vulnerable' IUCN Red List status for the Trindade petrel (Birdlife International 2016a) is based on its limited breeding range and therefore its susceptibility to stochastic events. The presence of this species in other locations, namely Round Island, should be taken into account for the Trindade petrel, and similar genetic and tracking studies concentrating in the Pacific may also provide valuable information for conservation efforts.

Acknowledgements

We thank the Mauritian Wildlife Foundation staff and volunteers and the Mauritian Government National Parks and Conservation Service for their assistance in providing ringing data, assistance with fitting geolocators and blood sampling. In particular, thanks to Vimul Nundlaul, Nicolas Zuel, Martine Goder, Richard Baxter, Tom Churchyard, Pat Banville, Lucy Rouse and Helen Gath in facilitating the petrel research programme on Round Island. Norman Ratcliffe (British Antarctic Survey) provided valuable insight on the use of geolocators. Additional blood samples and associated data were kindly provided by Ruth Brown (Round Island petrel). We thank Simon Tollington for helping with the organisation and transport of petrel samples and Dada Gottelli for helping with museum sample DNA extraction. Thanks also to Terry Burke and Jinliang Wang for their invaluable advice on genetic analyses. We would also like to thank the subject editor and anonymous reviewers for their helpful comments, which improved this manuscript. Microsatellite development and genotyping was performed at the Natural Environment Research Council (NERC) Biomolecular Analysis Facility (NBAF) at the University of Sheffield under grants NBAF446 and NBAF844. Andy Krupa and Rachel Tucker provided technical support. The petrel tracking programme was supported by the Natural Environmental Research Council (UK) (Grant NE/H5081500). Katherine Booth Jones was supported by NERC, UK (Award reference: 1230649). The authors have no conflict of interest to declare.

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Data Accessibility

- The microsatellite sequences isolated are available via the ENA-EBI EMBL sequence database (Accession numbers: AM072445-AM072447, LT608685, LT608692, LT608694, LT608717, LT608720, LT608733, LT608738, LT608740, DV575298, DV948691, CK307697).

- Microsatellite genotyping data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.fg86p

- Tracking data are available via the BirdLife International Tracking Ocean Wanderers database: http://www.seabirdtracking.org/. They are identified as data set 1326. This can be found by selecting the species Pterodroma arminjoniana and then clicking on the datasets 'view list' button and selecting 1326 from the resulting pop-up table.

Author Contributions:

KABJ deployed geolocators, collected blood and museum samples, performed the microsatellite genotyping, all analyses and wrote the manuscript; MACN devised the experiment, deployed and recovered geolocators, collected blood and museum samples and provided comments on the manuscript; CR characterised and validated the microsatellite markers and co-wrote the Supporting Information; DAD provided guidance on marker development, validation and genotyping and analyses, and provided comments on the manuscript, HH provided guidance on data analysis and comments on the manuscript; GJH isolated the microsatellite sequences and supported marker development and genotyping ; JJG devised the experiment, assisted with initial microsatellite library and genetic work and advised on the development of NERC funding applications; SMHI provided blood samples from Kermadec petrels of the Kermadec Islands and provided comments on the manuscript; PS facilitated access to the Whitney South Seas Expedition petrel skin collection at the American Museum of Natural History; CJ and VT facilitated access to Round Island and data on Round Island petrels through the Mauritian Wildlife Foundation; KR facilitated access to Round Island and data on Round Island petrels through the National Parks and Conservation Service (NPCS) and KN devised the experiment, deployed and recovered geolocators, collected blood and museum samples and provided comments on the manuscript.

Tables and Figures

Table 1: Number and origin of samples. N_S = Number of samples, N_G = Number genotyped at >75% of 12 microsatellite markers.

Putative species	Geographic location	Region	N_S	Type	N_G
Unknown	Round Island	Indian Ocean	561	blood	484
Trindade petrel (Pterodroma arminjoniana)	Trindade Islands	Atlantic Ocean	52	blood	45
Herald petrel (Pterodroma heraldica)	Ducie Atoll	Pacific Ocean	30	museum	28
	Marquesas Islands	Pacific Ocean	23	museum	23
	Oeno Island	Pacific Ocean	21	museum	21
Kermadec petrel (Pterodroma neglecta)	Ducie Atoll	Pacific Ocean	30	museum	25
	Juan Fernandez Islands	Pacific Ocean	30	museum	28
	Kermadec Islands	Pacific Ocean	29	museum	29
	Kermadec Islands	Pacific Ocean	41	blood	24

	Rapa Island (Bass Islands)	Pacific Ocean	30	museum	29
Murphy's petrel (Pterodroma ultima)	Marotiri Island	Pacific Ocean	28	museum	26
	Oeno Island (Bass Islands)	Pacific Ocean	30	museum	30
Phoenix petrel (Pterodroma alba)	Christmas Island (Kiritimati)	Pacific Ocean	30	museum	30
	Pitcairn Islands	Pacific Ocean	21	museum	18
	Marquesas Islands	Pacific Ocean	29	museum	29
	Phoenix Islands	Pacific Ocean	16	museum	16
Total			1001		885

Table 2: Characteristics of the 12 microsatellite markers genotyped in each of the potential species found at Round Island. Each species group only includes individuals from the native range of the species, and not from the Round Island population. Size: allele size range, N_A: number of alleles, Ar: allele richness, H_O: observed heterozygosity, H_E: expected heterozygosity, P-value: P-value for Hardy-Weinberg test.

	All spp.		**Trindade petrel**					**Herald petrel**					**Kermadec petrel**				
Locus	**Size**	**N_A**	**N_A**	**Ar**	**H_O**	**H_E**	**P-value**	**N_A**	**Ar**	**H_O**	**H_E**	**P-value**	**N_A**	**Ar**	**H_O**	**H_E**	**P-value**
Calex01	229–244	8	2	2.00	0.35	0.40	0.02	3	2.20	0.15	0.17	0.06	5	2.64	0.12	0.13	0.02
Parm22	199–207	6	2	2.00	0.21	0.22	0.52	4	2.87	0.10	0.17	<0.01	5	4.14	0.49	0.68	<0.01
Parm29	130-146	6	1	1.00	0.00	0.00	-	4	2.71	0.19	0.22	0.02	3	1.62	0.05	0.05	1.00
Parm31	91–107	10	2	1.95	0.13	0.19	0.06	3	2.81	0.21	0.26	0.01	7	3.65	0.29	0.30	0.13
Phel12	157–177	9	5	4.46	0.40	0.63	<0.01	6	5.61	0.68	0.76	<0.01	6	3.78	0.28	0.33	0.04
Phel15	86–90	3	1	1.00	0.00	0.00	-	2	1.37	0.03	0.03	1.00	3	2.66	0.22	0.22	0.87
Phel28	230–254	8	5	3.75	0.67	0.58	0.65	5	3.43	0.17	0.28	<0.01	5	3.63	0.30	0.47	<0.01
Phel33	116–164	13	9	7.85	0.81	0.84	0.59	10	7.89	0.83	0.86	0.01	11	8.14	0.71	0.74	0.01
Phel35	124–182	11	9	7.67	0.83	0.86	0.58	9	6.44	0.75	0.77	0.09	8	6.98	0.82	0.83	0.08
TG03-002	123–129	4	3	2.43	0.15	0.14	1.00	3	1.97	0.10	0.09	1.00	3	2.47	0.39	0.37	0.92
TG13-009	185–199	5	3	2.50	0.27	0.24	1.00	4	2.76	0.14	0.16	0.06	4	1.94	0.05	0.08	0.01
Tgu06	152–169	11	5	4.75	0.51	0.63	0.04	9	4.51	0.69	0.61	0.43	7	4.23	0.47	0.57	<0.01
Mean		7.83	3.92	3.45	0.36	0.39	-	5.17	3.71	0.34	0.36	-	5.58	3.82	0.35	0.40	-

Table 2 continued.

	All spp.		Murphy's petrel					Phoenix petrel				
Locus	**Size**	**N_A**	**N_A**	**Ar**	**H_O**	**H_E**	**P-value**	**N_A**	**Ar**	**H_O**	**H_E**	**P-value**
Calex01	229–244	8	3	2.61	0.23	0.40	<0.01	2	1.19	0.01	0.01	<0.01
Parm22	199–207	6	3	3.00	0.00	0.61	<0.01	2	1.43	0.03	0.03	1.00
Parm29	130-–46	6	3	2.69	0.21	0.20	1.00	6	4.39	0.53	0.57	0.04
Parm31	91–107	10	7	6.63	0.77	0.81	0.12	4	3.39	0.52	0.46	0.18
Phel12	157–177	9	6	4.38	0.50	0.64	<0.01	7	5.65	0.79	0.78	0.89
Phel15	86–90	3	1	1.00	0.00	0.00	-	3	1.82	0.05	0.06	0.07
Phel28	230–254	8	4	3.57	0.54	0.63	0.06	3	2.65	0.19	0.22	0.05
Phel33	116–164	13	8	5.42	0.79	0.72	0.69	11	7.49	0.83	0.83	0.34
Phel35	124–182	11	8	7.41	0.88	0.84	0.02	8	5.93	0.76	0.76	0.09
TG03-002	123–129	4	3	2.27	0.29	0.30	0.70	4	3.01	0.28	0.25	1.00
TG13-009	185–199	5	3	1.91	0.07	0.07	1.00	3	2.26	0.10	0.12	0.01
Tgu06	152–169	11	7	4.87	0.70	0.66	0.84	5	3.74	0.45	0.53	0.25
Mean		7.83	4.67	3.81	0.41	0.49	-	4.83	3.58	0.38	0.39	-

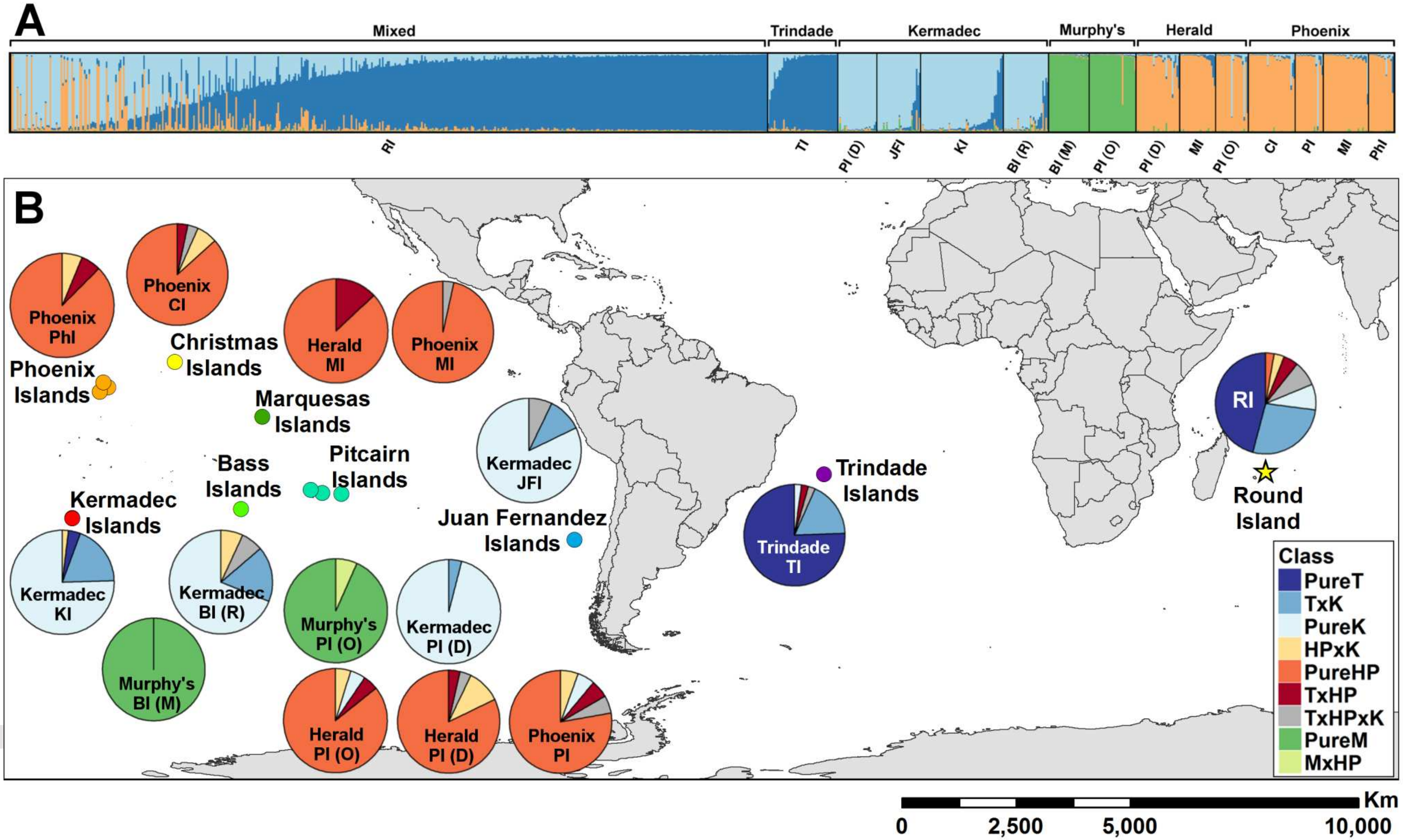
A
Mixed
Trindade
Kermadec
Murphy's
Herald
Phoenix
RI
TI
PI (D)
JFI
KI
BI (R)
BI (M)
PI (O)
PI (D)
MI
PI (O)
CI
PI
MI
PhI
B
Phoenix PhI
Phoenix CI
Herald MI
Phoenix MI
Christmas Islands
Phoenix Islands
Marquesas Islands
Bass Islands
Pitcairn Islands
Kermadec JFI
Kermadec Islands
Juan Fernandez Islands
Trindade Islands
Trindade TI
RI
Round Island
Kermadec KI
Kermadec BI (R)
Murphy's BI (M)
Murphy's PI (O)
Kermadec PI (D)
Herald PI (O)
Herald PI (D)
Phoenix PI
Class
PureT
TxK
PureK
HPxK
PureHP
TxHP
TxHPxK
PureM
MxHP
Km
0
2,500
5,000
10,000

Figure 1A: Plot showing STRUCTURE analysis results, where K (number of clusters) equalled 4. Each vertical bar represents a single individual and colours in each bar represent the proportion of an individual's ancestry assigned to a particular cluster. Individuals are grouped by putative species (above the plot) and island of origin (below the plot, separated by black vertical lines), with the exception of Round Island, where species is unknown. **B:** The global distribution of islands sampled. West to east, KI = Kermadec Islands, PhI =Phoenix Islands, CI = Christmas Islands, BI (R) = Bass Islands- Rapa Island, BI (M) = Bass Islands- Marotiri, MI = Marquesas Islands, PI = Pitcairn Islands, all, PI (O) = Pitcairn Islands- Oeno, PI (D) = Pitcairn Islands- Ducie, JFI = Juan Fernández Islands, TI = Trindade and Martim Vaz Islands, and Round Island (star). Pie charts represent individuals separated by recorded species at sampling time and source-island the sample originated from (for sample numbers at each location see Table S3). The class assignment of petrels in each pie chart is derived from estimated membership to each of the four potential clusters identified using STRUCTURE analysis, e.g. PureT = Trindade type, PureK = Kermadec type, PureHP = Herald/Phoenix type, PureM = Murphy's type, TxK = two-way hybrid between Trindade type and Kermadec type, TxHPxK = three-way hybrid between Trindade, Herald/Phoenix and Kermadec types.

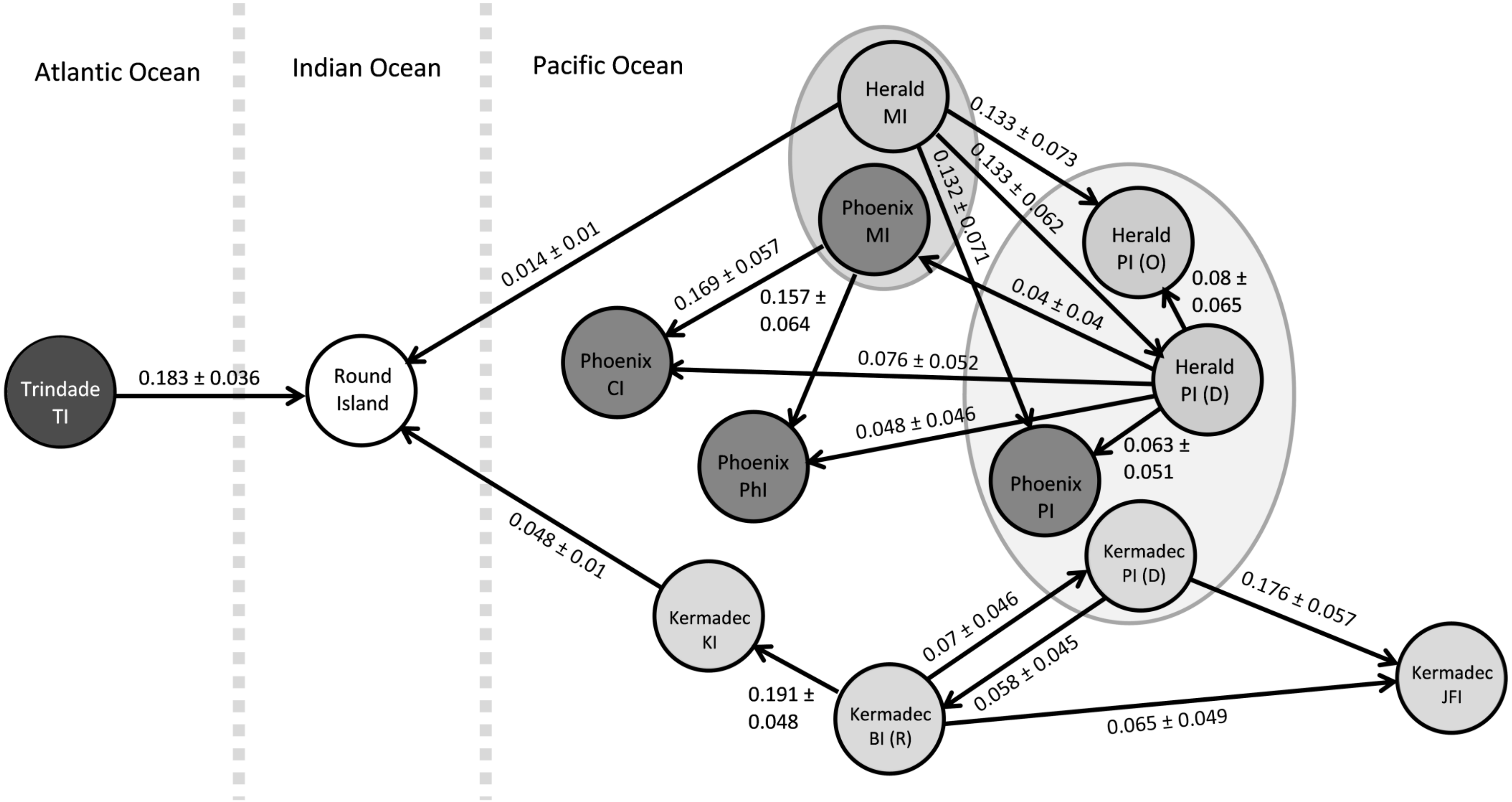

Figure 2: Migration rates (proportion of migrants from population x in population y per generation) between island populations, ± confidence intervals (1.96 x the standard deviation, as in Rannala (2012)). For example, 0.183 (18%) ± 0.036 of the Round Island population originates from the Trindade petrel population per generation. Black arrows represent the direction of movement. Dotted lines indicate divides between oceans. Each island population of a species is represented by a circle, with a species and island label. Island abbreviations are the same as in Figure 1. Grey background ovals represent populations from the same island group: dark grey = Marquesas Islands, light grey = Pitcairn Islands.

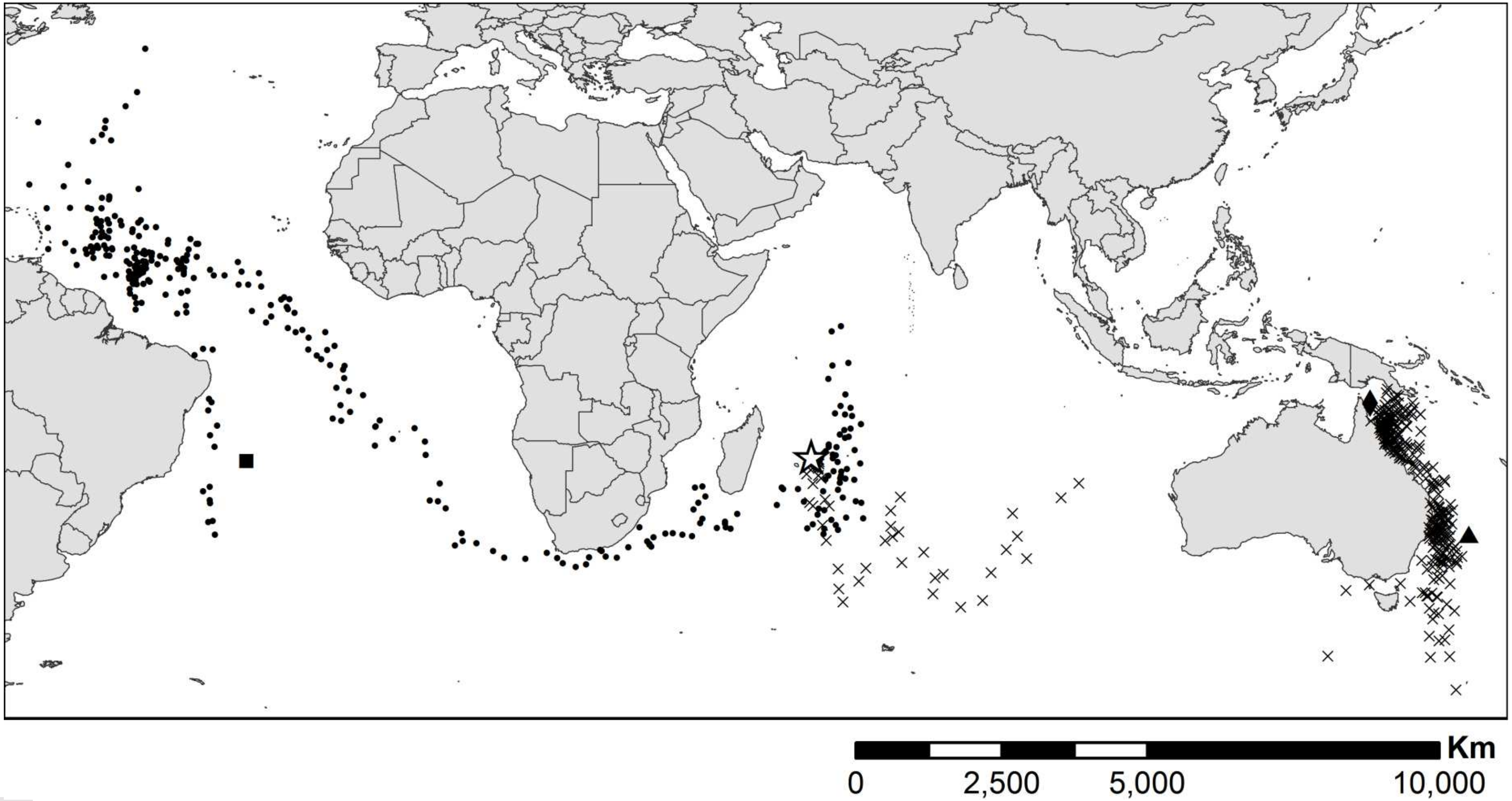

Figure 3: Recorded movement of individuals between oceans. Two individual petrels fitted with geolocators that departed from Round Island in the Indian Ocean and migrated into the Atlantic and Pacific Oceans. Black circular points represent locations from petrel 5H41919 (female) between 02/10/2012 - 11/03/2013. Crosses represent locations from petrel 5H41524 (male) between 19/02/2010 - 18/08/2010. Star = Round Island. Black square = Trindade Islands (Brazil), the only other known colony of the Trindade petrel. Diamond = Raine Island (Australia), where Herald petrel 061-39302 was ringed as a breeding adult in 1984 before it subsequently was found breeding on Round Island between 2006 – 2012. Triangle = Lord Howe Island, the closest known Pacific Kermadec petrel colony to Round Island.