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Phylogeography of the Galápagos hawk (*Buteo galapagoensis*): A recent arrival to the Galápagos Islands

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Abstract

Galápagos hawks (*Buteo galapagoensis*) are one of the most inbred bird species in the world, living in small, isolated island populations. We used mitochondrial sequence and nuclear minisatellite data to describe relationships among Galápagos hawk populations and their colonization history. We sampled 10 populations (encompassing the entire current species range of nine islands and one extirpated population), as well as the Galápagos hawk's closest mainland relative, the Swainson's hawk (*B. swainsoni*). There was little sequence divergence between Galápagos and Swainson's hawks (only 0.42% over almost 3 kb of data), indicating that the hawks colonized Galápagos very recently, likely less than 300,000 years ago, making them the most recent arrivals of the studied taxa. There were only seven, closely related Galápagos hawk haplotypes, with most populations being monomorphic. The mitochondrial and minisatellite data together indicated a general pattern of rapid population expansion followed by genetic isolation of hawk breeding populations. The recent arrival, genetic isolation, and phenotypic differentiation among populations suggest that the Galápagos hawk, a rather new species itself, is in the earliest stages of further divergence.

Keywords: *Buteo galapagoensis*; Galápagos hawk; Minisatellite DNA; Mitochondrial DNA; Phylogeography

1. Introduction

Island archipelagos have long been valuable for understanding evolutionary processes (Darwin, 1859; Grant, 1998; Whittaker, 1998). The relatively small size and isolation of populations on archipelagos often results in the occurrence of multiple, closely related yet distinct lineages on neighboring islands. There are numerous examples of radiations occurring in a variety of taxa on island systems around the world (e.g., Wagner and Funk, 1995). The refinement of phylogenetic techniques has opened up new avenues of investigation of these systems (Emerson, 2002; Grant, 2001), revealing mainland source populations and colonization patterns within archipelagos (e.g., Warren et al., 2003).

The Galápagos Islands, located on the equator 1000 km west of mainland Ecuador, are one of the most isolated archipelagos in the world and thus have a high degree of endemism. Almost a third of the plant species and half of the insect species are endemic (Tye et al., 2002). Fifty-nine percent of the vertebrates are endemic, including all of the native reptile and terrestrial mammal (rats) taxa (Tye et al., 2002). Endemism is high among the native terrestrial birds (84%) also, but it is much lower among the seabirds (26%) and shorebirds (23%; Tye et al., 2002). Though many taxa have speciated from their mainland ancestors, radiations within the Galápagos archipelago are relatively rare compared to other, older archipelagos where taxa have had more time to speciate (Tye et al., 2002).

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The islands in the Galápagos archipelago form over a mantle hotspot and drift in a southeasterly direction with the movement of the Nazca plate. The current islands range from less than half a million years old in the west up to 4 million years old in the east (White et al., 1993); however, older, now submerged islands indicate that islands have been present over the hotspot for at least 17 million years (Christie et al., 1992; Werner and Hoernle, 2003).

Radiations within Galápagos vertebrate lineages are skewed toward the reptiles and mammals, with few occurring among the birds (Table 1). There are about 40 recognized reptile taxa (including species and subspecies, depending on the latest taxonomic revisions). These 40 likely arose from only 9 or 10 original lineages from the mainland. The species and subspecies within taxa are generally isolated on different islands or volcanoes within an island. Within the mammals, the rice rats underwent a radiation, while neither of the two bat species have done so.

The pattern among the terrestrial birds is distinctly different from that of the reptiles. Only two of the founding bird lineages radiated into multiple species on the archipelago: the finches and the mockingbirds (Table 1). Two subspecies of Galápagos dove have been recognized (Swarth, 1931), but the rest of the taxa (even though they are all present on multiple islands) have not been subdivided. So, the 30 distinct lineages of terrestrial birds present now arose from only 14 colonizing lineages. This is a 2:1 ratio of current to colonizing lineages, whereas the reptiles are about 4:1. The 2:1 ratio is highly skewed by the finch radiation, the complexity of which is unique among Galápagos birds. Excluding the finches, the relationship drops to 1.4:1. None of the 32 lineages of seabird or aquatic/shorebird have radiated within the Galápagos Islands. This striking difference between birds and reptiles has two possible explanations. First, birds are obviously more mobile, and so gene flow among populations might be preventing further divergence. Second, most of the bird species might have colonized the archipelago more recently and thus have not had time to diverge. Both explanations are supported by the lower degree of endemism seen among the birds, especially the waterbirds. It is possible that the lack of differentiation within bird lineages is due to their being not as well studied as the reptiles, but most Galápagos vertebrate lineages have been recognized for decades from extensive museum collections (long before genetic studies on particular taxa).

1.1. Galápagos hawk

Here, we characterize the population genetic structure and colonization history of one of these terrestrial bird

Table 1
Summary of terrestrial vertebrate taxa of Galápagos, including the number of lineages that evolved on the archipelago, the number of colonizing species from which they evolved, and whether they are endemic

Class	Taxa	Number of lineages	Number of founding taxa	Endemic
Reptilia	Giant tortoises (*Geochelone nigra*)	11 subspecies	1 (Caccone et al., 1999)	Yes
	Marine (*Amblyrhynchus cristatus*) and land (*Conolophus subcristatus, C. pallidus*) iguanas	7 subspecies (marine), 2 species (land)	1 (Rassmann, 1997)	Yes
	Lava lizards (*Microlophus* spp.)	7 species	2 (Kizirian et al., 2004)	Yes
	Geckos (*Phyllodactylus* spp.)	6 species	2 (Wright, 1983)	Yes
	Snakes (*Philodryas hoodensis*, *Antillophis slevini*, *A. steindachmeri*, *Alsophis biseralis* subspp.)	3 species, 3 subspecies	At most 4	Yes
Total		40	10	
Mammalia	Rice rats (*Oryzomys* spp., *Nesoryzomys* spp., *Megaoryzomys curiori*)	At least 8 species	3	Yes
	Bats (*Lasiurus brachyotis*, *L. cinerius*)	2 species	2	Yes (*L. brachyotis*)
Total		10	5	
Aves	Darwin's finches (*Geospiza* spp., *Camarhynchus* spp., *Cactospiza* spp., *Platyspiza crassirostris*, *Certhidea olivacea*)	13 species	1 (Sato et al., 1999; Burns et al., 2002)	Yes
	Galápagos mockingbirds (*Nesomimus* spp.)	4 species	1	Yes
	Galápagos dove (*Zenaida galapagoensis*)	2 subspecies	1	Yes
	Galápagos hawk (*Buteo galapagoensis*)	1 species	1 (this study)	Yes
	Barn owl (*Tyto alba punctatissima*)	1 subspecies	1	Subspecies
	Short-eared owl (*Asio flammeus galapagoensis*)	1 subspecies	1	Subspecies
	Galápagos martin (*Progne modesta*)	1 species	1	Yes
	Yellow warbler (*Dendroica petechia aureola*)	1 subspecies	1 (Collins, 2003)	Subspecies
	Galápagos flycatcher (*Myiarchus magnirostris*)	1 species	1	Yes
	Vermilion flycatcher (*Pyrocephalus rubinus*)	1 species	1	No
	Dark-billed cuckoo (*Coccyzus melacoryphus*)	1 species	1	No
	Galápagos rail (*Laterallus spilonotus*)	1 species	1	Yes
	Paint-billed crake (*Neocrex erythrops*)	1 species	1	No
	Common gallinule (*Gallinula chloropus*)	1 species	1	No
Total		30	14	

Only native, resident taxa are listed (i.e., no introduced species or seasonal migrants), and lineages that arose in Galápagos but have since gone extinct are included. There are references listed where genetic studies have determined the likely number of founding events; otherwise, the numbers reflect what is believed based on morphological characters.

species, the endemic Galápagos hawk (*Buteo galapagoensis*). The islands' only diurnal raptor, this hawk is widely distributed within the archipelago, currently inhabiting nine islands: Española, Santa Fe, Pinzón, Santiago, Isabela, Fernandina, Marchena, Pinta, and Santa Cruz. Once the "center of abundance" of the species distribution (Gifford, 1919), the Santa Cruz breeding population may now be extinct, though juveniles are occasionally seen there (Bollmer et al., 2005). To our knowledge, hawks have never existed on Genovesa, and their populations on Floreana (Steadman and DeLeon, 1999) and San Cristóbal were extirpated due to human activities. Morphological studies have been inconclusive as to the putative mainland sister species of the Galápagos hawk, focusing on several New World *Buteo* species (Brown and Amadon, 1968; Mayr and Short, 1970; Voous and de Vries, 1978). Molecular phylogenetic studies suggest that Galápagos hawks are most closely related to the Swainson's hawk (*B. swainsoni*; Fleischer and McIntosh, 2001; Riesing et al., 2003), a Neotropical migrant which breeds in North America but migrates annually to southern South America (Fuller et al., 1998). Swainson's hawks are generally smaller and more slender than Galápagos hawks, and Swainson's adults have three color morphs as opposed to one dark morph in adult Galápagos hawks (Ferguson-Lees and Christie, 2001).

Island-populations of Galápagos hawks have extremely low levels of genetic variability as evidenced by mean similarity indices between 0.66 and 0.96 at hypervariable minisatellite loci, and genetic variation is positively correlated with island area, an index of population size (Bollmer et al., 2005). There is a significant amount of genetic differentiation among most populations; only two populations (Fernandina and Isabela) are statistically indistinguishable at minisatellite loci (Bollmer et al., 2005). Galápagos hawk populations vary behaviorally and morphologically (Bollmer et al., 2003; de Vries, 1973). The hawks breed in cooperatively polyandrous groups consisting of one female and up to eight males (DeLay et al., 1996; Faaborg and Patterson, 1981), and mean group size varies across islands (Bollmer et al., 2003). Galápagos hawks also vary in overall body size and shape across islands, with female mass in the smallest-bodied population averaging 22% less than in the largest-bodied population (26% in males; Bollmer et al., 2003).

In this study, we described the phylogeographic and population genetic structure of the Galápagos hawk, a species we know to be genetically monomorphic within populations but divergent between populations at nuclear loci. We collected mitochondrial sequence data from all nine extant populations of Galápagos hawk. We were also able to obtain sequence data from a San Cristóbal hawk (a population now extirpated) collected during the 1905–1906 California Academy of Sciences expedition. In addition, we sampled migratory Swainson's hawks and investigated the degree of divergence between the two species to determine when the Galápagos lineage likely colonized the archipelago. Within Galápagos hawks, we examined relationships among different island populations at mitochondrial loci, using multilocus minisatellite data as a nuclear comparison, with the goal of elucidating the colonization history of the hawks in the archipelago.

2. Materials and methods

2.1. Field methods

We visited the Galápagos Islands for two to three months between May and August of each year from 1998 to 2003 and sampled 541 Galápagos hawk individuals from all nine extant populations (Table 2). We captured hawks using balchatri traps baited with rats (Berger and Mueller, 1959) and rope nooses on poles. We banded each hawk and took morphological measurements (see Bollmer et al., 2003) and two 50 μl blood samples via venipuncture. In addition, we captured and sampled 34 Swainson's hawks using balchatri traps placed in agricultural fields near the town of Las Varillas, in Córdoba province (Central Argentina) during January 2003.

The California Academy of Sciences in San Francisco, California has a single Galápagos hawk specimen collected

Table 2
Sample sizes of Galápagos and Swainson's hawks sequenced at mitochondrial loci and fingerprinted at minisatellite loci

Species	Population	No. sequenced at all regions	No. sequenced at variable regions	No. fingerprinted at minisatellite loci
Galápagos hawk	Española	2	10	10
	Santa Fe	2	9	9
	Santa Cruz	4	4	4
	Santiago	2	21	20
	Pinzón	2	10	10
	Marchena	2	15	15
	Pinta	2	13	12
	Isabela	4	20	19
	Fernandina	2	20	20
	San Cristóbal	0	1	0
Swainson's hawk		4	29	0
Total		26	152	119

A total of 26 hawks were sequenced at all four mitochondrial regions (CYB, CR, COI, and ND2). An additional 126 hawks were then sequenced at the two variable regions (COI 3′ and CR) for a total of 152 hawks sequenced at those regions, though the San Cristóbal hawk sequence is incomplete.

in 1905 from the now extirpated San Cristóbal population. In order to obtain genetic data from this population, we visited the Academy in June 2004 and excised a toe pad from that specimen.

2.2. Laboratory methods

For most populations, we used a subset of the individuals in the genetic analyses (Table 2). When possible, we preferentially limited our pool of individuals to territorial, breeding adults, the class most likely to be genetically representative of the population and consist of nonrelatives [individuals within groups are unrelated (Faaborg et al., 1995)]. On Pinzón and Santa Cruz, however, we captured only juveniles and used all of them in the analyses. Initially, we sequenced 26 hawks (Table 2) at four mitochondrial regions comprising 2860 bp. This included complete NADH dehydrogenase subunit 2 (ND2) sequences (1041 bp), 320 bases at the 3′ end of cytochrome *b* (CYB), 72 bp between CYB and the control region (CR), including tRNAthr, 415 bp of the 5′ end of CR (66 bp of the 5′ end of CR were problematic to sequence and are excluded from analyses), and 516 bp near the 5′ end and 496 bp near the 3′ end of cytochrome oxidase (COI). Among the Galápagos hawks sampled, most regions were invariant in this initial sample; therefore, we sampled 126 additional individuals (Table 2; 123 Galápagos and 29 Swainson's hawks) at only the variable 3′ end of COI and 415 bp of the CR.

The majority of sequences were single-stranded, though we obtained double-stranded sequences from those individuals where all gene regions were amplified, and for sequences where there were uncertainties. Table 3 lists the primers used to amplify and sequence the CYB-CR, COI, and ND2 regions. Unless noted, primers are named to indicate light (L) or heavy (H) strand and the 3′ position of the primer numbered according to the complete mitochondrial genome of *Gallus gallus* (Desjardins and Morais, 1990). The CYB-CR region was amplified with L15662 and H15414 (name indicates the 3′ end of the primer numbered according to the complete mitochondrion of *Buteo buteo*). To double-strand sequences, we used the internal primers H16065 and L15004 (name indicates the 3′ end of the primer numbered according to the complete mitochondrion of *Buteo buteo*). COI was amplified in two reactions. The 5′ region was amplified with L6615 and H7539, and sequencing was done using L6615 or H7181. The 3′ region of COI was amplified with L7201 and H8214; sequencing was done using L7651 and H8214. ND2 sequences were obtained by amplifying and sequencing with primers L5216 and H6313. Sequences were double-stranded with internal primers L5716 and H5766.

PCR amplification followed standard protocols. We purified amplicons by precipitation using an equal volume of PEG:NaCl (20%:2.5 M) and washing with 70% ethanol. We sequenced purified amplicons using either ABI BigDye Terminator v.1.0, BigDye Terminator v.3.1, or Beckman DTCS Quickstart chemistries. Manufacturers' recommendations were followed, except reaction volumes were cut to 1/2–1/6 of the recommended volume. Sequences were analyzed on an ABI Prism 310, ABI Prism 3100-Avant genetic analyzer (PE Applied Biosystems), or a CEQ 8000 (Beckman–Coulter) genetic analysis system.

The 100-year-old San Cristóbal sample was processed in a laboratory dedicated to working with ancient DNA at the Florida Museum of Natural History located at the University of Florida. We extracted DNA from the toe pad and amplified the appropriate regions in the ancient DNA laboratory. Due to the poorer quality of the ancient DNA, we needed to sequence the regions in smaller segments using additional primers designed from Galápagos hawk sequences (primer sequences available from RTK upon request).

We performed multilocus minisatellite DNA fingerprinting using the restriction endonuclease *Hae*III and Jeffreys' probe 33.15 (Jeffreys et al., 1985) following procedures described in general in Parker et al. (1995) and specifically for Galápagos hawks in Bollmer et al. (2005). We visualized hybridized fingerprints using a Storm 820 Phosphorimager.

Table 3
Primers used in this study to amplify and sequence three hawk mitochondrial regions

Region	Primer	Source	Sequence (5′–3′)	T_M
CYB-CR	L15662	Kimball et al. (1999)	CTAGGCGACCCAGAAAACTT	54 °C, 30 s
	H15414	This study	CAAGTAGTGCTAGGGGTTTAGG	
	L15004	This study	CACATATCATGAACTATTATGGG	Seq. only
	H16065	Kimball et al. (1999)	TTCAGTTTTTGGTTTACAAGAC	Seq. only
COI	L6615	Modified from Sorenson et al. (1999)	TCTGTAAAAAGGACTACAGCC	52 °C, 30 s
	H7539	Sorenson et al. (1999)	GATGTAAAGTAGGCCGGGTGTCTAC	
	H7181	This study	TACGAATAGGGGTGTTTGG	Seq. only
	L7201	This study	ACCAAACACCCCTATTCGTATG	54 °C, 30 s
	H8214	This study	ATGCRGYTGGCTTGAAACC	54 °C, 30 s
	L7651	This study	GGAACTATCAAATGAGACCC	Seq. only
ND2	L5216	Sorenson et al. (1999)	GCCCATACCCCRAAAATG	52 °C, 30 s
	H6313	Sorenson et al. (1999)	CCTTATTTAAGGCTTTGAAGGC	
	L5716	This study	CCCTACTYACCYTCCTAGCAAT	Seq. only
	H5766	Modified from Sorenson et al. (1999)	GATGARAAGGCTAGGATYTTTCG	Seq. only

We fingerprinted a total of 119 of the 122 Galápagos hawks sequenced at the variable mitochondrial loci (Table 2). From the resulting banding patterns, we created a presence–absence matrix of bands (alleles) encompassing all individuals.

2.3. Data analysis

We examined and compared sequences using Sequencher 4.1 (Gene Codes). We used DnaSP v. 4.0.5 (Rozas et al., 2003) to calculate within-population genetic diversity indices: haplotype diversity (Nei, 1987) and nucleotide diversity (π; Nei, 1987). We generated a 95% statistical parsimony-based haplotype network using TCS v. 1.18 (Clement et al., 2000). Mean genetic distances (number of variable sites and uncorrected *p*-distances) within and between species were calculated using *MEGA* v. 2.1 (Kumar et al., 2001). Standard errors were calculated via bootstrapping (500 replicates). When the level of genetic differentiation between populations was ambiguous, we used pairwise differences to calculate F_{ST} values in Arlequin version 2.000 (Schneider et al., 2000).

To estimate divergence times, we assumed the mitochondrial protein-coding regions were diverging at 2% per million years (Shields and Wilson, 1987). There were six differences between Galápagos and Swainson's hawks (sites invariant within each species but variable between them) in the 2373 bp of protein-coding data used to determine divergence time: 3 in ND2, 1 in CYB, 1 in COI 5′, and 1 in COI 3′. There were other variable sites where some individuals from both species shared the same nucleotide, but these were not used to calculate the divergence between the two species. We estimated a 95% confidence interval for the divergence time assuming a Poisson model of evolution (e.g., Braun and Kimball, 2001). While this method does not correct for ancestral polymorphism, we were primarily interested in setting an upper limit on divergence time, making a correction unnecessary.

For the nuclear minisatellite data, pairwise similarity values were calculated from the presence–absence matrix (based on 46 characters) using the program GELSTATS v. 2.6 (Rogstad and Pelikan, 1996). Similarity values, the proportion of bands shared between any two individuals (Lynch, 1990), were converted to distances (1 − similarity value). We used the distances to construct a neighbor-joining tree in *PAUP** v. 4.0b10 (Swofford, 2002), using midpoint rooting and constraining it to non-negative branch lengths.

3. Results

3.1. Haplotype variation within and between Galápagos and Swainson's hawks

Sequence data are available in GenBank Accession Nos. AY870866–AY870892. For the 26 individuals sequenced at the four mitochondrial regions, polymorphic sites were present in only two of those regions, the CR and the 3′ end of COI (911 bp total), while the other regions (1949 bp total) were invariant within each species, differing by 5 bp between species. Among the 151 individuals (excluding the San Cristóbal hawk) sequenced for the two variable regions, there were only 27 variable sites across all individuals: 6 found only within the 122 Galápagos hawks sampled, 16 only within the 29 Swainson's hawks, 3 in both species, and 2 monomorphic within species but variable between them (Table 4). There were a total of 19 haplotypes sequenced, 7 among the 122 Galápagos hawks and 12 among the 29 Swainson's hawks, indicating greater genetic variability in the Swainson's hawks (Tables 4 and 5). The seven Galápagos hawk haplotypes differed from each other by an average of 3.14 ± 1.07 (SE) bases (mean uncorrected *p*-distance of 0.003 ± 0.001), while the 12 Swainson's hawk haplotypes differed by an average of 4.55 ± 1.10 bases (mean *p*-distance of 0.005 ± 0.001). The *p*-distances within Galápagos hawks ranged from 0 to 0.007, while they ranged from 0 to 0.011 in the Swainson's hawks. Including all the sampled individuals, the mean uncorrected *p*-distance was 0.002 ± 0.001 within Galápagos hawks and 0.003 ± 0.001 within Swainson's hawks. Galápagos and Swainson's hawk haplotypes differed from each other by an average of 10.43 ± 2.46 bases, with a mean *p*-distance of 0.011 ± 0.003, and *p*-distances ranged from 0.005 to 0.015. The smallest

Table 4
The polymorphic sites within the variable COI 3′ and CR regions of the Galápagos and Swainson's hawk mitochondrial DNA

	1224	56666	66677	77777	7777777
	27004	71111	56700	01112	2223467
	21173	30268	68778	92490	1471440
Galápagos hawks					
▼	CTGAT	CACCA	TGTCT	TGAGA	CGTTTAC
■	TTGGT	CACCA	TGTCT	TGAGA	CGTTTAC
△	TTGGT	CGTCA	TGTCT	TGAGA	CGTTTAC
□	TTAGT	CGCCA	TGTCT	TGAGT	TGTTTAC
●	TTAGT	CGCCA	TGTCT	TGAGA	CGTTTAC
▲	TTGGT	TGCCA	TGTCT	TGAGA	CGTTTAC
+	TTGGC	TGCCA	TGTCT	TGAGA	CGTTTAC
Swainson's hawks					
A	TTGGC	CACCA	TGTCT	TAGGA	CATCTGT
B	TTGGC	CACTG	TGTCT	TGGGA	TATTTGT
C	TTGGC	CACCA	TGTCT	TAAGA	CATTTGT
D	TCGGC	CACCA	TGTTT	CAAGA	CATTTGT
E	TTGGC	CACCA	TATTC	TAAGA	CATTCGT
F	TTGGC	CACCA	TGCTC	TAAGA	CATTCGT
G	TTGGC	CACCA	TGCTC	TAAGA	CACTCGT
H	TTGGC	CACCA	CGCTC	TAAGA	CATTCGT
I	TTGGC	CACCA	TGCTC	TAAGT	CATTCGT
J	TTGGC	CACCA	TGCTC	TAAAA	CATTCGT
K	TTGGC	TACCA	TGCTC	TAAGA	CATTCGT
L	TTGGC	CACCA	TGCTC	TAAGA	CGTTCGT

Of the 911 bp sequenced at the COI 3′ and CR regions, there were 27 variable sites. The sites are numbered according to their position within our combined COI and CR dataset; positions 1–496 are COI sites and positions 497–911 are CR sites. Each Galápagos hawk haplotype is labeled with a symbol corresponding to the symbols in Figs. 1 and 2. Each Swainson's hawk haplotype is labeled with a letter corresponding to the letters in Fig. 1.

Table 5
Genetic variability at five mitochondrial regions within Galápagos ($N = 122$; excluding the San Cristóbal hawk) and Swainson's ($N = 29$) hawks

	CYB, ND2, COI 5′ (1949 bp)	COI 3′ (496 bp)	CR (415 bp)	COI 3′/CR combined (911 bp)
B. galapagoensis				
No. of polymorphic sites	0	4	5	9
Nucleotide diversity	0	0.0017	0.0019	0.0018
No. of haplotypes	1	4	5	7
Haplotype diversity (±SD)	0	0.578 ± 0.023	0.625 ± 0.025	0.671 ± 0.030
B. swainsoni				
No. of polymorphic sites	0	1	18	19
Nucleotide diversity	0	0.0001	0.0059	0.0028
No. of haplotypes	1	2	12	12
Haplotype diversity (±SD)	0	0.069 ± 0.063	0.766 ± 0.081	0.766 ± 0.081

p-distance between Galápagos and Swainson's hawks (0.005) is less than the largest distance within either one of them (0.007 in Galápagos and 0.011 in Swainson's hawks). Including all the sampled individuals, Galápagos and Swainson's hawks differed by an average of 10.20 ± 2.75 bases, with a mean *p*-distance of 0.011 ± 0.003.

Using DnaSP, we inferred the amino acid sequences from 492 of the 496 bp at the 3′ end of COI, which resulted in 164 codons in an open reading frame. Interestingly, within the 122 Galápagos hawks, of the five nucleotide substitutions, four were nonsynonymous and one was synonymous. Within the 29 Swainson's hawks, the only mutation in this region was synonymous.

Using a divergence rate of 2% per million years for the 2373 bp of coding DNA (Shields and Wilson, 1987), Galápagos and Swainson's hawks diverged approximately 126,000 years ago, with a 95% confidence interval between 51,000 and 254,000 years ago. While there is a large amount of error in molecular clock estimates (Arbogast et al., 2002; Lovette, 2004), our estimate still indicates that Galápagos hawks arrived in Galápagos very recently, likely less than 300,000 years ago.

3.2. Divergence among Galápagos hawk populations

There were only seven mitochondrial haplotypes present across the nine extant Galápagos hawk populations; multiple haplotypes were present in two populations (Isabela and Santa Cruz), while the other seven populations were fixed (Fig. 1). Three haplotypes were present on multiple islands. One (black circles in Fig. 1) was found in all individuals from the northern and central islands of Pinta, Marchena, Santiago, and Santa Fe, and in two of the four Santa Cruz birds. The second haplotype (black triangles) was shared among all Pinzón individuals, as well as five individuals from Isabela and one from Santa Cruz. The third haplotype (black squares) was found in all Fernandina individuals, the majority of the sampled individuals from Isabela, and the San Cristóbal individual (see below). The remaining four haplotypes were unique to individual islands: one present in all Española individuals, one in a single Santa Cruz individual, and two in three Isabela individuals. Interestingly, one Isabela haplotype was more similar to the common haplotype present on the five central and northern islands than it was to other Isabela haplotypes. The genetic distances between populations were small, with the average number of base pair differences ranging from 0 to 4.25 (mean uncorrected *p*-distances ranging from 0 to 0.005).

Due to the degraded nature of the San Cristóbal sample, we sequenced a subset of the COI 3′ and CR regions. We were able to sequence 281 of the 496 bp of COI 3′ and 308 of the 415 bp of the CR, covering 65% of the 911 bp sequenced from the other individuals. These two fragments encompassed all but one of the sites that were variable in the other Galápagos hawks; the one missing site was a site that separated the Española haplotype from all the rest of the haplotypes, including the Swainson's haplotypes (site number 22 in Table 4). At the regions sequenced, the San Cristóbal haplotype was identical to the Fernandina/Isabela haplotype. While we cannot rule out possible variable sites in the 311 bp not sequenced for the San Cristóbal hawk, the rest of the Galápagos haplotypes were all monomorphic at those sites (except for site 22). It is likely that this individual is representative of the former population on San Cristóbal given that seven of the other nine populations were fixed for a single haplotype.

We calculated F_{ST} values between Isabela and Fernandina and Isabela and Pinzón, because Fernandina and Pinzón were each fixed for haplotypes present on Isabela, though Isabela had additional haplotypes. Both Fernandina ($F_{ST} = 0.216$, $P < 0.01$) and Pinzón ($F_{ST} = 0.451$, $P < 0.01$) were significantly differentiated from Isabela.

The minisatellite data indicated some differentiation among populations (Fig. 2). Española and Santa Fe individuals formed independent, distinct clusters. Most of the Pinzón individuals also clustered, though not as distinctly as those from Española and Santa Fe. Marchena and Pinta individuals generally clustered together, with some differentiation between them. Only individuals from Santiago, Isabela, and Fernandina, the three largest and most variable populations, were indistinguishable from each other.

The four Santa Cruz birds were widely distributed in the tree. One individual fell within the Santa Fe cluster, having a banding pattern identical to four Santa Fe individuals. Another fell within the Pinzón cluster. These two birds also

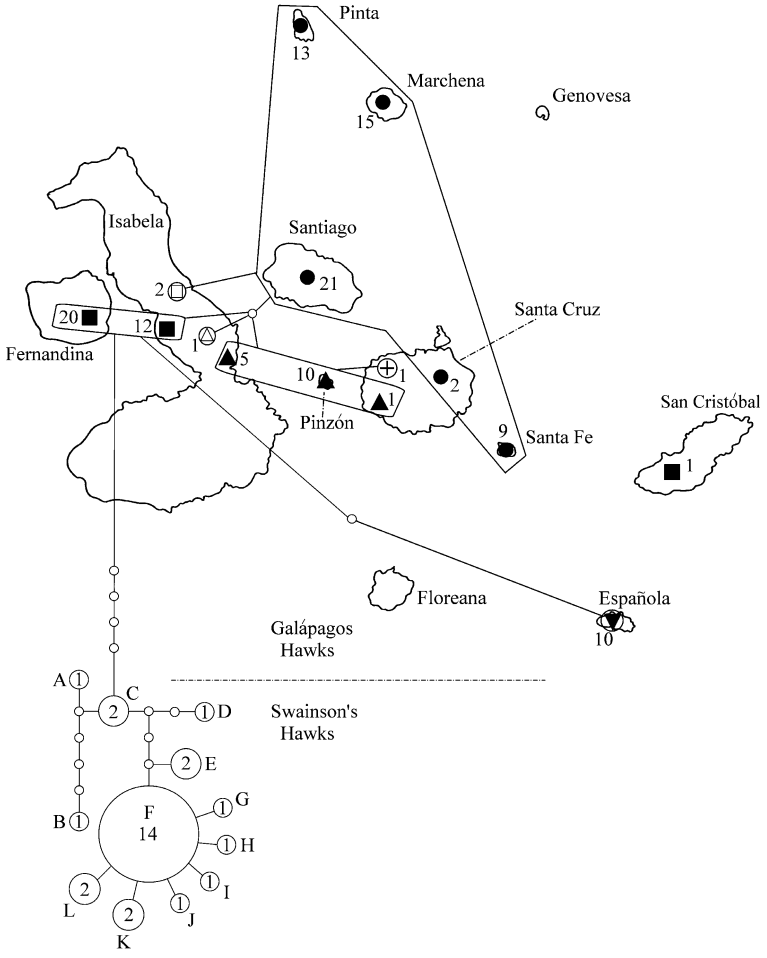

Fig. 1. Mitochondrial haplotype network of Galápagos and Swainson's hawks. Within the Galápagos hawks, each haplotype is represented by a different symbol (corresponding to symbols in Table 4 and Fig. 2), and the Swainson's hawks haplotypes are represented by different letters (corresponding to those in Table 4). Only one haplotype was found in each Galápagos hawk population except for Isabela (four haplotypes) and Santa Cruz (three haplotypes). The number of individuals with each haplotype is listed next to the corresponding symbol. It should be noted that while the Swainson's hawk haplotypes are drawn connecting to the Fernandina/Isabela haplotype, that same haplotype is also present on San Cristóbal, though it is based on fewer sequenced sites.

shared haplotypes with Santa Fe and Pinzón, respectively, suggesting that these birds were born on those islands and subsequently dispersed to Santa Cruz. The other two Santa Cruz birds were not closely associated with any particular population.

The program TCS will estimate the root of a haplotype network based on the position of a haplotype in the tree and its frequency, which correlate with haplotype age (Castelloe and Templeton, 1994). When Swainson's hawk haplotypes were not included, TCS estimated that the most likely root of the Galápagos hawk haplotypes was the common one shared by Pinta, Marchena, Santiago, Santa Fe, and Santa Cruz. When Swainson's hawks were included, TCS still estimated that the most common Galápagos haplotype was the root, because the program does not take into consideration information about outgroups. The haplotype network (Fig. 1) created by TCS, though, identified the haplotype shared by the Fernandina, Isabela, and San Cristóbal populations as the one most closely related to Swainson's hawks, indicating it is the oldest of the Galápagos hawk haplotypes.

4. Discussion

4.1. Recent divergence between Galápagos and Swainson's hawks

The mitochondrial data indicated that Galápagos hawks form a monophyletic clade; thus, there was likely a single colonization event. They showed remarkably little divergence from their mainland sister species, the Swainson's hawk, differing by only 0.42% over almost 3 kb of data. The divergence between Swainson's and Galápagos hawks is on average greater than that within either of them. There is overlap, however, in the ranges of the genetic distances; the maximum divergence among Swainson's hawk lineages and among Galápagos hawk lineages is greater than the minimum divergence between the two species (Fig. 1). It may be that if we sampled Swainson's hawks more broadly and included additional outgroups, we would find that Swainson's hawks are paraphyletic.

Although the genetic divergence between Galápagos and Swainson's hawks is minimal, their morphological differences are great enough to have prevented their earlier

Fig. 2. A midpoint rooted neighbor-joining tree of Galápagos hawk populations based on minisatellite distances (1 − similarity). Populations are identified with abbreviations: E, Española; F, Fernandina; I, Isabela; M, Marchena; PT, Pinta; PZ, Pinzón; SA, Santiago; SC, Santa Cruz; and SF, Santa Fe. The symbols following the population abbreviations represent mitochondrial haplotypes and correspond to those on the haplotype network (Fig. 1). The four Santa Cruz individuals are in boxes.

identification as sister species (e.g., Brown and Amadon, 1968; de Vries, 1973). Many studies have found significant morphological differentiation between species that show little if any mitochondrial divergence (e.g., Freeland and Boag, 1999; Seutin et al., 1995; Piertney et al., 2001). In an analysis of Old World *Buteo* lineages, Kruckenhauser et al. (2004) also found little mitochondrial divergence among morphologically distinct species and subspecies. The life histories of Swainson's and Galápagos hawks (migratory vs. sedentary, prey base) differ greatly in ways that affect their morphology, especially their wings and talons. In addition to selection, the rapid morphological differentiation could be the result of genetic bottlenecks and ongoing drift in small island populations. Swainson's and Galápagos hawks are not necessarily less divergent than other *Buteo* sister species. Using sequence data from Riesing et al. (2003) sequence data for the mitochondrial gene nd6, we calculated a *p*-distance of 0.008 between Swainson's and Galápagos hawks and an average *p*-distance of 0.010 ± 0.002 (SD) within five other well-supported (based on bootstrap values) pairs of *Buteo* sister species. There are few other raptor mitochondrial studies; however, Groombridge et al. (2002) found similarly low levels of divergence between some kestrel species.

The extremely low level of divergence between the Galápagos and Swainson's hawks indicates that they separated only very recently (less than 300,000 years ago). Of the native Galápagos fauna studied to date, Galápagos hawks appear to be the most recently arrived lineage. Some taxa predate the current islands. The endemic land (*Conolophus*) and marine (*Amblyrhynchus*) iguanas are sister taxa, likely having diverged 10–20 million years ago (MYA) on the now sunken islands (Rassmann, 1997; Wyles and Sarich, 1983). Lava lizards (*Microlophus* spp.) likely colonized the islands multiple times between 6 and 20 MYA (Kizirian et al., 2004; Lopez et al., 1992; Wright, 1983), and *Galapaganus* weevils separated from their mainland relatives approximately 11 MYA (Sequeira et al., 2000). Other lineages arrived in Galápagos more recently, colonizing the current islands. The oldest divergence among the 11 extant Galápagos tortoise (*Geochelone nigra*) subspecies occurred 1.5–2 MYA (Caccone et al., 1999, 2002). Sato et al. (2001) estimated that Darwin's finches diverged from their closest mainland relative around 2.3 MYA, likely arriving in Galápagos from the Caribbean (Burns et al., 2002). The yellow warbler (*Dendroica petechia aureola*) diverged from the mainland form approximately 2.5 MYA (Collins, 2003).

4.2. Galápagos hawk phylogeography

Most Galápagos lineages underwent further differentiation as they colonized multiple islands, and, in many taxa, older lineages occur on the older eastern islands (San Cristóbal, Española, and Floreana) and younger lineages on the western islands (e.g., Beheregaray et al., 2004; Rassmann et al., 1997; Sequeira et al., 2000). For example, six of the 11 tortoise subspecies occur on different islands (the rest inhabiting the five volcanoes of Isabela), and mitochondrial and microsatellite data indicate significant genetic differentiation among them (Caccone et al., 2002; Ciofi et al., 2002). There should be greater genetic diver-

gence among the older lineages due to a longer period of isolation. In the tortoises, differences among populations explain 97% of mitochondrial molecular variance for older islands and only 60% for younger islands (Beheregaray et al., 2004). Within geckos (*Phyllodactylus* spp.) and lava lizards, Wright (1983) found that the populations on the central and western islands tended to have higher allozyme similarities than the more divergent populations to the east.

The Galápagos hawk haplotype network shows a striking pattern of genetic monomorphism within populations and short genetic distances among populations at the mitochondrial loci. Four different populations (Santa Fe, Santiago, Marchena, and Pinta) comprising 58 sampled individuals were fixed for a single haplotype. Fernandina, Pinzón, and Española were also fixed but for different haplotypes. Only the populations on Isabela and Santa Cruz had any variability. Española hawks in the east have the highest mean genetic distance from the other populations; however, Española is not necessarily the oldest population, but instead may have become the first population to be isolated from the rest. The paucity of different haplotypes and the small genetic distances among them suggests the hawks spread across the archipelago relatively quickly, with subsequent lineage sorting resulting in different haplotypes on different islands. The pattern on Isabela, with haplotypes that are not most closely related to each other, and the presence of the same haplotype on San Cristóbal as on Fernandina (at opposite ends of the archipelago) further supports this. It is difficult to say from which direction the initial hawk colonization of the archipelago occurred; the Swainson's hawks were most closely related to the Fernandina/Isabela/San Cristóbal haplotype that was located on the far eastern and western islands. Limitations due to lineage sorting and possible homoplasy prevent a more definitive determination of the colonization pattern. Our understanding is also hindered by the missing information from the extirpated Floreana population, and our four samples from Santa Cruz (the most central island) are likely not representative of the former population there (see next section).

The role of genetic drift in these island populations was also demonstrated by the finding that the majority of nucleotide substitutions in the 3′ end of COI within Galápagos hawks were nonsynonymous. This finding is unsurprising from a theoretical perspective, given that slightly deleterious mutations with respect to fitness are expected to drift to fixation at a higher rate within small populations relative to larger populations (reviewed in Johnson and Seger, 2001). This qualitative interpretation is supported further by an empirical study by Johnson and Seger (2001) which found elevated rates of nonsynonymous substitutions on lineages of island bird taxa compared to their mainland relatives. Finally, the fact that Galápagos hawks have very small island populations, the majority of which are genetically isolated (Bollmer et al., 2005) also lends support for the role of drift in generating these patterns.

4.3. Mitochondrial vs. nuclear differentiation among populations

Mitochondrial and nuclear markers can often be used in conjunction to draw more accurate conclusions about genetic structure. The eastern population on Española was clearly genetically isolated at both mitochondrial and minisatellite loci. The central and northern populations (Santa Fe, Santiago, Marchena, and Pinta) share a common mitochondrial haplotype even though our pairwise F_{ST} estimates show significant differentiation among them at the more rapidly evolving minisatellite loci (Bollmer et al., 2005). The western populations of Fernandina and Isabela, less than 5 km apart, were statistically indistinguishable at minisatellite loci (Bollmer et al., 2005) and shared a mitochondrial haplotype; moreover, one female hawk banded as a juvenile on Isabela (Volcan Alcedo) in 1998 was observed in a territorial group on Fernandina in 2003, though we do not know which is its natal island (Bollmer et al., 2005). The presence of other haplotypes on Isabela, however, resulted in a significant F_{ST} value between them for the mitochondrial data. This discrepancy between the nuclear and mitochondrial data could be due to male-biased gene flow, though we have no other evidence that this occurs. Another explanation is that it is due to the differing natures of the two markers. Santiago, Isabela, and Fernandina are the largest of the hawk populations and have retained the most genetic variability. The fact that they are more distinguishable at mitochondrial loci than at minisatellite loci could be attributed to the shorter coalescent time of the mitochondrial loci, thus allowing significant genetic structuring to arise more quickly.

The combined mitochondrial and nuclear data can also be used to determine the populations of origin of dispersers, which is of potential conservation importance, both from the perspective of disease transmission and population management. Given the apparent absence of a breeding population on Santa Cruz, both the mitochondrial and the minisatellite data suggest that the four Santa Cruz juveniles are likely dispersers from different islands. One was very likely born on Pinzón and one on Santa Fe; both their minisatellite and mitochondrial profiles are consistent with that. The origin of the other two individuals is less clear. Neither of them is closely associated with any of the more inbred populations at the minisatellite loci, leaving Fernandina, Isabela, and Santiago as possible source populations. One shares the same haplotype as Santiago; the other has a unique haplotype that is most closely related to the one shared by Isabela and Pinzón. Given the genetic monomorphism on Pinzón, the latter bird more likely originated on Isabela.

Taking both the nuclear and mitochondrial data into account, the overall pattern among Galápagos hawk populations is one of genetic isolation. The Santa Cruz population is certainly an exception in that juveniles appear to be dispersing there, and there may be gene flow between

Fernandina and Isabela, since they are indistinguishable at the nuclear loci (though not at the mitochondrial loci). All the other populations show statistically significant divergence at nuclear or mitochondrial loci or both. This, combined with the morphological differentiation among populations and the recentness of its arrival, may mean that the Galápagos hawk is in the very early stages of speciation. The much older finch colonization of the archipelago resulted in fourteen morphological species; however, mitochondrial data only distinguished four groups (Sato et al., 1999), and interspecific genetic distances at microsatellite loci were generally lower among sympatric populations than among allopatric populations, likely due to introgressive hybridization (Grant et al., 2005). Galápagos hawks are less vagile, and most of their populations, like those of other sedentary species in the archipelago (e.g., tortoises, lava lizards), appear to be on separate evolutionary trajectories. Although the colonization history of the Galápagos hawk remains unclear, reconstructing the genealogies of its parasites (de Vries, 1975; Whiteman and Parker, 2005) may yield insight into the hosts' movements within the archipelago.

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