ORNITOLOGIA NEOTROPICAL 26, 349–362, 2015

GENETICS AND DEMOGRAPHY OF KELP GULLS IN PATAGONIA

Amanda C. Lyons[1], Nora Lisnizer[2], Pablo Garcia-Borboroglu[2,3], Pablo Yorio[2,4], & Juan L. Bouzat[1,5]

[1]Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA.

[2]Centro Nacional Patagónico, CENPAT-CONICET, Puerto Madryn, Chubut U9120ACV, Argentina.

[3]Department of Biology, 24 Kincaid Hall, Box 351800, University of Washington, Seattle, WA 98195-1800, USA.

[4]Wildlife Conservation Society, Amenábar 1595, P 2, Office 19, C1426AKC, Buenos Aires, Argentina.

[5]Corresponding author. *E-mail*: jbouzat@bgsu.edu

Abstract. – The Kelp Gull (*Larus dominicanus*), a common seabird in coastal Patagonia, has recently increased in overall abundance and distribution, creating some concerns for the protection and conservation of other native, threatened species. We combined genetic and demographic data of four large Kelp Gull colonies distributed along 1800 km of the northern Patagonian coast of Argentina to further understand patterns of population growth and migration of the species. DNA analysis of variable intron sequences of two separate genes (myelin proteolipid protein, β-fibrinogen) revealed similar intra-colony levels of DNA sequence diversity. Pairwise F_{ST} comparisons revealed significant differentiation of the northernmost colony, Islote La Pastosa, from the two southern colonies, Punta Tombo and Isla Vernaci Sudoeste ($p < 0.05$). Indirect estimates of gene flow suggest significant mixing among colonies ($Nm > 6$). Demographic estimates revealed that Islote La Pastosa showed an increase in the number of breeding individuals over time ($\lambda = 1.075$), representing an area of potential population expansion. This is consistent with previous studies that have suggested that the demographic connectivity among Kelp Gull colonies in Patagonia may follow a source-sink dynamics, where growth rates of growing colonies are fueled by the immigration of individuals from nearby colonies. The observed pattern also reflects the overall expansion of the species in the Patagonian region. This study suggests that proper management strategies for the Kelp Gull should take into account the genetic and demographic dynamics of this species.

Resúmen. – La Genética y demografía de la Gaviota Cocinera en Patagonia. – La Gaviota Cocinera (*Larus dominicanus*), un ave marina de las costas de Patagonia, ha incrementado su abundancia y distribución, creando problemas para la protección y conservación de otras especies nativas. En éste trabajo combinamos datos genéticos y demográficos de cuatro colonias de gaviotas cocineras distribuidas a lo largo de 1800 km de la costa norte de la Patagonia argentina para mejorar el conocimiento sobre los patrones de crecimiento poblacional y migración de la especie. Análisis de secuencias de ADN de intrones de dos genes (el gen de la Proteína Proteolipídica de la Mielina y el gen del β-fibrinógeno) revelaron niveles similares de variabilidad genética intra-colonial. Comparaciones apareadas del F_{ST} entre colonias indicaron una diferenciación genética significativa entre la colonia más septentrional, Islote La Pastosa, y las colonias del sur, Punta Tombo e Isla Vernaci Sudoeste ($p < 0.05$). Estimadores indirectos del flujo génico indican un intercambio migratorio significativo entre las colonias reproductivas ($Nm > 6$).

Estimadores demográficos revelaron que Isolate La Pastosa mostró un incremento en el número de individuos reproductivos a lo largo del tiempo (λ = 1.075), representando un área potencial de expansión poblacional. Estos resultados son consistentes con estudios previos que sugieren que la conectividad demográfica entre las colonias de la gaviota cocinera en la Patagonia sigue una dinámica metapoblacional de fuente-sumidero, donde las colonias en crecimiento reciben individuos inmigrantes de colonias cercanas. Los patrones observados también reflejan la expansión general de la especie en la región. Este trabajo sugiere que las estrategias de manejo para la gaviota cocinera debieran tomar en cuenta la dinámica genética y demográfica de ésta especie.

Key words: Demography, genetic diversity, gene flow, *Larus dominicanus*, metapopulation, population growth, seabird colonies.

Handling Editor: Kaspar Delhey; **Receipt:** 21 May 2015; **First decision:** 21 July 2015; **Final acceptance:** 23 November 2015.

INTRODUCTION

Genetic studies can provide important information to complement demographic assessments and help to better understand the structuring of breeding populations in the wild. Specifically, measures of genetic diversity can help to assess the overall health and future evolutionary potential of populations and species (Frankham *et al.* 2002, Bouzat 2010). Genetic markers may also reveal evidence of genetic differentiation, genetic structuring, and levels of genetic connectivity among local populations and breeding colonies (Avise 2000). This is especially relevant in seabirds, where breeding colonies can potentially represent genetically and demographically independent units of conservation concern (e.g., Roeder *et al.* 2001, Morris-Pocock *et al.* 2012, Taylor & Friesen 2012). Although seabirds are able to migrate and disperse over long distances, several studies have shown that population differentiation can be strong (see review by Friesen *et al.* 2007). Several factors can potentially influence population genetic structure in seabirds, including physical barriers, geographic distance between colonies, colony dispersal, and non-breeding distribution (Amos & Harwood 1998, Friesen *et al.* 2007). Thus, knowledge of the degree of genetic connectivity and levels of differentiation among colonies is a key factor for the identification and implementation of species-specific management plans, (e.g., development of adequate conservation or pest-control strategies).

Among seabirds, gull species tend to be generalist and opportunistic foragers (Burger & Gochfeld 1996). This feeding strategy allows gulls to take advantage of food sources of anthropogenic origin, which in many cases have increased population growth (Blokpoel & Spaans 1991, Garthe *et al.* 2006, Oro *et al.* 2013, Tyson *et al.* 2015). In some cases, population increases have resulted in negative effects on other coastal species, promoting the development of management actions aimed at reducing gull abundances (Thomas 1972, Belant 1997, Coulson & Coulson 2009; but see Oro & Martínez-Abraín 2007). Effective management of gull populations requires broad scale approaches, so as to take into account the potential connectivity among breeding colonies (Lisnizer *et al.* 2015). Genetic data are particularly useful to explore population connectivity in widely distributed species because it is typically difficult to measure dispersal directly through banding techniques over large spatial scales (Lowe & Allendorf 2010).

The Kelp Gull (*Larus dominicanus*) is a widely distributed seabird breeding throughout the Southern Hemisphere (Jiguet *et al.* 2012). This species is the most abundant gull breeding along the Atlantic Ocean coast of Argentina, from Buenos Aires (36º20'S) to

Tierra del Fuego (54°58'S), with a population estimated to exceed 105,000 breeding pairs distributed in ~ 140 colonies (Yorio *et al.* in press). Recent demographic trends suggest that this species is rapidly increasing in terms of both the number of breeding colonies and overall population size along the coasts of northern Patagonia, Argentina (Lisnizer *et al.* 2011). Spatial and temporal variations in population trends reported by Lisnizer *et al.* (2011, 2015) revealed that breeding colonies differ significantly both in size and growth rates. Population expansion has been also reported in other regions of the Southern Hemisphere (Coulson & Coulson 1998, Whittington *et al.* 2006), including South America (Yorio *et al.* in press). The Kelp Gull population increase in Patagonia is believed to be driven by metapopulation dynamics among colonies, influenced by the availability of human refuse and fisheries waste (Yorio *et al.* 1998a, Lisnizer *et al.* 2011). The population expansion of the Kelp Gull in northern Patagonia is a matter of concern given their predation on globally threatened species [e.g., Magellanic Penguin (*Spheniscus magellanicus*), Olrog´s Gull (*Larus atlanticus*), and White-headed Steamer Duck (*Tachyeres leucocephalus*)], potential hazards to the commercial aviation industry, and threats to human health (Yorio *et al.* 1998a, 2005). As a consequence, rapid changes in Kelp Gull abundance and regional distribution have created interest in developing management policies in this region with the intention of protecting other, more vulnerable species (Sironi *et al.* 2009).

Although several studies have assessed levels of genetic differentiation among populations of seabirds with wide geographic distributions (e.g., Friesen *et al.* 2007, Bouzat *et al.* 2009, Morris-Pocock *et al.* 2012), there is relatively limited information on the genetic structuring of Kelp Gull colonies. Most genetic studies on Kelp Gulls are focused on the phylogeny and taxonomy of *Larus* spp. (e.g., Liebers *et al.* 2004, Pons *et al.* 2005), and the development of molecular markers to assess the degree of genetic variation and differentiation among Kelp Gull populations from different continents (e.g, Liebers *et al.* 2001, Dantas *et al.* 2009). Only a few studies were aimed at assessing levels of genetic variability and structuring among Kelp Gull breeding colonies on local and regional scales (Dantas *et al.* 2006, 2012).

In this study, we estimated size, growth rate, population connectivity, and levels of genetic diversity within and among four Kelp Gull breeding colonies located along 1800 km of the southern Atlantic shores of Argentina. The combination of genetic and demographic information from these colonies provides important insights into the genetic structuring of breeding colonies and the population dynamics of this species. Results from this study are discussed in the context of recent metapopulation modeling of the demographic dynamics and the overall expansion of Kelp Gulls in northern Patagonia. We suggest that proper management strategies for the Kelp Gull should take into account the demographic dynamics and levels of connectivity among breeding colonies.

METHODS

Study area. The studied Kelp Gull colonies are located along the northern Patagonian coast of Argentina (Fig. 1). These include the colonies of Islote La Pastosa (41°25'S, 65°02'W), Punta Leon (43°04'S, 64°29'W), Punta Tombo (44°02'S, 65°11'W), and Isla Vernaci (45°11'S, 66°31'W). These colonies are part of a network of 68 colonies distributed along 1770 km of Atlantic Ocean shoreline, with a total estimate of 72,600 breeding pairs (Lisnizer *et al.* 2011). This coastal area encompasses more than 65% of the Kelp Gull breeding population of Argentina. The median number of breeding pairs per colony

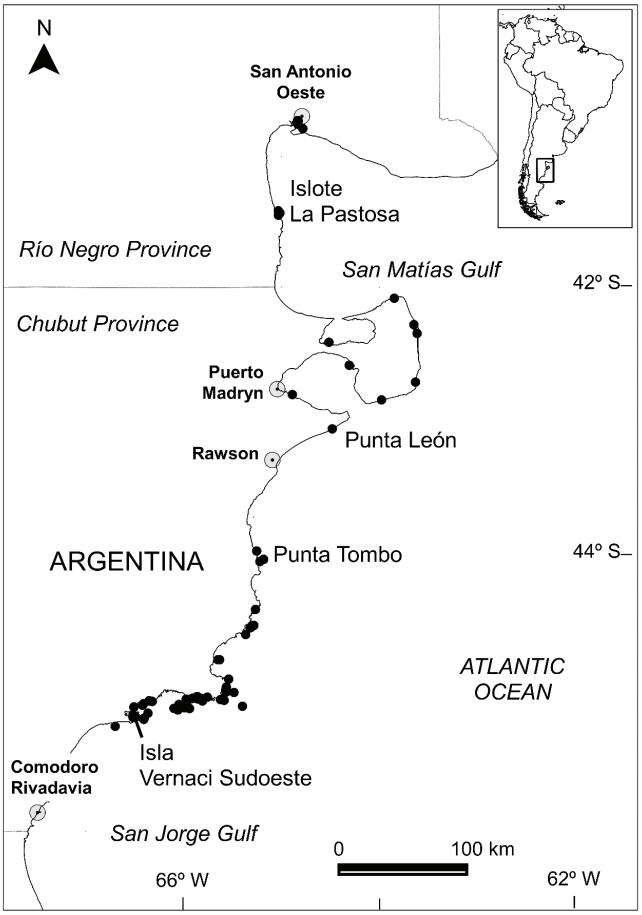

FIG. 1. Geographic distribution of Kelp Gull breeding colonies (represented by black dots) along the coasts of northern Patagonia, Argentina. The four focal colonies used in this study (Islote La Pastosa, Punta Leon, Punta Tombo, and Isla Vernaci Sudoeste) are identified. Gray circles indicate four major coastal cities in the region.

in this area is 454 (range: 1–11,300; Lisnizer *et al.* 2011).

The four focal Kelp Gull breeding colonies (Fig. 1) were selected because of their historically and consistently large population sizes (> 3000 breeding pairs) and their location in four coastal sectors where Kelp Gulls have shown variable demographic trends (Lisnizer *et al.* 2011). Selected colonies are all located within protected areas and are separated from each other by at least 140 km (Islote La Pastosa–Punta Leon = 288.5 km; Punta Leon–Punta Tombo = 146.7 km; Punta Tombo–Isla Vernaci = 249.6 km).

Sampling of breeding colonies and estimates of demographic parameters. The sizes of the colonies (i.e., number of breeding pairs) were estimated during 2006–2008 either by full nest counts or by sampling counts in circular plots

of 100 m^2, depending on the colony (Bibby *et al.* 1992). Plots were placed randomly throughout the colony, so as to capture differences in the density of nests and habitat. We considered a nest to be active when it contained an egg, chick, or signs of recent use, such as fresh nesting material. Counts were made during late incubation. Egg laying begins in early October at Islote La Pastosa and Punta León (Yorio *et al.* 1994; NL unpub. data), in early November at Punta Tombo (Bertellotti & Yorio 1999), and in mid-November at Isla Vernaci Sudoeste (Yorio & García-Borboroglu 2002).

We evaluated abundance trends from 1994 to 2008 using the counts conducted during this study and values obtained from published literature (Yorio *et al.* 1998b, Yorio *et al.* 2005). We estimated annual population growth rates for each colony by simple log-linear regression of counts against time, based on the population model $N_t = N_0 e^{rt}$, where N_t refers to the population size at time *t*, N_0 is the population size at time 0, *r* is the intrinsic rate of growth, and *t* refers to time in number of generations. The slope of the regression line corresponds to estimates of lambda (λ), the population growth rate (Caughley 1977). Confidence intervals for lambda were estimated by bootstrapping the residuals of the linear regression between population size and time (Efron & Tibshirani 1993). This method assumed a deterministic population growth model and attributed all variability in the data to errors in the estimates of population size (i.e., observation error model). We estimated levels of population connectivity indirectly as the number of neighboring colonies within a circle area with a radius of 114 km around each of the studied colonies. The diameter of the sampling area (228 km) represents the average distance between the four studied colonies. We also report the number of colonies within 50 km from the focal colonies to assess potential connectivity with neighboring colonies. Colonies located less than 2 km from each other were considered one unit (Yorio *et al.* 1998b).

Blood samples for genetic analysis were collected during the 2008 breeding season from 18–20 Kelp Gull chicks randomly selected from different nests from each target colony (77 individuals in total). Blood samples were obtained by puncture of the brachial vein and collection of five to ten blood drops onto a Whatman paper, and stored dry until further analysis.

DNA extractions and sequencing of intron markers. Total DNA was extracted from blood samples using the QIAGEN QIAamp® DNA Mini extraction kit (Valencia, CA), following the manufacturer's instructions. Quality and quantity of extracted DNA was tested by agarose gel electrophoresis. Extracted DNA was re-suspended in sterile water at a concentration of 50–100 ng/μl for PCR amplification.

For this study, two nuclear introns previously described in *Larus* species (Dantas *et al.* 2009) were selected to assess levels of genetic diversity and structuring among the four focal colonies. For the Kelp Gull, nuclear introns have found to be more variable than mitochondrial markers (Dantas *et al.* 2012), showing a significantly higher number of SNPs, and thus making them more appropriate for population-level studies. Furthermore, the mode of inheritance of nuclear introns reflects both maternal and paternal demographic patterns, in contrast to the typical maternal inheritance of mitochondrial DNA. Intron 4 of the myelin proteolipid protein (MPLPR) gene was amplified using primers located in exon 3 and 4 of the MPLPR gene (F-Primer: 5'- ACA TCT ACT TTA ACA CCT GGA CCA CCT G -3'; R-Primer: 5'- TTG CAG ATG GAG AGC AGG TGG GAG CC -3'; Primmer *et al.* 2002, Dantas *et al.* 2009), which resulted in an amplification product of 286 base-pairs (bp). In addition, a

725 bp section of the β-fibrinogen gene, including the complete intron 7, was amplified using primers located in exons 6, 7, and 8 (F-Primers: 5'- CAG GAC AAT GAC AAT TCA C -3' and 5'- TTG CAA AGA GTG GAG GGA AG -3'; R-Primer: 5'- CCA TCC ACC ACC ATC TTC TT -3'; Prychitko & Moore 1997, Hackett *et al.* 2008, Dantas *et al.* 2009). PCR conditions and amplification protocols were based upon those described previously (Prychitko & Moore 1997, Primmer *et al.* 2002, Hackett *et al.* 2008, Dantas *et al.* 2009).

PCR amplification products were directly sequenced by the University of Chicago DNA Sequencing and Genotyping Facility by cycle sequencing reactions using fluorescent dye terminators and one of the amplification primers as a sequencing primer. The DNA of each individual analyzed was subject to a minimum of two independent sequencing reactions per locus. Individual DNA with sequences indicating heterozygote genotypes (i.e., double peaks in electropherograms), in which individual alleles could not be defined, underwent subsequent amplification by PCR, cloning, and DNA sequencing using the Promega pGEM-T Easy cloning kit (Promega Corp., Madison, WI) and fluorescent cycling sequencing of individual clones. This allowed for the characterization of individual alleles and assignment of genotypes for each locus in all individuals studied.

Data analysis. DNA sequences from each marker were aligned using CLUSTAL X (Larkin *et al.* 2007) and unique haplotypes were identified and named following the format *Ldom*0## (*Larus dominicanus* [haplotype number]), with MPLPR- or βFib- as a prefix for the MPLPR and β-fibrinogen locus, respectively. For the genetic analyses, sequences were trimmed to exclude terminal DNA sections flanking intron sequences and ambiguous sequence information due to direct sequencing. The data analysis was therefore based on the partial exon 3 (69 bp) and complete intron 4 (217 bp) of the MPLPR gene and ~80% of intron 7 of the β-fibrinogen gene (725 bp).

DnaSP (Librado & Rozas 2009) was used to identify haplotypes and estimate measures of genetic diversity, including nucleotide diversity, number of nucleotide differences, and number of polymorphic sites per locus. Arlequin (Excoffier *et al.* 2005) provided estimates of genotype diversity (heterozygosity), sequence diversity (nucleotide diversity), and a hierarchical Analyses of Molecular Variance (AMOVA), clustering colonies into North (Islote La Pastosa, Punta Leon) and South (Punta Tombo, Isla Vernaci Sudoeste) groups. The AMOVA allowed partitioning of the total genetic variation into both within- and among-colony components, as well as a variation component between the defined regions. Pairwise F_{ST} comparisons were performed to assess genetic differentiation between colonies. Indirect estimates of gene flow (*Nm*) were calculated based on the inverse relationship between F_{ST} and *Nm* ($F_{ST} = 1 / 4Nm$), with *Nm* representing an estimate of the number of migrants between any two colonies per generation (Slatkin 1987).

Haplotype networks were characterized for each intron marker to assess relationship patterns among haplotypes and potential structuring of haplotypes across colonies. Minimum Spanning Networks were determined using Arlequin (Excoffier *et al.* 2005) and recreated using HapStar10 (Teacher & Griffiths 2011).

RESULTS

Demographic parameters. Colony size, estimated as the number of breeding pairs, ranged from 2935 for Islote La Pastosa to 7445 for Isla Vernaci Sudoeste (Table 1). Annual growth rates from 1994 to 2008 varied among colo-

TABLE 1. Colony size (number of breeding pairs), onset of egg laying period, population growth rate λ (with 95% confidence intervals and number of years used for growth rate estimates in parentheses), and indirect estimates of population connectivity as measured by the number of neighboring colonies within 50 and 114 km of each of the four Kelp Gull breeding colonies studied in northern Patagonia (see Methods).

Colony	Colony size	Year of census	First egg laying	Growth rate λ (95% CI; n)	Colonies within 50 km	Colonies within 114 km
Islote La Pastosa	2935	2008	October	1.075 (1.075–1.076; 3)	0	6
Punta León	5813	2007	October	0.981 (0.955–1.007; 7)	2	8
Punta Tombo	6457	2007	November	1.006 (0.989–1.022; 5)	2	23
Isla Vernaci Sudoeste	7445	2006	November	1.016 (0.990–1.043; 4)	12	28

nies, with Islote La Pastosa showing an increase in the number of breeding pairs (λ = 1.075), increasing from 1140 in 1995 to 1881 in 2002 and to 2935 in 2008. In contrast, annual growth rates estimated for the other colonies remained relatively stable, with λ values not being significantly different from 1 (Table 1). The uneven distribution of Kelp Gull breeding colonies throughout the study area indicates that the four focal colonies have different levels of population connectivity, as measured by the number of neighboring colonies (Fig. 1, Table 1). Overall, Islote La Pastosa and Punta Leon are located in regions of low colony density, having only six and eight neighboring colonies within a 114 km radius, respectively. Furthermore, Islote La Pastosa showed no neighboring colonies within the nearest 50 km. In contrast, the southern colonies Punta Tombo and Isla Vernaci Sudoeste are located in areas of high colony density, with 23 and 28 neighboring colonies within a radius of 114 km, respectively.

Genetic diversity. Direct DNA sequencing of amplification products from the MPLPR and β-fibrinogen introns of all studied individuals identified multiple haplotypes and the presence of heterozygote genotypes exhibited by the detection of two distinct amplified DNA sequences. Further cloning and sequencing of individual clones provided the complete characterization of intron diversity, which resulted in the detection of eight and 12 distinct haplotypes for the MPLPR and the β-fibrinogen introns, respectively (GenBank accession numbers: KT449802–KT449821; localities of individual birds and corresponding haplotypes are reported as Supplementary Material Online).

Intron markers showed relatively high levels of diversity both at the DNA sequence and genotype levels, with nucleotide diversity (π) estimates of 0.005 and 0.004, and observed heterozygosities (H_O) of 0.662 and 0.714 for the MPLPR and the β-fibrinogen introns, respectively (Table 2). There were no significant differences in the average levels of DNA sequence diversity between colonies ($0.055 \leq p \leq 0.816$). For both intron markers, most haplotypes were detected in multiple colonies. However, some colonies had a number of unique haplotypes (i.e., haplotypes not found in any other colony). In particular, Islote La Pastosa revealed five unique β-fibrinogen haplotypes and in Punta Tombo, two unique MPLPR haplotypes were detected.

TABLE 2. Genetic diversity estimates for the myelin proteolipid protein (MPLPR) and the β-fibrinogen gene introns in four Kelp Gull breeding colonies from northern Patagonia. N = sample size; # of haps = number of haplotypes; S = number of polymorphic sites; π = nucleotide diversity; H_O = observed heterozygosity; H_E = expected heterozygosity

Colony	N	MPLPR					β-fibrinogen				
		# of haps	S	π	H_O	H_E	# of haps	S	π	H_O	H_E
Islote La Pastosa	18	6	5	0.0049	0.389	0.537	10	14	0.0039	0.889	0.770
Punta León	19	4	5	0.0047	0.684	0.661	6	9	0.0034	0.526	0.693
Punta Tombo	20	7	6	0.0050	0.900	0.665	5	9	0.0039	0.750	0.712
Isla Vernaci Sudoeste	20	4	5	0.0044	0.650	0.596	5	9	0.0044	0.700	0.722
Mean	19.25	5.25	5.25	0.0047	0.656	0.615	6.50	10.25	0.0039	0.716	0.724
Total	77	8	6	0.0047	0.662	0.621	12	16	0.0039	0.714	0.732

Levels of heterozygosity in all colonies were relatively high (H_O > 0.389), with the highest average across loci found in Punta Tombo (H_O = 0.825). Observed and expected heterozygosities revealed that none of the markers analyzed showed significant deviations from Hardy-Weinberg expectations in any of the studied colonies (Table 2). Haplotype networks for both intron markers revealed a star-shaped pattern with no structuring of haplotypes across colonies (Fig. 2).

Genetic structuring. At regional level, AMOVA results indicated no significant genetic structuring between northern and southern colony groups (F_{ST} = 0.027; p = 0.333), with most variation (98%) being explained by differences between individuals. Variation among populations within groups was also not significant (p = 0.610).

Pairwise F_{ST} between colonies revealed, however, significant genetic differentiation (p < 0.05) between the northernmost colony Islote La Pastosa and the colonies of Punta Tombo and Isla Vernaci Sudoeste in the south (F_{ST} = 0.040 and 0.031, respectively; Table 3). Indirect estimates of gene flow (*Nm*) based upon pairwise F_{ST} values ranged from six to 110 individuals per generation (Table 3), with an average *Nm* estimate of 35 migrants per generation across colonies.

DISCUSSION

Estimates of genetic diversity within and among Kelp Gull breeding colonies indicated relatively high levels of genetic variation at the two nuclear intron markers studied, and limited genetic structuring across 1400 km of Atlantic Ocean shoreline in northern Patagonia. Both northern and southern colony groups and colonies within groups were not significantly different, suggesting considerable genetic mixing among breeding colonies. This is consistent with a demographic study conducted in northern Patagonia indicating that the Kelp Gull exhibits metapopulation dynamics, with significant transfer of individuals among breeding colonies (Lisnizer *et al.* 2015). Similar demographic and genetic patterns have been reported for Kelp Gull colonies from southern Brazil (Dantas *et al.* 2012),

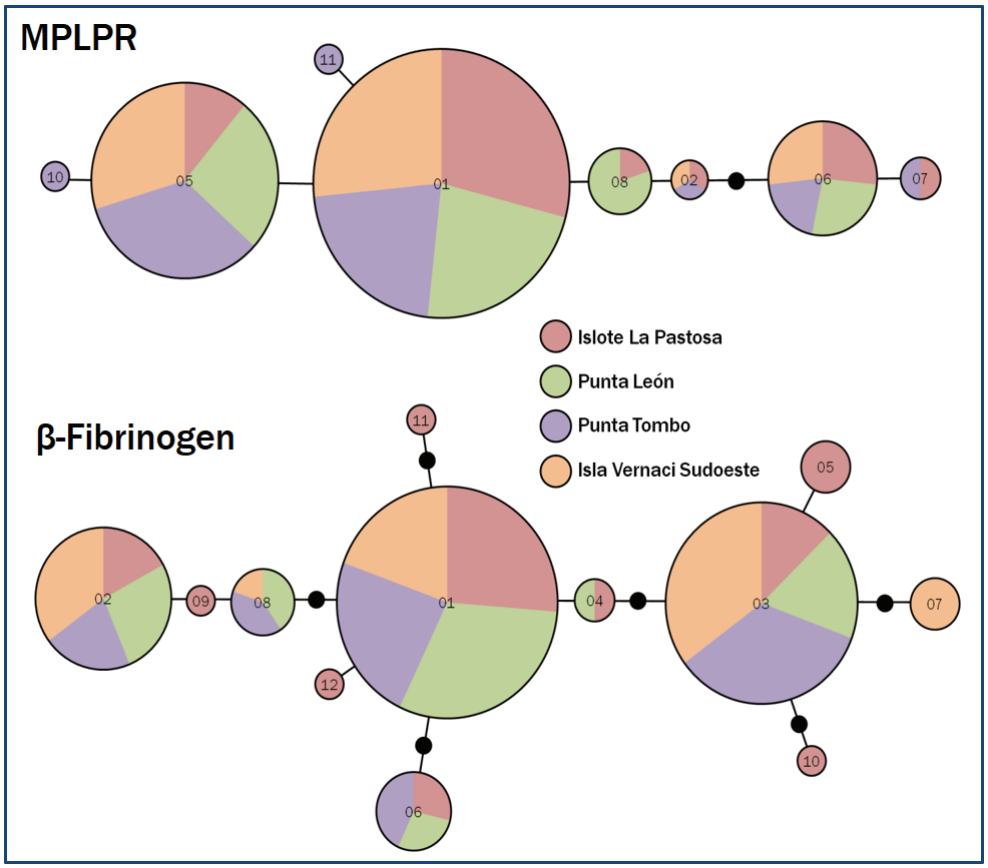

FIG. 2. Haplotype networks of MPLPR and β-fibrinogen intron markers. Size of circles indicates relative haplotype frequency and colors correspond to the proportion of the haplotype found in the corresponding breeding colony.

other gull species (Oro & Pradel 1999, Genovart *et al.* 2003, Doxa *et al.* 2013), and other colonial seabirds (Friesen *et al.* 2007, Bouzat *et al.* 2009).

Pairwise F_{ST} values, however, indicated some degree of genetic differentiation between Islote La Pastosa in the north and the colonies at Punta Tombo and Isla Vernaci Sudoeste (~ 3–4%; $p < 0.05$), which are located 680 and 1260 km south from Islote La Pastosa, respectively. This result is consistent with our estimates of population connectivity (Table 1), which showed that Islote La Pastosa is located in a region with a relatively small number of neighboring colonies (Fig. 1). In fact, there were only six colonies detected within a radius of 114 km from Islote La Pastosa, and none of these colonies were found within 50 km from this focal colony. Distance between colonies has been shown to be one of the most important determinants of dispersal within the Audouin's Gull (*Larus audouinii*) metapopulation, although for this species a low rate of dispersal was recorded even between the most distant colonies (Oro & Pradel 1999). Indirect estimates of gene

TABLE 3. Breeding colony pairwise F_{ST} (below diagonal) and pairwise estimates of gene flow (*Nm*) as number of individuals per generation (above diagonal). * $p < 0.05$, ** $p < 0.009$.

	Islote La Pastosa	Punta León	Punta Tombo	Isla Vernaci Sudoeste
Islote La Pastosa	-------	110	6	8
Punta León	0.002	-------	46	19
Punta Tombo	0.040**	0.005	-------	20
Isla Vernaci Sudoeste	0.031*	0.013	-0.012	-------

flow are consistent with the levels of genetic differentiation found in our study, as Islote La Pastosa revealed an average estimate of seven migrants per generation with Punta Tombo and Isla Vernaci Sudoeste, compared to the overall average of 35 migrants per generation estimated across all colonies. The observed pattern of differentiation was not consistent with a pattern of isolation by distance, since a Mantel test of genetic and geographic distances between colonies was not significant (data not shown). However, a non-significant Mantel test could have resulted from low statistical power, since only four colonies were analyzed in this study.

Although estimates of gene flow are consistent with our indirect measures of population connectivity between colonies, these do not suggest a high degree of genetic structuring within the spatial scale studied. As shown by other studies, Kelp Gulls have a cohesive gene pool with limited genetic differentiation at regional and global scales (Dantas *et al.* 2009, 2012). In our case, genetic differences between Islote La Pastosa and the two southernmost colonies are likely due to the presence of unique haplotypes, as this colony showed the highest number of haplotypes for both genetic markers analyzed (Table 2). The genetic differentiation of Islote La Pastosa from southern colonies does not preclude the existence of significant demographic inputs from other colonies in the North. Demographic estimates revealed that, compared to the other studied colonies, Islote La Pastosa had a population growth rate ($\lambda = 1.075$) above the replacement rate. In a survey of 68 colonies, Lisnizer *et al.* (2015) have also shown that the growth rate of Islote La Pastosa was within the maximum growth rate reference value estimated for this species in Patagonia, indicating that this colony is located in an area of population expansion. These results are consistent with the idea that the demographic connectivity among Kelp Gull colonies in Patagonia follows a source-sink dynamics, where growth rates of growing colonies are fueled by the immigration of individuals from nearby colonies acting as sources (Lisnizer *et al.* 2014, 2015). This may explain the presence of unique haplotypes at Islote La Pastosa, which may result from the recruitment of individuals from other, genetically differentiated neighboring colonies located in the North.

The observed pattern is also consistent with an overall expansion of breeding colonies throughout the species's range. The star-shaped haplotype networks with no apparent phylogeographic patterns suggest a recent demographic expansion of the species in this region, promoting a cohesive gene pool. This is also consistent with a previous demographic study of Kelp Gulls by Lisnizer *et al.* (2011), which revealed positive growth rates ($\lambda > 1$) in 70% of the colonies, an increase in overall abundance by 37%, and the establishment of ten new colonies between 1994 and

2008. A recent genetic study by Dantas *et al.* (2012) has shown a similar demographic expansion of Kelp Gulls along the Brazilian coast.

Our study revealed considerable levels of intra-population genetic variation across breeding colonies of the Kelp Gull in northern Patagonia and some level of genetic differentiation of specific colonies. This is an unexpected result for a widespread seabird species with relatively high demographic connectivity; however, it can be explained by a metapopulation dynamics within an expanding population context. The genetic differentiation of the Islote La Pastosa population from southern colonies may be driven in part by distinct ecological attributes associated with the San Matias Gulf region and its potential demographic connectivity with colonies in the north. This is consistent with observed mismatches in the phenology of northern colonies, where breeding takes place at least one month in advance compared to the two southernmost colonies (Table 1). Additional genetic studies of gull colonies north of Islote La Pastosa would be required to further uncover the potential regional pattern of genetic structuring.

Results from this study suggest that the demographic dynamics of Patagonian Kelp Gull breeding colonies should be studied under a metapopulation framework within a scenario of recent population expansion. Over the last few decades, there has been a considerable debate about culling as a potential management tool for controlling the number of breeding gulls in Europe and North America (Duncan 1978, Coulson 1991, Bosch *et al.* 2000, Oro & Martínez-Abraín 2007). Although some local authorities and nongovernmental organizations in northern Patagonia have proposed culling as a potential strategy to minimize the negative effects of Kelp Gulls on endangered species, such as the southern right whale, *Eubalaena australis* (Sironi *et al.* 2009), there has been some concern about the effectiveness of this management option. Our results suggest that in the case of the Patagonian Kelp Gull a thorough understanding of metapopulation dynamics may be essential before the implementation of management policies, since the demography of individual colonies, their potential levels of genetic differentiation/distinctiveness, and their connectivity to neighboring colonies may have significant effects on the outcomes of this strategy (Pulliam 1988). Our study shows the importance of combining genetic and demographic data to assess the population dynamics of seabirds, and thus plan effective management strategies for seabirds.

ACKNOWLEDGMENTS

We would like to thank Kaspar Delhey, Gisele Dantas, Dorit Liebers-Helbig, and an anonymous reviewer for their constructive suggestions on an earlier version of the manuscript. Financial support for this study was provided by Agencia Nacional de Promoción Cientifica Tecnológica/PICT 33611-Argentina, Wildlife Conservation Society, Bowling Green State University, and CENPAT-CONICET. We thank the Río Negro and Chubut provincial authorities for issuing permits to study the four focal Kelp Gull colonies and collect samples for genetic analysis. We also thank Joseph Schalk (Bouzat Lab) for assisting with DNA extractions and primer optimization.

REFERENCES

Amos, W., & J. Harwood. 1998. Factors affecting levels of genetic diversity in natural populations. Philos. Trans. R. Soc. Lond. B Biol. Sci. 353: 177–186.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard Univ. Press, Cambridge, Massachussetts, USA.

Bertellotti, M., & P. Yorio. 1999. Spatial and temporal patterns in the diet of the Kelp Gull in

northern Chubut, Patagonia. Condor 101: 790–798.

Bibby, C. J., N. D. Burgess, & D. A. Hill. 1992. Bird census techniques. Academic Press, London, UK.

Belant, J. L. 1997. Gulls in urban environments: landscape-level management to reduce conflict. Landsc. Urban Plann. 38: 245–258.

Blokpoel, H., & A. L. Spaans. 1991. Introductory remarks: superabundance in gulls: causes, problems and solutions. Pp. 2361–2363 *in* Bell, B. D., R. O. Cossee, J. E. C. Flux, B. D. Heather, R. A. Hitchmough, C. J. R. Robertson, & M. J. Williams (eds). Proc. XX. Int. Ornithol. Congress, 2–9 December 1990, Christchurch, New Zealand.

Bosch, M., D. Oro, F. J. Cantos, & M. Zabala. 2000. Short-term effects of culling on the ecology and population dynamics of the Yellow-legged Gull. J. Appl. Ecol. 37: 369–385.

Bouzat, J. L. 2010. Conservation genetics of population bottlenecks: the role of chance, selection, and history. Conserv. Genet. 11: 463–478.

Bouzat, J. L., B. G. Walker, & P. D. Boersma. 2009. Regional genetic structure in the Magellanic Penguin (*Spheniscus magellanicus*) suggests metapopulation dynamics. Auk 126: 326–334.

Burger, J., & M. Gochfeld. 1996. Family Laridae (gulls). Pp. 572–623 *in* del Hoyo J., A. Elliott, & J. Sargatal (eds). Handbook of the birds of the world. Volume 3: Hoatzin to Auks. Lynx Edicions, Barcelona, Spain.

Caughley, G. 1977. Analysis of vertebrate populations. Wiley, New York, New York, USA.

Coulson, J. C. 1991. The population dynamics of culling Herring Gulls and Lesser Black-backed Gulls. Pp. 479–497 *in* Perrins C. M., J.-D. Lebreton, & G. J. M. Hirons (eds). Bird population studies. Oxford Univ. Press, Oxford, UK.

Coulson, R., & G. Coulson. 1998. Population change among Pacific, Kelp and Silver Gulls using natural and artificial feeding sites in south-eastern Tasmania. Wildl. Res. 25: 183-198.

Coulson, J. C., & B. A. Coulson. 2009. Ecology and colonial structure of large gulls in an urban colony: investigations and management at Dumfries, SW Scotland. Waterbirds 32: 1–15.

Dantas, G. P. M., A. V. L. Rueda, F. P. Campos, J. B. Olinto, & J. S. Morgante. 2006. Genetic variability and sex ratio in the Kelp Gull: implications for management and conservation of seabirds. J. Ornithol. 147: 230–231.

Dantas, G. P. M., R. Godinho, J. S. Morgante, & N. A. Ferrand. 2009. Development of new nuclear markers and characterization of single nucleotide polymorphisms in Kelp Gull (*Larus dominicanus*). Mol. Ecol. Res. 9: 1159–1161.

Dantas, G. P. M., D. Meyer, R. Godinho, N. A. Ferrand, & S. M. Morgante. 2012. Genetic variability in mitochondrial and nuclear genes from *Larus dominicanus* (Charadriiformes: Laridae) at Brazilian coast. Genet. Mol. Biol. 35: 874–885.

Doxa, A., A. Besnard, A. Bechet, C. Pin, J. D. Lebreton, & N. Sadoul. 2013. Inferring dispersal dynamics from local population demographic modelling: the case of the Slender-billed Gull in France. Anim. Conserv. 16: 684–693.

Duncan, N. 1978. The effects of culling Herring Gulls (*Larus argentatus*) on recruitment and population dynamics. J. Appl. Ecol. 15: 697–713.

Efron, B., & R. J. Tibshirani. 1993. An introduction to the bootstrap. Chapman & Hall, New York, New York, USA.

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

Frankham, R., J. D. Ballou, & D. A. Briscoe. 2002. Introduction to conservation genetics. Cambridge Univ. Press, Cambridge, UK.

Friesen, V. L., T. M. Burg, & K. D. McCoy. 2007. Mechanisms of population differentiation in seabirds. Mol. Ecol. 16: 1765–1785.

Garthe, S., C. J. Camphuysen, & R. W. Furness. 2006. Amounts of discards by commercial fisheries and their significance as food for seabirds in the North Sea. Mar. Ecol. Prog. Ser. 136: 1–11.

Genovart, M., D. Oro, & F. Bonhomme. 2003. Genetic and morphological differentiation between the two largest breeding colonies of Audouin's Gull *Larus audouinii*. Ibis 145: 448–456.

Hackett, S. J., R. T. Kimball, S. Reddy, R. C. K. Bowie, E. L. Braun, M. J. Braun, J. L. Chojnowski, W. A. Cox, K. L. Han, J. Harsh-

man, C. J. Huddleston, B. D. Marks, K. J. Miglia, W. S. Moore, F. H. Sheldon, D. W. Steadman, C. C. Witt, & T. Yuri. 2008. A phylogenomic study of birds reveals their evolutionary history. Science 320: 1763–1768.

Jiguet, F., P. Capainolo, & A. Tennyson. 2012. Taxonomy of the Kelp Gull *Larus dominicanus* Lichtenstein revisited with sex-separated analyses of biometrics and wing tip patterns. Zool. Stud. 51: 881–892.

Larkin, M. A., G. Blackshields, N. P. Brown, R. Chenna, P. A. McGettigan, H. McWilliam, F. Valentin, I. M. Wallace, A. Wilm, R. Lopez, J. D. Thompson, T. J. Gibson, & D. G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947–2948.

Librado, P., & J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Liebers, D., A. J. Helbig, & P. De Knijff. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans–fuscus* group (Aves: Charadriiformes). Mol. Ecol. 10: 2447–2462.

Liebers, D., P. Knijff, & A. J. Helbig. 2004. The Herring Gull complex is not a ring species. Proc. R. Soc. Lond. B Biol. Sci. 271: 893–901.

Lisnizer, N., P. García-Borboroglu, & P. Yorio. 2011. Spatial and temporal variation in population trends of Kelp Gulls in northern Patagonia, Argentina. Emu 111: 259–267.

Lisnizer, N., P. García-Borboroglu, & P. Yorio. 2014. Demographic and breeding performance of a new Kelp Gull *Larus dominicanus* colony in Patagonia, Argentina. Ardeola 61: 3–14.

Lisnizer, N., P. García-Borboroglu, M. Pascual, & P. Yorio. 2015. Transfer processes drive population dynamics of Kelp Gull colonies in Patagonia: Implications for management strategies. Mar. Biol. Res. 11: 738–746.

Lowe, W. H., & F. W. Allendorf. 2010. What can genetics tell us about population connectivity? Mol. Ecol. 19: 3038–3051.

Morris-Pocock, J. A., J. C. Hennicke, & V. L. Friesen. 2012. Effects of long-term isolation on genetic variation and within-island population genetic structure in Christmas Island (Indian Ocean) seabirds. Conserv. Genet. 13: 1469–1481.

Oro, D., & R. Pradel. 1999. Recruitment of Audouin's Gull to the Ebro Delta colony at metapopulation level in the western Mediterranean. Mar. Ecol. Prog. Ser. 180: 267–273.

Oro, D., & A. Martínez-Abraín. 2007. Deconstructing myths on large gulls and their impact on threatened sympatric waterbirds. Anim. Conserv. 10: 117–126.

Oro, D., M. Genovart, G. Tavecchia, M. S. Fowler, & A. Martínez-Abraín. 2013. Ecological and evolutionary implications of food subsidies from humans. Ecol. Lett. 16: 1501–1514.

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

Primmer, C. R., T. Borge, J. Lindell, & G. P. Sætre. 2002. Single-nucleotide polymorphism characterization in species with limited available sequence information: high nucleotide diversity revealed in the avian genome. Mol. Ecol. 11: 603–612.

Prychitko, T. M., & W. S. Moore. 1997. The utility of DNA sequences of an intron from the ß-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). Mol. Phylogenet. Evol. 8: 193–204.

Pulliam, R. 1988. Sources, sinks and population regulation. Am. Nat. 132: 652–661.

Roeder, A. D., R. K. Marshall, A. J. Mitchelson, T. Visagathilagar, P. A. Ritchie, D. R. Love, T. J. Pakai, H. C. McPartlan, N. D. Murray, N. A. Robinson, K. R. Kerry, & D. M. Lambert. 2001. Gene flow on the ice: genetic differentiation among Adélie Penguin colonies around Antarctica. Mol. Ecol. 10: 1645–1656.

Sironi, M., V. Rowntree, C. Snowdon, L. Valenzuela, & C. Marón. 2009. Kelp Gulls (*Larus dominicanus*) feeding on southern right whales (*Eubalaena australis*) at Península Valdés, Argentina: updated estimates and conservation implications. Paper SC/61/BRG19 presented to the International Whaling Commission Scientific Committee, Portugal, June 2009 (unpublished). Available from the IWC Office at https://iwc.int/document_1780.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236: 787–792.

Taylor, S. A., & V. L. Friesen. 2012. Use of molecular genetics for understanding seabird evolution, ecology and conservation. Mar. Ecol. Prog. Ser. 451: 285–304.

Teacher, A. G. F., & D. J. Griffiths. 2011. HapStar: an automated haplotype network layout and visualization. Mol. Ecol. Res. 11: 151–153.

Thomas, G. J. 1972. A review of gull damage and management methods at nature reserves. Biol. Conserv. 4: 117–127.

Tyson, C., J. Shamoun-Baranes, E. E. Van Loon, K. C. J. Camphuysen, & N. T. Hintzen. 2015. Individual specialization on fishery discards by Lesser Black-backed Gulls (*Larus fuscus*). ICES J. Mar. Sci. 72: 1882–1891.

Whittington, P. A., A. P. Martin, & N. T. W. Klages. 2006. Status, distribution and conservation implications of the Kelp Gull (*Larus dominicanus vetula*) within the Eastern Cape region of South Africa. Emu 106: 127–139.

Yorio, P., & P. García Borboroglu. 2002. Breeding biology of Kelp Gulls (*Larus dominicanus*) at Golfo San Jorge, Patagonia, Argentina. Emu 102: 257–263.

Yorio, P., M. Bertellotti, & P. García Borboroglu. 2005. Estado poblacional y de conservación de gaviotas que reproducen en el litoral Argentino. Hornero 20: 53–74.

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

Yorio, P., E. Frere, P. Gandini, & G. Harris (eds). 1998b. Atlas de distribución reproductiva de aves marinas en el litoral patagónico argentino. Plan de Manejo Integrado de la Zona Costera Patagónica. Fundación Patagonia Natural, Wildlife Conservation Society, Instituto Salesiano de Artes Gráficas, Buenos Aires, Argentina.

Yorio, P., F. Quintana, C. Campagna, & G. Harris. 1994. Diversidad, abundancia y dinámica espacio temporal de la colonia mixta de aves marinas en Punta León, Patagonia. Ornitol. Neotrop. 5: 69–77.

Yorio, P., J. O. Branco, J. Lenzi, G. Luna-Jorquera, & C. Zavalaga. In press. Distribution and trends in Kelp Gull (*Larus dominicanus*) coastal breeding populations in South America. Waterbirds: – .

SUPPLEMENTARY MATERIAL

Sampling localities of individual birds and corresponding haplotypes can be accessed at: