

Evidence for gene flow differs from observed dispersal patterns in the Humboldt penguin, *Spheniscus humboldti*

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Abstract The Humboldt penguin, once common throughout its range, is today listed as Vulnerable by the IUCN. Mark-recapture and telemetry studies indicate that adult Humboldt penguins are sedentary, suggesting strong genetic differentiation between colonies. We developed genotypes for 336 individuals at 12 microsatellite loci sampled at four different localities spanning the entire range of this species. Results show that long-term gene flow has occurred but appears to be affected by geographic distance as pairwise F_{ST} comparisons involving the colony at Punta San Juan (Peru) and the two colonies at Algarrobo (central Chile) and Puñihuil (southern Chile) are significant. Bayesian estimates of recent migration rates indicate substantial dispersal among all colonies. Despite the dramatic decline in numbers, we did not observe a bottleneck in any population. Furthermore, we did not detect a founder effect in the recently discovered colony at Puñihuil. As our indirect estimates signal strong gene flow

between populations, we suggest that Humboldt penguin colonies need to be managed as a metapopulation rather than as discrete management units.

Keywords Humboldt penguin · Gene flow · Dispersal · Population structure · Microsatellites

Introduction

A central issue in wildlife conservation is the relationship between dispersal and gene flow. In order to manage a species effectively in situ it must be known how philopatric a species is, how much individual movement can be expected among populations, and what consequences these movements will have on population genetic structure and evolutionary potential (Crandall et al. 2000; Fraser and

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Bernatchez 2001). Direct methods such as capture-mark-recapture (CMR) have been employed to study dispersal, but these methods systematically bias estimates of dispersal downward (Koenig et al. 1996). Further, while direct methods may detect movement, its potential consequences on population genetic, and hence evolutionary, processes cannot be revealed using such approaches. These limitations can be overcome using indirect methods that estimate both recent migration and gene flow from molecular marker data (Koenig et al. 1996; Wilson and Rannala 2003). Indirect methods of estimating dispersal are not affected by many of the factors that limit direct estimates (e.g., restricted study areas and strong sampling limitations) and also allow determination of the influences dispersal may have on either short- or long-term rates of gene flow (Wilson and Rannala 2003). Since the detection of individuals in non-source populations does not necessarily equate with gene flow, comparisons of patterns of recent and long-term gene flow provide combined data that afford a more realistic view of movement that equates with the evolutionary potential of individual populations (Whitlock and McCauley 1999; Wilson and Rannala 2003), which is of greater conservation importance than individual movement patterns (Crandall et al. 2000). Gene flow between populations maintains the overall genetic variability, presumably allowing species to respond to changing selection pressures (Petit et al. 1998), a crucial issue for the long term survival of a species.

The Humboldt penguin (*Spheniscus humboldti*) is endemic to the coastal areas affected by the Humboldt Current in the South eastern Pacific. The species breeding range extends from Isla Foca (5°S), Peru, to Puñihuil (42°S), Chile (Fig. 1). Like most penguin species, Humboldt penguins are restricted to coastal areas, ground nest in colonies and forage in nearby coastal waters. *S. humboldti* probably numbered in the hundreds of thousands at the end of the nineteenth century (Johnson 1965), but is currently listed as Vulnerable due to population size reductions attributed to exploitation or habitat alteration (Birdlife International 2004). The current population size is estimated to be at 48,000 (Araya and Bernal unpubl.). Direct estimates of Humboldt penguin's dispersal patterns suggest they form distinct breeding populations with little opportunity for among-colony gene flow (e.g., Wallace et al. 1999). Adults show strong colony fidelity: 1,000 penguins banded as adults at Punta San Juan, Peru, were observed breeding only at this location (Araya et al. 2000). Wallace et al. (1999) banded 400 adult penguins at Algarrobo, Chile, but a mere nineteen birds were sighted at other locations, the majority of which were dead penguins recovered at sea mostly within 50 km of Algarrobo. A single marked bird was recorded by Wallace et al. (1999) at the Cachagua colony, 88 km north of Algarrobo, but it is unknown if this individual bred at this location or eventually

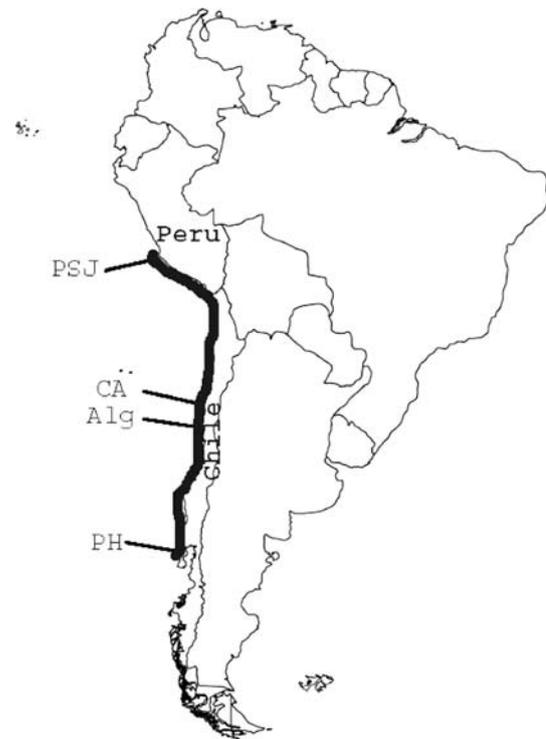


Fig. 1 Map of South America indicating the entire range of the Humboldt penguin and sampling locations (PSJ: Punta San Juan, CA: Cachagua, A: Algarrobo, PH: Puñihuil)

returned to Algarrobo without reproducing at Cachagua. Telemetry and satellite data developed from 12 tagged and breeding Humboldt penguins showed that most foraging during the breeding season occurs within 35 km of the colony (Culik and Luna-Jorquera 1997a; Culik et al. 1998), while five individuals followed outside of the breeding season foraged within 90 km of the colony (Culik and Luna-Jorquera 1997b). Adults also show extreme nest site fidelity. A multiyear study of reproductive behavior determined that approximately 60% of recaptured adult Humboldt penguins returned to the same nest occupied the previous breeding season. The remaining 40% either moved to a nest nearby (~30%) or moved to a new area within the colony (~10%, Teare et al. 1998). Polyandrous mating does occur within a breeding colony, but extra-pair copulations do not result in extra-pair fertilization (Schwartz et al. 1999). It is perhaps due to the combination of colony fidelity and nest site fidelity that proposed conservation recommendations to limit penguin bycatch in local fisheries are designed to protect areas immediately adjacent to individual breeding colonies and do not take into account dispersal routes among colonies (Culik and Luna-Jorquera 1997a; Taylor et al. 2002).

These direct data are limited in scope, both geographically and temporally. Useful CMR data for Humboldt penguins is difficult to obtain for several reasons: (1) Their

breeding range is prohibitively large along the major axis of the species range; (2) many breeding colonies are difficult to access, or completely inaccessible to researchers (i.e., Paredes et al. 2003); (3) reproduction is not predictable and strongly influenced by El Niño events (Araya and Todd 1988; Zavalaga and Paredes 1997; Simeone et al. 2002), and; (4) surveys to date are not standardized (Araya et al. 2000). Furthermore, dispersal of juvenile Humboldt penguins has not been studied until recently (Simeone et al. 2006) and therefore, only very limited data is available. Because it is extremely difficult to overcome these challenges in the field, molecular approaches for estimating dispersal could provide useful information for effective management of Humboldt penguin colonies effectively.

Our research was designed to study dispersal and gene flow indirectly by investigating population structure across four breeding colonies spanning the entire range of this species (Fig. 1). Based on observational data summarized above, we expected to find limited evidence of gene flow and recent dispersal among breeding colonies. Because at least one of our sampling locations is reputed to be a newly founded breeding colony, we sought evidence of recent founding events in all our study locations (Araya and Todd 1988).

Materials and methods

Sampling locations

We collected samples from four breeding colonies encompassing the species range of *S. humboldti* (Fig. 1). In Peru, Humboldt penguin colonies have been reported at 22 different locations, but breeding activities, i.e., nesting or nests with chicks, have only been observed at 14 sites (Paredes et al. 2003). Our sample population, Punta San Juan (15°22' S, 75°12' W), was, and continues to be, the largest breeding area in Peru (Paredes et al. 2003). In the mid-1990s the population was estimated at 1,800 breeding pairs (Zavalaga and Paredes 1997). In Chile, there are at least 14 known breeding sites for the Humboldt penguin (Ellis et al. 1998) of which Cachagua (32°35' S, 71°27' W) and Algarrobo (33°21' S, 71°41' W) were the two major breeding sites in Central Chile at the time of collection (Simeone and Bernal 2000). Puñihuil (41°43' S, 74°02' W) is located at the southernmost tip of the Humboldt penguin's range. There are no published records of any significant breeding colonies between Algarrobo and Puñihuil. Puñihuil is a recently discovered breeding colony which has led some workers to hypothesize that birds displaced during recent El Niño events founded this colony, extending the range of the species over 900 kilometers south (Wilson et al. 1995; Araya and Todd 1988).

Sample collection and DNA extraction

As part of a larger study that also included serological surveys for diseases, Humboldt penguin blood samples were collected during breeding seasons between 1992 and 1997. All sampled birds, with the exception of Cachagua, were permanently marked either by banding a wing and/or inserting a tiny transponder chip under the skin at the rostral portion of the top of the head. This ensured that no individual was sampled for DNA more than once. At Cachagua, samples were collected at the onset of the breeding season as males started to arrive and sampled birds were marked temporarily with water resistant color markers to avoid re-sampling. Blood (5 ml) was collected from the jugular vein using a 22-gauge needle and a 5 ml syringe. At Cachagua, blood was obtained from the medial metatarsal vein with 23-gauge butterfly blood collection tubing and a 5 ml syringe.

An aliquot of each blood sample was stored in long-term storage buffer (100 µM Trizma, 100 mM EDTA and 2% SDS, pH 8.0) for genetic analysis at the Brookfield Zoo Lab. Genomic DNA was extracted from blood following the protocol of Sambrook et al. (1989). Extracted samples were cleaned using three successive washes (equilibrated phenol, phenol/chlorophorm/isoamyl alcohol (25:24:1), and chloroform/isoamyl alcohol (24:1)), followed by precipitation with 3 M sodium acetate and 100% ethanol.

Microsatellite analyses and validation

Microsatellite primers were developed as described in Schlosser et al. (2003) and Garner et al. (2000). We screened 28 potential microsatellite primer pairs and found 12 loci with a dinucleotide repeat to be polymorphic (Table 1 describes five new loci, for the remaining seven loci see Schlosser et al. 2003). Forward primers were fluorescently labeled with WellRed Beckman Coulter dyes. PCR amplification and genotyping was performed as outlined in Schlosser et al. (2003). We used DNA Analysis System Software, version 4.3.9 (Beckman Coulter, Inc.) to visualize and characterize all genotypes. PCR amplification and genotyping was repeated for samples with unique alleles and at least twice randomly for a few individuals throughout the study and at each capillary change to ensure fragment migration time has not shifted.

A large panel of genotypes was also developed from captive Humboldt penguins that were known first order relatives. We used allelic inheritance patterns and the known pedigrees of these captive family groups to validate genotype scores and to detect null alleles.

Table 1 Microsatellite primer names, expected fragment sizes based on original sequenced clone, annealing temperatures (T), total number of alleles detected in this study (A), and GenBank accession numbers for five previously unpublished microsatellite loci

Locus name	Primer sequence 5'-3'	Size (bp)	T (°C)	A	GenBank accession number
Sh2Ca31	F:ATCACAGCTCCCCCTTTCTC R:AAGGCAAACAGAGTGGGATG	116	64	11	AY435087
Sh2Ca40	F:AGCAGCACGCCCTCCCTC R:TCTCCAGGAAAGCAGGAATC	90	63	16	AY435088
Sh2Ca49	F:GCTTTTCCACCAGCTCTTCC R:TTCTGTTCAAAGCGTGGTTG	122	63	9	AY435089
Sh2Ca55	F:TGAGTCTGAGTGCTCAGTTGG R:AGGGTCTGAAGGACAGCTACC	115	63	14	AY435090
Sh2Ca58	F:TACAGCAATGCAGCGTGTGT R:ACCTGGTAGAGGGCAGTAGT	106	63	4	AY435091

All forward primers were labeled and optimized as described by Schlosser et al. (2003)

Genetic variation within and among colonies

Observed and expected heterozygosities for each microsatellite locus between all pairs of loci were calculated for each year within each population using Genepop v. 3.1d (Raymond and Rousset 1995). We used exact tests to examine each locus for fit with the expectations of Hardy–Weinberg equilibrium (HWE). For those loci that deviated from HWE expectations, Mann–Whitney *U*-tests were used to test for heterozygote deficiency or excess. Significance was determined using the Markov chain method with all parameters left at default settings (Guo and Thompson 1992). Allelic and genotypic differentiation and F_{ST} among populations and years within Punta San Juan and Algarrobo was evaluated using Genepop. Allelic richness, number of alleles, genetic disequilibrium and F_{IS} were calculated using FSTAT 2.9.3.2 (Goudet 2002). We used allelic richness rather than allele frequencies to account for variation in sample sizes among our populations.

We tested for deviations from mutation/drift equilibrium using BOTTLENECK (Cornuet and Luikart 1996). Two models of microsatellite evolution were tested: stepwise mutation model (SMM) (Ohta and Kimura 1973) and two-phase model (TPM) (Di Rienzo et al. 1994). As suggested by Piry et al. (1997) the proportion of single-step mutations in TPM was set at 95%. The probability of a bottleneck in each population was assessed with the application of the one tailed Wilcoxon's test for heterozygosity excess as it is the most powerful and robust test offered by this program when using less than 20 loci (Piry et al. 1997).

Gene flow among colonies

We did not detect any significant population structure among years within Punta San Juan and Algarrobo, consequently, all further analyses and among-population

comparisons were performed using pooled samples for these two sites.

We investigated gene flow among colonies using three approaches. (1) We tested for isolation by distance along the linear distribution of this species by comparing half matrices of pairwise geographic distances and population genetic distances ($F_{ST}/(1 - F_{ST})$) using a one dimensional model Mantel test in Genepop (Rousset 1997) with 1,000 permutations and using a Mantel Nonparametric Test Calculator Shareware V. 2.0 with 10,000 permutations (Liedloff 1999). (2) The number of migrants, N_m , was estimated based on private alleles (Slatkin 1985), a method that is less sensitive to reverse mutations common in microsatellites (Allen et al. 1995). (3) To estimate migration rates between populations within the past few generations we used BayesAss 1.2 (Wilson and Rannala 2003). This model uses a Markov chain Monte Carlo technique to estimate the proportion of immigrants into a population. It assumes low levels of migration and sets the maximum allowed proportion of immigration into a population at ~30% per generation. This method provides a highly accurate estimate of migration rate given sufficient genetic differentiation between populations and a sufficient number of loci (for theoretical aspects see Wilson and Rannala 2003). We estimated recent migration rates using 3×10^6 iterations and a sampling frequency of 2000.

Results

Genotypic validation

Patterns of allelic inheritance among captive penguins (163 individuals from 42 family groupings) confirmed that individuals were correctly identified as heterozygotes or homozygotes in parent/offspring analyses and that

microsatellite alleles were codominantly inherited. The analyses also revealed possible null allele(s) at three loci (Sh1Ca12, Sh2Ca22, and Sh2Ca55) in two sire/offspring combinations. However, for two of these loci, loci Sh2Ca22 and Sh2Ca55, null alleles were detected in one family for which no DNA from the dam was available for comparison. In this family, the chick and sire were homozygous for different alleles.

A total of 22 individuals from among our wild samples (13 from Algarrobo, two from Puñihuil, and seven from Punta San Juan) were not assigned a genotype at locus Sh2Ca40 because PCR amplification consistently generated multiple peaks within the confirmed allele size range that could not be resolved. As this affected such a small fraction of our samples (Bonin et al. 2004) we included this marker in our study and present the data of all 12 loci unless otherwise stated.

Genetic variation within and among colonies

In total, 336 individuals from four different colonies were genotyped. The total number of alleles detected at a locus ranged from four (locus Sh2Ca58) to 16 (both Sh1Ca16 and Sh2Ca40), averaging 11.5 over all loci (Table 2). Allelic richness varied little among years within colonies, and among colonies (Table 2). Private and rare alleles (alleles found in no more than two populations) were observed in all populations (frequencies of ≤ 0.034 for private alleles and ≤ 0.058 for rare alleles, Appendix 1). Multi-locus tests revealed heterozygote deficiency at Algarrobo and Punta San Juan after Bonferroni correction, but no colony F_{IS} within years or pooled samples within sites were significant after Bonferroni correction (Table 2). Across all populations, two pairs of loci (Sh1Ca16/

Sh2Ca40 and Sh2Ca22/Sh2Ca55) were in genetic disequilibrium after Bonferroni correction ($P < 0.001$).

Genic and genotypic differentiation over all loci among years within sites was non significant except for allelic differentiation between Punta San Juan 1993 and 1994 ($P < 0.05$). The allelic distribution across all four colonies, with years pooled, was significantly ($P < 0.05$) different at seven of the 12 loci (Sh1Ca9, Sh1Ca12, Sh1Ca17, Sh2, Ca22, Sh2Ca58, Sh2Ca49 and ShCa31). Significant genotypic differentiation was detected after Bonferroni correction ($P < 0.0125$) at six of the 12 loci (Sh1Ca9, Sh1Ca12, Sh1Ca17, Sh2Ca31, Sh2Ca49 and Sh2Ca58). Over all loci, both allelic and genotypic differentiation were highly significant after Bonferroni correction ($P < 0.001$).

Locus Sh2Ca22, and Sh2Ca40 were excluded from all F_{ST} analyses because they were shown to be in linkage disequilibrium with locus Sh2Ca55 and Sh1Ca16, respectively. Within colonies, pairwise Weir and Cockerham (1984) F_{ST} values (θ) were low and not significant ($P > 0.05$) between years, at Algarrobo F_{ST} was 0.0017 between 1994 and 1995 and at Punta San Juan F_{ST} ranged from 0.0010 to 0.0086. When samples were pooled for each colony, F_{ST} values were also very low, ranging from -0.0010 to $+0.0104$ and pairwise F_{ST} were significant ($P < 0.05$, Table 3) between Punta San Juan and Algarrobo, Punta San Juan and Puñihuil, and Algarrobo and Puñihuil. All F_{ST} analyses were also run without locus Sh1Ca12, Sh2Ca22 and Sh2Ca55 to control for effects potentially caused by null alleles that may be operating at these loci. As this did not affect the outcome of the F_{ST} analyses, we present the results for 10 loci. To determine if differences in sample size were having an effect, the same pairwise differentiation tests were run with a randomly selected sample size of 50 individuals from Algarrobo. These analyses yielded the same results.

No bottleneck or founder effect was detected in any population, including Puñihuil, under both the SMM and TPM. Under the assumptions of these two models all four colonies were in mutation-drift equilibrium.

Table 2 Descriptive statistics of microsatellite polymorphisms for four Humboldt penguin colonies

Sample site	Year	<i>N</i>	<i>A</i>	<i>R</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>
Punta San Juan	1992	11	5.7	5.7	0.74	0.71	-0.041
	1993	49	7.6	5.9	0.72	0.75	0.031
	1994	26	8.1	6.3	0.73	0.77	0.053
	Total	86	9.5	6.0	0.73	0.75	0.034
Cachagua	1992	21	6.6	5.5	0.72	0.72	0
Algarrobo	1994	114	8.8	5.6	0.73	0.73	0
	1995	86	9.3	5.8	0.71	0.75	0.048
	Total	200	10	5.7	0.72	0.73	0.021
Puñihuil	1997	29	8.2	6.0	0.69	0.73	0.06

Statistics partitioned by year and total, allelic richness based on a minimum sample of 11 individuals. *N* = Number of samples, *A* = average number of alleles, *R* = allelic richness, *H_O* = average observed heterozygosity, *H_E* = average expected heterozygosity, and *F_{IS}*

Table 3 Pairwise geographic distance (km) and F_{ST} (θ) values for all possible colony combinations

	Punta San Juan	Cachagua	Algarrobo	Puñihuil
Punta San Juan	–	1,952	2,031	2,932
Cachagua	0.0083	–	88	1,041
Algarrobo	0.0078*	-0.0010	–	953
Puñihuil	0.0104*	0.0020	0.0020*	–

Geographic distance is located above the diagonal, and F_{ST} below. * indicates a significant F_{ST} value after Bonferroni correction ($P < 0.05$)

Table 4 Indirect estimates of recent migration rate, *m*

	Punta San Juan	Cachagua	Algarrobo	Puñihuil
Punta San Juan	0.76 (0.73,0.80)	0.17 (0.10,0.24)	0.25 (0.22,0.27)	0.17 (0.11,0.23)
Cachagua	0.00 (0.00,0.01)	0.68 (0.67,0.72)	0.00 (0.00,0.01)	0.01 (0.00,0.03)
Algarrobo	0.22 (0.19,0.26)	0.14 (0.07,0.21)	0.75 (0.07,0.21)	0.14 (0.08,0.21)
Puñihuil	0.01 (0.00,0.03)	0.01 (0.00,0.05)	0.00 (0.00,0.01)	0.68 (0.67,0.71)

Source populations are given in rows, recipient populations in columns

Values in bold along the diagonal are self-recipient rates. Values in parentheses are 95% confidence intervals. Standard deviations for all distributions were <0.05

Gene flow among colonies

Results from the Mantel test using the Mantel statistic *Z* (Liedloff 1999) showed a significant association between genetic and geographic distance ($r = 0.98$, $P < 0.05$) and the results from the rank correlation analysis of Rousset (1997) in Genepop are almost significant ($P = 0.07$). These tests suggest that gene flow among populations is affected by distance. The overall number of migrants (N_m), when calculated using Slatkin's private allele method (1985) and corrected for size, was estimated to be 9. The greatest amount of migration estimated using this method was between Punta San Juan and Algarrobo with 10 migrants per generation. Estimates of recent migration using the algorithm of Wilson and Rannala (2003) indicated levels of natal philopatry between 68 and 76% (Table 4). These results also suggest that Punta San Juan serves as a source population, with a migration rate of at least 0.17 into the three populations to the south.

Discussion

While it is assumed that young marine birds “make active decisions about where to breed” before they reproduce for the first time, many young are philopatric and will return to their natal site for reproduction (Coulson 2002). In most penguin species, adults breed within a few hundred meters of the nest-site where they hatched (Williams 1995) and field observations of Humboldt penguins suggest strong colony fidelity (e.g., Teare et al. 1998). This type of behavior should inevitably lead to significant population genetic differentiation among all breeding locales and strong global population genetic structure.

Population structure

Certainly, our analyses revealed long term structure among colonies, as we detected significant global differences in allelic and genotypic distributions across populations and three significant pairwise F_{ST} comparisons. The significant pairwise comparisons involved Punta San Juan and the two colonies farthest south of it, Algarrobo and Puñihuil. Cachagua, a major Chilean breeding locale in central Chile, was not significantly different from any colony and also lacked unique alleles. However, the sample size from this location was small and taken at the onset of breeding season, raising the possibility that our patterns of differentiation for this colony may be affected by sampling bias and proximity to the Algarrobo population.

Although the number of Humboldt penguins has decreased dramatically over the past 200 years microsatellite data do not show evidence of a bottleneck. None of the four colonies showed any significant heterozygosity excess under the SMM and TPM and when pooled, the total sample appeared to be in mutation-drift equilibrium. Whitehouse and Harley (2001) advised caution when performing this kind of analysis after they failed to detect population size reduction in post-bottleneck elephant populations. Nevertheless, we detected no difference in allelic richness between colonies and rare and/or unique alleles were detected in all colonies save Cachagua, which would not be the case if recent population declines had affected population genetic variability. Observed and expected heterozygosity was also equally high at all four locations. All these results indicate that while some of the Humboldt penguin colonies have decreased in size and the species is in decline (Zavalaga and Paredes 1997), the current population is still large enough to maintain high genetic diversity.

Cassens et al. (2005) reported similar findings for the Peruvian population of the dusky dolphin (*Lagenorhynchus obscurus*). This species has also declined in population size along the Peruvian coast due to combined forces of El Niño fluctuations and human factors, but appears to be maintaining high levels of genetic variation. While these two studies indicate that genetic diversity can be maintained during population decline, the long-term effects of these external factors could be substantial and warrant further monitoring.

Finally, the lack of evidence for a bottleneck, the presence of rare alleles and high levels of observed and expected heterozygosity refute the hypothesis that Puñihuil was founded by penguins displaced during a recent El Niño event. Instead, the population at Puñihuil has probably been overlooked in past censuses, as suggested by Araya and Todd (1988).

Gene flow among colonies

Direct estimates of dispersal through CMR suggest very limited rates and have been primarily made following adult birds. However, Simeone et al. (2006) recently showed that 1.9% or five of 267 Humboldt penguins marked as chicks at their natal colony in Algarrobo returned to breed to the same colony, while 1.5% or four of the birds were found nesting elsewhere. Two more birds (0.8%) were seen prospecting the natal colony, but no breeding was confirmed. First-year mortality in *Spheniscus* penguins is rather high, reaching up to 80% (e.g., Williams 1995; Whittington et al. 2005) possibly explaining the low re-sighting rates. However, indirect estimates of recent migration from this study indicate that movement among colonies is greater than reported by using direct measures of adult dispersal (Araya et al. 2000; Wallace et al. 1999). We estimated an overall migration rate of 9 migrants per generation using Slatkin's method (1985), a frequency that has a strong likelihood of going undetected using current direct methods in Humboldt penguins. Our data from the Mantel tests suggest that dispersal and gene flow are affected by the distance between colonies. The significance level is not high, probably due to the limited number of colonies included in this study.

The level of genetic structure among these four populations of Humboldt penguins is surprising given the level of gene flow among populations indicated by this study. An estimated average of 9 migrants per generation is sufficient to minimize genetic differentiation (Slatkin 1985). In a similar large-scale population genetics study on Adélie penguins, Roeder et al. (2001) expected to find among-colony genetic structure. Population history, current distribution patterns and behavioral and ecological observations of Adélie penguins (*Pygoscelis adeliae*) all suggested strong population differentiation. Instead, they found no heterogeneity among colonies (F_{ST} values of <0.02), and overall, nine migrants per generation among 13 sampled colonies. Large stable population sizes as well as migration are thought to contribute to the lack of genetic structure among colonies for this species.

Pairwise F_{ST} estimates among geographically distant populations such as Punta San Juan and Puñihuil are significant, yet our Bayesian analysis suggests that there is asymmetric gene flow into Puñihuil from all populations, including Punta San Juan ($m = 0.17$). However, unsampled ghost populations and small population size at this location could influence these results (Fraser et al. 2007; Johnson et al. 2007; Slatkin 2005). Algarrobo and Punta San Juan are also significantly differentiated at microsatellite loci, yet they have similar migration rates

between them of 0.22–0.25. Very little migration was detected from a large population at Cachagua, possibly due to the limited sample size and timing of sample collection there.

For the Humboldt penguin, migration rates and direction of dispersal might not correlate with annual breeding cycles, but rather are influenced by sharp episodic fluctuations in climate, such as El Niño events and human disturbances that change the relationship between breeding location, habitat quality and demography over the short term for all colony locations. Population counts have shown that numbers of penguins in breeding colonies dropped during El Niño events in 1982/1983 but colony size increased to about 50% of the original over the next few years (Araya and Todd 1988). More recently, during the 1997–1998 El Niño, Simeone et al. (2002) observed in central Chile a decrease in breeding Humboldt penguins of up to 85% and an increase by 42–58% following this event. In Algarrobo, there have been rapid and significant changes in colony sizes. The numbers of breeding individuals were 530 in 1984, 1,000 in 1985, 200 in 1986, 200 in 1995–1996, and 1,600 in 1996 (Ellis et al. 1998). While a higher mortality rate might contribute to the lower observed density, adults are known to leave the colony to feed farther away and stay out to sea for a longer time span during periods of poor environmental quality, such as El Niño. Dispersal to other colonies during these events that are not affected is possible. Also during those periods of high density, it might be better to disperse rather than attempt to reproduce in an overcrowded environment. Movement out of Algarrobo, in particular, might be explained by direct human habitat modification and a subsequent reduction in the quality of breeding locations or human disturbance. A recent study shows that Humboldt penguins are extremely sensitive to disturbance by humans (Ellenberg et al. 2006). Temporal and spatial fluctuations in habitat quality and high and chaotic population densities are both known to positively influence dispersal propensities (Dieckmann et al. 1999; Johnson and Gaines 1990).

Current research on wide ranging species such as Adélie penguins (Roeder et al. 2001), dusky dolphins (Cassens et al. 2005), gyrfalcons (Johnson et al. 2007) as well as this study, demonstrates the importance of genetic studies for species conservation, where direction and magnitude of migration and population structure that are difficult to observe in the wild can be measured. To designate management units, conservationists need to know whether a species, or part of it, consists of one or more metapopulations or of multiple genetically distinct populations.

Conservation implication

The Humboldt penguin is considered as threatened with extinction due to demographic decline (Birdlife International 2004) and is protected in Chile and Peru. Our observed patterns of long term and short term gene flow show that colonies can no longer be viewed as separate entities. The depletion of genetic variability, which limits evolutionary potential, and other forms of inbreeding are unlikely to be current issues for the conservation of this species and the loss of a single colony should not be viewed as a tragedy for the species as long as the overall number of colonies is not decreasing. Incidental catches of penguins in fishing nets are common and in some areas they are the main threat to this species (Simeone et al. 1999; Taylor et al. 2002). To reduce the number of penguins killed in fishing nets Culik and Luna-Jorquera (1997a) suggested protective zones of 35 km be established around breeding colonies to assure that penguins can forage safely. After studying the foraging pattern of breeding Humboldt penguins, Taylor et al. (2002) recommended that in areas where penguins forage, commercial fisheries should not use surface nets during the night and during the day they should avoid setting nets at depths of 0–30 m. While these recommendations might help the survival of colonies in areas where they are implemented it does not guarantee the colony's persistence if dispersal and gene flow are inhibited. Conservation efforts should certainly focus on protecting colonies, especially source populations such as Punta San Juan, but dispersal corridors that allow reinforcement of declining colonies or the establishment of new colonies need to be implemented. Marine reserves that include several breeding and foraging sites that allow immigration and emigration rather than colony buffer zones need to be established to take into account the fact that Humboldt penguins may disperse over thousands of kilometers. Within the boundaries of marine reserves, fishing and other human activities need to be regulated so that they do not interfere with penguin migration routes (Lubchenco et al. 2003). Properly designed, these reserves would not only benefit penguins and other seabirds but also local fishing industries, as conservation measures within marine reserves have been shown to increase fishing yields outside the reserves (Lubchenco et al. 2003; Palumbi 2003).

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Appendix

Appendix 1 Allele frequencies, number of samples (n), observed (H_o) and expected heterozygosity (H_e) for each locus in each population

Locus	Allele	PSJ $n = 86$	CA $n = 21$	Alg $n = 200$	PH $n = 29$	
Sh1Ca9	123	0.000	0.000	0.000	0.034	
	125	0.017	0.024	0.025	0.000	
	127	0.238	0.214	0.285	0.224	
	129	0.006	0.000	0.000	0.000	
	131	0.006	0.000	0.000	0.000	
	133	0.058	0.048	0.055	0.069	
	135	0.488	0.667	0.560	0.621	
	137	0.163	0.024	0.052	0.052	
	139	0.023	0.024	0.013	0.000	
	141	0.000	0.000	0.010	0.000	
	H_o	0.570*	0.429	0.550	0.655	
	H_e	0.678	0.518	0.600	0.566	
	Sh1Ca12	117	0.029	0.000	0.023	0.017
		121	0.012	0.000	0.000	0.000
123		0.058	0.048	0.038	0.034	
125		0.012	0.000	0.005	0.017	
127		0.116	0.119	0.170	0.207	
129		0.145	0.262	0.243	0.207	
131		0.128	0.071	0.085	0.069	
133		0.058	0.000	0.007	0.000	
135		0.174	0.167	0.125	0.190	
137		0.000	0.000	0.002	0.034	
139		0.052	0.024	0.007	0.034	
141		0.174	0.286	0.240	0.121	
143		0.012	0.000	0.025	0.052	
145		0.012	0.024	0.030	0.017	
147	0.017	0.000	0.000	0.000		
H_o	0.884	0.857	0.825	0.828		
H_e	0.882	0.819	0.830	0.867		
Sh1Ca16	90	0.006	0.000	0.000	0.000	
	92	0.047	0.048	0.035	0.034	
	96	0.000	0.000	0.005	0.000	
	98	0.366	0.333	0.347	0.241	
	100	0.145	0.071	0.087	0.103	
	102	0.151	0.238	0.168	0.224	
	104	0.023	0.000	0.043	0.052	
	106	0.058	0.071	0.065	0.052	
	108	0.087	0.119	0.115	0.138	
	110	0.052	0.048	0.045	0.052	
112	0.035	0.000	0.043	0.017		
114	0.017	0.071	0.035	0.017		
116	0.006	0.000	0.010	0.000		
118	0.000	0.000	0.002	0.017		

Appendix 1 continued

Locus	Allele	PSJ <i>n</i> = 86	CA <i>n</i> = 21	Alg <i>n</i> = 200	PH <i>n</i> = 29	
Sh1Ca17	120	0.006	0.000	0.000	0.017	
	122	0.000	0.000	0.000	0.034	
	<i>H_o</i>	0.826	0.714	0.77*	0.897	
	<i>H_e</i>	0.809	0.818	0.866	0.820	
	97	0.006	0.000	0.002	0.034	
	101	0.000	0.000	0.002	0.000	
	10	0.000	0.000	0.000	0.034	
	105	0.099	0.000	0.075	0.069	
	107	0.395	0.429	0.270	0.224	
	109	0.081	0.214	0.225	0.121	
	111	0.099	0.095	0.090	0.052	
	113	0.058	0.071	0.100	0.121	
	115	0.058	0.095	0.075	0.155	
	117	0.012	0.000	0.025	0.052	
	119	0.186	0.095	0.087	0.103	
	121	0.006	0.000	0.045	0.034	
	123	0.000	0.000	0.002	0.000	
<i>H_o</i>	0.744	0.809	0.835	0.862		
<i>H_e</i>	0.780	0.756	0.839	0.887		
Sh2Ca12	97	0.017	0.000	0.002	0.000	
	99	0.203	0.262	0.203	0.241	
	101	0.198	0.167	0.213	0.155	
	103	0.140	0.071	0.083	0.138	
	107	0.407	0.452	0.435	0.466	
	109	0.006	0.024	0.018	0.000	
	111	0.029	0.024	0.040	0.000	
	113	0.000	0.000	0.007	0.000	
	<i>H_o</i>	0.733	0.714	0.740	0.724	
	<i>H_e</i>	0.737	0.710	0.718	0.694	
	Sh2Ca21	114	0.169	0.214	0.220	0.241
118		0.076	0.167	0.115	0.069	
120		0.558	0.571	0.522	0.586	
122		0.006	0.000	0.002	0.017	
124		0.093	0.048	0.060	0.034	
126		0.029	0.000	0.018	0.000	
128		0.023	0.000	0.015	0.000	
130		0.041	0.000	0.043	0.017	
134		0.006	0.000	0.005	0.034	
<i>H_o</i>		0.581	0.524	0.670	0.517	
<i>H_e</i>		0.646	0.612	0.661	0.601	
Locus		Allele	<i>n</i> = 96	<i>n</i> = 21	<i>n</i> = 200	<i>n</i> = 29
Sh2Ca22		94	0.337	0.381	0.407	0.466
		96	0.064	0.000	0.055	0.069
	100	0.000	0.000	0.002	0.000	
	104	0.047	0.048	0.027	0.017	

Appendix 1 continued

Locus	Allele	<i>n</i> = 96	<i>n</i> = 21	<i>n</i> = 200	<i>n</i> = 29
	108	0.041	0.048	0.030	0.000
	112	0.000	0.000	0.005	0.000
	114	0.262	0.190	0.245	0.293
	116	0.029	0.000	0.013	0.034
	118	0.169	0.262	0.183	0.103
	120	0.000	0.024	0.020	0.000
	122	0.000	0.000	0.010	0.017
	124	0.023	0.024	0.002	0.000
	128	0.029	0.024	0.000	0.000
	<i>H_o</i>	0.686*	0.857	0.715*	0.414*
	<i>H_e</i>	0.784	0.762	0.737	0.692
Locus	Allele	<i>n</i> = 85	<i>n</i> = 21	<i>n</i> = 200	<i>n</i> = 29
Sh2Ca31	108	0.006	0.000	0.000	0.000
	114	0.000	0.000	0.002	0.034
	116	0.024	0.000	0.000	0.017
	118	0.224	0.214	0.333	0.310
	120	0.118	0.024	0.035	0.034
	122	0.141	0.095	0.130	0.052
	124	0.224	0.405	0.228	0.224
	126	0.047	0.048	0.020	0.034
	128	0.165	0.167	0.180	0.293
	130	0.047	0.048	0.058	0.000
	132	0.006	0.000	0.015	0.000
<i>H_o</i>	0.765	0.809	0.765	0.724	
<i>H_e</i>	0.839	0.767	0.785	0.774	
Locus	Allele	<i>n</i> = 79	<i>n</i> = 21	<i>n</i> = 187	<i>n</i> = 27
Sh2Ca40	84	0.019	0.048	0.008	0.000
	86	0.025	0.048	0.027	0.037
	90	0.032	0.000	0.013	0.019
	92	0.354	0.286	0.361	0.259
	94	0.158	0.071	0.088	0.093
	96	0.139	0.238	0.160	0.204
	98	0.032	0.000	0.040	0.056
	100	0.044	0.071	0.067	0.056
	102	0.108	0.119	0.107	0.111
	104	0.044	0.048	0.045	0.074
	106	0.019	0.000	0.043	0.019
	108	0.013	0.071	0.032	0.019
	110	0.006	0.000	0.005	0.000
	112	0.000	0.000	0.003	0.019
	114	0.006	0.000	0.000	0.019
	116	0.000	0.000	0.000	0.019
<i>H_o</i>	0.861	0.714	0.759*	0.926	
<i>H_e</i>	0.816	0.845	0.815	0.871	

Appendix 1 continued

Locus	Allele	<i>n</i> = 86	<i>n</i> = 21	<i>n</i> = 200	<i>n</i> = 29	
Sh2Ca49	100	0.006	0.000	0.000	0.000	
	108	0.000	0.000	0.005	0.000	
	110	0.250	0.143	0.210	0.276	
	114	0.105	0.095	0.105	0.017	
	116	0.163	0.405	0.310	0.397	
	118	0.058	0.071	0.035	0.069	
	120	0.209	0.095	0.172	0.086	
	122	0.110	0.119	0.065	0.052	
	124	0.099	0.071	0.097	0.103	
	<i>H_o</i>	0.895	0.857	0.815	0.724	
	<i>H_e</i>	0.836	0.792	0.806	0.754	
	Sh2Ca55	94	0.000	0.000	0.005	0.000
		96	0.406	0.381	0.450	0.534
102		0.000	0.000	0.002	0.000	
106		0.047	0.048	0.027	0.017	
110		0.051	0.048	0.038	0.000	
114		0.012	0.000	0.007	0.000	
116		0.247	0.190	0.243	0.293	
118		0.029	0.000	0.013	0.034	
120		0.165	0.262	0.185	0.103	
122		0.000	0.024	0.018	0.000	
124		0.006	0.000	0.010	0.017	
126		0.018	0.024	0.002	0.000	
128		0.012	0.000	0.000	0.000	
130		0.018	0.024	0.000	0.000	
<i>H_o</i>		0.694*	0.857	0.735*	0.414	
<i>H_e</i>		0.746	0.762	0.703	0.627	
Sh2Ca58		105	0.000	0.000	0.005	0.052
	107	0.424	0.548	0.498	0.448	
	109	0.576	0.452	0.498	0.466	
	113	0.000	0.000	0.000	0.034	
	<i>H_o</i>	0.500	0.524	0.455*	0.586	
	<i>H_e</i>	0.491	0.507	0.506	0.589	

* indicates heterozygote deficiency after Bonferroni correction ($P < 0.0042$)

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