

## RESEARCH UPDATE

# SURVIVING ATLANTIS: THE MOLECULAR EVOLUTION OF THE GALAPAGOS IGUANAS

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**T**he Galapagos archipelago is situated about 1000 km west of Ecuador, South America, the nearest mainland. It is of volcanic origin and has never been in contact with the American continent. The biota of the Galápagos is related to that of the Americas, but the species diversity, that is, the number of different species in the archipelago, is much smaller. The species composition is unbalanced, with few terrestrial mammals and no amphibians inhabiting the islands. Reptiles, on the other hand, dominate the scene. Capable of enduring weeks in tropical sun without fresh water, iguana, lizard, gecko and tortoise species are thought to have reached the islands on vegetation rafts, carried from the American coast by the waters of the El Niño or Humboldt current.

The Galápagos Islands are famous for the quick adaptive radiation of some of their taxa.

The 13 Darwin finch species, for example, evolved apparently from a single ancestral species that colonized the islands not more than 5 million years (MY) ago (Grant, 1994). The Galápagos iguanas, on the other hand, display a very different pattern of evolution. The two endemic genera seem to have diverged long before the finches, but the Galápagos land iguana comprises only two species (*Conolophus pallidus* and *C. subcristatus*) and the marine iguana one (*Amblyrhynchus cristatus*). Morphological data suggest that the land and marine iguana are sister taxa, that is, like the Darwin finches they probably share a direct common ancestor (de Queiroz, 1987). Thus, it is possible that this ancestral iguana species colonized the archipelago, where it then diverged into the land and the marine iguana lineages. Yet, the oldest of the present Galápagos islands emerged only 5 MY ago,



**The Galápagos marine iguana** (*Amblyrhynchus cristatus*). "It is a hideous-looking creature, of a dirty black color, stupid and sluggish in its movements" (Charles Darwin).  
Photograph: Kornelia Rassmann

or less, while protein-based studies indicate a much longer separation time between the two Galápagos iguana genera (e.g. Wyles and Sarich, 1983). Are the Galápagos iguana lineages perhaps much younger? Or did they originate elsewhere and the archipelago was colonized at a later stage with iguanas from two independent ancestral stocks?

DNA based technologies offer a powerful tool to analyze the phylogenetic or evolutionary history of a species, as well as its current demography, such as the level of migration or gene flow among the populations. Since 1991, I have been working on the molecular evolution of the Galápagos iguanas, attempting to infer their present and past mode of evolution from their genetic make-up. The first part of the study centered on the phylogenetic background of the Galápagos iguanas, specifically on the question of where and when the two genera separated (Rassmann, 1997). Second, I evaluated the level and pattern of genetic variation within both the land and the marine iguanas, addressing two main questions: 1) how genetically related are populations from different parts of the archipelago; and 2) are their genetic relationships determined by evolutionary history (for example the sequential colonization of the islands) or by contemporary processes (such as migration among the populations)?

(Rassmann *et al.*, 1997). Finally, the genetic data were used to derive suggestions aiding the conservation of the Galápagos iguanas (Rassmann, 1996).

### Phylogeny of the Galápagos Iguanas

Mitochondrial DNA (mtDNA) sequence data (c. 1 kb of the 16S and the 12S genes) were employed to re-evaluate the phylogenetic relationships among the nine genera of Iguanidae (sensu Frost and Etheridge, 1989), namely the Galápagos marine and land iguanas (*Amblyrhynchus* and *Conolophus*), the black or spiny-tailed iguana (*Ctenosaura*), *Enyaliosaurus* (often included in *Ctenosaura*), the green iguana (*Iguana*), the West Indian rock iguana (*Cyclura*), the chuckwalla (*Sauromalus*), the Fiji or banded iguana (*Brachylophus*), the desert iguana (*Dipsosaurus*), and as an outgroup the Malagasy iguana (*Oplurus*). The phylogenetic analyses largely confirmed the findings of the morphological studies. The desert iguana (*Dipsosaurus*) appeared to be the most basal lineage among the nine Iguanidae, followed by the Fiji iguana (*Brachylophus*) as the sister taxon to the seven remaining taxa, the so-called Iguanini. The molecular data suggested further that *Enyaliosaurus* and *Ctenosaura* were the closest living relatives of the Galápagos iguanas and that the latter shared a

**The Galápagos land iguana** (*Conolophus subcristatus*). "These lizards, like their brothers the sea-kind, are ugly animals; and from their low facial angle have a singularly stupid appearance" (Charles Darwin).  
Photograph: Kornelia Rassmann



direct common ancestor. Hence, the molecular phylogenetic results agreed with the hypothesis that this ancestral iguana species possibly colonized the archipelago, where the land and marine iguana then evolved in situ.

Rate comparisons between the iguanid sequences and a corresponding set of sequences from ungulates (such as the cow and the sheep) with known evolutionary ages were used to estimate the separation time among the Galápagos iguanas. Such separation time estimates are only crude approximations, particularly when the rate of sequence evolution is adopted from a different taxon group. However, it has been shown that the rate of mtDNA sequence evolution is faster in endotherms, such as the ungulates, than in ectotherms, such as the iguanids (Rand, 1994). Thus, using an endotherm rate probably gave an underestimate of the Galápagos iguanas' separation time, and it seemed safe to conclude that their separation took place 10 MY ago, or more.

The molecular analyses strengthened the notion that the speciation of the Galápagos iguanas happened long before the emergence of today's Galápagos islands. Yet, new geological data reveal that this does not necessarily exclude their origin within the archipelago. Christie *et al.* (1992) analyzed submarine sea mounts southeast of the present

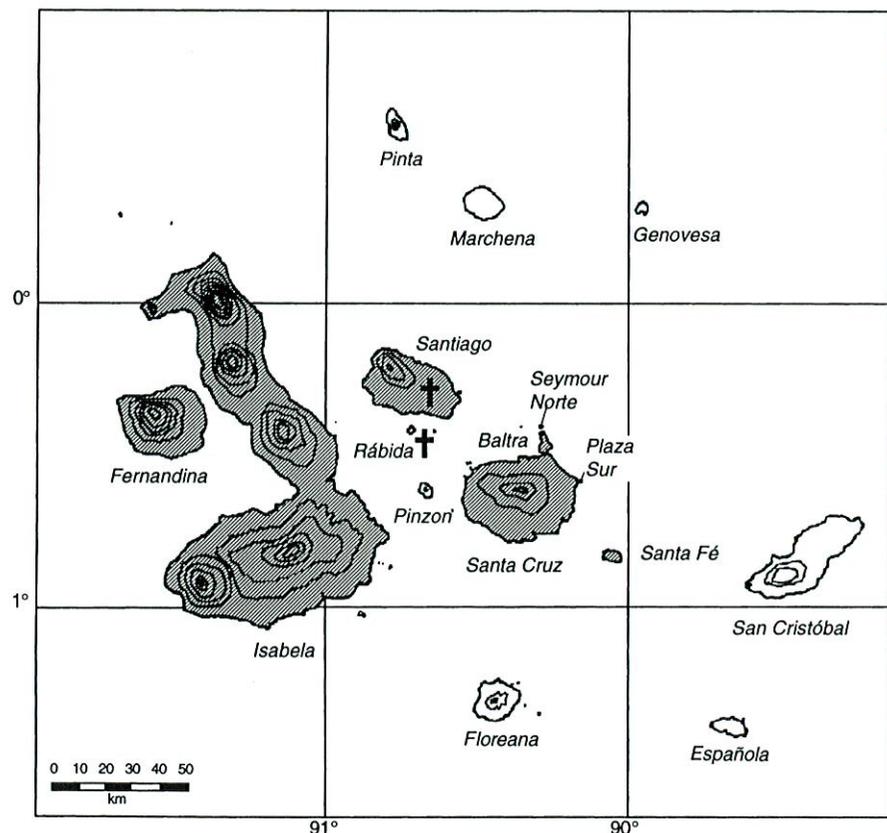
islands and suggested that some represent former, now drowned volcanic islands of the archipelago. The oldest of the studied sunken volcanoes was active over the Galápagos hotspot about nine to eleven MY ago, and Christie *et al.* suspect that islands may have appeared and disappeared for much longer. Thus, it may be that evolutionarily old Galápagos taxa, such as the iguanas, inhabited these former islands of the Galápagos and experienced more time for evolution within the archipelago than previously thought.

### Microevolution of the Galápagos Iguanas

Employing again mtDNA sequence analyses (c. 450 nucleotides of the cytochrome b gene), I also tested the level of genetic divergence within both Galápagos iguana genera, including 150 marine iguanas from 15 islands and 46 land iguanas from five islands (Figure 1). The maximum sequence divergence within both genera was low (< 2%), that is, even among the most diverged land iguana or marine iguana sequences I detected only a few nucleotide changes. Such low

**Figure 1. The Galápagos archipelago.**

While marine iguanas are widely distributed throughout the archipelago, land iguanas inhabit only the central islands (Santa Cruz, Seymour Norte, Plaza Sur) and the western islands (Isabela and Fernandina). Two further land iguana populations from Santiago and Rabida are today extinct.



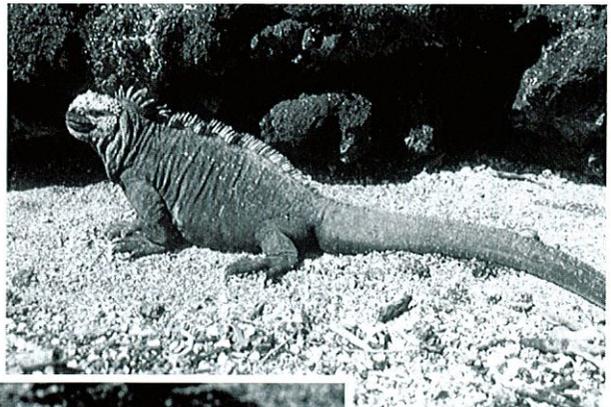
levels of mtDNA sequence divergence can accumulate within a relatively short period of time, possibly within the time frame the present Galápagos islands existed.

Given that the Galápagos iguanas experienced a long evolutionary history in the archipelago, the low level of genetic divergence within both genera seemed surprising. However, the distribution of the different variants of mitochondrial sequences (or haplotypes) in the archipelago suggested a possible explanation for this. None of the haplotypes detected in the western populations were also found in the eastern populations, and vice versa. Thus, there is apparently no mitochondrial gene flow among western and eastern iguana populations. Because only females transmit mtDNA to their offspring, this finding implies that female land and marine iguanas migrate little among different islands. With such low levels of mitochondrial gene flow among island populations, one would expect that only few mitochondrial variants from the former, now extinct island populations were passed on to today's populations. Hence, most of the observed mitochondrial variants originated probably within the present archipelago. The maximum mitochondrial sequence divergence among today's iguana populations is therefore probably limited by the age range of the surfaced islands, not the former islands of the archipelago.

To test the level and pattern of genetic differentiation among the populations in more detail, the marine iguanas were also analyzed using microsatellite DNA fingerprinting. Microsatellite fingerprinting detects variation in the nuclear genome. The technique is well-used today, for example, in forensic work, where it can serve to identify a specific individual among others from the same population. In the marine iguanas, however, the micro-

satellite analyses detected surprisingly little nuclear genetic differences among the different island populations. Although the fingerprints revealed a large number of different nuclear DNA markers, many were shared among the island populations, even among those that had no mitochondrial markers in common.

Did the mitochondrial or the nuclear data convey a wrong picture? The answer may be much simpler. Unlike mitochondrial DNA, nuclear DNA is inherited from both the mother and the father. In other words, males that reproduce in a foreign population transmit their nuclear genes to their offspring, but not their mitochondrial genes. For the marine iguanas this means that males probably migrate relatively frequently among different island populations, and thus spread nuclear alleles throughout large parts of the archipelago, whereas females migrate little, leading to the observed geographically restricted distribution of the mitochondrial haplotypes. A similar analysis of nuclear markers in the land iguanas is currently being conducted in collaboration with Melanie



**ABOVE:**  
The Galápagos marine iguana (*Amblyrhynchus cristatus*). Photograph: Kornelia Rassmann



**LEFT:** The Galápagos land iguana (*Conolophus subcristatus*). Photograph: William Hayes

Markmann and Diethard Tautz at the University of Munich, Germany.

### Conservation Genetics in the Galápagos Iguanas

Because of their isolation and late colonization by humans, the Galápagos Islands are still relatively pristine and their biota undisturbed. However, in some parts of the archipelago, the human impact over the last few centuries has taken its toll. Many of the Galápagos vertebrate populations are threatened by predation from feral animals, habitat destruction and competition for resources by plants and animals introduced by humans. The most urgently needed steps toward the rescue of Galápagos biodiversity are probably immediate ecological actions and the enforcement of political decisions. However, there is also potential in the data accumulating from molecular evolutionary studies in the archipelago.

The discipline of conservation genetics aims to document the contemporary and future level and pattern of genetic variation in endangered species and offers suggestions to aid their preservation. One of its applications is to rank the conservation values of populations or taxa based on their degree of genetic deprivation or their phylogenetic distinctiveness. This allows the targeting of limited conservation means toward specific projects and can lend support to political decisions.

During the sampling of marine iguanas in 1991 and 1993, Linda Cayot, Fritz Trillmich and I observed low population sizes and an absence of juveniles on islands with introduced predators, such as Isabela and San Cristobal (Cayot et al., 1994). The consequences of artificially increased levels of predation on natural populations, however, are not easily assessed when information on a population's demography and their genetic relatedness to other populations is missing. It may be, for example, that migration among different subpopulations from the same or neighboring islands is sufficiently high to make up for losses.

The microsatellite fingerprinting and mitochondrial sequence data of marine iguanas were employed to evaluate the relative level of genetic variation within each population and the level of

genetic relatedness among the populations. The population from Punta Pitt on San Cristobal clearly had the highest genetic distance to all other populations and also the lowest level of nuclear and mitochondrial DNA variation. Based on the molecular findings, and bearing in mind the low number of individuals, the absence of hatchlings in this population, and the conspicuous signs of predators, we strongly recommended the immediate eradication of feral animals and protection of the habitat in the Punta Pitt area. 

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# Iguana Times

THE JOURNAL OF THE INTERNATIONAL IGUANA SOCIETY  
\$6.00

VOLUME 5, NUMBER 4  
DECEMBER 1996



6½ year-old male Grand Cayman  
Blue Iguana, *Cyclura nubila lewisi*.  
Photograph: Robert W. Ehrig