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The Rate of Mitochondrial 12S rRNA Gene Evolution Is Similar in Freshwater Turtles and Marsupials

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Abstract. Assertions that the "conventional" rate of mitochondrial DNA (mtDNA) evolution is reduced in poikilotherms in general and turtles in particular were tested for side-necked turtles (Pleurodira: Chelidae). Homologous data sets of mitochondrial 12S rRNA gene sequences were used to compare the average divergence between the Australian and South American species for two Gondwanan groups: the chelid turtles and the marsupials. The mean nucleotide divergences between continental groups for both the turtles and the marsupials are remarkably similar. These data suggest that the rate of evolution of mitochondrial 12S rRNA gene is not substantially slower in turtles than in the homeothermic marsupials.

Key words: Rate — Molecular — Evolution — Chelid — Turtles — Marsupials

Introduction

Evidence is accumulating that the rate of evolution of mitochondrial DNA is lower in turtles than in other vertebrates (Lamb et al. 1989, 1994; Avise et al. 1992; Bowen et al. 1993). In one study, Avise et al. (1992) inferred the rate of sequence evolution in the mitochondrial genome across five testudine taxa. Estimated rates give an average of 0.25%/MY, eightfold lower (range two- to 14-fold) in Testudines than the "conventional" rate of vertebrates initially suggested by Brown et al. (1979) for primates. Other recent studies have questioned the universality of this "conventional" rate across vertebrate taxa, suggesting that the rate of change in mtDNA is slower in poikilotherms than in homeotherms (Kocher et al. 1989; Thomas and Bechenbach 1989; Martin et al. 1992; Canatore et al. 1994) and is influenced by body size, generation time and metabolic rate (Martin and Palumbi 1993).

The reduction in the rate of mtDNA evolution in poikilotherms in general, and turtles in particular, has been widely adopted in the calculation of divergence times (for example, Encalada et al. 1996). However, it may be premature to apply this reduced rate without further substantiation. We present an example in which the rate of mtDNA evolution in freshwater turtles is not reduced.

In the present study, we use a biogeographical approach to compare the rate of evolution of presumed homologous mitochondrial 12S rRNA gene sequence data for two Gondwanan groups, the side-necked turtles (Pleurodira: Chelidae) and the marsupials. The Chelidae are a family of freshwater, side-necked turtles for which fossil and extant species are found only in the Southern Hemisphere, reflecting their presumed Gondwanan origins (Pritchard 1979; Gaffney 1981). Phylogenetic

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analysis of the 12S rRNA gene sequence data suggests that the Australian Chelidae form a monophyletic clade (Seddon et al. 1997). A similar biogeographical pattern is seen in the distribution and phylogency of the marsupials of Australia and the Americas (Kirsch 1984; Springer et al. 1994).

It is proposed that the Australian and South American species for both the Chelidae and the marsupials diverged at approximately the time of the separation of the continents upon the breakup of Gondwana. Therefore a roughly similar divergence time for both groups can be assumed but not necessarily estimated. The relative rates of mitochondrial evolution can be assessed by comparing the divergence of the Australasian and South American species for both chelid turtles and marsupials. In this manner, the reliance on estimates of the time of cladogenesis can be avoided.

Sequence data were derived from PCR-amplified fragments of the 12S rRNA gene for 16 species of Chelidae (nine Australasian, seven South American; Genbank accession numbers U40392, U40633–U40640, U40645–U40648, U40650–U40651, U62017). Sequences of 17 species of marsupials (12 Australian, five American) were extracted from Genbank (accession numbers U02576, U02587, U21167–U21170, U21174, U21176, U21178–U21179, U21181, U21183, U21184, U21187–U21190). Sequences were aligned in Clustal W (Thompson et al. 1994) and the alignment was adjusted by eye. The sequences were presumed to be homolgous.

Tamura-Nei distances (Tamura and Nei 1993) based on transversions only were calculated on 407 (Chelidae) and 399 (marsupial) nucleotides of the 12S rRNA gene (Table 1). Transversions only were used for distance estimates because the graph of transitions vs. transversions for the Chelidae (not shown) reaches a plateau, indicating saturation of transitional changes (Mindell and Honeycutt 1990). Tamura-Nei distances were calculated to account for the observed nucleotide bias. The nucleotide frequencies in the Australian and South American taxa of the Chelidae are similar to those of the marsupials (chelid/marsupial, from sites informative under parsimony: A 33%/36, T 23%/26%, C 29%/24%, G 16%/25%).

The mean divergence between the Australian and South American species was determined for both the chelids and the marsupials (Table 2). The mean divergence between the continental groups is 1.4-fold higher for chelids than for marsupials (1.3–1.5-fold difference taking into account the standard errors). Our data do not support the hypothesis that the rate of evolution at the 12S rRNA gene is reduced in turtles.

Possible Explanations

Several explanations can be postulated to account for our data. Patterns of evolutionary change at the 12S rRNA gene may not be equivalent in turtles and marsupials. However, several indicators suggest that the pattern of evolution is not disparate in one species. The 12S rRNA gene sequences from both turtles and marsupials fitted well to the stem-loop structure of published secondary structure models for domain III (Hickson et al. 1996). In addition, mean divergences were calculated using gamma distances (Tamura-Nei model, $\alpha = 0.5$, transversions only), which takes into account rate heterogeneity among sites. The difference between species was similar to the above comparison (chelid/marsupial 0.131/0.089; 1.5-fold difference) indicating that rate heterogeneity among sites was not strongly biasing one species group.

Unequal branch lengths within the clades following separation of the fauna can introduce error in calculation of mean divergence due to the nonindependence of pairwise comparisons (Hillis et al. 1996). In an attempt to examine the influence of potential errors, taxa were removed to equalize the branch lengths on a neighbor-joining tree. Five chelid taxa and one marsupial taxon separated from their close relatives by short branch lengths were removed. The mean divergence recalculated thus (Table 2) remains greater for turtles than for marsupials (1.2-fold difference).

Another possible explanation of these data is that marsupials may also exhibit a reduced rate of sequence evolution. However, a relative rate test using monotremes as the outgroup to marsupials and placentals shows that transversions for 12S rRNA in marsupials are the same as placentals (Springer et al. 1994). Therefore, the similarity of rate between chelid turtles and marsupials does not reflect a slowdown in these two lineages but an overall similarity with Theria.

Biogeographical calibrations of rates of evolution have been criticized for their dependence on the assumption of species divergence with the separation of land masses, an assumption which has occasionally been invalidated (for example, Cannatella and de Sa 1993). In our comparison, this assumption of species divergence occurring with the separation of continents could be incorrect as some support for a paraphyly of marsupials has been found. In particular, the relationships of *Dromiciops* are controversial (Springer et al. 1994 and references therein).

The "effective" separation time of the species provides a source of variation—for example, with continued movement of homeotherms between continents via Antarctica. However, even this variation is unlikely to be sufficient to account for a reduction in the rate of mitochondrial evolution in turtles—for example, in the order of eightfold as suggested by Avise et al. (1992). An eightfold reduction in rate would require a divergence of the Australian and South American species within the Chelidae at approximately 400 MY. Such an estimate is unreasonable as fossil chelids extend only to the Eocene

Table 1. Pairwise distance matrix for Australian and South American species of a) Chelidae and b) Marsupials[a]

a)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 ***Pseudemydura umbrina***		0.1600	0.1778	0.1597	0.1713	0.1498	0.1859	0.2253	0.1874	0.2065	0.2927	0.2919	0.3495	0.3184	0.3605	0.2617
2 ***Rheodytes leukops***	0.0361		0.0703	0.0419	0.0743	0.0624	0.1388	0.1771	0.1660	0.1614	0.2384	0.2943	0.2934	0.2718	0.2644	0.1998
3 ***Elusor macrurus***	0.0356	0.0217		0.0646	0.0855	0.0885	0.1507	0.1693	0.1432	0.1898	0.2483	0.3107	0.2885	0.2799	0.3086	0.2156
4 ***Emydura macquarii***	0.0390	0.0137	0.0135		0.0474	0.0505	0.1451	0.1756	0.1649	0.1737	0.2363	0.2790	0.2887	0.2650	0.2687	0.2042
5 ***Elseya latisternum***	0.0332	0.0220	0.0189	0.0192		0.0805	0.1418	0.1643	0.1610	0.1793	0.2585	0.2997	0.2879	0.2705	0.2792	0.2035
6 ***Elseya dentata***	0.0390	0.0192	0.0245	0.0220	0.0248		0.1528	0.1923	0.1611	0.1556	0.2363	0.2843	0.2851	0.2643	0.2802	0.2076
7 ***Chelodina longicollis***	0.0505	0.0420	0.0415	0.0421	0.0419	0.0361		0.0789	0.0661	0.1745	0.2434	0.3133	0.3265	0.2930	0.3370	0.2458
8 ***Chelodina oblonga***	0.0574	0.0516	0.0508	0.0515	0.0513	0.0485	0.0243		0.0880	0.2137	0.2489	0.3235	0.3265	0.2712	0.3474	0.2664
9 ***Chelodina rugosa***	0.0447	0.0422	0.0416	0.0451	0.0421	0.0391	0.0159	0.0243		0.1880	0.2224	0.2952	0.3030	0.2415	0.3494	0.2397
10 *Hydromedusa tectifera*	0.0567	0.0539	0.0502	0.0571	0.0539	0.0450	0.0617	0.0716	0.0621		0.2024	0.2866	0.2695	0.2629	0.3006	0.2269
11 *Chelus fimbriata*	0.0848	0.0760	0.0689	0.0697	0.0823	0.0760	0.0773	0.0756	0.0841	0.0925		0.2993	0.3151	0.2158	0.3230	0.2506
12 *Platemys platycephala*	0.1356	0.1365	0.1276	0.1265	0.1333	0.1293	0.1398	0.1284	0.1237	0.1257	0.1265		0.2265	0.2480	0.2928	0.2381
13 *Acanthochelys pallidopectoris*	0.1153	0.1164	0.1046	0.1202	0.1164	0.1092	0.1234	0.1116	0.1173	0.0965	0.1040	0.1061		0.2477	0.2511	0.2328
14 *Phrynops (Phrynops) geoffroannus*	0.1280	0.1153	0.1103	0.1157	0.1188	0.1184	0.1126	0.1076	0.1033	0.1216	0.0963	0.1323	0.1119		0.2543	0.2087
15 *Phrynops (Mesoclemmys) gibbus*	0.1502	0.1339	0.1385	0.1304	0.1443	0.1331	0.1448	0.1471	0.1381	0.1479	0.1381	0.1411	0.1276	0.1325		0.1035
16 *Phrynops (Batrachemys) nasuta*	0.0961	0.0973	0.0858	0.0873	0.0871	0.0868	0.0953	0.0967	0.0890	0.0885	0.0920	0.1109	0.1089	0.0140	0.0689	

b)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1 ***Phascolarctos cinereus***	0.2014	0.1290	0.1725	0.1439	0.1980	0.2472	0.1668	0.1737	0.2340	0.1962	0.2049	0.2163	0.2291	0.2120	0.1939	0.1810	
2 ***Phascogale tapoatafa***	0.0756		0.1802	0.2218	0.1587	0.1645	0.2181	0.1614	0.1959	0.2158	0.1969	0.2330	0.2050	0.2529	0.2125	0.1713	0.1730
3 ***Tarsipes rostratus***	0.0486	0.0784		0.1715	0.1300	0.1336	0.1994	0.1491	0.1529	0.1511	0.1471	0.1525	0.1864	0.1701	0.1956	0.1433	0.1506
4 ***Trichosurus vulpecula***	0.0752	0.0942	0.0902		0.1402	0.1640	0.1549	0.1451	0.1636	0.2089	0.1666	0.2091	0.1856	0.2363	0.1962	0.1660	0.1702
5 ***Dendrolagus inustus***	0.0486	0.0463	0.0716	0.0516		0.1363	0.1793	0.1238	0.1376	0.1627	0.1342	0.1809	0.1759	0.1821	0.1585	0.1340	0.1254
6 ***Distoechurus pennatus***	0.0752	0.0670	0.0692	0.0728	0.0349		0.1674	0.1543	0.1785	0.1533	0.1352	0.1795	0.1198	0.1778	0.1757	0.1261	0.1426
7 ***Isoodon macrourus***	0.0902	0.0910	0.0902	0.0578	0.0752	0.0724		0.1851	0.2087	0.1915	0.1932	0.2481	0.1594	0.1950	0.1914	0.1452	0.1520
8 ***Vombatus ursinus***	0.0455	0.0547	0.0399	0.0811	0.0485	0.0544	0.0808		0.1510	0.1638	0.1513	0.1885	0.1733	0.2066	0.1865	0.1540	0.1454
9 ***Notoryctes typhlops***	0.0604	0.0761	0.0724	0.0820	0.0548	0.0637	0.0754	0.0459		0.1764	0.1823	0.2383	0.1930	0.2243	0.1950	0.1846	0.1646
10 ***Petaurus breviceps***	0.1051	0.0724	0.0774	0.0965	0.0628	0.0573	0.0869	0.0594	0.0632		0.1799	0.2099	0.1720	0.1967	0.2039	0.1814	0.1849
11 ***Pseudocheirus cupreus***	0.0543	0.0607	0.0602	0.0724	0.0486	0.0576	0.0871	0.0428	0.0546	0.0628		0.2086	0.1868	0.2184	0.1805	0.1519	0.1545
12 ***Cercartetus caudatus***	0.0836	0.0910	0.0599	0.1031	0.0776	0.0722	0.1123	0.0596	0.1031	0.0954	0.0836		0.2437	0.1643	0.2344	0.1953	0.2020
13 *Monodelphis domestica*	0.0720	0.0850	0.0838	0.0874	0.0628	0.0635	0.0907	0.0659	0.0908	0.0778	0.0810	0.0900		0.1503	0.1308	0.1304	0.1343
14 *Didelphis virginiana*	0.0968	0.1075	0.0813	0.1171	0.0905	0.0910	0.1262	0.0839	0.1164	0.0900	0.0996	0.0685	0.0752		0.0794	0.1626	0.1691
15 *Lutreolina crassicaudata*	0.0691	0.0820	0.0750	0.0939	0.0689	0.0666	0.1095	0.0689	0.0846	0.0748	0.0688	0.0931	0.0486	0.0348		0.1523	0.1460
16 *Caenolestes fuliginosus*	0.0486	0.0576	0.0570	0.0722	0.0456	0.0575	0.0810	0.0485	0.0635	0.0776	0.0486	0.0776	0.0599	0.0874	0.0630		0.0425
17 *Rhynocholestes raphanurus*	0.0402	0.0576	0.0599	0.0693	0.0373	0.0546	0.0720	0.0456	0.0548	0.0746	0.0514	0.0806	0.0456	0.0844	0.0601	0.0129	

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[a] Upper right matrix: Tamura-Nei distances; lower left matrix: Tamura-Nei distances based on transversions only; Australian taxa in bold

Table 2. Nucleotide divergences between Australian and South American taxa for Chelid Turtles and Marsupials from 12S rRNA gene sequence data

Species	Mean divergence[a] ± standard error	Mean divergence (reduced taxa)[b] ± standard error
Turtles	0.104 ± 0.003	0.097 ± 0.005
Marsupials	0.075 ± 0.004	0.079 ± 0.003

[a] Tamura-Nei distance based on transversions only

[b] Calculation of mean divergence after removal of *Phrynops (Mesoclemmys) gibbus, Rheodytes leukopus, Emydura macquarii, Elseya latisternum, Chelodina longicollis* and *Rhynocholestes raphanurus*

(Benton 1993), and micro-complement fixation (MC′F) data and fossil dating give the divergence time for the family Chelidae from their sister family, Pelomedusidae, at the Cretaceous/Paleocene boundary (65MY; Chen et al. 1980). In contrast, the latest movement of the marsupials through Antarctica may be as late as 42 MY, although climatic restrictions make such movement improbable. Given the extremes of this combination, the data support only a 1.1-fold slowdown in turtles.

Another study also failed to find a reduced rate of change in 12S rRNA gene in the crocodile relative to birds or mammals (Mindell et al. 1996). It is possible that the apparent slow rate of evolution in Testudines observed by others may be occurring in regions of the mitochondrial genome other than the 12S rRNA gene and consequently would influence RFLP studies but not 12S rRNA sequence data. This situation supports the body temperature theory (Thomas and Bechenbach 1989), which explains the reduction in the rate of evolution in poikilotherms as a relaxation of selective constraints on protein-coding genes of homeothermic vertebrates. Such differential selective constraints on mitochondrial protein-coding and rRNA genes would account for the slowdown in mtDNA detected by RFLP studies but not in 12S rRNA gene sequencing studies. The effects of differential selection in poikilotherms could be tested by extending a study such as this to include a mitochondrial protein-coding region.

Conclusion

By comparison of a homologous region of mtDNA in two groups of fauna with a similar distribution and presumed similar origins, the similarity in rates of mitochondrial evolution between the chelid turtles and the marsupials has been demonstrated without recourse to an absolute date of divergence for calibration. The comparison, while not suggesting that the rates of mitochondrial evolution of turtles and marsupials are identical, indicates that proposed reductions in the rate of evolution should not be broadly applied to poikilotherms in general nor to turtles in particular without further substantiation.

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