PHYLOGEOGRAPHY AND HISTORY OF GIANT GALÁPAGOS TORTOISES

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Abstract.—We examined the phylogeography and history of giant Galápagos tortoise populations based on mitochondrial DNA sequence data from 161 individuals from 21 sampling sites representing the 11 currently recognized extant taxa. Molecular clock and geological considerations indicate a founding of the monophyletic Galápagos lineage around 2–3 million years ago, which would allow for all the diversification to have occurred on extant islands. Founding events generally occurred from geologically older to younger islands with some islands colonized more than once. Six of the 11 named taxa can be associated with monophyletic maternal lineages. One, *Geochelone porteri* on Santa Cruz Island, consists of two distinct populations connected by the deepest node in the archipelago-wide phylogeny, whereas tortoises in northwest Santa Cruz are closely related to those on adjacent Pinzón Island. Volcan Wolf, the northernmost volcano of Isabela Island, consists of both a unique set of maternal lineages and recent migrants from other islands, indicating multiple colonizations possibly due to human transport or multiple colonization and partial elimination through competition. These genetic findings are consistent with the mixed morphology of tortoises on this volcano. No clear genetic differentiation between two taxa on the two southernmost volcanoes of Isabela was evident. Extinction of crucial populations by human activities confounds whether domed versus saddleback carapaces of different populations are mono- or polyphyletic. Our findings revealed a complex phylogeography and history for this tortoise radiation within an insular environment and have implications for efforts to conserve these endangered biological treasures.

Key words.—Galápagos, *Geochelone nigra* (*elephantopus*), giant tortoises, mitochondrial DNA, molecular phylogenies, phylogeography.

Biota inhabiting relatively young, remote, oceanic archipelagoes are prized by ecologists and evolutionary biologists because they often display transparent cases of processes not readily observable in more complex and mature mainland ecosystems. Several special features of island species can be identified. First, on remote oceanic islands, species are isolated from their mainland progenitors and often evolve into new, endemic forms. Second, for species that are not strong swimmers or fliers, populations occupying different islands are free to independently evolve, thus providing natural replicates of the evolutionary process. Third, the geological record of island formation can often provide important information for reconstructing the biogeographic history of species.

Several examples of such island species reside on the Galápagos Archipelago including Darwin's finches, marine and land iguanas, and the subject of this report, giant tortoises. The Galápagos are volcanic islands located 1000 km west of the coast of Ecuador and have never been connected to mainland South America. The islands were formed as the Nazca Plate passed west to east over a hotspot such that the islands in the east (Española and San Cristóbal) are the oldest with progressively younger islands to the west (Fig. 1). The oldest lava flows on eastern islands have been aged to no more than 4 million years, whereas the youngest islands, Fernandina and Isabela, are less than 0.5 million years old (White et al. 1993).

The taxonomic history of Galápagos tortoises is complex

and even today remains unsettled (Ernst and Barbour 1989; Pritchard 1996; Zug 1997). Up to 15 species or subspecies have been described, yet no clear consensus exists as to their taxonomic rank. When referred to as a single species, the names *Geochelone nigra* or *G. elephantopus* are in most use today. Of the 15 described taxa, most correspond to tortoises on a single island; the exception is the largest island, Isabela, which has five named taxa occupying each of its five major volcanoes (Fig. 1). Four of the 15 named taxa no longer exist, and the remaining 11 are in various stages of imperilment. Because of the unsettled taxonomy of these tortoises and because our initial genetic studies indicated genetic differentiation may not always correspond to taxonomic status (Caccone et al. 1999), here we will refer to population samples and after having presented the results, will attempt to relate them to the prevailing taxonomy. In a future publication, we intend to integrate our ongoing genetic studies with previous morphological, ecological, and behavioral data in an attempt to arrive at a logical modern taxonomy, but this issue is not the purview of this paper.

Here we report on our ongoing work on the genetics and phylogenetics of these tortoises. Our previous work identified the relatively small Chaco tortoise (*G. chilensis*) on mainland South America as the likely closest living relative of Galápagos tortoises (Caccone et al. 1999). We also demonstrated that the Galápagos tortoises are monophyletic and, based on molecular clock estimates, the differentiation of the distinct populations could have occurred in situ on the extant islands.
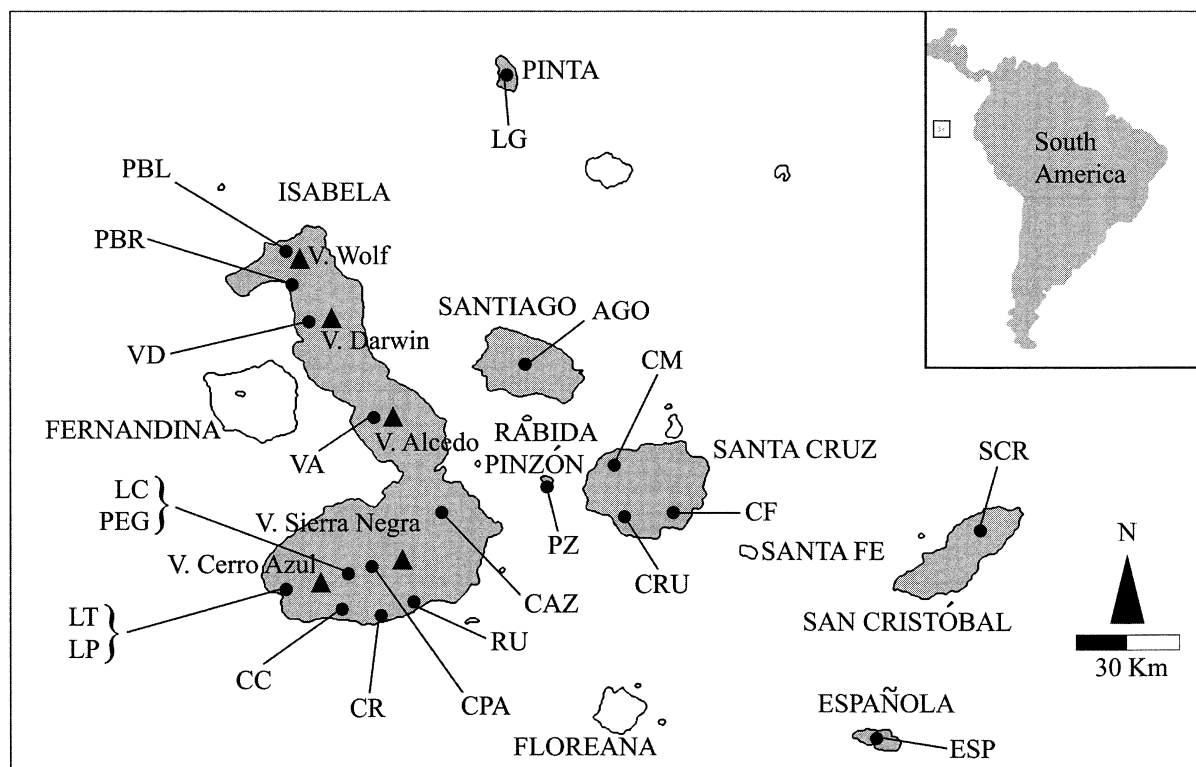
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FIG. 1. Map of the Galápagos Islands with an inset showing their location with respect to South America. Each island name is shown together with the approximate location of the populations sampled (dots) and its abbreviation (see Table 1 for details). Black triangles represent the tops of volcanoes on Isabela. Shaded islands have extant populations of tortoises.

Here we expand our sampling of both individuals and genes from the various populations and report on variation in the nucleotide sequence of about 4.5 kb of mitochondrial DNA (mtDNA) in 161 individuals. We address the issues of how genetically distinct and cohesive are the different populations, how they are related to one another, how the geological history of the islands lends insight into their phylogeography, and how these genetic findings relate to the current systematics for the group.

MATERIALS AND METHODS

We studied 161 individuals belonging to 21 populations representing all the extant named taxa of Galápagos tortoises. See Table 1 and Figure 1 for information on the taxonomic classification, location and number of the individuals studied from each site, and abbreviations we use to refer to collections. We analyzed three to 17 tortoises for each site except for sites where only one individual exists (LG) or was available (CPA and CM). Two individuals each were sequenced for all three extant South American *Geochelone* species and used as outgroups: *G. chilensis* (Chaco tortoise), *G. denticulata* (yellow-footed tortoise), and *G. carbonaria* (red-footed tortoise).

DNA was extracted from blood stored at 4°C as in Caccone et al. (1999). We obtained DNA sequences for six mtDNA regions: 12S (430 bp) and 16S rRNA (553 bp) genes, the cytochrome *b* (cyt*b*, 416 bp) gene, the control region (934 bp), and the ND5 (1790 bp) and ND6 (520 bp) genes. Primers and polymerase chain reaction (PCR) conditions for the

rDNA genes, the cyt*b*, and the control region are described in Caccone et al. (1999), where results from a small subset of individuals were reported. The entire ND5 and ND6 genes (approximately 2400 bp) were amplified in a single PCR reaction with primers designed for the flanking conserved regions located at the ends of the fragment. PCR conditions and PCR and sequencing primers for all the gene fragments are available from the authors.

Sequences were determined with an automated sequencer (Applied Biosystems 377, Foster City, CA) following the manufacturer protocols. To promote accuracy, both strands were sequenced. Sequences were edited using the program Sequencer 3.1.1 (Gene Codes Corporation, Ann Arbor, MI). GenBank accession numbers are AY097476–AY098441. Alignments were done by eye and also checked using CLUSTAL W (Thompson et al. 1994). They are available from the authors.

Network analysis to estimate gene genealogies were carried out using the TCS program (Clement et al. 2000) which implements the Templeton et al. (1992) statistical parsimony procedure. Input data were individual mtDNA sequences. This program collapses sequences into haplotypes, calculates haplotype frequencies in the sample, and produces a network linking different haplotypes only if they have a 95% probability of being justified by the parsimony criterion.

Phylogenetic analyses were carried out using maximum parsimony (MP; Farris 1970), maximum-likelihood (ML; Felsenstein 1981), neighbor-joining (NJ; Saitou and Nei 1987), and Bayesian methods (Larget and Simon 1999; Huel-

TABLE 1. List of the populations of Galápagos tortoises studied. Names of each subspecies and their estimated population size (MacFarland et al. 1974) are reported together with the carapace morphology (Fritt 1983, 1984). The island and location where the samples were collected is also shown. The last two columns report the abbreviations used for each population and its sample size.

Taxa	Estimated population size	Carapace morphology[1]	Island	Locality	Population abbreviation[2]	Sample size
abingdoni	1	S	Pinta	—	LG	1
chatamensis	500–700	S	San Cristóbal	Media Luna	SCR	12
darwini	500–700	sS	Santiago	—	AGO	14
ephyppium	150–200	S	Pinzón	Central	PZ	13
hoodensis	15 (native)	S	Española	Research Station	ESP	16
porteri	2000–3000	D	Santa Cruz	Caseta	CRU	12
		D	Santa Cruz	Cerro Fatal	CF	9
		S	Santa Cruz	Cerro Montura	CM	1
becki	1000–2000	D/S	Isabela-Volcan Wolf	Piedra Blanca	PBL	12
			Isabela-Volcan Wolf	Puerto Bravo	PBR	10
microphyes	500–1000	D	Isabela-Volcan Darwin	Calleta Tagus	VD	17
vandenburghi	3000–5000	D	Isabela-Volcan Alcedo	—	VA	7
guntheri/vicina	100–300	D	Isabela-Sierra Negra	Cazuela	CAZ	5
			Isebela-Sierra Negra	Cerro Paloma	CPA	1
			Isabela-Sierra Negra	Cabo Rosa	CR	5
			Isabela-Sierra Negra	Roca Union	RU	4
	400–600	D	Isabela-Cerro Azul	Cincos Cerros	CC	3
			Isabela-Cerro Azul	Los Crateres	LC	4
			Isabela-Cerro Azul	Los Pampas	LP	4
			Isabela-Cerro Azul	Las Tablas	LT	4
			Isabela-Cerro Azul	Los Pegas	PEG	7

[1] D, domed; S, saddleback; and sS, semisaddleback carapace morphology.
[2] Each population is identified by a code in capital letters, which refers to their location.

senback 2000). MP, ML, and NJ analyses were carried out starting from consensus sequences for each of the 21 samples, using PAUP* 4.0b8 (Swofford 2001). MP searches were done by coding variable sites within a sample as polymorphic and by treating gaps as missing data. Heuristic searches were run using ACCTRAN character-state optimization, 100 random stepwise additions, and the TBR branch-swapping algorithm. Searches were performed using various weighting methods: all substitutions unweighted, transversions (Tv) weighted three times more than transitions (Ti), or using only Tv. MP analyses were also performed excluding Ti from third positions of all codons and excluding all changes in third codon position. ML analyses were carried out using empirically determined Ti/Tv ratio (Ti/Tv = 15.933). Rates were assumed to be variable following a gamma distribution with an empirically determined shape parameter ($\gamma = 0.208$). For the NJ analysis, Tamura and Nei (1993) distances (TN) were calculated using the same empirically determined gamma parameter.

We used Markov chain Monte Carlo (MCMC) methods within a Bayesian framework to estimate the posterior probability of phylogenetic trees (Larget and Simon 1999). The MCMC procedure ensures that trees are sampled in proportion to their probability of occurrence under the model of gene-sequence evolution. We employed the general time reversible (GTR) model of sequence evolution, combined with gamma rate heterogeneity and rate variation partitioned by gene and by codon positions to estimate the likelihood of each tree. Bayesian analyses were done on the combined dataset using MrBayes (Huelsenback 2000). This program was run for 2 million generations with a sampling frequency of 100 generations. From the 20,000 trees obtained, the first 10% were discarded to avoid including any tree that might have been sampled before convergence of the Markov chain. A 50% majority-rule consensus tree was constructed from the remaining trees using PAUP*. Alternative tree topologies were tested using the Templeton (1983) two-tailed Wilcoxon rank test (Larson 1994) and the one tailed Shimodaira-Hasegawa (1999) log-likelihood test as implemented in PAUP*. The robustness of the phylogenetic hypotheses was also tested by bootstrap (Felsenstein 1985).

Rate homogeneity among lineages was tested for each gene and for the combined data set by using the maximum-likelihood ratio test (Goldman 1993) as implemented in PAUP*. The null hypothesis (all lineages evolving at the same rate) was rejected if $P < 0.05$.

RESULTS

Sequence Variation

We sequenced 4481 bp of mtDNA for each of 161 Galápagos tortoises plus representatives of the three continental South American species. The length of the alignment for the combined dataset is 4642 bp. This is due to some 3-bp insertions in the ND5 gene in the outgroup species, and one large insertion of 147 bp in the control region of *G. denticulata*. The mtDNA regions include fragments of the 12S and 16S rDNA genes, the first half of the cyt*b* gene, the entire ND5 and ND6 genes, and 73% of the control region. Because both strands were analyzed, this corresponds to approximately 1.5 million bases of DNA sequencing.

Levels of sequence variation and number of phylogenetically informative sites vary quite substantially among gene fragments (Table 2). As expected ribosomal genes display the least amount of variation (11.0–12.8%), whereas the highest levels of variation were found in third codon positions

TABLE 2. Percent variable and informative sites by gene and codon positions across the 25 taxa of tortoises studied. A + T and G percentages for the same regions are also listed.

Gene and codon positions	No. of sites	% variable	% informative	% A + T	% G
12S	429	12.8	5.4	56.4	17.6
16S	553	11.0	4.1	55.6	19.4
cytb-first	139	9.4	2.9	56.1	20.0
cytb-second	139	2.2	0	61.1	16.5
cytb-third	138	43.5	21.7	53.8	3.3
cytb-all	416	18.3	8.2	57.1	13.3
ND6-first	174	13.8	6.9	55.6	6.0
ND6-second	173	6.4	1.7	58.7	15.6
ND6-third	173	32.4	14.4	66.9	2.0
ND6-all	520	17.5	7.7	60.4	7.9
ND5-first	597	16.6	4.9	58.5	17.2
ND5-second	597	11.6	2.7	59.3	10.3
ND5-third	597	47.4	18.8	58.6	2.3
ND5-all	1791	25.2	8.8	58.8	10.0
Control region	933	28.0	14.0	65.8	14.6
All	4642	21.4	8.8	59.4	15.5

of the ND5 gene (47.4%) and in the control region (28.0%). Coding regions for all three coding fragments display a deficit in guanine in third codon positions typical of mtDNA coding genes. Ti outnumber Tv in the whole dataset, even for the most distant comparisons involving the mainland outgroups. This, together with inspection of saturation plots for each gene and position (data not shown), suggests that Ti-saturation is not a major problem in this dataset. Ti/Tv ratios

(computed via maximum likelihood) are 15.9 for all genes combined and, individually, are 6.1 (rRNA genes), 14.0 (cytb), 17.5 (ND6), 13.5 (ND5), and 60.4 (control region).

Figure 2 presents average and range of percent sequence divergence for each DNA fragment and for the combined data. Average percent sequence divergences within populations range from 0% and 0.2% in the rRNA genes to 0.6% in the control region. Average percent sequence divergence
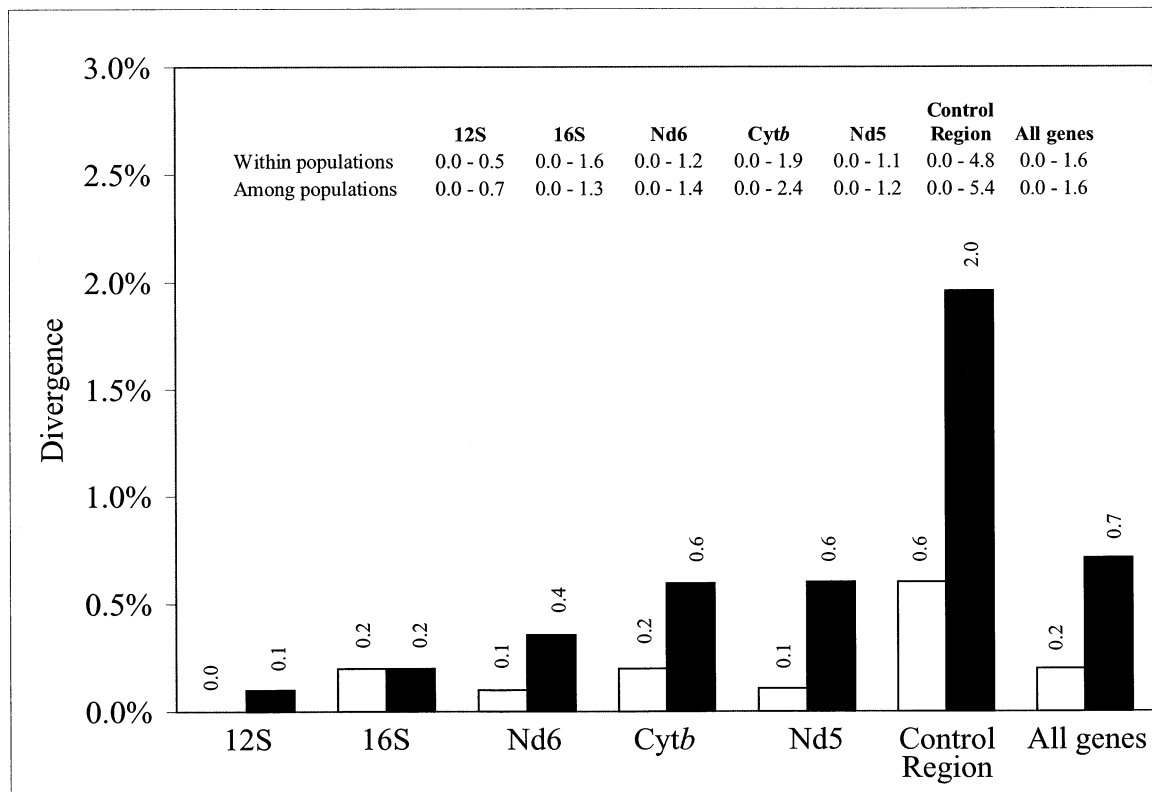
FIG. 2. Evolutionary rates among the six genes studied and for the combined dataset. Plotted are the averages of percent sequence divergence within (white bars) and between populations (black bars) of Galápagos tortoises. The average for each comparison is reported on top of the bars. The ranges are provided at the top of the figure. Ranges for the comparisons with the closest outgroup (*Geochelone chilensis*) are provided in the text.

between populations ranges from 0.1% to 0.2% for the rRNA genes to 2% for the control region. Uncorrected nucleotide divergence between the Galápagos tortoises and *G. chilensis* are 3.6% (12S), 3.9% (16S), 6.8% (cyt*b*), 8.9% (ND6), 13.8% (ND5), 11.6% (control region), and 11% (all genes combined).

Haplotype Distribution

The distribution of the variable sites for each of the 85 distinct haplotypes found within the Galápagos tortoises is available via http://pantheon.yale.edu/~sv54/consgalap.html. Table 3 reports haplotype frequencies in each population for the combined dataset. The designation of haplotypes in this table, mtDNA followed by a number, allows identification of the specific sequence from the information on the website. The number of distinct haplotypes present in each population from which more than one individual was sampled ranges from three to 10. The only exception is the population from Española (ESP), where all the 15 breeders studied have a single mtDNA haplotype. Most populations are characterized by groups of very closely related haplotypes (differing by no more than 17 substitutions in 4.5 kb, with an average of three). This is not true for four populations on Isabela (PBL, PBR, VD, and RU). Of the 161 tortoises analyzed, 10 tortoises belonging to these four populations stand out as being aberrant in that they differ from individuals from the same location by 27 to 70 substitutions while they differ by a few substitutions from haplotypes found only in geographically distinct populations, often located on different islands. We will refer to these as ''aliens.''

Table 4 shows as an example of the extent of the differences found between the individuals of one of these four populations (Volcan Darwin, VD). Most individuals have identical or nearly identical haplotypes (none to four substitutions in 4.5 kb of DNA). One individual (VD4) clearly stands out differing by 64–67 substitutions from all the others. Interestingly VD4 has a haplotype (mtDNA78, Table 3) that is only found in PBL (Volcan Wolf). A similar pattern led us to identify eight other tortoises from Volcan Wolf (samples PBL and PBR). These individuals have haplotypes that are very different from the majority found in the locality where they were collected (54–70 substitutions), but very similar (if not identical) to haplotypes found only in other populations. Individual PBL20 has a haplotype (mtDNA84) that differs by only five to seven differences from haplotypes found only on San Cristóbal (SCR). PBL37 (mtDNA80) has three to six differences from haplotypes found only on Volcan Alcedo. A similar situation is observed in the other population from Volcan Wolf (PBR). Individuals PBR1, PBR2, and PBR27 have the same haplotype (mtDNA81) that is separated by two substitutions from PBR14 (mtDNA82). These two haplotypes are clearly distinct from the other haplotypes on PBR (>57 substitutions), and very similar to the single haplotype found on Española (five to seven substitutions). Two other PBR tortoises (PBR25 and PBR26) share the same haplotype (mtDNA85), which differs by only five substitutions from a haplotype (mtDNA69) found in a single individual (RU66) from Sierra Negra, Southern Isabela. RU66 is 26–27 steps away from the other RU samples. For these three individuals (PBR25, PBR26, and RU66) we can only say that their haplotypes are clearly different from the majority of haplotypes in the population from which they were sampled, but we cannot identify an obvious source population as we could for the other seven tortoises.

Network and Phylogenetic Analyses

Figure 3 shows the relationship among all the mtDNA haplotypes obtained from the network analysis on a trimmed dataset (151 tortoises) that excluded the 10 individuals we provisionally classified as aliens. The network was obtained using statistical parsimony (Templeton et al. 1992) as implemented in the TCS program (Clement et al. 2000). This analysis identifies five distinct groups of haplotypes (I to V in Fig. 3). Cluster I is represented by haplotypes found in the two populations sampled on Volcan Wolf (northern Isabela) and Santiago (mtDNA1–7, mtDNA34, mtDNA51 and 52, and mtDNA78, 79, and 83; see Table 3). Cluster II (mtDNA36 and 41) groups all the individuals from Española with the sole survivor of the population from Pinta (Lonesome George). Cluster III (mtDNA15–18 and mtDNA72 and 73) includes all the individuals from San Cristóbal linked with all the individuals of one of the three Santa Cruz populations (CF). Cluster IV includes all the individuals from Pinzón (PZ) linked to the only individual we studied from the CM population on Santa Cruz (mtDNA57–66 and mtDNA35). The most complex is cluster V, which includes two major subclusters of haplotypes separated by at least 17 steps. The first of these subclusters includes all the haplotypes (mtDNA25–33 and mtDNA70 and 71) found in the CRU population on Santa Cruz. This cluster links to the other major subgroup, which includes haplotypes found on central and southern Isabela, via haplotypes found only on Volcan Darwin (VD, central Isabela; mtDNA49 and 50 and mtDNA77). The haplotypes (mtDNA74–76) found only on Volcan Alcedo (central Isabela) cluster next, together with all the remaining haplotypes found in the nine samples from southernmost part of Isabela (Sierra Negra and Cerro Azul).

Figure 4 shows the ML tree for the combined dataset on consensus sequences for each sample (excluding the aliens) and summarizes the results of the other phylogenetic analyses. MP trees (using either unordered or ordered characters), the NJ tree based on TN distances, and the Bayesian tree have topologies that are statistically indistinguishable from the ML tree, as indicated by the results of both the Templeton (1983) and Shimodaira and Hasegawa (1999) tests. We also analyzed phylogenetically the six mtDNA regions separately and found no significant incongruence with the topology in Figure 4 (data available from authors). As expected, the relatively slowly evolving rRNA genes, cyt*b,* and the ND6 provide good support for the deepest branches, whereas the faster evolving ND5 gene and the control region produced robust resolution at the tips of the tree. The Chaco tortoise, *G. chilensis*, is the closest living relative of the monophyletic Galápagos complex. The branch separating the Galápagos clade from the South American tortoises is much longer (175 steps in the MP tree, about 19% TN distance) than the branches within the Galápagos complex. The deepest node in the Galápagos lineage is the one linking the two easternmost pop-

ulations (ESP and SCR) and their respective sister taxa (LG and CF) to a clade that includes all the other populations. Within the latter cluster the northernmost populations from Isabela and Santiago (PBL, PBR, and AGO) form a well-supported clade distinct from the populations from the central islands of the archipelago (Pinzón and Santa Cruz) and from the populations on the central and southern part of Isabela. The position of the branch leading to the Pinzón/CM clade is not well supported. The next well-supported clade includes one population from Santa Cruz (CRU), which is basal to a monophyletic clade containing all the populations from central and southern Isabela. Within this cluster there is no resolution among the 11 populations that belong to four named taxa (*microphyes*, *vandenburghi*, *guntheri*, and *vicina*) from Isabela.

The results of the phylogenetic analysis complement the results of the TCS analysis on individual haplotypes (cf. Figs. 3 and 4). Both analyses identify the same relationships between groups of individuals and/or haplotypes from the same location. They also similarly support the existence of sister taxa relationships between populations that would be assigned to different named taxa based on their geographic location: LG/ESP, SCR/CF, and PZ/CM. The network analysis does not link the five TCS clusters, whereas the phylogenetic analysis does. A minor but interesting difference between the two approaches is the resolution of the relationship of one of the populations on Santa Cruz (CRU) to all the populations from central and southern Isabela. The TCS network (Fig. 3) strongly identifies the three haplotypes from the population on Volcan Darwin (VD) as the closest haplotypes to the Caseta (CRU) population from Santa Cruz. This relationship suggests that the population on Volcan Darwin is not only distinct, but also is basal to the other taxa from the central and southern parts of Isabela.

Network and phylogenetic analyses were also carried out including the 10 aliens (results available on the website cited above). The addition of the aliens produced a TCS network with essentially the same topology as the one shown in Figure 3, but with an increased amount of reticulation among the haplotypes from Southern Isabela. Each of the aliens was identical to, or linked with 95% confidence to haplotypes or groups of haplotypes not found in their sample. MP, ML, and NJ trees on the consensus sequences from each sample plus the aliens resulted in the same topology as in Figure 3 with most aliens forming a strongly supported clade (bootstrap values > 90%) with samples from a different locality than where they were collected. Thus, the TCS network and the phylogenetic analyses support the existence in a few samples of maternal lineages unrelated to the most common haplotypes found in each location and in seven of 10 cases they are identical to or very closely related to haplotypes exclusive to other localities (see above).

DISCUSSION

Tortoise Movement

Understanding how tortoises migrate among islands is important in interpreting our findings. While not good swimmers, Galápagos tortoises float with their heads above water and can live without food or water for up to six months; thus, drifting on currents could convey them long distances. The arrival of tortoises on Galápagos 2–3 million years ago (see below) predates the origin of many of the islands they occupy today, so additional drifting events must have occurred within Galápagos. Currents responsible for mainland-archipelago drifting events include the Humboldt Current, which runs north along mainland South America and then diverts westward at Equatorial latitudes on which the Galápagos Archipelago lies. Within Galápagos prevailing currents run in a northwesterly direction, going from the older to younger islands (Pak and Zaneveld 1973).

A second source of transport has been translocation of tortoises by humans. From the 17th to 19th centuries, whalers and buccaneers collected tortoises as a source of fresh meat; records exist for the taking of some 40,000 tortoises (Townsend 1925), but as many as 200,000 may have been removed (MacFarland et al. 1974). Tortoises were occasionally stashed on various islands for safe-keeping and even tossed overboard in large numbers in nearshore areas to lighten cargo during flight or battle (Capt. David Porter 1812, quoted in Bauer 1889).

Aliens

The distinct morphological and genetic differentiation among populations of Galápagos tortoises could have occurred only if migration among the islands had been very rare in the thousands of generations of tortoise evolution that occurred before humans arrived; thus, although not impossible, we feel it unlikely that our small sample sizes would have detected a natural migrant (this assumes that natural migration has been more or less constant over time). Human transport is perhaps more likely for the presence of alien genotypes in our sample. Five of these rare occurrences of a maternal lineage typical of one population in an otherwise homogeneous set of lineages in another population may well involve interisland transport. All occur on Volcan Wolf, the northern most volcano of Isabela; four are Española-related haplotypes and one is a San Cristóbal-related haplotype. Moreover, all the interisland alien haplotypes were found on the west and northwest slopes of Volcan Wolf—a geographic pairing (Fig. 1) unlikely to be accounted for by natural drifting. The frequent passage of whaling ships by Volcan Wolf sailing toward fertile whaling sites to the north and west of the Galápagos Archipelago may have made this site an ideal location for whaling crews to deposit excess tortoises collected elsewhere to be retrieved upon return.

Other aliens could be due to migration over land. This could account for the presence on Vocan Darwin of an individual with a maternal lineage identical to those found on the adjacent Volcan Wolf (PBL). The presence on Volcan Wolf of an individual with a maternal lineage linked to the Volcan Alcedo (VA) population is more difficult to explain by land migration; human-associated transport may also have been involved here or relatively short drifting.

The coexistence of domed and saddleback populations as a result of habitat segregation may provide an alternative explanation for these patterns (Fritts 1983; T. H. Fritts, unpubl. mss.). Early in island formation arid conditions with sparse xerophilic vegetation predominate, an environment

TABLE 3. Haplotype frequencies in the 21 Galápagos tortoise populations. Population abbreviations are defined in Table 1.

Haplotype										Populations											
	LG	PBL	PBR	SCR	AGO	PZ	CAZ	CPA	CR	RU	ESP	VD	CRU	CF	CM	VA	CC	LC	LP	LT	PEG
mtDNA1	—	—	—	—	0.07	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA2	—	—	—	—	0.36	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA3	—	—	—	—	0.14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA4	—	—	—	—	0.07	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA5	—	—	—	—	0.07	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA6	—	0.25	0.40	—	0.07	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA7	—	—	—	—	0.14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA8	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA9	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA10	—	—	—	—	—	—	0.40	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA11	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.33	—	—	—	—
mtDNA13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.33	—	—	—	—
mtDNA14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.33	—	—	—	—
mtDNA15	—	—	—	—	—	—	—	—	—	—	—	—	—	0.67	—	—	—	—	—	—	—
mtDNA16	—	—	—	—	—	—	—	—	—	—	—	—	—	0.11	—	—	—	—	—	—	—
mtDNA17	—	—	—	—	—	—	—	—	—	—	—	—	—	0.22	—	—	—	—	—	—	—
mtDNA18	—	—	—	0.58	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA19	—	—	—	—	—	—	—	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA20	—	—	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA21	—	—	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA22	—	—	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA23	—	—	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA24	—	—	—	—	—	—	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA25	—	—	—	—	—	—	—	—	—	—	—	—	0.17	—	—	—	—	—	—	—	—
mtDNA26	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA27	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA28	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA29	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA30	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA31	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA32	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA33	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA34	—	—	—	—	0.07	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA35	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	—	—	—	—	—	—
mtDNA36	—	—	—	—	—	—	—	—	—	—	1.00	—	—	—	—	—	—	—	—	—	—
mtDNA37	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—	—
mtDNA38	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—	—
mtDNA39	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—	—
mtDNA40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—	0.29
mtDNA41	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA42	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	0.25	0.14
mtDNA43	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—
mtDNA44	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—
mtDNA45	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—	—
mtDNA46	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—
mtDNA47	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—
mtDNA48	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.25	—
mtDNA49	—	—	—	—	—	—	—	—	—	—	—	0.12	—	—	—	—	—	—	—	—	—
mtDNA50	—	—	—	—	—	—	—	—	—	—	—	0.47	—	—	—	—	—	—	—	—	—
mtDNA51	—	0.25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE 3. Continued.

Haplotype	LG	PBL	PBR	SCR	AGO	PZ	CAZ	CPA	CR	RU	ESP	VD	CRU	CF	CM	VA	CC	LC	LP	LT	PEG
mtDNA52	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA53	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14
mtDNA54	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14
mtDNA55	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14
mtDNA56	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14
mtDNA57	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA58	—	—	—	—	—	0.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA59	—	—	—	—	—	0.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA60	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA61	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA62	—	—	—	—	—	0.15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA63	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA64	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA65	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA66	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA67	—	—	—	—	—	—	—	—	—	0.25	—	—	—	—	—	—	—	—	—	—	—
mtDNA68	—	—	—	—	—	—	—	—	—	0.50	—	—	—	—	—	—	—	—	—	—	—
mtDNA69	—	—	—	—	—	—	—	—	—	0.25	—	—	—	—	—	—	—	—	—	—	—
mtDNA70	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA71	—	—	—	—	—	—	—	—	—	—	—	—	0.08	—	—	—	—	—	—	—	—
mtDNA72	—	—	—	0.33	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA73	—	—	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA74	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14	—	—	—	—	—
mtDNA75	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.71	—	—	—	—	—
mtDNA76	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.14	—	—	—	—	—
mtDNA77	—	—	—	—	—	—	—	—	—	—	—	0.35	—	—	—	—	—	—	—	—	—
mtDNA78	—	0.08	—	—	—	—	—	—	—	—	—	0.06	—	—	—	—	—	—	—	—	—
mtDNA79	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA80	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA81	—	—	0.30	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA82	—	—	0.10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA83	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA84	—	0.08	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
mtDNA85	—	—	0.20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Populations

TABLE 4. Pairwise comparisons of the absolute number of differences over 4.5 kb of mitochondrial DNA sequence for all the individuals within the Isabela populations from Volcan Darwin (VD).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1-VD26	—																
2-VD27	0	—															
3-VD28	3	3	—														
4-VD29	3	3	0	—													
5-VD16	3	3	0	0	—												
6-VD21	3	3	0	0	0	—											
7-VD22	3	3	0	0	0	0	—										
8-VD23	3	3	0	0	0	0	0	—									
9-VD24	3	3	0	0	0	0	0	0	—								
10-VD25	3	3	0	0	0	0	0	0	0	—							
11-VD17	4	4	1	1	1	1	1	1	1	1	—						
12-VD19	4	4	1	1	1	1	1	1	1	1	0	—					
13-VD7	4	4	1	1	1	1	1	1	1	1	0	0	—				
14-VD2	4	4	1	1	1	1	1	1	1	1	0	0	0	—			
15-VD3	4	4	1	1	1	1	1	1	1	1	0	0	0	0	—		
16-VD13	4	4	1	1	1	1	1	1	1	1	0	0	0	0	0	—	
17-VD4	65	67	67	64	64	64	64	64	64	64	65	65	65	65	65	65	—

that favors colonization by saddleback tortoises. At higher elevations on larger islands, moister conditions predominate, favoring colonization by domed tortoises and coexistence of both shell types. For example, the extinct San Cristóbal population existed in a moister environment, had access to high elevations, and was much more domed than the extant saddleback population on San Cristóbal now confined to a lower and drier environment. Similarly, the isolated saddleback Cerro Montura population on Santa Cruz exists in a drier habitat than the other Santa Cruz populations and may represent the remnants of the initial colonization of this island, with domed tortoises having replaced the saddlebacks on most of the island. Curiously, this Santa Cruz saddleback population may have given rise to the Pinzón population rather than vice versa. As a final example, the northernmost volcano on Isabela, Volcan Wolf, represents a mosaic of dry and moist environments, which may explain why Volcan Wolf is occupied by both saddlebacks (aligned with Española and/or San Cristóbal populations) and semisaddlebacks (aligned with Santiago). Our data cannot reject this alternative interpretation.

Genetics and Taxonomy

Removal of the 10 aliens from our sample of 161 individuals reveals several geographically distinct and genetically cohesive units. Of the 11 extant taxa, six correspond to genetically distinct monophyletic maternal lineages: *hoodensis* on Española, *chatamensis* on San Cristóbal, *ephippium* on Pinzón, *darwini* on Santiago, *microphyes* on Volcan Darwin, and *abingdoni* on Pinta. Additionally, two maternal lineages derived from distinct haplotype groups on Santiago can be identified on Volcan Wolf. The majority of individuals sampled on Volcan Wolf (16 of 22) belong to these two lineages, arguably the taxon described from Volcan Wolf as *becki*. However, the presence of other maternal lineages typical of other taxa, as well as the morphological variation on Volcan Wolf, make it difficult to assign a single taxon to this locality. That is, the majority of tortoises on this volcano are genetically unique (albeit not monophyletic) and closely related

to adjacent Santiago tortoises (cluster I in Fig. 3), but are not the exclusive occupants of this locale.

Tortoises from the two largest populations on Santa Cruz (Caseta, CRU; Cerro Fatal, CF) have a similar domed morphology and have been considered a single taxon, *porteri*. Phylogenetically, however, these two populations are connected through the deepest node within Galápagos tortoises and are more genetically distinct than most recognized taxa (Fig. 4). The population from Cerro Fatal is most closely related to San Cristóbal (cluster III in Fig. 3; see also Fig. 4), despite the distinct morphological differences between present day populations. The third small isolated population in northwest Santa Cruz (CM) is very closely related to tortoises from Pinzón (cluster IV in Fig. 3), consistent with the geographic proximity of Pinzón to this site (Fig. 1). Unlike all other Santa Cruz tortoises, the CM population has saddlebacked carapaces as do Pinzón tortoises.

Populations of tortoises on the southern volcanoes of Isabela present the most difficult taxonomic problems. Traditionally, populations on each of the Isabela volcanoes have been assigned to separate taxa. Our initial analysis, based on smaller regions of mtDNA could not genetically differentiate any of the four southern Isabela named taxa (Caccone et al. 1999). However, with the increase to 4.5 kb of sequence, we can now detect some degree of genetic differentiation for two of these taxa. The maternal lineages we found on Volcan Darwin form a unique grouping (VD in cluster V in Fig. 3) and thus the subspecies *microphyes* can be defended as a genetically distinct taxon. MtDNA haplotypes in this populations are distinct from, but very similar to, the maternal lineages on the three southern volcanoes. From the TCS analysis (Fig. 3) it appears that Volcan Darwin was colonized by migrants from the Caseta population (CRU) on Santa Cruz. Our sample from Volcan Alcedo (*vandenburghi*) contains unique haplotypes not found elsewhere, but they do not form a monophyletic clade (Fig. 3).

Despite increase sampling of both individuals and amount of mtDNA analyzed, there is no clear pattern of genetic differentiation of tortoise populations on the two southernmost
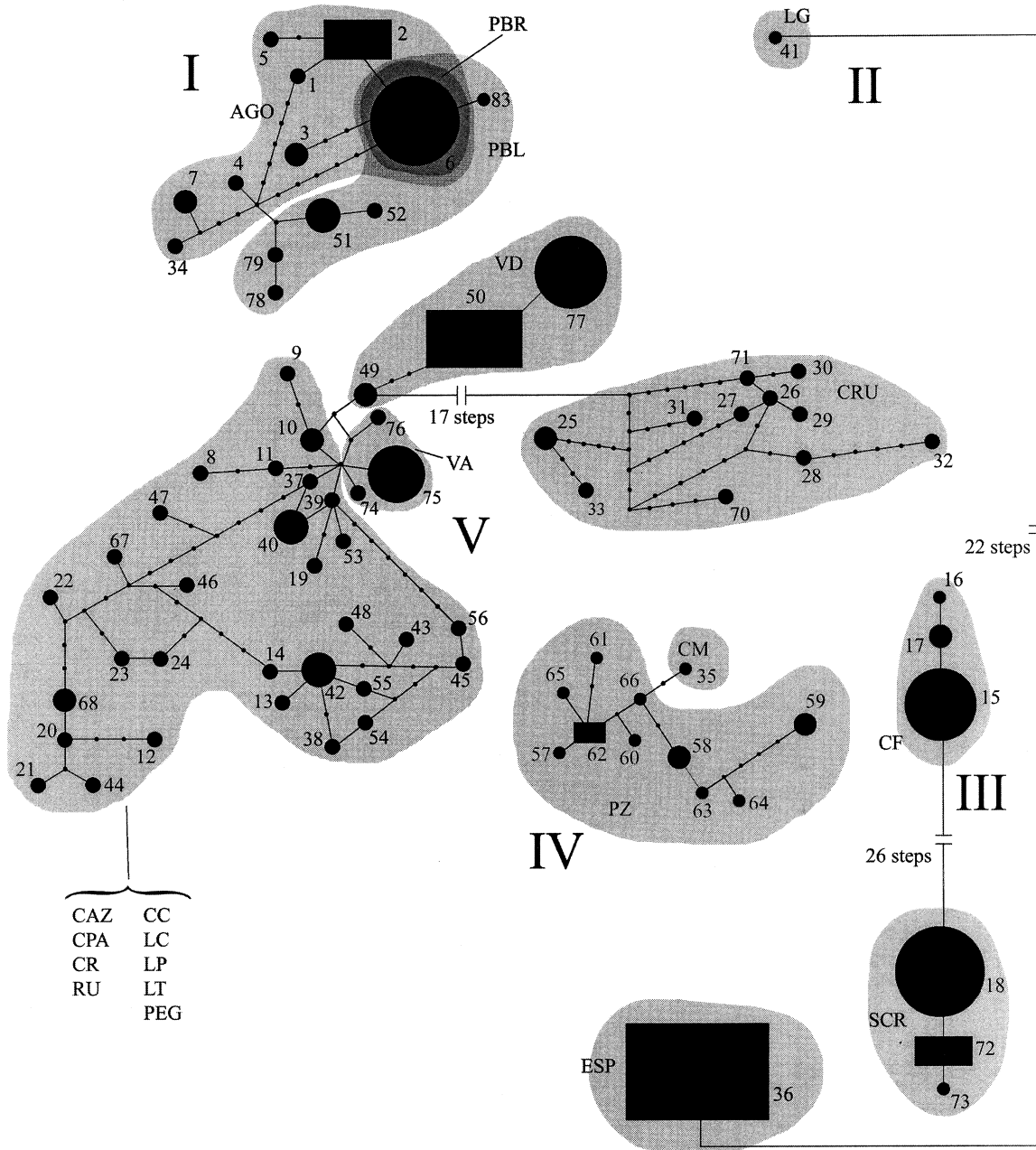
FIG. 3. Network analysis based on statistical parsimony (Templeton et al. 1992) showing the genealogical relationship of the 85 haplotypes found in 151 individuals of Galápagos (all six genes combined). The maximum number of steps connecting parsimoniously two haplotypes is depicted. Only connections with a probability higher than 95% are shown. Roman numerals label isolated clusters not connected to other clusters. Haplotypes considered ancestral according to coalescence principles (Donnelly and Tavaré 1986) are boxed. The other haplotypes are shown as circles. The size of the squares or the circles corresponds to relative haplotype frequency. The number in each square or circle identifies the haplotype in Table 3 and on the website cited in the text. The number of nucleotide substitutions between haplotypes is given by the number of dots connecting them, connections are bracketed when the number of steps was higher than 12. Only a limited amount of reticulation is present (loops), indicating low amounts of homoplasy in the dataset. Shadings encompassing groups of haplotypes identify the population in which the haplotypes are found (identified with its letter code as in Table 1). Overlapping shadings represents groups of haplotypes present in more than one populations. Positioning of samples roughly approximates geographic relationships (e.g., Fig. 1).

volcanoes. Tortoises from Sierra Negra have been assigned to the taxon *guntheri* and those from Cerro Azul to *vicina*. If we assign our samples based on locality of collection (i.e., Sierra Negra vs. Cerro Azul), then clearly we cannot genetically distinguish these two taxa. Early explorers found no evidence of separation between the populations of tortoises on Cerro Azul and Sierra Negra; it was only in 1925 that a volcanic eruption produced a lava flow that today partially separates tortoises on these two volcanoes (MacFarland et al. 1974). However, the distribution of these named taxa may
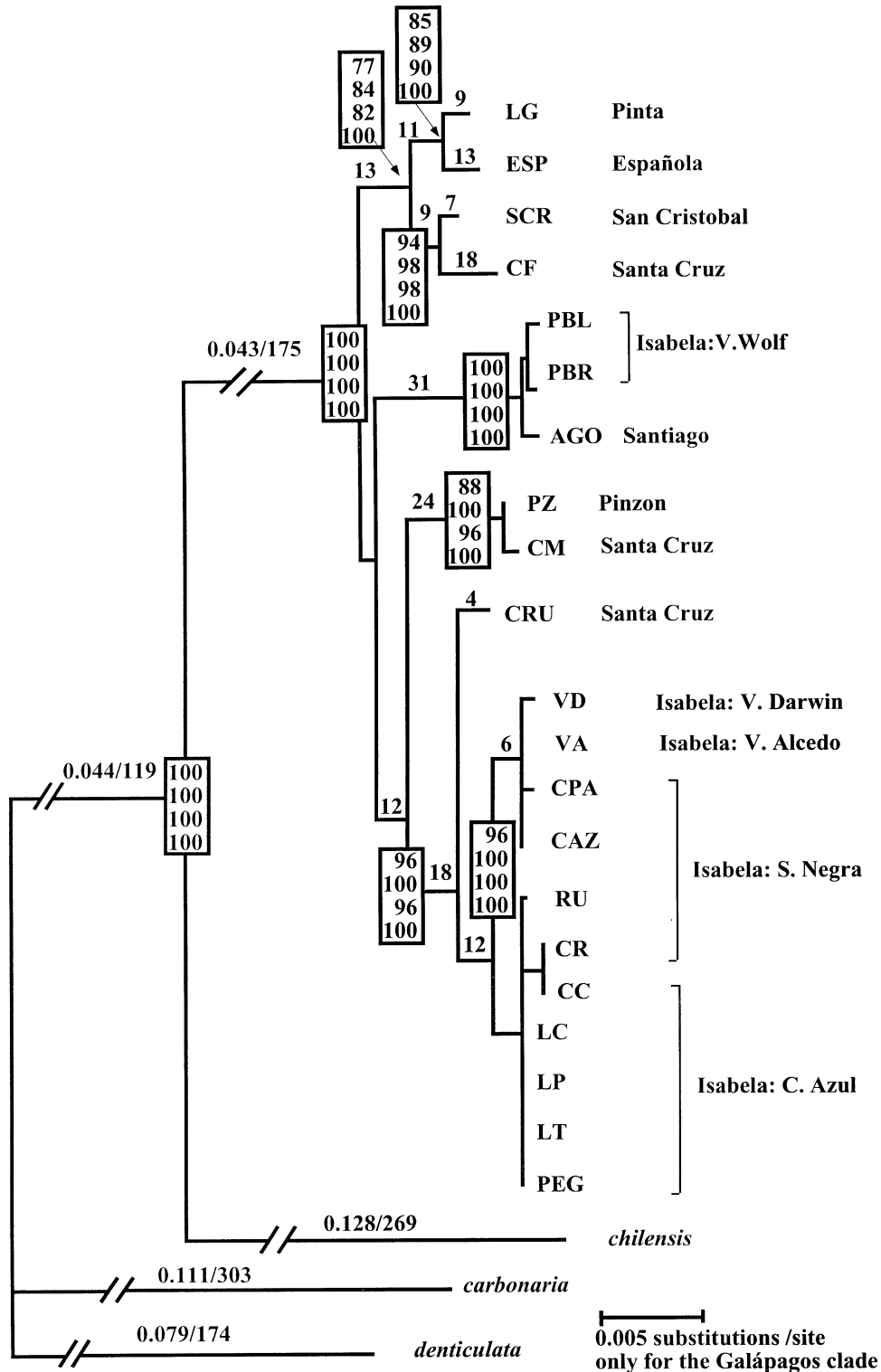
FIG. 4. Maximum-likelihood tree based on the combined mtDNA dataset for 21 populations of Galápagos tortoises and the three continental South American *Geochelone* species. The tips of the tree are labeled with both the population designation and island (see Fig. 1, Table 1). Branch lengths are proportional to the number of substitutions per site only within the Galápagos clade. For the outgroup species the branch lengths have been bracketed (actual value reported above it). Numbers above branches are the branch lengths of the maximum-parsimony consensus tree (all characters unordered); only branch lengths > 5 are shown. The first three boxed numbers are bootstrap values for the maximum-likelihood (100 replicates), maximum-parsimony (all characters unordered, 1000 replicates), and neighbor-joining trees (based on Tamura and Nei distances, 1000 replicates). We report bootstrap values for a particular node only if all three phylogenetic methods support the node at 75% or greater. The last value in each box is the proportion of trees supporting that node using the Bayesian Monte Carlo Markov chain method.
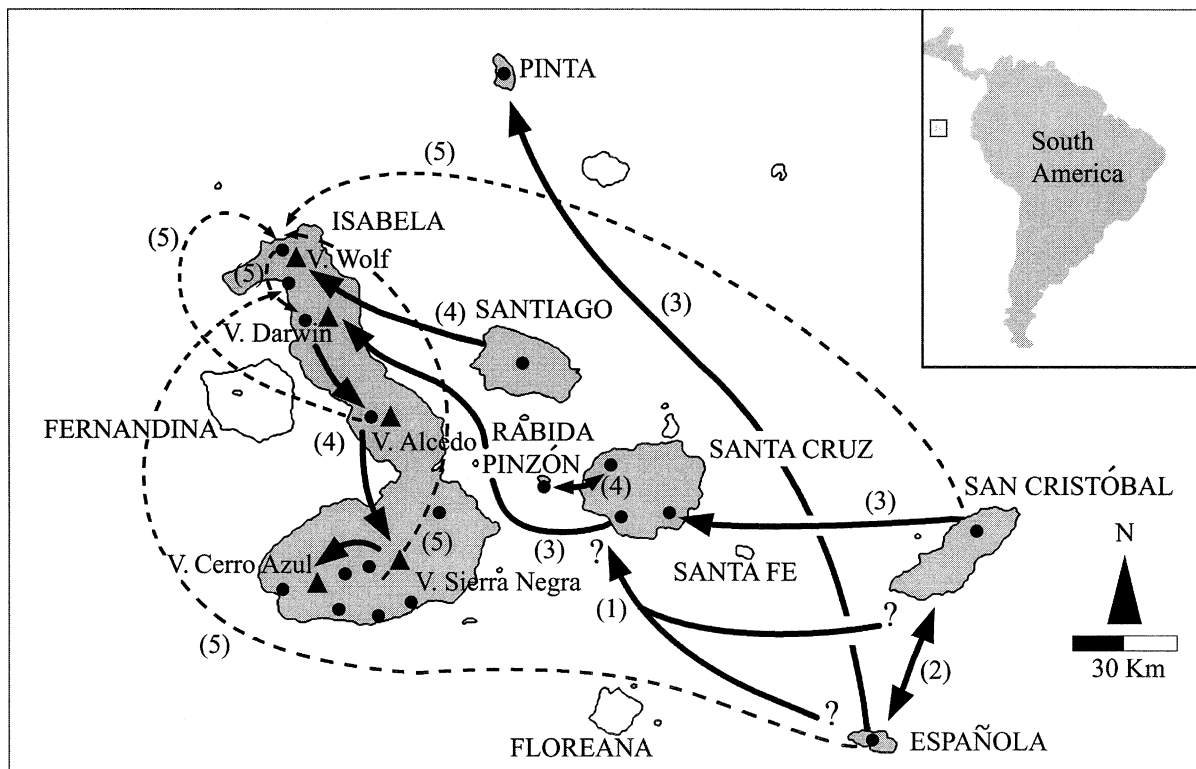
FIG. 5. Schematic of proposed phylogeographic history of Galápagos tortoises. The older islands of San Cristóbal and Española are the likely first islands colonized from mainland progenitors, but the genetic data cannot identify which. The arrows represent colonization events within Galápagos with the numbers indicating very approximate temporal order. We hypothesize the solid arrows were natural colonization events and the dashed arrows possibly human-induced translocations. See text for detailed discussion.

be much more complex than simple separation between volcanoes (e.g., altitudinal segregation) and there may well be areas where the taxa overlap and potentially form hybrid zones (Fritts 1984). Our samples do not contain unique sets of haplotypes, that is, maternal lineages from individual samples from Sierra Nigra and Cerro Azul are interspersed in cluster V in the network shown in Figure 3 (cf. Table 3) and thus either our samples contain a mixture of these two taxa or they have not been separated long enough to have evolved distinctive mtDNA haplotypes.

Based on morphology and field observations, Pritchard (1996) suggested that all four southern Isabela subspecies should be synonymized into a single taxon. Our mtDNA results argue that *microphyes* (Volcan Darwin) and *vandenburghi* (Volcan Alcedo) are genetically distinct. Our data cannot refute the suggestion that the two southern most subspecies are not genetically distinct. Microsatellite data from these populations do indicate some degree of isolation among populations, although the pattern is complex (Ciofi et al. 2002).

Phylogeography and History

Phylogenetic relationships as they relate to biogeography (phylogeography sensu Avise et al. 1987) are complex for Galápagos tortoises; Figure 5 summarizes the following discussion. Evidently there has not been a simple linear migration from older to younger islands, yet many aspects of the phylogenetic patterns do make clear biogeographical sense.

For example, the deepest node in the phylogeny connects the populations on the oldest islands (Española and San Cristóbal) to populations on younger islands (Fig. 4). Founding migrant(s) likely colonized one of these older islands before the younger islands were either formed or had developed adequate vegetation to support a tortoise population. This is consistent with our molecular clock estimates (see later). Also implied is no (detectable) back migration from younger islands to Española and San Cristóbal. The lack of mtDNA variation in the 15 survivors of the Española population is further evidence of complete isolation of this population, that is, no migration from other islands. This scenario is consistent with the prevailing northwesterly currents.

The oldest populations on Española and San Cristóbal have given rise at least three times to populations on younger islands. The oldest migration event (at the deepest node in Fig. 4) evidently led to several other populations: Pinzón, one of the Santa Cruz populations (CRU), Santiago, and all the Isabela populations. It is not clear from the TCS analysis (Fig. 3) nor the phylogenetic tree (Fig. 4) which of the three central islands, Santa Cruz, Pinzón, or Santiago, was colonized first. Given the ages, geographic locations, and sizes, it is most likely that Santa Cruz was the first of these islands to be colonized, a scenario not rejected statistically by our data. The oldest basalt from Santa Cruz dates to about 2.2 million years ago and the oldest basalt from Pinzón and Santiago date to about 1.5 million years ago (White et al. 1993).

There appear to have been two more recent migration

events from the older islands, one each from San Cristóbal and Española. A second population on Santa Cruz, Cerro Fatal, was founded much more recently than the Caseta population, evidently by migrants from San Cristóbal. This is the first strong evidence that the two populations on Santa Cruz, always considered a single taxon, are genetically distinct (see also Beheregaray et al. 2002). Despite this relatively recent founding, the Cerro Fatal population has a dome morphology similar to the Caseta animals and is clearly morphologically distinct from the extant saddleback population on San Cristóbal. A second relatively recent migration from the Española population was to Pinta. Geographically this seems an unlikely migration event (Figs. 1, 5), yet prevailing currents are certainly in the correct direction (Pak and Zaneveld 1973). The single survivor from Pinta, Lonesome George, as well as preserved skins of tortoises collected on Pinta in 1906 (Caccone et al. 1999) have yielded DNA sequences indicating Española lineages are Lonesome George's closest relatives. However, there is some degree of genetic differentiation (Fig. 3) consistent with the notion the Pinta population predates human arrival and was a truly natural population.

Was Pinta colonized directly from Española? Volcan Wolf on Isabela has two maternal lineages closely related to that from Española as well as having tortoises with saddleback carapaces typical of Española and Pinta and is geographically closer to Pinta. If the origin of the Española-like lineages on Volcan Wolf predates the founding of Pinta and directly gave rise to Pinta, they should be as genetically diverged from Española as is Lonesome George, which they clearly are not. Lonesome George is equally diverged from the single extant Española mtDNA lineage and the two Española-like lineages on Volcan Wolf (23 and 22 substitutions, respectively). Furthermore, the single mtDNA lineage representing Española is much more closely related (four or five substitutions) to the lineages on Volcan Wolf. Finally, if the Española-like lineages on Volcan Wolf are human-induced transports, this does not allow enough time for the Pinta population to have accumulated so many substitutions if it was founded by these Volcan Wolf tortoises. One caveat relates to the bottleneck that the Española population has experienced, resulting in what appears to be a single maternal lineage and very low microsatellite variation (Ciofi et al. 2002); possibly Lonesome George represents a now extinct Española-related maternal lineage that also exists (or existed at one time) on Volcan Wolf, but has not appeared in our sample.

Morphology

Shell shape (domed vs. saddledbacked carapaces) is the most distinctive morphological differences among populations. A genetic basis for shell morphologies is demonstrated by observations of tortoises reared in the same environment in captivity, although ontological changes also occur and the degree of expression of saddleback morphology is sexually dimorphic (Fritts 1983, 1984). Saddlebacked tortoises are found on dry, low-elevation islands on which resources are scarce and food limiting; the saddleback morphology allows longer upward extension of the neck permitting grazing on higher perennial vegetation and would also provide an ad-

vantage during antagonistic display associated with conflict for scarce food or mates. Domed tortoises live on the upper slopes of islands with higher volcanic cones where conditions are cooler and moister and have relatively abundant food closer to the ground.

It is difficult to determine definitively whether the dome versus saddleback morphologies are monophyletic or polyphyletic traits. Tortoises that first arrived on Galápagos were likely domed, a morphology typical of almost all tortoises other than those on some islands of Galápagos and extinct lineages on dry islands in the Mascarenes (Arnold 1979). The populations of tortoises on the oldest islands (San Cristóbal and Española), likely the oldest populations, are saddlebacked today. These form a monophyletic clade with another extreme saddleback population from Pinta. However, the fourth extreme saddleback population on Pinzón would, judging by the relationship of extant populations (Fig. 4), be an independent evolutionary event. However, the node indicating the exact position of Pinzón with regard to the semisaddleback population of Santiago and mixed population of Volcan Wolf, is the least well supported in the whole phylogeny.

A crucial population in determining the evolution of carapace shape is, unfortunately, the extinct population on San Cristóbal. From photographs in Van Denburgh (1914) and examination of skeletons (T. H. Fritts, pers. obs.), this population was domed (or at least semidomed). Thus it is conceivable that San Cristóbal was the initial colonization site with ancestrally domed tortoises occupying moister localities and saddlebacks having evolved to occupy drier habitats of this relatively large and ecologically complex island. Descent of all present day saddlebacked and domed populations from these two distinct populations on San Cristóbal could explain the close relatedness of the domed Cerro Fatal population on Santa Cruz to maternal lineages on San Cristóbal (assuming that the two populations on San Cristóbal were not entirely genetically isolated so that mtDNA continued to flow between the populations despite morphological differentiation related to habitat segregation). The recently extinct taxon that once inhabited Floreana and one that may have inhabited Santa Fe could also be crucial in understanding the history of these tortoises. Given their geographic locations (Figs. 1, 5), they could have been stepping stones in the radiation of these tortoises. DNA extracted from adequately preserved museum specimens could potentially provide important insights into the history of Galápagos tortoises.

Molecular Evolution and Clocks

As with other ectothermic vertebrates and Chelonians in particular (Avise et al. 1992), mtDNA evolution in the Galápagos tortoises is apparently slow relative to other vertebrates. An average rate of mtDNA evolution in ectotherms has been estimated at about 0.5% per million years since last common ancestor (Avise et al. 1992) compared to 1–2% per million years for endothermic vertebrates. Using the maximum-likelihood tests of the clocklike behavior of DNA evolution, we could not reject the clock for the combined dataset within the Galápagos lineage. It is well known that estimates of coalescence of genes are older than the separation of populations due to ancestral polymorphism (reviewed in Edwards

and Beerli 2000) and correction for this is especially important for very recently diverged taxa, as is the case here. Various methods to correct for this factor have been proposed depending on a number of variables (Edwards and Beerli 2000). For our data, corrections based on either the mean or maximum divergence of alleles within a taxon would seem most appropriate. For those samples (usually corresponding to a named taxon, although CF and CRU were treated separately and the southern Isabela samples were excluded due to ambiguous taxonomic status) for which we sequenced at least 10 individuals, the mean divergence of alleles within a sample averaged 0.10% and mean maximum divergence 0.20%. Because two lineages are involved between the gene split and population split the correction is half this estimate, 100,000 or 200,000 years. Using the overall rate of 0.5% per million years gives an estimate of the deepest split in the Galápagos lineage at about 2.5–2.6 million years ago corrected for intrapopulation divergence.

Using an overall rate for mtDNA divergence, however, is not particularly satisfying given ample evidence that different mtDNA genes evolve at very different rates (evident in Fig. 2) and that most of the divergence in our data is due to the fastest evolving region, the control region. Thus the 2.5–2.6 million years estimate of the deepest node is probably an overestimate. Furthermore, the deepest split involves populations on islands with a maximum age of 2.2 million years (Santa Cruz). Using rates suggested for rDNA and cyt*b* in another ectothermic vertebrate, salamanders (Caccone et al. 1997), leads to estimates of the deepest divergence at about 1.2 million years ago (corrected). Given the vagaries in these estimates, we suggest an estimate of the initial split at 1.5–2.0 million years ago is reasonable. This also leads to the estimate of colonization of the youngest island Isabela at about 0.2–0.3 million years ago, consistent with the oldest lava flow on this island dated to no more than 0.5 million years ago. Once volcanic activity subsided, colonization by tortoises could only occur after sufficient vegetation developed to support a population.

It is important to point out that this estimate of 1.5–2.0 million years ago for the deepest node does not indicate when tortoises arrived on the islands. Assuming the initial founding of the Galápagos was a single event on one island (either Española or San Cristóbal), the founding population could have existed for some time before initiation colonization of other islands. When did the first tortoises become established in Galápagos? If the direct ancestor to Galápagos tortoises existed we could estimate this. Although we have identified the closest living relative, *G. chilensis*, it is doubtful that this is the direct ancestor of Galápagos tortoises, but rather *G. chilensis* is the sole survivor of a larger lineage that gave rise to Galápagos tortoises. Others (e.g., Pritchard 1996) have made good arguments that the gigantism of the surviving tortoises on remote archipelagos was a prerequisite for long-distance drifting in oceans. This is particularly clear in Aldabra (Indian Ocean), where giant tortoises repeatedly colonized the islands after rising sea levels submerged them (Braithwaite et al. 1973). Giant fossil tortoises, up to 1.5 m in length, were common on many continents including South America up to and during the Pleistocene (Auffenberg 1974). Thus giant tortoises existed on the South American mainland

at a time when many of the extant Galápagos Islands were formed. What we can conclude is that Galápagos tortoises were in existence by 1.5–2.0 million years ago (the oldest split within the Galápagos lineage) and are unlikely to have arrived more than about 3 million years ago as the oldest lava flow in the Galápagos (Española) is dated to about 3.2 million years ago (White et al. 1993). While it is possible that the Galápagos tortoise lineage is older than the oldest extant island if tortoises arrived on islands now eroded and submerged (as seems to be the case for the endemic Galápagos iguanas; Rassman 1997), our molecular genetic dating does not require explanations that invoke islands older than the extant ones.

Synopsis

The giant tortoises inhabiting Galápagos are spectacular reptiles and were a crucial inspiration for arguably the most profound revolution in biology, Darwin's theory of evolution by natural selection. They represent one of only two surviving lineages of once widespread giant tortoises. Moreover, they are the only lineage with genetically and morphologically differentiated populations. Our genetic and phylogenetic studies are beginning to reveal fascinating and complex patterns of diversification. Some findings have been quite unexpected, such as the existence of two genetically distinct populations on Santa Cruz and the close relationship of the sole survivor from Pinta to the most distant population on Española. Other findings have shed insight into otherwise paradoxical observations such as the origin of the small isolated saddleback population in northwest Santa Cruz and the reason for the mixed morphology of the Volcan Wolf population. The molecular genetic studies reported here can serve as an important guide, along with empirical information on Galápagos tortoise morphology, behavior, ecology, and evolution, for setting conservation priorities or revising taxonomy of this prominent and imperiled group.

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