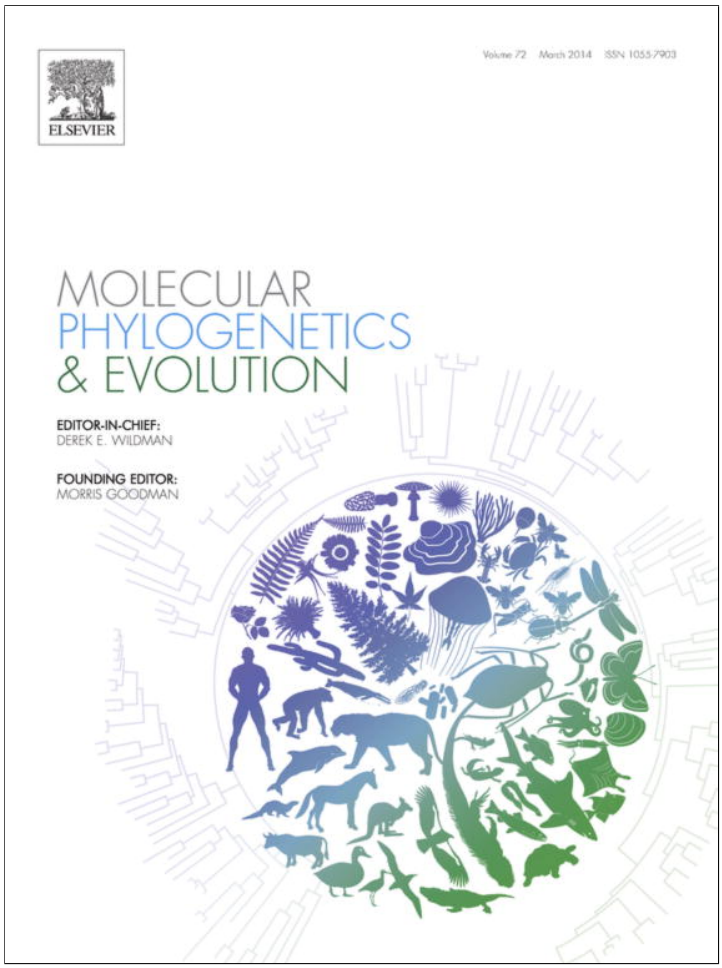

Molecular Phylogenetics and Evolution 72 (2014) 42–53

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeography and genetic structure of two Patagonian shag species (Aves: Phalacrocoracidae)

Luciano Calderón [a,*], Flavio Quintana [b,c], Gustavo S. Cabanne [a], Stephen C. Lougheed [d], Pablo L. Tubaro [a]

[a] Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", CONICET, Av. Angel Gallardo 470, C1405DJR Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina
[b] Biología y Manejo de Recursos Acuáticos, Centro Nacional Patagónico, CONICET, Blvd. Brown 2915, U9120ACD Puerto Madryn, Chubut, Argentina
[c] Wildlife Conservation Society, Amenabar 1595, C1426AKC Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina
[d] Queen's University, Department of Biology, Kingston, Ontario K7L3N6, Canada

ARTICLE INFO

Article history:
Received 19 June 2013
Revised 24 December 2013
Accepted 31 December 2013
Available online 10 January 2014

Keywords:
Phylogeography
Genetic structure
Physical barriers
Non-physical barriers
Gene flow
Seabirds

ABSTRACT

We compared the phylogeographic and genetic structure of two sympatric shag species, *Phalacrocorax magellanicus* (rock shag) and *Phalacrocorax atriceps* (imperial shag), from Patagonia (southern South America). We used multilocus genotypes of nuclear DNA (microsatellite loci) from 324 individuals and mitochondrial DNA sequences (ATP*ase*) from 177 individuals, to evaluate hypotheses related to the effect of physical and non-physical barriers on seabird evolution. Despite sharing many ecological traits, the focal species strongly differ in two key aspects: *P. magellanicus* has a strong tendency to remain at/near their breeding colonies during foraging trips and the non-breeding season, while *P. atriceps* exhibits the converse pattern. Both species showed similar mtDNA genetic structure, where colonies from the Atlantic Coast, Pacific Coast and Fuegian region were genetically divergent. We also found similarities in the results of Bayesian clustering analysis of microsatellites, with both species having four clusters. However population differentiation (e.g. F_{st}, Φ_{st}) was higher in *P. magellanicus* compared to *P. atriceps*, and average membership probabilities of individuals to specific clusters (*Q*-values) were also higher in the former. *Phalacrocorax magellanicus* has strong phylogeographic structure, consistent with the impact of Pleistocene glaciations, with diagnostic haplotypes associated with each of the three mentioned regions. The same pattern was not as evident for *P. atriceps*. Migration rate estimators were higher for *P. atriceps* than for *P. magellanicus*; however both species followed an *n*-island-like model of gene flow, this implies that dispersal occurs across the continental land mass that separates Atlantic and Pacific Oceans. Our results supported the hypothesis that non-physical barriers are important drivers of the genetic and phylogeographic structure in seabirds, and also that physical barriers constitute effective but not absolute impediments to gene flow.

1. Introduction

Gene flow is one of the main forces that opposes genetic differentiation among populations (Wright, 1931). Thus, identifying factors that modulate gene flow provides insight into the forces that influence species evolutionary trajectories.

Even though seabirds are considered to be highly mobile organisms, physical barriers impact their genetic structuring in the same way as in less mobile or non-volant organisms. For example, the presence of the narrow Isthmus of Panama may serve as an effective physical barrier to gene flow for seabirds (Steeves et al., 2005). Physical barriers affecting such species may be either historical (e.g. advance of glaciers) or contemporary (e.g. land masses) although these categories are not mutually exclusive (Friesen et al., 2007a).

Many seabird species exhibit strong genetic structure even in the absence of obvious physical barriers (reviewed in Friesen et al. (2007a)). Hence, factors other than extrinsic barriers must be responsible for the existence of this structure. Indeed, factors related to species life histories, termed non-physical barriers, have been suggested as important in affecting evolutionary trajectories of some seabirds (Friesen et al., 2007a). Examples of such so-called non-physical barriers are foraging range and non-breeding season distribution (e.g. Burg and Croxall, 2004; Friesen et al., 2007a, 2007b; Morris-Pocock et al., 2008, 2010b). Species with wide foraging ranges and/or that disperse during the non-breeding season might be less likely to be genetically structured, as a consequence of higher rates of gene flow.

Here, we focus on evaluating the potential impacts of both physical and non-physical barriers for two seabirds from the

* Corresponding author. Address: División de Ornitología, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Av. Angel Gallardo 470, C1405DJR Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.
E-mail address: lucianocalderon@yahoo.com.ar (L. Calderón).

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http://dx.doi.org/10.1016/j.ympev.2013.12.011

Patagonian region of southern South America. The biogeographic history of this region is particularly marked by the advance and retreat of continental ice sheets during the Pleistocene (e.g. Clapperton, 1993; Rabassa et al., 2005). Ice sheets covered most of southwestern Patagonia and the Isla Grande de Tierra del Fuego during the Great Patagonian Glaciation (GPG), approximately one million years before present (Mercer, 1976; Rabassa and Clapperton, 1990). Several other glacial cycles affected this region before and after the GPG (e.g. Rabassa et al., 2005, 2011), and these have been identified as responsible for causing vicariant events in many Patagonian plants and terrestrial vertebrates (reviewed in Sérsic et al. (2011)). Major phylogeographic divisions found in this region separate northern from southern populations (Sérsic et al., 2011). This is compatible with geological and paleoclimatological evidence, suggesting that certain regions of Tierra del Fuego and the northern Patagonia remained free of ice during glaciations (Rabassa et al., 2005; Rabassa and Clapperton, 1990). Alternatively, the current topology of Patagonia presents a striking longitudinal barrier to movement of seabirds, with the Andes mountain range on the Pacific littoral, and the continental land mass itself separating the Atlantic and Pacific Oceans. These two geographical constraints may have affected coastal seabird dispersal, because such species require aquatic environments for feeding and to enable flight (Friesen et al., 2007a). In summary, both historical and contemporary physical factors could have been important in the evolution of coastal seabirds. However, it is unclear how such barriers might have affected coastal seabirds in this area, in part because DNA studies of these species are scarce (but see Bouzat et al., 2009).

In the present study we focus on two shag species (Pelecaniformes: Phalacrocoracidae), *Phalacrocorax magellanicus* (rock shags) and *P. atriceps* (imperial shags), to evaluate the hypothesis that life history traits relate to genetic structure in seabirds, and to test predictions related to the biogeographic history of Patagonia. These shags are sister species (Kennedy et al., 2000; Holland et al., 2010), endemic to Patagonia (Orta, 1992; Nelson, 2005), and dependent on marine environments for feeding (Punta et al., 2003; Bulgarella et al., 2008; Yorio et al., 2010). They have overlapping breeding distributions that mainly encompass the coastlines of Chilean and Argentine Patagonia, including the Islas Malvinas (Falklands) (Fig. 1). *Phalacrocorax atriceps* colonies are also found in some fresh water lakes (Nelson, 2005). Some of these freshwater populations are morphologically and/or ecologically divergent in relation to their marine counterparts, including individuals from Lakes Yehuin (Rasmussen, 1994) and Nahuel Huapi (Casaux et al., 2010). Both species show plumage color variation throughout their distribution. *Phalacrocorax magellanicus* has no named subspecies, despite showing plumage color differences in both adults and juveniles in different geographic regions (Rasmussen, 1987). *Phalacrocorax atriceps* has the nominate subspecies (more abundant in Santa Cruz and Pacific Coast) and *P. a. albiventer* (more abundant in Chubut and Tierra del Fuego provinces) (Rasmussen, 1991; Orta, 1992; Nelson, 2005), which are diagnosable using plumage color characters only (Devillers and Terschuren, 1978). These subspecies do not exhibit diagnostic differences in behavior, morphometry or allozyme (Siegel-Causey, 1986; Rasmussen, 1991, 1994).

Despite being broadly ecologically similar, *P. magellanicus* and *P. atriceps* exhibit two key differences in their foraging range and non-breeding distribution, that could have affected their respective evolutionary trajectories. *Phalacrocorax magellanicus* is mainly an inshore forager (Quintana, 2001; Sapoznikow and Quintana, 2003; Frere et al., 2008), while *P. atriceps* typically has a wider foraging range, including offshore waters (Sapoznikow and Quintana, 2003; Quintana et al., 2011; Harris et al., 2012). *Phalacrocorax magellanicus* is also a year round resident, with most individuals remaining at or near their colonies during the non-breeding season

Fig. 1. Distribution of *P. magellanicus* and *P. atriceps*. Dark gray indicates the joint breeding distribution for both species, and light gray indicates the non-breeding distribution (modified from Nelson (2005)).

(Punta and Saravia, 1993; Punta et al., 2003; Sapoznikow and Quintana, 2008), while *P. atriceps* disperse; individuals abandon their colonies during the non-breeding season (Rasmussen, 1994; Punta et al., 2003; Harris et al., 2013). We thus predict that *P. magellanicus* will exhibit lower migration rates, with a stronger genetic and phylogeographic structure compared to *P. atriceps*.

We further expect that contemporary physical barriers would have affected the genetic structure of both focal species. We predict that the southern South American land mass and the Andes will hamper trans-continental dispersion of both species, and promote stronger gene flow along coastlines in a stepping-stone fashion. This should be evident in isolation by distance and lack of direct gene flow between colonies on the Pacific and Atlantic Coasts.

Earlier allozyme studies found genetic differentiation in *P. magellanicus* (Siegel-Causey, 1997), but not in *P. atriceps* (Rasmussen, 1994). For *P. magellanicus*, Atlantic Coast (AC), Fuegian region (FR) and Pacific Coast (PC) colonies showed shallow genetic differences, supporting the notion that vicariant events affected coastal seabirds as a consequence of past glacial advance within the breeding range (Siegel-Causey, 1997). This also raises the possibility that these regions could have been refugia during Pleistocene glaciations (Siegel-Causey, 1997). Based on these and previous findings in other taxa (e.g. Sérsic et al., 2011), we expect to find the genetic signatures of refugia in northern Patagonia on the Atlantic Coast (AC) and Pacific Coast (PC), as well as in the Fuegian region (FR). Evidence for such refugia should include genetic differences among the aforementioned regions and higher levels of genetic diversity within them (e.g. Sérsic et al., 2011).

Here we analyze and compare the phylogeographic structure of *P. magellanicus* and *P. atriceps*. We address predictions related to the effect of physical and non-physical barriers on seabird evolution and include considerations on the biogeographic history of Patagonia. We also evaluate the evolutionary and conservation status of both species, particularly of *P. atriceps* subspecies and inland colonies. Although neither species is considered to be of conservation concern (BirdLife International 2013a,b), some colonies have

declined in abundance (e.g. Casaux et al., 2010). We use mitochondrial and nuclear DNA to assess the evolutionary history of both species.

2. Materials and methods

2.1. Sampling

Our sampling covered most of the distribution of both species. Samples were either blood or pectoral muscle stored in 96% ethanol. Blood was taken from adults' jugular veins. Sampling was performed during reproductive seasons; one individual per nest was sampled to avoid sampling relatives. To preclude duplicate sampling, individuals sampled in the same colony were marked with ink before release. Samples of *P. atriceps* were taken between October 2007 and February 2008, whereas samples of *P. magellanicus* were taken during December 2008. Those samples ($n = 168$) are housed in the tissue collection of the Ornithology Division of the Museo Argentino de Ciencias Naturales (MACN-Or-ct). Muscle samples were loaned by the Kansas University Natural History Museum (KUNHM, $n = 139$) and by the American Museum of Natural History (AMNH, $n = 17$). Samples loaned by the KUNHM have been used for previous biogeographic studies (Rasmussen, 1994 and Siegel-Causey, 1997). In total we used 151 samples from *P. magellanicus* and 173 from *P. atriceps* (Tables 1 and 2 provide details of sampling localities).

2.2. DNA extraction and microsatellite genotyping

DNA extractions followed the silica-based protocol proposed in Ivanova et al. (2006), adapted for spin columns (Epoch Life Sciences, Missouri City, TX, USA). A total of 12 microsatellite loci were successfully crossamplified in both species: PcT1, PcD2, PcT3, PcT4, PcD5, PcD6 (originally developed for *Phalacrocorax carbo* by Piertney et al., 1998); and Cor01, Cor05, Cor06, Cor30, Cor43 and Cor45 (originally developed for *Phalacrocorax auritus* by Fike et al., 2009). Although all markers amplified successfully, some loci were discarded from subsequent analyses. PcT1 was not informative and excluded in both species because of hypervariability; each individual showed a different pair of alleles. Another subset of loci was discarded because they were monomorphic: PcD6, Cor30, Cor43 in *P. magellanicus* and Cor45, and Cor01, Cor30 and Cor45 in *P. atriceps*. Thus for our final microsatellite datasets we had genotypes for seven polymorphic microsatellite loci for 151 *P. magellanicus*, and genotypes at eight polymorphic loci for 173 *P. atriceps* individuals. Forward primers were modified by adding the M13 sequence to their 5′ ends, to allow for binding of the fluorescent-labeled M13 primer (Neilan et al., 1997). Annealing temperatures were 55° C for both species across all markers (more details about PCR conditions in Supplementary material 1a).

2.3. Mitochondrial marker amplification

We found evidence of a tandem duplication involving the mitochondrial control region in *P. atriceps*, but not in *P. magellanicus*, and thus did not use this marker for our analyses. This has also been reported for other seabird species (e.g. Abbott et al., 2005; Morris-Pocock et al., 2010a). We obtained partial sequences (657 bp) of the ATP synthase gene, subunits 8 and 6 (hereafter ATPase). We sequenced 83 individuals for *P. magellanicus* (GenBank accession numbers: KF983947–KF984029) and 90 for *P. atriceps* from across each species range (GenBank accession numbers: KF983857–KF983956). We designed specific primers to our species using sequences published in Kennedy et al. (2000) as templates. The forward primer (FATP) sequence is: 5′-CACTCCTAATCCAACCAA AGC-3′ and the reverse primer (RATP) sequence is: 5′-ATTATGGC TACTGCTACTTCTA-3′. Annealing temperatures were 56 °C for both species (more details about PCR conditions in Supplementary material 1a).

2.4. Testing variability of microsatellite loci

We calculated observed heterozygosity, expected heterozygosity, and number of alleles, for every locus and colony using ARLEQUIN v3.5 (Excoffier and Lischer, 2010). Tests for departure from Hardy-Weinberg (HW) equilibrium expectations within each cluster identified by the program STRUCTURE (see below) were also performed in ARLEQUIN. Null allele frequencies for each locus

Table 1
P. magellanicus sampling localities details and genetic diversity per colony. Number of individuals analyzed (n), averaged values of observed heterozygosity (Ho) and expected heterozygosity (He), mean number of alleles (Na), allelic richness (R) and number of private alleles (Pa). Nucleotidic diversity (π) and haplotipic diversity (Hd). AC, FR and PC indicate colonies that were included in Atlantic Coast, Fuegian region and Pacific Coast groups for AMOVA analysis (based on microsatellites).

Locality	Ref.	Lat	Long	Microsatellite diversity						mtDNA diversity		
				n	*Ho*	*He*	*Na*	*R*	*Pa*	*n*	π	*Hd*
AC												
Punta Arco (Chubut)	PA	−42.50	−64.50	4	0.64	0.67	3.14	2.72	2	4	0.0007	0.5
Las Charas (Chubut)	CH	−42.70	−64.98	10	0.59	0.60	4.14	2.60	2	4	0.0007	0.5
Punta Loma (Chubut)	PL	−42.81	−64.88	20	0.55	0.62	4.86	2.65	0	7	0	0
Roca Malaspina (Chubut)	RM	−45.18	−66.51	10	0.60	0.60	4.29	2.76	0	7	0	0
Vernaci Este (Chubut)	VE	−45.18	−66.48	10	0.61	0.61	4.00	2.62	0	7	0	0
Puerto Melo (Chubut)	PM	−45.05	−65.84	8	0.36	0.51	3.00	2.33	0	–	–	–
Cabo Blanco (Santa Cruz)	CB	−47.20	−65.75	13	0.49	0.48	3.67	2.36	1	8	0	0
Bahía Uruguay (Santa Cruz)	UR	−47.64	−66.01	5	0.46	0.58	3.00	2.38	0	–	–	–
Isla Elena (Santa Cruz)	IE	−47.75	−65.93	10	0.40	0.49	3.71	2.43	1	8	0	0
Isla Pingüino (Santa Cruz)	IP	−47.90	−65.71	7	0.39	0.52	3.43	2.46	0	–	–	–
Monte León (Santa Cruz)	ML	−50.33	−68.88	12	0.66	0.68	4.29	2.88	0	9	0.0016	0.2
FR												
Lively Island (Malvinas, Falklands)	IM	−52.02	−58.46	3	0.57	0.76	3.29	3.19	1	3	0	0
Bahía Ushuaia (Tierra del Fuego)	BU	−54.84	−68.25	12	0.73	0.70	5.43	3.10	4	9	0.0016	0.5
Isla Navarino (XII Region)	IN	−54.93	−67.65	10	0.55	0.63	4.50	3.15	0	–	–	–
Strait of Magellan (XII Region)	EM	−52.47	−69.57	3	0.62	0.58	3.00	2.83	0	3	0	0
PC												
Llanquihue (X Region)	LL	−41.80	−76.66	14	0.56	0.57	3.71	2.54	1	14	0	0
Total/Average	–	–		151	0.54	0.6	3.84	2.69	–	83	0.0029	0.54

Table 2
P. atriceps sampling localities details and genetic diversity per colony. Other details and abbreviations as in Table 1. AC, FR and PC indicate colonies that were included in Atlantic Coast, Fuegian region and Pacific Coast groups for AMOVA analysis (based on microsatellites). When included in the analysis, Lake Yehuin colony (YE) was considered as a separate group.

Locality	Ref.	Lat	Long	Microsatellite diversity						mtDNA diversity		
				n	*Ho*	*He*	*Na*	*R*	*Pa*	*n*	π	*Hd*
AC												
Punta León (Chubut)	PL	−43.03	−64.28	15	0.73	0.72	6.86	3.55	0	10	0.0021	0.71
Roca Malaspina (Chubut)	RM	−45.18	−66.51	16	0.77	0.79	7.57	3.86	1	10	0.0009	0.2
Puerto Melo (Chubut)	PM	−45.05	−65.84	8	0.76	0.78	6.14	3.88	0	–	–	–
Isla Arce (Chubut)	IA	−45.00	−65.50	14	0.67	0.76	6.71	3.69	2	–	–	–
Roca Foca (Santa Cruz)	RF	−47.73	−65.83	9	0.62	0.72	6.29	3.58	0	–	–	–
B. Oso Marino (Santa Cruz)	BO	−47.91	−65.78	8	0.80	0.74	6.14	3.72	0	–	–	–
Isla Chata (Santa Cruz)	IC	−47.93	−65.73	10	0.71	0.73	6.57	3.66	3	6	0.0023	0.73
Pico Quebrado (Santa Cruz)	PQ	−50.25	−68.63	9	0.65	0.71	5.57	3.48	0	8	0.0024	0.75
Monte León (Santa Cruz)	ML	−50.33	−68.86	5	0.73	0.70	4.14	3.33	0	–	–	–
FR												
New Island[a] (Malvinas, Falklands)	IM	−54.86	−68.23	12	0.87	0.84	8.57	4.25	2	8	0.0003	0.25
Isla Navarino (XII Region)	IN	−54.95	−67.00	7	0.68	0.77	6.00	3.88	0	–	–	–
Isla Bridges (Tierra del Fuego)	BR	−54.93	−67.65	6	0.69	0.69	5.13	3.54	1	–	–	–
Becasses (Tierra del Fuego)	BE	−51.71	−61.28	11	0.69	0.80	7.86	4.06	2	11	0.0005	0.32
Punta Arenas (XII Region)	PA	−52.47	−69.57	3	0.76	0.70	3.43	3.43	0	3	0.002	0.66
YE												
Lake Yehuin[b] (Tierra del Fuego)	YE	−54.41	−67.70	14	0.55	0.61	5.50	2.96	3	12	0	0
PC												
Lake Vintter[b] (Chubut)	LV	−43.93	−71.60	4	0.75	0.73	4.00	3.52	1	4	0	0
Llanquihue (X Region)	LL	−41.80	−76.66	15	0.68	0.71	7.13	3.60	1	11	0	0
Lake N. Huapi[b] (Rio Negro)	NH	−40.98	−71.50	7	0.73	0.68	4.25	3.12	0	7	0.0017	0.57
Total/Average	–	–		173	0.71	0.73	5.99	3.61	–	90	0.002	0.76

[a] New Island locality from Islas Malvinas (Falklands), includes two samples obtained in Lively Island (colony 11 in Fig. 3, −52.02 −58.46), these samples were pooled and analyzed as one locality (IM).
[b] Indicates inland colonies.

were estimated with FREENA (Chapuis and Estoup, 2007). Tests for linkage disequilibrium were done with GENEPOP v4.0 (Rousset, 2008). GENALEX v6 (Peakall and Smouse, 2006) was employed to estimate the number of private alleles per colony. Significance levels were adjusted for multiple comparisons with Benjamini–Yekutieli (Benjamini and Yekutieli, 2001) modified false discovery rate method (B–Y FDR), following Narum (2006). ADZE (Szpiech et al., 2008) was used to quantify allelic richness standardizing sample sizes (*g*).

2.5. Population differentiation based on microsatellite

We used Bayesian clustering analyses to estimate the optimal number of genetic clusters (*K*), employing STRUCTURE v2.3.3 (Pritchard et al., 2000; Falush et al., 2003). STRUCTURE was run using the admixture model, with localities as priors (Hubisz et al., 2009) and assuming correlated allelic frequencies (recommended by Falush et al., 2003). Short preliminary runs, testing from $K = 1$ to K equaling the total number of sampled colonies in each species ($K = 16$ in *P. magellanicus* and $K = 18$ in *P. atriceps*) showed that values of $K > 10$ had very low probabilities for each species. Final outputs were obtained after 20 independent runs, testing $K = 1$ to $K = 9$; each run had 100,000 cycles of burn-in and 300,000 cycles of MCMC. STRUCTURE HARVESTER (Earl and vonHoldt, 2012) was used to detect the most probable number of partitions using Evanno's method (ΔK) (Evanno et al., 2005). Membership probabilities (i.e. *Q*-values) of the 20 runs for each value of $K = 2$ to $K = 5$ were averaged using CLUMPP v1.2 (Jakobsson and Rosenberg, 2007) and displayed using DISTRUCT v1.1 (Rosenberg, 2003).

Traditional estimators of global genetic population differentiation, F_{st} (Weir and Cockerham, 1984) and R_{st} (Slatkin, 1995), were obtained with MSATANALYZER (Dieringer and Schlötterer, 2003) and RSTCALC (Goodman, 1997), respectively. We also calculated pairwise estimates of F_{st} and R_{st} between all colonies with sample sizes $\geqslant 7$. Significance values were corrected with the B–Y FDR method (Narum, 2006). F_{st} is more appropriate than R_{st} when numbers of loci and individuals are limited (Balloux and Goudet, 2002). However, when loci follow the stepwise mutation model R_{st} is more sensitive in detecting population differentiation, typically providing higher values (Balloux and Lugon-Moulin, 2002). Pairwise F_{st} values were used to build neighbor-joining (NJ) trees using PHYLIP 3.6 (Felsenstein, 2005).

We tested for significant differentiation among groups of colonies from: Atlantic Coast (AC), Fuegian region (FR), and Pacific Coast (PC) employing the hierarchical AMOVA approach in ARLEQUIN. We estimated F_{ct} (i.e. the percentage of the total genetic variation attributable to among geographical regions) as *Va*/*Vt*, where *Va* is the molecular variance among groups and *Vt* is the total variance (Excoffier et al., 1992). Colonies were grouped as indicated in Tables 1 and 2. For *P. atriceps*, analyses were performed with and without the inland colonies, because the latter comprise a small percentage of the global population and we wanted to test their effect on global genetic variation. When included, Lakes Vintter and Nahuel Huapi were grouped in the Pacific Coast group based on their geographic proximity, and Lake Yehuin was considered as a separate group. The Isla Navarino colony was not included in the AMOVAs for either species because of missing data.

Mantel tests (Mantel, 1967) were performed with GENALEX v6 to test for isolation by distance among colonies. Genetic distance was measured as linearized F_{st}, and geographic distances between colonies were estimated from geographical coordinates with the algorithm implemented in GENALEX, a modification of the Haversine Formula (Sinnott, 1984). Significance for each test was obtained using 10,000 permutations. We used two different approaches to construct the geographical matrixes: (i) considering Euclidean distances between colonies, and (ii) using corrected distances, where distance between colonies was calculated as if they were all distributed along a straight line following the coastline.

The contrast between the two allowed us to evaluate the role of the continental land mass as a physical barrier.

2.6. Demographic history, gene flow model selection and estimates based on microsatellite loci

We tested for recent reduction of the effective population size using BOTTLENECK v1.2.02 (Cornuet and Luikart, 1996). A Wilcoxon's signed-rank test was used to detect significant excess of heterozygotes, one of the signatures of a recent bottleneck. We employed the two-phase mutation model (TPM) assuming 95% stepwise mutation model and 5% multiple-step mutations (recommended by Piry et al. (1999)). This test was performed on each of the clusters obtained with STRUCTURE (Figs. 2 and 3).

We used a model-based approach to test how well our microsatellite data fit different models of gene flow. We used only the microsatellite data for this analysis because they were more variable and informative than mtDNA data. We compared the following models: (i) gene flow occurs between adjacent populations along the coastlines in a stepping-stone fashion (Kimura and Weiss, 1964), (ii) gene flow occurs in an *n*-island-like way (Wright, 1943) with different degrees of connectivity among populations, and (iii) panmixia where all populations collapse into a single population in the first generation. Supplementary material S5 shows schematic representations of all tested models for both species. Data were simulated for each microsatellite separately using BAYESSC (Anderson et al., 2005) a modification of the software SIMCOAL v1.0 (Excoffier et al., 2000). Locus PcD6 was not included in the *P. atriceps* analysis because it equally rejected all models, lowering final probabilities. Tested models differed in their migration matrices; parameters used for simulations were set as continuous intervals. Migration rate (m) was set as symmetrical (for sake of modeling simplicity) with a low-moderate rate [0.001–0.01] and the mutation rate (μ) for microsatellite loci was set as 5×10^{-3}–5×10^{-5} per locus/generation (Estoup et al., 2002). For *P. magellanicus* we used four groups testing five gene flow models, and for *P. atriceps* we used three populations testing three models. In both species, clusters obtained in STRUCTURE were used as populations to obtain the observed statistics, excluding inland colonies in *P. atriceps*. We performed 1000 simulations per microsatellite locus per model. We used three independent summary statistics to test each model. For each simulated dataset, the number of alleles and expected heterozygosity were obtained with BAYESSC, and the F_{st} estimators per loci were obtained with ARLEQUIN. These same summary statistics were obtained for the observed data using ARLEQUIN (per locus data were only used for simulation analysis and are not shown). To evaluate the goodness of fit of the observed data to the simulated data, we used an empirical likelihood of each summary statistic (Richards et al., 2007; Knowles, 2009). First, we estimated the proportion *P* of simulated values equal to or higher than the observed summary statistics and then, we obtained an overall *p*-value for each microsatellite locus for each species. All microsatellite loci *p*-values were combined using the on-line application METAP (Whitlock, 2005) to obtain final probabilities for each model.

The multilocus Bayesian method implemented in BAYESASS v3.0 was used (Wilson and Rannala, 2003) to estimate recent migration rates (*m*). This method requires fewer assumptions than long-term migration rate estimators, and can be applied legitimately to populations that are not in equilibrium (Wilson and Rannala, 2003). Most seabirds, and these shag species in particular, are likely not to be in equilibrium, because of the metapopulation

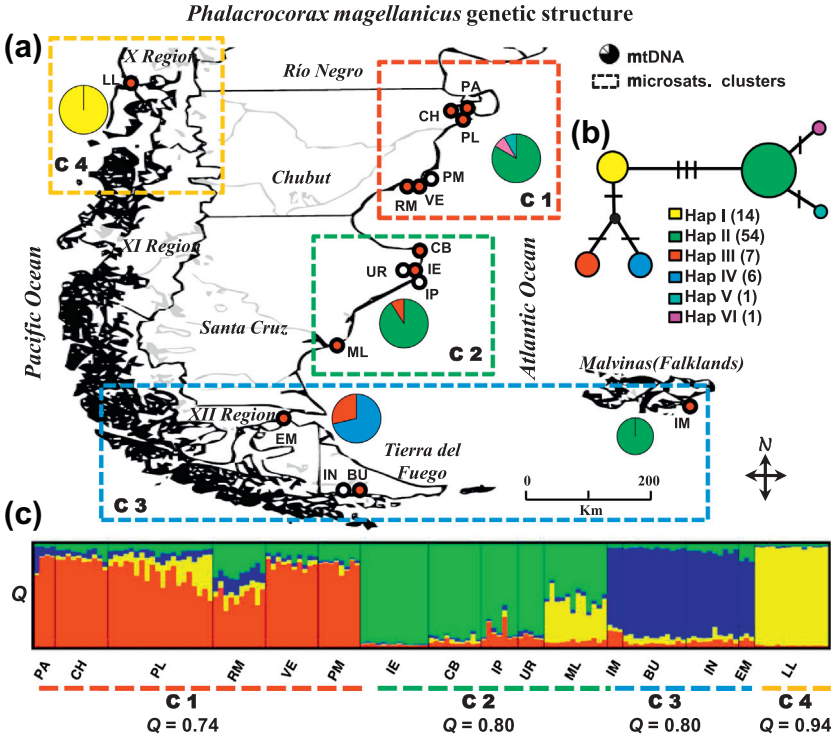

Fig. 2. *P. magellanicus* genetic structure. (a) Sampling localities: filled red circles indicate colonies that were analyzed for both, mtDNA and microsatellites, open circles represent colonies that were analyzed for microsatellites only. Locality abbreviations and references are defined in Table 1. Pie charts show mitochondrial haplotype frequencies, neighboring colonies were grouped into a single pie chart for simplicity. Dashed squares circumscribe the clusters obtained with STRUCTURE. (b) Reduced median-joining network of mtDNA obtained with NETWORK. Hash-marks crossing line connections represent mutational steps. Haplotype frequencies are shown in parentheses. (c) Microsatellite loci bar-plot obtained with STRUCTURE, $K = 4$. *Q* values indicate membership probability of an individual to a cluster. Average *Q* values to the cluster with the highest membership probability are shown beneath each cluster (C1, C2, C3 and C4). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

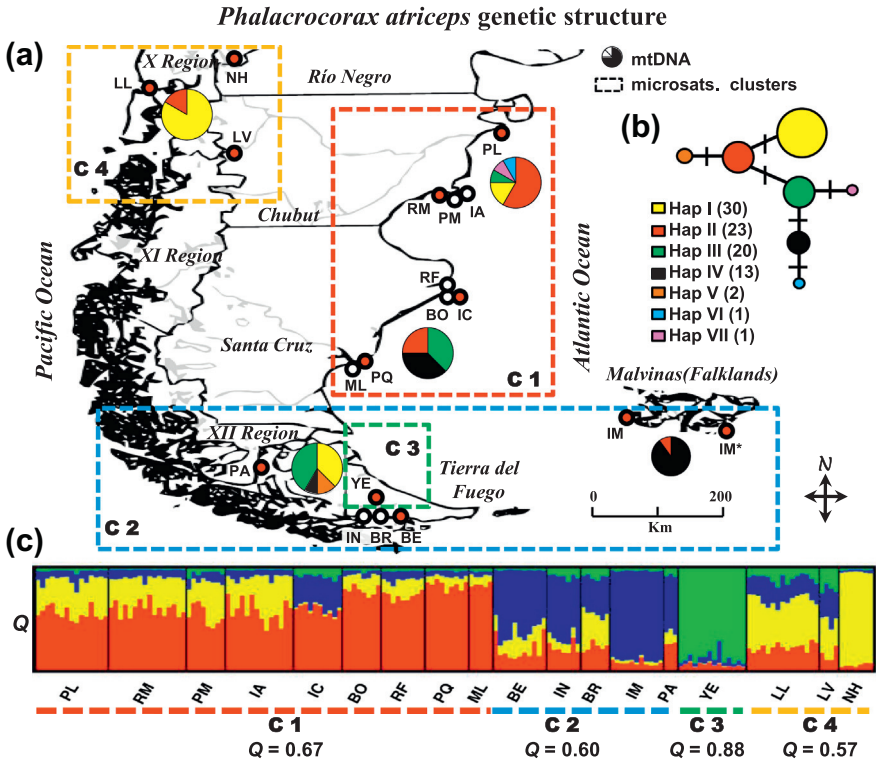

Fig. 3. *P. atriceps* genetic structure. Locality abbreviations and references are defined in Table 2. Other details as in Fig. 2.

dynamics typical of colony breeders (e.g. Oro, 2003). Several preliminary short runs were performed to adjust the acceptance rate for the main parameters (i.e. migration rate, inbreeding coefficient and allele frequencies), and to ensure sufficient mixing of chains. Convergence was assessed by checking the trace files in TRACER v1.4.1 (Rambaut and Drummond, 2007). Final parameters estimates were obtained after performing three independent runs using different starting random seed numbers. Each run consisted of 5×10^7 iterations, a burn-in period of 5×10^6 and a sampling interval of 500 genealogies. The same methodology was applied to both species.

2.7. Mitochondrial DNA analyses

We employed DNASP v4.5.3 (Rozas et al., 2003) to obtain the standard measures of genetic diversity. Hierarchical AMOVAs were performed in ARLEQUIN v3.5, with the same grouping used for our microsatellite analyses (except for Islas Malvinas (Falklands) colonies that were included in the Atlantic Coast in both species). Here we estimated the parameter Φ_{ct} (comparable to F_{ct}) and pairwise Φ_{st} distances between colonies using the Kimura 2-Parameter (K2P) substitution model. Colonies with sample size <7 were not included in the pairwise analysis. NETWORK v4.6.1 (http://www.fluxus-engineering.com) was used to obtain haplotypes median-joining (MJ) networks (Bandelt et al., 1999). The best model of evolution for subsequent phylogenetic analysis was obtained with JMODELTEST v0.1.1 (Posada, 2008), using the Bayesian information criterion (BIC). Maximum-likelihood (ML) trees were constructed using PHYML (Guindon and Gascuel, 2003) with nodal support assessed using 100 bootstrap pseudoreplicates. For *P. magellanicus*, we used two *P. atriceps* haplotypes as outgroup, and *vice versa*. The package BEAUTi/BEAST v1.7.4 (Drummond and Rambaut, 2007) was used to estimate the TMRCA (time to the most recent common ancestor) for clades observed in the *P. magellanicus* ML-tree for which we found a phylogenetic structure (see below). This package is appropriate for estimating intraspecific divergence times when sampled populations are not connected by gene flow (Heled and Drummond, 2010); with exception of one individual showing a typical FR haplotype found in the AC (ML colony, see Fig. 2), no other shared haplotypes among regions were found in *P. magellanicus*. Runs consisted of 5×10^8 MCMC cycles, with a burn-in of 5×10^7. A strict clock was implemented and the substitution rate was obtained dividing the average genetic distance between *Sula sula* and *P. atriceps* + *P. magellanicus* by 35 million years, the estimated divergence time of Sulidae and Phalacrocoracidae based on the only available fossil record (Morris-Pocock et al., 2011; Patterson et al., 2011). Such an ancient calibration may underestimate substitution rates (Ho et al., 2005) and therefore overestimate divergence times. We performed three independent runs with different random starting seeds to obtain final estimates.

3. Results

3.1. Microsatellite genetic diversity

All pairs of loci were in linkage equilibrium within colonies for both species. In *P. magellanicus* we found evidence for departures from HW equilibrium at: PcD2 in Santa Cruz (cluster 2), PcT4 in Chubut (cluster 1) and PcD5 in Chubut and Santa Cruz (Supp. material 1b). Genetic diversity is summarized in Tables 1 and 2 for *P. magellanicus* and *P. atriceps*, respectively.

In *P. magellanicus* low allelic richness was evident (Table 1). When colonies were grouped according to the clusters obtained in STRUCTURE and sample sizes (g) standardized to 20, Chubut and the Fuegian region showed the highest allelic richness and expected heterozygosity. These also had greater numbers of private alleles than other sampled colonies when considering all loci together. Estimated proportion of null alleles was <7% for all surveyed loci within each cluster, with the number of alleles per locus ranging from 5 to 18.

After correction, *P. atriceps* deviated from HW equilibrium only for Cor43 on the Pacific Coast (Supp. material 1c). The highest

values of allelic richness and expected heterozygosity were found in the Fueguian region colonies, a pattern that remained even after standardization ($g = 20$). Estimated null allele frequencies were <4% for all the surveyed loci within each cluster, and the number of observed alleles per locus ranged from 2 to 21.

3.2. Population differentiation

For both species results from STRUCTURE were ambiguous. Evanno's method (ΔK) showed a bimodal distribution and Ln$Pr(X|K)$ supported a value of $K = 4$ (see Supp. material 3a). Figs. 2 and 3 show bar plots of individual Q-values for STRUCTURE when $K = 4$ for *P. magellanicus* and *P. atriceps*, respectively. For *P. magellanicus* most individuals showed high membership probability to a cluster. In *P. atriceps*, most individuals exhibited high admixture levels. Supplementary material 3b shows bar plots from STRUCTURE analyses from $K = 2$ to 5 for both species.

Apportionment of genetic diversity among the proposed genetic regions (Atlantic Coast, Pacific Coast and Fuegian region) was tested using AMOVAs. In *P. magellanicus* the highest F_{ct} value was obtained when Atlantic Coast colonies were grouped together (see Table 1) $F_{ct} = 0.082$ ($p < 0.01$). When inland colonies of *P. atriceps* were included F_{ct} was 0.046 ($p < 0.01$); when inland colonies were excluded F_{ct} was 0.021 ($p < 0.01$), showing the importance of the inland colonies to global among population differentiation.

Global values of population differentiation were significant in both species for both estimators, but in all cases, higher in *P. magellanicus* than in *P. atriceps* (Table 3). When inland colonies were excluded in *P. atriceps*, these values decreased by almost half (see Table 3) showing the importance of the inland colonies when estimating among population genetic differentiation. For both species a greater number of R_{st} than F_{st} values were not significant after B–Y FDR corrections (Supp. material 2a and 2b). Balloux and Goudet (2002) suggested that F_{st} is a better estimator than R_{st} when sample sizes are limited and population differentiation is moderate. The unrooted neighbor-joining tree based on F_{st} distance in *P. atriceps* showed the clear differentiation of two of the three analyzed inland colonies, Lakes Nahuel Huapi (NH) and Yehuin (YE) (Supp. material 2c).

Both species exhibited significant isolation by distance. In *P. magellanicus*, the matrix correlation between Euclidean geographic distances and the F_{st} linearized distances was $r = 0.397$ ($p = 0.01$). When distance along colonies was corrected for the presence of the land mass, the correlation was stronger, $r = 0.68$ ($p = 0.0001$). In *P. atriceps*, the matrix correlation using Euclidean distances was significant ($r = 0.34$, $p = 0.001$); when distances among colonies were corrected, the correlation was similar ($r = 0.31$, $p = 0.027$).

3.3. Demographic history and gene flow

We found evidence of a bottleneck in only a single cluster in *P. magellanicus* – cluster four (C4), corresponding to the Pacific Coast colony, Wilcoxon test = $p < 0.05$. In both species the gene flow model with the highest probability was *n*-island-like. The panmixia model had the lowest probability (Table 4). For *P. magellanicus* a four-population model was tested, and the highest probability was for the model that considered two trans-continental connections, but did not allow for direct connection between Chubut and the Fuegian region (see Supp. material 4). The fact that an *n*-island like models showed higher probability than the stepping-stone models in both species implies that gene flow across the continental land mass may occur.

Average migration rates (m) were higher in *P. atriceps* than in *P. magellanicus* (Fig. 4). Average migration rate for *P. magellanicus* was 0.032 (CI 95%: −0.017 to 0.081), with values ranging from 0.0106 (CI 95%: −0.009 to 0.03) to 0.0971 (CI 95%: −0.016 to 0.21). Average migration rate for *P. atriceps*, when inland colonies were excluded was 0.105 (CI 95%: 0.07–0.14), with values ranging from 0.0059 (CI 95%: −0.0053 to 0.016) to 0.2881 (CI 95%: 0.238–0.337); and when inland colonies were included, average migration rate diminished to 0.056 (CI 95%: 0.021–0.091), driven by the low migration rates between Yehuin colony and all the other populations (data not shown). We consider the migration values estimated without Yehuin colony to be more representative of the *P. atriceps* global population and use these for discussion below. Despite the different magnitude of the migration rates, both species showed evidence of recent unidirectional trans-continental gene flow in a Pacific to Atlantic direction. For both species, the Fuegian region appeared as a source of migrants for the Pacific and the Atlantic Coasts colonies.

3.4. Mitochondrial DNA results

In *P. magellanicus*, we found six haplotypes, all defined by transitions (see Table 1 for summaries of mtDNA diversity). Results of our AMOVA grouping colonies from the Pacific Coast (PC), the Atlantic Coast (AC) and the Fuegian region (FR) were as follows: $\Phi_{ct} = 0.91$ ($p < 0.01$) and global $\Phi_{st} = 0.92$ ($p < 0.01$). Indeed, only one shared haplotype was observed among these regions, with a typically Fuegian region haplotype (Hap. III) found in the Monte Leon colony (i.e. Atlantic Coast) (Fig. 2). We found a significant correlation between genetic and geographic distance, both for Euclidean and corrected geographic distance matrices: $r = 0.49$ ($p = 0.013$) and 0.64 ($p < 0.01$), respectively. The median-joining network showed that haplotypes from the Atlantic Coast were more closely related to those of the Pacific Coast than to haplotypes from the Fuegian region, with three mutational steps separating Pacific and Atlantic haplotypes (Fig. 2b). The maximum-likelihood tree obtained using the Hasegawa-Kishino-Yano 85 (HKY85) model of evolution (Hasegawa et al., 1985), showed the same genealogical relationship (Supp. material 5). The deepest split with highest bootstrap support (100%) observed separated the AC and PC clades from a clade comprised of FR haplotypes. AC and PC where also separated from each other, but with lower bootstrap

Table 3
Summary of all population differentiation estimators for both species, including for *P. atriceps* the treatment without inland colonies (i.e. Nahuel Huapi, Vintter and Yehuin lakes).

Species	Population differentiation estimators				
	Bayesian clustering methods		Classical estimators		
	ΔK[a] (STRUCTURE)	Ln$Pr(X\|K)$[a] (STRUCTURE)	F_{st}[a]	R_{st}[a]	Φ_{st}[b]
P. magellanicus	2/4	4	**0.10**	**0.14**	**0.92**
P. atriceps	2/4	4	**0.048**	**0.06**	**0.56**
P. atriceps (without inland colonies)	2	2	**0.024**	**0.031**	**0.49**

Bayesian clustering shows the optimal number of genetic clusters obtained with each method.
Bold values, in classical estimators, indicate significance at the $p < 0.01$ level.
[a] Based on microsatellite data.
[b] Based on mitochondrial data.

Table 4
Ranking of probabilities for all tested gene flow models. Four groups were considered in *P. magellanicus*, and three in *P. atriceps*.

Species	Model	Combined *p*-value
P. magellanicus	***n* – island (2 t-c connections)**	**0.28**
	n – island (full connections)	0.25
	Stepping-stone	0.13
	n – island (1 t-c connection)	0.12
	Panmixia	0.0001
P. atriceps	***n* – island**	**0.25**
	Stepping-stone	0.20
	Panmixia	0.0001

Combined *p*-value for each model is a combination of all *p*-values for that model. t-c = trans-continental.

support (67%) (Supp. material 5). Fuegian region haplotypes were estimated to have diverged from the Pacific and Atlantic Coast haplotypes approximately one million years ago (HPD: 490,000–1,610,000), while Pacific and Atlantic coasts haplotypes diverged approximately 643,000 years ago (HPD: 253,000–1,080,000). The two haplotypes found in Fuegian region diverged approximately 702,000 years ago (HPD: 280,000–1,182,000).

P. atriceps had a total of seven haplotypes, all of them again defined by transitions (Fig. 3). Colonies showing the highest haplotype diversity were Punta Leon, Isla Chata and Pico Quebrado. Punta Leon colony had two unique haplotypes not found in any other sampled colony, as did the Isla Becasses colony. The AMOVA results indicated that a significant portion of the genetic variance was explained by differences among the three regions (i.e. AC, FR and PC), $\Phi_{ct} = 0.26$ ($p < 0.05$) and $\Phi_{st} = 0.56$ ($p < 0.01$). When inland colonies where excluded from the analysis: $\Phi_{ct} = 0.28$ ($p = 0.06$) and $\Phi_{st} = 0.49$ ($p < 0.01$). Mantel tests did not suggest significant isolation by distance using either Euclidean or the corrected geographic distances matrices. Neither the median-joining networks nor the maximum-likelihood trees, showed evidence of phylogeographic structure in this species. In maximum-likelihood tree haplotypes from the PC, AC and FR formed a polytomy, with a single divergent haplotype from Punta León (Supp. material 5).

4. Discussion

We used genetic data from two Patagonian shag species to evaluate hypotheses on the role of physical and non-physical barriers in seabirds evolution. Our mtDNA data supported the existence of the three predicted genetic groups in both species: Atlantic Coast (AC), Pacific Coast (PC) and Fuegian region (FR). AMOVA results based on mtDNA and microsatellite loci showed significant differences when colonies were grouped as mentioned. However, Bayesian clustering analyses based on microsatellites suggested the existence of four genetic clusters in both species. An unexpected cluster in *P. magellanicus* is related to the Atlantic Coast group, and in *P. atriceps* to fresh water colonies. We also found inconsistency in respect to the Islas Malvinas (Falklands) colonies grouping in both species. These points are discussed below.

4.1. The effect of non-physical barriers on seabird genetic structure

Even though there were similarities in the genetic structure of both species, there were also many contrasting aspects between them. As expected from life history attributes, *P. magellanicus* showed higher levels of population differentiation than *P. atriceps* (Table 3). Although direct comparison of F_{st} across different taxa is challenging (e.g. Jost, 2008; Meirmans and Hedrick, 2011), the following comparison does highlight the contrasting patterns found in our focal species. Population genetic structures of three other Phalacrocoracidae species have been studied using microsatellite loci and global F_{st} values are available: *P. auritus*, $F_{st} = 0.050$ (Mercer, 2008), *P. harrisii*, $F_{st} = 0.097$ (Duffie et al., 2009) and *P. aristotelis*, $F_{st} = 0.055$ (Barlow et al., 2011). The values found in our study bracket this range, with *P. magellanicus* exhibiting the highest and *P. atriceps* the lowest global F_{st} values (0.10 and 0.048, respectively). We also found strong differences in the admixture pattern using STRUCTURE, and thus the individual probabilities of membership (i.e. *Q*-values). Despite finding equivalent number of clusters for both species, *P. atriceps* had higher admixture levels than *P. magellanicus* (Figs. 2 and 3), usually associated with higher gene flow rates (e.g. Latch et al., 2006).

Life history thus appears to be a major factor in the evolution of genetic population structure of both shag species. The stronger genetic structure evident in *P. magellanicus* reflects the tendency of individuals to remain at or near their colonies during the non-breeding season (Punta et al., 2003; Sapoznikow and Quintana, 2008) and during foraging trips (Quintana, 2001; Sapoznikow and Quintana, 2003; Frere et al., 2008). The very low migration rates we found (Fig. 4) would be mediated mostly by juvenile males, given the few shared mtDNA haplotypes found among the

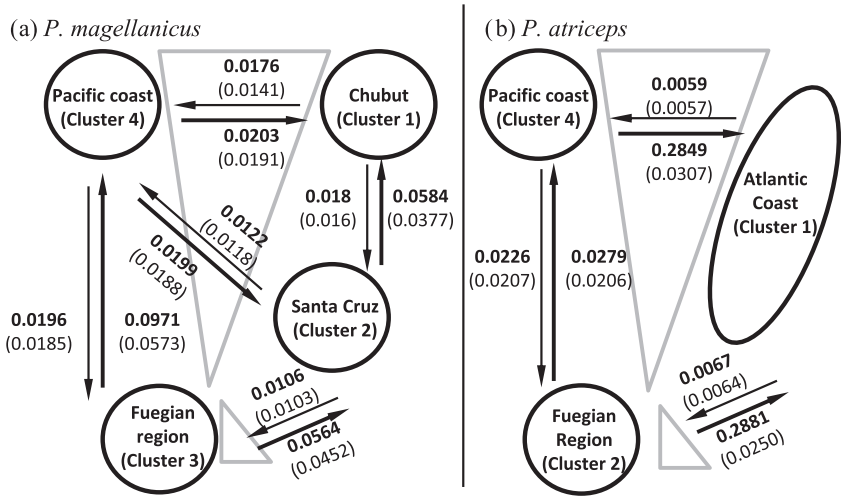

Fig. 4. Recent migration rate (m) estimators, obtained with BAYESASS. Triangles in light gray represent the Patagonian region. In parentheses below each m value there is the standard deviation. Thicker lines indicate in which direction gene flow occurs with more intensity.

three main geographic regions (only one shared haplotype between AC and FR, Fig. 2). Moreover, juveniles have been reported to abandon their colony during non-breeding seasons (Sapoznikow and Quintana, 2008). For *P. atriceps*, their wider foraging range (Sapoznikow and Quintana, 2003; Quintana et al., 2011; Harris et al., 2012) and greater propensity to disperse during the non-breeding season (Rasmussen, 1994; Punta et al., 2003; Harris et al., 2013) likely underlies elevated levels of gene flow (Fig. 4) and weaker population structure (Fig. 3).

Because both non-breeding distribution and foraging range would result in recruitment of individuals to reproduce in non-natal colonies, it is difficult to disentangle their relative contribution. Friesen et al. (2007a) suggested that non-breeding distribution is the most important predictor of genetic and phylogeographic structure in seabirds. In our focal species, individuals may overlap in their foraging range depending on the geographic location of colonies or when the resources are abundant (Sapoznikow and Quintana, 2003). These observations suggest that the non-breeding distribution is the main non-physical barrier responsible for the observed differences between these species.

The dispersal behavior of *P. atriceps* may have contrasting consequences on genetic diversity, depending on the spatial scale considered. For example inland colonies clearly contribute significantly to among-colony genetic differentiation, yet represent a very small percentage of the global population (Orta, 1992; Nelson, 2005). While dispersal and concomitant gene flow tends to homogenize genetic variation when considering only marine colonies, when we consider the overall distribution (including inland colonies) it is evident that it also affords opportunities to colonize new environments (e.g. fresh water), resulting in genetic diversification. Such scale-dependent conclusions have also been reported in passerine birds (Burney and Brumfield, 2009).

4.2. Influence of physical barriers on Patagonian coastal seabirds

In agreement with our hypothesis, *P. magellanicus* was the only of the two analyzed species that exhibited strong phylogeographic structure, with diagnostic mtDNA haplotypes associated with each of the three predicted geographic regions (Fig. 2 and Supp. mat. 5). The deepest split between the found haplotypes occurs between northern and southern groups; consistent with the direction of other documented phylogeographic breaks found in other Patagonian organisms (Sérsic et al., 2011). For *P. magellanicus* this break maps onto the Strait of Magellan, between FR haplotypes and a clade containing both PC and AC haplotypes. This split dates to approximately one million years ago, coinciding with the GPG (Mercer, 1976), when ice sheets reached the Atlantic Coast in southern continental Patagonia and northern Tierra del Fuego (Rabassa et al., 2011). The presence of a PC clade and an AC one is, to our knowledge, the first evidence of a break between east and west (longitudinal) populations in the Patagonian region and dates to ca. 640,000 years ago. We cannot attribute this vicariant event to the orogenesis of the southern Andes, as this uplift dates to the Miocene (7–23 million years ago) (Ramos and Ghiglione, 2008). The two haplotypes in the Fuegian region (Hap. III and IV, Fig. 2) diverged ca. 700,000 years ago, suggesting the possibility of multiple vicariant events within this region, promoted by several advances and retreats of glaciers that affected this region during the Pleistocene (e.g. Rabassa et al., 2011).

Despite the observed phylogeographic structure in *P. atriceps* was not as strong as in *P. magellanicus*; we described significant genetic differences among the three regions that were supported by AMOVA results based on both microsatellites loci and mtDNA. We also found evidence that *P. atriceps* shared historical refugia with *P. magellanicus* in the northeastern Patagonian lowlands and in the Fuegian region. The former was supported by the presence of unique mtDNA haplotypes in both species (Figs. 2 and 3), but microsatellites data supported this refugium only in *P. magellanicus*, where high allelic richness and expected heterozygosity were observed. The same microsatellite variation pattern was also observed in the Fuegian region for both species, and is a signature of a historical refugium. Paleoclimatological evidence indicates that both regions were among the least affected during Pleistocene glaciation cycles (Clapperton, 1993; Rabassa et al., 2005, 2011). It thus seems reasonable that many organisms used these areas as refugia (Sérsic et al., 2011). It can also be asserted, based on the differences in the phylogeographic structure, that the effects of historical physical barriers (i.e. glaciers) that affected both species equally, are accentuated in the less mobile species.

In regard to the effect of contemporary physical barriers on Patagonian coastal seabirds, we found in *P. magellanicus* that colonies from Chubut and Santa Cruz provinces comprise separate genetic clusters (Fig. 2). Although the coastlines of these provinces are adjacent, the San Jorge Gulf (148 km in length) located in between them appears to be an effective physical barrier to gene flow. This gulf coast is flat and sandy in most of its extension, therefore unsuitable for the settlement of the cliff-nesting *P. magellanicus* (Nelson, 2005).

Analysis of our microsatellites data show that dispersal across the continental land mass occurs in both species. Despite finding significant pairwise population differentiation between PC and AC colonies, other coalescent and Bayesian clustering analyses suggested trans-continental gene flow. For example, our Bayesian clustering results implied that AC colonies have high shared ancestry with PC in both species, in *P. magellanicus* this is very clear for PL and ML colonies (Fig. 2) and in *P. atriceps* for PL, RM, PM and IA colonies (Fig. 3). Coalescent simulations indicated a high probability for the *n*-island-like model (Table 4), and migration rate estimators (m) showed evidence of trans-continental gene flow (Fig. 4). Our microsatellite data also showed evidence of isolation by distance in both species, consistent with a stepping-stone model of gene flow. This is especially striking in *P. magellanicus* where Mantel's test based on mtDNA also supports this contention. The evidence together indicates that dispersal across the continent occasionally occurs in both species, but more frequently in *P. atriceps* than in *P. magellanicus*. Moreover a stronger effect of continental land mass as a physical barrier to gene flow was observed for the latter. Similar patterns have been found in other seabird species, where contemporary physical barriers in general and land masses in particular, have proved to be effective but not absolute impediments to gene flow (e.g. *Sula leucogaster*, Morris-Pocock et al., 2011; *Oceanodroma leucorhoa leucorhoa*, Bicknell et al., 2012).

At the same time, despite the low migration rate observed in *P. magellanicus* (Fig. 4), two common directional gene flow patterns were evident from our analyses (i) the Pacific Coast region acts as a source of migrants for the Atlantic Coast colonies; (ii) the Fuegian region provides migrants for the Pacific and Atlantic coasts. The former pattern could be explained by the topography of southwest Patagonia, where numerous fjords and canals penetrate the continent (Coronato et al., 2008), perhaps facilitating access for strong flyers and water-dependent organisms to Andean lakes and to major rivers (e.g. Chubut and Santa Cruz rivers) that flow eastward across Patagonia. The prevalent west to east direction of the winds in this region (Gaiero, 2007) might also promote unidirectional dispersal. Supporting this pattern, we found some records of *P. atriceps* in the middle of the continent, far from marine coastlines (Svagelj and Quintana, pers. comm.). The west to east movement of birds, even across the high Andes, has been previously reported for the Peruvian pelican, *Pelecanus thagus* (Elías, 2008) and for the borrowing parrot, *Cyanoliseus patagonus* (Masello et al., 2011). The south to north pattern of gene flow that we observed in both species can be explained by their tendency to

move towards lower latitudes during non-breeding seasons, reaching as far as the Uruguay and occasionally southern Brazilian coasts (Orta, 1992; Nelson, 2005). This movement may increase the chance of birds from higher latitudes to be recruited to breed in lower latitudes colonies.

4.3. Taxonomic and conservation implications

Although both analyzed shag species are abundant along the Patagonian shelf (Frere et al., 2005) and are considered of "least concern" by BirdLife International (2013a,b), we think there is room for some insights into conservation and taxonomy.

For *P. magellanicus*, we found a geographically coincident pattern of genetic and plumage color variation. The genetic differences observed between Atlantic Coast, Pacific Coast and Fuegian region, reflect the pattern observed by Rasmussen (1987), where five categories of juvenile plumage were described. According to this study juveniles from Pacific Coast have whiter ventral feathering than those from the Atlantic Coast, while the Fuegian region exhibits the highest juvenile plumage variation with representatives of almost all categories. Rasmussen (1987) also observed an adult coloration polymorphism, in which AC individuals have whiter head and neck than those from PC and FR.

For *P. atriceps*, we found strong genetic differentiation in two of the three analyzed inland colonies, Lakes Nahuel Huapi and Yehuin. This was evident from microsatellite data (Supp. material 2b and 2c); moreover Yehuin colony appeared as a separate cluster in the STRUCTURE analysis (Fig. 3c). The genetic distinction of Yehin colony is mirrored by morphological differentiation, with individuals from this colony having the smallest body size and nasal-glands relative to their conspecifics, consistent with adaptation to fresh water (Rasmussen, 1994). The demographic status of Yehuin colony is not known, but our data suggest no recent bottleneck, although we recognize the methods employed by the program BOTTLENECK may have limited statistical power (Peery et al., 2012). Individuals from the Nahuel Huapi colony also exhibit phenotypic differences in nesting and feeding habits, swapping the usual preferred flat surfaces for cliffs (Rasmussen, 1994), and shifting to crustaceans from fish as the main prey (Casaux et al., 2010). This colony is better known and is considered to be locally endangered (Casaux et al., 2010). These apparent adaptations to freshwater environments, the significant differentiation in putatively neutral DNA markers, and their highly localized distributions imply genetic isolation of the fresh water *P. atriceps* populations from their marine counterparts. We recommend these two inland colonies to be considered as distinct management units (*sensu* Moritz, 1994). Moreover, all available evidence for the Lake Yehuin colony suggests that it merits subspecies status as proposed by Rasmussen (1994).

In contrast, we found no genetic evidence supporting *P. atriceps atriceps* and *P. a. albiventer*. Neither of these subspecies emerged as a separate cluster in Bayesian analysis, nor appeared as clades in our mtDNA analyses (Supp. material 5b). There is no correlation between genetic structure and the geographic distributions of these putative subspecies; *P. a. albiventer* is more frequent in Chubut and Tierra del Fuego coasts, while *P. a. atriceps* is more frequent in Santa Cruz and the Pacific Ocean coasts (Rasmussen, 1991). Our results are concordant with Rasmussen (1994) and support the observation that these "subspecies" comprise color morphs of *P. atriceps*. We also found no compelling evidence to suggest that the Islas Malvinas (Falklands) colonies comprise separate subspecies, as postulated by Devillers and Terschuren (1978), although we did find significant pairwise F_{st} values between this and other colonies (Supp. material 2b). Moreover, both analyzed species showed ambiguities when grouping Islas Malvinas (Falklands) colonies. In both cases mtDNA grouped these colonies within Atlantic Coast group, indicating historical colonization of these islands from the AC (Figs. 2 and 3). In contrast, microsatellite evidence showed high levels of admixture between the Islas Malvinas (Falklands) and Tierra del Fuego colonies (Figs. 2c and 3c), suggesting recent gene flow between them.

5. Conclusions

Our comparative analyses of two broadly co-distributed sister species from Patagonia in southern South America show that even though they are affected by the same contemporary physical landmarks and have been impacted by the same geological events, their phylogeographic and genetic structuring patterns are divergent in some aspects. The contrasting life histories of these species clearly impact on their migration rates. We also found that, although physical barriers are important, they are not absolute constraints to gene flow occurrence within seabird. Our work thus contributes to the growing literature showing the importance of non-physical barriers on seabirds evolution.

Data accessibility

DNA sequences in GenBank. Accession numbers: KF983947–KF984029 and KF983857–KF983946.

Acknowledgments

We would like to thank people that contributed with fieldwork and samples collection: M. Uhart, A. Raya Rey, S. Harris, R. Matus, J. Masello and P. Quilfeldt. To museum curators P. Sweet (AMNH) and M. Robbins (KUNHM) for providing key samples. Research was supported by the National Research Council of Argentina (CONICET) (PIP No. 5387/05 to F.Q. and PIP No. 112-200801-00741 to P.L.T.), UBACyT (X114 2008-2010 to P.L.T.), the International Development Centre of Canada (IDRC), the Richard Lounsbery Foundation and the Wildlife Conservation Society. Part of the laboratory work was supported by a Natural Science and Engineering Research Council of Canada Discovery grant to S.C.L. L. Campagna supplied valuable advice during initial laboratory stages and data analyses. L.C. was supported by a doctoral fellowship from CONICET.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2013.12.011.

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