Genetic analyses reveal an unexpected refugial population of subantarctic snipe (*Coenocorypha aucklandica*)

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ABSTRACT: Auckland Island snipe (*Coenocorypha aucklandica aucklandica*) are presumed to have occurred throughout the Auckland Island archipelago but became restricted to a subset of the islands following mammal introductions. Snipe were known to have survived on Adams Island, Ewing Island, and Disappointment Island. However, it is uncertain whether snipe were continually present on Enderby Island and/or adjacent Rose Island. These islands lie near Ewing Island, and both hosted a suite of introduced mammals until the last species were eradicated in 1993. Using SNPs generated by ddRAD-Seq we identified four genetically distinct groups of snipe that correspond to the expected three refugia, plus a fourth comprised of Enderby Island and Rose Island. Each genetic group also exhibited private microsatellite alleles. We suggest that snipe survived *in situ* on Rose and/or Enderby Island in the presence of mammals, and discuss the conservation implications of our findings.

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Introduction

The New Zealand region is the centre of diversity for the austral snipes (*Coenocorypha*: Scolopacidae). These are small birds (*c.* 100 g body mass) of forest, shrubland, and tussock grassland habitats. Snipe were formerly widespread throughout New Zealand (Worthy *et al.* 2002; Roberts & Miskelly 2003), but their small size and unwillingness to fly makes them extremely vulnerable to introduced predators such as cats (*Felis catus*) and rats (*Rattus* spp.) (Miskelly *et al.* 2006). Since Polynesian arrival, three of the six currently recognised New Zealand snipe species have become extinct and the remaining species have suffered range contractions and population extinctions (Worthy & Holdaway 2002; Baker *et al.* 2010).

Subantarctic snipe (*Coenocorypha aucklandica*) occur on the Auckland, Campbell, and Antipodes Islands (Baker *et al.* 2010). The nominate subspecies on the Auckland Islands (Fig. 1) is estimated to occupy less than 20% of its former range owing to the introduction of pigs (*Sus scrofa*) and cats to the main island in the group (Roberts & Miskelly 2003). There have been no confirmed records of snipe on the main Auckland Island since the establishment of cats there some time before 1840 (Miskelly *et al.* 2020 – Chapter 2 in this book). By 1920, Auckland Island snipe were thought to have been restricted to Adams, Disappointment, and Ewing Islands (Fig. 2), which were the only substantial islands lacking introduced mammals at the time (Moore & McClelland 1990; Miskelly *et al.* 2006; Baker *et al.* 2010). However, snipe were occasionally detected on Enderby Island and Rose Island prior to eradication of browsing mammals in 1993 (Torr 2002; Miskelly & Taylor 2020 – Chapter 1 in this book; Miskelly *et al.* 2020 – Chapter 2). Snipe were reported from Enderby Island in 1840, 1874–75, 1890, and 1891, and from Rose Island in 1875 and 1890 (Miskelly & Taylor 2020 – Chapter 1; Miskelly *et al.* 2020 – Chapter 2). Despite numerous landings on both islands from 1941 onwards, there were no subsequent records until 1972 on Enderby Island and 1985 on Rose Island (Miskelly *et al.* 2020 – Chapter 1; Miskelly & Taylor 2020 – Chapter 2). Snipe were reported

FIGURE 1. Auckland Island snipe, Enderby Island, January 2018. *Image: Colin Miskelly.*

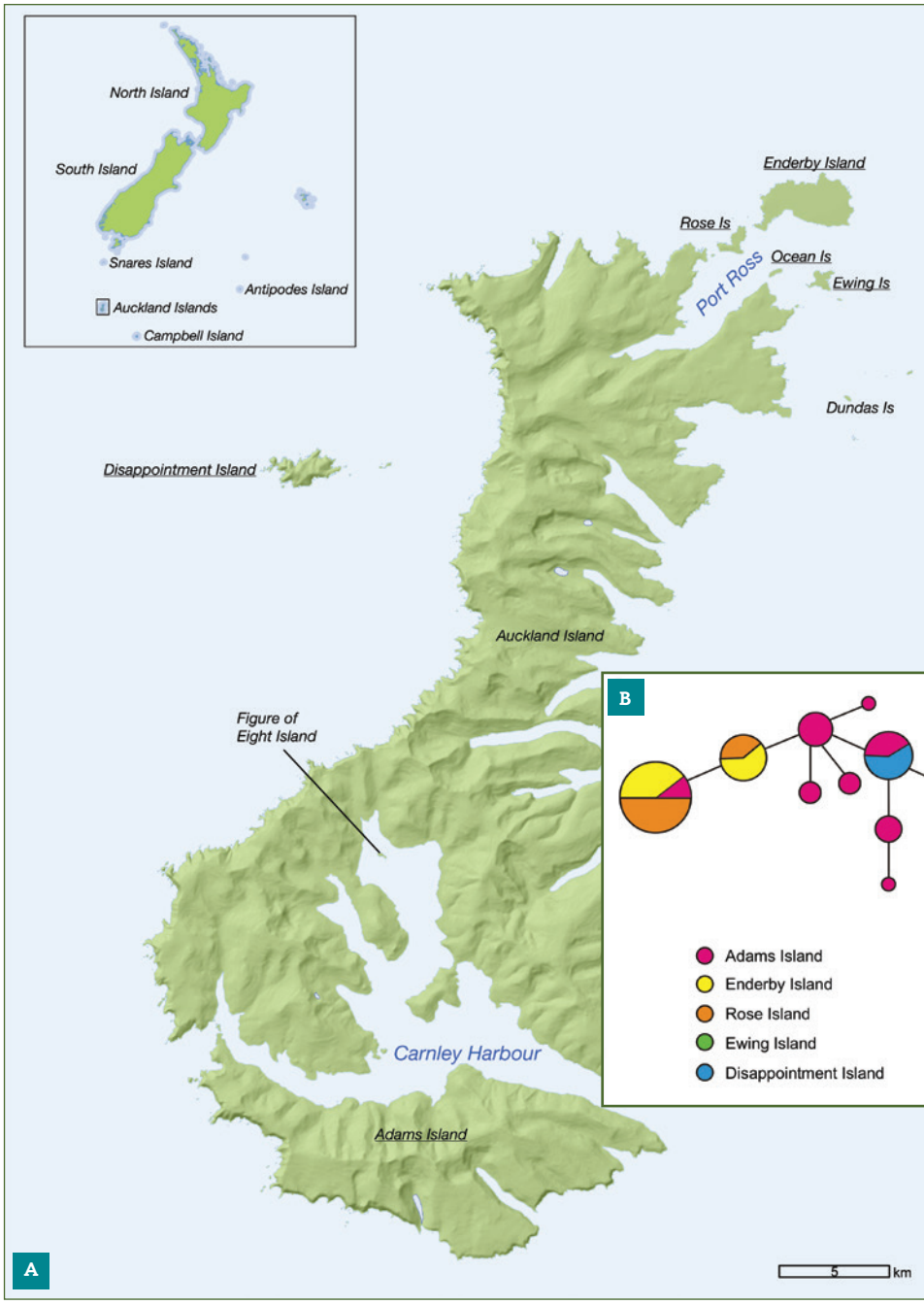

FIGURE 2. A. Map of the Auckland Islands. Islands on which snipe are now present have underlined names; all of these were sampled for the present study except Ocean Island (12 ha). **B.** Median-joining network of the relationships between Auckland Island snipe mitochondrial DNA haplotypes. Each haplotype is separated by a single mutation. The size of each circle is proportional to haplotype frequency.

on Rose Island on four occasions between 1985 and 1991 (including a nest on 24 Dec 1991), and almost annually on Enderby Island between 1980 & 1993 (Miskelly *et al.* 2006; French *et al.* 2010 – Chapter 4 in this book; CMM, *unpubl. data*). Since human arrival, browsing mammals and fire have caused significant changes to the vegetation of Rose and Enderby Islands, with much of the original southern rātā (*Metrosideros umbellata*)-dominated forest replaced by scrub and closely cropped grassland (Taylor 1971). This reduced the available habitat for snipe, as well as making them more vulnerable to predation by New Zealand falcon (*Falco novaeseelandiae*) and southern skua (*Catharacta antarctica*) (French *et al.* 2020 – Chapter 4).

Enderby Island had at least eight species of introduced mammal between 1842 and 1900, but only mice (*Mus musculus*), rabbits (*Oryctolagus cuniculus*), and cattle (*Bos taurus*) after 1900 (Russell *et al.* 2020 – Chapter 6 in this book). Rabbits, cattle, and sheep (*Ovis aries*) were introduced to Rose Island, but rabbits only were present after 1945 (Russell *et al.* 2020 – Chapter 6). Since mammals were eradicated from Rose and Enderby Islands in 1993, snipe have been regularly recorded from both islands (Miskelly *et al.* 2020 – Chapter 2; French *et al.* 2020 – Chapter 4). However, it is unclear whether snipe recolonised from other islands after 1966, or if there was a small overlooked resident population that had persisted since the 1890s (Moore & McClelland 1990; Miskelly *et al.* 2006).

A genetic study of snipe from the Auckland Islands, Campbell Island, and Antipodes Island showed that although mitochondrial DNA (mtDNA) haplotypes were shared, birds from each archipelago were distinguishable by microsatellite DNA markers (Baker *et al.* 2010). Within the Auckland Island archipelago, Baker *et al.* (2010) sampled snipe from three islands (Adams, Enderby, and Rose), with samples from the two islands at Port Ross (Rose and Enderby) pooled for analyses owing to their small sample sizes. Adams Island had the highest genetic diversity of any snipe population, and showed some differentiation with microsatellites from the Port Ross samples.

In this study we perform genetic analyses, for the first time, on snipe from Ewing and Disappointment Islands. One aim was to determine whether these previously unsampled snipe populations harbour novel genetic variation, since these islands were understood to be refugia for snipe. A clear understanding of the distribution of Auckland Island snipe genetic variation is important for their conservation management. We also tested whether Ewing Island and/or Disappointment Island had been the source of the snipe on Rose and Enderby Islands. We added new samples from Rose and Enderby Islands, enabling the data from these islands to be analysed separately. For our new samples, we produced mtDNA sequences and microsatellite genotypes, enabling comparison with the Baker *et al.* (2010) dataset. We also generated single-nucleotide polymorphisms (SNPs) for all sampled populations of Auckland Island snipe using ddRAD-Seq (Peterson *et al.* 2012). SNPs may be superior to microsatellites for resolving

TABLE 1. Sampling details for Auckland Island snipe and genetic data used in this study.

Locality	Sampling date	*n*	mtDNA	microsatellites	ddRAD-Seq
Adams Island	2001	10	Y	Y	Y
	2006	13	Y	Y	–
Rose Island	2001	5	Y	Y	–
	2018	10	Y	Y	Y
Enderby Island	2006	4	Y	Y	–
	2018	10	Y	Y	Y
Ewing Island	2018	10	Y	Y	Y
Disappointment Island	2018	6	Y	Y	Y

population structuring (Vendrami *et al.* 2017).

Materials and methods

Sample collection and DNA extraction

Blood from four populations of subantarctic snipe from the Auckland Islands (Enderby Island (n = 10), Rose Island (n = 10), Ewing Island (n = 10), and Disappointment Island (n = 6)) was collected under permit in 2018 and stored in 95% ethanol prior to DNA extraction (Table 1). DNA was extracted from 10 μL of blood using a DNeasy Blood and Tissue kit (Qiagen), following manufacturer's instructions but eluting in a final volume of 60 µL of Buffer AE.

Mitochondrial DNA (mtDNA) sequencing

We amplified and sequenced the four protein coding genes analysed by Baker *et al.* (2010). COI was ampli-fied with the primers AWCFI and AWCR6 (Patel *et al.* 2010). ATPase6 and 8 were amplified together using LysL (5'-AGCCTTTTAAGCTAGAGA-3') and A2Univ (5'-GGNCGGATNAGNAGRCTTGTTGTTTC-3'). COII was amplified with L8205 (5'-CAAGAAAGGAAGGAATCGAACC-3') and LysH (5'-TCTCTAGCTTAAAAGGCT-3'). PCR amplifications were performed in 12 µL reactions with 1×MyTaq mix (Bioline, Australia), 5 pmol of each primer and between 10 ng and 100 ng DNA. PCR thermocycling conditions followed Baker *et al.* (2010).

PCR products were visualised by agarose gel electrophoresis. Amplification products were purified by digestion with 0.5 U shrimp alkaline phosphatase (SAP, USB Corp.) and 2.5 U exonuclease I (*Exo*I, USB Corp.) at 37°C for 15 min, followed by inactivation of the enzymes at 80°C for 15 min. Sequencing was performed on an ABI 3730xl DNA sequencer (Macrogen, Seoul, Republic of Korea) and sequences were edited with Sequencer version 5.2.4 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences contained no indels and were aligned by eye to the published sequences from Baker *et al.* (2010).

A median-joining network (Bandelt *et al.* 1999) was constructed to determine the relationship between the mitochondrial haplotypes with Network v4.613 (www.fluxus-engineering.com). Transitions and transversions were equally weighted.

Microsatellite genotyping

The newly collected samples were genotyped with the nine microsatellite loci developed for snipe by Baker *et al.* (2010). An M13 tag (TGTAAAACGACGGCCAGT) was added to the 5' end of the forward primer of each locus, thus enabling a fluorescent dye to be added by PCR (Schuelke 2000). Amplifications were performed in 10 µL PCR reactions containing 1 µL of diluted template DNA, 2 pmol forward primer, 8 pmol each of reverse primer and M13 primer (labelled with either FAM, NED, PET or HEX fluorescent tags), and 1× MyTaq mix. PCR thermocycling conditions followed Baker *et al.* (2010) and PCR products were visualised by agarose gel electrophoresis.

Genotyping was performed on an ABI 3130xl Genetic Analyzer at the Massey Genome Service (Massey University, Palmerston North, New Zealand). Alleles were sized using the internal size standard GeneScan 500 LIZ (Applied Biosystems) and scored using Geneious version 10.2.3 (Biomatters Ltd., Auckland, New Zealand).

A subset of samples from the Baker *et al.* (2010) study were genotyped in the Te Papa laboratory in order to standardise scoring between the two laboratories. This allowed the newly generated microsatellite data to be combined with the Baker *et al.* (2010) dataset. Possible scoring errors caused by null alleles, stutter and allelic dropout were assessed with MICROCHECKER v2.2.3 (van Oosterhout *et al.* 2004).

GenAlEx 6.5 (Peakall & Smouse 2012) was used to calculate descriptive statistics for each population, including observed (H_O) and expected (H_E) heterozygosity, number of alleles (A), and number of private alleles (P_A). Inbreeding coefficients (F_{IS}) were calculated in Arlequin v3.5.2.2 (Excoffier & Lischer 2010) and statistical significance was tested by 10,000 permutations. Given the temporal separation of samples from Enderby and Rose Islands with our STRUCTURE analyses of the microsatellite data and the inability of these data to distinguish the two islands (see Results section), microsatellite summary statistics for snipe from these two islands were analysed in several ways: (1) Enderby and Rose Island snipe

TABLE 2. Summary of ddRAD-Seq data assembled for Auckland Island snipe with ipyrad following duplicate merging. * = after excluding loci with depth <6.

Sample	Population	Number of raw reads	Clusters	Loci assembled	Average depth*	Heterozygosity
Adams1	Adams Island	1,921,077	783,394	1,991	9.09	0.00239
Adams2	Adams Island	1,046,974	499,207	1,226	8.55	0.00285
Adams3	Adams Island	685,962	380,567	448	9.02	0.00314
Adams4	Adams Island	1,010,226	527,622	840	9.21	0.00291
Adams5	Adams Island	608,516	351,921	360	9.65	0.00391
Adams7	Adams Island	1,216,307	550,695	1,360	8.77	0.00282
Adams8	Adams Island	906,220	496,414	678	9.09	0.00364
Adams9	Adams Island	2,295,495	924,115	2,155	8.88	0.00270
Adams10	Adams Island	1,846,937	731,001	2,086	8.85	0.00289
Rose1	Rose Island	881,977	470,646	738	8.84	0.00289
Rose2	Rose Island	2,231,940	980,214	2,121	8.66	0.00355
Rose3	Rose Island	1,683,087	838,773	1,559	8.53	0.00224
Rose4	Rose Island	788,334	472,609	372	9.21	0.00234
Rose5	Rose Island	2,574,284	1,054,258	2232	8.58	0.00342
Rose6	Rose Island	912,882	524,990	529	9.03	0.00230
Rose7	Rose Island	1,664,995	745,664	1,888	8.37	0.00336
Rose8	Rose Island	642,234	357,880	471	9.50	0.00230
Rose9	Rose Island	1,486,283	718,384	1,546	8.62	0.00386
Rose10	Rose Island	657,913	383,598	333	9.22	0.00233
Enderby1	Enderby Island	2,548,446	718,384	2,162	8.47	0.00210
Enderby2	Enderby Island	831,095	466,747	337	9.15	0.00348
Enderby3	Enderby Island	3458742	966,127	2,420	9.28	0.00370
Enderby4	Enderby Island	2,484,346	818,878	2,265	8.70	0.00220
Enderby5	Enderby Island	1,568,913	635,688	1,543	8.39	0.00221
Enderby6	Enderby Island	3,245,428	1,132,421	2,412	9.36	0.00255
Enderby7	Enderby Island	1,605,043	655,021	1,624	8.38	0.00221
Enderby8	Enderby Island	1,617,932	696,632	1,423	8.39	0.00242
Enderby9	Enderby Island	1,942,218	764,437	1,758	8.41	0.00233
Enderby10	Enderby Island	942,172	512,026	491	9.55	0.00234
Ewing1	Ewing Island	2,057,010	867,853	1,793	8.37	0.00210
Ewing2	Ewing Island	1,108,098	550,301	845	8.85	0.00306
Ewing3	Ewing Island	1,537,708	672,930	1,486	8.28	0.00184

Sample	Population	Number of raw reads	Clusters	Loci assembled	Average depth*	Heterozygosity
Ewing4	Ewing Island	1,289,934	672,930	888	8.64	0.00288
Ewing5	Ewing Island	726,659	407,258	303	9.60	0.00416
Ewing6	Ewing Island	2,148,003	963,580	1,727	8.35	0.00203
Ewing7	Ewing Island	961,644	511,298	602	8.97	0.00312
Ewing8	Ewing Island	2,081,608	850,629	2,038	8.41	0.00200
Ewing9	Ewing Island	2,660,564	1,034,632	2,331	8.86	0.00157
Ewing10	Ewing Island	2,601,661	938,088	2,345	8.99	0.00200
Disappointment1	Disappointment Island	2,640,003	1,481,423	2,427	9.64	0.00201
Disappointment2	Disappointment Island	2,848,722	1,133,778	2,163	8.56	0.00197
Disappointment3	Disappointment Island	909,770	462,258	539	9.29	0.00310
Disappointment4	Disappointment Island	2,351,254	966,182	1,959	8.31	0.00226
Disappointment5	Disappointment Island	1,778,850	821,905	1,396	8.52	0.00230
Disappointment6	Disappointment Island	3,354,528	1,067,436	2,371	9.08	0.00200

were analysed separately, with samples collected on each island at different time periods combined; (2) Enderby and Rose Island snipe were analysed independently, with temporally separated samples also distinguished; and (3) all Rose and Enderby Island samples were combined (Port Ross population in Baker *et al.* 2010).

Loci were tested for deviation from Hardy-Weinberg equilibrium (HWE) with GenAlEx 6.5. The sequential Bonferroni correction was used to correct the significance values for multiple tests (Holm 1979). Genetic differentiation was then estimated by calculating pairwise F_{ST} values between all sample groupings with more than five samples with Arlequin v3.5.2.2. Statistical significance was tested in Arlequin by 10,000 permutations.

Population structure was examined with STRUCTURE v2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2007), without prior grouping assumptions. The number of genetic clusters (K) was set between 1 and 5, with 10 permutations performed for each value of K. We used the admixture model with correlated allele frequencies, and performed 100,000 generations of burn-in, followed by 500,000 Markov Chain Monte Carlo iterations. The optimal number of genetic clusters (K) was obtained by calculating the ΔK statistic (Evanno *et al.* 2005 in STRUCTURE HARVESTER web v.0.6.94 (Earl & vonHoldt 2012)), but we also examined all clustering results that warranted biological interpretation, following Meirmans (2015). The CLUMPAK online server (http://clumpak.tau.ac.il/contact.html; Kopelman *et al.* 2015) was used to average iterative runs of K and view the results.

ddRAD-Seq

Double-digest restriction-site-associated sequencing (ddRAD-Seq) libraries (Peterson *et al.* 2012) were prepared for 45 snipe from the Auckland Islands (Table 2), plus 17 samples from Campbell Island and Antipodes Island from Baker *et al.* (2010), with five libraries processed in duplicate as technical replicates. Libraries were prepared following the method used by Shepherd *et al.* (2019) and described at protocols.io (dx.doi.org/10.17504/protocols.io.x2afqae).

Briefly, for each sample, 300 ng of DNA was digested with two restriction enzymes, following

the manufacturer's instructions. We tested AvaII paired with MspI or MseI because we already had the appropriate adaptors for these enzymes from other projects in the laboratory. AvaII and MspI were selected because they produced a large number of fragments within the desired size range for the test samples. Adaptors containing sample-specific barcodes and Illumina indices were ligated to each sample. Samples were pooled into three index pools, and 300–500 bp fragments were size-selected by excision from an agarose gel, followed by extraction with a Qiaquick gel extraction kit (Qiagen). Illumina indices were then added by PCR to each size-selected sample pool with Phusion flash high-fidelity PCR master mix (Thermo Scientific). Each pooled sample was purified and concentrated with a MinElute kit (Qiagen), quantified with a Qubit dsDNA HS (high sensitivity) assay kit (Thermo Fisher Scientific), and combined in equimolar amounts. The library was sequenced across a quarter of a lane of an Illumina HiSeq 2500 to generate 2 × 125 bp reads.

ipyrad v0.7.28 (Eaton & Overcast 2017) was used to demultiplex the paired-end reads, remove adaptors, then merge and assemble the reads into *de novo* loci. To confirm that each of the five technical replicates grouped with their duplicate, a preliminary analysis was performed clustering the merged reads at 90% similarity, with a minimum depth of coverage of six. Only loci present in at least 50% of the samples were retained. A NeighborNet network (see below) was constructed from this initial assembly, which confirmed that the technical replicates grouped together, apart from one replicate in which very few loci were assembled and that was placed on an internal node in the network. The reads from the technical replicates were pooled for subsequent analyses and a final dataset reassembled as described above.

The final output from ipyrad (.vcf file) was imported into Stacks 2.41 (Catchen *et al.* 2011) in order to calculate population genetic statistics for each of the Auckland Island populations. The number of private alleles, observed and expected heterozygosity, nucleotide diversity (π), and inbreeding coefficients (F_{IS}) were calculated for all SNPs, except those that had more than two alleles, with the POPULATIONS program in Stacks.

Networks were constructed with the NeighborNet algorithm (Bryant & Moulton 2004), implemented in SplitsTree4 v4.14.8 (Huson & Bryant 2006), in order to assess the phylogenetic signal present in the ddRAD-Seq data. Indels were excluded from the dataset, then uncorrected

TABLE 3. Genetic diversity of Auckland Island snipe populations based on nine microsatellite markers. *n* = number of samples, A and P = number of alleles and private alleles, respectively. H_o and H_e = observed and expected heterozygosity, respectively. F_{IS} = inbreeding coefficient, calculated for populations with n ≥ 5 individuals. *p < 0.01.

Population	*n*	A	P	H_O	H_E	F_{IS}
Adams Island	23	40	13	0.46 ± 0.09	0.56 ± 0.09	0.219*
Enderby Island	14	27	2	0.32 ± 0.07	0.37 ± 0.08	0.133
Enderby <2007	4	18	0	0.28 ± 0.10	0.32 ± 0.07	–
Enderby 2018	10	24	2	0.33 ± 0.08	0.33 ± 0.08	0.104
Rose Island	15	24	1	0.30 ± 0.07	0.37 ± 0.08	0.242*
Rose <2007	5	20	1	0.36 ± 0.10	0.26 ± 0.07	–0.224
Rose 2018	10	21	0	0.28 ± 0.08	0.29 ± 0.09	0.002
Rose & Enderby	29	30	4	0.31 ± 0.06	0.39 ± 0.08	0.206*
Ewing Island	10	19	1	0.26 ± 0.09	0.34 ± 0.09	0.394*
Disappointment Island	6	20	1	0.30 ± 0.08	0.37 ± 0.08	0.219

p-distances and the equal-angle algorithm were used to construct the networks. Networks were constructed for all the subantarctic snipe sampled (Auckland Islands, Campbell Island, and Antipodes Island) and for only the snipe from the Auckland Islands.

Population structuring of the Auckland Islands snipe ddRAD-Seq data was examined using STRUCTURE v2.3.4, as described for the microsatellite dataset. A single SNP was randomly selected from each ddRAD locus (.ustr file) using ipyrad v0.7.28.

Results

mtDNA sequencing

The newly generated mitochondrial sequences have been deposited in GenBank (accession numbers MK889241 to MK889349). The relationships between the mtDNA haplotypes are shown in the median-joining network in Fig. 2B based on 1,980 bp of the four protein-coded genes concatenated. No additional haplotypes to those found by Baker *et al.* (2010) were discovered. Snipe from Ewing and Disappointment Islands (which were not sampled by Baker *et al.* 2010) each had a different single mtDNA haplotype. Both of these haplotypes were also found on Adams Island. Two haplotypes were found in snipe from Enderby and Rose Islands, with one of these haplotypes also detected on Adams Island.

Microsatellites

MICROCHECKER found no evidence of large allele dropout or stuttering in the microsatellite data. Possible null alleles were inferred for two loci, each in a single population (locus SN6 on Rose Island and locus SN11 on Ewing Island). The null allele frequencies were estimated to be low (>0.25) and, because previous research has shown that low-frequency null alleles have little influence on the detection of genetic differentiation (Carlsson 2008), we retained these loci for subsequent analyses.

TABLE 4 (RIGHT). Pairwise F_{ST} values for snipe populations with n ≥ 5 individuals based on nine microsatellite loci. Significant values are shown in bold type ($P < 0.01$).

Population	Adams Island	Enderby Island	Enderby 2018	Rose Island	Rose <2007	Rose 2018	Rose & Enderby	Ewing Island	Disappointment Island
Adams Island	–								
Enderby Island	**0.241**	–							
Enderby 2018	**0.279**	–	–						
Rose Island	**0.203**	0.043	–	–					
Rose <2007	**0.226**	–	**0.395**	–	–				
Rose 2018	**0.260**	–	0.168	–	**0.378**	–			
Rose & Enderby	**0.227**	–	–	–	–	–	–		
Ewing Island	0.246	**0.124**	**0.121**	**0.102**	**0.412**	0.059	**0.246**	–	
Disappointment Island	**0.210**	**0.170**	**0.175**	**0.169**	**0.397**	**0.174**	**0.206**	**0.184**	–

Auckland Island snipe exhibited a total of 46 microsatellite alleles across the nine loci, with 2–10 alleles per locus. All populations had at least one private microsatellite allele, with Adams Island exhibiting the greatest number of private alleles (13; Table 3). Adams Island also had the highest total number of microsatellite alleles, with 40, while the other populations had 19–27 alleles each. Adams Island had the highest H_O and H_E values (0.46 and 0.56, respectively), with values of 0.26–0.39 calculated for the other islands. Four sample groupings had significant F_{IS} values, indicating inbreeding: Adams, Rose, Rose & Enderby, and Ewing Islands, although combining samples across time periods may have contributed to the significant values for the first three sites.

Following sequential Bonferroni correction, no loci significantly deviated from Hardy-Weinberg equilibrium. The F_{ST} values were significant for all pairwise comparisons of populations except for the comparisons between Rose and Enderby Islands, both for samples collected in 2018, and for Rose Island (both time periods pooled) versus Enderby Island (both time periods pooled) and

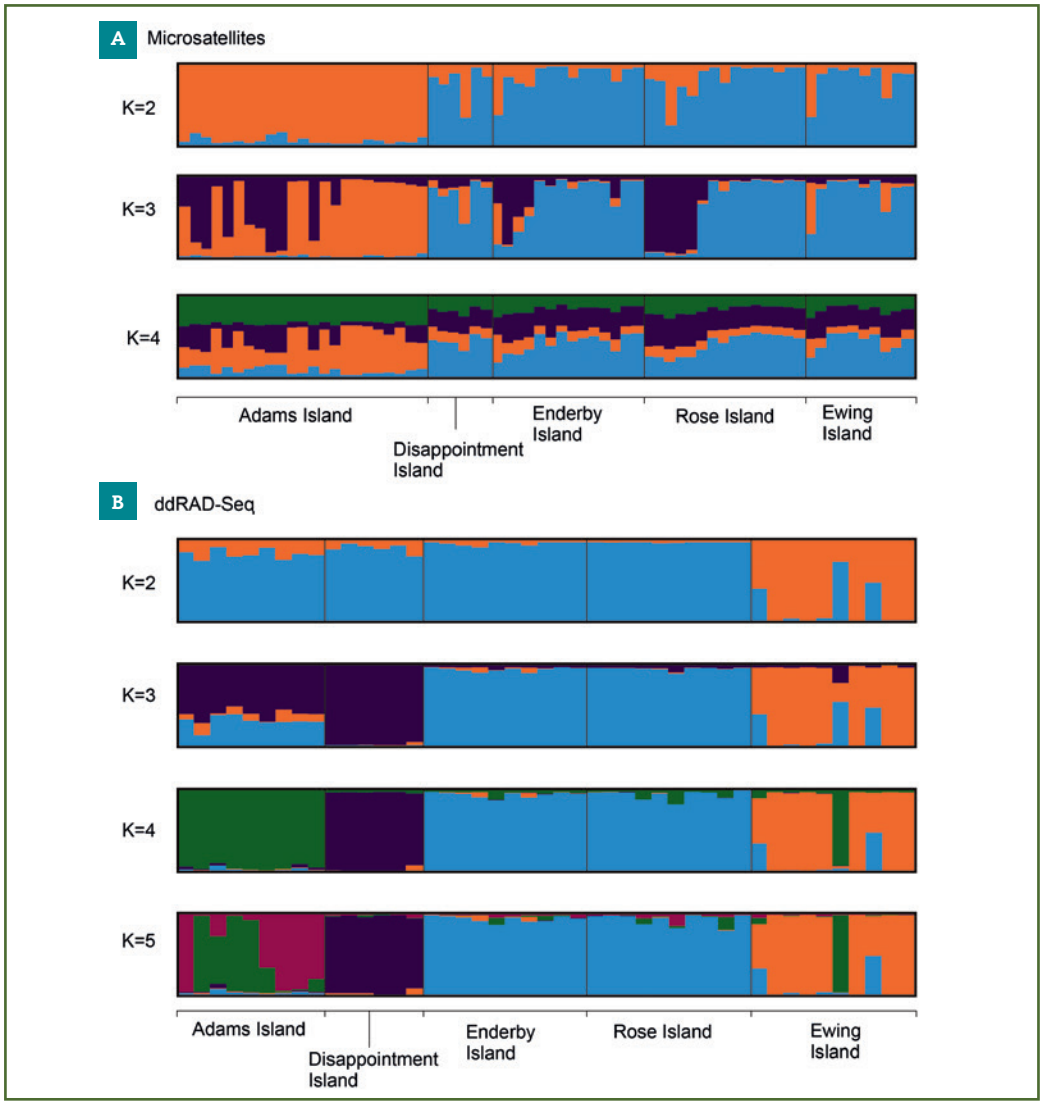

FIGURE 3. Structure plots for Auckland Island snipe for (**A**) microsatellites and (**B**) SNPs developed using ddRAD-Seq.

TABLE 5. Genetic diversity of Auckland Island snipe populations based on both fixed and variant SNPs generated by ddRAD. ***n*** = number of samples; P = number of private alleles; H_O and H_E = observed and expected heterozygosity, respectively; □ = average nucleotide diversity; F_{IS} = inbreeding coefficient.

Population	*n*	P	H_O	H_E	□	F_{IS}
Adams Island	9	737	0.195 ± 0.003	0.208 ± 0.002	0.241	0.094
Rose Island	10	350	0.166 ± 0.003	0.175 ± 0.002	0.203	0.073
Enderby Island	10	430	0.174 ± 0.002	0.193 ± 0.002	0.211	0.083
Ewing Island	10	472	0.157 ± 0.002	0.160 ± 0.002	0.179	0.048
Disappointment Island	6	592	0.188 ± 0.002	0.193 ± 0.002	0.222	0.069

the Rose Island samples from 2018 versus Ewing Island (Table 4).

The Structure Harvester analysis of ΔK indicated that the optimal K was 2. However, this method cannot evaluate the ΔK value for K = 1 and so we visually confirmed that the K = 2 plot made biological sense. At K = 2 the Adams Island snipe were partitioned into a single cluster with high probability (Fig. 3A). Snipe from the remaining islands (Enderby, Rose, Ewing, and Disappointment Islands) were largely assigned to a second cluster, but some individuals from these islands exhibited mixed ancestry to both clusters. At K = 3 a third cluster partitioned samples that were collected from Rose Island in 2001, Enderby Island in 2006, and some of the Adams Island samples collected in 2001, although apart from the Rose Island samples, assignments mostly had moderate probabilities. This separation may reflect differences in microsatellite scoring between laboratories, despite our attempts at standardising scoring. However, there were no apparent allele 'size shifts' (Ellis *et al.* 2011), and most of the differences between sampling periods appeared to result from loci that were fixed for a single allele in the older samples showing variation in the more recently collected samples, and vice versa. Alternatively, the differences may have arisen by chance sampling of alleles owing to small sample sizes. At values of K > 3, individuals were partitioned with low to moderate Q values across groups (e.g. K = 4; Fig. 3A).

ddRAD-Seq

The Illumina sequencing of the 45 snipe ddRAD libraries, with duplicates combined (see below), resulted in 76.4M paired reads after initial quality filtering, with an average of 1.7M paired reads per individual (Table 2). Fastq sequence files have been deposited in the GenBank short-read archive database (accession number: PRJNA541383). The final datasets, clustered in ipyrad with a 90% similarity threshold, comprised 2,490 loci with 8,390 SNPs for the Auckland Island samples only, and 1,758 loci with 6,870 SNPs for the dataset that also includes samples from Campbell and Antipodes Islands snipe.

The summary statistics for the ddRAD data for each population (Table 5) indicate that the Adams Island population is the most diverse, with the highest number of private alleles, highest nucleotide diversity, and highest observed and expected heterozygosities. Ewing Island snipe were the least diverse.

In the NeighborNet network of the Auckland Island snipe (Fig. 4) the Rose and Enderby Island samples together formed a well-defined cluster, but each of these two islands was not distinguishable. Apart from sample Ewing5, which clustered with the Adams Island snipe, the Ewing Island snipe also formed a distinct cluster. The samples from Disappointment Island grouped together except for sample Disappointment3, which fell in an intermediate position between the remaining Disappointment Island samples and sample Adams7 from Adams Island.

In the NeighborNet network of all the subantarctic snipe sampled, the Antipodes Island snipe and Campbell Island snipe were each recovered as distinct clusters separated from snipe from the Auckland Islands (Fig. 5). Snipe from both the Antipodes Island and Campbell Island were less diverse than those from the Auckland Islands.

For the STRUCTURE analyses the optimal K was 2, as determined by the STRUCTURE HARVESTER analysis of ΔK. At K = 2 most of the Ewing Island snipe were partitioned from

the remaining Auckland Island snipe (Fig. 3B). At K = 3 the Ewing Island snipe formed a cluster; a second cluster included the Enderby and Rose Island snipe; and snipe from Disappointment Island formed a third cluster. Adams Island snipe showed mixed ancestry to all three clusters, but with highest membership coefficients to the Disappointment Island cluster. At K = 4 Adams and Disappointment Island snipe were each assigned with high probability to distinct clusters. Enderby and Rose Island snipe were assigned with high probability to a single cluster. Most of the Ewing Island snipe were assigned to a fourth cluster but two individuals (Ewing1 and Ewing7) showed mixed ancestry between the Ewing cluster and the Rose & Enderby cluster, and a third Ewing Island individual (Ewing5) was assigned with high probability to the Adams Island cluster. At K = 5 Enderby and Rose Island snipe were still not distinguished, but the Adams Island samples were assigned across two clusters (Fig. 3B).

Discussion

Unexpected survival of snipe in the presence of mammals

Our genetic data support the survival of snipe in at least four refugia in the Auckland Island archipelago: Adams Island, Disappointment Island, and Ewing Island, plus Rose Island and/

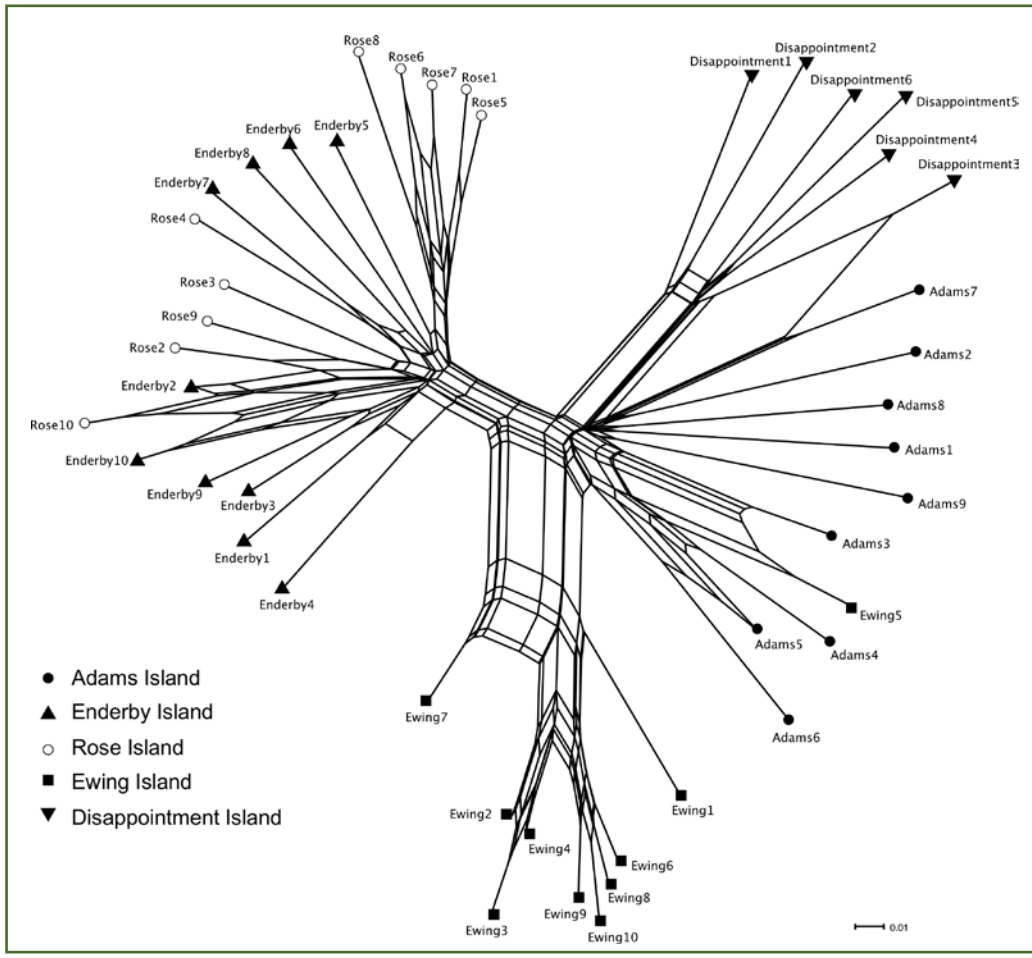

FIGURE 4. NeighborNet phylogenetic network for Auckland Island snipe based on 8,390 SNPs.

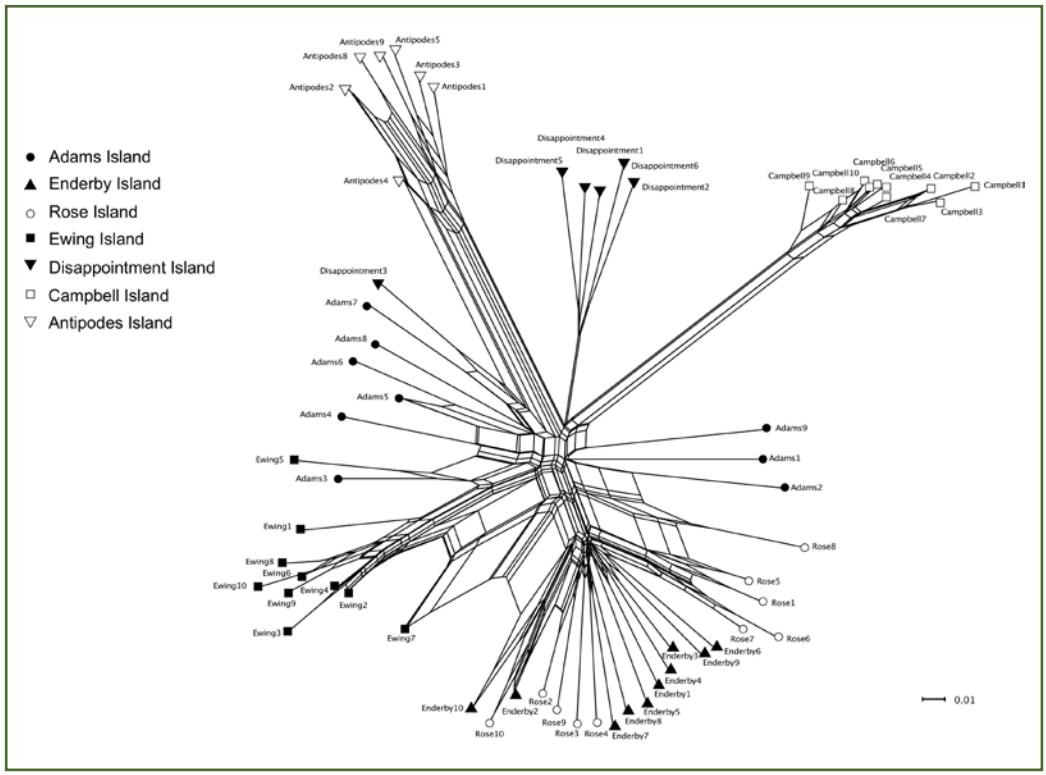

FIGURE 5. NeighborNet phylogenetic network for subantarctic snipe based on 6870 SNPs.

or Enderby Island. Snipe from each of these locations exhibited private microsatellite and ddRAD-Seq alleles, and each island was distinguished in the STRUCTURE analyses of the SNP dataset, except for Rose and Enderby, which were distinct from the other islands but not differentiated from each other.

Determining when this genetic differentiation arose is difficult. It is likely that Auckland Island snipe existed as a panmictic population prior to the establishment of introduced mammals in the past 220 years, with the exception of Disappointment Island snipe, which occur on the most distant island. The extinction of snipe on the main Auckland Island, which is centrally located in the archipelago, would have reduced any gene flow between islands. The isolation of the remaining snipe on the small islands in Port Ross, plus the reduction of their population sizes owing to predation (aggravated by the removal of ground-cover vegetation by introduced browsers; French *et al* 2020 – Chapter 4; Russell *et al*. 2020 – Chapter 6), may have led to their genetic differentiation as a result of genetic drift.

Alternatively, snipe populations may have became isolated on islands as a result of sea-level rise following the Last Glacial Maximum (LGM). During the LGM, sea level was significantly lowered so that the islands of the Auckland Island archipelago were connected, with the landmass at that time approximately ten times greater than at present (fig. 518 in Craig *et al*. 2012). Both scenarios could conceivably produce the observed genetic patterns.

The distinctiveness of Rose and Enderby Island snipe compared with the other snipe populations was surprising because snipe were thought to have disappeared from these islands owing to the introduction of browsing mammals (Taylor 1971; Roberts & Miskelly 2003; Miskelly *et al*. 2006). They could not have recolonised from nearby Ewing or Disappointment Islands, as snipe from these islands exhibited different mtDNA haplotypes to those found on Rose and

Enderby Islands. Rose and Enderby Island snipe did share one of their two mtDNA haplotypes with snipe from the distant Adams Island, but it seems unlikely that snipe would have colonised from there when much closer source populations were present on Ewing and Disappointment Islands. Instead it seems likely that snipe managed to survive on Enderby and/or Rose in the presence of mammals, and despite the vegetation changes caused by herbivorous mammals and fire. Enderby Island had eight species of mammal prior to the 1993 eradication, including pigs, which were common there in the mid-1850s and mid-1880s (Russell *et al.* 2020 – Chapter 6). Pigs have been observed to prey on seabirds and their eggs and likely also preyed on ground-nesting land birds such as snipe (Russell *et al.* 2020 – Chapter 6). During times of high pig densities, snipe may have been restricted to Rose Island, subsequently dispersing back to Enderby Island across the ~400 m of ocean separating the two islands when pigs died out.

There are few records of snipe on Enderby and Rose Islands prior to 1993 but, following mammal eradication, snipe quickly become abundant in the densely vegetated areas of these islands (Miskelly *et al.* 2006, 2020 – Chapter 2). Surprisingly, snipe from Enderby and Rose Islands do not show reduced genetic variation relative to Ewing and Disappointment Islands, which never lost their snipe populations, and, in the case of Disappointment Island, never had introduced mammals. The four islands exhibited similar microsatellite heterozygosity estimates, and the combined Rose and Enderby population demonstrates both a greater number of microsatellite alleles and private alleles than detected from Ewing and Disappointment snipe (30 total alleles and four private alleles on Rose and Enderby, versus 20 total and one private allele on Disappointment Island, and 19 total and one private allele(s) on Ewing Island). This result suggests that snipe must have survived in reasonable numbers on Enderby and Rose Islands to maintain the observed genetic diversity.

Dispersal of snipe between islands

Although snipe are generally considered poor flyers, they are capable of sustained flight. Snipe are thought to have dispersed from the Auckland Islands to the Campbell and Antipodes Islands in the past 10,000 years (Baker *et al.* 2010). In the early 2000s, following the removal of Norway rats in 2001, snipe rapidly recolonised Campbell Island from Jacquemart Island 1 km offshore (Miskelly & Fraser 2006). There is also some evidence that snipe have dispersed within the Auckland Island archipelago. In the 1980s, snipe tracks were found on Dundas Island, 5 km south of Ewing Island, and Figure of Eight Island, 10 km north of Adams Island, and a snipe was seen on Dundas Island in 1999 (Miskelly *et al.* 2006, 2020 – Chapter 2). These small islands are unlikely to support breeding populations of snipe, and so these individual birds are likely to have dispersed from nearby islands.

Our inability to genetically distinguish the snipe from Rose and Enderby Islands indicates that sufficient dispersal of snipe has occurred, or continues to occur, across the *c.* 400 m water gap separating these two islands to homogenise their gene pools. The genetic data contained several hints of snipe dispersal between other islands, although none is convincing. One sample from Disappointment Island, Disappointment3, fell in an intermediate position between the remaining samples from Disappointment Island and samples from Adams Island in the NeighborNet. However, this sample clearly clustered with the rest of the Disppointment Island samples in the STRUCTURE analysis of the ddRAD data. Sample Ewing5 from Ewing Island grouped with Adams Island snipe in both the NeighborNet and STRUCTURE analyses of the SNP data, which may indicate that it is a migrant. However, this sample had a high level of missing data, which may have resulted in its misclassification. Two other Ewing Island snipe (Ewing1 and Ewing7) showed mixed ancestry between the Enderby/Rose cluster and the Ewing cluster in the STRUCTURE analysis. It is possible that these are descendants of snipe that have migrated to Ewing from Enderby or Rose Islands. Since these samples exhibit the mtDNA haplotype found in Ewing Island snipe, which was not detected on Enderby or Rose Islands, it would suggest any dispersers were male.

Conservation implications

The distinctiveness of each of the populations of Campbell Island, Antipodes Island, and Auckland Island snipe in the network, combined with the

morphometric and plumage differences that occur between them (Worthy *et al*. 2002; Miskelly & Fraser 2006), supports their recognition as separate subspecies. None of the snipe populations from the Auckland Islands are as distinct as the Campbell and Antipodes Island populations.

If the Department of Conservation undertakes a planned eradication of pigs, cats, and mice from the main Auckland Island, then it is expected that snipe will eventually naturally recolonise. In order to avoid a population deriving from a small number of founders, which may put populations at risk of inbreeding depression (Frankham *et al*. 2002), conservation managers may opt to assist in the colonisation process. Translocations from Ewing, Disappointment, Adams, and Rose/Enderby Islands would be required to ensure that all the genetic variation detected in the Auckland Island archipelago is represented. If only one source of snipe can be chosen for translocation to Auckland Island, then Adams Island would be the obvious choice because it harbours the most genetic diversity.

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