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ORIGINAL ARTICLE

Late Pleistocene colonization of South Georgia by yellow-billed pintails pre-dates the Last Glacial Maximum

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ABSTRACT

Aim Glacial cycles had a major influence on the distribution of high-latitude species, and while genetic consequences of these cycles have been well studied in the Circumpolar North, notably fewer studies have been undertaken in the Antarctic. Here, we use multilocus genetic data from the South Georgia pintail (*Anas georgica georgica*) as a proxy to study the presence and timing of ice-free refugia on South Georgia, a glaciated subantarctic island in the South Atlantic Ocean that has been the subject of intense geomorphological and palaeoenvironmental study.

Location South Georgia.

Methods Multilocus DNA sequence data from five nuclear loci and the mitochondrial DNA control region were analysed for South Georgia pintails ($n = 60$) and the neighbouring population of yellow-billed pintails (*A. georgica spinicauda*) in Argentina ($n = 64$). Population genetic structure and gene flow were examined using Φ_{ST}, assignment tests, and multilocus coalescent analyses.

Results Isolation-with-migration (IM) analysis revealed that the South Georgia pintail population was founded by pintails dispersing from South America. Although the confidence intervals on divergence dates inferred from genetic data are generally wide and there may be time-dependency in rate calibrations, our analysis suggests that this founding event probably occurred *c.* 34,000 years ago, prior to the Last Glacial Maximum (LGM). Our findings further suggest that South Georgia pintails might have experienced a bottleneck coinciding with complete replacement of mitochondrial DNA prior to 8700 years ago following the final advance of glaciers.

Main conclusions These findings suggest that ice-free refugia existed earlier in the chronology of deglaciation in contrast with earlier studies, but in agreement with observations that the plant community was also established on South Georgia prior to the end of the Pleistocene. Like other recent studies that have utilized genetic data to date dispersal and vicariance events in the Antarctic, our results provide a constraint on the extent of ice sheets, suggesting that past ice coverage on South Georgia through the LGM was overestimated.

Keywords
Anas georgica, Anatidae, Argentina, ducks, glacial refugia, island biogeography, multilocus genetic data, Patagonia, Subantarctic, waterfowl.

INTRODUCTION

Glacial cycles have had a major influence on the distribution of species and genetic diversity in high-latitude regions. Climatic conditions were highly variable during the Pleistocene, with more than 20 different cycles of glacial advance and retreat (Hewitt, 1996). These glacial cycles led to vicariance and changes in species ranges as populations contracted into refugia during glacial maxima and expanded into ice-free areas during interglacial periods (Taberlet & Cheddadi, 2002;

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Provan & Bennett, 2008; Shafer *et al.*, 2010). Genetic signatures of population demography are consistent with these effects in that they often show evidence of both the occupancy of glacial refugia and increases in population sizes for many Arctic species following recent deglaciations (Fedorov & Stenseth, 2002; Galbreath & Cook, 2004; Sonsthagen *et al.*, 2011). Genetic data have also provided evidence of the locations of glacial refugia and divergence times between populations inhabiting recently deglaciated habitats (Weir & Schluter, 2004; Waltari *et al.*, 2007; Parducci *et al.*, 2012).

At high latitudes in the Southern Hemisphere, there is also a rich literature describing late Pleistocene palaeoenvironmental changes and the timing and extent of ice cover (Rabassa & Clapperton, 1990; Petit *et al.*, 1999; Blunier & Brook, 2001; Anderson *et al.*, 2002; Doran *et al.*, 2002; Van der Putten *et al.*, 2010). Prior to the Last Glacial Maximum (LGM) ice sheets were both thicker and more expansive throughout the region, and one perception that has arisen is that these ice sheets wiped out most terrestrial habitats for Antarctic species (Convey *et al.*, 2008). However, there is mounting evidence that many Antarctic and subantarctic species survived these periods of deep glaciation within ice-free refugia for tens of thousands and even millions of years (Rogers, 2007; Convey *et al.*, 2008). Among these findings are series of recent phylogenetic and phylogeographical studies utilizing genetic data and molecular clock dating approaches to infer the timing of past dispersal and vicariance events in terrestrial and freshwater taxa ranging from chironomid midges (Allegrucci *et al.*, 2006), springtails (Stevens *et al.*, 2006), mites (Mortimer *et al.*, 2011), and beetles (Grobler *et al.*, 2011) to benthic cyanobacteria in Antarctic lakes (Taton *et al.*, 2006).

Here, we use multilocus genetic data from a common South American dabbling duck (family Anatidae), the first terrestrial vertebrate to be studied in this context in the Antarctic, as a proxy to study the presence and timing of ice-free refugia on South Georgia. Using DNA sequence data from five nuclear loci and the mitochondrial DNA control region, we infer the timing of colonization of South Georgia by pintail ducks dispersing from the nearest continental source population, South America, relative to dates of deglaciation chronicled in glacial deposits and lake and peat sediments.

The island of South Georgia (54.0–55.0° S, 35.5–38.5° W) lies isolated in the South Atlantic Ocean 1300 km east-south-east of the Falkland Islands. The closest continental land areas are South America and the Antarctic Peninsula, at distances of 1800 and 1500 km, respectively. The island sits at the junction of the Pacific and Atlantic plates on the Scotia Arc, a mid-oceanic ridge that extends east from Tierra del Fuego. The island is very mountainous, with peaks rising to 2935 m. Approximately 58% of the island is still covered with ice (Clapperton, 1971; Headland, 1984), and glaciers have carved fjords and valleys on all sides of the island. Ice-free areas at lower elevations are dominated by tundra-like vegetation, peat bogs and small ponds, with a notable absence of trees and a low number of vascular plants (Van der Putten *et al.*, 2009). The climate of South Georgia is cool and wet, with a mean annual temperature of 2.0 °C, and its weather is dominated by polar cyclones that traverse the Southern Ocean.

Because it lies south of the Polar Frontal Zone in the Antarctic Circumpolar Current, South Georgia has long been of interest to glacial geologists and palaeoclimate scientists (Bentley *et al.*, 2007a). Studies indicate that the island experienced extensive glaciation and that ice caps extended to the continental shelf (Clapperton, 1971; Sugden & Clapperton, 1977; Clapperton *et al.*, 1978; Graham *et al.*, 2008; Fretwell *et al.*, 2009). While the ultimate extent of the ice cover is not debated, the extent of the ice during the LGM and the timing of deglaciation through this period are still uncertain. Graham *et al.* (2008) suggested that the continental shelf morphology may date back to the Miocene, but left open the possibility that extensive glaciation also occurred during the LGM. The timing of deglaciation is thus also still a matter of debate. Based on a unique lake record from Husvik Fjord, Rosqvist *et al.* (1999) proposed that deglaciation commenced prior to 18.6 ka. They also found that colder conditions prevailed for several thousand years, beginning 14.8–14.2 ka, with warming to post-glacial conditions occurring between 8.4 and 6.5 ka. Their findings contrast with the results of Bentley *et al.* (2007a), who found using isotopic signatures that the outermost morainal deposits at 15 sites across the north-eastern coastline of South Georgia, including Husvik Fjord, were deposited approximately 12 ka. If ice was restricted to the inner fjords and the sea level was much lower than it is today, however, these conditions might still have permitted the existence of ice-free refugia. Finally, palaeobotanical studies indicate that the plant biota of South Georgia was mostly well established by the start of the Holocene, further suggesting the existence of ice-free glacial refugia on the island during the LGM (see Van der Putten & Verbruggen, 2005, 2007; Bentley *et al.*, 2007b; Van der Putten *et al.*, 2009, 2010).

Here, we examine evidence for the persistence of incomplete ice cover and glacial refugia through the LGM using multilocus genetic data from the South Georgia pintail (*Anas georgica georgica* Gmelin, 1789), a non-migratory, island endemic population of the yellow-billed pintail (*Anas georgica spinicauda* Vieillot, 1816). The South Georgia pintail is morphologically distinct in both body size (smaller) and plumage colour (darker) from the yellow-billed pintail, which ranges widely over southern South America and is the most common waterfowl species in that region, probably numbering more than one million individuals (Wetlands International, 2006). On South Georgia, by contrast, pintails number approximately 6000 pairs (Prince & Poncet, 1996; Martin & Prince, 2005; Clarke *et al.*, 2012). Like other endemic high-latitude island ducks, South Georgia pintails are predominately intertidal feeders, as freshwater ponds freeze and snow blankets the island to the shoreline during much of the year. Even during summer, individuals infrequently stray far from the coast, and because less than 50% of the

island is deglaciated, available inland freshwater habitat remains limited.

Using sequences from five independently assorting nuclear genes and one mitochondrial gene, we employed coalescent methods to investigate how the timing of colonization of South Georgia by pintails relates to the LGM and the published chronology of deglaciation events (Rosqvist *et al.*, 1999; Bentley *et al.*, 2007a). Although confidence intervals on divergence date estimates derived from multilocus genetic data are generally large, analyses of island endemic, terrestrial birds such as the South Georgia pintail can contribute new information to our understanding of the chronology of deglaciation in the Subantarctic and South Polar regions.

MATERIALS AND METHODS

Specimen collection, PCR and DNA sequencing

South Georgia pintails were banded by A.R.M. at various sites on the island between 1998 and 2002 (n = 60; Fig. 1). Yellow-billed pintails (n = 64; Fig. 1) were collected by K.G.M. and R.E.W. in Argentina between 2001 and 2005 (Fig. 1). Total genomic DNA was isolated from web punches and muscle tissue, respectively, using standard protocols with DNeasy Tissue Kits (Qiagen, Valencia, CA, USA). Six gene regions including the mitochondrial DNA (mtDNA) control region and five nuclear loci were sequenced (Table 1). Methods describing PCR and DNA sequencing protocols are described in McCracken *et al.* (2009). Sequences and specimen voucher informa-

Table 1 Genes sequenced from South Georgia pintails (*Anas georgica georgica*) and yellow-billed pintails (*A. g. spinicauda*) and their chromosomal positions in the chicken (*Gallus gallus*) genome.

Locus	Base pairs sequenced	Chicken chromosome
mtDNA control region (mtDNA)	977–981	mtDNA
Ornithine decarboxylase intron 5 (*ODC1*)	352	3
α enolase intron 8 (*ENO1*)	314	21
β fibrinogen intron 7 (*FGB*)	246	4
N-methyl D aspartate 1 glutamate receptor intron 11 (*GRIN1*)	328–330	17
Phosphoenolpyruvate carboxykinase intron 9 (*PCK1*)	345–351	20

Figure 1 Map illustrating the localities of South Georgia pintail samples (*Anas georgica georgica*, n = 60) and yellow-billed pintail samples (*A. g. spinicauda*, n = 64) on South Georgia and in Argentina, respectively.

tion, including geo-referenced localities, are available in GenBank (accession numbers FJ617817–FJ618512 and KC987596–KC987946).

Allelic phase determination

Multilocus, allele-phased nuclear DNA sequences provide a useful tool for studying gene flow, historical phylogeography, and population genetics. The allelic phase of each nuclear sequence that was heterozygous at two or more nucleotide positions was determined using allele-specific priming and the software PHASE 2.1 (Stephens *et al.*, 2001). PHASE uses a Bayesian algorithm to infer haplotypes from diploid genotypic data while incorporating recombination and the decay of linkage disequilibrium (LD) with genetic distance, and it is as effective or better than cloning for resolving highly polymorphic sequences (Harrigan *et al.*, 2008). We first analysed each composite sequence, the consensus of both alleles, using the default software settings (100 main iterations, 1 thinning interval, 100 burn-in) followed by 1000 main iterations and 1000 burn-in (-×10 option) for the final iteration. The PHASE algorithm was run five times (-×5 option) from different starting points, selecting the result with the best overall goodness of fit. For individuals with allele pair probabilities < 80%, we then designed allele-specific primers to selectively amplify a single allele (Peters *et al.*, 2012a). The resulting haploid allele sequence was then subtracted from the diploid composite sequence to obtain the gametic phase of the second allele. Each data set was then analysed five more times using PHASE and the additional known allele sequences (-k option). The gametic phase of each autosomal sequence was identified experimentally or with greater than 95% posterior probability for > 96% of the individuals included in each data set.

Analysis of genetic differentiation

To characterize genetic differentiation between pintail populations on South Georgia and in Argentina and examine how these patterns varied among loci, we calculated Φ_{ST} using the software ARLEQUIN 3.5 (Excoffier & Lischer, 2010) and illustrated networks for each locus using the median-joining algorithm in the software NETWORK 4.6 (Bandelt *et al.*, 1999; Fluxus Technology Ltd., Clare, Suffolk, England). We also calculated other measures of genetic diversity such as the total number of polymorphic positions, frequency of the most common allele in each population, and nucleotide diversity (π/site).

We next used STRUCTURE 2.2 (Pritchard *et al.*, 2000) to compute the probability of assignment of each individual to the South Georgia and continental populations. The six-locus data set (including mtDNA) was first converted to numerical genotypic data using the software COLLAPSE (Posada, 2006), with missing data coded in place of a second allele for the mtDNA. A simple two-population model ($K = 2$) was conducted using the admixture model ($\alpha = 1$) with independent allele frequencies ($\lambda = 1$) and no a priori population information (POPFLAG = 0).

Isolation-with-migration (IM) analysis

We used the two-population isolation-with-migration model in IM (Hey & Nielsen, 2004) to estimate the effective population size parameters (Θ), rates of gene flow (M), and time since divergence (t) between pintail populations on South Georgia and Argentina. Because the IM model assumes that all sequences are free from intralocus recombination, we tested for recombination using the four-gamete test (Hudson & Kaplan, 1985) implemented in DNASP 4.10 (Rozas *et al.*, 2003) and truncated each sequence to include the longest fragment with no apparent recombination ($R_M = 0$ in the four-gamete test). For *ODC1* this included positions 1–187, *ENO1* positions 1–172, and *GRIN1* positions 75–185. The infinite sites model was used in the IM analysis because no locus had more than two alleles segregating at any site. The mtDNA was analysed separately using the HKY model (Hasegawa *et al.*, 1985).

IM was first run with wide uninformative priors. Analyses were then conducted with more narrow uniform priors that encompassed the full posterior distributions from the preliminary runs. For the five nuclear loci, we specified $\Theta = 15$ and 1, $M = 100$, and $t = 0.1$ as the priors. For the mtDNA, we specified $\Theta = 1000$ and 10, $M = 2$ and $t = 3.0$. The upper bound for t was selected based on the assumption that time since divergence could not exceed initial estimates of time to the most recent common ancestor (TMRCA). The Markov chain Monte Carlo was run for 130 million steps, sampling the posterior distribution every 50 steps, with a burn-in of 500,000 steps. All runs included 20 chains with a geometric heating scheme. Autocorrelation was monitored during the run, and analyses were continued until the effective sample sizes (ESS) were > 190 for all parameter estimates. Each analysis was conducted with and without the splitting parameter (s), which is bounded by zero and one and measures the percentage of the ancestral population contributing to population 1 at the time of divergence. In this study, a value of s that peaks abruptly at 1.0 would indicate colonization of South Georgia by pintails from Argentina.

Substitution rate estimates

Following the IM analyses, parameter estimates for t, Θ and M were converted to biologically meaningful values of T in years (t/μ) and $4N_em$ representing the number of effective immigrants per generation. To determine T in years before present and incorporate uncertainty in lineage-specific substitution rates, we estimated substitution rates (μ) based on comparison between sequences from pintails and representatives from four distant waterfowl genera that diverged contemporaneously. Ducks (Anatinae) and geese (Anserinae) probably diverged sometime during the Oligocene (23–34 Ma; Howard, 1964), as numerous fossils from these two

subfamilies have been recovered during this time period and thereafter but not prior (Howard, 1964; Livezey, 1997; Louchart *et al.*, 2005). Three additional lineages comprising two extant monotypic genera, *Biziura* and *Malacorhynchus*, and the stiff-tailed ducks and allies (Oxyurinae) also diverged about this time, although the order in which these lineages diverged remains unresolved (Harshman, 1996; Sraml *et al.*, 1996; McCracken *et al.*, 1999; Hackett *et al.*, 2008; Gonzalez *et al.*, 2009). Together with the Anatinae, which includes the genus *Anas*, these lineages compose a five-way polytomy near the base of the waterfowl phylogeny and make a useful reference point for calibrating substitution rates.

To incorporate stochasticity among nuclear loci in our estimate of μ and obtain 95% confidence limits for *T*, we performed a multilocus coalescent analysis in BEAST 1.6 (Drummond & Rambaut, 2007). Specifically, we calculated the root height of the five-locus species tree across the descendants of this five-way polytomy using one representative each of *Anas georgica*, *Anser caerulescens*, *Biziura lobata*, *Malacorhynchus membranaceus* and *Oxyura jamaicensis* (Peters *et al.*, 2012a) and the HKY nucleotide substitution model. We then divided the mean root height (*d*) by 28 Ma, the approximate midpoint of the Oligocene; $\mu = d/\tau$. To incorporate uncertainty associated with the fossil dates, we simulated a lognormal distribution of *T* (lognormal mean = 15.9, SD = 0.5, offset by 20 Ma) following Fulton *et al.* (2012) based on the premise that divergence among these five taxa had a 95% probability of occurring 23–42 Ma. The uncertainty that mutation rates were either slower or faster was thus also incorporated into our analysis. To obtain *T* we divided *t* per site as obtained from IM by μ per site from the BEAST analysis. Using 10,000 random samples drawn from the full posterior distribution of *t*, μ and τ we then computed the upper and lower 95% confidence intervals for *T*.

For the mtDNA, we used the point estimate of the substitution rate and confidence intervals published by Peters *et al.* (2005): 4.8×10^{-8} substitutions/site/year [95% confidence interval (CI) = 3.1 to 6.9×10^{-8} substitutions/site/year]. More precise estimates of μ for the mtDNA control region are difficult to obtain because unequal rates among sites and a high frequency of insertions and deletions in this gene result in high levels of homoplasy, which in turn makes the control region difficult to align among outgroup taxa.

Finally, the number of effective immigrant individuals per generation was obtained by multiplying Θ ($4N_em$) by *M* (m/μ) to obtain $4N_em$. The 95% confidence intervals for $4N_em$ were calculated as described above using 10,000 random samples from the posterior distribution of Θ and *M*.

RESULTS

Genetic differentiation

South Georgia pintails exhibited significant mtDNA differentiation from pintails in Argentina ($\Phi_{ST} = 0.47$; Table 2). Thirty-seven mtDNA haplotypes were found in Argentina, whereas five haplotypes were sampled on South Georgia (Fig. 2). No mtDNA haplotypes were shared; the South Georgia population thus possessed five private haplotypes. Nucleotide diversity (π/site) was approximately an order of magnitude smaller on South Georgia.

In contrast to the mtDNA, the five nuclear loci showed less population differentiation. Φ_{ST} values ranged from 0.05 to 0.31 with a mean of 0.18 and were significant for all loci ($P < 0.000001$; Table 2). For each nuclear locus the most common allele on South Georgia also occurred at moderately high frequency in Argentina (Fig. 3). Three private alleles were observed for *ODC1* on South Georgia, and one each were found in *FGB* and *PCK1* (Fig. 2). Most nuclear alleles were thus shared between South Georgia and Argentina. However, pintails from Argentina had many private alleles not found on South Georgia.

Based on the STRUCTURE analysis, the average assignment probability (± SD) for individuals banded on South Georgia was 0.985 ± 0.004, and no individuals had $P < 0.975$ (Fig. 4). In contrast, pintails from Argentina were assigned to the continental population with lower probability and wider variance, 0.802 ± 0.208. Seven individuals collected in Argentina were assigned to the mainland with $P < 0.5$, and 21 individuals were assigned with $P > 0.95$.

Effective population size, gene flow and time since divergence

As is typical of small populations, Θ for South Georgia pintails was approximately two orders of magnitude smaller

Table 2 Genetic diversity measures and Φ_{ST} between South Georgia pintail (*Anas georgica georgica*) and yellow-billed pintail (*A. g. spinicauda*) populations on South Georgia and in Argentina, respectively.

Locus	Variable sites	Alleles	Nucleotide diversity (π/site)	Shared alleles	Private alleles on South Georgia	Φ_{ST}
mtDNA control region	38/5	37/5	0.004110/0.000529	0	5	0.47
Ornithine decarboxylase	24/19	16/6	0.011831/0.002802	3	3	0.28
α enolase	12/1	13/2	0.004723/0.000590	2	0	0.08
β fibrinogen	6/3	6/3	0.004446/0.003973	2	1	0.20
N-methyl D aspartate 1 glutamate receptor	31/6	34/3	0.009000/0.002236	3	0	0.05
Phophoenolpyruvate carboxykinase	14/2	9/3	0.002004/0.001150	2	1	0.31

All Φ_{ST} values were significant ($P < 0.000001$). Tamura & Nei (1993) substitution model was used to calculate Φ_{ST}.

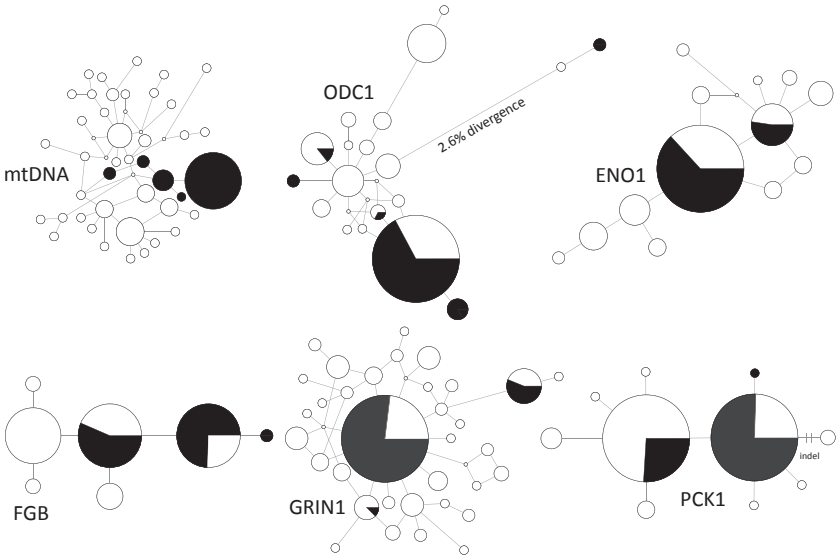

Figure 2 Networks for six genetic loci sequenced from pintails. Alleles sampled from South Georgia pintails (*Anas georgica georgica*) are illustrated in black, and alleles sampled from yellow-billed pintails (*A. g. spinicauda*) in Argentina are illustrated in white. Circle area is proportional to the total number of alleles.

than for the pintail population in Argentina, both for the five nuclear genes and for mtDNA (Fig. 5a). The ancestral Θ estimates for both genomes were smaller than Θ for Argentina, suggesting that pintail populations in Argentina have experienced a recent increase in population size.

The splitting parameter (*s*) peaked sharply at 1.0 (Fig. 5b) both for the five nuclear genes and for mtDNA, confirming that South Georgia was probably colonized by pintails dispersing from South America. Gene flow estimates to and from South Georgia based on the five nuclear loci were approximately equal and broadly overlapping, suggesting that post-divergence gene flow has been 7.4- and 12.7-fold greater than the mutation rate (mean = 37.3 and 24.2, respectively, Fig. 5c). Based on the small size of the recipient population, the number of effective immigrants into the South Georgia population has probably averaged less than one per generation ($4N_em = 0.06$; 95% CI = 0.05–1.14). Gene flow for mtDNA, which is maternally inherited, peaked at zero in both directions. MtDNA gene flow into Argentina peaked sharply, whereas the posterior distribution for gene flow into South Georgia was long and flat (i.e. uninformative), suggesting that mtDNA gene flow into South Georgia could be greater than zero, or there is insufficient information in these data to obtain a good estimate for this parameter.

Based on the five nuclear loci, time since divergence between the South Georgia and Argentina populations was estimated to be approximately 34,000 years ago (95% CI = 13,800–577,000 years; Fig. 5d). Sources of variation in the 95% CI for *T* include variance in *t* ($t/site_{IM} = 0.0000272$; 95% CI = 0.0000111–0.0004629) and the mutation rate (8.02×10^{-10}; 95% CI = 5.30×10^{-10} to 1.08×10^{-9} substitutions/site/year), sampled from the 95% upper and lower posterior distribution of each parameter. Time since divergence for mtDNA was calculated to be 8700 years ago (95% CI = 5300–45,700 years) based on the point estimate of the substitution rate of 4.8×10^{-8} substitutions/site/year published by Peters *et al.* (2005). Considering slower or faster substitution rates of 3.1 or 6.9×10^{-8} substitutions/site/year, respectively (Peters *et al.*, 2005), time since divergence for the mtDNA could be as recent as 6000 years ago or as old as 13,500 years ago.

DISCUSSION

Population genetics

South Georgia pintails exhibit the classic genetic signatures of a founder event and island colonization followed by restricted gene flow and a small effective population size. Based on the coalescent analysis, colonization was inferred to have resulted from yellow-billed pintails dispersing from South America, as the splitting parameter peaked sharply at 1.0. The Falkland Islands can probably be ruled out as a source population because yellow-billed pintails are extremely rare in the Falkland Islands, and have never been abundant since their presence was first recorded (Woods & Woods, 1997). Based on nuclear DNA, subsequent gene flow between South America and South Georgia was inferred to be greater than zero but low, with an average of < 1.0 effective immigrant per generation. The mtDNA in contrast, was consistent with zero gene flow, suggesting that gene flow is primarily male-mediated, as appears to be true for other species of waterfowl (Anderson *et al.*, 1992; Sonsthagen *et al.*, 2010; Peters *et al.*, 2012b). These findings are consistent with what we know about pintail population biology, as the mainland subspecies *A. g. spinicauda* is occasionally observed on South Georgia as well as elsewhere in the Subantarctic, including the Antarctic Peninsula, King George Island, South Orkney Islands, and the South Shetland Islands (Weller, 1980; Prince & Croxall, 1996; Young, 2005). Yellow-billed pintails in particular are thus known to wander widely, and this periodic dispersal facilitates gene flow, as indicated by a recent study comparing yellow-billed pintail populations inhabiting low- and high-elevation habitats in the Andes

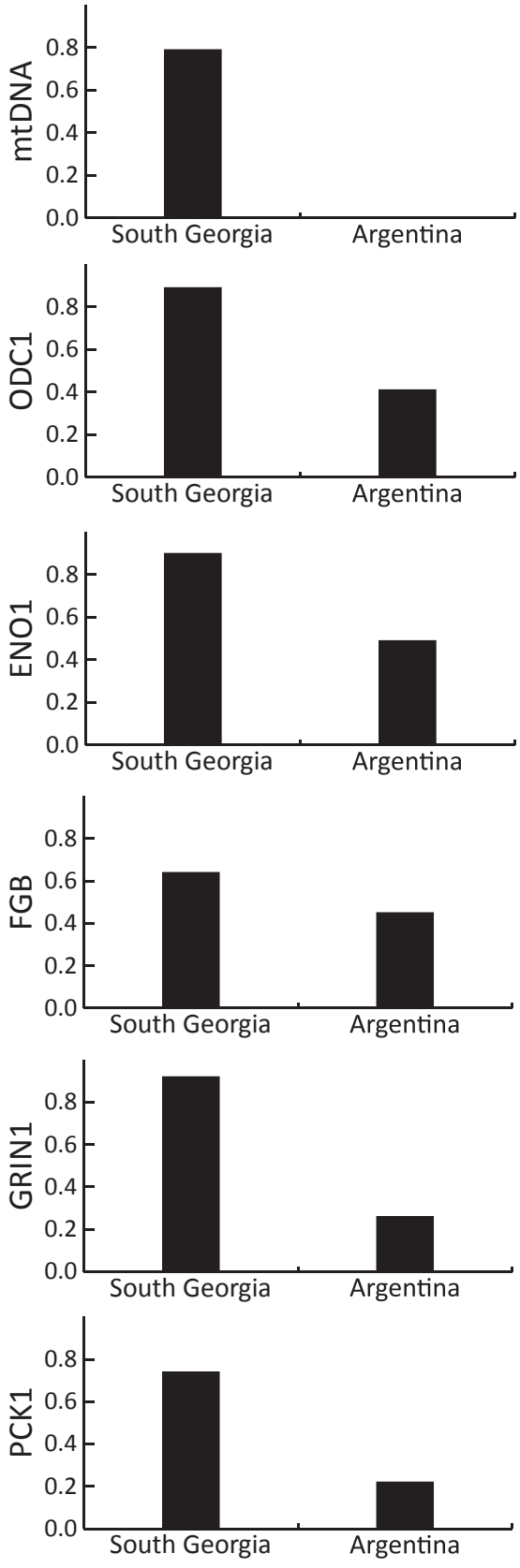

Figure 3 Frequency of the most common pintail allele on South Georgia (*Anas georgica georgica*) and in Argentina (*A. g. spinicauda*).

(McCracken *et al.*, 2009). The South Georgia population in contrast is clearly resident year-round and non-migratory (Martin & Prince, 2005).

Finally, while South Georgia pintails were accurately assigned to the island population with high probability in the STRUCTURE analysis, pintails in South America were not. This probably reflects the effects of genetic drift, the strength of which is an inverse function of the effective population size (Wright, 1931). On South Georgia, most individuals were homozygous for a few common alleles, whereas most individuals in Argentina were heterozygous. Linkage disequilibrium was thus high for the population with low diversity, but low for the population with high diversity. For each nuclear locus, the most common allele on South Georgia also occurred at low frequency in South America; see also Pruett & Winker (2005) for similar empirical examples in island populations of the song sparrow (*Melospiza melodia*). Argentine pintails that carried alleles that are common in the South Georgia population could thus be assigned to either population without large deviations from Hardy–Weinberg equilibrium, especially if they carried common alleles at two or more loci. Genetic drift and a founder event have thus decreased genetic diversity on South Georgia, whereas time since divergence has not been sufficiently long to evolve reciprocal monophyly in the large continental population because the effects of genetic drift are weaker.

Timing of colonization of South Georgia relative to deglaciation chronology

The isolation-with-migration analysis demonstrated that the South Georgia pintail population was established by individuals dispersing from South America. Further, the two-population IM analysis gave the peak estimate of time since divergence as 34 ka, prior to the LGM, which spanned 26.5–19 ka (Clark *et al.*, 2009). Although the confidence intervals are wide and the lower 95% confidence interval overlaps the LGM, our findings suggest that ice-free areas existed on South Georgia earlier in the chronology than previously reported (Clapperton *et al.*, 1989). This finding is consistent with the observation that the plant community of South Georgia was well established by the start of the Holocene (Van der Putten & Verbruggen, 2005; Van der Putten *et al.*, 2009, 2010).

Van der Putten *et al.* (2010) provide evidence for the presence of LGM refugia on South Georgia and other subantarctic islands based on plant macrofossil and pollen analysis. Although there exists consensus for most subantarctic islands that ice-free refugia were present through the LGM, for South Georgia it has been suggested that the flora is a result of post-glacial colonization as extensive LGM ice cover would have wiped out all biota. Van der Putten *et al.* (2010) concluded that the majority of the plant species were present at the onset of accumulation of post-glacial organic (peat) sediment (early Holocene) and that there is no evidence for the arrival of new immigrants during the subsequent period,

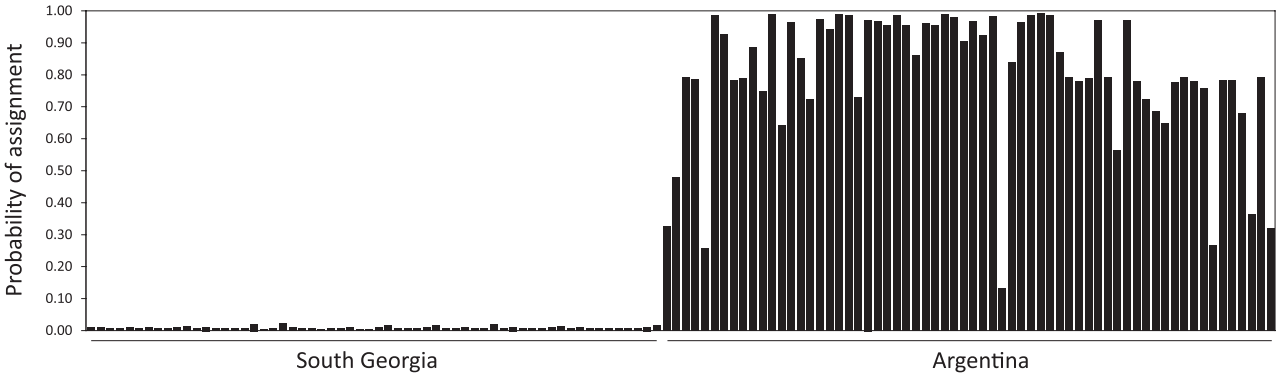

Figure 4 Assignment probabilities for pintails from South Georgia (*Anas georgica georgica*) and Argentina (*A. g. spinicauda*) for $K = 2$ populations.

suggesting that plants survived the LGM on South Georgia. These observations support findings by Bentley *et al.* (2007a) that LGM-ice cover may have been restricted to fjords, and that ice-free lake sites were already in existence 18.6 ka (Rosqvist *et al.*, 1999).

In sum, while South Georgia was probably glaciated to the continental shelf, ice-free areas in the littoral zone would have provided suitable intertidal foraging habitat for vagrant pintails dispersing from South America during time points in the deglaciation. Our results, which indicate that the founding of the South Georgia pintail population could have occurred 34,000 years ago point in the same direction as the previous paleobotanical studies and support the existence of ice-free refugia on South Georgia at that time and all the way through the LGM.

We found a mismatch, however, between peak estimates in the dates of divergence inferred from nuclear DNA and mitochondrial DNA. Time since divergence for mtDNA was estimated to be 8700 years, a date that is in closer agreement with the final stages of ice retreat on South Georgia (Rosqvist *et al.*, 1999; Bentley *et al.*, 2007a) and elsewhere in neighbouring Patagonia (Rabassa & Clapperton, 1990; Rabassa *et al.*, 2005; Turner *et al.*, 2005). There are several factors, however, that could render the date inferred from mtDNA divergence, the date from the nuclear DNA, or both sets of dates misleading.

First, in the case of mtDNA, substitution rates are difficult to infer for the control region, which exhibits high rate variation among sites (Tamura & Nei, 1993). The mtDNA substitution rates we obtained, while carefully calibrated, are approximations based on comparison to the more slowly evolving cytochrome *b* (Peters *et al.*, 2005), as direct alignment of mtDNA control region sequences beyond the genus *Anas* is problematic due to the high frequency of indels. Second, mtDNA has been shown to be subject to selective sweeps (Niki *et al.*, 1989; Mishmar *et al.*, 2003; Fontanillas *et al.*, 2005), which can lead to the fixation of a single haplotype. Third, a similar pattern could also result from a post-divergence bottleneck. Although bottlenecks will generally act to increase net population divergence, this would be unlikely to occur if a single ancestral or mainland-origin mtDNA haplotype became fixed in the population by chance. For example, if the population declined sharply following a subsequent re-advance of the glaciers, as appears to have happened 14.8–14.2 ka (Rosqvist *et al.*, 1999), it is possible that a prolonged period of small population size could have coincided with the complete replacement of mtDNA from the mainland following the initial divergence. Under this scenario, diversity of mtDNA would be preferentially reduced compared to nuclear DNA because it has one-quarter of the effective population size, potentially resulting in a mismatch for time since divergence between nuclear and mitochondrial loci. This scenario is thus a distinct possibility for South Georgia pintails, but it is important to interpret the results of this study cautiously, as the upper confidence interval for the mtDNA overlapped the peak estimate for nuclear DNA.

Second, it has been shown that substitution rates are time dependent and that extrapolation of rates across different time-scales can result in invalid divergence date estimates (Ho *et al.*, 2005; Ho & Larson, 2006). Although most of this literature has focused on mtDNA and not nuclear DNA, calibrations using time points deep in the fossil record (> 1–2 Ma) will underestimate short-term substitution rates and therefore overestimate time since divergence. While stochasticity in the lineage sorting process is probably not a problem in this case because we used multiple outgroup species in the BEAST analysis, the 28 Ma calibration point for the duck–goose split or even 5 Ma for the *Anser–Branta* split (Paxinos *et al.*, 2002; Peters *et al.*, 2008; Fulton *et al.*, 2012) are less than ideal reference points for South Georgia pintails. A better fossil calibration point would incorporate DNA sequences from duck lineages that diverged within the last 10 to 100 ka. While these data do not yet exist, comparison with several radiocarbon-dated, ancient mtDNA sequence data sets (Shapiro *et al.*, 2004; Ho *et al.*, 2007; Saarma *et al.*, 2007) suggests that the long-term substitution rate of 4.8×10^{-8} substitutions/site/year for the mtDNA control region could be as much 4–6 times underestimated. On the other hand, calibration points at 430,000–500,000 ka based

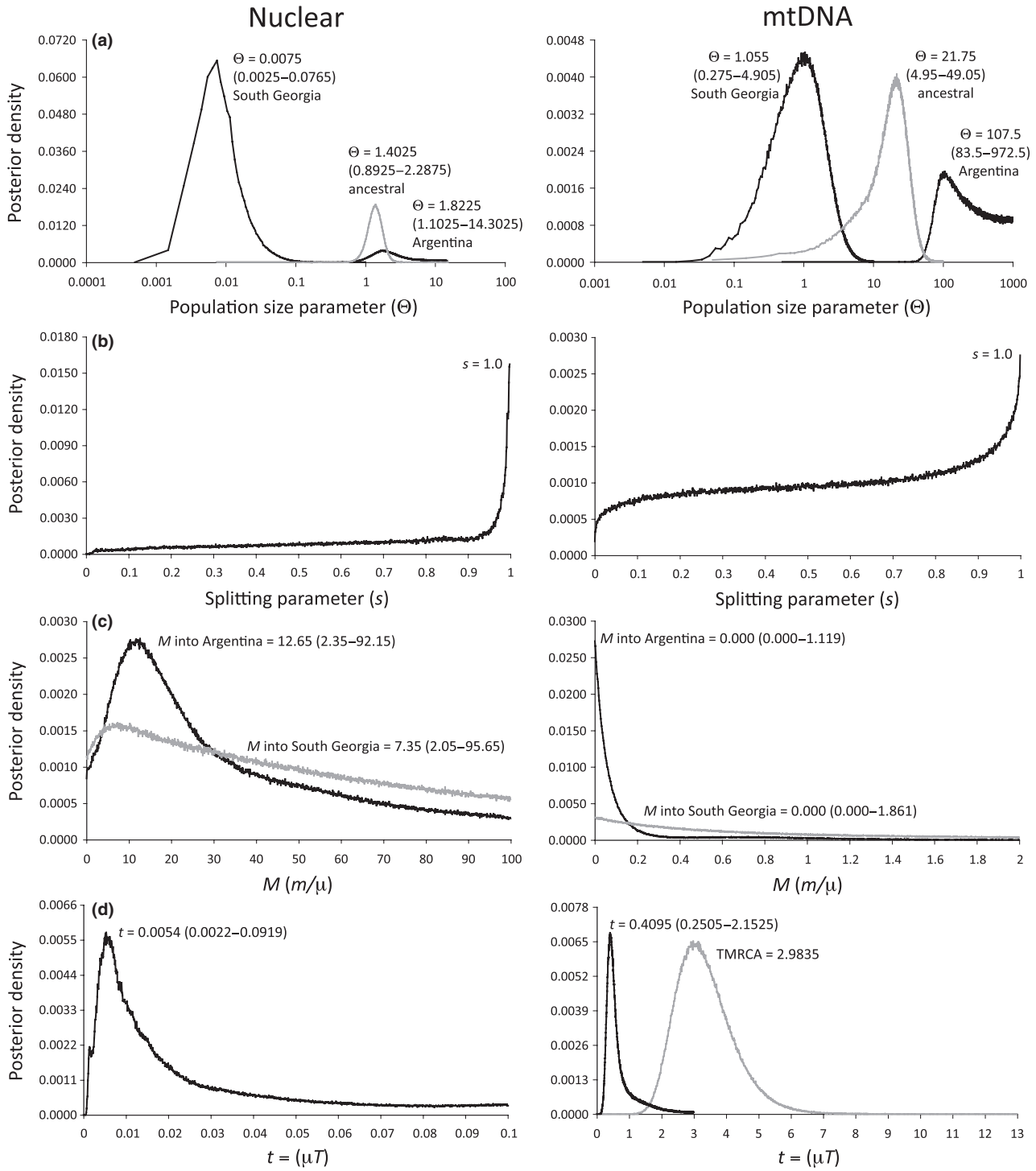

Figure 5 Parameter estimates from the two-population isolation-with-migration analysis of South Georgia pintails (*Anas georgica georgica*) and yellow-billed pintails (*A. g. spinicauda*) for nuclear (left) and mitochondrial DNA (right) for (a) effective population size (Θ), (b) splitting (*s*), (c) gene flow (*M*), and (d) time since divergence (*t*). TMRCA = time to the most recent common ancestor.

on potassium–argon dating from lava flows and ancient DNA from the extinct Hawaiian goose radiation (Paxinos *et al.*, 2002) suggest a similar rate for the mtDNA control region as that obtained by Peters *et al.* (2005). In sum, the nuclear DNA substitution rate we obtained from the multilocus BEAST analysis could also be underestimated. Taking these considerations into account for either the mitochondrial or nuclear DNA, it is therefore possible that South Georgia pintails diverged more recently within the Holocene, and the apparent conflict between mtDNA and nuclear divergence times could be an artefact of our uncertainty in rate calibrations.

CONCLUSIONS

Our study has shown that the South Georgia pintail exhibits the classic genetic signatures of island colonization and while we cannot definitively determine the date at which this occurred, the founding of this population probably pre-dates the LGM. This information contributes to a growing body of biological literature describing the extent of ice cover and timing of deglaciation on South Georgia. Our findings are also in agreement with a broader trend of recent studies indicating that despite widespread glaciation and continental ice sheet formation, ice-free refugia persisted in various locations throughout the Antarctic. As such, multilocus coalescent analyses such as those presented here offer a useful tool for further testing chronological hypotheses about the constraints and timing of deglaciation. Numerous endemic species, populations, and subspecies of terrestrial plants and animals exist on the island; the Sough Georgia pintail is the first to be studied in a multilocus genetic context.

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BIOSKETCH

Kevin G. McCracken studies population genetics and molecular evolution of waterfowl and the evolutionary and physiological process by which they have adapted or acclimatized to high-latitude and high-elevation ecosystems.

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