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Proc. R. Soc. B published online 22 September 2010
doi: 10.1098/rspb.2010.1342

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FirstCite® e-publishing

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Published online

Long-term isolation of a highly mobile seabird on the Galapagos

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The Galapagos Islands are renowned for their high degree of endemism. Marine taxa inhabiting the archipelago might be expected to be an exception, because of their utilization of pelagic habitats—the dispersal barrier for terrestrial taxa—as foraging grounds. Magnificent frigatebirds (*Fregata magnificens*) have a highly vagile lifestyle and wide geographical distribution around the South and Central American coasts. Given the potentially high levels of gene flow among populations, the species provides a good test of the effectiveness of the Galapagos ecosystem in isolating populations of highly dispersive marine species. We studied patterns of genetic (mitochondrial DNA, microsatellites and nuclear introns) and morphological variation across the distribution of magnificent frigatebirds. Concordant with predictions from life-history traits, we found signatures of extensive gene flow over most of the range, even across the Isthmus of Panama, which is a major barrier to gene flow in other tropical seabirds. In contrast, individuals from the Galapagos were strongly differentiated from all conspecifics, and have probably been isolated for several hundred thousand years. Our finding is a powerful testimony to the evolutionary uniqueness of the taxa inhabiting the Galapagos archipelago and its associated marine ecosystems.

Keywords: Galapagos endemism; microsatellites; morphological differentiation; mtDNA; nuclear introns; philopatry

1. INTRODUCTION

Darwin was strongly influenced by the uniqueness of many Galapagos taxa when he conceived *On the origin of species* [1]. He hypothesized that many Galapagos endemics arose from *in situ* radiations, following initial colonization of the archipelago by ancestral species. For numerous taxa, this view has received support from morphological and molecular studies (reviewed in [2]). However, Darwin noted that '... it is obvious that marine birds could arrive at these (Galapagos) islands much more easily and frequently than land-birds...', and thus show a much lower degree of endemism ([1], p. 348). Indeed, while all native reptiles and terrestrial mammals and 84 per cent of terrestrial birds are endemic [3], only 37 per cent (7 out of 19) of Galapagos seabird species are currently classified as endemic. Because seabirds and other marine species forage in the pelagic zone, which is the isolating agent for terrestrial species, the 1000 km of open ocean separating the Galapagos archipelago from the mainland could link archipelago to continental populations, especially in highly dispersive species.

Species predicted to be least susceptible to isolation effects on the Galapagos would be far-ranging in the pelagic zone, and habitat generalists with a widespread occurrence in the surrounding coastal and marine environments of South and Central America. Such species residing on the Galapagos would encounter suitable habitat should they disperse back to the mainland. Further, in species exhibiting gene flow across large geographical distances, one would predict recurrent arrival of immigrants to the Galapagos, counteracting allopatry and potentially swamping out local adaptation.

Some of the endemic seabird taxa of the Galapagos Islands have no flight capabilities (e.g., Galapagos penguin, flightless cormorant). The most capable flyers among seabirds that breed on the Galapagos are probably the albatrosses and frigatebirds. Albatrosses perform long-distance foraging trips [4] and most albatross species exhibit extensive gene flow across vast geographical distances [5]. However, weak prevailing winds around the inner tropical convergence zone are thought to restrict the flight patterns of albatrosses, which have relatively high wing loading, or relatively small wings for their body weight [6,7]. Indeed, only four albatross species occur outside the Southern Hemisphere oceans, and their ranges are very restricted, including that of the Galapagos-endemic waved albatross (*Phoebastria irrorata*).

Magnificent frigatebirds are perhaps the least likely of Galapagos species to be subject to geographical isolation. These tropical seabirds are widely distributed along the Atlantic and Pacific coasts of Central and South America, and on neighbouring archipelagos, including

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2010.1342 or via http://rspb.royalsocietypublishing.org.

Received 8 July 2010
Accepted 2 September 2010

Table 1. Genetic variation in magnificent frigatebird populations across three mtDNA regions (n, sample size; N_H, number of unique haplotypes; HD and π, gene and haplotype diversities, respectively). Belize populations are HC (Halfmoon Caye) and MW (Man O'War Caye).

region	population	n	N_H	HD $\pm$ s.d.	$\pi \pm$ s.d.
Galapagos	North Seymour Islands	20	3	0.195 $\pm$ 0.115	0.00012 $\pm$ 0.00007
eastern Pacific	(overall)	36	11	0.867 $\pm$ 0.031	0.00143 $\pm$ 0.00089
	Panama	25	9	0.863 $\pm$ 0.040	0.00128 $\pm$ 0.00012
	toepads	11	8	0.927 $\pm$ 0.066	0.00187 $\pm$ 0.00037
Atlantic	(overall)	175	26	0.760 $\pm$ 0.030	0.00121 $\pm$ 0.00076
	Bahamas	29	5	0.421 $\pm$ 0.110	0.00076 $\pm$ 0.00020
	Florida	29	8	0.675 $\pm$ 0.087	0.00104 $\pm$ 0.00019
	British Virgin Islands	21	12	0.852 $\pm$ 0.071	0.00133 $\pm$ 0.00018
	Jamaica	30	10	0.897 $\pm$ 0.027	0.00152 $\pm$ 0.00009
	Cayman Islands	30	9	0.786 $\pm$ 0.0065	0.00135 $\pm$ 0.00017
	Belize (HC)	13	5	0.795 $\pm$ 0.076	0.00111 $\pm$ 0.00014
	Belize (MW)	23	6	0.708 $\pm$ 0.090	0.00089 $\pm$ 0.00016

Table 2. Genetic variability in magnificent frigatebird populations at eight microsatellite markers (n, sample size (number of individuals); AR, rarefied allelic richness [22]; HE and HO, unbiased expected and observed heterozygosity, respectively).

region	population	n	AR	HE $\pm$ s.d.	HO $\pm$ s.d.
Galapagos	North Seymour Islands	20	4.6	0.54 $\pm$ 0.11	0.58 $\pm$ 0.04
eastern Pacific	Panama	25	5.6	0.62 $\pm$ 0.09	0.61 $\pm$ 0.04
Atlantic	Bahamas	29	6.3	0.68 $\pm$ 0.09	0.69 $\pm$ 0.03
	Florida	29	6.0	0.68 $\pm$ 0.08	0.68 $\pm$ 0.03
	British Virgin Islands	21	6.0	0.65 $\pm$ 0.09	0.69 $\pm$ 0.04
	Jamaica	28	5.9	0.65 $\pm$ 0.09	0.67 $\pm$ 0.03
	Cayman Islands	30	5.6	0.65 $\pm$ 0.09	0.65 $\pm$ 0.03
	Belize (HC)	13	6.0	0.66 $\pm$ 0.09	0.65 $\pm$ 0.05
	Belize (MW)	24	5.7	0.63 $\pm$ 0.09	0.58 $\pm$ 0.04

the Galapagos. They are observed as vagrants far north along the eastern and western coasts of North America, and have even reached western Europe and Africa, usually after big storms [8]. The species has the lowest wing loading (i.e. smallest body mass relative to the area of its wings [9]) among birds and is known for its soaring behaviour. It uses thermal winds to reach high altitudes, and can travel hundreds of kilometres at slow speed, even while tending an active nest [9]. This combination of life-history traits makes the magnificent frigatebird especially suitable for studying gene flow and isolation in highly mobile species of the Galapagos.

Here we present data from three classes of genetic markers (mitochondrial DNA, microsatellites and nuclear introns) surveyed in magnificent frigatebirds from across their distribution. The markers reflect both (i) maternally and biparentally inherited lineages and (ii) rapidly and slowly evolving genomic regions, providing a comprehensive view of genetic differentiation. We also provide morphological data that enable us to investigate patterns of phenotypic differentiation within the species, and how they relate to the patterns of genetic variation. Based on widespread sampling across the species's distribution range, we investigate whether gene flow among non-Galapagos colonies is extensive. We then determine whether geographical structuring of genetic and morphological variation supports or rejects a scenario of allopatric isolation of magnificent frigatebirds on the Galapagos.

2. MATERIAL AND METHODS

(a) *Sampling*

We sampled 232 individuals from nine populations across the range of the magnificent frigatebird (tables 1, 2 and figure 1), including 221 fresh samples and 11 samples from toe-pads of museum specimens collected between 1895 and 1986 (electronic supplementary material, table S1). We collected fresh blood or plucked feathers from nestlings or adults on active nests, ensuring that resident birds were sampled. Birds were individually marked during sampling, and we did not sample offspring and adults from the same nest. Samples are therefore presumably unrelated, at least with regard to the present generation. Blood samples were stored in lysis buffer and frozen once in the laboratory. Toe-pad samples were from Pacific localities, extending our sampling in a geographical region otherwise covered only by Galapagos and Panamanian samples. Very small pieces of toe-pads were cut from the museum specimens using clean scalpel blades and stored dry until extraction.

(b) *Laboratory methods*

Following digestion with Proteinase K, DNA was extracted from modern samples using standard phenol–chloroform, salt precipitation or Qiagen kit (Qiagen, Valencia, USA) methods. DNA from museum toe-pads was extracted in a facility solely dedicated to 'ancient' DNA work. We followed stringent protocols to avoid and detect potential contamination (see [10,11]).

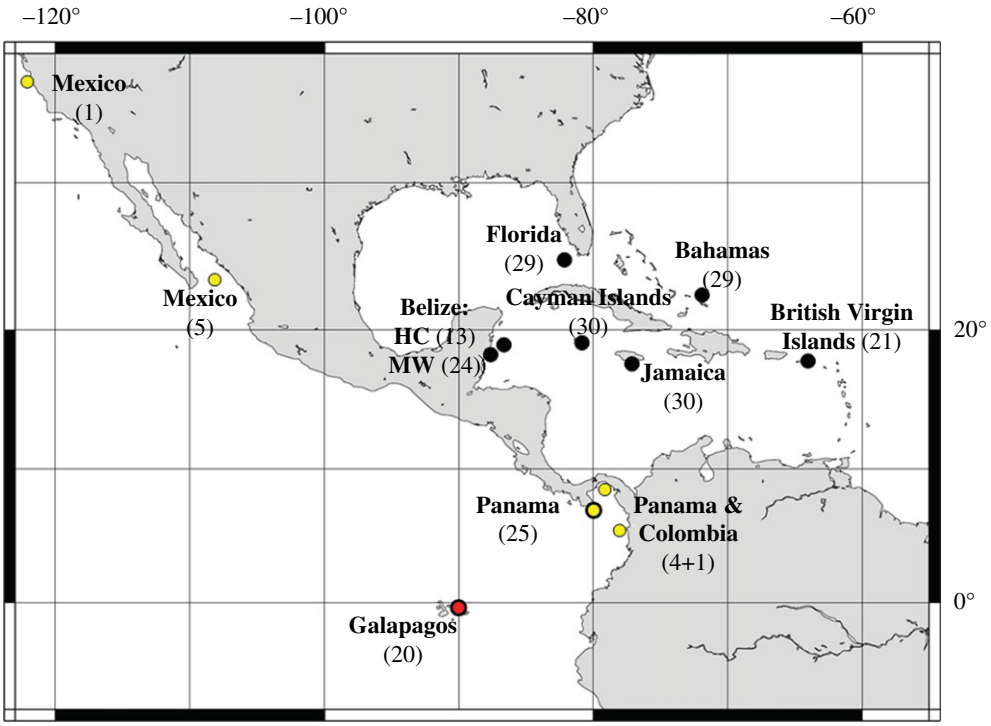

Figure 1. Sampling locations and sample sizes of magnificent frigatebirds analysed in this study. Small yellow dots denote toepad samples. Large dots denote fresh samples: red, Galapagos; yellow, eastern Pacific; black, Atlantic. HC, Halfmoon Caye (Belize); MW, Man O'War Caye (Belize).

(i) *Mitochondrial DNA*

We amplified fragments of three genes, *ATP6* (531 base pairs (bp)), *cytochrome b* (550 bp) and *ND2* (555 bp; sequence lengths do not include the primers). Details of the PCRs are given in the electronic supplementary material. All PCRs of museum material were set up in an 'ancient' DNA laboratory, and negative and positive controls were used throughout (details in the electronic supplementary material). PCR products were cleaned using EXOSAP (USB Scientific, Cleveland, USA). Both strands of DNA were cycle-sequenced with the PCR primers using BigDye v. 3.1 (Applied Biosystems, Foster City, USA), followed by an ethanol or Sephadex clean-up. Sequences were run on an ABI 3130xl instrument and assembled in Sequencher v. 4.8 (Gene Codes, Ann Arbor, USA).

(ii) *Microsatellite markers*

Following initial assessment of multiple microsatellite markers (see electronic supplementary material), we selected eight loci that exhibited multiple alleles, showed reliable amplification and could be scored consistently: *Fmin02*, *Fmin11*, *Fmin12*, *Fmin14*, *Fmin15*, *Fmin16*, *Fmin17* and *Fmin18* [12]. The loci were amplified in three multiplex PCR reactions using fluorescently labelled forward primers (electronic supplementary material, table S3) and run on an ABI 3130xl instrument. Genotypes were scored in Genemapper v. 4.0.

(iii) *Nuclear introns*

For a subset of samples (electronic supplementary material, table S4) we amplified four introns [13,14] from the nuclear genes α-enolase (*ENOL*), glyceraldehyde-3-phosphate dehydrogenase (GAPD), myelin proteolipid protein (MPP) and ornithine decarboxylase (OD), in total 1595 bp. PCR products were cleaned and sequenced on both strands as described above. Intron sequences heterozygous for indels were analysed and phased using CHAMPURU [15] and INDELLIGENT [16].

All sequences obtained in this study have been submitted to the GenBank database (accession numbers: FR691079–FR691320).

(c) *Data analysis*

To visualize the genealogical relationships among haplotypes, we generated statistical parsimony networks of mitochondrial and nuclear sequences using TCS [17]. For evolutionary calculations based on mitochondrial DNA (mtDNA) and whenever implemented in the software, we chose the HKY model of sequence evolution; transition–transversion ratio was set to 47, as estimated using the AIC test in Jmodeltest v. 0.1.1 [18]. Otherwise, we used the next simplest model available, which at divergence levels below 1 per cent (see §3) has only a minor effect on the outcome. Standard nuclear diversity indices (haplotype and nucleotide diversity) were calculated in DnaSP v. 5 [19] and Arlequin v. 3.5.1.2 [20]. The mean net nucleotide distance among groups was calculated in MEGA v. 4.1 [21] using the K2P model; standard errors were estimated based on 1000 bootstrap replicates across sites.

GENEPOP on the web (http://genepop.curtin.edu.au/) was used for standard population genetic data quality assessment tests, including tests for heterozygote deficit/excess and linkage disequilibrium, applying sequential Bonferroni correction. To account for differences in sample size among locations, we calculated the rarified mean number of alleles per locus using HP-RARE [22]. Principal coordinates analysis (PCA) of individual genotypes was performed in GenAlEx [23]; *F*-statistics were calculated in Genetix [24]. The latter provide a measure of genetic differentiation (fixation index) that quantifies the genetic distance among populations, with larger values indicating higher differentiation. Assignment tests based on multi-locus

microsatellite genotypes were performed in GENECLASS v. 2.0 [25] using the Bayesian algorithm of Rannala & Mountain [26], and the same data were evaluated in a Bayesian genotype clustering procedure in STRUCTURE v. 2.3.3 [27]. We employed default settings in the newly implemented *Locprior* model [28], which is designed for cases of especially weak population structure, and assumed correlated allele frequencies. For each value of K (number of demes assumed for the clustering procedure), we performed two long runs of 500 000 iterations each (after a burn-in of 200 000 steps) and averaged the results. Multiple additional shorter runs were performed using different settings (admixture model, no-admixture model) to check for convergence and to assess the importance of model choice.

The three datasets were analysed separately using a Bayesian coalescent-based framework in MIGRATE v. 3.0.7 [29,30], a procedure that jointly estimates Θ (a measure of effective population size) and unidirectional migration rates among populations. To limit the number of parameters to be estimated, we grouped all samples *a priori* into three geographical regions (Galapagos, eastern Pacific, Atlantic). Runs were initiated based on starting values from F_{ST} values and used wide uniform priors. Multiple additional runs were performed using results from earlier runs as starting conditions, still using flat priors but longer chains (see electronic supplementary material, table S5 for details).

To estimate the mtDNA phylogeny and to date the ages of the splits among main clades, we employed the Bayesian-relaxed (uncorrelated lognormal) molecular clock approach implemented in the program BEAST v. 1.5.3 [31]. Trees were rooted with the sister taxon *Fregata aquila* (GenBank accession numbers EU166963, EU166990, AY369064 [32]). Settings included a Yule prior to model lineage birth, a normal distribution of substitution rate (mean $2.13 \pm 0.065\%$ divergence per million years; see [33]). We also calibrated the tree using an assumed maximum age of separation from the sister taxon *F. aquila*, of 1 Myr, based on geological dating of the emergence of Ascension Island [34]. BEAST analyses were run for up to 300 million generations, and convergence was checked in TRACER v. 1.5 (available from http://beast.bio.ed.ac.uk/Tracer) and by comparing results from independent runs.

(d) *Morphological measurements*

We collected a series of morphometric measurements from specimens in museum collections (electronic supplementary material, table S6). We measured *wing* (length of the unflattened first primary), *inner tail* and *outer tail* (innermost and outermost tail feather, respectively) *culmen* length (starting at the end of feather cover at the bill origin), *bill depth* and *bill width* (measured at the starting point of *culmen*), and the length of the *middle toe* (taken from the end of the skin towards the claw, to the third joint counting from the claw; electronic supplementary material, figure S2). All measurements were recorded to the nearest millimetre using a calliper, except for wing length, which was measured to the nearest 0.5 mm using a ruler. All measurements were taken by the same person (F.H.), using five males and five female individuals from the Galapagos (roughly two-thirds of all Galapagos specimens available in US museums). For comparison, we measured 16 males and 11 female museum specimens from eastern Pacific and Atlantic locations. Body size measurements were compared statistically using *U*-tests in R [35]. R was also used to perform linear discriminant function analysis, following log-transformation of all measurements.

3. RESULTS

Basic information and statistics on the variability of the employed markers are given in the electronic supplemental material.

(a) *Population genetic structure*

(i) *Mitochondrial DNA*

A statistical parsimony network of mtDNA sequences (figure 2) showed a deep split into two main lineages, separated by 14 nucleotide changes, or a mean net sequence divergence of $0.88 \pm 0.24\%$ (s.e.; same result for Kimura two-parameter and Tamura-Nei model distances). One lineage consisted of individuals from the Atlantic and eastern Pacific populations (together referred to as 'non-Galapagos'), while the second lineage was confined to the Galapagos (electronic supplementary material, tables S7 and S8). Consistent with its wider geographical distribution, the former lineage harboured more genetic diversity (33 haplotypes, $\pi = 0.00126 \pm 0.00006$) than the Galapagos lineage (three haplotypes, $\pi = 0.00012 \pm 0.00018$). Pairwise Φ_{ST} values among localities (electronic supplementary material, table S9) confirmed this finding: all comparisons between Galapagos and non-Galapagos populations were larger than 0.90 and statistically significant. In contrast, all comparisons among non-Galapagos populations yielded Φ_{ST} values smaller than 0.20; most of these were non-significant, even between ocean basins.

Non-Galapagos birds exhibited extensive haplotype sharing among populations (figure 2). The two most frequent haplotypes (BMF01, BMF06) were present in every sampled population except the Galapagos, and found in almost 60 per cent of those individuals. Frequent haplotypes were shared among eastern Pacific and Atlantic populations, and only rare haplotypes were confined to one or two populations.

A relaxed molecular clock model in BEAST indicated that the Galapagos and non-Galapagos lineages diverged several hundred thousand years ago. The geometric mean of the posterior distribution was 247 200 years before present (YBP), and the 95 per cent higher posterior density spanned 82 800–657 400 YBP. Despite the potential drawbacks associated with divergence dating based on mtDNA [36], this indicates with high certainty that the two lineages split during the Middle or Late Pleistocene, well before the last glacial maximum (around 22 000 YBP).

(ii) *Microsatellites*

Genetic diversity within populations was relatively similar among sampling locations, except for the less variable Galapagos population (table 2). As for mtDNA, analyses of population structure recovered two strongly differentiated main groups. PCA clearly separated the Galapagos samples from all others (figure 3). Non-Galapagos genotypes showed little or no geographical structuring, even between ocean basins: eastern Pacific and Atlantic individuals overlapped almost completely in the PCA, and STRUCTURE did not provide any additional resolution (electronic supplementary material,

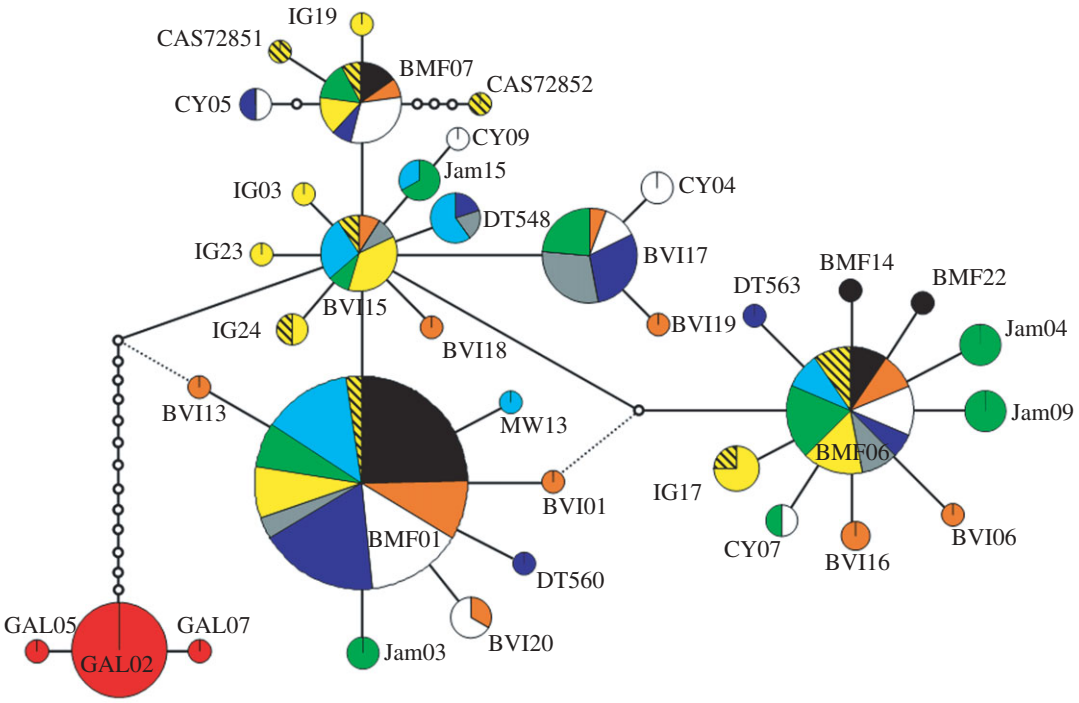

Figure 2. Statistical parsimony network of mtDNA sequences (1636 bp). Pie charts and filled circles correspond to haplotypes, circular area being proportional to their frequency. Inferred intermediate steps are shown as small open circles, dotted lines are less likely genealogical pathways (based on haplotype frequencies). Red, Galapagos; yellow, Panama; yellow with black lines, Pacific toepads; black, Bahamas; orange, British Virgin Islands; white, Cayman Islands; violet, Florida; green, Jamaica; grey, Belize (HC); blue, Belize (MW). Haplotypes are named as in electronic supplementary material, table S7. BMF, Bahamas; BVI, British Virgin Islands; CY, Little Cayman; DT, Dry Tortugas (FL, USA); Gal, Galapagos (Ecuador); IG, Isla Iguana (Panama); Jam, Jamaica.

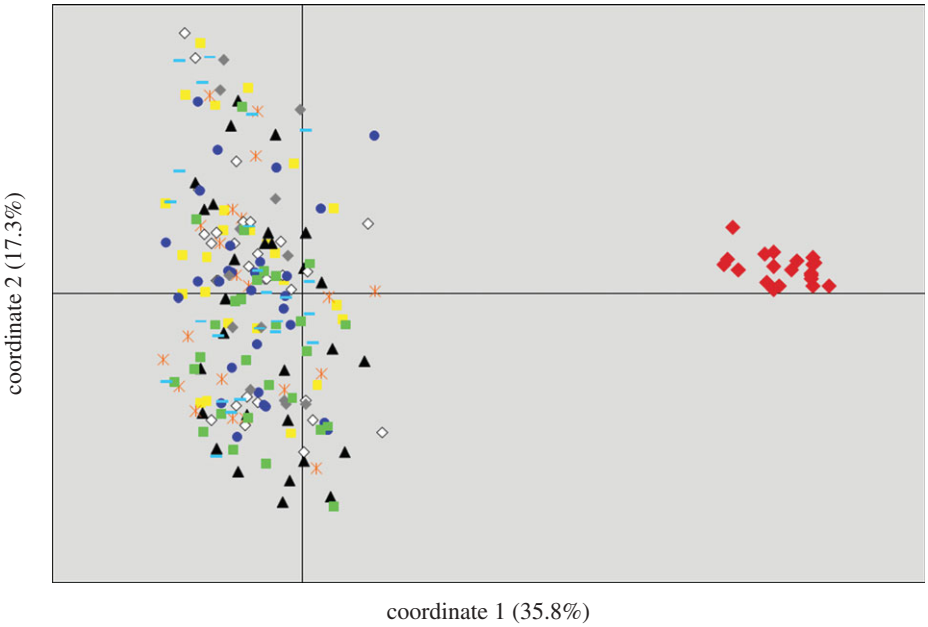

Figure 3. Principal coordinate analysis of microsatellite genotypes. Symbols denote individuals with their multi-locus genetic ancestry scaled on two axes. Red diamonds, Galapagos; yellow squares, Pacific; black triangles, Bahamas; orange asterisks, British Virgin Islands; violet circles, Florida; green squares, Jamaica; grey diamonds, Belize (HC); blue dashes, Belize (MW).

figure S1). Similarly, all pairwise F_{ST} values involving the Galapagos were larger than 0.34 and significant, while the remaining values were smaller than 0.05 and non-significant in all but three cases, including most cross-isthmus comparisons (electronic supplementary material, table S10). An assignment test in GENECLASS provided perfect resolution between Galapagos and non-Galapagos samples, but poor resolution among the non-Galapagos populations (electronic supplementary material, table S11).

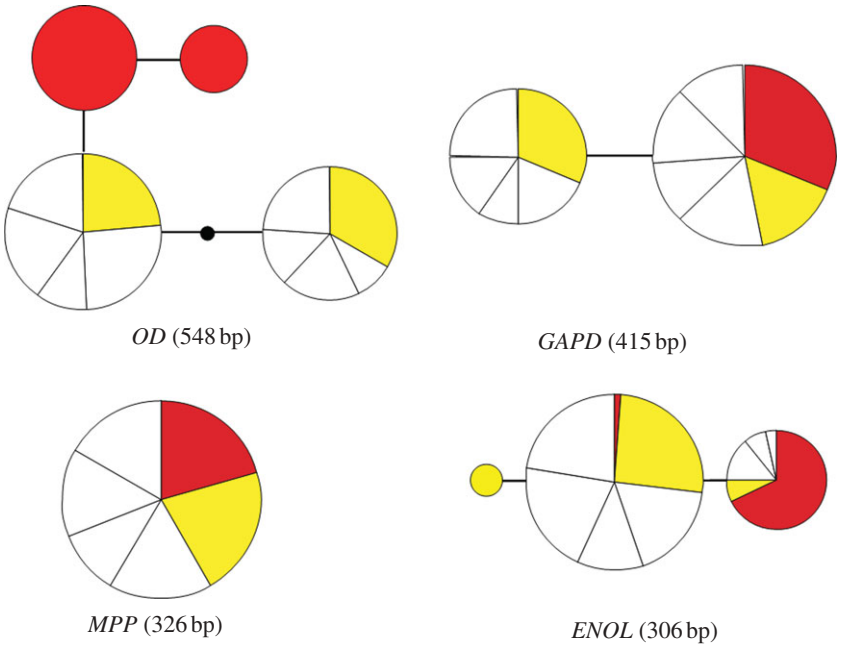

Figure 4. Statistical parsimony networks of sequence variation in four nuclear introns. Pie charts and filled circles denote haplotypes, black dots are inferred intermediate steps. Red, Galapagos; yellow, Panama. For clarity, the four Atlantic populations are all shown in white (see electronic supplementary material, table S4).

Table 3. Morphometric measurements of magnificent frigatebird museum specimens. Numbers given are mean ± s.d. Significant differences within sexes among regions are marked by asterisks ($p < 0.01$, *U*-test).

	wing (cm)	outer tail (cm)	inner tail (cm)	culmen (mm)	bill depth (mm)	bill width (mm)	middle toe (mm)
males							
Galapagos ($n = 5$)	64.0 ± 0.9*	49.1 ± 2.2*	21.8 ± 1.3*	109.6 ± 4.2	30.2 ± 1.5	29.8 ± 1.9	42.0 ± 2.0
non-Galapagos ($n = 16$)	61.8 ± 1.3	45.8 ± 3.3	18.2 ± 1.3	107.5 ± 3.3	28.9 ± 1.2	29.3 ± 1.2	41.1 ± 1.1
females							
Galapagos ($n = 5$)	68.8 ± 0.8*	54.7 ± 1.5*	22.1 ± 3.4*	125.2 ± 2.2*	32.4 ± 1.1	31.2 ± 0.8	43.8 ± 0.4
non-Galapagos ($n = 11$)	64.7 ± 1.2	47.4 ± 2.1	18.0 ± 0.5	119.8 ± 3.1	31.7 ± 1.6	32.2 ± 1.2	43.7 ± 0.8

(iii) *Nuclear intron markers*

Assessment of haplotypes (figure 4 and electronic supplementary material, table S4) revealed a diagnostic character at the *OD* locus, separating the Galapagos from all other individuals. Large and significant frequency differences between Galapagos and all other samples were found at *GAPD* and *ENOL*.

For all three marker systems, Bayesian coalescent simulations in MIGRATE indicated a much lower Θ (effective population size) value for the Galapagos than for non-Galapagos populations, and suggested the absence of gene flow among Galapagos and continental populations (mode at zero), despite wide posterior credibility intervals. No gene flow was indicated in an eastward direction across the isthmus by all marker systems, but analyses of mitochondrial and microsatellite data indicated significant westward gene flow from Atlantic into eastern Pacific populations. The posterior distributions for all migration estimates had a clear maximum at zero, except the estimate from Atlantic into the eastern (non-Galapagos) Pacific, which showed a peak at 25 (mtDNA) and 433 (microsatellites). Demographic analyses (electronic supplementary material, tables S12 and S13) indicated pronounced recent population growth of Galapagos as well as non-Galapagos lineages.

(b) *Morphological measurements of museum specimens*

Three to four size measurements (depending on the sex) indicated that Galapagos birds were significantly larger than those from the mainland ($p < 0.05$; table 3). Those measurements included *wing*, *inner tail* and *outer tail* (both sexes), and *culmen* (females only). A multivariate discriminant function analysis performed separately for males and females correctly classified 100 per cent of individuals to their region of origin (Galapagos

or non-Galapagos), and a subsequent leave-one-out cross-evaluation procedure classified about 80 per cent of individuals correctly. The latter may relate to our limited sample size, or indicate only subtle inter-regional differences at the surveyed morphometric characters.

4. DISCUSSION

All marker types indicated extensive gene flow across most of the range of the magnificent frigatebird, but pronounced population structure separating the Galapagos from all other populations. This signal was also reflected in significant morphological differences between Galapagos and mainland birds. The Galapagos archipelago has long received attention for its high degree of endemism and has been recognized as a showcase for evolutionary processes (e.g. [2]). A new case documenting endemism on the Galapagos is thus not surprising *per se*. However, the behaviour and ecology of magnificent frigatebirds render them one of the least likely of Galapagos taxa to have evolved in isolation from its conspecifics.

Magnificent frigatebirds are renowned for their wide-ranging behaviour [9]. Finding little or no genetic structure among continental populations, despite the use of high-resolution genetic markers, is consistent with this high dispersal capability. Importantly, our results reveal signatures, at all three classes of genetic markers, of extensive gene flow even between Atlantic and Pacific colonies. This is consistent with field observations ([37]; Frank Hailer 2007, personal observation). The Isthmus of Panama closed approximately 2.8 Myr ago and has since posed a major barrier to gene flow in numerous marine species [38,39], including highly dispersive taxa (e.g. [40]). To our knowledge, the magnificent frigatebird is thus the first tropical seabird for which extensive natural gene flow across the Isthmus of Panama has been suggested.

(a) *Explanations for the uniqueness of magnificent frigatebirds on the Galapagos*

Many seabirds show pronounced natal and breeding philopatry (i.e. a tendency to return to breed at the location they were born or had bred previously). Long-term field data are lacking for magnificent frigatebirds, but short-term data suggest some degree of philopatry also in this species [8]. The ultimate causes for such philopatry are not known. Among several factors, familiarity with natal and/or previous breeding habitats has been suggested as a driver of philopatry [41]. However, the inherent contrast in our findings between the Galapagos and the non-Galapagos range suggests that a factor unique to the Galapagos population may be promoting evolutionary isolation on the archipelago. One potential mechanism is the presence of some barrier to movement between the Galapagos and the mainland [42]. Alternatively, a behavioural mechanism related to the elaborate courtship rituals of frigatebirds [8] could be causing allopatric isolation.

The Galapagos archipelago is located approximately 1000 km from the South American mainland. Galapagos seabirds have been reported to forage predominantly to the west of the archipelago, attracted by local upwelling of cold, nutrient-rich waters that lead to higher prey availability [43]. Seabirds from the South American mainland, however, tend to forage in the nearby and highly productive upwelling zone along the continental shelf [41], so many of them may not venture out far from the coast. A recent review of seabird population structuring [42] found that most populations occupying separate ranges during the non-breeding season also display population genetic structure. Our results regarding the Galapagos population could thus be explained by geographical/foraging range isolation. For instance, magnificent frigatebirds could be avoiding dispersal across the open ocean, despite their far-ranging behaviour [9], and despite our genetic results from the non-Galapagos lineage. Extensive dispersal in the non-Galapagos range under this scenario might be oriented along coastlines and among more proximate islands [44].

However, magnificent frigatebirds banded in Galapagos have been recovered as dead and/or emaciated vagrants in Central America (Carlos Valle, Galapagos Academic Institute for the Arts and Sciences 2010, personal communication), demonstrating movement of individuals across the potential barrier. Similarly, recent data from frigatebird *Haemoproteus* blood parasites suggest that there may be physical interactions between Galapagos and continental frigatebirds (Levin *et al.*, unpublished data). In the Nazca booby (*Sula grantii*), banding records have demonstrated reproduction of Galapagos-banded individuals on the mainland [45].

Surprisingly, and in contrast to this movement data, our results indicate long-term isolation on the Galapagos, probably for several hundred thousand years. Over those time frames, the global climate has changed cyclically, with marked fluctuations of trade wind patterns [46], water nutrient levels [47], sea level [48], sea surface temperature [49] and circulation patterns [50], implying vast changes to marine habitats. Tropical seabirds have thus experienced significant spatio-temporal fluctuations of the available marine nutrients (and thus of their prey), which probably influenced their foraging patterns. Given their capacity for long-distance flight, magnificent frigatebirds have had ample opportunity to move between the Galapagos and the continent, calling for consideration of adaptive scenarios to explain the lack of gene flow between those regions.

Magnificent frigatebirds and great frigatebirds *F. minor* occur in sympatry on the Galapagos. Typically, only one of the two frigatebird species is found breeding at a given location (but see [51] for another rare, and possibly recent [52], instance of sympatry between those species). If interspecific hybridization is disadvantageous, selection should favour behavioural avoidance of mating between magnificent and great frigatebirds. While very rare hybridization between the two species has been anecdotally reported, such field observations are difficult because of the complex plumage maturation patterns of frigatebirds (Carlos Valle 2010, personal communication; [8]). Genetic data from Galapagos great frigatebirds lack signals of introgression and thus indicate reproductive isolation (Hailer *et al.*, unpublished data). As a by-product of increased selectiveness for mates, magnificent frigatebirds on the Galapagos may thus reject their conspecifics from the mainland (i.e. character displacement). More data on individual movement and mechanisms of mate choice in frigatebirds on the Galapagos are necessary to evaluate this hypothesis.

Future studies may reveal the exact mechanism of how such a highly dispersive species maintains long-term genetic differentiation on the Galapagos.

The evolutionary distinctiveness of the Galapagos population of the magnificent frigatebird necessitates separate management. This population encompasses approximately 1000 pairs, distributed across four islands [53]. Possible catastrophic events, along with recent human impacts, could seriously threaten its survival, especially during El Niño years, which are associated with dramatic population size reductions in Pacific seabirds [54]. Current classification of the Galapagos population as Least Concern [55] should therefore be revisited.

For permits to collect blood samples and facilitation of fieldwork, we thank (i) Bahamas: Department of Agriculture and the Gerace Research Center; (ii) Belize: Ministry of Natural Resources and the Environment, Toledo Institute for Development and Environment, and Belize Audubon Society; (iii) British Virgin Islands: Department of Environment and Fisheries, National Parks Trust, Henry Jarecki and Skip Lazell; (iv) Cayman Islands: Department of the Environment and National Trust; (v) Ecuador: Galapagos National Park, Charles Darwin Foundation; (vi) Jamaica: National Environment and Planning Agency, The Nature Conservancy, Nathalie Zenny, Ann Sutton and Brandon Hay; (vii) Panama: Autoridad Nacional del Ambiente, Smithsonian Tropical Research Institute, George Angehr and Jim Kushlan; and (viii) Dry Tortugas, Florida, US: National Park Service and Sonny Bass. The California Academy of Sciences and National Museum of Natural History are gratefully acknowledged for granting permission to access and sample their collections. We thank Gary Schenk, Jon Beadell, Judy Pierce, Bill Hayes, Mike Favazza, Cheryl McKinley and Anita Carrion for assistance in the field. We thank Thomas Mueller, Don Dearborn, Storrs Olson, Carlos Valle, Peter Beerli, Andreanna Welch, Sharyn Hood, Heather Lerner and Amy Wilson for discussions. Part of this work was carried out by using the resources of Cornell University's Computational Biology Service Unit, which is partially funded by Microsoft Corporation. Galapagos sampling was funded by the Field Research for Conservation programme of the Saint Louis Zoo and the Des Lee Professorship in Zoological Studies at the University of Missouri—St Louis and the Saint Louis Zoo. The Smithsonian's James Bond Fund, Marine Science Network, and the Center for Conservation and Evolutionary Genetics, T. L. Cross and Seabird Research Inc. provided funding.

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