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ORIGINAL ARTICLE

Demographic history inferred from genome-wide data reveals two lineages of sheldgeese endemic to a glacial refugium in the southern Atlantic

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ABSTRACT

Aim The Malvinas/Falkland Islands (MFI) constitute the largest archipelago in the southern Atlantic, and harbour endemic lineages that presumably evolved after sea-level rise, associated with glacial periods, isolated ancestral populations. We investigate the role of the MFI in isolating populations from continental counterparts of two highly vagile species: the sheldgeese *Chloephaga picta* and *Chloephaga rubidiceps*.

Location Patagonia and the Malvinas/Falkland Islands.

Methods We sampled *C. picta* and *C. rubidiceps* on the continent and MFI. Using a reduced-representation genomic approach, we quantified the genetic differentiation between insular and continental populations of both species, and used coalescent-based analyses to model their demography.

Results The MFI harbour independently evolving lineages of *C. picta* and *C. rubidiceps*, which diverged from their continental counterparts during the Middle-Late Pleistocene and have since experienced negligible gene flow.

Main conclusions The *c.* 450 km that separate the archipelago from the continent are sufficient to isolate populations of these putatively highly vagile species. Ancestral lineages may have reached the MFI refugium during glacial cycles. Without conservation measures, the drastic decline of the morphologically, behaviourally and ecologically distinct continental population of *C. rubidiceps*, to < 1000 individuals, may lead to the extinction of an independently evolving taxon.

Keywords
***Chloephaga*, conservation genetics, demographic modelling, endangered species, island endemism, Malvinas/Falkland Islands, Patagonia, Pleistocene refugium, taxonomy**

INTRODUCTION

Isolated environments that harbour endemic lineages, such as archipelagos, sky islands and crater lakes, illustrate the processes that influence diversification and speciation (MacArthur & Wilson, 1967; Whittaker & Fernandez Palacios, 2007; Losos & Ricklefs, 2009). A combined effect of geographical isolation on reducing gene flow, founder effects that accentuate genetic drift, the availability of vacant niches and novel selective pressures have led to new species and also generated spectacular radiations that are central to the study of evolutionary biology (e.g. Darwin's finches: Grant, 1999). An organism's dispersal ability (or vagility) increases the chance that it may colonize a remote area, but will also have an effect on the degree of continued gene flow from a source population that may limit differentiation once the coloniza-

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tion event has occurred. Evolutionary shifts in the ability to disperse provide a possible solution to this paradox, and have been cited as a driver of diversification in the species-rich white-eyes (Aves, Zosteropidae; Moyle *et al.*, 2009). The dispersal capacity of most birds, and the fact that a significant proportion of the world's extant avifauna (*c.* 17% of all species) evolved in association with archipelagos (Johnson & Stattersfield, 1990), suggests that this mechanism is plausible.

Fluctuations in the level of the sea generated by climatic oscillations during the Pleistocene have affected the connectivity between islands and continents, and coupled with changes in the distribution of suitable habitat have promoted the diversification of various species (Hosner *et al.*, 2014). The Malvinas/Falkland Islands (hereafter MFI), the largest archipelago in the southern Atlantic (52° S; 12,000 km^2 in area), are on the Argentine continental shelf, *c.* 450 km from mainland Tierra del Fuego. Despite the close proximity to the continent and the effects of the Pleistocene glaciations on climatic conditions at these high latitudes (Rabassa *et al.*, 2011), the MFI contain a large number of endemic species including invertebrates (McDowall, 2005; Papadopoulou *et al.*, 2009), vascular plants (Woods, 2000; McDowall, 2005), two bird species (the Falkland steamer duck, *Tachyeres brachypterus*, Woods & Woods, 2006; Fulton *et al.*, 2012; and Cobb's wren, *Troglodytes cobbi*, Campagna *et al.*, 2012) and the extinct Falkland Islands wolf (*Dusicyon australis*, Slater *et al.*, 2009). This biota is derived from that of Patagonia (McDowall, 2005; Morrone & Posadas, 2005) and is thought to have reached the MFI during the Pleistocene glaciations, when sea levels were lower and a large proportion of Patagonia was covered by ice sheets (McDowall, 2005; Rabassa *et al.*, 2011). The avifauna of the MFI is composed of *c.* 240 species (Woods, 1988), and all but the two aforementioned endemics have populations on the continent. A genetic analysis of all passerines that breed in the MFI found mostly low genetic differentiation with respect to continental populations, suggesting either very recent colonization or ongoing gene flow (Campagna *et al.*, 2012). McCracken & Wilson (2011) used genetic data to study the connectivity between continental and insular populations of speckled teal (*Anas flavirostris*), finding significant differentiation despite low levels of gene flow into the MFI, in the direction of the prevailing winds. Preliminary molecular analyses of sheldgeese in the genus *Chloephaga* (Eyton, 1838) also suggest that the MFI are sufficiently isolated from the continent to promote the diversification of populations of putatively highly vagile species (Bulgarella *et al.*, 2014).

Chloephaga comprises five species of waterfowl (Anatidae) endemic to mainland South America and the MFI. Three species, the ashy-headed goose (*C. poliocephala*; Sclater, 1857), the upland goose (*C. picta*; Gmelin, 1789), and the ruddy-headed goose (*C. rubidiceps*; Sclater, 1861), have continental populations that breed in Patagonia and Tierra del Fuego and migrate northwards to winter in central Argentina (Fig. 1). *Chloephaga picta* and *C. rubidiceps* possess non-migratory populations in the MFI (Woods, 1988). There are two recognized subspecies for *C. picta*: *C. p. picta* in the mainland and *C. p. leucoptera* in the MFI (Carboneras, 1992). No subspecies of *C. rubidiceps* are currently recognized, although differences in morphology, behaviour (differences in vocalizations) and ecology (resident population in the MFI versus migratory population in the continent) have been suggested between populations (Chebez, 2008). Insular and continental populations of sheldgeese of both species show opposing demographic trends. In the MFI populations are stable, with 42,000–81,000 individuals of *C. rubidiceps*

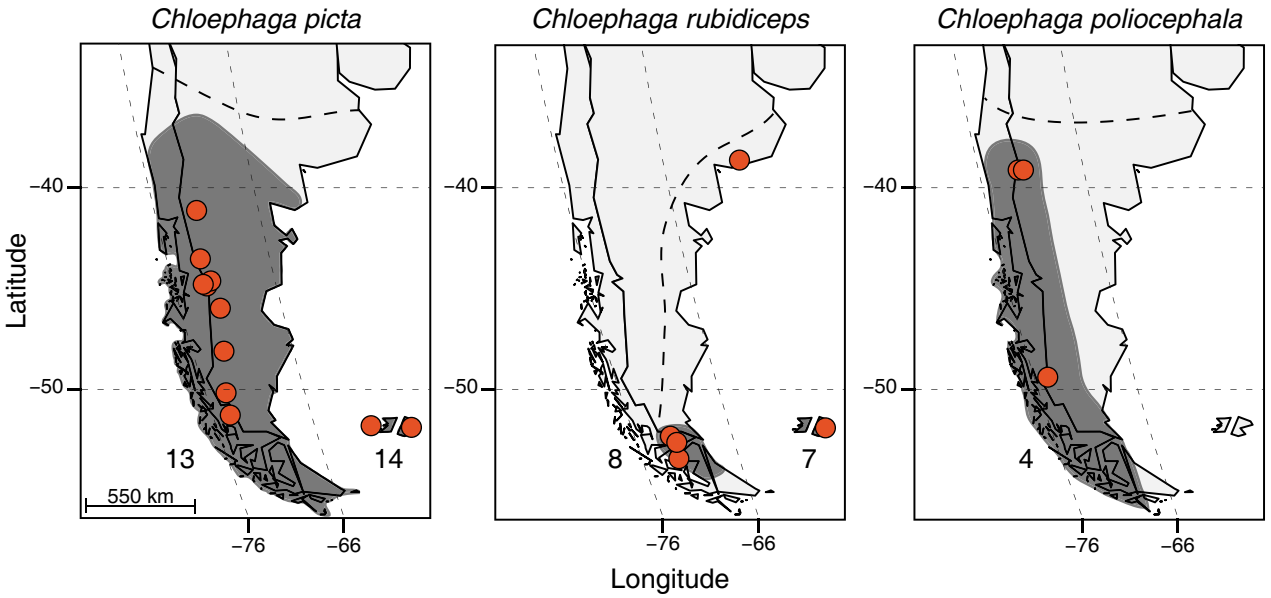

Figure 1 Range maps (Gilbert projection) for the three species of *Chloephaga* analysed in this study. Breeding grounds are in dark grey and the northern limits of the wintering area are indicated by a dashed line. Circles represent sampling localities and the number of continental/insular samples is shown at the bottom of each map.

and 138,000–255,000 individuals of *C. picta leucoptera* (Wetlands International, 2012), possibly because of the extinction of the only terrestrial top predator in the archipelago, the Falkland Islands wolf (by *c.* 1876). In contrast, all continental populations of sheldgeese have declined by at least 50% in the past 30 years, to a large extent due to anthropogenic causes (Wetlands International, 2012). Sheldgeese were declared an agricultural pest in Argentina in 1931 because they were thought to damage wheat crops in their wintering area in the province of Buenos Aires, an assumption that has recently been shown to be incorrect (Petracci, 2011). This motivated early measures to reduce their numbers, such as the destruction of up to 150,000 eggs per breeding season in Tierra del Fuego (Weller, 1975), and hunting of adult birds (Pergolani de Costa, 1955). Moreover, the South American grey fox (*Lycalopex gymnocerus*) that was introduced to Tierra del Fuego and the islands of the Strait of Magellan to control populations of the exotic European rabbit (*Oryctolagus cuniculus*), also had a negative impact on the populations of sheldgeese (Carboneras, 1992). In addition, the introduced American mink (*Neovison vison*) reached northern Tierra del Fuego, the main breeding area of *C. rubidiceps*, increasing predation on continental sheldgeese (Valenzuela *et al.*, 2013). The continental populations of *C. rubidiceps* show the steepest decline, going from a breeding population in Tierra del Fuego of several thousand in the 1950s (Delacour, 1954) to currently fewer than 300 individuals (Petracci *et al.*, 2014). The total number of continental *C. rubidiceps* individuals (including Argentina and Chile) was estimated to be fewer than 1000 and decreasing (Madsen *et al.*, 2003; Wetlands International, 2012).

In a recent study, Bulgarella *et al.* (2014) used mitochondrial control region DNA sequence data to study the phylogenetic affinities among *Chloephaga* species and to quantify the genetic divergence between two sheldgoose species that possess insular and continental populations (*C. picta* and *C. rubidiceps*). *Chloephaga rubidiceps* and *C. picta* showed 1.0% and 0.6% sequence divergence, respectively, when comparing insular to continental populations within each species. In both cases, mainland and insular populations were reciprocally monophyletic and did not share mtDNA haplotypes, suggesting that these populations are genetically distinct and that at least female-mediated gene flow is restricted. However, male-based dispersal is common in Anatidae (Rohwer & Anderson, 1988), and provides a mechanism by which differences in mtDNA may arise despite continued nuclear gene flow (Peters *et al.*, 2012). Using nuclear markers to determine the relationship between insular and continental populations of *C. rubidiceps* is thus of vital importance for the design of appropriate conservation programs for the rapidly declining continental population. If indeed genetic differentiation is only limited to mtDNA, it would be possible to design an *ex-situ* breeding program and/or to reinforce the continental population with individuals from MFI where the population is increasing in number (Ewen *et al.*, 2012). These strategies might not be appropriate if divergence between these populations proves to be genome-wide and deep, but instead, it would mean that the continental clade should be managed as a critically endangered taxon in need of urgent conservation measures (and should be categorized as a threatened species by IUCN).

Here, we use a reduced-representation genomic approach (ddRAD, Peterson *et al.*, 2012) to sample thousands of markers across the genomes of *C. rubidiceps* and *C. picta*. We assessed the levels of differentiation between insular and continental populations and modelled demographic history to investigate whether the MFI harbour independently evolving lineages of these potentially highly vagile species of sheldgeese. We expect a signal of genome-wide differentiation to reflect a history of isolation among populations, in which case the *c.* 450 km that separate the continent from the archipelago will have generated sufficient isolation to reduce gene flow in two species that are capable of undergoing medium distance migration. If this holds true, immediate conservation actions will be required to avoid the disappearance of the drastically declining continental populations of *C. rubidiceps*, which would represent the extinction of an independently evolving lineage worthy of a different taxonomic status from the insular population. On the other hand, lack of nuclear genetic differentiation may indicate ongoing gene flow and/or incomplete lineage sorting between insular and continental populations. In the latter case, differentiation in mtDNA could be a product of female philopatry.

MATERIALS AND METHODS

Sampling and molecular methods

We obtained blood or pectoral muscle samples from 46 *Chloephaga* individuals: 13 *C. picta picta* (Argentina), 14 *C. picta leucoptera* (MFI), eight continental *C. rubidiceps* (Argentina and Chile), seven insular *C. rubidiceps* (MFI) and four *C. poliocephala* (Argentina). We included *C. poliocephala* individuals because this species is sister to *C. rubidiceps* (Bulgarella *et al.*, 2014), and provides a point of comparison for the extent of differentiation between populations of *C. rubidiceps*. Details of the samples used for this study are in Appendix S1 in Supporting Information. We isolated genomic DNA using the DNEasy blood and tissue kit (Qiagen, Valencia, CA, USA) and generated double-digest restriction-site associated DNA markers (ddRADtags) following the protocol outlined by Peterson *et al.* (2012) and described in detail in Campagna *et al.* (2015). Briefly, *c.* 500 ng of genomic DNA were digested using SbfI High Fidelity and MspI (New England Biolabs, Ipswich, MA, USA), and ligated to P1/P2 adapters. The P1 adapters included 5–7 bp inline barcodes for multiplexing, allowing samples with unique P1 barcodes to be pooled into three different indexing groups. Fragments of between 400–700 bp were selected using Blue Pippin (Sage Science, Beverly, MA, USA) and finally Illumina TruSeq adapters were incorporated into the library by performing low cycle number polymerase chain reaction. The

three indexing groups were combined in equimolar proportions and sequenced on an Illumina HiSeq 2500 lane at the Cornell University Institute for Biotechnology, obtaining single-end 101 bp sequences.

Assembly of reads into RAD loci and variant calling

We obtained *c.* 110 million reads from the Illumina run and assessed their general quality using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We trimmed all sequences to 97 bp to avoid the lower quality base pairs at the 3′ end of the sequences using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Subsequently, we filtered out any read that had at least a single base with a Phred quality score below 20 (99.9% call accuracy). The 'process_radtags' program from the Stacks 1.20 bioinformatics pipeline (Catchen *et al.*, 2011) was used to demultiplex reads and conduct further quality filtering. We filtered out reads that had not passed the Illumina chastity filter, did not contain a SbfI cut site, had barcode contamination in the 3′ end, and/or did not contain one of the unique barcodes form the P1 adapter in the 5′ end.

The 1.12 ± 0.37 million quality-filtered sequences per individual were trimmed to 90 bp, the length of the shortest sequences after the inline barcodes were removed. We conducted a *de novo* assembly of RAD loci, including sequences from all the species in our study. The Stacks bioinformatics pipeline consisted of 'ustacks/cstacks/sstacks' (controlled by the 'denovo_map' program), followed by the 'rxstacks' error correction module and, finally, a second iteration of 'cstacks/sstacks'. This pipeline aligns reads at the individual level and generates loci when a minimum coverage is reached (set to 50 using the parameter *m*); we allowed up to three differences between alleles of the same locus (controlled by the parameter *M*). Subsequently, a catalogue of all loci is generated, and we allowed three differences among aligned loci of different individuals (parameter *n*). Variants (single nucleotide polymorphisms; SNPs) are identified by matching loci from an individual against the catalogue. The error correction module examines SNP and haplotype calls across populations and makes corrections at the individual level by (1) removing loci from repetitive areas of the genome that match more than one catalogue locus, (2) eliminating excess haplotypes that are uncommon at the population level and may be erroneous and (3) eliminating loci according to a log likelihood threshold (in this case log likelihood = −20, determined from the distribution across all loci). Once errors in each individual are corrected, a new catalogue is generated and variants are identified again. The combination of parameters mentioned above produced a catalogue with 13,563 loci. To explore the sensitivity of the assembly to alternative combinations of parameters we reran the pipeline with all 12 possible combinations of $m = 5$, 10, 50; $M = 1$, 10 and $n = 1$, 10. We found that the assembly was most sensitive to the *m* parameter; lower values of *m* produced higher numbers of loci in the catalogue. We compared different assemblies by conducting a principal coordinates analysis (PCoA) using GenAlEx 6.5 (Peakall & Smouse, 2012), an analysis of molecular variance (AMOVA) in Arlequin 3.5 (Excoffier & Lischer, 2010) and by running structure 2.3.4 (Pritchard *et al.*, 2000). We obtained very similar results and consequently decided to present the data generated by our original assembly.

Finally, we exported SNP and haplotype data using the program 'populations' from the Stacks pipeline, running it separately for taxa relevant to the comparisons of interest: *C. picta picta* versus *C. picta leucoptera*; insular versus continental *C. rubidiceps*, together with the sister species *C. poliocephala*. We filtered out loci with more than 20% missing data in any population/species and that did not reach a minimum coverage of 20× per individual. These settings produced 2172 RAD loci for the *C. picta picta* and *C. picta leucoptera* comparison, 1127 of which had at least one SNP. For the *C. rubidiceps* insular/continental and *C. poliocephala* comparison we obtained 2034 loci; 1074 of which were variable. These data were used for all downstream analysis.

Population genomic analyses

Assessing levels of genomic differentiation

The overall level of differentiation among species/populations was assessed by (1) calculating an F_{ST} value for each SNP and also an average F_{ST} value across all loci for each pairwise comparison in Stacks; (2) using haplotype information to calculate a Φ_{ST} value for each RAD locus and an average Φ_{ST} value per pairwise comparison in Stacks; (3) by performing an AMOVA in Arlequin (statistical significance was evaluated by comparing to 10,000 random permutations); and (4) by conducting a PCoA using GenAlEx.

Bayesian assignment test

We used structure to assign individuals to genetic clusters exploring values of $K = 1$ through 6 (conducting 10 iterations per *K* value). We implemented the admixture ancestry model and calculated 90% probability intervals for admixture coefficients. We used the correlated allele frequencies model and ran the program for 300,000 generations after discarding the initial burn-in of 200,000. We ran structure twice, once for each comparison (*C. picta picta* versus *C. picta leucoptera*; *C. rubidiceps* insular versus continental, plus *C. poliocephala*). To avoid including closely linked SNPs we used only the first SNP from each RAD locus. The method of Evanno *et al.* (2005), as implemented in structure Harvester 0.6.94 (Earl & vonHoldt, 2012), was used to determine the most likely *K* value. We combined different runs from the optimal *K* in Clumpp 1.1.2 (Jakobsson & Rosenberg, 2007).

Demographic inferences

We co-estimated divergence times, effective population sizes, and gene flow using the Generalized Phylogenetic

Coalescent Sampler (G-PhoCS) version 1.2.2 (Gronau *et al.*, 2011). G-PhoCS is based on a full coalescent isolation-with-migration model, and requires a population phylogeny, allowing non-symmetric gene flow between each pair of populations. We inferred the demographic history of *C. picta picta* and *C. picta leucoptera* using sequence data from 2172, 84 bp RAD loci. Invariant loci were included and the SbfI cut site was trimmed from the 5′-end of each sequence. The model had six free parameters: two migration rates, two current and one ancestral effective population size and one divergence time. We used sequence data from 2034 RAD loci to infer the demographic history of the sister populations (continental and insular) of *C. rubidiceps*, with *C. poliocephala* as outgroup. The latter model included 15 free parameters: eight migration rates, three current and two ancestral effective population sizes and two divergence times. G-PhoCS was run for 500,000 generations using the default settings for the Markov chain Monte Carlo described in Gronau *et al.* (2011), discarding the initial 50,000 as burn-in; runs showed adequate mixing and convergence under these conditions after inspection in Tracer 1.6 (Rambaut *et al.*, 2014). We assumed an approximate mutation rate of 10^{-9} mutations per bp per generation (Kumar & Subramanian, 2002) to convert the posterior distribution for the estimates of different parameters from mutation scale to generations (τ) and individuals (θ). A similar value has been used for RAD loci in other Anatidae species (Lavretsky *et al.*, 2016). Because this mutation rate is a rough approximation, we are cautious to interpret the absolute values of the estimated parameters, and focus mainly on relative comparisons, which are not impacted by assumptions on the mutation rate. We measured gene flow using the total migration rate, which for small values approximates the probability that a lineage experienced migration in the past. This value is obtained by multiplying the per-generation migration rate by the number of generations over which gene flow is assumed to take place.

RESULTS

Genomic divergence between continental and insular populations of sheldgeese

Despite the geographical proximity of the MFI to the continent (*c.* 450 km) and the potential high vagility of sheldgeese, the insular populations of *C. rubidiceps* and *C. picta leucoptera* showed strong evidence of genetic isolation from their respective continental populations. Insular individuals of *C. rubidiceps* clustered separately from their continental counterparts in a PCoA based on 1706 SNPs sampled across the genome (Fig. 2a), in which *C. poliocephala* individuals also formed a separate cluster. structure assigned individuals to three different genetic clusters using 1074

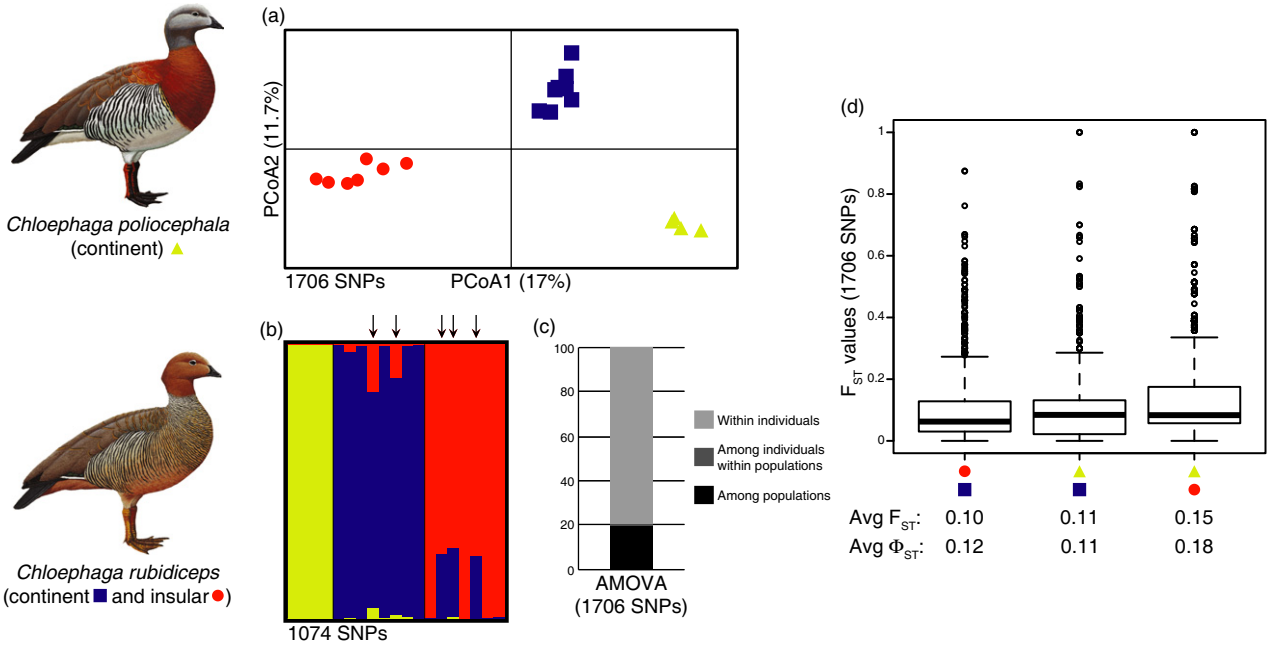

Figure 2 Genomic divergence between *Chloephaga poliocephala*, and continental and insular populations of *C. rubidiceps*. (a) A principal coordinates analysis (PCoA) displaying differences at 1706 single nucleotide polymorphisms (SNPs) among *C. poliocephala* and continental and insular populations of *C. rubidiceps* (the percentage of variation explained by each axis is in parenthesis). (b) A structure plot derived from 1074 SNPs (one per RAD locus). Individuals marked with arrows have admixture coefficients with 90% Bayesian CI that do not overlap with zero or one. (c) Results from an analysis of molecular variance (AMOVA) showing the distribution of genetic variation; 19.6% of which can be explained by differences among species/populations. (d) Boxplots showing the distribution of F_{ST} values across 1706 SNPs for each pairwise comparison (colour coded). There are three fixed SNPs between *C. poliocephala* and the continental population of *C. rubidiceps*, and six fixed SNPs between insular *C. rubidiceps* and *C. poliocephala* (superimposed).

SNPs (one per RAD locus), showing low levels of admixture (Fig. 2b; see Appendix S2 for overall likelihood and selection of the optimal model *sensu* Evanno *et al.*, 2005). Approximately 20% of the genetic variation in the *C. rubidiceps*/*C. poliocephala* comparison could be explained by differences among populations/species (Fig. 2c). The overall level of genomic differentiation between continental and insular populations of *C. rubidiceps* was comparable in magnitude to that of either population with their sister species, *C. poliocephala* (Fig. 2d). We did not find fixed SNPs between insular and continental populations of *C. rubidiceps*. There were three fixed differences between *C. poliocephala* and continental *C. rubidiceps*, and six different SNPs were fixed between the former species and insular *C. rubidiceps* (Fig. 2d).

Chloephaga picta leucoptera individuals were also genetically isolated from their continental counterparts, *C. picta picta*. Individuals from both populations clustered separately in a PCoA based on 1862 SNPs (Fig. 3a). Using one SNP per RAD locus (total of 1127), STRUCTURE assigned individuals to two different genetic clusters (Fig. 3b; see Appendix S2), with only one *C. picta leucoptera* individual showing evidence of admixture. Subspecies accounted for *c.* 14% of the genetic variation (Fig. 3c), and we found only one fixed difference (Fig. 3d). The average level of genomic differentiation between subspecies of *C. picta* was smaller than that found between insular and continental populations of *C. rubidiceps* (Fig. 3d, cf. Fig. 2d).

Demographic history of differentiated sheldgeese populations

To better understand the history of sheldgeese populations in the MFI, we used our sequence data to co-estimate divergence times, effective population sizes, and gene flow using G-PHOCS. We found evidence for a split between continental and insular populations of *C. rubidiceps*, followed by levels of gene flow that could not be distinguished from zero (Fig. 4; see Appendix S3). Despite the extremely low estimated current population size (< 1000 individuals) of the continental *C. rubidiceps* population, we found the current effective population size of continental *C. rubidiceps* to be around twice as large as that of the insular population. This pattern was confirmed by larger numbers of private alleles (0.158 vs. 0.081 in the MFI), higher levels of heterozygosity (0.191 vs. 0.165 in the MFI) and a larger value of theta (indicating a larger effective population size; 0.242 vs. 0.194 in the MFI) estimated in ARLEQUIN for the continental population of *C. rubidiceps*. This suggests the *C. rubidiceps* population in the continent retains genetic diversity, reflecting its historical and not its contemporary census size. The split within *C. rubidiceps* was approximately seven times more recent than the divergence time with respect to *C. poliocephala*, and we also inferred low levels of recent gene flow with this species. The demographic history of *C. picta leucoptera* and *C. picta picta* was similar to that of insular and

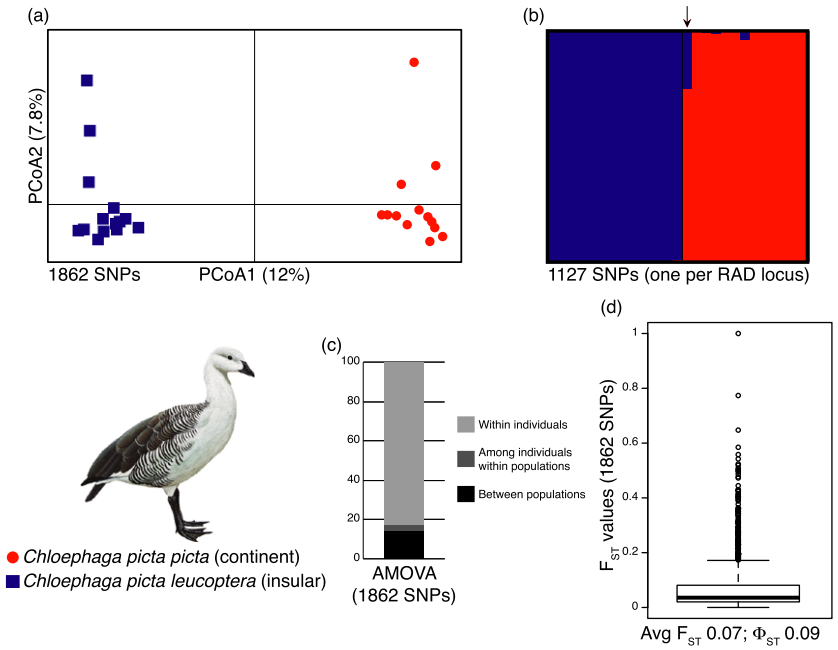

Figure 3 Genomic divergence between continental and insular populations of *Chloephaga picta*. (a) A principal coordinates analysis (PCoA) derived from 1862 single nucleotide polymorphisms (SNPs) between *C. picta picta* (continental population; red circles) and *C. picta leucoptera* (insular population; blue squares). The percentage of variation explained by each axis is in parenthesis. (b) STRUCTURE plot derived from 1127 SNPs with individuals colour coded as in (a). The arrow indicates the only individual (which belongs to *C. picta leucoptera*) with admixture coefficients that do not have 90% credible intervals that overlap with zero or one. (c) A bar plot showing the distribution of genetic variation as measured by an analysis of molecular variance; 14.3% of which can be explained by differences between populations. (d) A boxplot showing the distribution of F_{ST} values across 1862 SNPs. We found one fixed SNP between *C. picta picta* and *C. picta leucoptera* in our data set.

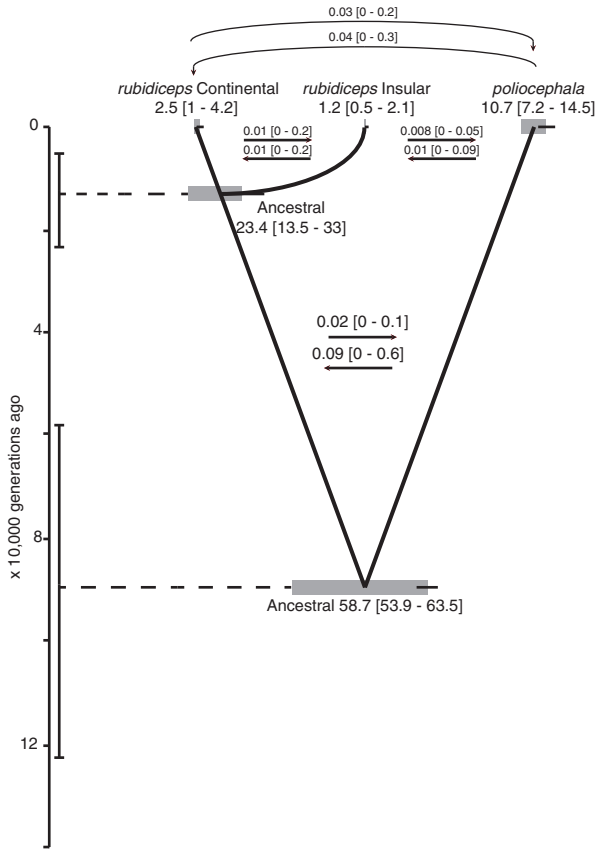

Figure 4 Demographic inference performed using G-PHOCS. Grey bars represent effective population sizes and 95% Bayesian CI for *Chloephaga poliocephala* and both continental and insular populations of *C. rubidiceps*. Numbers are expressed in ×10,000 individuals. The inferred splitting time (and 95% CI) between these populations is expressed in ×10,000 generations. Absolute values should be interpreted with caution as they are based on an approximate mutation rate of 10^{-9} mutations per bp per generation. Migration (indicated with arrows) is shown as mean and 95% CI values for the total migration rate: the per-generation migration rate times the number of generations migration has taken place.

continental populations of *C. rubidiceps*. We found support for a split between subspecies of *C. picta* that was followed by levels of gene flow that could not be distinguished from zero (Fig. 5; see Appendix S3). The effective population size of the continental *C. picta picta* was approximately three times as large as that of the insular subspecies.

DISCUSSION

Here, we find evidence of genome-wide differentiation in two sheldgoose species (*C. rubidiceps* and *C. picta*) that are co-distributed across the southern portion of South America and the MFI. Our results from demographic modelling show that gene flow among populations of the same species has been negligible, suggesting that the *c.* 450 km that separate the archipelago from the continent are sufficient to isolate populations of these potentially highly vagile species. We

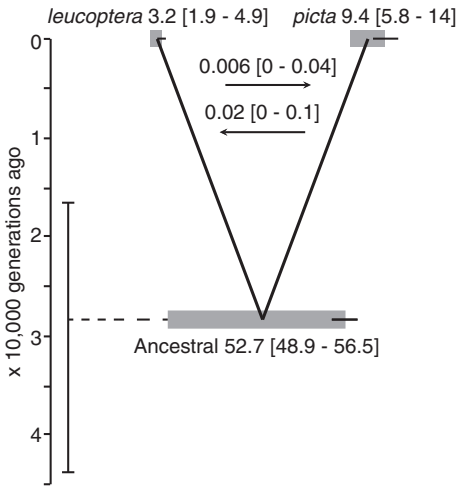

Figure 5 Demographic inference for *Chloephaga picta leucoptera* and *C. picta picta* performed using G-PHOCS. Details as in Fig. 4.

discuss our results with respect to the possible drivers of diversification as well as the conservation implications for the rapidly declining continental *C. rubidiceps* population.

The Malvinas/Falkland Islands as a glacial refugium in the southern Atlantic

Throughout the Pleistocene a large proportion of Patagonia was covered by glaciers, which expanded and retracted from the Andes Mountains during several cycles. The most dramatic of these events is known as the Great Patagonian Glaciation and occurred *c.* 1 Ma; the Last Glacial Maximum took place *c.* 24,000 years before present. This complex geological history modified the landscape and generated isolation among habitats, covering a large proportion of the continent with ice sheets, while other areas remained without glaciers (Clapperton, 1993; Rabassa *et al.*, 2011). The glacial cycles of the Pleistocene have shaped the Patagonian-Fueguian biota (reviewed by Sersic *et al.*, 2011). Many taxa that inhabit the region are thought to have colonized from lower latitudes after the glaciations (Lessa *et al.*, 2010; Pardiñas *et al.*, 2011). Others have persisted in multiple refugia (e.g. rodents: Lessa *et al.*, 2010; Pardiñas *et al.*, 2011; fish: Ruzzante *et al.*, 2008; the Patagonian otter: Vianna *et al.*, 2011; frogs: Nuñez *et al.*, 2011; cormorants: Calderón *et al.*, 2014). The MFI are located on the shallow continental shelf and were likely connected to the continent by land bridges at times when sea levels were low due to the glacial cycles (Ponce *et al.*, 2011). Parts of the MFI were not covered by ice (Hall, 2004), and populations of different species (e.g. the Falkland Island wolf, Cobb's wren and the Falkland steamer duck) are thought to have persisted in this refugium where they diverged from their continental relatives.

It is likely that both *C. picta leucoptera* and insular populations of *C. rubidiceps* diverged from their continental counterparts in isolation in the MFI during the Pleistocene.

Generation times in *Chloephaga* sheldgeese have been estimated to be in the order of 8.5 years, based on adult survival rates and expected age of maturity (BirdLife International, 2016). Combined with our approximate value of genome-wide mutation rate (*c.* 10^{-9}, Kumar & Subramanian, 2002), we obtain a 95% CI for divergence times of 140–373 kyr BP for the *C. picta picta*/*C. picta leucoptera* pair, and 44–196 kyr BP for both populations of *C. rubidiceps*. These approximate time estimates suggest that different glacial cycles that took place between the Great Patagonian Glaciation and the Last Glacial Maximum could have facilitated the isolation of ancestral lineages in the MFI refugium.

Our results raise the question of why organisms that are capable of undergoing medium distance migrations (at least their continental populations) have remained isolated in the MFI. Fulton *et al.* (2012) studied the evolution of steamer ducks (*Tachyeres*) and could not distinguish flying steamer ducks (*T. patachonicus*) from the MFI from flightless Falkland steamer ducks (*T. brachypterus*) using a small portion of the mitochondrial control region. The authors estimated that insular species had been isolated from continental steamer duck species for *c.* 2.2–0.6 Myr, suggesting flightlessness in *T. brachypterus* evolved in the MFI. Isolation of sheldgoose species in the MFI is presumably more recent, and although the insular populations of these species are able to fly, they have become sedentary and do not migrate (Summers, 1985; Woods, 1988). The frequency of flightless birds is higher in aquatic species when compared to all other birds (Roff, 1994), suggesting that in this group evolutionary shifts in the ability to disperse (*sensu* Moyle *et al.*, 2009) could contribute to restricting gene flow following colonization of isolated environments. Moreover, because the prevailing winds are from Argentina to the MFI, they would hinder bird movement from the MFI to the mainland (Summers & McAdam, 1993). If gene flow from the MFI to the continent is restricted by strong winds, this explanation does not account for the low gene flow in the opposite direction (which could be facilitated by the wind). It is also possible that the *c.* 450 km of ocean that separate the continent from the MFI are responsible for restricting the movement of sheldgeese in either direction.

Conservation status of the rapidly declining continental *C. rubidiceps* population

Using DNA sequences from the mitochondrial control region Bulgarella *et al.* (2014) found insular and continental populations of *C. rubidiceps* to be reciprocally monophyletic, with *c.* 1% sequence divergence. The authors could not distinguish between restricted gene flow between populations and/or female philopatry as possible explanations for this result. As females tend to be the sex that shows higher philopatry in waterfowl (Rohwer & Anderson, 1988), the question of whether continental *C. rubidiceps* populations represent an evolutionarily distinct lineage remained unanswered.

Here, using a genomic approach we were able to confirm that insular and continental populations of *C. rubidiceps* represent distinct lineages that have most likely not experienced detectable levels of gene flow for a substantial time, with an average level of differentiation (as measured by F_{ST}) similar to that observed when comparing either group to the sister species, *C. poliocephala*. Our STRUCTURE analysis detected low levels of admixture between populations of *C. rubidiceps*, which could be due to incomplete lineage sorting and/or gene flow. Because our GPhoCS analysis did not detect significant levels of gene flow, it is more likely that incomplete lineage sorting is responsible for the low admixture levels inferred by STRUCTURE. Similarly, continental *C. picta picta* and insular *C. picta leucoptera* populations have not experienced detectable levels of gene flow. While the latter two populations are considered subspecies, the populations of *C. rubidiceps* show slightly higher levels of differentiation (Fig. 2d, cf. Fig. 3d) and are not recognized as different taxonomic units. Based on our results, we suggest that continental and insular populations of the ruddy-headed goose (*C. rubidiceps*) should be considered different taxa. Differences in morphology, behaviour and ecology have also been suggested between these putatively different taxa (Chebez, 2008) and require further study. Such differences may be a product of local adaptations to different environments, and could lead to low hybrid fitness and ultimately reproductive isolation (see Frankham *et al.*, 2012).

Numerous examples exist of endangered species that have not been the focus of conservation actions until their taxonomic status has been resolved (e.g. Frankham *et al.*, 2010; Coetzer *et al.*, 2015). Designating the continental population of *C. rubidiceps* as a separate taxon from the insular population is thus a critical first step to conserving it. Despite a difference in census size of over 40-fold in favour of insular *C. rubidiceps* when compared to the continental population, our demographic modelling analysis found an effective population size twice as large on the continent. This suggests that despite the severe reduction in population size, the continental *C. rubidiceps* population has not undergone a bottleneck and retains genetic variation that reflects its historical size and might be restored. Currently, this population is considered critically endangered in Argentina and Chile and conservation measures include a ban on hunting that has been in place since 1998. However, illegal hunting events still occur (Petracci *et al.*, 2014) and could have a very high impact on the overall population, given its low numbers. Our results taken together with the demographic information (e.g. populations census sizes) show that the distinct continental *C. rubidiceps* population is highly endangered, and urgent management actions are needed to avoid its extinction in the near future. We recommend detecting and conserving breeding sites, to protect actively nesting attempts from predation by introduced species, and to reinforce hunting controls in wintering areas. At the same time we recommend the development of a management plan that includes a group of continental *C. rubidiceps* individuals involved in a

captive or semi-captive breeding program until the threats can be mitigated.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Data of the individuals included in this study.
Appendix S2 Likelihood values averaged across ten runs for $K = 1$ to $K = 6$ and delta K for all the studied populations of *Chloephaga*.
Appendix S3 Trace plots for pairs of bidirectional migration bands between populations and/or species.

DATA ACCESSIBILITY

For details on the individuals sampled see Appendix S1. Input files for the analyses conducted in this study are stored in Dryad (doi: 10.5061/dryad.9fg2r).

BIOSKETCH

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