Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 573–576, January 1989
Evolution

An odyssey of the green sea turtle: Ascension Island revisited

(mitochondrial DNA/intraspecific phylogeny/gene flow/genetic distance)

BRIAN W. BOWEN*, ANNE B. MEYLAN†, AND JOHN C. AVISE*‡

*Department of Genetics, University of Georgia, Athens, GA 30602; †Florida Institute of Marine Research, Department of Natural Resources, Saint Petersburg, FL 33701

Communicated by Wyatt W. Anderson, October 17, 1988 (received for review September 23, 1988)

ABSTRACT **Green turtles (*Chelonia mydas*) that nest on Ascension Island, in the south-central Atlantic, utilize feeding grounds along the coast of Brazil, more than 2000 km away. To account for the origins of this remarkable migratory behavior, Carr and Coleman [Carr, A. & Coleman, P. J. (1974) *Nature (London)* 249, 128–130] proposed a vicariant biogeographic scenario involving plate tectonics and natal homing. Under the Carr–Coleman hypothesis, the ancestors of Ascension Island green turtles nested on islands adjacent to South America in the late Cretaceous, soon after the opening of the equatorial Atlantic Ocean. Over the last 70 million years, these volcanic islands have been displaced from South America by sea-floor spreading, at a rate of about 2 cm/year. A population-specific instinct to migrate to Ascension Island is thus proposed to have evolved gradually over tens of millions of years of genetic isolation. Here we critically test the Carr–Coleman hypothesis by assaying genetic divergence among several widely separated green turtle rookeries. We have found fixed or nearly fixed mitochondrial DNA (mtDNA) restriction site differences between some Atlantic rookeries, suggesting a severe restriction on contemporary gene flow. Data are consistent with a natal homing hypothesis. However, an extremely close similarity in overall mtDNA sequences of surveyed Atlantic green turtles from three rookeries is incompatible with the Carr–Coleman scenario. The colonization of Ascension Island, or at least extensive gene flow into the population, has been evolutionarily recent.**

Green turtle (*Chelonia mydas*) nesting grounds (surf-built beaches) and feeding grounds (protected shallow-water marine pastures) are often spatially separate, necessitating seasonal migrations between the two (1–4). For example, tagging data have demonstrated that green turtles nesting on Ascension Island utilize feeding grounds along the coast of Brazil, 2000 km distant. Tagging studies have also shown that adult females usually return faithfully to the same beach, or even section of beach, for nesting in successive seasons (5). This nest-site fidelity has led researchers to suggest that females return to their natal site (6). However, due to the difficulties of marking hatchlings with a tag that persists to adulthood, this hypothesis remains untested. In principle, natal homing could be achieved by population-specific genetic programming, environmental imprinting of hatchlings, or a combination of these factors (6, 7). If green turtles return to breed and nest at their natal beach, each rookery would represent an independent breeding unit. The evolutionary consequences of natal homing therefore include genetic isolation, and expected genetic divergence between nesting colonies.

To explain the origin of the remarkable migratory circuit between Ascension Island and Brazil, Carr and Coleman (8) proposed a gradualistic scenario in which nesting turtles tracked a series of progressively distant volcanic islands. The fossil record indicates that turtles of the family Cheloniidae inhabited the proto-Atlantic prior to the separation of Africa and South America, about 70 million years ago (9, 10). These cheloniid turtles may have nested on islands formed on the mid-Atlantic ridge, at that time adjacent to shallow South American feeding grounds. As these island chains were gradually removed from South America by the action of sea-floor spreading, at a rate of about 2 cm/year, nesting turtles may have developed a progressively longer migratory route, culminating in the contemporary migration to Ascension Island. Thus, the Carr–Coleman hypothesis is an attempt to integrate ideas from behavioral biology (natal homing) and geology (sea-floor spreading) to account for the origin of this isolated nesting colony. This vicariant scenario was subsequently challenged by Gould (11), who proposed a rare and possibly recent colonization event to explain the presence of the Ascension Island rookery. Here we empirically test these alternative hypotheses.

We chose mtDNA for this test of the Carr–Coleman hypothesis for two major reasons. First, an earlier protein electrophoretic survey of *Chelonia mydas* (involving 23 nuclear loci, but not including the Ascension rookery) revealed no genetic differences between populations in separate ocean basins (12). In many vertebrate species, the more sensitive mtDNA assays often reveal substantial geographic population structure, even where allozyme surveys have failed to do so (13, 14). Second, mtDNA is inherited maternally, through the egg cytoplasm (15, 16). Green turtle tagging studies have focused primarily on adult females, because only they ascend nesting beaches and are readily captured. Males also migrate to nesting areas, and matings occur offshore, but there are considerably fewer data available on male fidelity to nesting areas. It is possible that males provide an avenue for nuclear gene flow between rookeries. Using mtDNA, we can set aside the question of male dispersal, and examine the natal homing hypothesis in its simplest form. If females have homed faithfully to their natal beach on Ascension Island over the evolutionary time-spans proposed under the Carr–Coleman scenario, the consequences should be reflected in extensive mtDNA sequence divergence.

MATERIALS AND METHODS

During the 1987 nesting season, 46 green turtle nests were sampled from the following numbered locations: 1, French Frigate Shoals, Hawaii (n = 12); 2, Hutchinson Island, Florida (n = 10); 3, Aves Island, Venezuela (n = 8); 4, Ascension Island, United Kingdom (n = 16) (see Fig. 1). A maximum of three eggs or one hatchling was collected from each nest. Eggs were incubated for at least 4 weeks to allow deposition of sufficient tissue for analysis.

Restriction analysis of mtDNA samples was accomplished with procedures routinely used in our laboratory for discrim-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

‡To whom reprint requests should be addressed.

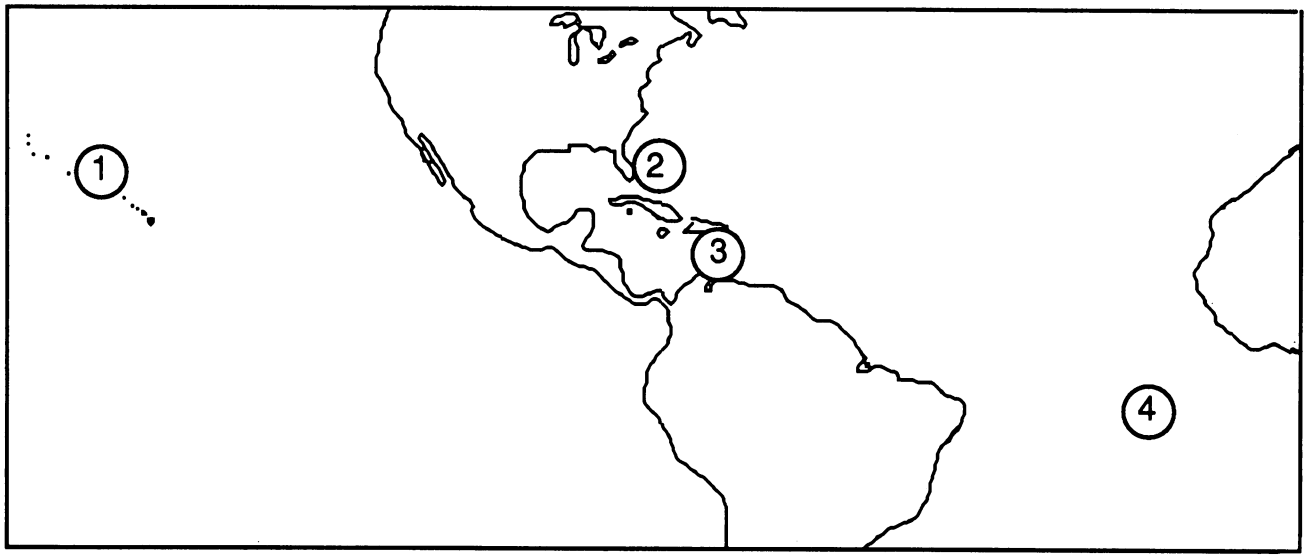

FIG. 1. Collection locales for *Chelonia mydas*. 1, French Frigate Shoals, Hawaii (n = 12 different nests); 2, Hutchinson Island, Florida (n = 10); 3, Aves Island, Venezuela (n = 8); 4, Ascension Island, United Kingdom (n = 16).

ination of maternal lineages (17). mtDNAs were isolated from soft tissues and purified in closed circular form by CsCl density gradient centrifugation. Each sample was digested with 13 informative restriction enzymes (*Ava* II, *Bcl* I, *Dde* I, *Eco*RV, *Hin*cII, *Hin*dIII, *Mbo* I, *Msp* I, *Nde* I, *Pvu* II, *Spe* I, *Sst* II, and *Stu* I). The resulting fragments were end-labeled with ^{35}S-labeled nucleotides (18). mtDNA fragments were separated on the basis of molecular weight by electrophoresis through 1.0–1.7% agarose gels and visualized by autoradiography. No attempt was made to score fragments smaller than 0.4 kilobases (kb). An additional nine restriction enzymes (*Bam*HI, *Bgl* I, *Bgl* II, *Bst*EII, *Cla* I, *Kpn* I, *Pst* I, *Sac* I, and *Xba* I) did not produce multiple cuts in our digests of green turtle mtDNA, and hence they were not included in the estimates of sequence divergence. All fragment profile changes could be accounted for by specific restriction site gains or losses. Thus genetic distances (base substitutions per nucleotide) between mtDNA genomes were estimated by the site approach (19).

RESULTS

The genetic results are straightforward. mtDNAs sampled from the three Atlantic rookeries were closely related, as judged by the 93–95 restriction sites scored per individual. Within the Atlantic, three mtDNA genotypes were observed—a "common" pattern, and two variant patterns due to single *Hin*cII and *Spe* I restriction site changes (Fig. 2). Thus all Atlantic samples shared at least 93 of 95 restriction sites, with the three observed genotypes distinguished from one another by only one or two assayed mutation steps.

The three genotypes observed in the Atlantic were not randomly distributed among assayed rookeries. The common *Spe* I genotype characterized 100% of Ascension Island samples and 87% (seven of eight) of the Aves Island samples, but was absent from the Florida samples (Fig. 2 *Upper*). The other *Spe* I type, found in all Florida samples and one Aves Island sample, was characterized by an additional restriction site, cleaving a 3.4-kb fragment into fragments 2.7 and 0.7 kb in size (see Fig. 2 *Upper*). The common *Hin*cII pattern (which is probably ancestral because it is present in Hawaiian samples) was found at 100% frequency in Florida and Ascension Island samples, but at significantly lower frequency (12.5%; one of eight) in Aves Island samples. The derived *Hin*cII pattern, observed only in Aves Island samples, is characterized by the loss of a restriction site, combining a 1.9-kb fragment and a 0.6-kb fragment (Fig. 2 *Lower*).

With the exception of one individual (an Aves Island specimen with a "Florida" genotype), samples from each of the three Atlantic rookeries were characterized by a fixed restriction site pattern not observed in the other surveyed rookeries. This geographic distribution of genotypes suggests a contemporary restriction of female-mediated gene flow between Atlantic rookeries.

All nests sampled in the Pacific rookery (Hawaii) were identical at 95 mtDNA restriction sites scored per individual. However, Hawaiian samples were readily distinguished from Atlantic specimens with five restriction enzymes (*Ava* II, *Nde* I, *Pvu* II, *Spe* I, and *Stu* I). The Hawaiian *Ava* II pattern was two restriction site changes removed from the Atlantic pattern, and the other diagnostic enzymes produced profiles which reflected a single restriction site gain or loss from the common Atlantic pattern (see example in Fig. 2 *Upper*).

DISCUSSION

In mammals and other vertebrates (including reptiles) mtDNA is known to accumulate base substitutions at a rapid pace (20–23). Debate has centered on the exact calibration of the mtDNA evolutionary "clock" and its generality across taxa, but a conventional estimate is about 2% sequence divergence per million years. If the Ascension Island population of green turtles had been evolving independently for 40 million years or more, as proposed by Carr and Coleman, the mtDNA genome should be saturated with base-substitutional differences from other populations. Yet the Ascension Island green turtles are genetically close to those from other Atlantic rookeries. These data suggest that the species *Chelonia mydas* consists of at least two phylogenetic units, corresponding to major ocean basins, and that the Atlantic is further subdivided into contemporary breeding units. While the genetic evidence indicates that the Ascension Island population is differentiated from other Atlantic rookeries, the level of mtDNA sequence divergence is vastly lower than would be expected under the Carr–Coleman hypothesis.

It is possible that mtDNA evolution is much slower in green turtles [perhaps due to the exceptionally long generation time of 10–60 years (24)]. In fact, our data do suggest a considerable deceleration in mtDNA evolution. *Chelonia*

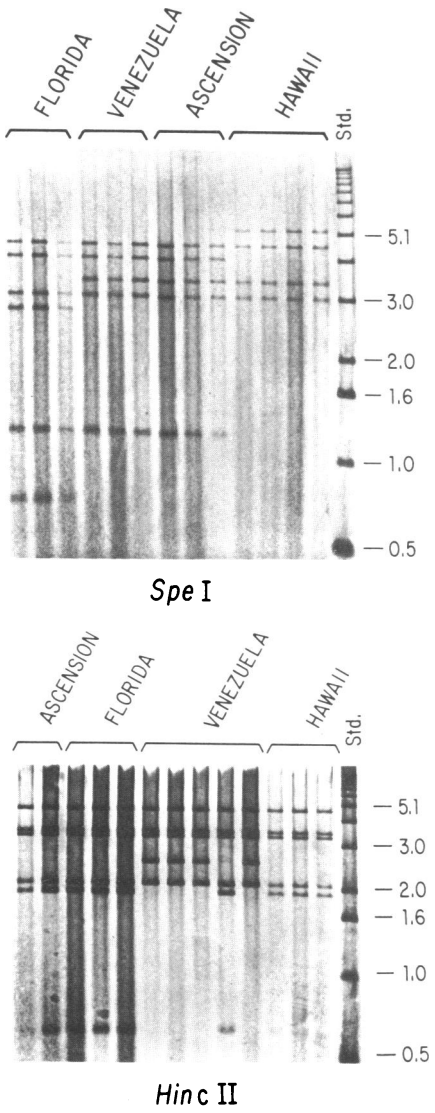

FIG. 2. Restriction endonuclease digests of mtDNA from representative green turtles from one Pacific and three Atlantic rookeries. The rightmost lanes contain a molecular weight standard, with selected sizes (in kb) indicated. (*Upper*) *Spe* I digests. Inspection of mtDNA fragment sizes shows that the *Spe* I patterns in Florida and Hawaii samples differ from those observed in Venezuela and Ascension Island by a single site gain and site loss, respectively. (*Lower*) *Hin*cII digests. Inspection of mtDNA fragment profiles shows that the common *Hin*cII pattern observed in Venezuelan samples is characterized by a single restriction site loss from the pattern found in other assayed rookeries.

mydas in the Atlantic and Pacific oceans have presumably been separated (by the Isthmus of Panama) for at least 3 million years, over which time we observe a nucleotide sequence divergence of $P \approx 0.006$ (after correction for within-ocean mtDNA divergence). This yields a rate of mtDNA evolution of 0.2% sequence divergence per million years, or about 1/10th the "conventional" pace. Even at this decreased rate, we would expect to have observed numerous restriction site changes (more than 20 with our sample of enzymes) over the 40 million years or more of proposed isolation of the Ascension Island rookery.

Our results demonstrate that in terms of overall matriarchal phylogeny, the Ascension Island rookery is closely allied to other Atlantic green turtle populations. To accommodate this finding with the significant mtDNA genotype frequency shifts observed within the Atlantic, and with the results of tagging studies showing strong nest-site fidelity in adult females, two classes of (nonexclusive) hypotheses related to natal homing may be advanced. First, perhaps natal homing occurs, but with some low error rate. Tag recoveries document at least the rare breakdown of nesting beach fidelity. Of the tens of thousands of females tagged on nesting beaches, a few have subsequently been observed nesting at alternative sites. For example, a female tagged at the Aves Island rookery 200 km west of Dominica was later observed nesting at Mona Island, Puerto Rico, 560 km distant (25); and a female tagged on Tromelin Island in the Indian Ocean was subsequently observed nesting on Europa Island, over 2000 km away (26). Nonetheless, any such genetic "leakage" between our assayed rookeries must be low, given the observed shifts in mtDNA genotypic frequency. From a theoretical treatment of gene flow, Slatkin showed that an average of one or more individuals exchanged per generation between any two populations is sufficient to prevent different neutral alleles from becoming nearly fixed (27).

An alternative explanation involving episodic rookery extinction might better reconcile the mtDNA sequence similarity with the significant mtDNA genotype shifts among Atlantic rookeries. Nesting sites require a specific suite of factors, including an appropriate temperature regime (for incubation and sex determination), oceanic currents (for hatchling transport), and beach stability (28). Appropriate nesting beaches must be ephemeral over evolutionary time, continually arising and disappearing with catastrophic events such as hurricanes and with long-term changes in physical environment (such as sea level and climate) and biotic environment (such as presence of nest predators). These environmental perturbations could cause a periodic restructuring of green turtle populations through rookery extinctions and colonizations. The low mtDNA variability in Atlantic green turtles may itself be evidence of historically small effective population sizes of females (29), perhaps due to such an extinction/colonization cycle. Whether through occasional dispersal events or episodic population restructuring, Atlantic green turtle rookeries are closely related. The genetic data are not consistent with a vicariant hypothesis for the origin of the Ascension Island colony; they are consistent with a recent origin, perhaps the result of a rare colonization event.

With the current survey of three widely separated Atlantic rookeries, we cannot establish the geographic scale of population structuring. It is possible that natal homing operates on a regional rather than a rookery-specific basis, such that clusters of neighboring nesting colonies could constitute a single population. In the case of Ascension Island, 2000 km removed from the nearest alternative nesting habitat, regional dispersal by nesting females is presumably diminished by the extreme physical isolation.

In light of the transient nature of nesting beaches and the shallow evolutionary separations of Atlantic breeding units, it seems unlikely that specific migratory routes are genetically programmed (but see ref. 30 for an example of very rapid evolution of migratory behavior in birds). Although a predisposition to utilize environmental cues must surely have a genetic basis, the positional information essential for navigation is probably learned (imprinted) rather than inherited. Marine turtles are known to possess a high degree of olfactory discrimination (31), and orientation mechanisms involving an olfactory component have been proposed (32). Navigation might also be accomplished with celestial, inertial, Coriolis force, or geomagnetic guidance mechanisms (33). Regardless of the orientation mechanism employed, imprinted homing behavior would allow a more flexible response to altered nesting conditions, such that a new migrational circuit could be established by a single female in a single generation. Imprinting on a new habitat requires no

genetic modification and is consistent with a successful colonization strategy.

Green turtle nesting colonies were historically more numerous (prior to decimation by man) in the Atlantic and Caribbean and occurred on islands suspected to be only a few thousand years old. Emerging nesting habitats must be colonized at some reasonable frequency by turtles hatched elsewhere. Imperfect natal homing provides a mechanism for such colonization. That natal homing predominates, however, is suggested by both the migration and mtDNA data and by the fact that rookeries extirpated in the 17th and 18th centuries (such as at Grand Cayman and Bermuda) (34) have not yet been recolonized. Imperfect natal homing, resulting in occasional colonization of newly opened nesting habitat, may have provided a flexibility in migrational behavior that so far has circumvented the extinction of *Chelonia mydas*.

We dedicate this paper to the memory of Prof. Archie Carr, whose death in 1987 ended a life-long career devoted to the study of marine turtles and to conservation biology. We thank George Balazs, R. Eric Martin, Glenda Medina, and Ross Witham for help with obtaining samples. Carol Reeb and Bill Nelson provided excellent technical help. Permits or logistic support were provided by Michael Blick, Cecilia de Blohm, Burma Campbell, David Carr, Carol Carson, Guy Childress, Guillermo Cruz, Marion McDowell, Jim Richardson, Earl Possardt, Jack Woody, the U.S. Fish and Wildlife Service, the U.S. Air Force, the National Marine Fisheries Service, the Fundacion para La Defensa de la Naturaleza, the Caribbean Conservation Corporation, the Florida Department of Natural Resources, and the Georgia Department of Natural Resources. Work was supported by the National Geographic Society and by a grant from the National Science Foundation (BSR-8603775).

1. Carr, A., Carr, M. H. & Meylan, A. B. (1978) *Bull. Am. Mus. Nat. Hist.* **162,** 1–46.
2. Carr, A. (1975) *Copeia* **1975,** 547–555.
3. Pritchard, P. C. H. (1973) *Anim. Behav.* **21,** 18–27.
4. Meylan, A. (1982) in *Biology and Conservation of Sea Turtles*, ed. Bjorndal, K. (Smithsonian Institution Press, Washington, D.C.), pp. 91–100.
5. Carr, A. & Carr, M. H. (1972) *Ecology,* **53,** 425–429.
6. Carr, A. (1967) *So Excellent A Fishe* (Natural History Press, Garden City, NY).
7. Owens, D. W., Grassman, M. A. & Hendrickson, J. R. (1982) *Herpetologica* **38,** 124–135.
8. Carr, A. & Coleman, P. J. (1974) *Nature (London)* **249,** 128–130.
9. Zangerl, R. (1980) *Am. Zool.* **20,** 585–596.
10. Zangerl, R. (1960) *Fieldiana Geol. Mem.* **3** (5), 280–312.
11. Gould, S. J. (1978) *Nat. Hist.* **87,** 22–28.
12. Bonhomme, F., Salvidio, S., LeBeau, A. & Pasteur, G. (1987) *Genetica* **74,** 89–94.
13. Avise, J. C. (1986) *Philos. Trans. R. Soc. London Ser. B* **312,** 325–342.
14. Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. & Saunders, N. C. (1987) *Annu. Rev. Ecol. Syst.* **18,** 489–522.
15. Avise, J. C. & Vrijenhoek, R. C. (1987) *Mol. Biol. Evol.* **4,** 514–525.
16. Gyllensten, U., Wharton, D. & Wilson, A. C. (1985) *J. Hered.* **76,** 321–324.
17. Lansman, R. A., Shade, R. O., Shapira, J. F. & Avise, J. C. (1981) *J. Mol. Evol.* **17,** 214–226.
18. Brown, W. M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3605–3609.
19. Nei, M. & Li, W.-H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5269–5273.
20. Brown, W. M., George, M., Jr., & Wilson, A. C. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1967–1971.
21. Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Jr., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, R. G., Palumbi, S. R., Prager, E. M., Sage, R. D. & Stoneking, M. (1985) *Biol. J. Linn. Soc.* **26,** 375–400.
22. Shields, G. F. & Wilson, A. C. (1987) *J. Mol. Evol.* **24,** 212–217.
23. Moritz, C., Dowling, T. E. & Brown, W. M. (1987) *Annu. Rev. Ecol. Syst.* **18,** 269–292.
24. Zug, G. R., Wynn, A. H. & Ruckdeschel, C. (1986) *Smithsonian Contrib. Zool.* **427,** 1–34.
25. Kontos, A., Eckert, S., Eckert, K., Gomez, J. L., Lee, R. & Dam, R. V. (1988) *Mar. Turtle Newsl.* **42,** 10–11.
26. LeGall, J.-Y. & Hughes, G. R. (1987) *Amphibia-Reptilia* **8,** 277–282.
27. Slatkin, M. (1987) *Science* **236,** 787–792.
28. Limpus, C. J. (1985) Ph.D. Dissertation (Univ. of Queensland, Brisbane, Australia).
29. Avise, J. C., Ball, R. M. & Arnold, J. (1988) *Mol. Biol. Evol.* **5** (4), 331–344.
30. Berthold, P. (1988) *J. Evol. Biol.* **1,** 195–209.
31. Manton, M. L., Carr, A. & Ehrenfeld, D. W. (1972) *Brain Behav. Evol.* **5,** 188–201.
32. Koch, A. L., Carr, A. & Ehrenfeld, D. (1969) *J. Theor. Biol.* **22,** 163–169.
33. Carr, A. (1967) in *Animal Orientation and Navigation*, ed. Storm, R. M. (Oregon State Univ. Press, Corvallis, OR), pp. 35–53.
34. Parsons, J. J. (1962) *The Green Turtle and Man* (Univ. Florida Press, Gainesville, FL).