

# Higher Ribosomal RNA Substitution Rates in Bacillariophyceae and Dasycladales than in Mollusca, Echinodermata, and Actinistia-Tetrapoda

Ulf Sorhannus

Department of Biology, Edinboro University of Pennsylvania

Molecular evolutionary rates within two protistan and three metazoan taxa were estimated using divergence times derived from fossil records. The results indicate that the small-subunit rRNA sequences within Dasycladales (Chlorophyta) and Bacillariophyceae evolved at a rate approximately two to three times faster than that estimated within Echinodermata, Mollusca, and Actinistia-Tetrapoda. It was concluded that this twofold discrepancy demonstrates actual taxonomic differences in the fixation rate of mutations in the small-subunit rRNA.

## Introduction

The universal molecular clock (e.g. Zuckerkandl and Pauling 1965; Wilson, Carlson, and White 1977; Ochman and Wilson 1987), which proposes an approximately constant rate of nucleotide substitution per unit time within and between different groups of organisms, continues to be widely discussed (e.g., Langley and Fitch 1974; Gillespie 1986, 1992; Nei 1987; Clegg and O'Brien 1990; Scherer 1990; Li and Grauer 1991). Evidence for and against this hypothesis of rate constancy has been drawn from either multicellular eukaryotic genes (e.g., Wu and Li 1985; Britten 1986; Li, Tanimura, and Sharp 1987; DeSalle and Templeton 1988; Sharp and Li 1989; Eastal 1990; Shapiro 1991; O'hUigin and Li 1992; Pamilo and Bianchi 1993; Cantatore et al. 1994; Gibbs and Dugaiczky 1994) or prokaryotic (e.g., Ochman and Wilson 1987) and viral genes (e.g., Gojobori, Moriyama, and Kimura 1990). The analyses of nucleotide substitution rates for both unicellular and multicellular eukaryotes are included in only a few comparative studies (e.g., Philippe et al. 1994; Sorhannus 1994). This may be partly due to the fact that many protistan groups lack reliable fossil records. Thus, in order to make possible further testing of the universal rate constancy hypothesis for a particular gene, it is necessary to incorporate representatives of both protistan and metazoan taxa in the same analysis. For instance, diatoms and green algae are groups that serve this purpose well. The former is characterized by a well-documented fossil record (e.g., Jouse 1978; Simonsen 1979; Tappan 1980), particularly in the Cenozoic period, and certain lineages, such as representatives of Dasycladales, within the latter group have good paleontological records (e.g., Berger and Kaever 1992; Olsen et al. 1994).

In this study, the substitution rates of nuclear-encoded small-subunit ribosomal RNA were compared between representatives of the following monophyletic groups: Bacillariophyceae, Dasycladales (Chlorophyta), Echinodermata, Mollusca, and Actinistia-Tetrapoda

**Key words:** small-subunit ribosomal RNA, evolutionary distance, divergence time, variable positions, taxonomic nucleotide substitution rate, molecular clock, Bacillariophyceae, Dasycladales, Actinistia-Tetrapoda, Echinodermata, Mollusca.

Address for correspondence and reprints: Ulf Sorhannus, Department of Biology, Edinboro University of Pennsylvania, Edinboro, Pennsylvania 16444. E-mail: usorhanns@edinboro.edu.

Mol. Biol. Evol. 13(7):1032-1038. 1996

© 1996 by the Society for Molecular Biology and Evolution. ISSN: 0737-4038

(sensu Maisey 1988). Divergence times derived from the fossil record were used to infer the substitution rates within each taxon. The results indicate that the largest rate discrepancy exists between the protistan and metazoan groups.

## Materials and Methods

The entire nuclear-encoded small-subunit ribosomal RNA gene (18s rRNA) has been sequenced for representatives of a number of diatoms, green algae, echinoderms, molluscs, and vertebrates. Evolutionary lineages within these groups are characterized by a well-documented geological history. The following diatom sequences, which represent all the major taxonomic groups, were used in this study: *Nitzschia apiculata*, *Rhizosolenia setigera*, *Stephanopyxis* cf. *broschii*, *Thalassionema nitzschioides*, and *Rhaphoneis belgica*. The first three taxa were obtained from Bhattacharya et al. (1992) and the last two species from the EMBL data bank (accession numbers X77702 and X77703). The 18s rRNA data of *Paramecium tetraurelia* were taken from Sogin and Elwood (1986), whereas those of the Dasycladales lineages (*Acetabularia crenulata*, *Polyphysa peniculus*, *Polyphysa parvula*, and *Chlorocladus australasicus*) have been sequenced by Olsen et al. (1994). The nucleotide sequences of *Alligator mississippiensis*, *Heterodon platyrhinos*, *Mus musculus*, *Oryctolagus cuniculus*, and *Latimeria chalumnae* have been published by Hedges, Moberg, and Maxson (1990), Hedges, Moberg, and Maxson (1990), Raynal, Michot, and Bachellerie (1984), Rairkar, Rubino, and Lockard (1988), and Stock et al. (1991), respectively. The small-subunit rRNA data of *Antedon serrata*, *Asterias amurensis*, *Ophioplocus japonicus*, and *Strongylocentrotus intermedius*, which represent different extant echinoderm classes, were obtained from Wada and Satoh (1994), whereas those of *Argopecten irradians* (accession number L11265), *Limicola kambeul* (accession number X66374), and *Acanthopleura japonica* (accession number X70210), which are placed in the mollusc classes Bivalvia, Gastropoda, and Polyplacophora, respectively, were retrieved from the EMBL Data Bank.

Alignment of nucleotides between the 22 taxa considered in this study was performed using the multiple alignment algorithm in CLUSTAL V (Higgins and Sharp 1989; Higgins, Bleasby, and Fuchs 1992). In order to maximize similarity between sites within Actin-

istia-Tetrapoda, Echinodermata, Dasycladales, Bacillariophyceae, and Mollusca, the result of the multiple alignment was manually adjusted by taking the published taxon-specific alignments into consideration (see Hedges, Moberg, and Maxson 1990; Bhattacharya et al. 1992; Olsen et al. 1994; Wada and Satoh 1994). Because the original data matrix of Mollusca was not available, maximum correspondence between the rRNA sequences of *A. irrudians*, *L. kambeul*, and *A. japonica* was achieved by using CLUSTAL V and by considering the secondary structure of *Plagopecten magellanicus* (Gutell 1994).

Nucleotide sites characterized by substantial intertaxic variability and by missing (not sequenced) information (412 sites) were discarded from the analysis. Because of biasing effects the elimination of highly variable sites may have on rate analysis, two different data matrices were generated, one in which unambiguously aligned regions were retained (874 nucleotide sites) and another in which a number of highly variable sites (1,002 nucleotide sites) with uncertain alignments were kept. In the former case, the author wanted to ensure the elimination of potential saturation effects, that is, genetic distances that are not proportional to the true number of substitution events (see Philippe, Chenuil, and Adoutte 1994; Philippe et al. 1994).

Three different substitution models were used for deriving distance matrices from the 1,002 and 874 nucleotide characters. The most general of these three approaches is the two-parameter model, which allows for both differences in transition/transversion rates and unequal base frequencies (see Hasegawa, Kishino, and Yano 1985; Felsenstein 1993). The second model is a special case of the first one, that is, the model of Kimura (1980), which allows for differences in transition/transversion rates but assumes the nucleotide base frequencies to be equal. The third one, developed by Palumbi (1989), is a modified version of the Kimura model (1980), which takes into account the potential biasing effects differences in the proportion of nucleotide sites free to vary between each group may have on taxonomic distance estimates. The maximum likelihood approach (Hasegawa, Kishino, and Yano 1985) to calculating genetic distances between taxa was used to evaluate the distorting effects differences in nucleotide composition among taxa may have on the substitution rate (see Palumbi 1989). Estimates of the three different kinds of distances were obtained by using the DNADIST program in the PHYLIP package (Felsenstein 1993). The author calculated standard errors of the Kimura corrections as suggested in Kimura (1980). Derivation of the dendrogram from the Kimura (K) distance matrix was accomplished by the neighbor-joining method (Saitou and Nei 1987). The actual computations were carried out by the NEIGHBOR and DRAWGRAM programs in PHYLIP (Felsenstein 1993). I subjected the nucleotide sequences to a bootstrap analysis (1,000 iterations) by using the SEQBOOT, DNADIST (Kimura corrections), NEIGHBOR, and CONSENSE programs in PHYLIP (Felsenstein 1993).

The absolute divergence rates were obtained by dividing the distance estimates derived from the three different models by the approximate time period the compared taxa have evolved as independent lineages (divergence time).

## Results

Table 1 displays three different evolutionary distances obtained from the 874 nucleotide characters (K = Kimura corrections, MI = maximum likelihood model, and K' = Kimura corrections adjusted according to proportions of nucleotides free to vary) and the resulting absolute divergence rates. Noteworthy is the fact that K and MI distances are the same. The former and the latter parameters were estimated by using the average empirical transition/transversion ratio of 3.5, in addition to the empirical base frequencies required for the maximum likelihood calculations. K' distances and the resulting absolute rates are much higher than the same values for K and MI. According to Palumbi (1989), this is also an expected result because K' is not "diluted" by all the constant sites. The fractions of positions free to vary (=x) for the 874 nucleotides in Bacillariophyceae, Dasycladales, Actinistia-Tetrapoda, Echinodermata, and Mollusca were estimated to be 0.18, 0.26, 0.15, 0.19, and 0.19, respectively. Because maximum nucleotide divergence within a taxon is the poorest known value for x, outgroups were used that are older than the considered groups to estimate the proportion of positions free to vary (see Palumbi 1989). The outgroups of Mollusca, Echinodermata, Actinistia-Tetrapoda, Bacillariophyceae, and Dasycladales were representatives of Cnidaria, Mollusca, ciliates, and diatoms, respectively.

Table 2 summarizes the taxonomic rate comparisons. With regard to K and MI distances, the diatom-metazoa ratios ranged from 2.2 to 3 and the Dasycladales-metazoa ratios varied between 3.2 and 4.3. Within protista and metazoa, the ratios are much lower. By comparing the lineage-specific divergence rates ( $\pm$ SE) derived from the K distances within diatoms and Dasycladales with that in vertebrates, echinoderms, and molluscs, it appears that only two absolute substitution rates within Bacillariophyceae show an overlap with some of the metazoan values (see table 1). Evolutionary rates derived from K' show a lower discrepancy between protistan and metazoan groups in relation to the K and MI derived rates. This is particularly evident in the Dasycladales-metazoa comparison (see table 2). The overall result indicates a two- to threefold faster absolute divergence rate within the protistan taxa than within Metazoa (table 2).

The same methods as the ones employed for the smaller data set were used to derive evolutionary distances from the 1,002 sites. The results (not shown) indicate that the distance estimates between the taxa are higher than the ones derived from the more conservative data matrix. The taxonomic rate comparisons also exhibit somewhat higher rate discrepancies in relation to those shown in table 2, that is, the two protistan groups appear to have diverged at slightly higher rates in relation to the metazoan taxa. Thus, it can be concluded that adding 128 highly variable

**Table 1**  
**Absolute Divergence Rates Within Bacillariophyceae, Dasycladales (Chlorophyta), Actinistia-Tetrapoda, Echinodermata, and Mollusca**

TAXA	DISTANCES			TIME	ABSOLUTE RATES		
	K	MI	K'		K	MI	K'
Bacillariophyceae							
Nitzsch.-Thalass.	1.5 (0.4)	1.5	8.8	80	0.019 (0.005)	0.019	0.110
Rhaphon.-Thalass.	1.4 (0.4)	1.4	8.1	80	0.018 (0.005)	0.018	0.101
Rhaphon.-Nitzsch.	2.0 (0.5)	2.0	11.9	80	0.025 (0.006)	0.025	0.150
Rhizoso.-Stephan.	3.4 (0.7)	3.4	21.7	180	0.019 (0.004)	0.019	0.121
Rhizoso.-Rhaphon.	3.7 (0.8)	3.7	23.4	200	0.018 (0.004)	0.018	0.117
Rhizoso.-Thalass. . . .	3.3 (0.7)	3.3	20.8	200	*0.016 (0.004)	0.016	0.104
Rhizoso.-Nitzsch.	3.5 (0.7)	3.6	22.5	200	0.018 (0.004)	0.018	0.112
Stephan.-Rhaphon.	3.4 (0.7)	3.4	21.6	200	0.017 (0.004)	0.017	0.108
Stephan.-Thalass.	2.8 (0.7)	2.8	17.4	200	*0.014 (0.004)	0.014	0.087
Stephan.-Nitzsch.	3.4 (0.7)	3.4	21.6	200	0.017 (0.004)	0.017	0.108
Dasycladales							
Acetabu.-Poly.pe.	1.4 (0.5)	1.4	5.6	36	0.039 (0.014)	0.039	0.155
Acetabu.-Poly.pa.	3.7 (0.8)	3.7	15.7	135	0.027 (0.006)	0.027	0.116
Poly.pa.-Poly.pe.	4.5 (0.8)	4.5	19.7	135	0.033 (0.006)	0.033	0.146
Chloroc.-Acetabu.	5.4 (0.9)	5.4	23.7	290	0.019 (0.003)	0.019	0.082
Chloroc.-Poly.pe.	6.1 (0.9)	6.1	27.6	290	0.021 (0.003)	0.021	0.095
Chloroc.-Poly.pa.	4.9 (0.9)	4.9	21.4	290	0.017 (0.003)	0.017	0.074
Actinistia-Tetrapoda							
Mus mus.-Oryctol.	0.1 (0.1)	0.1	0.9	60	0.002 (0.002)	0.002	0.015
Heterod.-Alligat.	1.1 (0.4)	1.1	7.6	260	0.004 (0.002)	0.004	0.029
Heterod.-Mus mus.	3.1 (0.7)	3.1	24.0	310	0.010 (0.002)	0.010	0.077
Heterod.-Oryctol.	3.0 (0.7)	3.0	22.6	310	0.010 (0.002)	0.010	0.073
Alligat.-Mus mus.	2.6 (0.6)	2.6	19.4	310	0.008 (0.002)	0.008	0.062
Alligat.-Oryctol.	2.4 (0.6)	2.4	18.3	310	0.008 (0.002)	0.008	0.059
Latimer.-Mus mus.	3.7 (0.8)	3.7	29.4	405	0.009 (0.002)	0.009	0.072
Latimer.-Oryctol.	3.6 (0.7)	3.6	28.1	405	0.009 (0.002)	0.009	0.069
Latimer.-Alligat.	2.7 (0.6)	2.7	20.3	405	0.007 (0.001)	0.007	0.050
Latimer.-Heterod.	3.5 (0.9)	3.5	27.5	405	0.009 (0.002)	0.009	0.068
Echinodermata							
Strongy.-Asteria.	3.2 (0.7)	3.2	18.6	500	0.006 (0.002)	0.006	0.037
Ophiopl.-Strongy.	4.1 (0.8)	4.1	25.6	523	0.008 (0.001)	0.008	0.049
Ophiopl.-Asteria.	4.4 (0.8)	4.4	27.7	523	0.008 (0.002)	0.008	0.053
Antedon-Strongy.	3.3 (0.7)	3.3	19.3	555	0.006 (0.001)	0.006	0.035
Antedon-Asteria.	3.9 (0.8)	3.9	23.9	555	0.007 (0.001)	0.007	0.043
Antedon-Ophiopl.	4.5 (0.8)	4.5	27.7	555	0.008 (0.002)	0.008	0.050
Mollusca							
Argopect.-Limicol.	3.5 (0.7)	3.5	21.2	600	0.006 (0.001)	0.006	0.035
Limicol.-Acantho.	3.4 (0.7)	3.4	20.4	600	0.006 (0.001)	0.006	0.034

NOTE.—The distance values K and K' represent number of nucleotide substitutions/100 nucleotides. K' is estimated based only on sites that are free to vary, whereas K is derived from all the sites considered in the study. MI = maximum likelihood estimates. The standard errors of the K estimates are shown within parentheses. Time (divergence time) = millions of years. Rate = number of nucleotide substitutions/100 nucleotides/million years. \* = overlapping rates with respect to some of the metazoan rates. The divergence times for Bacillariophyceae, Dasycladales, Actinistia-Tetrapoda, Echinodermata, and Mollusca were obtained from Tappan (1980), Berger and Kaever (1992), Benton (1990), Smith (1988), and Runnegar and Pojeta (1974), respectively.

sites (1,002 sites) had no major impact on the results of the taxonomic rate comparisons.

The neighbor-joining method (Saitou and Nei 1987) yielded the phylogenetic tree that is displayed in figure 1 from the Kimura data matrix consisting of 874 nucleotides. The branching order of some taxa within diatoms, vertebrates and echinoderms as well as the phylogenetic relationship between Mollusca, Echinodermata, and Actinistia-Tetrapoda are unconventional.

## Discussion

### Phylogenetic Tree

The dendrogram in figure 1 displays some unconventional evolutionary relationships, that is, the phylo-

genetic positions of *L. chalmunae*, *R. belgica*, *L. kambeul* and the relative branching order of the lineages within Echinodermata as well as the sister-group relationship of Actinistia-Tetrapoda. A bootstrap analysis (1,000 iterations) indicates relatively low support (bootstrap proportions ranging from 39% to 75%) for the unexpected phylogenetic relationships, whereas the traditional evolutionary affinities receive values ranging from 80% to 100%. Thus, for the purpose of rate analysis, it was assumed that *L. chalmunae*, *R. belgica*, *L. kambeul* forms a sister-taxon relationship with the tetrapods, (*T. nitzschoides*, *N. apiculata*) and (*A. irradians*, *A. japonica*), respectively. Within Echinodermata, the branching order is presumed to be the following: (*A.*

**Table 2**  
**Summary of the Taxonomic Rate Comparisons**

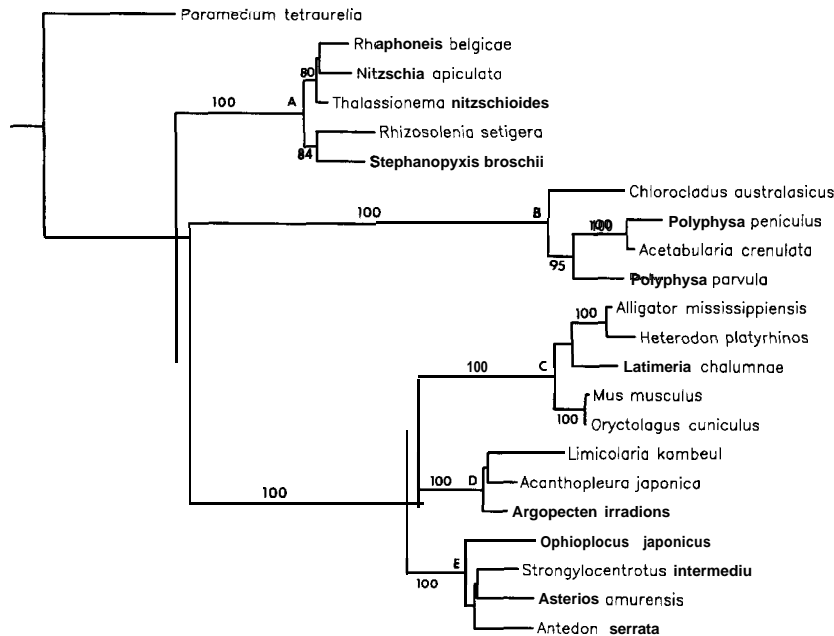
TAXA	AVERAGE TAXONOMIC RATE RATIO	
	K (MI)	K'
Bacillariophyceae/Actinistia-Tetrapoda.	0.018 (0.006)/0.008 (0.002) = 2.2	0.112/0.057 = 2.0
Bacillariophyceae/Echinodermata	0.018 (0.006)/0.007 (0.002) = 2.6	0.112/0.044 = 2.5
Bacillariophyceae/Mollusca	0.018 (0.006)/0.006 (0.001) = 3.0	0.112/0.034 = 3.3
Dasycladales/Actinistia-Tetrapoda.	0.026 (0.014)/0.008 (0.002) = 3.2	0.111/0.057 = 1.9
Dasycladales/Echinodermata	0.026 (0.014)/0.007 (0.002) = 3.7	0.111/0.044 = 2.5
Dasycladales/Mollusca	0.026 (0.014)/0.006 (0.001) = 4.3	0.111/0.034 = 3.3
Dasycladales/Bacillariophyceae.	0.026 (0.014)/0.018 (0.006) = 1.4	0.111/0.112 = 1.0
Actinistia-Tetrapoda/Echinodermata	0.008 (0.002)/0.007 (0.002) = 1.1	0.057/0.044 = 1.3
Actinistia-Tetrapoda/Mollusca	0.008 (0.002)/0.006 (0.001) = 1.3	0.057/0.034 = 1.7
Echinodermata/Mollusca	0.007 (0.002)/0.006 (0.001) = 1.3	0.044/0.034 = 1.3
Protista/Metazoa	0.021/0.007 = 3.0	0.112/0.050 = 2.2

NOTE.—The absolute rate values derived from K and K' represent the average number of nucleotide substitutions/100 nucleotides/million years. K' is estimated based only on sites that are free to vary, whereas K is derived from all the sites considered in the study. MI = maximum likelihood estimates (same values as K). The maximum standard error ranges observed for the K estimates of each taxon are shown within parentheses.

*serrata* (*O. japonicus* (*S. intermedius*, *A. amurensis* (see Wada and Satoh 1994).

By examining the relative lengths of the internodes and terminal branches in the phylogenetic tree presented in figure 1, one can conclude either that the rRNA gene evolved much slower in Bacillariophyceae than in the other taxonomic groups or that there should not be a substantial rate difference between lineages leading to the animal and Dasycladales taxa. Such inferences are inconsistent with the results obtained from divergence dates. In this study, nucleotide substitution rates were compared between the monophyletic taxa seen in figure 1. Consequently, the internodes leading to these groups

are not and should not be included in the rate comparisons because the internodes are not part of the considered monophyletic taxa. The taxonomic units that were compared and are of interest in this analysis originated from the nodes ranging from A to E and not from the nodes that they have in common, that is, for instance, the one that unites Dasycladales and Metazoa (fig. 1). If one was concerned about nucleotide substitution rate variability between the (Dasycladales, Metazoa) group and Bacillariophyceae, it would be pertinent to include the long internodes from their common node (ancestor). Thus, if the long internodes are not part of the analysis and only the branch lengths and the relative divergence



**FIG. 1.**—A dendrogram derived by the neighbor joining procedure from corrected nucleotide substitutions estimated by the two-parameter method of Kimura (1980). Horizontal distances are proportional to evolutionary distances (K), whereas the vertical distances are arbitrary. The scale bar corresponds to 2 substitutions per 100 nucleotides. Nodes A, B, C, D, and E represent Bacillariophyceae, Dasycladales (Chlorophyta), Actinistia-Tetrapoda, Mollusca, and Echinodermata, respectively. Bootstrap proportions are indicated on those internodes which received relatively strong support (80%–100%).

times used within each group are considered, the discrepancy between the rate estimates and the phylogenetic tree structure disappears.

The branch lengths within each monophyletic taxon also indicate that there is rate variability between the lineages. This shows that rRNA nucleotide substitution rates are lineage specific within each group, but the within-taxon variability appears to be, on average, less than between-group variability when one compares the protistan taxa (Dasycladales or/and Bacillariophyceae) with the metazoan taxa (table 2).

The results of the rate analysis suggest that a slowdown of the rRNA substitution rates has taken place within Acintistia-Tetrapoda, Echinodermata, and Mollusca relative to that in Bacillariophyceae and Dasycladales. This is supported by nonoverlapping rate estimates between most of the protistan and metazoan taxa (tables 1 and 2).

### Divergence Times

A major problem with the fossil record in evolutionary rate studies is that divergence dates between different lineages can be either over- or underestimated. This phenomenon gives rise to spurious nucleotide substitution rates. In fact, the approximately threefold higher molecular divergence rate (K and Ml) observed in Dasycladales and Bacillariophyceae in relation to that in the metazoan groups could be explained by the divergence dates being highly underestimated for the protistan lineages. The important question is the degree divergence times have to be underestimated in order to eliminate the observed discrepancies between the compared taxa. This problem can be addressed by computing the divergence times between the investigated diatom and the Dasycladales lineages based on the average substitution rate in, for instance, the Actinistia-Tetrapoda group, that is, what the evolutionary separation times between the protistan lineages would be if they evolved at the same rate as the vertebrate lineages. Such a calculation assumes that the paleontological record and the inferred average substitution rate within the Actinistia-Tetrapoda group are fairly accurate and that there are no major discrepancies in the rRNA evolutionary rates between the protistan and metazoan taxa (a universal rRNA clock). The first two assumptions appear to be rather reasonable, whereas the third one is questionable but can be used to elucidate whether the underestimations necessary for the universal molecular clock to be valid are reasonable and in general agreement with the fossil record.

If one considers the *R. belgica*/*T. nitzschioides*-*N. apiculata*, *R. belgica*-*T. nitzschioides*, and *R. setigera*-*S. broschii* divergences and assumes that these lineages evolved at the same average rate as the considered vertebrates, their divergence times are  $2.0/0.008 = 250$ ,  $1.5/0.008 = 188$ ,  $1.4/0.008 = 175$ , and  $3.4/0.008 = 425$  million years ago, respectively (see tables 1 and 2 for values). With regard to the Dasycladales lineages, the corresponding values are 175 million years for *A. crenulata*-*P. peniculus*, 462 million years for *A. crenulata*-*P. parvula*, and 675 million years for *A. crenulata*-*C.*

*australasicus* (see tables 1 and 2 for values). Thus, the results of these estimations (predictions) show that the divergence dates of 80 and 180 million years within diatoms must be underestimated by about 95-245 million years for the universal rRNA clock to be valid. These values are even higher for Dasycladales, that is, ranging from 139 to 385 million years. If all these predicted times were correct, one would have to assume that the considered diatom and Dasycladales lineages occurred at times when, according to the fossil record, no representatives of these taxa existed (see table 1 for values). Even if the considered diatom and Dasycladales divergence events (80 and 180 Myr/36, 135, and 290 Myr) were underestimated by, for instance, 20 million years, the rRNA rate discrepancies between the protistan taxa and Actinistia-Tetrapoda would still differ by a factor of about 2.

This argument can also be turned around by assuming that the protistan divergence times are approximately correct and the metazoan fossil record is overestimated, that is, the separation times should be more recent than indicated in the literature. To achieve similar absolute rates (taxonomic rRNA clock) as the diatom and Dasycladales lineages, one has to presume that the metazoan divergence times are overestimated by about 120-200 million years. For instance, the alligator-snake divergence should have taken place some time in the Late Cretaceous, which is unlikely because of the fact that these lineages clearly existed before that time (Benton 1990).

The evidence for taxonomic rate differences is, of course, made weaker when applying a similar analysis to the twofold discrepancy in the K'-derived absolute divergence rates between protista and metazoa. Thus, the K'-derived rates are more sensitive to uncertainties in the fossil record than those derived from Kimura corrections.

### Factors Biasing Substitution Rates

Variation in taxonomic divergence rates derived from, for instance, Kimura corrections for multiple hits (K) are often assumed to reflect differences in mutation rates (Palumbi 1989). However, factors such as taxonomic variation in the proportion of positions free to vary, varying transition-transversion ratios, and different nucleotide compositions among lineages have biasing effects on the distance estimates and, consequently, on the number of substitutions per unit time (Palumbi 1989; see also Galtier and Gouy 1995). In this study, particular attention has been given to the proportion of positions free to vary within each taxonomic group because of the strong effect it may have on DNA divergence rates (see Palumbi 1989 for in depth discussion). With regard to the other biasing factors, the results indicate that nucleotide composition does not seem to be a major determinant of the estimated Kimura corrections (K) nor the transition-transversion ratio because it is relatively small (3.5). A large transition/transversion bias "slows down" the divergence rates as it approaches maximum nucleotide divergence (Palumbi 1989).

According to Kimura and Ohta (1974), the rate of molecular evolution is expected to be approximately clocklike, provided that the function and the secondary/tertiary structure of a molecule has remained unchanged throughout the taxonomic spectrum (time). There is now evidence for the secondary/tertiary structure of ribosomal RNA having changed over evolutionary time (Hori and Osawa 1987), and, consequently, differences in absolute divergence rates between taxa can be expected. The idea of varying lineage-specific functional constraints of 18s rRNA is also supported by the inferred taxonomic differences in the proportion of nucleotide positions free to vary ( $x$ ) (see tables 1 and 2). When the proportion of sites free to vary is relatively low, which is the case here, evolutionary distances ( $K$ ) are severely underestimated (Palumbi 1989), but this does not necessarily imply that proportional relationships between the values have to change much. Thus, the important question for this comparative study was how the taxonomic discrepancies in the  $K$ -derived absolute divergence rates are affected by taking lineage-specific variation in proportion of sites free to vary into consideration. By examining tables 1 and 2, it becomes apparent that the taxonomic molecular rate differences derived from  $K'$  estimates are not as high as those obtained from the Kimura corrections. Thus, by adjusting  $K$  by the proportion of variable sites, the overall differences in divergence rates between the protistan and metazoan lineages become lower (table 2). This equalizing effect is particularly evident between Bacillariophyceae and the Dasycladales lineages (table 2). The implication of the results are that, despite  $K$  being corrected by  $x$ , an approximately twofold absolute rate discrepancy still remains and can, thus, be attributable to taxonomic differences in the fixation rate of mutations. An alternative interpretation is that the approximately threefold discrepancy in the 18s rRNA evolutionary rate (derived from  $K$  estimates) between the protistan and metazoan groups can be explained, in part, by the considered taxa displaying different fractions of nucleotide sites free to vary.

## Conclusion

The results of the analysis indicate an approximate two- to threefold higher absolute substitution rate within two protistan taxa than within the three metazoan groups. The higher value can be interpreted as being a result, in part, of the considered taxa differing in the fractions of nucleotide sites free to vary, whereas the lower figure most likely expresses the actual taxonomic differences in the fixation rate of mutations in the small-subunit rRNA. Under- and overestimations of divergence times between the considered protistan and metazoan lineages have to be substantial in order to render the threefold difference in the absolute nucleotide substitution rates similar, whereas the twofold rate difference is more sensitive to uncertainties in paleontological information.

## Acknowledgments

I thank three anonymous reviewers for providing valuable comments that improved the content of this article. The data matrices are available from the author on request.

## LITERATURE CITED

- BENTON, M. J. 1990. Phylogeny of the major tetrapod groups: morphological data and divergence dates. *J. Mol. Evol.* 30: 409-424.
- BERGER, S., and M. J. KAEVER. 1992. *Dasycladales*: an illustrated monograph of a fascinating algal order. Thieme, Stuttgart.
- BHATTACHARYA, D., L. MEDLIN, I? O. WAINRIGHT, E. ARIZTIA, C. BIBEAU, S. STICKEL, and M. SOGIN. 1992. Algae containing chlorophylls a + c are paraphyletic: molecular evolutionary analysis of the Chromophyta. *Evolution* 46: 1801-1817.
- BRITEN, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. *Science* 231: 1393-1398.
- CANTATORE, F!, M. ROBERTI, G. PESOLE, A. LUDOVICO, F. MILLELLA, M. N. GADALETA, and C. SACCONI. 1994. *J. Mol. Evol.* 39:589-597.
- CLEGG, M. T., and S. J. O'BRIEN. 1990. Evolutionary analysis of cytochrome b sequences in some perciformes: evidence for a slower rate of evolution than in mammals. *J. Mol. Evol.* Wiley-Liss, New York.
- DESALLE, R., and A. R. TEMPLETON. 1988. Founder effects and the rate of mitochondrial DNA evolution of Hawaiian *Drosophila*. *Evolution* 42: 1076-1084.
- EASTEAL, S. 1990. The pattern of mammalian evolution and the relative rate of molecular evolution. *Genetics* 124: 165-173.
- FELSENSTEIN, J. 1993. PHYLIP (Phylogeny Inference Package), version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- GALTIER, N., and M. GOUY. 1995. Inferring phylogenies from sequences of unequal base compositions. *Proc. Natl. Acad. Sci. USA* 92:11317-11321.
- GIBBS, P., and A. DUGAICZYK. 1994. Reading the molecular clock from the decay of internal symmetry of a gene. *Proc. Natl. Acad. Sci. USA* 91:3413-3417.
- GILLESPIE, J. H. 1986. Rates of molecular evolution. *Ann. Rev. Ecol. Syst.* 17:637-665.
- . 1992. *The causes of molecular evolution*. Oxford University Press, New York.
- GOJOBORI, T., E. N. MORIYAMA, and M. KIMURA. 1990. Molecular clock of viral evolution, and the neutral theory. *Proc. Natl. Acad. Sci. USA* 87:10015-10018.
- GUTELL, R. R. 1994. Collection of small subunit 16S- and 16S-like ribosomal RNA structures: 1994. *Nucleic Acids Res.* 22:3502-3507.
- HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22:160-174.
- HEDGES, S. B., K. D. MÖBERG, and L. MAXSON. 1990. Tetrapod phylogeny inferred from 18S and 28S ribosomal RNA sequences and a review of the evidence for amniote relationships. *Mol. Biol. Evol.* 7:607-633.
- HIGGINS, D. G., and I? M. SHARP. 1989. Fast and sensitive multiple sequence alignments on a microcomputer. *Cabios* 5:151-153.
- HIGGINS, D. G., A. J. BLEASBY, and R. FUCHS. 1992. CLUSTAL V: improved software for multiple sequence alignment. *Cabios* 8: 189-191.

- HORI, H., and S. OSAWA. 1987. Origin and evolution of organisms as deduced from 5S ribosomal RNA sequences. *Mol. Biol. Evol.* **4**:445-472.
- JOUSE, A. I? 1978. Diatom biostratigraphy on the generic level. *Micropaleontology* **3**:316-26.
- KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111-120.
- KIMURA, M., and T. OHTA. 1974. On some principles governing molecular evolution. *Proc. Natl. Acad. Sci. USA* **71**: 2848-2852.
- LANGLEY, C. H., and W. M. FITCH. 1974. An examination of the constancy of the rate of molecular evolution. *J. Mol. Evol.* **3**:166-177.
- LI, W.-H., and D. GRAUER. 1991. *Fundamentals of molecular evolution*. Sinauer Associates, Sunderland, Massachusetts.
- LI, W.-H., M. TANIMURA, and P. M. SHARP. 1987. An evaluation of the molecular clock hypothesis using mammalian DNA sequences. *J. Mol. Evol.* **25**:330-342.
- MAISEY, J. G. 1988. Phylogeny of early vertebrate skeletal induction and ossification patterns. Pp. 1-30 in M. K. HECHT, B. WALLACE, and G. T. PRANCE, eds. *Evolutionary biology*. Vol. 22. Plenum Press, New York.
- NEI, M. 1987. *Molecular evolutionary genetics*. Columbia University Press, New York.
- OCHMAN, H., and A. C. WILSON. 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genome. *J. Mol. Evol.* **26**:74-86.
- O'UIGIN, C., and W.-H. LI. 1992. The molecular clock ticks regularly in murid rodents and hamsters. *J. Mol. Evol.* **35**: 377-384.
- OLSEN, J. L., W. T. STAM, S. BERGER, and D. MENZEL. 1994. 18S rDNA and evolution in the *Dasycladales* (Chlorophyta): modern living fossils. *J. Phycol.* **30**:729-744.
- PALUMBI, S. R. 1989. Rate of molecular evolution and the fraction of nucleotide positions free to vary. *J. Mol. Evol.* **29**: 180-187.
- PAMILO, I?, and N. BIANCHI. 1993. Evolution of the Zfx and Zfy genes: rates and interdependence between the genes. *Mol. Biol. Evol.* **10**:271-281.
- PHILIPPE, H., A. CHENUIL, and A. ADOUTTE. 1994. Can the Cambrian explosion be inferred through molecular phylogeny. *Development Suppl.* 15-25.
- PHILIPPE, H., U. SORHANNUS, A. BAROIN, R. PERASSO, F. GASSE, and A. ADOUTTE. 1994. Comparison of molecular and paleontological data in diatoms suggests a major gap in the fossil record. *J. Evol. Biol.* **7**:247-265.
- RAIRKAR, A., H. M. RUBINO, and R. E. LOCKARD. 1988. Revised primary structure of rabbit 18S ribosomal RNA. *Nucleic Acid Res.* **16**:311-313.
- RAYNAL, F., B. MICHOT, and J. P. BACHELLERIE. 1984. Complete nucleotide sequence of mouse 18S rRNA gene: comparison with other available homologs. *FEBS Lett.* **167**: 263-268.
- RUNNEGAR, B., and J. POJETA. 1974. Molluscan phylogeny: the paleontological viewpoint. *Science* **186**:311-317.
- SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**:406-425.
- SCHERER, S. 1990. The protein molecular clock: time for a reevaluation. Pp. 83-106 in M. K. HECHT, B. WALLACE, and R. J. MACINTYRE, eds. *Evolutionary biology*. Vol. 24. Plenum Press, New York.
- SHAPIRO, S. G. 1991. Uniformity in the nonsynonymous substitution rates of embryonic B-globin genes of several vertebrate species. *J. Mol. Evol.* **32**: 122-127.
- SHARP, P., and W.-H. LI. 1989. On the rate of DNA sequence evolution in *Drosophila*. *J. Mol. Evol.* **28**:398-402.
- SIMONSEN, R. 1979. The diatom system: ideas on phylogeny. *Bacillaria* **2**:9-71.
- SMITH, A. B. 1988. Fossil evidence for the relationship of extant echinoderm classes and their times of divergence. Pp. 85-97 in C. PAUL and A. B. SMITH, eds. *Echinoderm phylogeny and evolutionary biology*. Clarendon Press, Oxford.
- SOGIN, M. L., and H. J. ELWOOD. 1986. Primary structure of the *Paramecium tetraurelia* small-subunit rRNA coding region: phylogenetic relationships within the Ciliophora. *J. Mol. Evol.* **23**:53-60.
- SORHANNUS, U. 1994. Relative-rate tests versus paleontological divergence data for diatoms and vertebrates. *Acta Palaeontol. Polon.* **38**: 199-214.
- STOCK, D. W., K. D. MOBERG, G. S. WHITT, and L. R. MAXSON. 1991. Phylogenetic implications of the 18S ribosomal RNA sequence of the coelacanth *Latimeria chalumnae* (Smith). *Environ. Biol. Fishes* **32**:99-118.
- TAPPAN, H. 1980. *The paleobiology of plant protists*. W. H. Freeman and Co., San Francisco.
- WADA, H., and N. SATOH. 1994. Phylogenetic relationships among extant classes of echinoderms, as inferred from sequences of 18S rDNA, coincide with relationships deduced from the fossil record. *J. Mol. Evol.* **38**:41-49.
- WILSON, A. C., S. S. CARLSON, and T. J. WHITE. 1977. Biochemical evolution. *Ann. Rev. Biochem.* **46**:573-639.
- WU, C. I., and W.-H. LI. 1985. Evidence for higher rates of nucleotide substitution in rodents than in man. *Proc. Natl. Acad. Sci. USA* **82**:1741-1745.
- ZUCKERKANDL, E., and L. PAULING. 1965. Evolutionary divergence and convergence in proteins. Pp. 97-166 in V. BRYSON and H. VOGEL, eds. *Evolving genes and proteins*. Academic Press, New York.

TAKASHI GOJOBORI, reviewing editor

Accepted May 24, 1996