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RESEARCH ARTICLE

Low genetic diversity and lack of population structure in the endangered Galápagos penguin (*Spheniscus mendiculus*)

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Abstract Long-term monitoring of the endangered Galápagos penguin (*Spheniscus mendiculus*) has indicated poor reproductive periods and severe population fluctuations in association with El Niño – Southern Oscillation events. An earlier mark and recapture study indicated that adults exhibit some degree of breeding-site and mate fidelity, and that juveniles potentially move more frequently than adults; however, the extent to which migrants and gene flow occur between islands within the Galápagos archipelago is largely unknown. This study tested the hypothesis that geographic isolation and adult breeding philopatry has led to a degree of genetic differentiation between island subpopulations within the archipelago. We examined the genetic diversity within and among different subpopulations and the extent to which gene flow occurs between island subpopulations. Estimates of allelic richness and gene diversity were not significantly different between subpopulations. Tests to detect genetic heterogeneity failed to reject the H_0 of no difference in allele frequencies for chi-square ($P = 0.28$) and Fisher's exact test ($P = 0.19$). All pairwise values of the F_{ST} variant θ were not significant, while a power analysis revealed a >99% probability of detecting a biologically true F_{ST} of 0.05. Migration estimates in BAYESASS+ suggest symmetrical gene flow throughout the species' distribution. Our results indicate a low level of genetic diversity throughout the population and a seemingly high level of gene flow between subpopulations. We argue that the Galápagos penguin should be managed as one panmictic population and we discuss the risk of disease threats in the archipelago.

Keywords Conservation · Galápagos Islands · Galápagos penguin · Population genetic structure *Spheniscus mendiculus*

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Introduction

Of the four penguins in the genus *Spheniscus*, the Galápagos penguin (*Spheniscus mendiculus*) is the most northern species, residing on the equator within the Galápagos archipelago. The survival and distribution of the Galápagos penguin is dependent upon the oceanic upwelling that occurs when the eastward Cromwell current hits the Galápagos plateau (Boersma 1976). Approximately 95% of the population inhabits the western islands of Fernandina and Isabela, with smaller populations on Santiago, Bartolomé, and Floreana (Fig. 1) (Vargas et al. 2005). Long-term monitoring of the Galápagos penguin, which is listed as Endangered (BirdLife International 2005), has indicated poor reproductive periods and severe population fluctuations in association with El Niño – Southern Oscillation (ENSO) events (Boersma 1998). These events within the archipelago have been associated with disrupted wind and ocean current patterns, warmer sea

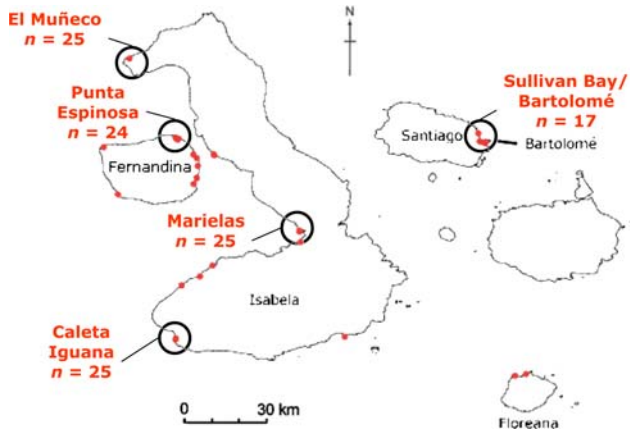

Fig. 1 Sampling sites of the Galápagos penguin; subpopulations included in analysis are indicated

surface temperatures, and increased patterns of rainfall (Glynn 1988). This causes variability in marine productivity and in the abundance of aquatic food resources (Barber et al. 1983), having drastic consequences for seabird populations (Jaksic 2004).

It is estimated that the El Niño of 1982–1983 effectively reduced the Galápagos penguin population from over 3000 birds to 699 birds; similarly, the 1997–1998 El Niño reduced the population from 2252 to 779 birds (Vargas et al. 2005). Estimates place the current Galápagos penguin population at 1500 individuals, approximately 50% smaller than pre-1982/83 El Niño population sizes (Vargas and Wiedenfeld 2004). Population recovery has been slow, possibly due to poor recruitment and high juvenile mortality coincident with a period of weak El Niño events (Vargas et al. 2006). The occurrence of ENSO events in Galápagos is not merely a recent phenomenon. Sediment-core analysis indicates that 435 moderate-very strong ENSO events have occurred over the last 7,100 years (Riedinger et al. 2002). However, there is evidence that the frequency and intensity of these events has increased over the last 30 years (Trenberth and Hoar 1996, 1997).

A mark and recapture study conducted over 2 years on banded individuals indicated a moderate level (68% of males and 76% of females recaptured at banding site, with 4% and 1% recaptured at different localities, respectively) of site fidelity amongst adults where both sexes frequently return to previous nesting sites, even when not breeding; pair-fidelity between mates over subsequent breeding periods also appears high (89%) (Boersma 1976). Juveniles are believed to move more and suffer greater mortality than adults, with 19% of banded juveniles being recaptured at the same banding site; however, further study regarding the degree of natal philopatry is still needed (Boersma 1976; Vargas et al. 2006). Annual censuses and analysis of an ongoing Passive Integrated Transponder (PIT) tag study (2003–2006) has revealed movement between subpopulations along islands, but data on the number of migrants between Fernandina and Isabela, and between both of these islands and Santiago, Bartolomé, and Floreana is lacking (Matamoros et al. 2006; Vargas et al. 2007). Similarly the extent of gene flow between islands is largely unknown.

Endangered species characteristically have low genetic diversity, often exhibiting about half the genetic variation of related non-endangered species (Frankham et al. 2002). Additionally, small populations typically possess less variation than larger populations (Frankham 1996). A 2002 study comparing the genetic diversity of the Galápagos penguin to its congener, the Magellanic penguin (*Spheniscus magellanicus*), observed microsatellite heterozygosity values of 3% and 46% for each species, respectively (Akst et al. 2002). Similar studies comparing populations of endangered and common bird congeneric species found heterozygosity values for the Mariana crow (*Corvus kubaryi*) and American crow (*C. brachyrhynchos*) at 16% and 68% (Tarr and Fleischer 1999), and the Mauritius kestrel (*Falco punctatus*) and European kestrel (*F. tinnunculus tinnunculus*) at 10% and 61% (Nichols et al. 2001). Thus, the Galápagos penguin appears to exhibit severely low genetic diversity, even when compared to other endangered bird populations.

The previous population study of the Galápagos penguin (Akst et al. 2002) indicated severely low levels of variation at five microsatellite loci, but did not test for population structure between islands. This study was restricted to a portion of Fernandina and Isabela islands and did not include the geographically disjunct subpopulations located on Santiago, Bartolomé, and Floreana. Therefore, these results are uninformative regarding an analysis of population structure in the archipelago. Further, the degrees to which the Galápagos penguin migrates throughout its range and the reproductive success of migrating adults are unclear. We examined the hypothesis that geographic isolation and adult breeding philopatry has led to a degree of genetic differentiation between island subpopulations within the archipelago. Our study quantified genetic diversity using microsatellite markers for loci previously untested in the Galápagos penguin. Based on what little is known about individual movements (Boersma 1976; Vargas et al. 2006), we predicted the following: (1) genetic diversity will be low due to genetic drift, particularly on islands such as Santiago and Bartolomé that have smaller populations; (2) the degree of divergence between subpopulations will be positively correlated with geographic distance between colonies; and (3) given current movements within islands but no large-scale migrations observed between islands, gene flow will be greater among subpopulations within islands than among those on different islands.

Methods

Field methods

Breeding colonies of the Galápagos penguin are primarily found on the northeastern coast of Fernandina, along the southwestern coast of Isabela, and smaller colonies on northeastern Santiago and northern Floreana (Fig. 1) (Vargas et al. 2005). Blood samples were collected from the subpopulations at Caleta Iguana, Marielas, El Muñeco, and Punta Espinosa along the islands of Isabela and Fernandina during the periods of 8–13 August 2003, and 10–16 March and 5–11 August 2004. Additionally, the subpopulations at Sullivan Bay, Santiago, and the islands of Bartolomé and Floreana were sampled on 13 February to 17 March 2005.

Individuals were captured and blood samples (100 µl) collected using jugular venipuncture. Blood samples were stored in a lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; Longmire et al. 1988) for DNA extraction and genetic analysis. These samples were collected as part of a regular census and monitoring project of the Galápagos penguin and the flightless cormorant conducted by the Charles Darwin Research Station, the Galápagos National Park, the St. Louis Zoo, and the University of Missouri-St. Louis. In total there were 432 samples collected from the entire population. For this analysis 116 adult individuals from five subpopulations at Caleta Iguana (n = 25), Marielas (n = 25), El Muñeco (n = 25), Punta Espinosa (n = 24), and Sullivan Bay (n = 17) were selected to examine the extent of population genetic structure across the archipelago (Fig. 1). Samples collected at Sullivan Bay and Bartolomé were pooled due to the close proximity of the islands and inferred movement between subpopulations (Vargas et al. 2005). Individuals from Floreana were excluded due to small adult sample size (n = 2).

DNA extraction and microsatellite typing methods

Samples were incubated overnight at 65°C with 300 µg Proteinase K and DNA was extracted using a phenol–chloroform procedure followed by dialysis in 1 x TNE_2 (10 mM Tris–HCl, 10 mM NaCl, 2 mM EDTA). Genotypes were identified at five polymorphic microsatellite loci (Table 1), *G3-6* developed for the Galápagos penguin (Akst et al. 2002), and *Sh1Ca12*, *Sh1Ca16*, *Sh1Ca17*, and *Sh2Ca21*, developed for the Humboldt penguin (*Spheniscus humboldti*; Schlosser et al. 2003). Polymerase chain reactions (PCR) of 10 µl were prepared that included 75 ng of whole genomic DNA, 0.1 mM dNTP's, 1.0 µl of 10× reaction buffer, 0.25 mM $MgCl_2$, 0.5 µg of both forward and reverse primer, 0.1 µl of BSA, and 0.5 units of *Taq* DNA polymerase (Biolase Red). The reaction conditions were as follows: 3 min at 95°C, 35 cycles at 95°C for 30 s, 40 s at 53–60°C, and 40 s at 72°C, and a final extension at 72°C for 10 min (Akst et al. 2002; Schlosser et al. 2003). Forward primers were fluorescently labeled and fragment analysis of the amplified product was conducted on an ABI Prism™ 3100-Avant genetic analyzer (PE Applied Biosystems). Data were analyzed and multilocus microsatellite genotypes were assigned using the software program GeneMapper™ (Applied Biosystems).

Statistical analysis

Genetic diversity and differentiation were examined within and between colonies at five microsatellite loci with the program FSTAT version 2.9.3.2 (Goudet 2002). Allelic richness was calculated by rarefaction analysis to account for uneven sample sizes (El Mousadik and Petit 1996; Petit et al. 1998). Genetic diversity was quantified by numbers of alleles per locus and by Nei's gene diversity statistics, an

Table 1 Total number of alleles (N), Nei's unbiased gene diversity (h), and estimates of allelic richness (R_S for each subpopulation and locus, R_T over all subpopulations) are reported at five microsatellite loci for five subpopulations of the Galápagos penguin

	Caleta Iguana (n = 25)			El Muñeco (n = 25)			Marielas (n = 25)			Punta (n = 24)			Espinosa	Sullivan Bay (n = 17)			Total (n = 116)		
Locus	N	h	R_S	N	h	R_S	N	h	R_S	N	h	R_S	N	h	R_S	N	h mean	R_T
G3-6	2	0.04	1.68	2	0.12	1.97	2	0.12	1.97	2	0.08	1.92	2	0.17	2.00	2	0.10	1.86
Sh1Ca12	2	0.39	2.00	2	0.46	2.00	2	0.30	2.00	2	0.42	2.00	2	0.37	2.00	2	0.39	2.00
Sh1Ca16	4	0.65	3.68	3	0.67	3.00	4	0.67	3.90	3	0.67	3.00	3	0.65	3.00	4	0.66	3.38
Sh1Ca17	2	0.45	2.00	2	0.30	2.00	2	0.48	2.00	3*	0.53	2.92	2	0.50	2.00	3	0.45	2.27
Sh2Ca21	4	0.60	3.00	4	0.65	3.97	4	0.70	3.90	3	0.58	3.00	3	0.56	3.00	4	0.62	3.55
All loci	14	–	–	13	-	–	14	–	–	14	–	–	14	–	–	15	–	–
Mean	2.8	0.43	2.47	2.6	0.44	2.59	2.8	0.45	2.76	2.6	0.46	2.57	2.4	0.45	2.40	3	0.44	2.61

The total number of individuals genotyped (n) is given in parentheses. Allelic richness is based on rarefaction analysis of 17 individuals. A * indicates private allele among subpopulations

unbiased estimator of heterozygosity (Nei 1987). Samples were tested for Hardy–Weinberg equilibrium using F_{IS} after 1,000 randomizations. Linkage disequilibrium was tested using the log-likelihood ratio *G*-statistic after 1,000 permutations. Sequential Bonferroni corrections were applied to account for multiple comparisons (Rice 1989).

Genetic differentiation between sampled sites was measured in two ways. The first method tested the hypothesis of allele frequency heterogeneity between each pair of subpopulations. The test suggested as most appropriate for combining information from multiple loci varies across studies (Ryman et al. 2006). Therefore, tests were conducted using both Pearson's traditional contingency chi-square and Fisher's exact test as implemented by the program CHIFISH (Ryman 2006). The chi-square approach sums the chi-square test statistics and their associated degrees of freedom and gives the *P*-value for this sum; it is particularly robust in situations dealing with few alleles per locus (Ryman and Jorde 2001, Ryman et al. 2006). Similar to the STRUC routine in the program GENEPOP (Raymond and Rousset 1995), the *P*-values for each locus-comparison in Fisher's exact test are estimated with CHIFISH using an unbiased Markov chain algorithm and are then combined by means of Fisher's method (Ryman 2006). The *P*-values from Fisher's exact test were obtained using 10,000 dememorization steps and a total of 500,000 iterations (100 batches with 5,000 iterations/batch).

Secondly, genetic differentiation between subpopulations was estimated using Weir and Cockerham's (1984) variant of F_{ST}, θ, in FSTAT. This measure summarizes the distribution of variation within and among populations and also accounts for variable sample and population sizes (Weir and Cockerham 1984). The analog of F_{ST} developed specifically for microsatellites, R_{ST}, is excluded in this analysis as F_{ST} is a better index when the number of loci is less than $\sim$20 (Gaggiotti et al. 1999). To test for a relationship between the extent of genetic differentiation and geographical distance between subpopulations, the nonparametric Mantel test using the program IBD (Bohonok 2002) was used to test for isolation by distance. Geographical distance was measured as the shortest distance between two subpopulations and distances were log-transformed before analysis.

Traditional estimates of gene flow within subdivided populations have often relied upon Wright's (1951) equation 4 Nm = 1/F_{ST} − 1, where m corresponds to migration rate. However, this model is based on several unrealistic assumptions including constant population sizes and symmetrical rates of migration. Therefore, in this study, estimates of recent migration rates between subpopulations were determined using a molecular assignment program that relies on a non-equilibrium Bayesian method through Markov chain Monte Carlo techniques, as implemented in BAYESASS+ (Wilson and Rannala 2003). This program estimates asymmetrical rates of migration between populations over the last several generations. The program was run after 3,000,000 MCMC iterations, with a burn-in of 1,000,000 iterations and a sampling frequency of 2000; delta was set to 0.15 (the default value). In addition to levels of gene flow, BAYESASS+ reports 95% confidence intervals for each estimate.

Finally, statistical power was analyzed with the program POWSIM (Ryman and Palm 2006). This program estimates power specifically for chi-square and Fisher's exact tests when testing for genetic homogeneity between subpopulations. Computer simulations mimic sampling from populations at various levels of expected divergence under a classical Wright–Fisher model without migration or mutation. Simulations were run to test this study's power to detect an expected divergence of F_{ST} = 0.05 between subpopulations. During the simulation the initial base population was programmed based on five microsatellite loci that exhibited defined allele frequencies as observed in our study-population; the base population was programmed to divide into five subpopulations each with N_e = 500 after 51 generations of random drift and were sampled for 25, 25, 25, 24, and 17 individuals. The latter parameters allow the simulation to distribute marker loci alleles over subpopulations that then result in a predetermined F_{ST}, and they are not meant to reproduce actual demographic or evolutionary history of the study-population. The default parameters for the number of dememorizations, batches, and iterations per batch were kept at 1000, 100, and 1000, respectively.

Results

Genetic diversity

The total number of alleles and estimates of gene diversity and allelic richness are presented in Table 1. In total, 15 alleles were identified at the five loci examined. Within each subpopulation, there were no deviations from Hardy–Weinberg equilibrium ($P > 0.215$) and pairwise comparisons of loci revealed no linkage disequilibrium (in all cases $P > 0.02$, adjusted critical value = 0.001). Two loci, *Sh1Ca16* and *Sh2Ca21*, had the highest estimates for allelic richness and gene diversity (Table 1) across subpopulations, although there were no significant differences in allelic richness (Friedman's test; $\chi^2_4 = 1.40$, $P = 0.84$) or Nei's unbiased gene diversity (Friedman's test; $\chi^2_4 = 2.80$, $P = 0.59$) among subpopulations.

Genetic differentiation and population structure

The results of the analysis with CHIFISH failed to reject the H_0 of no difference in allele frequencies between

subpopulations for both the chi-square ($P = 0.28$) and Fisher's exact test ($P = 0.19$). Similarly, there was no evidence for genetic differentiation at each individual locus (in all cases $P > 0.05$). The largest pairwise value of the F_{ST} variant θ was 0.0405 between Caleta Iguana and Punta Espinosa (Table 2). However, none of the pairwise comparisons for genetic heterogeneity were significant (in all cases $P > 0.007$, adjusted nominal level = 0.005). The results of the Mantel test using IBD also did not detect a significant relationship between genetic differentiation and geographic distance ($P = 0.927$).

Migration rates

The results of the migration rates estimated in BAYESASS+ suggest a consistent level of gene flow throughout the archipelago (Table 3). The average migration rates in all pairwise comparisons were between 0.03 and 0.100. Each value represents the proportion of individuals that is derived from a corresponding source population each generation.

Power analysis

The analysis of statistical power to detect a hypothetical degree of true differentiation of $F_{ST} = 0.05$ was estimated as 0.9975. Frankham et al. (2002) indicates that a value of 0.05 for F_{ST} is an appropriate indicator of genetic structure, were it to biologically occur. The simulations run in POWSIM allow each population to diverge to some predefined F_{ST}, where populations are then sampled and tested for genetic homogeneity. The results of this analysis in POWSIM report a proportion of significant outcomes ($P < 0.05$) for summed/combined test statistics as = 0.9975 for both chi-square and Fisher's exact test. Therefore, power analysis revealed that five microsatellite loci were sufficient to provide a >99% probability of detecting an F_{ST} of 0.05 when analyzing a total of 116 specimens distributed over five sampled subpopulations.

Discussion

One of the goals of conservation genetics is to identify populations at risk and to provide information regarding management-strategies and conservation decision-making (Frankham et al. 2002). This becomes especially relevant when assessing the genetic variation of geographically isolated species, understanding population connectivity, and recommending fragmented populations as separate management units (DeSalle and Amato 2004). Genetic diversity represents the potential upon which a species may adapt and respond to changing environments (Frankham et al. 2002). Preserving genetic diversity is recognized as one of three global conservation priorities (McNeely et al. 1990). Therefore, there is a need to identify genetic diversity and how it is distributed throughout the archipelago.

This study presents evidence for the lack of population structure among subpopulations of the Galápagos penguin throughout the Galápagos archipelago. Our results indicate a low level of genetic diversity throughout the population and a seemingly high level of gene flow between subpopulations. There were no deviations from Hardy–Weinberg or linkage equilibrium, suggesting complete random mating within and among populations. Further, measures of allelic richness and gene diversity were not significantly different between subpopulations. The chi-square, Fisher's exact test, and Weir and Cockerham's θ all failed to reject the H_0 of genetic homogeneity among the sampled populations.

The results of this study are strengthened by the inclusion of an analysis of power. The concept of power involves the probability of obtaining a statistically significant result given a biologically real effect (Lowe et al. 2004). Power allows the researcher to examine whether a non-significant result is due to lack of biological effect or because of limitations in the experimental design. This concept has been previously overlooked in population genetics studies to the potential detriment of conservation and management decisions (Taylor and Dizon 1999). Several factors affecting power include sample sizes, the

Table 2 Estimate of genetic differentiation among subpopulations of the Galápagos penguin

Island	Caleta Iguana	El Muñeco	Marielas	Punta Espinosa	Sullivan Bay
Caleta Iguana	–	0.0083	0.0105	0.0405	0.0148
El Muñeco	0.10 (123)	–	0.0328	0.0373	0.0340
Marielas	0.19 (74)	0.02 (90)	–	0.0068	0.0099
Punta Espinosa	0.01 (87)	0.01 (38)	0.17 (55)	–	−0.0025
Sullivan Bay	0.22 (155)	0.04 (135)	0.14 (225)	0.29 (173)	–

Pairwise values of the F_{ST} variant θ are displayed above the diagonal. *P*-values are below (with geographic distance in km in parentheses) the diagonal. No values were significant (Bonferroni corrected *P*-value at = 0.005)

Table 3 Estimates of migration rates (proportion of individuals) among subpopulations of Galápagos penguins, derived by BAYESASS+

		Migration from				
		Caleta Iguana	El Muñeco	Marielas	Punta Espinosa	Sullivan Bay
Migration into	Caleta Iguana	0.735 (0.669–0.926)	0.063 (0.001–0.207)	0.075 (0.000–0.229)	0.062 (0.00–0.234)	0.066 (0.00–0.224)
	El Muñeco	0.100 (0.001–0.253)	0.740 (0.668–0.938)	0.048 (0.000–0.169)	0.069 (0.000–0.234)	0.043 (0.000–0.200)
	Marielas	0.096 (0.003–0.215)	0.033 (0.000–0.135)	0.726 (0.668–0.868)	0.086 (0.001–0.219)	0.059 (0.000–0.213)
	Punta Espinosa	0.040 (0.000–0.169)	0.034 (0.000–0.155)	0.068 (0.000–0.234)	0.782 (0.671–0.971)	0.078 (0.000–0.265)
	Sullivan Bay	0.089 (0.000–0.232)	0.042 (0.000–0.176)	0.040 (0.000–0.182)	0.081 (0.000–0.256)	0.748 (0.669–0.939)

Means of the posterior distributions of *m*, the migration rate into each population, are shown. The populations from which each individual was sampled are listed in the rows, while their populations from which they migrated are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Values in parentheses below migration rates are 95% CI

magnitude of biological divergence, and the number and type of loci assayed; therefore the complexity of power analysis has typically led to a lack of quantitative assessments in population genetic studies (Ryman and Palm 2006).

The Galápagos penguin has reported low genetic diversity at microsatellite loci, particularly in comparison with its congener the Magellanic penguin (Akst et al. 2002). Our study examined genetic diversity at four additional microsatellite loci and one locus (*G3-6*) that was included in the previous study. The amount of genetic diversity at these additional loci was higher than that detected in the previous analysis. Akst et al. (2002) reported that the average observed heterozygosity was 3% in the Galápagos penguin. Our study indicated an average observed heterozygosity of 45% at the microsatellite loci assayed. Analysis at these additional loci also indicated a trend of greater evenness amongst allele frequencies. While this number may be biased, as only polymorphic loci were considered, it does indicate a greater level of diversity than previously noted.

The previous genetic study of the Humboldt penguin, the closest relative to the Galápagos penguin (Baker et al. 2005), revealed greater allelic diversity at microsatellite loci than detected in our study of the Galápagos penguin. The number of alleles detected at these four loci, *Sh1Ca12, Sh1Ca16, Sh1Ca17, and Sh2Ca21*, in 24 individuals of the Humboldt penguin were 11, 9, 9, and 6, respectively (Schlosser et al. 2003). Additional comparisons of genetic diversity between the Galápagos penguin and closely related taxa are difficult due to the lack of published genetic studies on penguins. Roeder et al. (2001) did not calculate allelic richness or Nei's gene diversity in their genetic analysis of the Adélie penguin (*Pygoscelis adeliae*); however, the number of alleles detected at seven microsatellite loci ranged from 4 to 20, with an average of 9.9 alleles per locus. Similarly, the number of alleles detected at five microsatellite loci in the Magellanic penguin ranged from 1 to 19, with an average of 8.4 alleles per locus (Akst et al. 2002). In our study the number of alleles detected at five microsatellite loci ranged from 2 to 4, with an average of 3.0 alleles per locus. When looking strictly at the average number of alleles per locus, the Galápagos penguin has lower genetic diversity compared to other penguin species.

Comparisons of the extent of population structure between the Galápagos penguin and other penguin species indicate results similar to our study. Roeder et al. (2001) failed to detect structure in subcolonies of the Adélie penguin around the Antarctic continent (pairwise F_{ST} values were all ≤ 0.02). This result was unexpected due to the high degree of natal philopatry and large geographical range of the species, and it may be due to the large effective population size. Similarly, Schlosser et al. (*unpublished*) also failed to detect structure in Humboldt penguins, although a Mantel test indicated a significant level ($P < 0.05$) of isolation by distance. In regards to other seabirds in the Galápagos archipelago, there was no significant structure detected in populations of the waved albatross (*Phoebastria irrorata*) on the island of Española (Huyvaert and Parker 2006). However, analyses of populations of the Galápagos petrel (*Pterodroma phaeopygia*) point toward three genetic populations that are significantly structured between islands (Friesen et al. 2006).

The limited distribution of the Galápagos penguin is primarily based on oceanic currents and the major upwelling zones that occur in the western portion of the archipelago (Boersma 1976, 1998). While these currents and primary productivity may be limiting potential breeding colonies, there is no indication that these same currents affect the level of gene flow within the population. Migration rates were fairly consistent and suggest a symmetrical degree of gene flow between subpopulations. This is particularly remarkable in light of the most potentially isolated colony on eastern Santiago. Estimates for the number of migrants from Sullivan Bay to all other colonies were not disproportionately lower despite its seeming remoteness from other subpopulations. Therefore, geographic distance between islands does not seem to act as a

barrier to gene flow within the archipelago. The possibility that subpopulations constitute separate management units is not supported. Rather these data suggest that all populations may be important sources of migrants between subpopulations within the archipelago.

If the Galápagos penguin moves readily throughout its range, any introduced disease has a greater chance of spreading throughout the population. A previous hematological study has shown the Galápagos penguin to be seronegative for several avian diseases including West Nile virus and avian influenza (Travis et al. 2006). A species of microfilaria, likely the same detected at greater prevalence in the flightless cormorant (*Phalacrocorax harrisi*), has been detected in the Galápagos penguin (Merkel et al. 2007; Parker et al. 2006; Travis et al. 2006). Further, the presence of the mosquito *Culex quinquefasciatus*, a vector of avian malaria, has been confirmed (Whiteman et al. 2005), although thus far *Plasmodium* spp., which can have life threatening consequences for penguins in captivity, have not been detected (Parker et al. 2006).

The conclusion of panmixia does cause concern regarding the conservation of the Galápagos penguin. In 2005, a Population and Habitat Viability Assessment (PHVA) was conducted to determine the species' long-term viability (Matamoros et al. 2006). Introduced diseases were deemed one of the highest threats facing the population. The first baseline health study determined the penguins to be immunologically naïve for all viruses tested (Travis et al. 2006). The estimated mobility between subpopulations suggests that any introduced disease could potentially spread rapidly through the species' distribution. Similarly, Galápagos Doves (*Zenaida galapagoensis*) have been shown to have high rates of gene flow in addition to being a potential reservoir/vector of avian diseases (Santiago-Alarcon et al. 2006). Any risk of infection is limited by the presence of suitable arthropod vectors, by their feeding preferences, and by the specificity of the parasites for the avian host; penguins in general may have lower inherited resistance to the diversity of tropical vectors and parasitic infections (Jones and Shellam 1999). This factor and the combined stress of intense ENSO events within the archipelago may further compromise their immune function. Continual population censuses and disease monitoring (Parker et al. 2006), and the control of anthropogenic impacts, remain a priority in Galápagos penguin conservation (Vargas et al. 2006).

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