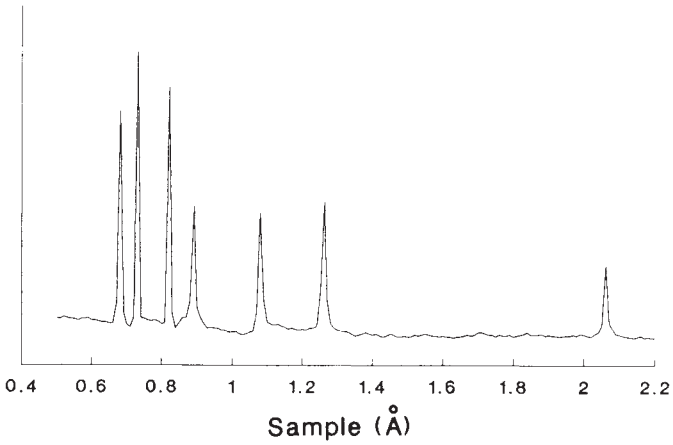

FIG. 1 Electron diffraction profile of the powder. The peaks correspond closely to the accepted values for diamond.

above yielded no residue, not even spinel or chromite, when extracted by the same process. We conclude that the white residue which survives the extraction procedure is confined to the boundary clay itself. This diamond fraction is distinct from the abundant soot found at the K/T boundary[18].

Badziag *et al.*[19] have proposed that, under conditions pertaining in the early Solar System, the energetically preferred form of elemental carbon is diamond, in the 3–5-nm size range discussed here, rather than graphite. Correspondingly, Huss[7] has shown that diamonds were primitively incorporated into all chondrite meteorite groups at concentrations of 500–1,000 μg g^{-1}. In addition, spinel and chromite are commonly found in chondrites[6,14–16], and they too were present in the boundary layer but absent above and below. Impact by one or more meteorites or comets, a possible cause for the event horizon at the K/T boundary which has strong support[2], may therefore be expected to distribute a diamond powder along with the other debris. The diamond powder is indeed present in the boundary clay, but absent a few centimetres above or below. The powder cannot have originated in volcanic action because the microdiamonds would have reverted to graphite or oxidized to carbon-dioxide at the temperatures and relatively low pressures characteristic of volcanic eruptions, nor can it have arrived directly from a supernova, for the diamonds would have burned up as micrometeors in the upper atmosphere. The possibility remains that the diamonds may have arisen by shock metamorphism of carbonaceous target rocks at the site of an impact. In principle, a good X-ray diffraction photograph could allow us to determine whether the diamonds were shock-produced, but the quality of our photographs is not adequate for this. The question of terrestrial or extraterrestrial origin can, however, best be addressed by an examination of isotope ratios, which we intend to study next.

In the meantime we note that the ratio of diamond to iridium (1.22:1) is close to that found in C2 chondritic meteorites[2]. Integrated over the rock column, the concentration of diamonds is 129 ng cm^{-2}, and that of iridium, which is smeared over a greater depth of the boundary rocks, may be estimated at 105 ng cm^{-2} (our own data), giving a diamond/iridium ratio of 1.22:1. The diamond concentration of the C2 chondritic meteorites Murrary and Murchison has been variously estimated[6,7] at 360 to 800 p.p.b., and that of iridium has been reported[1,2] at 300 to 650 p.p.b. Taking the ratio of the extremes of these measurements, we may estimate the diamond/iridium ratio in these C2 chondritic meteorites at 360:300 and 800:650 respectively, or 1.20:1 and 1.23:1 (ref. 2). □

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Evolutionary distinctiveness of the endangered Kemp's ridley sea turtle

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THE endangered Kemp's ridley sea turtle (*Lepidochelys kempi*) nests almost exclusively at a single locality in the western Gulf of Mexico, whereas the olive ridley (*L. olivacea*) nests globally in warm oceans. Morphological similarities between *kempi* and *olivacea*, and a geographical distribution that "... makes no sense at all under modern conditions of climate and geography"[1], raise questions about the degree of evolutionary divergence between these taxa. Analysis of mitochondrial (mt) DNA restriction sites shows that Kemp's ridley is distinct from the olive ridley in matriarchal phylogeny, and that the two are sister taxa with respect to other marine turtles. Separation of olive and the Kemp's ridley lineages may date to formation of the Isthmus of Panama, whereas the global spread of the olive ridley lineage occurred recently. In contrast to recent examples in which molecular genetic assessments challenged systematic assignments underlying conservation programmes[2–6], our mtDNA data corroborate the taxonomy of an endangered form.

From >40,000 adult females censused in a single mass nesting event in the late 1940s, the number of Kemp's ridley turtles nesting annually at the primary site in Tamaulipas, Mexico, has declined dramatically to only a few hundred in recent years[7–9]. Kemp's ridley, first recognized in 1880 (ref. 10), has had a troubled taxonomic history. Before the discovery of the principal nesting site, Kemp's ridley was commonly known as the bastard loggerhead, reflecting belief that it was a hybrid between the loggerhead (*Caretta caretta*) and either the hawksbill (*Eretmochelys imbricata*) or the green (*Chelonia mydas*) turtle[11]. Early in this century, some authors classified the Kemp's ridley as a loggerhead (*Caretta kempii*)[12] or as a subspecies of the olive ridley (*L. olivacea kempi*)[13]. A further complication has been that the Kemp's ridley is routinely misidentified as a loggerhead in museum collections. *L. kempi* is restricted in range to the Gulf of Mexico and the north Atlantic. *L. olivacea* occurs in the east and west Pacific, Indian Ocean, and both sides of the Atlantic, but does not overlap geographically with the Kemp's ridley[14].

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TABLE 1 Description and distribution of mtDNA genotypes in ridley and representative loggerhead turtles

Taxon	Number of turtles	mtDNA genotype																
L. kempi	4	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
L. kempi	2	C	C	C	C	C	C	C	C	C	C	C	C	B	C	C	C	C
L. olivacea	19	B	E	E	C	D	C	B	C	C	C	D	C	B	D	C	B	C
C. caretta (Atlantic)	5	—	E	—	D	—	—	E	B	E	D	A	C	D	—	C	—	E
C. caretta (Pacific)	5	—	E	—	E	—	—	E	B	F	D	A	C	E	—	C	—	E

Letters refer to mtDNA digestion profiles produced by (from left to right) *Ava*II, *Bcl*I, *Bst*EII, *Bst*NI, *Cla*I, *Eco*RI, *Eco*RV, *Eco*0109, *Hin*cII, *Hin*dIII, *Nde*I, *Pvu*II, *Sac*I, *Spe*I, *Sst*II, *Stu*I and *Xba*I: adjacent letters in the alphabet indicate that fragment profiles differ by a single restriction site; nonadjacent letters differ by at least two sites. Dashes indicate enzymes for which site comparisons were not made between loggerhead and ridley.

The international programme to protect Kemp's ridley represents the largest conservation effort for any marine turtle[15]. As in other conservation programmes, exceptional preservation efforts are motivated ultimately by the premise that the endangered population is a distinct phylogenetic entity. Here we use conventional mtDNA restriction site analyses to elucidate evolutionary relationships and phylogeny within *Lepidochelys*. Mitochondrial DNA from Kemp's ridley ($n = 6$) was compared with that from two widely separated populations of olive ridley (Suriname, West Atlantic, $n = 14$; Costa Rica, East Pacific, $n = 5$) after digestion with the 17 restriction endonucleases listed in Table 1. Loggerhead turtles were included as an outgroup, based on an accepted subfamily affiliation[16].

A mean of 80 restriction sites per individual, corresponding to 461 nucleotides in recognition sequence, was scored in the ridleys (Fig. 1). Two genotypes (separated by a single restriction enzyme site change) were observed in the Kemp's and one in the olive ridley. The latter genotype differed from those in the Kemp's ridley at 8–9 of the 17 digestion profiles (Table 1), involving changes at 10–11 assayed restriction sites. Genetic distances are summarized in Table 2.

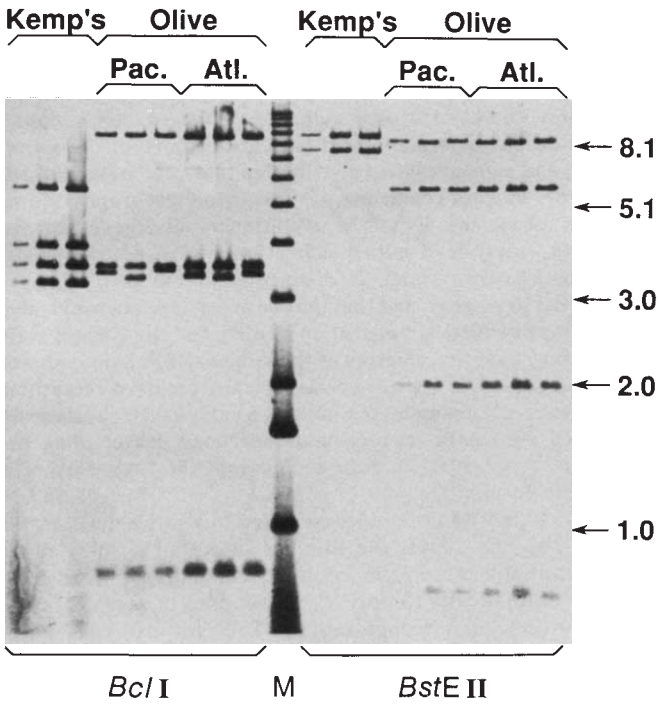

FIG. 1 Examples of mtDNA digestion profiles (produced by *Bcl*I and *Bst*EII) that are identical in olive ridley populations from the Atlantic (Atl.) and Pacific (Pac.) Oceans, but which each distinguish Kemp's from olive ridley by two restriction-site changes. Heart and liver samples of *L. kempi* were obtained from cold-stunned juveniles that perished near Long Island, New York from 1987–1989. Samples of *L. olivacea* consisted of eggs (one per female) taken during laying and incubated in the laboratory for two to eight weeks. MtDNA was isolated by CsCl density-gradient centrifugation[25]. Restriction fragments were end-labelled with ^{35}S-labelled radionucleotides, separated on 1.0–1.5% agarose gels and visualized by autoradiography[25]. In this gel the centre lane (*M*) is a molecular size standard; selected fragment sizes (in kilobases) are indicated to the right. Note that in olive ridleys, the *Bcl*I fragments near 3.3 kb also exhibit a size polymorphism (as judged by concordant differences in digestion profiles from other enzymes).

Phylogenetic summaries of mtDNA data (Fig. 2) are consistent with the current morphology-based taxonomy of the Kemp's and olive ridley. Atlantic and Pacific populations of the olive ridley, separated by about 25,000 km of ocean, were indistinguishable in our assays (sequence divergence, $p = 0.000$), whereas the Kemp's ridley showed substantial mtDNA differences from both ($p = 0.012 \pm 0.003$ (s.e.; ref. 17)). The mtDNA sequence divergence between Kemp's and olive ridleys is also greater than any estimated genetic distances in global surveys of either the green or loggerhead turtle (Fig. 2). On the basis of a provisional mtDNA clock calibrated from other marine turtles (0.2 to 0.4 per cent sequence divergence per million years (see legend to Fig. 2)), we estimate that the olive and Kemp's ridleys diverged about 3–6 million years ago, whereas the two widely separated olive ridley populations diverged recently. Although absolute time estimates must be interpreted with caution, mtDNA data are consistent with a suggestion that Kemp's and olive ridley were isolated by formation of the Isthmus of Panama[18] some three million years ago.

Molecular data are also consistent with the hypothesis that olive ridley populations in the Atlantic and Pacific Oceans are closely related[14]. Based on a synthesis of distributional data and comparative morphology, Pritchard[19] suggested that olive ridleys recently colonized the Atlantic via the Cape of Good Hope. Contemporary oceanic current patterns and the presence of olive ridleys in southeast Africa are both compatible with this view[20], and this route has been widely cited as a path of Atlantic colonization by other Indo-Pacific faunas[21]. Our data support Pritchard's[19] biogeographic scenario for the olive ridley, but a complete test should include additional population samples from spatially intermediate locales.

TABLE 2 Estimates of mtDNA nucleotide sequence divergence based on restriction site and fragment comparisons[22]

		L. kempi	*L. kempi*	*L. olivacea*	*C. caretta* (Atlantic)	*C. caretta* (Pacific)
I	*L. kempi*	—	0.001	0.011	0.038	0.042
II	*L. kempi*	0.001	—	0.012	0.040	0.039
III	*L. olivacea*	0.013	0.012	—	0.036	0.035
IV	*C. caretta* (Atlantic)	0.043	0.043	0.042	—	0.008
V	*C. caretta* (Pactific)	0.044	0.043	0.042	0.007	—

Estimates from restriction sites (above diagonal) are based on the enzymes listed in Table 1; those from fragment comparisons (below diagonal) are based on data from *Bcl*I, *Bgl*I, *Bst*EII, *Bst*NI, *Eco*RV, *Eco*0109, *Hin*cII, *Hin*dIII, *Nde*I, *Pvu*II, *Sac*I, *Spe*I, *Sst*II, *Stu*I and *Xba*I.

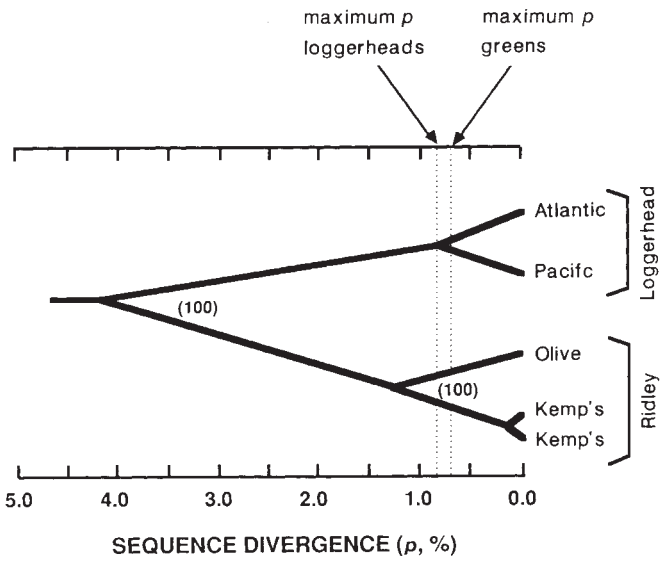

FIG. 2 UPGMA phenogram[26] summarizing mtDNA relationships among ridley and representative loggerhead turtles from Cumberland Island, Georgia, USA and Mon Repos, Queensland, Australia. An exhaustive computer search (using PAUP; ref. 27) identified a single most-parsimonious network, with topology identical to that of the phenogram. Among 1,000 bootstrap replicates, support for putative clades (expressed in %) are in parentheses. Sequence divergence, *p*, can be related tentatively to absolute time by assuming 0.2–0.4% sequence divergence between two lineages per million years. This calibration comes from analyses of several other turtle species (ref. 28, and our unpublished data), and represents a 5- to 10-fold deceleration compared with the 'conventional' vertebrate mtDNA clock[29]. Also indicated are largest mtDNA genetic distances observed among global collections of 213 green and 144 loggerhead turtles (ref. 28, and our unpublished data).

Lepidochelys kempi and *L. olivacea* are related more closely to one another in the mtDNA tree than either is to the loggerhead (Fig. 2). Using the 'site' and 'fragment' methods[22], intergeneric comparisons yielded estimates of sequence divergence (p) ranging from 0.035–0.044 (Table 2). Applying the aforementioned clock calibration, ridley and loggerhead ancestors seem to have diverged about 10–20 million years ago.

Under the terms of the US Endangered Species Act and parallel international regulations, legal protection may be extended to 'distinct' species, subspecies and populations, with the definition of these categories left to the purview of biologists and legal experts[5]. Phylogeographic data alone cannot establish whether the allopatric *kempi* and *olivacea* are isolated by intrinsic reproductive barriers, and hence qualify as distinct species under a biological species definition[23,24]. Nonetheless, the molecular analysis demonstrates that Kemp's ridley is phylogenetically distinct from assayed olive ridley populations, with mtDNA genetic distances greater than those typically observed among global populations of other marine turtle species. □

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Regulation of fast inactivation of cloned mammalian $I_K(A)$ channels by cysteine oxidation

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MODULATION of neuronal excitability by regulation of K^+ channels potentially plays a part in short-term memory[1] but has not yet been studied at the molecular level. Regulation of K^+ channels by protein phosphorylation[2–5] and oxygen[6] has been described for various tissues and cell types; regulation of fast-inactivating K^+ channels mediating $I_K(A)$ currents has not yet been described. Functional expression of cloned mammalian K^+ channels[7–13] has provided a tool for studying their regulation at the molecular level. We report here that fast-inactivating K^+ currents mediated by cloned K^+ channel subunits derived from mammalian brain expressed in *Xenopus* oocytes are regulated by the reducing agent glutathione. This type of regulation may have a role *in vivo* to link metabolism to excitability and to regulate excitability in specific membrane areas of mammalian neurons.

Three types of cloned rat brain $I_K(A)$ channels have been described based on K^+ channel proteins RCK4 (ref. 9), Raw3 (ref. 14) and the heteromultimer composed of RCK1 and RCK4 (RCK1,4; ref. 15). The time constants of inactivation observed for these three channel types exhibited an unusually wide scatter[14,15]. Figure 1*a* compares current traces from two *Xenopus* oocytes (cell K and cell G) expressing RCK4-1 tandem channels (described in Fig. 1 legend). They had markedly different time constants (by a factor of eight) of inactivation and a different percentage of steady-state current. As shown in Fig. 1*b*, the value of the time constant tends to be similar for patches from any oocyte. This suggests that inactivation might be regulated by an intracellular factor common to all patches on a particular oocyte.