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Genetic structure within and between island populations of the flightless cormorant (*Phalacrocorax harrisi*)

CAROLINE V. DUFFIE,* TRAVIS C. GLENN,†‡ F. HERNAN VARGAS,§¶ and PATRICIA G. PARKER*,**
*Department of Biology R223, University of Missouri-St Louis, 1 University Boulevard, St Louis, MO 63121-4499, USA, †Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA, ‡Department of Environmental Health Science, University of Georgia, Athens, GA 30602, USA, §Charles Darwin Foundation, Isla Santa Cruz, Galápagos, Ecuador, ¶Wildlife Conservation Research Unit, University of Oxford, Oxford, UK, **St. Louis Zoo, 1 Government Drive, St. Louis, MO 63110, USA

Abstract

We assessed colony- and island-level genetic differentiation for the flightless cormorant (*Phalacrocorax harrisi*), an endangered Galápagos endemic that has one of the most limited geographical distributions of any seabird, consisting of only two adjacent islands. We screened 223 individuals from both islands and nine colonies at five microsatellite loci, recovering 23 alleles. We found highly significant genetic differentiation throughout the flightless cormorant's range on Fernandina and Isabela Islands (global F_{ST} = 0.097; P < 0.0003) both between islands (supported by Bayesian analyses, F_{ST} and R_{ST} values) and within islands (supported only by F_{ST} and R_{ST} values). An overall pattern of isolation-by-distance was evident throughout the sampled range (r = 0.4169, one-sided $P \leq 0.02$) and partial Mantel tests of this relationship confirmed that ocean is a dispersal barrier (r = 0.500, one-sided $P \leq 0.003$), especially across the 5-km gap between the two islands. The degree of detected genetic differentiation among colonies is surprising, given the flightless cormorant's limited range, and suggests a role for low vagility, behavioural philopatry, or both to limit dispersal where physical barriers are absent. We argue that this population should be managed as at least two genetic populations to better preserve the species-level genetic diversity, but, for demographic reasons, advocate the continued conservation of all breeding colonies.

Keywords: Galápagos, Pelecaniformes, *Phalacrocorax harrisi*, microsatellite markers, population genetics, Bayesian statistics

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Introduction

The Galápagos archipelago is located approximately 1000 km west of the coast of South America and harbours a diversity of endemic bird taxa that have originated in isolation. The biodiversity of these islands remains largely intact (Gibbs *et al*. 1999); there are no documented extinctions of birds. In recent years, several authors have studied the population genetic structure of birds endemic to the Galápagos Islands (Galápagos hawk, *Buteo galapagoensis*, Bollmer *et al*. 2005; Galápagos finches, Geospiza and Certhidia species, Petren *et al*. 2005; Galápagos dove, Zenaida galapagoensis, Santiago-Alarcon *et al*. 2006; Galápagos petrel *Pterodroma phaeopygia*, Friesen *et al*. 2006; waved albatross *Phoebastria irrorata*, Huyvaert & Parker 2006; the Galápagos penguin, *Spheniscus mendiculus*, Nims *et al*. 2008). Taxon-specific physiological and behavioural traits interact to influence the population genetic structure among local populations of each species. For instance, Galápagos hawks, like most *Buteo* species (Kerlinger 1985), avoid flying over water gaps and have highly divergent island populations (Bollmer *et al*. 2005, 2006). In contrast, Galápagos doves are strong flyers and frequently move from island to island; consequently, gene flow is high and there is little genetic differentiation among island populations (Santiago-Alarcon *et al*. 2006).

Correspondence: Caroline Duffie, Division of Biological Infrastructure, The National Science Foundation, 4201 Wilson Boulevard, Arlington, VA 22230, USA; Fax: 703-292-9063; E-mail: caroline.duffie@gmail.com

Flightless cormorant

The flightless cormorant (Pelecaniformes; *Phalacrocorax harrisi*) is an endangered Galápagos endemic seabird (IUCN 2008)

that has evolved flightlessness during its history in the Galápagos (Johnsgard 1993). The time of the flightless cormorant's arrival to the archipelago is unknown, as are historical patterns of colonization within the archipelago; however, contemporary population trends and dispersal patterns have been well characterized over the last several decades using capture–mark–recapture techniques (e.g. Snow 1966; Harris 1979; Tindle 1984; Valle 1995; Vargas *et al*. 2005; Jiménez-Uzcátegui *et al*. 2006, 2007).

The entire population (*c*. 1800 individuals; Jimenez Uzcátegui & Vargas 2007) is distributed along less than 400 km of coastline on just two islands in the western archipelago, Isabela and Fernandina, which are separated by less than 5 km at the narrowest part. Colonies are most abundant on the western side of Isabela and on the eastern side of Fernandina (Fig. 1), presumably because these are places where food sources are most abundant and reliable (Harris 1979; Valle 1995). On the eastern side of Isabela, few colonies have formed successfully. Former studies of now-vanished colonies to the south of El Muerto revealed that reproduction attempts in those areas usually failed, most likely due to the lower food availability on this side of the island (Valle 1995).

Earlier studies of movement and behaviour have led some authors to characterize the population as sedentary (Harris 1979; Tindle 1984; but see Valle 1995). Flightless cormorants are resident at breeding colonies year round, and movement among colonies typically occurs by swimming since the birds cannot fly and are only capable of walking short distances. Within islands, dispersal distances of up to 20–30 km have been reported (Valle 1995; Vargas *et al*. 2005), although the majority of individuals stay within 2 km of their natal colony to breed (Harris 1979; Tindle 1984; Vargas *et al*. 2005). The birds also seem reluctant to venture into the open water; they have rarely been recorded more than 200 m offshore (Harris 1979; Valle 1995). However, two interisland migration events were recently recorded for the first time (Larrea 2007).

We use five variable microsatellite DNA markers to describe the genetic structure of the flightless cormorant population throughout its range. Observations of movement suggest that it is limited between islands, as well as among some colony pairs even within islands, particularly on Isabela, which is much larger than Fernandina. Thus, we predict that (i) the ocean is a dispersal barrier that promotes genetic structure between island populations, and that (ii) philopatry, low vagility, or both promote genetic differentiation even where physical barriers are absent.

Materials and methods

Sample collection and definition of sample unit

In August 2003, February–March 2004, August 2004, and February–March of 2005, we collected 19–31 genetic samples from each of six colonies on Isabela and each of three colonies on Fernandina for a total of 223 individuals representing most of the major breeding colonies in the flightless cormorant's range and over 12% of the entire population. We targeted adults that were in a stage of reproduction (i.e. courtship, nest construction, incubation, or with chicks), because these individuals represent the true breeding colony in each locale. We captured the birds by hand, and preserved 50 μL of blood in 500–700 μL of lysis buffer (Longmire *et al*. 1998). We then placed a unique transponder (AVID Microchip) on each captured individual subcutaneously over the left dorsal midphalangeal area and sealed the insertion site with tissue glue (3M Vetbond).

Some populations consisted of a single large colony (20–40 individuals), whereas others consisted of a cluster of 2–4 small colonies (4–16 individuals) separated by less than 15 km. In the latter case, we combined individuals from small colonies to achieve our target number of 20–30 samples per population, but confirmed the statistical validity of this approach prior to conducting the broad-scale analyses. Specifically, we evaluated the significance of F_{ST} for all pairs of small colonies within populations, and tested for the number of genetic populations using the software STRUCTURE (see below for methods). None of the pairwise comparisons were significant ($P > 0.05$; or where three colonies were pooled, $P > 0.013$, the adjusted critical value), but the statistical power was low due to small sample sizes (4–10 individuals) and only five scored loci. Furthermore, the analysis in STRUCTURE did not reveal distinct genetic subgroups on either island. Therefore, we will hereafter refer to 'population' as either a single, large colony or a cluster of 2–4 small colonies from which we combined individuals for analytical purposes.

Laboratory procedures

We incubated blood samples overnight in a proteinase K/SDS solution and then extracted DNA using phenol/chloroform following standard procedures (Sambrook *et al*. 1989) followed by dialysis against 1× TNE_2 (10 mM Tris–HCl, 10 mM NaCl, 2 mM EDTA). The concentration of each DNA sample was estimated by spectrophotometry and diluted to a working concentration of 20 ng/μL for use in polymerase chain reactions (PCR).

We screened a total of 31 microsatellite loci, found nine loci to be polymorphic, and amplified five in 223 individuals: PcT3 (*Phalacorocrax carbo*; Piertney *et al*. 1998), and PhC11, PhG12, PhB4, and PhB2 (Duffie *et al*. 2007), following methods therein. We amplified locus PcT3 using the following methods: PCR cocktails (7.7 μL) were prepared containing 20 ng whole genomic DNA, 1.1 μL of 10× reaction buffer (Bioline), 3.4 mM dNTPs, 3.2 mM $MgCl_2$, 0.13 μM forward primer labelled with fluorescent dye, 0.13 μM reverse primer, and 0.5 μL of *Taq* DNA polymerase (Bioline Red). PCR products were amplified using a standard protocol: initial

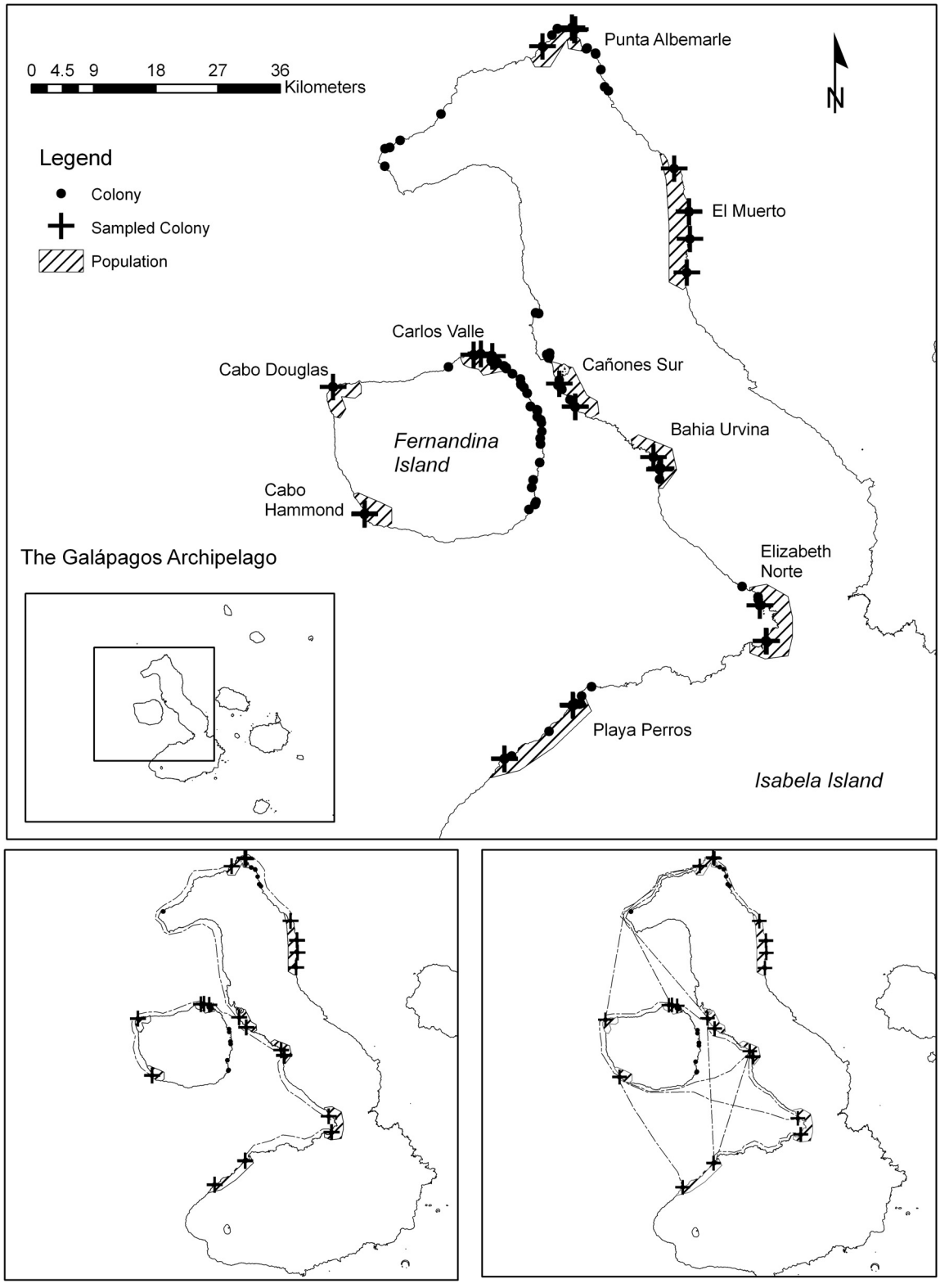

Fig. 1 Flightless cormorant colonies on Isabela and Fernandina. Sampled colonies are indicated with a cross symbol and unsampled colonies are indicated with a closed circle. Populations, defined in this study as a single large colony or 2–4 small colonies, are indicated by hatch marks. Sample sizes from southeastern Fernandina were too small to include in this study. Lower panels: hypothetical dispersal distances taken as coastline distance (left) and shortest swimming distance (right). Dispersal distances between populations can only be estimated in the absence of tracking data.

denaturation at 94 °C for 2 min, 30 cycles of 94 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s, and a final extension at 72 °C for 10 min. PCR products for all loci were resolved on an Applied Biosystems (AB) 3100 capillary sequencer and scored using GeneMapper (AB) software version 4.01.

Statistical analyses

We quantified genetic variation for each locus–population combination using numbers of alleles, genetic diversity (Nei 1973), and allelic richness (El Mousadik & Petit 1996; Petit *et al*. 1998). Significant deviations from Hardy–Weinberg (H–W) and linkage equilibria were evaluated for each population via log-likelihood ratio *G*-tests and randomization procedures, and Bonferroni corrections were applied in cases of simultaneous multiple comparisons of the data (Goudet *et al*. 1996; Goudet 1999). We also estimated null allele frequencies using the program CERVUS (Summers & Amos 1997; Marshall *et al*. 1998).

We assessed genetic differentiation using estimates of both F_{ST} (Weir & Cockerham 1984) and R_{ST} (Slatkin 1995). F_{ST} generally performs better than R_{ST} when limited numbers of loci and individuals are genotyped, especially when populations are weakly structured (Gaggiotti *et al*. 1999; Balloux & Goudet 2002). However, there are some cases where R_{ST} is superior to F_{ST}; for example, when the loci used follow the stepwise mutation model exactly (Balloux *et al*. 2000; Balloux & Lugon-Moulin 2002). Significance of F_{ST} was evaluated using a *G*-test and 1000 randomizations (Goudet *et al*. 1996) in the program FSTAT version 2.2.3 (Goudet 2002). R_{ST} values and their significance were evaluated in the program R_{ST} CALC (Goodman 1997).

We also employed a Bayesian clustering approach implemented in STRUCTURE version 2.1 to infer genetic differentiation without using information about the geographical location of sampled individuals (Pritchard *et al*. 2000). We determined the most probable number of populations, *K*, by evaluating the significance of the posterior probabilities (Pritchard *et al*. 2007), and by using the method described by Evanno *et al*. (2005) that examines Δ*K*, an *ad hoc* quantity related to the change in posterior probability between runs of different *K*. We ran the program using the admixture model and correlated allele frequencies option, which are considered most appropriate for detecting structure among populations that are likely to be similar due to migration or shared ancestry (Falush *et al*. 2003; Pritchard *et al*. 2007). We performed five runs at each *K* (*K* = 1–10) using a burn-in time of 10 000 followed by 50 000 iterations. The likelihood trace remained stationary after the burn-in time for all runs, and longer burn-in and run times did not significantly change the results (Fig. 2). Individuals were assigned probabilistically to populations, or clusters, in all cases where the proportion of membership, Q, was greater than 60%.

We explored the relationship between genetic and geographical distances to evaluate the potential for isolation-by-distance. Because flightless cormorants have not been observed to swim far from shore, coastline distances may best reflect the true dispersal path between colonies. Therefore, we measured geographical distances as the coastline distance between each pair of colonies, and placed the cross-over point between islands at the narrowest part of the channel separating the two islands [Fig. 1 (lower left panel)]. For comparison, we also measured the shortest swimming distance between colonies, which are much shorter in some cases [Fig. 1 (lower right panel)]. We converted genetic distances based on F_{ST} to Rousett's genetic distance (Rousset 1997), and evaluated the significance of the association with geographical distance using Mantel tests. We then used a partial Mantel test of this relationship to determine whether the ocean represents a dispersal barrier. All tests were based on 10 000 randomizations and were implemented in the web-based program IBDWS (Jensen *et al*. 2005).

Results

Tests of equilibrium and genetic diversity

We detected 23 alleles at five loci in 223 individuals. The number of alleles per locus ranged from three to eight (Supporting Information, Table S1). While no allele was private to a single population, three low frequency alleles occurred only in individuals sampled on Fernandina (8%, 13%, 16%), and one high frequency allele occurred only in individuals sampled on Isabela (30%). Within populations, we found no significant departures from H–W equilibrium ($P > 0.0011$, the adjusted critical value), no evidence for linkage among the five loci ($P > 0.00005$, the adjusted critical value, for each pair of loci across all populations), and only low frequencies for null alleles per locus ($f_o < 0.08$). We found consistently high levels of genetic diversity (mean expected heterozygosity ranged from 0.51 to 0.66) and low values for allelic richness (mean R_A ranged from 3.60 to 3.99) for all locus-colony combinations (Table S1). Significant differences among populations for either measure were rejected using a Friedman's test ($\chi^2 = 7.89$, $P = 0.44$; genetic diversity, and $\chi^2 = 9.31$, $P = 0.31$; allelic richness).

Population genetic structure

Our estimate of F_{ST} over all populations and loci was 0.097, which is highly significant ($P < 0.0003$; 95% bootstrap confidence intervals were 0.078 and 0.119). Our estimate of R_{ST} was 0.084, and was also highly significant ($P < 0.000001$; 95% bootstrap confidence intervals were 0.078 and 0.134). Only five of the 36 pairwise F_{ST} values were nonsignificant (Table 1). Of these, four corresponded to pairs of populations that are geographical neighbours. The only nonsignificant

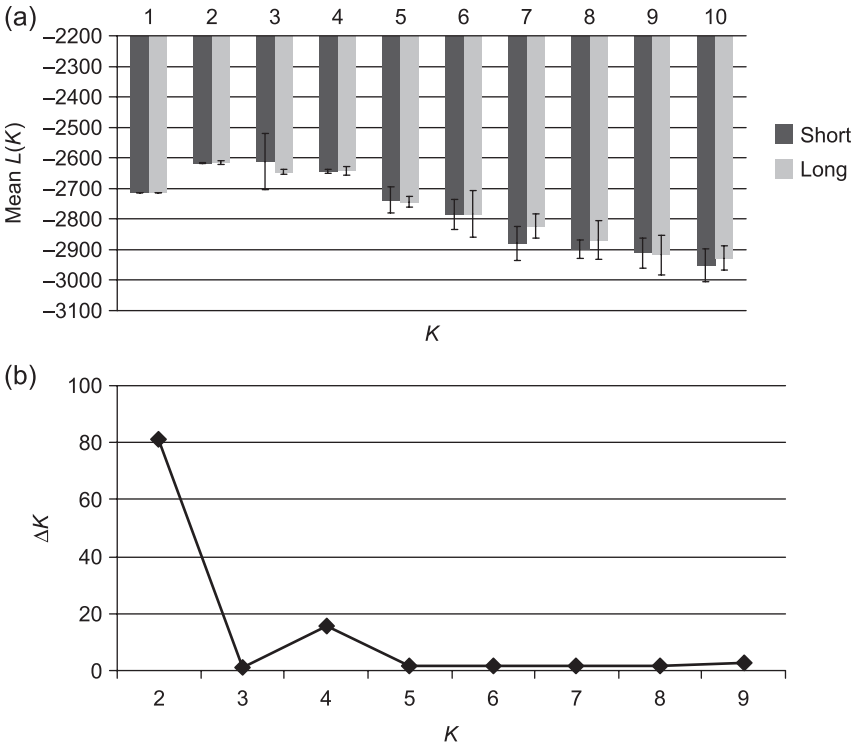

Fig. 2 (a) Plot of mean and standard deviation of the posterior probabilities, L(*K*), among runs for each value of *K*, 1–10. (b) Plot of Δ*K* vs. *K*.

Table 1 Estimates of genetic differentiation for flightless cormorants from six populations on Isabela and three populations on Fernandina. Pair-wise F_{ST} values are below, and R_{ST} values are above the diagonal. High***, medium**, and low* significance corresponds to significance at the 0.1%, 1%, and 5% nominal levels, respectively (Bonferroni corrected *P*-value at α = 0.05 was 0.001)

	Isabela						Fernandina		
	El Muerto	Punta Albemarle	Cañones Sur	Bahia Urvina	Elizabeth Norte	Playa Perros	Carlos Valle	Cabo Douglas	Cabo Hammond
El Muerto	—	0.00	0.09***	0.05	0.05	0.03	0.05	0.01	0.07
Punta Albemarle	0.02	—	0.11***	0.06	0.03	0.09*	0.13***	0.07***	0.16***
Cañones Sur	0.08***	0.07***	—	0.01	0.10*	0.09	0.12***	0.13*	0.25***
Bahia Urvina	0.07**	0.05**	0.00	—	0.03	0.10***	0.10*	0.06	0.15***
Elizabeth Norte	0.05	0.04*	0.04*	0.01	—	0.12***	0.16***	0.09*	0.17***
Playa Perros	0.05**	0.09***	0.1**	0.14***	0.13***	—	0.06	0.07*	0.16***
Carlos Valle	0.11***	0.13***	0.1***	0.11***	0.12***	0.1***	—	0.02	0.07
Cabo Douglas	0.11***	0.12***	0.09***	0.07***	0.1***	0.15***	0.02	—	0.02
Cabo Hammond	0.19***	0.18***	0.18***	0.16***	0.17***	0.21***	0.04**	0.03*	—

F_{ST} value to occur between non-neighbouring populations was between El Muerto and Elizabeth Norte, but while nonsignificant, the associated *P* value is small (*P* = 0.002, Bonferroni-corrected *P* value at α = 0.05 was 0.001). The pairwise R_{ST} values between El Muerto and all populations except Cañones Sur were all nonsignificant after Bonferroni corrections; however, before applying Bonferroni corrections, only pairs with Punta Albemarle (*P* = 0.30) and, interestingly Cabo Douglas (*P* = 0.266), were nonsignificant.

The Bayesian analysis strongly supported two genetic clusters (*P*~1), and strongly rejected a single genetic cluster or more than two clusters (*P*~0, *K* = 1, 3–5). Evaluation of Δ*K* (Evanno *et al*. 2005) identified a modal value at *K* = 2 and *K* = 4, but the signal was much stronger for *K* = 2 (Fig. 2). At higher values for *K*, the proportion of membership (Q) shifted for all individuals but much more markedly for individuals in Genetic Cluster 1. However, distinct subgroups within Genetic Cluster 1 were not apparent, as most individuals had

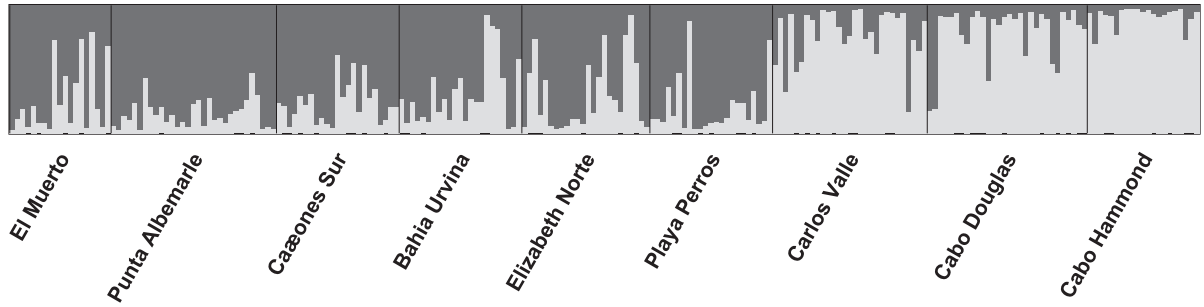

Fig. 3 Plot of posterior probability of assignment for 223 flightless cormorants (vertical lines) to two genetic clusters based on Bayesian analysis of variation at five microsatellite loci. Individuals are grouped by population, and the population names are indicated along the horizontal axis. Light grey = Genetic Cluster 1; dark grey = Genetic Cluster 2.

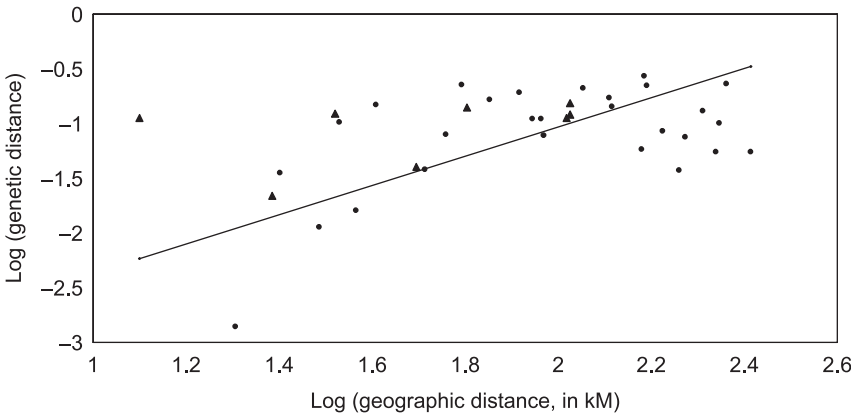

Fig. 4 Scatter plot of log (genetic) vs. log (geographic) distances for all population pairs overlaid by the RMA regression line. Data points representing populations paired with El Muerto are indicated by triangles rather than squares.

approximately symmetrical Q values for multiple clusters, and thus, could not be assigned. Therefore, we present results for $K = 2$, the number of populations that appears to capture the major genetic structure in our data set.

All runs at $K = 2$ produced identical clustering solutions and corresponded closely to the sampled populations on Isabela and Fernandina (Fig. 3). Out of 223 individuals, 201 individuals were assigned to either genetic cluster (i.e. had Q > 60%). Of the 143 individuals sampled on Isabela, 128 were assigned to Genetic Cluster 1, and 14 were assigned to Genetic Cluster 2. Of the 80 individuals sampled on Fernandina, 74 were assigned to Genetic Cluster 2 and four were assigned to Genetic Cluster 1. Sixty per cent of the individuals sampled on Fernandina and assigned to Genetic Cluster 2 had values for Q > 90%, indicating strong assignments, whereas only 38% of individuals sampled on Isabela and assigned to Genetic Cluster 1 had values of Q > 90%.

Isolation-by-distance

Analyses performed in IBD version 2.1 revealed that genetic distances show a significant, positive relationship with coastline distances ($r = 0.44$, one-sided $P \leq 0.010$ from 1000 randomizations), but not with the shortest swimming distances ($r = 0.038$, one-sided $P \leq 0.129$ from 1000 randomizations). Correlation of genetic distances and the island matrix was also highly significant ($r = 0.56$, one-sided $P \leq 0.001$ from 1000 randomizations). Finally, genetic distance show a significant, positive correlation with coastline distance, after controlling for islands ($r = 0.42$, one-sided $P \leq 0.020$ from 1000 randomizations). The r^2 value for the reduced major axis regression of log (genetic distance) vs. log (geographical distance) was 0.196 (Fig. 4). Therefore, these data show a significant, positive relationship between genetic and (coastline) geographical distance and reveal that the ocean is a dispersal barrier for this species.

Discussion

Insular organisms that shift from having high dispersal capabilities to sedentariness over time display potential for population differentiation at much smaller spatial scales (Blondel 2000). We present the flightless cormorant as an avian example for this trend, referred to as the 'island syndrome' (Blondel 2000). Our findings show that the flightless cormorant population is genetically differentiated (global $F_{ST} = 0.097$) throughout its extremely limited breeding range on Isabela and Fernandina Islands. Genetic differentiation of seabird populations within a single archipelago or island has been reported between seasonal (allochronous) populations

of band-rumped storm-petrel and Leach's storm-petrel (Friesen *et al*. 2007 and references therein), and among island populations of the Galápagos petrel (Friesen *et al*. 2006). However, this is one of the first studies to present evidence for significant genetic differentiation among synchronously breeding seabird populations within an archipelago.

Our results indicate that genetic structure is strongest between island populations. Most interisland pairwise F_{ST} and R_{ST} comparisons were highly significant, private alleles occurred at each island (one in high frequency), and Mantel tests confirmed that the ocean is a dispersal barrier. Further genetic subdivision within islands was also supported by F_{ST} and R_{ST} estimates and Mantel tests confirmed an overall pattern of isolation-by-distance, but these findings were not supported by Bayesian analysis in STRUCTURE (discussed below). No allele was private to a single population within either island; however, significant pairwise F_{ST} values occurred between Cabo Hammond and Carlos Valle on Fernandina and between most non-neighbouring populations on Isabela, with the population at El Muerto being the only exception. El Muerto is the most geographically isolated population on northeastern Isabela, but low F_{ST} and R_{ST} values between it and many of the other populations on Isabela and Fernandina indicate that it is not the most genetically isolated (Table 1). Historically, populations on the eastern side of Isabela have tended to have poor reproductive success (Valle 1995), and some colonies to the south of El Muerto have vanished. If the reproductive success of the El Muerto population is similarly low (unknown), then migrants from other populations would be necessary to maintain its numbers.

The overall pattern of these findings (apart from El Muerto) suggests that dispersal is limited in many parts of the range even where physical barriers do not exist, and that low vagility, behavioural philopatry, or both may serve to promote genetic differentiation as a function of distance. Philopatry is a common trait for seabirds and has been implicated as a factor to promote genetic differentiation among island populations of at least one seabird species (Galápagos petrel; Friesen *et al*. 2006), and even stronger patterns of isolation-by-distance have been reported but at much larger spatial scales (reviewed in Friesen *et al*. 2007).

Bayesian analysis performed in STRUCTURE confirmed the genetic differentiation observed between island populations but did not confirm further genetic differentiation within islands. Simulation studies have shown that the program STRUCTURE performs well to infer the number of clusters when clusters were not well differentiated (F_{ST} = 0.02–0.03; reviewed in Latch *et al*. 2006). However, there are several possibilities why STRUCTURE did not detect more subdivision among flightless cormorant populations within islands, some of which had pairwise F_{ST} values as high as 0.14. Generally speaking, the underlying model of STRUCTURE is not well suited to delineate distinct genetic groups in populations that reflect a relationship of isolation-by-distance (Pritchard *et al*. 2007) such as we found in the present study (discussed above). Also, the arrangement of populations into two hierarchical levels (between- and within-island) may present some challenges according to a simulation study (Evanno *et al*. 2005), which showed that only the uppermost hierarchical level was detected in populations for which multiple hierarchical levels of population structure were present.

Implications

Our results from STRUCTURE indicate that the two island populations in the flightless cormorant's range are genetically distinct and should be managed as separate conservation units (*sensu* Moritz 1994) to best preserve the genetic diversity at the species level. However, for demographic reasons we advocate the preservation of all major breeding colonies throughout the flightless cormorant's range. As an island endemic, the flightless cormorant is inherently more vulnerable to extinction than species with larger population sizes and broader distributions (Frankham 1997, 1998, 2005; Frankam & Kingsolver 2004), and thus the protection of all major colonies is warranted.

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References

Balloux F, Brünner H, Lugon-Moulin N, Jausser J, Goudet J (2000) Microsatellites can be misleading: an empirical and simulation study. *Evolution*, **54**, 1313–1422.

Balloux F, Goudet J (2002) Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Molecular Ecology*, **11**, 771–783.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology*, **11**, 155–165.

Blondel J (2000) Evolution and ecology of birds on islands: trends and prospects. *Vie et Milieu-Life and Environment*, **50**, 205–220.

Bollmer JL, Kimball RT, Whiteman NK, Sarasola JH, Parker PG (2006) Phylogeography of the Galápagos hawk (*Buteo galapagoensis*): a recent arrival to the Galápagos Islands. *Molecular Phylogenetics and Evolution*, **39**, 237–247.

Bollmer JL, Whiteman NK, Cannon M, Bednarz J, De Vries T, Parker PG (2005) Population genetics of the Galápagos hawk (*Buteo galapagoensis*): genetic monomorphism within isolated populations. *Auk*, **122**, 1210–1224.

Duffie C, Glenn T, Hagen C, Parker P (2007) Microsatellite markers isolated from the flightless cormorant (*Phalacrocorax harrisi*). *Molecular Ecology Resources*, **8**, 625–627.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic of Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, **164**, 1567–1587.

Frankam R, Kingsolver JG (2004) Response to environmental change: adaptation or extinction. In: *Evolutionary Conservation Biology* (eds Ferrier R, Dieckman U, Couvet D), pp. 85–100. Cambridge University Press, Cambridge, UK.

Frankham R (1997) Do island populations have less genetic variation than mainland populations? *Heredity*, **78**, 311–327.

Frankham R (1998) Inbreeding and extinction in island populations. *Conservation Biology*, **12**, 665–675.

Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.

Friesen V, Burg TM, McCoy KD (2007) Mechanisms of population differentiation in seabirds. *Molecular Ecology*, **16**, 1765–1785.

Friesen V, González JA, Cruz-Delgado F (2006) Population genetic structure and conservation of the Galápagos petrel (*Pterodroma phaeopygia*). *Conservation Genetics*, **7**, 105–115.

Gaggiottii OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513–1520.

Gibbs JP, Snell HL, Causton CE (1999) Effective monitoring for adaptive wildlife management: lessons from the Galápagos Islands. *Journal of Wildlife Management*, **63**, 1055–1065.

Goodman SJ (1997) R_{ST} Calc: a collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. *Molecular Ecology*, **6**, 881–885.

Goudet J (1999) An improved procedure for testing the effects of key innovations on the rate of speciation. *The American Naturalist*, **153**, 549–555.

Goudet J (2002) FSTAT, version 2.9.3.2. University of Lausanne, Lausanne, Switzerland. www2.unil.ch/popgen/sofwares/fstat.htm (accessed September 2006).

Goudet J, Raymond M, de Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics*, **144**, 1933–1940.

Harris MP (1979) Population dynamics of the flightless cormorant (*Nannopterum harrisi*). *Ibis*, **121**, 135–146.

Huyvaert KP, Parker PG (2006) Absence of population genetic structure among breeding colonies of the waved albatross. *The Condor*, **108**, 440–445.

IUCN (2008) *2008 IUCN Red List of Threatened Species*. www.iucnredlist.org. Last accessed 28 March 2009.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation-by-distance, web service. *BMC Genetics*, **6**, 13. v.3.15 http://ibdws.sdsu.edu/.

Jiménez Uzcátegui G, Vargas FH (2007) Censo del pingüino de Galápagos y cormorán no volador en 2007. Informe técnico para la Estación Científica Charles Darwin y el Parque Nacional Galápagos. Estación Científica Charles Darwin, Puerto Ayora, Isla Santa Cruz, Galápagos.

Jiménez Uzcátegui G, Vargas FH, Larrea C, Milstead B, Llerena W (2006) Galápagos penguin (*Spheniscus mendiculus*) and flightless cormorant (*Phalacrocorax harrisi*) survey. Informe técnico para la Estación Científica Charles Darwin. Estación Científica Charles Darwin, Puerto Ayora, Isla Santa Cruz, Galápagos.

Johnsgard PA (1993) *Cormorants, Darters, and Pelicans of the World*. Smithsonian Institution Press, Washington D.C.

Kerlinger P (1985) Water-crossing behavior of raptors during migration. *Wilson Bulletin*, **97**, 109–113.

Larrea C (2007) *Movimiento, dispersión, y éxito reproductivo del cormorán no volador (*Phalacrocorax harrisi*), en las islas Galápagos*. Licenciado en Ciencias Biológicas. Pontifica Universidad Católica del Ecuador, Quito, Ecuador.

Latch EK, Dharmarajan G, Glaubitz J *et al*. (2006) Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, **7**, 295–302.

Longmire JL, Lewis AK, Brown NC *et al*. (1998) Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2**, 14–24.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Moritz C (1994) Defining evolutionarily-significant units for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA*, **12**, 3321–3323.

Nims B, Vargas H, Gottdenker N, Parker PG (2008) Low genetic diversity and lack of population structure in the endangered Galápagos penguin (*Spheniscus mendiculus*). *Conservation Genetics*, **9**, 1413–1420.

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, **12**, 844–855.

Petren K, Grant PR, Grant BR, Keller LF (2005) Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Molecular Ecology*, **14**, 2943–2957.

Piertney SB, Goostrey A, Dallas JF, Carss DN (1998) Highly polymorphic microsatellite markers in the great cormorant (*Phalacrocorax carbo*). *Molecular Ecology*, **79**, 361–382.

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

Pritchard JK, Wen X, Falush D (2007) *Documentation for Structure Software*: Version 2.2. Available from http://pritch.bsd.uchicago.edu/software.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation-by-distance. *Genetics*, **145**, 1219–1228.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Santiago-Alarcon D, Tanksley SM, Parker PG (2006) Morphological variation and genetic structure of Galápagos dove (*Zenaida galapagoensis*) populations: issues in conservation for the Galápagos bird fauna. *Wilson Journal of Ornithology*, **118**, 194–207.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Snow BK (1966) Observations on the behaviour and ecology of the flightless cormorant (*Nannapterum harrisi*). *Ibis*, **108**, 265–280.

Summers K, Amos W (1997) Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. *Behavioral Ecology*, **8**, 260–267.

Tindle R (1984) The evolution of breeding strategies in the flightless cormorant (*Nannapterum harrisi*). *Biological Journal of the Linnean Society*, **21**, 157–164.

Valle CA (1995) Effective population size and demography of the rare flightless Galápagos cormorant. *Ecological Applications*, **5**, 601–617.

Vargas H, Steinfurth A, Larrea C, Jimenez G, Llerena W (2005) *Penguin and Cormorant Census 2005*. Report to the Charles Darwin Research Station and the Galápagos National Park Service, pp. 1–34. Charles Darwin Research Station, Puerto Ayora, Santa Cruz, Galápagos.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Caroline Duffie conducted the work reported here as part of her MS thesis in the Ecology, Evolution and Systematics program at the University of Missouri – St. Louis Department of Biology. Her advisor was Dr. Patricia Parker, an evolutionary ecologist in that program whose research group focuses on population genetics and phylogeography of Galapagos birds and their parasites. Dr. Hernan Vargas has conducted long-term population monitoring of some Galapagos seabirds and currently oversees studies of Neotropical raptors. Dr. Travis Glenn is a molecular ecologist who specializes in the development of molecular markers for application in studies such as this one.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Gene diversity/number of alleles/and allelic richness of flightless cormorants in nine breeding colonies on Isabela and Fernandina in the Galápagos Islands, Ecuador. Mean and standard deviation are reported

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