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ORIGINAL ARTICLE

Evolutionary history of the Kelp Gull (*Larus dominicanus*) in the southern hemisphere supported by multilocus evidence

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Abstract The high dispersal ability of seabirds and the absence of geographical barriers has led to high gene flow and reduced population differentiation. Nevertheless, some species with philopatric behavior have restricted gene flow among colonies, revealing a strong population structure. Gulls show widespread colonial behavior, and are long-lived species, which make them a good model for understanding evolutionary processes in seabirds. Previous genetic studies on the Kelp Gull (*Larus dominicanus*) have revealed low genetic variability in mitochondrial markers but relatively high genetic variation in a nuclear marker. These observations can be explained by the occurrence of a selective sweep on mtDNA, population genetic bottlenecks or a recent origin of the species. We used microsatellite data to further investigate these hypotheses, mainly by testing for bottleneck events. Low genetic variability ($H_o = 0.276$–0.570) was detected in Kelp Gulls. However, population genetic structure was observed among regions (Chile, Argentina and Brazil), and between continents (South America and Antarctica). The population of the Kelp Gull in South America may have differentiated due to isolation by distance ($r = 0.7273$, $p = 0.0013$), whereas the population in the Antarctic seems to be isolated by non-physical barriers. Bottleneck events were detected in 6 out of 14 colonies studied. These colonies are at the limits of the distribution of the Kelp Gull, and thus experience harsh survival conditions. We believe that the Kelp Gull has a complex history in the southern hemisphere, with a recent origin, followed by bottlenecks and then population

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expansion. Thus, the genetic diversity found in Kelp Gull is similar to that observed for other species of Laridae.

Keywords Microsatellites · Bottleneck · Recent population · Expansion · South America · Antarctic

Zusammenfassung

Die Evolutionsgeschichte der Dominikanermöwe (*Larus dominicanus*) auf der Südhalbkugel, gestützt durch Multilokus-Analysen

Die hohe Ausbreitungsfähigkeit von Seevögeln und das Fehlen geografischer Barrieren führen zu starkem Genfluss und verringerter Differenzierung von Populationen. Dennoch ist bei manchen Arten mit philopatrischem Verhalten der Genfluss zwischen den Kolonien eingeschränkt, was eine deutliche Populationsstruktur zutage treten lässt. Kolonieverhalten ist bei Möwen weit verbreitet, und es sind langlebige Arten, was sie zu einem guten Modell für das Verständnis evolutionärer Prozesse bei Seevögeln macht. Frühere genetische Untersuchungen an der Dominikanermöwe (*Larus dominicanus*) enthüllten eine niedrige genetische Variabilität mitochondrialer Marker, aber eine relativ hohe genetische Variation bei einem nukleären Marker. Diese Befunde lassen sich durch das Auftreten eines „selective sweep"(=selektionsbedingte Bereinigung) der mtDNA erklären, zum Beispiel populationsgenetische Flaschenhälse oder einen rezenten Ursprung der Art. Anhand von Mikrosatelliten-Daten untersuchten wir diese Hypothesen weiter, indem wir hauptsächlich auf Flaschenhals-Ereignisse testeten. Wir fanden eine niedrige genetische Variabilität ($H_o = 0.276$ bis 0.570) bei den Dominikanermöwen. Allerdings ließ sich eine genetische Populationsstruktur zwischen Regionen (Chile, Argentinien und Brasilien) sowie zwischen Kontinenten (Südamerika und Antarktis) beobachten. Die Dominikanermöwen-Population in Südamerika könnte sich aufgrund entfernungsbedingter Isolation differenziert haben ($r = 0.7273$, $p = 0.0013$), wohingegen die antarktische Population durch nicht-physikalische Barrieren isoliert zu sein scheint. Flaschenhals-Ereignisse konnten bei sechs der 14 untersuchten Kolonien nachgewiesen werden. Diese Kolonien befinden sich an den Rändern des Verbreitungsgebietes der Dominikanermöwe und unterliegen so unwirtlichen Überlebensbedingungen. Wir glauben, dass die Dominikanermöwe auf der Südhalbkugel eine komplexe Geschichte hat, mit einem rezenten Ursprung, gefolgt von Flaschenhals-Ereignissen und anschließender Populationsexpansion. Folglich ähnelt die bei der Dominikanermöwe beobachtete genetische Diversität den Befunden bei anderen Laridenarten.

Introduction

Seabirds exhibit philopatry and monogamic behavior, thus significant genetic structure should be expected (Milot et al. 2008; Friesen et al. 2007). However, seabirds are also typically strong flyers and can disperse over long distances, thus high gene flow among colonies would be expected to reduce genetic differentiation (Bicknell et al. 2012; Morris-Pocock et al. 2010). Furthermore, physical barriers to seabird dispersal are few or nonexistent, although nonvisible barriers, such as oceanic currents, have been shown to interfere with gene flow (Birt et al. 2011; Morris-Pocock et al. 2008, 2010; Friesen et al. 2007; Congdon et al. 2000). Other evolutionary forces that act on natural populations, such as genetic drift and inbreeding associated with demographic events (bottleneck and founder events) and selective pressures, can also influence the genetic structure of a species (Frankhan et al. 2010).

Bottlenecks and founder events are not easy to differentiate because both involve a reduction in population size. Newly founded populations typically have few alleles occurring at high frequency, which, in general, had been the most abundant alleles in the ancestral population (Templeton 2011). Genetic signals of founder events or bottlenecks are not expected in species with intense gene flows, since gene flow introduces new variations into a natural population and, consequently, decreases genetic differentiation (Friesen et al. 2007; Morris-Pocock et al. 2008). Signals of bottlenecks depend on the intensity of the reduction of the population, the dispersal abilities of the organisms, and breeding behavior (Allendorf and Luikart 2007).

The Kelp Gull (*Larus dominicanus*; Laridae) is a seabird that is widely distributed in the southern hemisphere, occurring in South America, southern Africa, Australia, New Zealand, in the Subantarctic islands and the Antarctic Peninsula (Burger and Gochfeld 1996; Higgins and Davies 1996). It is the only large white-headed gull occurring in the southern hemisphere. Gulls generally have broad distributions, exhibit colonial behavior, and are long-lived, all of which make them a good model for studying evolutionary processes in seabirds (Crochet et al. 2000). The only study of genetic diversity within Kelp Gulls found little genetic variability in mitochondrial markers and relatively high genetic variation in a nuclear marker (intron Fib7) (Dantas et al. 2012).

Different outcomes between nuclear and mitochondrial markers have been observed in other organisms (Gangloff et al. 2013; Welch et al. 2011) and have been attributed to low effective population size for mtDNA (N_e) compared to nuclear DNA ($\sim 4N_e$), resulting in faster lineage sorting of mtDNA than nDNA. Thus, mtDNA allows the detection of

more recent population events (Zink and Barrouclough 2008). Discrepancies between these markers have also been explained by different mutation rates or philopatric behavior of females (Gay et al. 2004). However, in birds, females usually disperse widely (Prugnolle and de Meeus 2002), and thus low genetic structure and high genetic diversity in mtDNA compared to nDNA markers are expected.

The results of Dantas et al. (2012) contrast with what is expected: they found higher diversity in nuclear DNA than in mitochondrial DNA. The authors proposed three hypotheses to explain these results: a selective sweep on the mitochondrial DNA; a population bottleneck, which would have caused a reduction in genetic variability; and a recent origin or founder effect followed by population expansion. Dantas et al. (2012) also found the origin of Kelp Gulls based on mtDNA (around ~241,200 years ago) and the occurrence of population expansion based on nDNA (10,000 years ago) to have been relatively recent. However, neither the prior genetic bottleneck nor the selective sweep hypothesis could be excluded.

In this context, multilocus studies can reveal important aspects of the evolutionary history of the Kelp Gull. Thus, the present study proposed to: (1) examine the distribution of genetic variation among populations in South America and the Antarctic region, including colonies in Brazil, Chile, Argentina, Marion Island and King George Island; (2) estimate the population structure of this species throughout its occurrence; and (3) seek evidence of population bottlenecks or founder events. To achieve these aims, we established the following assumptions. First, if a bottleneck were responsible for low genetic diversity in mtDNA, the same signal would be recovered in multilocus nuclear markers. Second, if a recent origin (founder event), followed by population expansion were responsible for low genetic diversity in mtDNA, genetic diversity found with nuclear markers should be similar to the genetic diversity found in closely related seabirds (Charadriiformes), with new alleles in the distribution of the Kelp Gull. Finally, if selection were responsible for low genetic diversity in mtDNA, we would expect to find high genetic diversity in nuclear markers.

Methods

During the breeding seasons from 2001 to 2009, we collected blood samples from 199 Kelp Gulls from nine islands off the Brazilian coast [São Pedro ($n = 12$), Queimadinha ($n = 9$), Guararitama ($n = 25$), Laje da Conceição ($n = 6$), Moleques do Sul ($n = 34$), Deserta ($n = 14$), Itacolomis ($n = 5$), Tambores ($n = 10$) and Lobos ($n = 14$)]; two islands in Argentina [Chaffers ($n = 21$) and Quiroga ($n = 20$)]; an island in Chile [Chiloé ($n = 7$)]; an island from the Antarctic Peninsula [King George ($n = 16$)]; and a Subantarctic island [Marion ($n = 6$)] (Fig. 1).

We extracted ~0.2 mL of blood from the brachial vein of captured individuals with an insulin syringe—a technique that caused neither injury nor death. Blood was stored in 100 % ethanol at 4 °C. DNA was extracted through conventional proteinase K digestion and subsequent purification with phenol/chloroform or with ammonium acetate (Sambrook et al. 2001).

We tested 14 SSR primers developed for other species of Laridae: HG16, HG18, HG25 and HG27 (Crochet et al. 2003); RBG13, RBG18, RBG20, RBG27, RBG28 and RBG29 (Given et al. 2002); and K6, K16, K32, K67 and K71 (Tirard et al. 2002). Polymerase chain reactions (PCRs) were performed on a final volume of 10 μL containing 1× buffer, 2 mM dNTP, 2.5 mM $MgCl_2$, 0.5 U Platinum Taq polymerase, 25 ng of DNA and varying amounts (from 1 to 4 pmol) of primer. Reactions were performed in thermal cyclers, maintaining 95 °C for 5 min, 35 cycles of 95 °C to 30 s, annealing temperature for 30 s, 72 °C for 1 min and a final extension of 10 min at 72 °C.

The PCR product was checked on 2 % agarose gel and good amplifications were diluted 1:15 for primers labeled with FAM and TET, or 1:5 for primers labeled with HEX; then, 0.5 μL of this diluted product was mixed with 9 μL formamide and 0.5 μL of ROX for genotyping in a PE Applied Biosystems automatic sequencer ABI-310. Results were analyzed using the Gene Scan program (ABI) to determine allele sizes.

Data analysis

Genotyping error or null alleles were tested for each colony using the program MicroChecker (Van-Oosterhout et al. 2004). We performed tests for Hardy–Weinberg and linkage equilibrium for each locus within each colony, as well as all colonies and all loci together with GENEPOP (Raymond and Rousset 1995). Allele frequencies, allelic richness, and observed and expected heterozygosity were estimated for each colony through GeneAlex (Peakall and Smouse 2005). The HP-Rare program (Kalinowski 2005) was used to calculate allelic richness with and without normalization of samples due to the lower number of individuals genotyped for a given locus in a given colony.

The distribution of genetic variation among colonies was subjected to analysis of molecular variance (AMOVA) using ARLEQUIN v.3.1 (Excoffier et al. 2005). Analyses were performed with non-clustered colonies and, subsequently, colonies clustered according to their country or region of origin. P values for all statistics were based on 10,000 randomization replicates. In addition, F_{ST} values

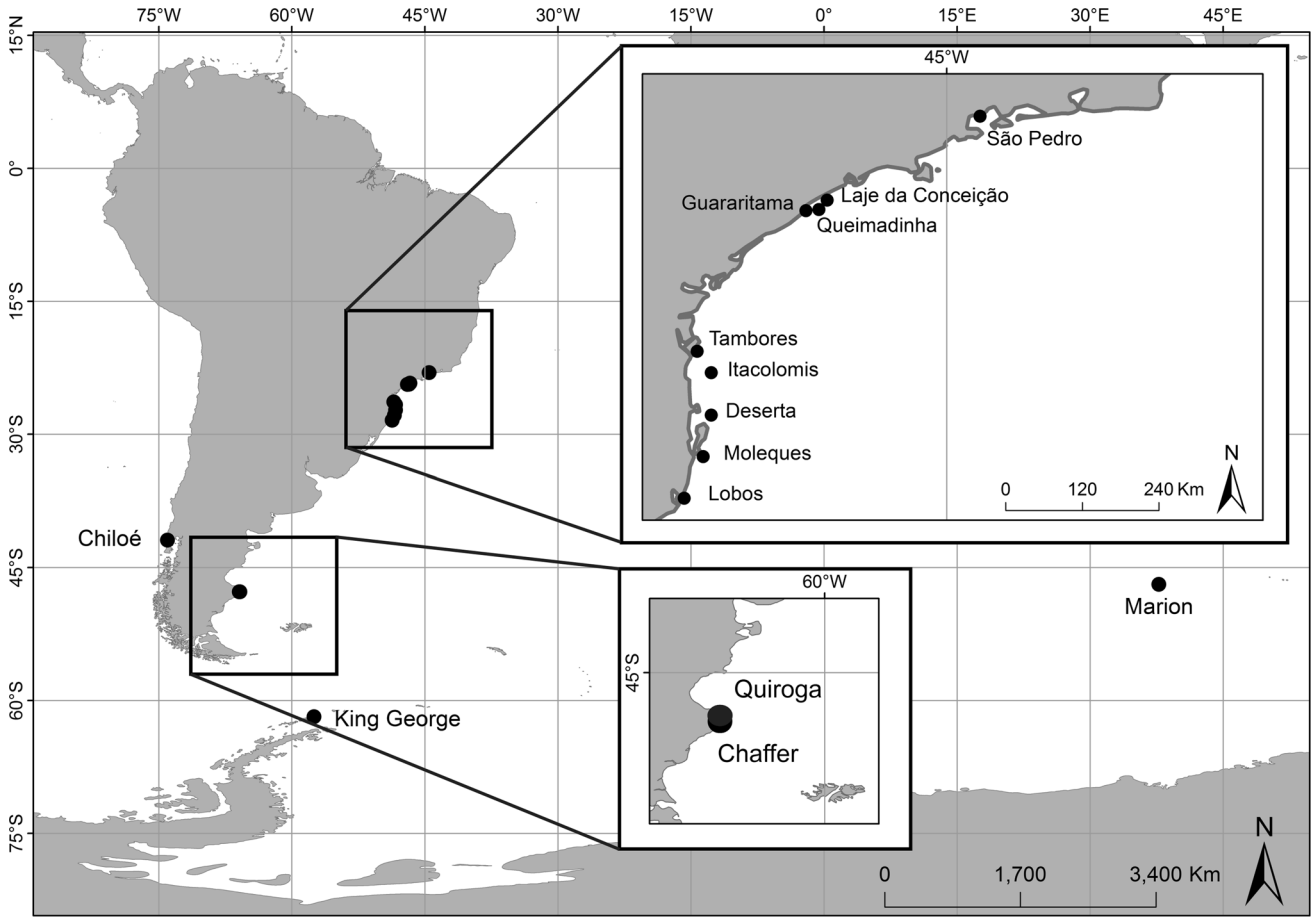


Fig. 1 Location of breeding colonies of Kelp Gulls (*Larus dominicanus*) sampled in the southern hemisphere: São Paulo (Laje Conceição, Guaraitama and Queimadinha), Santa Catarina (Tambores, Itacolomis, Deserta, Moleques and Lobos), Argentina (Isla Chaffers and Quiroga), Chile (Chiloé), Antarctic Peninsula and Marion Island

between all pairs of colonies were calculated using GeneAlex software. Although not developed for microsatellite analysis, it is less sensitive to high variance resulting from low number of individuals and loci (Raymond and Rousset 1995).

Correlations between F_{ST} and geographic distances between all pairs of colonies were tested using the Mantel nonparametric test calculator v.2.0 (Liedloff 1999) using 10,000 randomizations. Euclidean distances between colonies (islands) were calculated from geographic coordinates using the program SULCOM (http://www.sulcom.com.br), with the exception of distances between Brazil–Chile and Argentina–Chile. Since Kelp Gulls do not fly long distances over land, distances between these islands were calculated considering Isla Magdalena (52°55′S, 65°32′W) and Ushuaia (54°48′S, 68°14′W) as intermediate points along the coast from Chile to Argentina and Brazil.

To investigate population structure we used STRUCTURE v.2.3.3 (Pritchard et al. 2000) and BAPS v.5.3 (Corander et al. 2008) to cluster individuals and populations. In STRUCTURE, for each value of K (from 2 to 14), 20 runs were performed and the mean posterior probability was calculated for the data ['log probability of data', L (K)]. We determined the most probable number of populations, K, by evaluating the significance of the posterior probabilities (Pritchard et al. 2000), and by using the method described by Evanno et al. (2005). We ran the program using the admixture model and independent allele frequencies option for 10,000, 100,000 and 10,000,000 interactions after respective burn-in periods of 10,000, 100,000 and 10,000,000 interactions. In addition to K, the highest likelihoods were tested in order to verify the existence of any population structure. BAPS was performed to cluster individuals and populations, with five runs for each value of K from 2 to 14. The clusters of populations assignments from the mixture analysis of clustering of populations were then used to perform admixture analysis.

Once populations were identified through cluster analysis (STRUCTURE and BAPS), we then calculated migration rates. These rates were estimated in recent time by identifying migrants of up to the previous two

generations using the program Bayesass+ (Wilson and Rannala 2003). The strategy adopted was to compare rates of recent gene flow with observed genetic population structure, considering that recent differentiation could be masked by older panmixia of ancestral populations and population structure observed in nature may not reflect current gene flow.

Finally, each colony and each cluster were tested for genetic bottlenecks with BOTTLENECK v.1.2.02, using the TPM model, the values 1, 2, 4, 8 and 16 for sigma_m, and 2, 4, 6, 12, 20 and 30 % for p_g. We used the Wilcoxon test, which is more powerful and robust with small sample sizes (Luikart et al. 1999). Probabilities that excess heterozygosity occurred at random were calculated and plotted on a three-dimensional plot with the axes sigma_m × p_g × P (excess rate of heterozygosity). P values below 0.05 indicate no heterozygosity in excess of that by random, and indicating the possible occurrence of a recent bottleneck.

Results

Fourteen primers were tested and four microsatellites were found to be monomorphic for the Kelp Gull: HG27 ($n = 25$, n = number of individuals tested), K71 ($n = 37$), K6 ($n = 37$) and K16 ($n = 43$). In addition, microsatellite loci RBG29 and K67 were excluded from analyses for not having standard polymorphisms. Analyses performed by Microchecker showed evidence for null alleles at locus HG18 on King George Island (Antarctic Peninsula) and consequently this marker was also excluded from analysis. The seven remaining markers showed no evidence of the presence of null alleles, genotyping errors or alleles that do not amplify stochastically in heterozygotes, so they were used in the population analysis. Moreover, there were no deviations from Hardy–Weinberg and linkage equilibria.

Allelic richness (Ar) observed among colonies was around 2.0 (range 1.97–2.32), while observed heterozygosity varied between 0.276 and 0.570. The higher values of observed heterozygosity were observed in the Quiroga and Chaffers colonies (Table 1) and the lower values were observed in Marion Island. Allele frequencies were very similar among colonies (Fig. 2), which also reflects structure as detected using AMOVA when no clusters were considered, with 91.23 % ($p < 0.001$) of the variation being observed within populations and only 8.77 % ($p < 0.001$) among populations (Table 2). However, when samples sites were divided into five groups (Brazil, Chile, Argentina, Antarctic and Subantarctic), the AMOVA found 12.03 % of the variation to occur among groups, 1.31 % among populations and 86.66 % within populations (Table 2).

STRUCTURE results indicated that the number of populations with higher likelihood was $K = 2$, and BAPS indicated the number of populations with higher likelihood

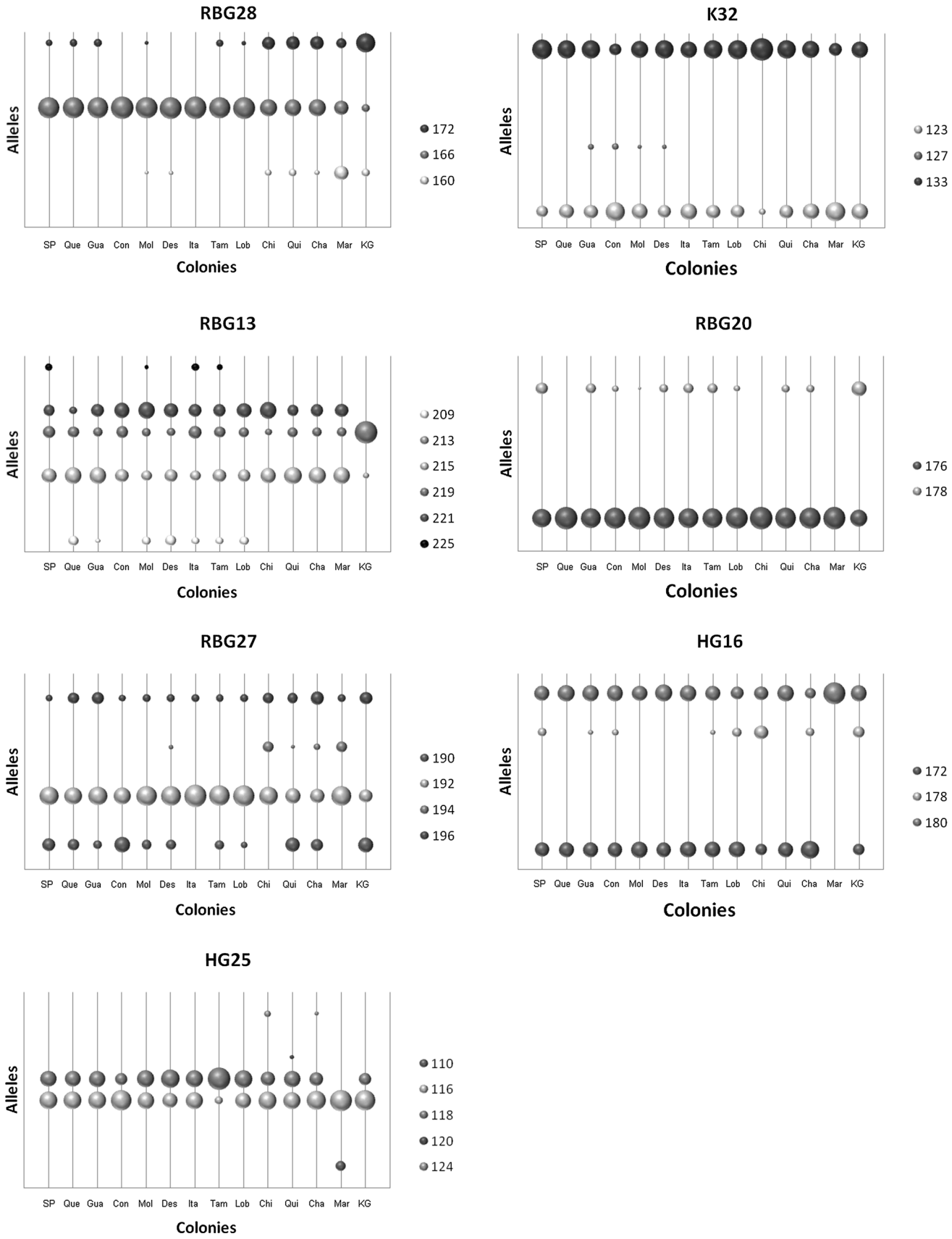
Table 1 Sample sizes (n) and genetic parameters for microsatellite markers obtained from Kelp Gulls (*Larus dominicanus*)

Population	Region	n	A (A_r)	H_o	H_e	Sensu (breeding pairs)
SP	RJ-BRA	11.29	2.57 (2.19)	0.489	0.463	–
Que	SP-BRA	9	2.29 (2.05)	0.460	0.416	–
Gua	SP-BRA	23.57	2.71 (2.14)	0.478	0.465	100 (Campos et al. 2004)
Con	SP-BRA	6	2.43 (2.10)	0.429	0.401	–
Mol	SC-BRA	33.29	2.86 (1.97)	0.396	0.395	177 (Branco 2003)
Des	SC-BRA	13.57	2.71 (2.10)	0.453	0.424	497 (Branco 2003)
Ita	SC-BRA	4.71	2.29 (2.07)	0.390	0.394	76 (Branco 2003)
Tam	SC-BRA	9.71	2.71 (2.13)	0.441	0.407	280 (Branco 2003)
Lob	RS-BRA	13.71	2.57 (2.04)	0.378	0.394	680 (Branco 2003)
Chi	CHI	5.86	2.57 (2.21)	0.471	0.429	50–2000 (Simeone et al. 2003)
Qui	ARG	17.86	2.86 (2.22)	0.555	0.500	400 (Yorio et al. 1998)
Cha	ARG	18	3.00 (2.32)	0.570	0.508	3270 (Yorio et al. 1998)
Mar	SUBANT	5.29	2.14 (1.97)	0.276	0.344	200 (Williams et al. 1979)
KG	ANT	15.43	2.43 (2.12)	0.488	0.457	47–144 (Lsinski 1993; Sander et al. 2006)

Values of n reflect the average sample size over the panel of seven loci used for analyses

A and A_r represent allelic richness and rarefied estimates of allelic richness. H_o and H_e are observed and expected heterozygosities, respectively

SP São Pedro (Brazil); *Que* Queimadinha (Brazil); *Gua* Guararitama (Brazil); *Con* Conceição (Brazil); *Mol* Moleques do Sul (Brazil); *Des* Deserta (Brazil); *Ita*Itacolomis (Brazil); *Tam* Tambores (Brazil); *Lob* Lobos (Brazil); *Chi* Chiloe (Argentina); *Qui* Quiroga (Argentina); *Cha* Chaffers (Chile); *Mar* Marion (Subantarctic); *KG* King George (Antarctic). *RJ* refers to colonies from the state of Rio de Janeiro, *SP* for colonies from the state of São Paulo, *SC* for colonies from the state of Santa Catarina and *RS* for colonies from the state of Rio Grande do Sul, all of Brazil



◀**Fig. 2** Distribution of allele frequencies at Kelp Gull colonies. *Colour of the circles* represents each allele and its size represents the frequency in the population. The colonies are represented by *numbers*: 1-São Pedro, 2-Queimadinha, 3-Guararitama, 4-Laje da Conceição, 5-Moleques do Sul, 6-Deserta, 7-Itacolomis, 8-Tambores, 9-Lobos, 10-Chiloé, 11-Quiroga, 12-Chaffers, 13-Marion, 14-King George

was $K = 12$, but results of both analyses were not consistent with the geographic distribution of sampling. On the other hand, when we performed the BAPS analysis considering the colony of origin for the formation of clusters, the results were informative and consistent with AMOVA, revealing four groups with genetic homogeneity: (1) Brazilian colonies; (2) Argentinian and Chilean colonies; (3) Marion Island; and (4) Antarctic Island (Fig. 3).

Pairwise F_{ST} values (Table 3) showed significant genetic differentiation of Marion and King George islands from all the other colonies. Colonies from the Argentinian coast showed no differentiation among themselves or in relation to a few colonies on the Brazilian coast. The Chilean colony showed differentiation from almost all colonies with the exceptions of Guararitama and Queimadinha (Brazil), and Quiroga (Argentina). Among the Brazilian colonies, only a few pairwise comparisons showed significant differentiation (Table 3).

The Mantel test revealed a significant positive correlation between genetic and geographic distances ($r = 0.7273$, $g = 3.2787$, $P < 0.0013$; Fig. 4). Despite this correlation, the highest pairwise F_{ST} values were observed between Brazilian colonies and King George (Fig. 4), but the greatest geographical distances occurred between Brazilian colonies and Marion Island. In addition, estimated migration revealed low rates among all groups, Brazil, Chile, Argentina, Subantarctic and Antarctic (Table 4).

Evidence for population bottlenecks was found for three colonies on the Brazilian coast (São Pedro, Queimadinha and Guararitama) (Fig. Sa–c), two colonies on the Argentinian coast (Fig. Sl, m) and an Antarctic colony (Fig. Sn). Except for Quiroga (Argentina), all colonies showed a bottleneck signature with significant P values for any combinations of sigma_m and p_g, indicating that the signature was not a bottleneck bias due to erroneous estimates of these parameters. Another colony from Brazil that showed a possible bottleneck signature was Itacolomis (Fig. Sh); however, the results were not uniform in this colony and a significant bottleneck signature was obtained only when low and intermediate sigma_m values were combined with high and intermediate p_g. So, although the colony of Itacolomis may have experienced a bottleneck, the results may also be due to the small sample size ($n = 5$).

Table 2 Molecular variance analysis (AMOVA) from seven microsatellites of Kelp Gull populations from an analysis without groups and another analysis with groups (Brazil, Chile, Argentina and Antarctic)

	Number of groups	Variance components	% Variation	*P* value
Without groups	1	Among populations	8.77	<0.0001
		Within population	91.23	<0.0001
With groups	5	Among groups	12.03	<0.0001
		Among populations	1.31	0.0101
		Within population	86.66	<0.0001

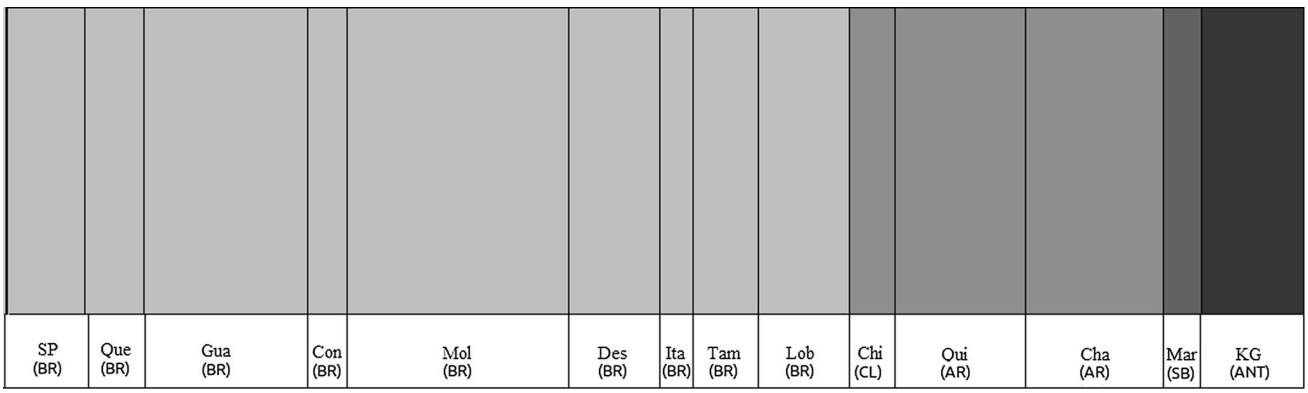


Fig. 3 Posterior probability of assignment of Kelp Gulls (*vertical lines*) to four genetic clusters based on Bayesian analysis of variation at seven microsatellite loci. Individuals are grouped by locality, which are indicated along the *horizontal axis*. *Light gray* Genetic Cluster 1: Brazilian group; *medium gray* Genetic Cluster 2: Chilean and Argentinian group; *dark gray* Genetic Cluster 3: Subantarctic group; *very dark gray* Genetic Cluster 4: Antarctic group

Table 3 Pairwise F_{ST} values among sampled colonies of Kelp Gulls

Population	Que	Gua	Com	Mol	Des	Ita	Tam	Lob	Chi	Qui	Cha	Mar	KG
SP	0.000	0.000	0.038	0.044*	0.001	0.000	0.027	0.010	0.041	0.020	0.045	0.194**	0.219**
Que	–	0.000	0.019	0.023	0.000	0.000	0.045*	0.017	0.059	0.001	0.025	0.155**	0.248**
Gua		–	0.031	0.027*	0.000	0.000	0.033	0.010	0.045	0.018	0.031*	0.157**	0.246**
Com			–	0.034	0.051	0.007	0.130*	0.073*	0.137**	0.060	0.044	0.136**	0.250**
Mol				–	0.000	0.000	0.045*	0.000	0.097**	0.082**	0.087**	0.207**	0.326**
Des					–	0.000	0.000	0.000	0.072**	0.049**	0.084**	0.203**	0.298**
Ita						–	0.000	0.000	0.087*	0.060*	0.065*	0.176**	0.260**
TAM							–	0.008	0.113**	0.069**	0.108**	0.289**	0.302**
Lob								–	0.050*	0.076**	0.077**	0.244**	0.313**
Chi									–	0.041	0.047*	0.173**	0.248**
Qui										–	0.008	0.130**	0.176**
Cha											–	0.162**	0.183**
Mar												–	0.267**

SP São Pedro, *Que* Queimadinha, *Gua* Guararitama, *Con* Conceição, *Mol* Moleques do Sul, *Des* Deserta, *Ita* Itacolomis, *Tam* Tambores, *Lob* Lobos, *Chi* Chiloé, *Qui* Quiroga, *Cha* Chaffers, *Mar* Marion, *KG* King George

* $p < 0.05$; ** $p < 0.01$

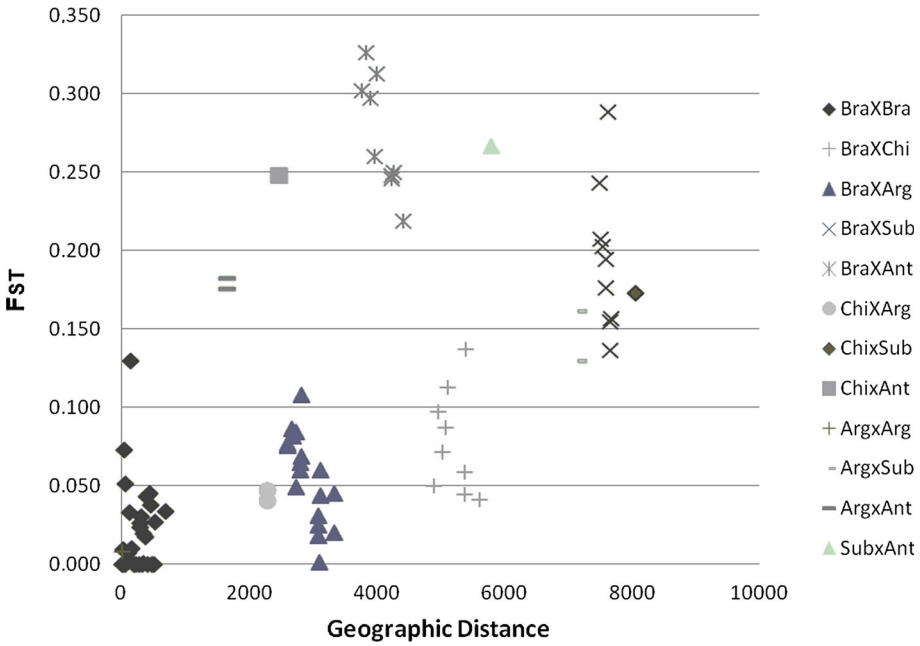


Fig. 4 Correlation between geographic distance and F_{ST} values for pairwise comparisons among Kelp Gull colonies. *Symbols* represent a pairwise colony combination according to country or region origins: *Bra* Brazil, *Arg* Argentina, *Chi* Chile, *Ant* Antarctic, *Sub* Subantarctic

Table 4 Estimates of migration rates of Kelp Gulls (proportion of individuals) among regions, derived by BayesAss+

	Destination				
	Brazil	Chile	Argentina	Subantarctic	Antarctic
Source					
Brazil		0.1125[a]	0.1188[a]	0.0438[a]	0.0086
Chile	0.0014		0.0094	0.0233	0.0061
Argentina	0.0026	0.1024[a]		0.0209	0.0085
Subantarctic	0.0012	0.0378[a]	0.0114		0.0077
Antarctic	0.0014	0.0305[a]	0.0088	0.024	

[a] Values with insufficient data

Discussion

Kelp Gulls showed genetic differentiation among regions (Antarctic, Chile, Argentina and Brazil). However, populations within each region showed low differentiation as is expected in panmictic populations. Similar results have been found in other seabird studies that showed differentiation among oceans (e.g., Patagonian Shag *Phalacrocorax magellanicus*, Calderon et al. 2013; boobies, *Sula dactylactra, S. sula* and *S. leucogaster*, Steeves et al. 2003; Morris-Pocock et al. 2010). These patterns could be related to the high dispersal ability of seabirds, which do not have physical barriers to their movements. Gene flow is an important homogenizing force for populations, which would be revealed by low population genetic structure displayed by neutral markers (Allendorf and Luikart 2007). However, within the same ocean basin, studies have demonstrated low structure. Faria et al. (2010) observed differentiation between Argentinian and Brazilian colonies of South American Terns (*Sterna hirundinacea)*, but no differentiation among Brazilian colonies. Bouzat et al. (2009) found significant differentiation among southern and northern populations of Magellanic Penguins (*Spheniscus magellanicus*) along the Atlantic coast of Argentina. In previous studies with Kelp Gulls, Dantas et al. (2012) found no genetic structure along the Brazilian coast.

There are few data about migration distances by Kelp Gulls, but the subspecies *L. d. vetula* travelled ~935 km from natal sites along the South African coast (Whittington et al. 2009). Thus, low genetic differentiation is expected at a small scale due to the dispersal abilities of Kelp Gulls.

In this context, we believe that Kelp Gulls in South America show isolation by distance (stepping-stone model) ($r = 0.7273$) (Fig. 4). In contrast, the Antarctic colony (King George) is the most different from all South America colonies, even more so than the Subantarctic colony (Marion Island), which is further from the South America colonies. One possible explanation is that the Antarctic current could be a barrier to dispersal of individuals between the continents of South America and Antarctic. Seabirds use wind for efficient foraging (Spear and Ainley 2008), so strong winds that form around the Antarctic continent could act as a barrier to birds, and this may explain the distinction between the subspecies from the Antarctic Peninsula *L. d. austrinus,* and South America, *L. d. docminicanus* (Jiguet et al. 2012).

Despite differentiation of populations among regions, low genetic diversity was observed throughout the Kelp Gull's distribution (allelic richness $A = 2.00$; Fig. 2). Low genetic variability is consistent with a previous study of this species (Dantas et al. 2012) which examined mitochondrial markers CytB and ATPase 6 and 8. In addition, low allelic richness has also been observed for other species of Chadriiformes, such as the Yellow-legged Gull (*Larus michaelis lusitanus*; Arizaga et al. 2006), Western Gull (*Larus occidentalis*; Pickes 2008), Herring Gull (*Larus argentatus*; Vigfúsdórttir et al. 2008); Glaucous Gull (*Larus hyperboreus*; Vigfúsdórttir et al. 2008), Least Tern (*Sternula antillarum*; Draheim et al. 2010) and Common Tern (*Sterna hirundo*; Sruoga et al. 2006). Large white-headed gulls have been shown to have recently diverged and radiated throughout the northern hemisphere giving origin to the Kelp Gull, a unique species of the group in South America (Liebers et al. 2004; Pons et al. 2005). The low genetic diversity observed in Kelp Gulls may be due to this recent origin and retained ancestral polymorphisms.

Another explanation for the low genetic diversity found in Kelp Gull could be bottleneck events. A bottleneck signature was observed at the northern limit of the species' distribution in the southern part of the Atlantic Ocean (São Pedro, Guararitama, Queimadinha islands), at its southern limit of distribution in the Antarctic (King George), and in Argentina (Chaffers and Quiroga islands). These bottleneck signatures seem to be local and related to the specific demographic histories of each colony. The colonies in northern part of the distribution in Brazil and in the Antarctic region are located at the limit of the Kelp Gulĺs distribution where conditions for survival are known to be harsher and population size fluctuates greatly (high instability) Austin (2007), which may explain bottleneck signatures.

The last glaciation may have also led to bottlenecks due to changes in sea level during glacial and interglacial periods. In this context, it is probable that colonies close to continents were more impacted than more distant colonies, as was observed in Argentina with regard to colonies on Chaffers and Quiroga islands located in the Ria Deseado (Isla et al. 2004). Rias were formed by the flooding of river valleys by a rising sea level during interglacial periods. Thus, Chaffers and Quiroga islands must have experienced great changes as a result of the retraction of the sea during glacial periods. Other studies have demonstrated that glacial periods have affected the distribution of seabirds (Birt et al. 2011; Calderón et al. 2013; Peña et al. 2014). For example, Sternkopf et al. (2010), Vigfíssdóttir et al. (2008) and Liebers et al. (2004) showed evidence that the Herring Gull complex from the northern hemisphere was isolated into two distinct refuges during a glacial period. Recent studies with the Patagonian Shag demonstrated that glaciation has dramatically affected its distribution and migration (Calderón et al. 2013).

Despite bottlenecks being a possible explanation for low genetic diversity found in Kelp Gulls, exclusive alleles in

low frequency due to high mutation rates of microsatellite markers were expected to be found throughout their distribution, but this was not the case (Luikart et al. 1999). Kelp Gulls showed low genetic diversity in microsatellites similar to that found among other seabirds, suggesting the retention of ancestral polymorphisms. No exclusive alleles were observed throughout the distribution of the Kelp Gull. Thus, the genetic variation of the Kelp Gull cannot be explained by a single cause, but is probably due to their recent origin followed by population expansion and bottleneck events resulting in the loss genetic diversity in the mitochondrial DNA and microsatellites of Kelp Gulls.

In summary, microsatellite and mitochondrial data show that the Kelp Gull has low genetic diversity throughout its entire distribution (Dantas et al. 2012). Despite this low genetic diversity, we were able to identify genetic structure between Pacific and Atlantic coastal populations, and among populations of South America and the Antarctic region. The differentiation in South America is probably due to isolation by distance (stepping-stone model), with high gene flow between closer colonies and low gene flow between distant colonies (Tables 3, 4). Breeding colonies in the Antarctic region seem isolated from the South America colonies probably because of the Antarctic current and the intense cyclones in this region. We believe that the Kelp Gull had a complex history in South America, with a recent origin (around 240,000 years ago; Dantas et al. 2012), followed by bottlenecks and population expansion, probably through glacial periods.

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References

Allendorf WA, Luikart GH (2007) Conservation and the genetics of populations. Blackwell, Malden

Arizaga J, Alda F, Doadrio F, Herrero A (2006) Análisis de la dispersión de la gaviota patiamarilla (*Larus michahellis lusitanius*) en Gipuzkoa. Relatorio de la Sociedad de Ciencias Aranzadi, Madrid

Austin M (2007) Species distribution models and ecological theory: a critical assessment and some possible new approaches. Ecol Model 200:1–19

Bicknell AWJ, Knight ME, Bilton D, Reid JB, Burke T, Votier SC (2012) Population genetic structure and long-distance dispersal among seabird populations: implications for colony persistence. Mol Ecol 21:2863–2876

Birt TP, Mackinnon D, Piatt JF, Friesen VL (2011) Genetic differentiation of the Kittlitźs Murrelet Brachyramphus brevirostris in the Aleurian Island and Gulf of Alaska. Mar Ornithol 39:45–51

Bouzat JL, Walker BG, Boersma PD (2009) Regional genetic structure in the Magellenic Penguin (*Spheniscus magellanicus*) suggests metatpopulatn dynamics. Auk 126:326–334

Branco JO (2003) Reprodução das aves marinhas nas ilhas costeiras de Santa Catarina. Rev Bras Zool 20:619–623

Burger J, Gochfeld M (1996) Laridae. In: del Hoyo JA, Elliott SJ (eds) Handbook of the birds of the world, vol 3. Lynx, Barcelona

Calderón L, Quintana F, Cabanne GS, Lougheed SC, Tubaro PL (2013) Phylogeography and genetic structure of two Patagonian shag species (Aves: phalacrocoracidae). Mol Phylogenet Evol 72:42–53

Campos P, Paludo D, Faria PJ, Martuscelli P (2004) Aves insulares marinhas, residents e migratórias, do litoral do Estado de São Paulo. In Branco JO (ed) Aves marinhas insulares brasileiras: bioecologia e conservação. Editora da Univali, Itajaí, Brazil, pp 57–82

Congdon BC, Piatt JF, Martin K, Friesen VL (2000) Mechanisms of population differentiation in marbled murrelets: historical versus contemporary processes. Evolution 54:974–986

Corander J, Marttinen P, Sirén J, Tang J (2008) Enhanced Bayesian modeling in BAPS software for learning genetic structures of populations. BMC Bioinform 9:539

Crochet PA, Bonhonne F, Lebreton JD (2000) Molecular phylogeny and plumage evolution in gulls (Larini). J Evol Biol 13:47–57

Crochet PA, Chen JJZ, Pons JM (2003) Genetic differentiation at nuclear and mitochondrial loci among large white-headed gulls: sex-biased interspecific gene flow? Evolution 57:2865–2878

Dantas GPM, Meyer D, Godinho R, Ferrand N, Morgante JS (2012) Genetic variability in mitochondrial and nuclear genes of *Larus dominicanus* (Charadriiformes, Laridae) from the Brazilian coast. Genet Mol Biol 35:874–885

Draheim HM, Miller MP, Baird P, Haig SM (2010) Subspecific statusand population genetic structure of least terns (*Sternula antillarum*) inferred by mitochondrial DNA control-region sequences and microsatellite DNA. Auk 127:807–819

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Laval G, Schneider S (2005) Arlequim (version 3.0): an integrated software package for population genetics data analysis. J Evol Bioinform 1:47–50

Faria PJ, Campos FP, Branco JO, Musso CM, Morgante JS, Bruford MW (2010) Population structure in the South American tern *Sterna hirundinacea* in the South Atlantic: two populations with distinct breeding phenologies. J Avian Biol 41:378–387

Frankhan R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetic. Cambridge University Press, Cambridge

Friesen VL, Burg TM, McCoy KD (2007) Mechanisms of population differentiation in seabirds. Mol Ecol 16:1765–1785

Gangloff B, Zino F, Shirihai H, Gonzalez-Solis J, Couloux A, Pasquet E, Bretagnolle V (2013) The evolution of north-east Atlantic gadfly petrels using statistical phylogeography. Mol Ecol 22:495–507

Gay L, Du Defos Rau P, Mondain-Monval J-Y, Crochet P-A (2004) Phylogeography of a game species: the red-crested pochard (*Netta rufina*) and consequences for its management. Mol Ecol 13:1035–1045

Given AD, Mills JA, Baker AJ (2002) Isolation of polymorphic microsatellite loci from the red-billed gull (*Larus novaehollandiae scopulinus*) and amplification in related species. Mol Ecol 2:416–418

Higgins PJ, Davies SJJF (1996) Handbook of Australian, New Zealand and Antarctic birds. In: Snipe to pigeons, vol 3. Oxford University Press, Melbourne

Isla F, Iantanos N, Estrada E (2004) Subtidal dynamics and environmental conditions of the Río Deseado estuary, Santa Cruz. Rev de la Asoc Geol da Argent 59:367–375

Jiguet F, Capainolo P, Tennyson A (2012) Taxonomy of the kelp gull, Larus dominincanus, Lichtenstein revisited with sex-separared analyses of biometrics and wing tip patterns. Zool Stud 51:881–892

Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189

Liebers D, Knijff P, Helbig AJ (2004) The herring gull complex is not a ring species. Proc R Soc Lond B 271:893–901

Liedloff AC (1999) Mantel nonparametric test calculator. Version 2.0. School of Natural Resource Sciences, Queensland University of Technology, Australia

Lsinski G (1993) Monitoring of birds and pinnipedians on King George Island (South Shetland Islands) in 1989/90. Pol Polar Res 14:75–89

Luikart G, Cornuet JM, Alendorf FW (1999) Temporal changes in allele frequencies provide estimates of population bottleneck size. Conserv Biol 13:523–530

Milot E, Weimerskirch H, Bernatchez L (2008) The seabird paradox: dispersal, genetic structure and population dynamics in a highly mobile, but philopatric albatross species. Mol Ecol 17:1658–1673

Morris-Pocock JA, Taylor SA, Birt TP, Damus M, Piatt JF, Warheit KI, Friesen VL (2008) Population genetic structure in Atlantic and Pacific Ocean common murres (*Uria aalge*): natural replicate tests of post-Pleistocene evolution. Mol Ecol 17:4859–4873

Morris-Pocock JA, Taylor SA, Birt TP, Friesen VL (2010) Concerted evolution of duplicated mitochondrial control regions in three related seabird species. BMC Evol Biol 10:14

Peakall R, Smouse P (2005) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Peña F, Poulin E, Dantas GPM, Gonzalez-Acuña D, Petry MV, Vianna JA (2014) Have historical climate changes affected Gentoo penguin (*Pygoscelis papua*) populations in Antarctica? PLoS ONE 9(4):e95375. doi:10.1371/journal.pone.0095375

Pickes C (2008) The effects of ocean climate variability on Western Gull (*Larus occidentalis*) ecology and population genetics. Pro Quest Dissertations and Theses

Pons J-M, Hassanin A, Crochet P-A (2005) Phylogenetic relationships within the Laridae (Charadriiformes: aves) inferred from mitochondrial markers. Mol Phylogenet Evol 37:686–699

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. Heredity 88:161–165

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283

Sambrook KJ, Russel DW, Sambrook J (2001) Molecular cloning: a laboratory mannual. CSHI, New York

Sander M, Carneiro APB, Mascarello NE, dos Santos CR, Costa ES, Balbão TC (2006) Distribution and status of the Kelp Gull, Larus dominicanus Lichtenstein (1823), at Admiralty Bay, King George Island, South Shetland, Antarctica. Polar Biol 29:902–904

Simeone A, Luna-Jorquera G, Bernal M, Garthe S, Sepúlveda F, Villablanca R, Ellenberg U, Contreras M, Muñoz J, Ponce T (2003) Breeding distribution and abundance of seabirds on islands off north-central Chile. Rev Chil Hist Nat 76:323–333

Spear LB, Ainley DG (2008) Flight behaviour of seabirds in relation to wind direction and wing morphology. Int J Avian Sci 139:221–233

Sruoga A, Butkauskas D, Prakas P, Paulauskas A (2006) Evaluation of the genetic structure of the breeding Common Tern (*Sterna hirundo*) population by means of microsatellite markers. Biologija 1:47–52

Steeves TE, Anderson DJ, McNally H, Kim MH, Friesen VL (2003) Phylogeography of Sula: the role of physical barriers to gene flow in the diversification of tropical seabirds. J Avian Biol 34:217–223

Sternkopf V, Liebers-Helbig D, Ritz MS, Zhang J, Helbig AJ, de Knijff P (2010) Introgressive hybridization and the evolutionary history of the herring gull complex revealed by mitochondrial and nuclear DNA. BMC Evol Biol 10:348

Templeton AR (2011) Population genetics and microevolutive theory. Sociedade Brasileira de Genética, Ribeirão Preto

Tirard C, Helfenstein F, Danchin E (2002) Polymorphic microsatellite in the Black-legged Kittiwake *Rissa tridactyla*. Mol Ecol Notes 2:431–433

Van-Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol 4:535–538

Vigfíssdóttir F, Pálsson S, Ingólfsson A (2008) Hybridization of glaucous gull (*Larus hyperboreus*) and herring gull (*Larus argentatus*) in Iceland: mitochondrial and microsatellite data. Philos Trans R Soc Lond B 363:2851–2860

Welch AJ, Yoshida AA, Fleischer RC (2011) Mitochondrial and nuclear DNA sequences reveal recent divergence in morphologically indistinguishable petrels. Mol Ecol 20:1364–1377

Whittington PA, Martin AP, Klages NTW, Schultz A (2009) Movements of the kelp gull Larus dominicanus vetula to, from and within Southern South Africa. Mar Ornithol 37:139–152

Williams AJ, Siegfried WR, Burger AE, Berruti A (1979) The Prince Edward Islands: a sanctuary for seabirds in the southern ocean. BioI Conserv 15:59–71

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191

Yorio P, Bertellotti, M, Gandini P, Frere E (1998) Kelp gulls Larus dominicanus breeding on the Argentine coast: population status and relationship with coastal management and conservation. Mar Ornithol 26:11–18

Zink RM, Barrouclough GF (2008) Mitochondrial DNA under siege in avian phylogeography. Mol Ecol 17:2107–2121