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Phil. Trans. R. Soc. Lond. B 1998 **353**, 1221-1237
doi: 10.1098/rstb.1998.0278

The Royal Society

The platypus is not a rodent: DNA hybridization, amniote phylogeny and the palimpsest theory

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We present DNA-hybridization data on 21 amniotes and two anurans showing that discrimination is obtained among most of these at the class and lower levels. Trees generated from these data largely agree with conventional views, for example in not associating birds and mammals. However, the sister relationships found here of the monotremes to marsupials, and of turtles to the alligator, are surprising results which are nonetheless consistent with the results of some other studies. The Marsupionta hypothesis of Gregory is reviewed, as are opinions about the placement of chelonians. Anatomical and reproductive data considered by Gregory do not unequivocally preclude a marsupial–monotreme special relationship, and there is other recent evidence for placing turtles within the Diapsida. We conclude that the evidential meaning of the molecular data is as shown in the trees, but that the topologies may be influenced by a base-compositional bias producing a seemingly slow evolutionary rate in monotremes, or by algorithmic artefacts (in the case of turtles as well).

Keywords: Chelonia; Crocodilia; Eutheria; Marsupialia; Marsupionta; molecular evolution

> 'Unyielding factualists have so set the style in taxonomy and morphology that, if their assertions were accepted, hardly any known type of animal could possibly have been derived even from any known past group.'
>
> Gregory (1947, p. 34)

1. INTRODUCTION

In connection with a DNA-hybridization investigation into the phylogeny of several avian orders, which successfully used the alligator as an out-group (Bleiweiss *et al.* 1995), we conducted some additional hybridization experiments using lizards and mammals in which we found that all four of these amniote groups could be discriminated. Encouraged by these results, we decided to explore the ability of the hybridization technique to distinguish the deeper branches of tetrapod phylogeny, and perhaps the limits of the technique, by carrying out a series of further hybridizations among representatives of the amniote classes and two out-group anurans. As shown herein, we found that discrimination was maintained over the entire group, with recovered relationships that were, in general, at least plausible. Thus, frogs were clearly separated from the amniotes, predicted terminal sister-pairs were supported at high bootstrap percentages, and there was no evidence of a bird–mammal clade. At the same time, we observed two notable departures from expectation: that turtles were placed among diapsids, close to the alligator exclusive of birds; and that the representative monotreme was sister to the marsupial instead of to a combined marsupial–placental group. While turtle relationships continue to be debated, 'everyone knows' that the latter result cannot be correct.

The orthodox view of the phylogeny of mammals regards monotremes (the living 'Prototheria') as the sister-group to marsupials + placentals (together, the extant 'Theria'). Evidence that the therians are more closely related to each other than to monotremes includes a wide range of anatomical and physiological features (Lillegraven 1975; Vaughn 1986; Carroll 1988; Starck 1995; Rougier *et al.* 1996). A minority opinion, that monotremes and certain marsupials might be each other's nearest relatives, was promoted by Gregory (1934, 1947, 1951). Gregory's inference of a monotreme–marsupial taxon resulted from application of his 'palimpsest' theory of phylogenetic inference (see also Camp 1923; Broom 1924; Colbert & Mook 1951). Noting resemblances between living monotremes and the Australasian diprotodontian marsupials, Gregory proposed that monotremes were derived relatively recently from the latter, invoking truncation of development as one possible explanation for the monotreme habit of egg-laying and some other features. His hypothesis was later endorsed (in a more general form, with monotremes sister to all marsupials) by Kühne (1973, 1977) on the basis of supposedly similar tooth-replacement patterns in marsupials and monotremes. Monotreme fossils discovered since Gregory's papers were written (Archer *et al.* 1985, 1993; Pascual *et al.* 1992; Flannery *et al.* 1995) are up to twice the inferred age of diprotodontians (Kirsch *et al.* 1997; Springer *et al.* 1997), and thus falsify the specific point of divergence argued by Gregory, but not a monotreme–marsupial sister-group relationship. It is such a more general affinity that is supported by our own results.

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Along with the monotreme–marsupial clade indicated by our data, our most surprising finding is the placement of the turtles within the Diapsida as sister-group to the alligator. Turtles have generally been thought to be among the earliest branches of the amniote tree (e.g. Gaffney 1980), but whereas a proposed association of turtles with diapsids as a whole is not entirely without precedent in the literature (Goodrich 1916), the suggestion of special relationship to crocodilians has, to our knowledge, only rarely been mooted (Bishop & Friday 1987; Goodman *et al*. 1987; Hedges *et al*. 1990). Because of these two unlikely relationships (turtles with crocodilians and monotremes with marsupials), we hesitated to present our results, yet for two general reasons we believe it is worth considering them now.

First, there is additional recent evidence that calls the orthodox views into question, or is at least consistent with our results. New anatomical analyses (Rieppel & deBraga 1996) question the received views of turtle relationships, and place chelonians amongst the Diapsida. In addition, Janke *et al*. (1996, 1997) present trees based on complete coding sequences of the mitochondria indicating that marsupials and the platypus (a monotreme) are more closely related to each other than the former are to placentals, a result the authors interpret as evidence for a monotreme–marsupial clade (similar to the Marsupionta of Gregory (1947) in content but not structure).

The second reason relates to possible algorithmic, sampling or biochemical biases, which require serious consideration in the larger context of the interpretation of any molecular systematic results. One of these biases is the familiar long-branch attraction problem (Felsenstein 1978), a difficulty exacerbated by the rather few exemplars of major taxa that have been compared for relationship in many studies (e.g. D'Erchia *et al*. 1996): species-poor trees may have strong support for nonetheless untrue relationships, and use of different exemplars of the same taxa may produce inconsistent topologies (Philippe & Douzery 1994). Another type of bias, only recently appreciated, may result from base-compositional effects (e.g. Loomis & Smith 1992; Pettigrew 1994).

Thus, in this paper we take a fresh look at the evidence and arguments that have been made for both turtle and, especially, monotreme affinities, and consider also possible chemical or computational explanations for their respective associations in our trees. However, the following is not intended as (nor could it be) an exhaustive treatment. Rather, at least as it concerns the monotremes, it is a sympathetic consideration of what might otherwise be considered merely an historic curiosity, prompted by the evidential (and possibly true) meaning of some new molecular data.

2. METHODS

We examined DNA extracts from the 11 amniotes and two anurans listed in table 1, labelling all of these but the python to provide a 12 × 13 matrix of pairwise comparisons. Procedures for extracting, fractionating, radiolabelling and hybridizing the samples were as reported previously (Kirsch *et al*. 1990; Bleiweiss *et al*. 1994). Distances among the taxa were indexed as NPHs (differences in percentages of hybridization, normalized against the homologous hybrids for each label).

A phylogenetic tree was generated from the data of table 1 (after symmetrization by the method of Sarich & Cronin (1976) and subsequent reflection of the distances to *Python regius*) by the FITCH program for generating additive trees in Felsenstein's (1993) PHYLIP package, version 3.5c, varying the input order 100 times and using the subreplicate and global branch-swapping options. The Cavalli-Sforza & Edwards (1967) least-squares option ($p=0$) was also used, as the correlation of standard deviations with distance ($r=0.15$) was not significant.

The tree was validated both by bootstrapping on the replicate measurements (Krajewski & Dickerman 1990) and by jackknifing on taxa (Lapointe *et al*. 1994). For the bootstrap, each pseudoreplicate matrix was separately symmetrized and missing reciprocals then reflected. We employed the jackknife for weighted trees of Lapointe *et al*. (1994), performing all possible single and multiple deletions of taxa (7813) and expressing the result as a least-squares tree calculated from the average pathlengths observed over all jackknife pseudoreplicates, while also noting discrepancies in the 'range consensus' (the strict consensus of trees based on the minimum and maximum pathlengths recovered).

To test whether the results from the 12 × 13 matrix might be due to sparse taxonomic sampling among mammals, we 'sutured' the completed data of table 1 with information on nine additional mammals from the 21-taxon set of comparisons reported in Kirsch *et al*. (1997). Three labels (of *Chaetophractus villosus*, *Didelphis virginiana*, and *Tachyglossus aculeatus*) were held in common between these added experiments and the 12 × 13 matrix, and we estimated the missing cells (37%) in the sutured 22-taxon matrix by the additive-reconstruction method of Landry *et al*. (1996). The resulting filled matrix was then used to generate a FITCH tree, which was validated by the jackknife. Because in this instance it was computationally impractical to carry out all possible deletions (*ca*. 4.2 million), we employed the alternative strategies of performing 1000 random deletions sampled proportionately, and of successive deletions of all single, all double, etc., deletions until the average-consensus topology stabilized (see Lapointe *et al*. (1994) for rationalization of these sampling strategies).

Finally, we added to the sutured matrix some one-way comparisons involving the platypus (*Ornithorhynchus anatinus*) as driver, with labelled *Procyon lotor*, *Tachyglossus* and five marsupials, and calculated a final FITCH tree of all 23 taxa, again after estimating missing cells.

3. RESULTS

Table 1 presents the data for the 12 × 13 matrix newly generated for this paper. Symmetrization was based on the scalar multipliers listed at the feet of columns, and missing reciprocals (the absent *Python* column) were then obtained by reflection. Data from Kirsch *et al*. (1997) included in the sutured matrix of 22 species are presented as T50Hs in table 2 of that paper, but unconverted NPHs were used in the present study. Values for the three common labels were weighted averages of the corresponding measurements in both contributing tables. The previously unpublished one-way comparisons ($n=1$ except as noted) with *Ornithorhynchus* as driver were: from

Table 1. *DNA-hybridization distances among 11 amniote and two anuran species* (*common names given in parentheses*), *indexed as* Δ*NPHs* (*number of hybrids* = *481; average replicates per cell* = *3.1*)

(Columns are labels, identified by first three letters of genus names, given in rows. First rows of cells give average Δs; second rows provide standard deviations (s.d.) and numbers of replicate measurements, separated by slashes. Average s.d. = 4.02; correlation of s.d. with distance = 0.15. Corrections at feet of columns are scalar multipliers (row:column ratios) used to correct data for asymmetry by method of Sarich & Cronin (1976); iterations (i.e. multiplication of column values followed by recalculation of row:column ratios) were continued until ratios reached unity. Distances from unlabelled *Python regius* were then inferred by reflection from known (symmetrized) reciprocals. Average table-wide asymmetry before correction (measured cells only), 8.19%; after correction, 2.75%.)

	Buf	*Xen*	*Did*	*Cha*	*Tac*	*Gal*	*Bub*	*All*	*Chr*	*Che*	*Cro*	*Tha*
Bufo marinus	0	54.73	56.39	52.67	44.13	73.40	68.50	59.70	62.95	54.93	45.63	53.23
(cane toad)	1.10/3	6.11/4	1.06/2	5.03/3	1.89/4	1.70/2	3.82/2	7.53/4	2.47/2	4.78/4	5.18/4	4.18/3
Xenopus laevis	49.40	0	55.94	54.23	53.53	68.10	68.13	59.50	63.50	58.03	48.85	55.65
(clawed frog)	6.22/2	1.33/4	na/1	2.78/3	8.30/4	na/1	8.60/3	2.69/2	na/1	2.89/4	3.13/4	1.63/2
Didelphis virginiana	56.33	61.90	0	56.45	42.38	72.90	73.50	67.38	67.13	60.88	48.93	61.03
(Virginia opossum)	2.42/3	1.86/4	7.17/7	4.03/2	3.20/4	2.40/2	8.01/3	3.29/4	3.22/3	1.54/4	5.12/4	2.35/3
Chaetophractus villosus	52.70	58.72	49.84	0	44.33	68.50	67.35	60.67	63.10	59.80	45.83	57.03
(hairy armadillo)	2.28/4	2.54/4	2.69/2	2.10/3	6.07/3	na/1	7.85/2	3.89/3	0.99/2	5.12/4	2.01/4	0.55/3
Tachyglossus aculeatus	48.97	60.80	42.54	53.63	0	75.25	63.20	59.93	59.20	59.15	45.12	56.07
(short-nosed echidna)	1.38/3	2.28/4	7.92/2	2.31/3	1.07/4	6.14/4	4.19/3	6.99/3	3.48/3	3.78/4	3.90/4	6.58/4
Gallus gallus	44.20	57.27	51.34	54.05	41.70	0	39.45	61.53	56.07	52.35	44.35	55.85
(chicken)	1.56/2	3.05/3	5.11/3	8.27/2	2.85/4	0.65/6	3.53/4	3.17/3	1.46/3	1.37/4	4.73/4	7.99/2
Bubo virginianus	53.27	60.07	56.04	57.70	46.35	44.37	0	64.25	62.73	56.63	48.15	60.37
(horned owl)	7.12/3	8.89/4	na/1	8.26/4	6.50/4	3.72/4	0.70/3	5.02/2	4.24/3	2.56/4	2.76/4	3.74/4
Alligator mississippiensis	53.00	60.10	57.34	49.65	48.35	61.70	54.80	0	52.90	47.33	46.40	46.20
(American alligator)	na/1	1.78/3	8.63/4	1.06/2	4.60/2	na/1	na/1	1.43/5	0.99/2	1.27/3	1.41/2	1.70/2
Chrysemys picta	53.72	59.07	49.44	56.50	45.08	67.00	62.37	52.87	0	8.18	45.95	55.22
(painted turtle)	3.47/4	3.08/4	9.48/2	2.88/4	2.74/4	2.83/2	7.41/3	2.69/4	1.43/4	1.39/4	4.53/4	3.70/4
Chelydra serpentina	53.40	64.45	61.17	58.20	51.90	64.90	64.73	64.30	7.40	0	48.63	59.67
(snapping turtle)	0.42/2	13.71/4	2.50/4	0.14/2	2.61/3	1.91/4	6.77/3	5.45/3	11.52/3	7.56/4	1.01/4	3.90/3
Crotaphytus collaris	53.87	58.80	52.64	59.85	44.79	68.37	68.85	65.13	61.10	63.25	0	59.12
(collared lizard)	2.09/4	3.50/4	6.34/3	4.84/4	1.22/7	3.97/3	5.59/2	1.12/3	1.43/4	2.71/4	2.89/8	4.22/4
Thamnophis sirtalis	50.80	59.00	53.42	50.40	48.93	72.05	63.80	61.88	69.50	59.00	47.50	0
(garter snake)	3.65/4	14.85/2	7.46/4	2.78/3	17.53/3	3.75/2	8.20/2	4.24/4	0.28/2	3.25/2	0.42/2	0.00/2
Python regius	63.15	60.45	61.04	55.55	48.10	71.30	63.30	58.20	56.40	52.85	38.95	41.90
(ball python)	11.38/2	3.18/2	na/1	2.47/2	2.26/2	1.83/3	na/1	na/1	6.36/2	3.89/2	7.99/2	1.41/2
correction	1.099	0.969	1.141	1.041	1.220	0.758	0.878	0.853	0.888	1.033	1.273	1.027

labelled *Procyon*, 67.00%; from *Tachyglossus*, 8.40% (*n* = 2); from *Caenolestes fuliginosus*, 48.20%; from *Dasyurus hallucatus*, 67.10%; from *Dromiciops gliroides*, 55.00%; from *Echymipera clara*, 51.10%; and from *Phalanger orientalis*, 54.30% (mean of the five measurements with marsupial labels was 55.14%).

Figure 1 shows a tree based on the symmetrized and reflected table 1 data. All depicted relationships are in accord or consistent with received wisdom about amniote relationships (mammals are separated from all others, snakes and the lizard are united, etc.) except for the placement of turtles with the alligator (by a short, but nonetheless well-supported, internode) and the sister-group relation of the echidna with the marsupial (where the internode separating these two taxa from the placental is both well-supported and substantial). However, the range consensus reduces the topology to a pentatomy among amniotes consisting of *Chaetophractus*, *Tachyglossus* + *Didelphis*, *Crotaphytus collaris*, the two snakes, and a group of all others.

Figure 2 is the average-consensus jackknife tree obtained from the data of table 1 combined with those for additional mammals (from Kirsch *et al.* 1997), after estimation of missing pairs of comparisons, and as validated by 1000 random deletions. In the conservative range consensus, amniote relationships collapse to a hexatomy: marsupials + the echidna, placental mammals, *Crotaphytus*, snakes, turtles + *Alligator mississippiensis*, and birds. Nevertheless, despite the inclusion of additional marsupials and placentals in figure 2, the echidna remains sister to the marsupials, and other amniote relations are the same as in figure 1 except for the irresolution of birds with respect to the clade of turtles with *Alligator*. The jackknife average-consensus trees of single, double and triple deletions of taxa were identical in topology to figure 2; given this stable arrangement, we did not proceed with further deletions. Moreover, owing to the large number of estimated cells (37%), it did not seem useful to attempt a bootstrap on the sutured matrix. The dashed line in figure 2 shows the position of (and length of the branch bearing) the

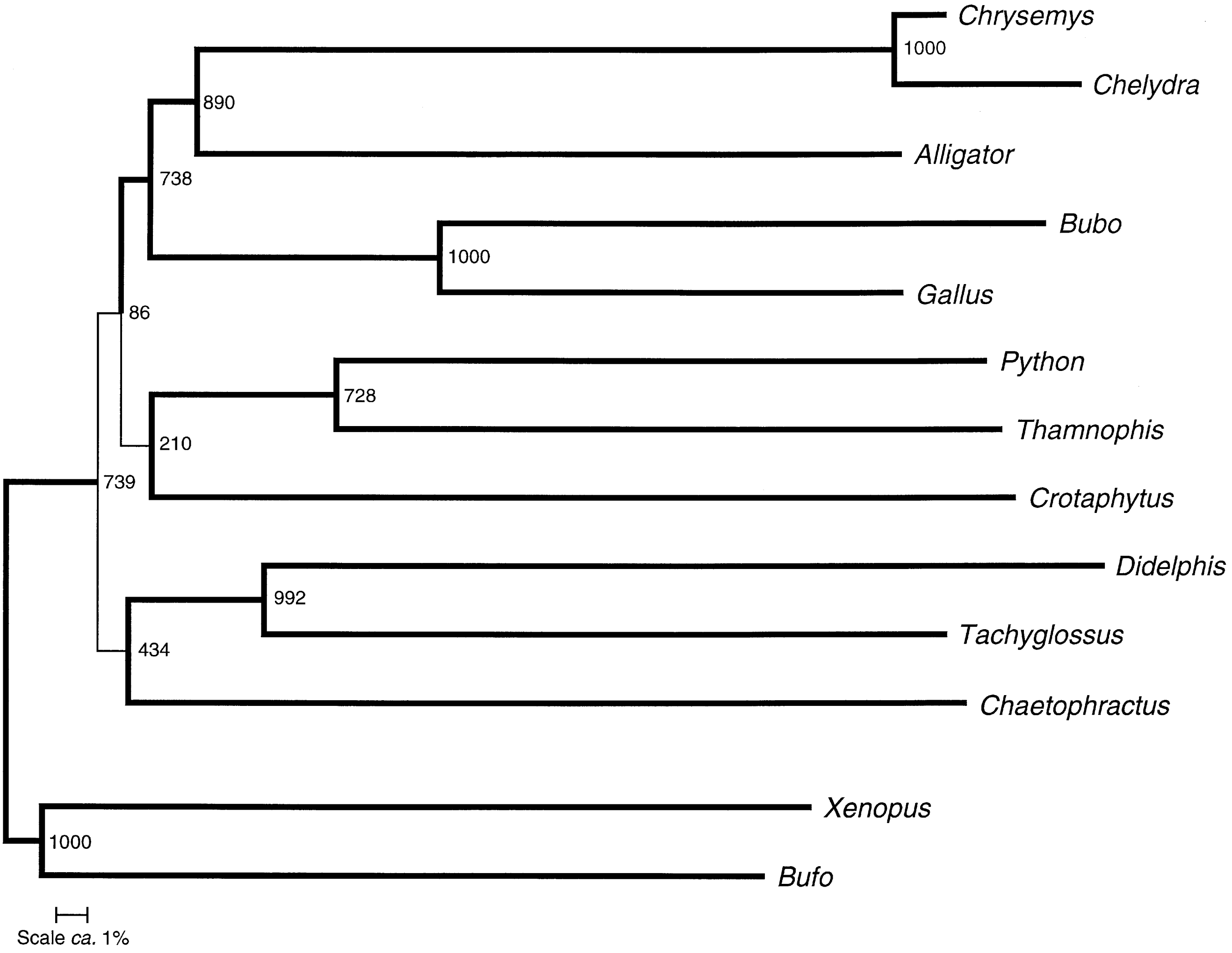

Figure 1. FITCH tree of relationships among 11 amniotes and two anurans, based on the symmetrized and reflected data of table 1. Topology and branch lengths shown are those of the average-consensus tree calculated from the mean pathlengths observed over all possible single- and multiple-deletion jackknife pseudoreplicate trees (7813). Discrepancies in trees from the range consensus of the minimum and maximum pathlengths are shown as thin lines emanating from nodes not supported in that consensus. Numbers at nodes are bootstrap pseudoreplicates (out of 1000) supporting these associations. The non-jackknifed but bootstrapped FITCH tree differed from figure 1 in placing the lizard, *Crotaphytus collaris*, with mammals in 222 of 1000 bootstrap trees.

platypus when data involving this species were added to the matrix and another tree calculated.

4. DISCUSSION

(a) *Data and trees*

Conventional wisdom (Hillis *et al.* 1996) holds that the technique of DNA hybridization is useful only over a relatively narrow range of divergence times, from about 5 to 50 million years (Ma) (in part because of the foreshortening of distances at or near criterion (incubation) temperature (Sheldon & Bledsoe 1989; Werman *et al.* 1996)), whether distances are indexed by the mode, the median or the median adjusted for per cent hybridization. The only index of thermal stability even potentially applicable below criterion temperature is the normalized per cent hybridization, which is subject to a great deal of experimental error (Bleiweiss & Kirsch 1993) and has therefore been little used. Kirsch *et al.* (1991) and Bleiweiss *et al.* (1995), however, found NPH useful in discriminating among marsupials and birds, respectively; and Kirsch *et al.* (1995) and Kirsch & Pettigrew (1998) obtained nearly identical topological results from modes and NPHs for their respective studies of opossums and bats. Because the NPH therefore appears to be a usable index despite its high variance, and the divergences we wished to estimate were very deep, we employed NPH in these experiments. In fact, our results demonstrate that NPH provides discrimination among taxa that diverged over 360 Ma before present (BP) (Benton 1990).

However, the most persuasive evidence that NPH is useful for our purposes is that figure 1 provides a picture of anuran and amniote-class interrelationships that is largely in accord with expectation: the two out-group frogs are united at a high bootstrap percentage, and are distinctly separated from the other taxa; all of the reptiles and the two birds are grouped in opposition to the mammals; and the only surprising non-mammalian association is of the alligator with the two turtles. On the other hand, judging by the bootstrap and conservative range

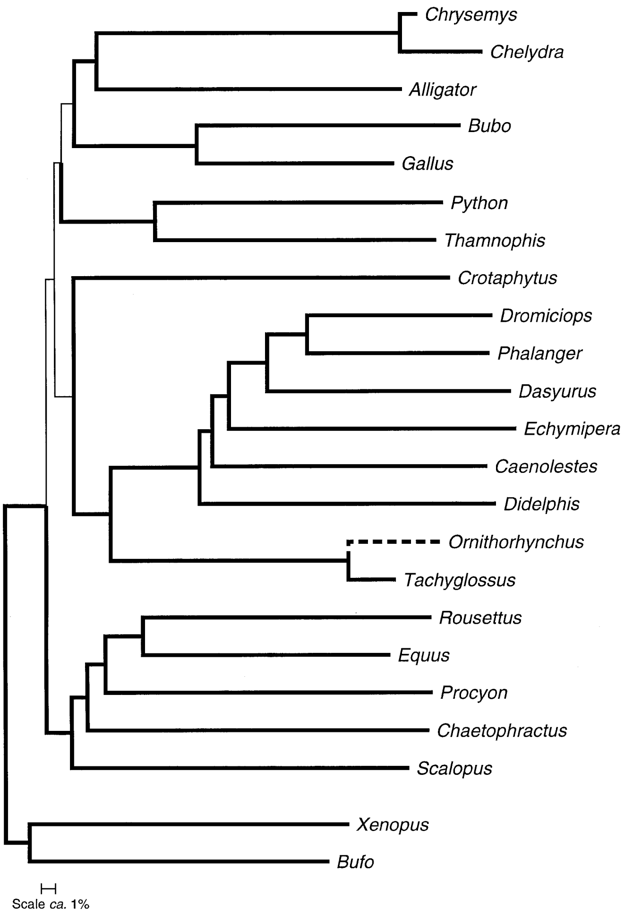

Figure 2. FITCH tree of relationships among 20 amniotes and two anurans, based on the data of table 1 'sutured' with information from table 2 in Kirsch *et al.* (1997). The combined table was completed by estimating missing pairs using the additive reconstruction method of Landry *et al.* (1996). Topology and branch lengths shown are those of the average consensus of pathlengths observed over 1000 random single- and multiple-deletion jackknife pseudoreplicate trees (this topology was also obtained for the average consensuses of all single (22), all double (231), and all triple (1540) deletions). Discrepancies in the range consensus of the minimum and maximum pathlengths are shown as thin lines emanating from nodes not supported in that consensus. Estimate of the position of *Ornithorhynchus* (dashed line) was made in a separate analysis, using the one-way distances from (labelled) *Tachyglossus aculeatus*, *Procyon lotor*, and five marsupials listed in § 3.

consensus of the jackknives, placements of the single lizard and the placental are less certain than most other affiliations. The high variance of NPH together with the probably brief and very remote periods of time involved in many class-level divergences undoubtedly contribute to the imperfect resolution of some nodes. This we expected, but we emphasize that our experiments do generally discriminate among the amniote classes and group them as anticipated. More significant and pertinent to the subject of this paper is that, in all but one case (the mammals) where more than a single terminal species represented an acknowledged group, the terminal sister-pairings are 'correct' and very well supported.

One particular area in which our data do emphatically support the received wisdom, but which is nonetheless worth a brief comment, is the absence of any evidence for a sister relationship between birds and mammals. Owen (1866) had united these two classes in the Haematotherma (not 'Haemothermia', as in some later citations), although it is clear that, even given Owen's somewhat equivocal views on evolution, he intended this assemblage to be a grade. At almost the same time, however, the special relationships on morphological grounds of both classes to specific reptile groups (the archosaurs and synapsids, respectively) came to be recognized (Huxley 1868, 1870; Owen 1876; Cope 1878). Later work has abundantly confirmed these relationships for both mammals (Crompton & Jenkins 1973, 1979; Kemp 1982; Hopson & Barghusen 1986) and birds (Heilmann 1926; Ostrom 1976; Witmer 1991; Novas & Puerta 1997), although debate about exactly which group within the synapsids or archosaurs is closest to their warm-blooded kin continues (Kemp 1988*a*; Hopson 1991; Martin 1991; Ostrom 1991; Luo 1994; Chiappe 1995; Feduccia 1996). The revival of Owen's concept as a clade (rather than a grade) by Gardiner (1982) and Løvtrup (1985; see also Jamieson & Healy (1992) and Gardiner (1993)) on diverse, but largely morphological, grounds was thus decidedly contrarian, and was noted as being 'remarkable' (Gauthier *et al.* 1988*a*) and 'rather revolutionary' (Benton 1991). Somewhat surprisingly, a number of molecular studies, of both protein and nucleic-acid sequences (Goodman *et al.* 1987; Bishop & Friday 1987, 1988; Hedges *et al.* 1990; Benton 1991), also supported this association. A vigorous critique on morphological grounds of the proposals of Gardiner and Løvtrup (Benton 1985; Gauthier *et al.* 1988*a*,*b*; Kemp 1988*b*), which led to a renewed understanding of the importance of fossils in phylogenetic inference (cf. Kirsch & Archer 1982), seemed to bring the consensus back nearer to the traditional view, as did later and more substantial molecular-sequence evidence (Hedges 1994; Seutin *et al.* 1994; Kumazawa & Nishida 1995; Mannen *et al.* 1997; Janke & Arnason 1997). Our results, especially from the 12 × 13 amniote matrix, also support the re-emergent traditional view. As can be seen in figure 1, the two birds, which are always united with one another, have a fairly high bootstrap association (738/1000) with a reptile clade that includes the alligator, which, as the only other archosaur in the matrix, would, under the refreshed orthodoxy (*sensu* Reig 1981), be the closest reptilian relative of the birds. This reptile–bird clade is also present in the average-consensus jackknife tree, and in the range-consensus jackknife (i.e. the tree resulting from collapse of the thin branches in figure 1) as well. In the 22-taxon sutured tree (figure 2), the same clade is present in the average-consensus jackknife, but, unlike for the previous tree, this collapses in the range consensus. Thus, with respect to the Haematotherma, our results accord with the most recent morphological and molecular conclusions.

In contrast, a notable exception to 'correctness' in figure 1 is the union of the monotreme and marsupial, with the representative placental their probable sister-taxon, agreeing with the similar results of Janke *et al.* (1996, 1997; see also Arnason *et al.* (1997) and Janke & Arnason (1997)). Molecular techniques would seem apt for resolving marsupial–placental–monotreme interrelationships, but most molecular systematists have assumed the correctness of the conventional view, using monotremes as an out-group for examination of relationships between and among marsupials and placentals (e.g. Westerman & Edwards 1992; Gemmell & Westerman 1994; Springer *et al*. 1994). In addition, because rates of DNA evolution appear to be markedly slower in Monotremata (Springer *et al*. 1994; Kirsch *et al*. 1997), molecular systematists have made the paradoxical observation that marsupials and placentals are each as close or closer to monotremes than they are to each other, a finding that obtains also (in part) because few molecular studies have included the non-mammalian out-groups necessary to resolve the tripartite division among Mammalia. However, if the presumption of a prototherian–therian dichotomy is relaxed, or if non-mammals are included, a different interpretation of mammalian relationships is clearly possible, as Janke *et al*. (1996, 1997) and we have found.

The opossum–echidna pairing in figure 1 begs the question of what result might have obtained had a greater number of mammals been included in our matrix. Although 37% of the cells were missing in the sutured table underlying figure 2, that tree does give an indication of the answer: in it, the monotreme remains sister to the six marsupials, whose interrelations are much as reported in previous papers (Kirsch *et al*. 1991, 1997). Addition of the platypus did not alter these mammalian interrelationships, and correction of the measured distance between the echidna and platypus (8.40%) for per cent sequence divergence and saturation (to 10.77%; see Kirsch *et al*. (1997) for details of such calculations) suggests a divergence date between the platypus and echidna of about 25 Ma BP (assuming the 0.44% Ma^{-1} mammalian rate reported by Kirsch *et al*. (1997)), which is very similar to the most-favoured estimates of Westerman & Edwards (1992) and Gemmell & Westerman (1994), inferred from DNA hybridization and 12S rRNA gene-sequences, respectively. This date may, however, be an underestimate if DNA evolution in monotremes is markedly slower than in other mammals (see § 4d). Our results and those of Janke *et al*. thus support the construct of a clade identical in composition, but somewhat different in internal arrangement, to Gregory's Marsupionta.

(b) *Deconstructing Gregory: how likely is the Marsupionta?*

(i) *What Gregory actually said*

In 1947, William King Gregory elaborated a notion he had entertained in a previous paper (Gregory 1934), that monotremes and (certain) marsupials are more closely related to each other than either group is to placental mammals, defining the 'Marsupionta' as 'didelphian, cloacate to trivaginate, oviparous or fetiparous, marsupiate mammals, typically with epipubic bones; primitively a large rhinarium (lost in tachyglossids); brain with hippocampus but without a corpus callosum; malleus with large anterior process (goniale)', and classifying living mammals thus (Gregory 1947, p. 46):

Class Mammalia
  Subclass Marsupionta
    Order Marsupialia
    Order Monotremata
  Subclass Monodelphia (Placentalia)

However, Gregory did not mean that Marsupialia and Monotremata are holophyletic sister-taxa (as a cladist might read this scheme), but rather that monotremes were derived relatively recently from within Marsupialia, i.e. that marsupials are paraphyletic. Gregory seems to have believed that placentals were also derived paraphyletically, but much earlier: 'The transition *from the earlier Marsupionta to the Monodelphia* may have occurred not by way of the monotremes but through the Mesozoic orders Triconodonta, Symmetrodonta, Pantotheria. From the construction of their jaws and teeth, I infer that the first two were essentially marsupionts, the third, primitive placentals' (Gregory 1947, p. 46, emphasis added; see figure 3 herein). Importantly, Gregory regarded ancestral marsupionts as being prototherian in grade, from which 'divergent specializations toward the monotreme and marsupial stages took place while the common ancestors of the marsupials and monotremes were still in a prototherian or pre-marsupial stage' (Gregory 1947, p. 35). One of the apparently more peculiar features of Gregory's phylogenetic analysis, the specification of particular homologies between Australasian diprotodontian marsupials and monotremes, appears less so when it is remembered that Diprotodontia represented for Gregory (1910; fig. 18.21 in Gregory 1951) a distinct, basal, and in many respects primitive group of marsupials, with no special affinity with any one of the other metatherian 'orders' and not far removed from the prototherian condition of the ancestral marsupiont. Thus 'monotremes may represent a semi-aquatic branch of ground-living derivatives of an old arboreal [marsupial] stock' (Gregory 1947, p. 46). At the same time, '[t]he Cretaceous to Recent American didelphids seem indeed to be the more direct heirs [than the monotremes] of the progressive mammal-like reptiles; their Jurassic predecessors may have given rise to the Palaearctic early marsupial stock; the latter in turn may have given off the Australasian diprotodonts and, later, the monotremes' (Gregory 1947, p. 45). This passage suggests that Gregory might have regarded 'marsupialism' as a grade (see § 4b(iv)), and because Gregory had a keen appreciation of heterobathmy (as the very title of his 1947 paper suggests), the derived features (e.g. presence of a fasciculus aberrans in the brain, syndactyly of the pes and procumbent lower incisors) of the monotremes' sister-group in his phylogeny, Diprotodontia, presented no difficulty to his monotreme–diprotodontian association, especially given his use of the habitus/heritage distinction (see § 4b(ii)). Figure 3 represents our interpretation of the overall view of mammalian evolution held by Gregory, with respect to branching, character evolution and classification.

(ii) *Gregory's analytical method*

In his monumental 1910 monograph on the orders of mammals, Gregory introduced (in a footnote, p. 111) the distinction (attributed to Camp (1923) by Moody (1985); cf. Estes (1988)) between 'caenotelic' and 'palaeotelic' characters, the former being the immediately adaptive features of an organism (or clade), and the latter the residue of past changes. Perhaps to capture this notion of character categories in a more accessible way, Gregory later (e.g. Gregory 1922; in addition, see Colbert & Mook 1951) suggested a roughly parallel distinction between

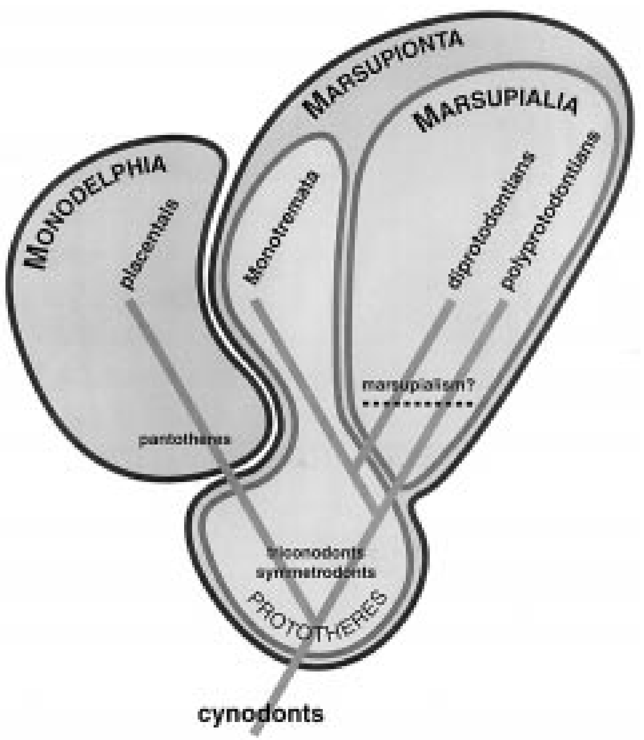

Figure 3. Representation of Gregory's (1947) view of the phylogeny of mammals, incorporating one of the alternatives for the evolution of reproduction considered by him. Circumscription of groups shown is based on the assumption that egg-laying is a retention in monotremes, such that 'marsupialism' (fetiparity) must have evolved twice, and so marsupials are polyphyletic with respect to this character. Under the assumption that egg-laying is a reversion to the prototherian (or reptilian) reproductive condition, marsupials would be paraphyletic as regards monotremes. If molecular evidence that monotremes are sister to all marsupials (rather than, as shown and inferred by Gregory, derived specifically from Australasian diprotodontian marsupials) is proven correct, marsupials would be holophyletic. However, live-bearing must have evolved independently in placentals whether monotremes evolved from within a primitively oviparous Marsupialia or simply dichotomously from a common egg-laying prototherian ancestor shared with (all) marsupials.

characters of 'habitus' and 'heritage'. Habitus characters (equivalent to caenotelic features) become in time transformed into, or at least included among, those of heritage as the collected adaptive wisdom of the lineage at more general levels, by a process of sequential adaptation (cf. Lull 1917, pp. 280–282). Habitus and heritage are thus 'correlative terms', so that 'the remainders of the successive habitus of the remote ancestors become incorporated into the heritage of later times' (Gregory 1947, p. 8). Heritage features are therefore of utmost importance in determining the broad affinities of a higher-category taxon, because they may be ones shared with a similarly inclusive but different group.

Gregory derived his Marsupionta hypothesis through application of this method of character analysis, which he called the 'palimpsest theory', because his object was—here as in so many other papers—essentially to distinguish the habitus features (apomorphies) of a taxon from the temporally accumulated heritage characters indicating possible relationships with other, collateral taxa. His metaphor is therefore that of a parchment that has been written upon more than once and imperfectly scraped clean between uses, such that a shadow of earlier meanings is seen through the most recent inscriptions.

Gregory's traversal of the evidence for and against various possible associations of monotremes is designed to demonstrate, first of all, that monotremes are not reptiles but true mammals (actually an uncontroversial conclusion even in 1910, much less in 1947; but cf. MacIntyre 1967), and that many of the superficially reptilian characters of monotremes in fact reflect the habitus (immediate adaptations) of the platypus and echidnas. His second aim is to show that much other evidence (more recently acquired but still heritage features) favours a special relationship between monotremes and certain marsupials. Gregory's third objective is mechanistic: to suggest how the derivation of monotremes from diprotodontians could have occurred by a combination of retention and developmental truncation (or arrest), the latter being crucial to one of his interpretations of the evolution of monotreme reproduction.

(iii) *Skeletal and other non-reproductive characters*

In his 1910 treatment of monotremes, Gregory provided long lists of ways in which monotremes resembled reptiles, mammals generally, and marsupials specifically; many of these points were considered again by him in 1947. Having disposed of the anatomical correlates of the peculiar postural and behavioural features of the aquatic platypus and digging echidnas as mere analogies with splayed-limb reptiles (they are habitus or immediately adaptive features), he is left largely with the pectoral girdle and some few cranial characters as indications of heritage shared with reptiles. The large (and unfused) coracoid, anterior coracoid in contact with the sternum, and interclavicle are all considered retentions from cynodonts. However, Gregory does not comment on the extensive reshaping of the scapula shared by marsupials and placentals (Klima 1987), which provides evidence contrary to his union of monotremes with marsupials.

More positively, Gregory presents several diagrams that draw point-for-point correspondences between monotreme (usually platypus) skulls and those of marsupials (usually wombats). Central to his inference of affinity is the supposed homology of the leathery skin of the platypus bill with that of the marsupial 'muffle' or rhinarium, an argument which he strengthens by citing Selenka (1887) and McCrady (1938) on the transitory appearance of an oral shield in developing Virginia opossums. On the other hand, Gregory is not deterred by the lack of a fasciculus aberrans or other diprotodontian habitus features in monotremes: these absences apparently represent for him expected plesiomorphies in one of the two sister-taxa.

Other features of the monotreme head or skull are similarly shown not to be inconsistent with the Marsupionta taxon: the detrahens mandibulae muscles and 'echidna pterygoids' (also found in the platypus) Gregory considers to be specializations (rather than legacies from reptiles, which they may be), consistent for him with derivation from diprotodontians. He does not mention the septomaxilla, a certain retention from cynodonts, perhaps because this bone is fused with the premaxilla at least in the adult platypus, although not in some fossil monotremes (see Archer *et al.* 1993). The structure of the sidewall of the braincase, which has figured greatly in hypotheses about mammalian interrelationships (see, for example, the discussions in Griffiths (1978), Kemp (1982), Maier (1987), and Novacek (1993)), might be expected to receive considerable attention. However, Gregory accepts the then-standard view (deriving from Watson (1916)) that much of this region represents an anterior extension of the periotic (= petrosal), and labels it as such in both the echidna and platypus. Kielan-Jaworowska (1971) used this (derived?) character, supposedly shared with Multituberculata, as evidence for a phylogenetic relationship between monotremes and multituberculates. (Although it was more usual at the turn of the century (e.g. Gregory 1910) to consider multituberculates as marsupials, at various times others have also suggested multituberculate relationship with monotremes. For example, Cope (1888, p. 259), in reporting enthusiastically that '... Mr. E. B. Poulton has discovered teeth in sections of the jaws of a young *Ornithorhynchus anatinus*,' remarks that '[t]he description reads like that of the dentition of the Plagiaulacid genus Ptilodus. *It renders it extremely probable that the Multituberculata are Monotremata*, and not Marsupialia, as has been supposed' (emphasis as in the original). Broom (1914) provided more substantial arguments, although most of his supporting characters appear to be primitive ones. Much current opinion, however, favours a general therian–multituberculate relationship (e.g. Rowe (1988, 1993), Simmons (1993), Wible (1990) and Hu *et al.* (1997), but not Wible & Hopson (1993), who like Cope and Broom discern a monotreme–multituberculate clade). But while Miao (1993) instead champions a Simpsonian view that multituberculates are an ancient lineage possibly sister to all other mammals, this seems unlikely to us now that Jenkins *et al.* (1997) have shown that late Triassic haramiyids are probably not related to multituberculates.) In fact, Griffiths (1978) elegantly showed that there is no such thing as 'the' monotreme condition, echidnas having, like therians, a strong alisphenoid contribution to the lateral wall of the braincase (actually hinted at by MacIntyre (1967)). Kemp (1982) endorsed (but oversimplified) Griffiths' arguments, and suggested an alternative definition of the sidewall, which emphasizes the pattern of ossification in the membranes of the secondary braincase, elements forming what becomes (by definition, through fusion) a periotic wing in (some) monotremes and the alisphenoid in therians. Kemp further suggested that the non-therian condition could be the primitive one for mammals. (Indeed, Kemp's controversial conclusion that monotremes are cladistically quite close to therians was in part based on this supposition.)

Not unrelated to this part of the debate is, of course, the vexed problem of the foramen ovale: whether it should be defined positionally as a hole through the alisphenoid, as in some therians (and therefore non-homologous—a 'foramen pseudovale'—when it penetrates or abuts any other bone), or functionally as a passage for the mandibular branch (or branches) of the trigeminal nerve, located wherever the fortuities of development (or phylogeny?) leave it. But as Novacek (1993, p. 488) observes, 'a plastic ontogenetic relationship between nerves and skeletal elements does not rule out homology for various components'. Another apparent distinguishing cranial feature of monotremes (or at least one that excludes them from membership in, or association with, any constituent taxon of Theria)—their only partly coiled cochlea—is one of a few non-reproductive features that Gregory supposes might represent developmental arrests (others include some features of the pharynx). (Hu *et al.* (1997) argue that such coiling of the cochlea as occurs in monotremes (of the membranous but not bony labyrinth) is convergent on the marsupial–placental condition, because in their view symmetrodonts (which probably had a straight cochlea) are more closely related to therians (and multituberculates) than are monotremes. But if there is convergence between therians and prototherians, why not within Theria itself?)

However, Gregory regards the topologies of the deciduous platypus molars as fundamentally like those of wombats, the special monotreme features being overlaid on an arrested diprotodontian–monotreme developmental stage. The dental formula and simplified tooth-replacement pattern shared with marsupials by ornithorhynchids also seem to provide positive evidence for association of monotremes and marsupials. Platypus dental replacement is, according to Green (1937), restricted entirely to a single pair of teeth, as it is in marsupials. It was Kühne's (1973, 1977) summary designation of the putatively replaced platypus teeth as premolars that this author claimed as a marsupial–monotreme synapomorphy, but Luckett & Zeller (1989) showed that Green's (1937) conclusions (and Kühne's interpretation and endorsement of them) were in error: the platypus has no tooth replacement whatsoever. Still, the reduction of monotreme dentition to a single series of teeth in *Ornithorhynchus* could be seen as a further simplification of the marsupial pattern and hence evidence of a special marsupial–monotreme relationship, especially now that it is known that Cretaceous marsupials showed the identical successional pattern to living metatherians (Cifelli *et al.* 1996) and that the eupantotherian dryolestids had an extensive (placental-like) diphyodont replacement (Martin 1997). At the same time, the problematic interpretation and use of reduction or loss to infer relationship—as of any autapomorphous or radically transformed condition—show the difficulty of determining the collateral relatives of a group so highly derived as Monotremata: out-groups may evolve no less than do in-groups (Camp 1923, p. 337). In addition, Archer *et al.* (1993) question the homology of therian and prototherian molars.

Gregory also suggests that the monotreme fossil record, which at his time (and until much later) was limited to the Quarternary of Australia, is consistent with inference of a monotreme–diprotodontian clade. But we now know that platypus-like animals were present in the Australian Early Cretaceous (Archer *et al.* 1985), as well as in the South American Eocene (Pascual *et al.* 1992). This newer palaeontological evidence (together with estimates of the times of divergence among extant marsupial orders of between only 60 and 72 Ma BP (Kirsch *et al.* 1997; Springer *et al.* 1997)) decisively falsifies any particular derivation of monotremes from an ancestor shared exclusively with diprotodontians. And, were Ornithorhynchidae and Tachyglossidae conceived of as holophyletic sister-taxa, this would force an even more general sister-group relationship of monotremes and marsupials back at least to the Early Cretaceous. However, Gregory frequently makes the point that the digging echidnas are best understood as derived (paraphyletically) from an aquatic platypus-like ancestor, remarking that echidnas merely swim in a more resistant medium, and so echoing Samuel Johnson's (1759) similar comment about flying and swimming birds. Estimates from genetic distances of the timing of the platypus–echidna divergence at around 25–30 Ma BP (Westerman & Edwards 1992; Gemmell & Westerman 1994; this paper) are also indicative of ornithorhynchid paraphyly, if assignments of the fossil teeth by Archer *et al.* (1985) and Pascual *et al.* (1992) to Ornithorhynchidae are correct.

(iv) *Reproduction and development*

Reproductive features, both anatomical and physiological, might be expected to present the greatest problems for the Marsupionta hypothesis. Regarding anatomy, the chief difficulty under any of the three scenarios would seem to be the marked differences in arrangement of the ureters and oviducts or uteri in females of the three mammalian groups and their various (correlated) connections with the bladder and urogenital sinus or cloaca. Briefly, the oviducts and medially placed ureters are entirely separate in monotremes, all emptying directly (and dorsally in the case of the ureters) into the cloaca. In marsupials, the ureters instead terminate more ventrally in the bladder, but still pass medially to the two lateral vaginae and are separated by a third, central birth canal connecting the distinct urogenital sinus with the anteriorly fused oviducts. In placentals, of course, the oviducts may fuse along the midline and the ureters always pass lateral to this sometimes single structure. Abbie (1941), whom Gregory cites approvingly, cleverly accounted for the differences between marsupials and placentals by suggesting that the marsupial lateral vaginae (and birth canal) represent remnants of the urogenital sinus 'perforated' by the migrating ureters (which stopped at the cloacal dorsum in monotremes); such remnants subsequently degenerated in evolution in placentals. But, and as might be expected, Gregory does question whether the monotreme condition is the necessary starting-point in Abbie's sequence or whether it could also be derived by ontogenetic arrest from the marsupial arrangement.

However, it had been known since long before Abbie's paper that the marsupial birth canal (or median vagina) is a usually temporary structure, established at first parturition (see the historical introduction in Tyndale-Biscoe & Renfree (1987)); it is not a caudad extension of the anteriorly fused (lateral) oviducts or even transversely homologous to them, much less a part of the urogenital sinus. Tyndale-Biscoe & Renfree (1987) state the situation best when they characterize the two therian conditions as strictly dichotomous, admitting of no polarity or intermediate state. Yet the entirely separate ductal system and its topological layout in the monotremes can perhaps be seen as 'closer to' the marsupial condition, the arrangements in the two taxa sharing a common phyletic origin that excludes the placental condition. Still, the explanation for the marsupial–placental difference may well be only slight differences in the timing of growth of the Müllerian and Wolffian ducts, such that the 'winners' in the race to reach the cloacal goal enjoy the medial position; the losers accept lateral placement. In this formal sense, contemporary theory agrees with Gregory (*ex* Abbie) in implying a developmental (i.e. heterochronic) mechanism to account for the marsupial–placental dichotomy, but not the one that he invoked.

It is also true that the reproductive physiology of marsupials, with a yolk-sac (or rarely, chorio-allantoic) placenta established only near the end of intrauterine development, is not unlike the completely oviparous 'prenatal' ontogeny of monotremes, in which some embryogenesis facilitated by uterine secretions takes place before laying of the egg(s) and a respiratory allantois is featured during later stages, after the egg is laid. Monotremes and marsupials also share a common radial cleavage pattern resulting in a bilaminar blastocyst by a quite different process than in placentals, although the marsupial–monotreme condition is a retained primitive feature shared with other amniotes (Hughes & Hall, this issue). Providing no ontogenetic arrest in Monotremata is involved, the union of monotremes and marsupials as sister-taxa (deriving from an oviparous common ancestor) to the exclusion of placentals of course mandates that live-bearing in placentals is not a further transformation of the marsupial (fetiparous) condition, but evolved separately from an earlier, presumably also egg-laying precursor. Sharman (1970) and Tyndale-Biscoe (1973)—and more recently Zeller (1997)—have argued for just such a dual origin of mammalian live-bearing but from an oviparous therian progenitor, and we note that viviparity has evolved several times in the Squamata, often with detailed resemblances to the placental condition (Blackburn *et al.* 1984; Zug 1993).

In fact, the details of both pre- and postnatal development in marsupials are profoundly different from those in placentals, although in ways that are often adaptively entrained by the truncated intrauterine development of, and emphasis on lactation in, marsupials. For example, Clark & Smith (1993) and Smith (1994, 1997) show in their superb studies of cranial development that marsupials depart markedly from the usual timing and order of structural changes, even violating the 'rule'—based mostly on studies of the laboratory mouse—that skeletal structures (e.g. of the braincase sidewall and mandible) must be established prior to, or simultaneously with, the muscles that attach to them (Smith 1994). Indeed, marsupials are derived among amniotes in that the development of the central nervous system (CNS) is delayed relative to that of somatic structures (Smith 1997). In particular, and understandably given the urgency of nipple attachment and suckling, ossification of the circumoral bones precedes that of any others, with the exception (also understandable, in view of the need for support of the head) of the exoccipital (Clark & Smith 1993). Monotremes show a similar anterior precociousness, including a somewhat accelerated development of the forelimbs shared with marsupials, but the oral bones of monotremes do not complete development as early as in marsupials (a circumstance that can be attributed to the lack of nipples in monotremes (Clark & Smith 1993)). Although the striking resemblances of monotreme hatchlings to marsupial neonates may seem 'only' to reflect common early requirements to reach and attach to the mammary area, and therefore may represent just shared primitive features (or convergent adaptations), it is well to remember Griffiths'

(1978) point that the essentially larval hatchling monotremes and neonatal marsupials are totally unlike the 'newborn' of reptiles, which begs the question of whether such extreme altriciality can possibly be primitive for mammals. Again, Smith's (1997) work suggests that, in view of the dramatic repatterning of development involved, it cannot be. Interestingly in this context, Huxley (1880), believing marsupials and placentals to be specially related, characterized his hypothetical 'Metatheria' in such a way that its members would lack almost every feature that we might, today, regard as definitive for marsupials: for Huxley this therian common ancestor or grade would have had no pouch, enjoyed extended intrauterine development (and therefore a relatively shorter period of lactation) and even possessed undivided vaginae! Thus, one might say that for Huxley, marsupials were actually reproductively derived from an ancestor much more like placentals (or, in some respects, viviparous reptiles).

It should be mentioned here that pouches are not necessarily correlated with extreme altriciality: the existence of a temporary broodpouch in echidnas but its lack in the platypus reflects the peripatetic habit of the former and the nest-depositing of eggs by the latter. So, as is true for marsupials (Reig *et al.* 1987; Springer *et al.* 1997), it is unlikely that the presence of a developed pouch is a primitive feature for monotremes, although 'marsupiate' is part of Gregory's definition of Marsupionta. Thus, notwithstanding that some of the shared marsupial–monotreme reproductive and developmental characters in question may be plesiomorphies, on the whole they seem not to be, and even the possibly primitive resemblances are of course not inconsistent with the union of (all) marsupials and monotremes as a monophyletic group apart from placentals. More convincingly for that association, marsupials and at least *Tachyglossus* share an exquisite control over lactation and sequential changes in milk composition that are practically unknown in placentals, even in those with markedly altricial young and therefore extended lactation (Tyndale-Biscoe & Renfree 1987). This last complex of features is difficult to view as plesiomorphic or convergent between monotremes and marsupials. Tyndale-Biscoe & Renfree (1987) usefully summarize their discussion of the evolution of mammalian reproductive features in a table of 38 characters, but attempts to categorize the variable states listed as primitive or advanced emphasize how much such interpretation depends upon a prior presumption of pairwise relationships among the three groups of mammals. Clearly, reproductive anatomy and physiology provide less decisive information on the true relationships of Monotremata than might be hoped or expected, in part because of the lack of relevant extant out-groups.

Nonetheless, the critical consideration in evaluating Gregory's derivation of monotremes from within marsupials versus a more general sister-group relationship of Monotremata and Marsupialia remains whether oviparity is primitive for a putative marsupial–monotreme clade or constitutes a secondary specialization of monotremes. Monotreme derivation from diprotodontians appears to mandate the reinvention of oviparity, or else a retention of that condition in the common ancestor shared with diprotodontians. Retention clearly means that marsupialism itself ('fetiparity', or live-bearing of highly altricial young) would be a grade, achieved independently in at least two lineages of marsupials (see figure 3). Gregory regards reinvention as a possibility (but does not commit himself to it), contingent upon a developmental truncation that left the oviducts entirely separate and entrained oviparity (Gregory 1947, p. 44):

> For the present writer the question is left open whether the complete separation of the right and left oviducts in monotremes is a direct inheritance from reptiles or whether it came via a prototherian stage from the common ancestors of monotremes and marsupials, or, finally, whether it is partly owing to: (1) arrest of development at a stage in which the right and left oviducts were still separate, and (2) subsequent specialization toward a secondary increase in yolk, large eggs, and oviparous rather than ovo-viviparous habits.

Therefore no development truncation need be involved to account for the monotreme condition (although the possibility of such arrest seems much less untestable 50 years after Gregory's paper, in the light of recent work revealing the extraordinary phylogenetic depth of many characters and their evokability after eons of genetic dormancy (Raff 1996)). Yet we stress that, in the absence of such truncation, fetiparity must have evolved at least twice among marsupials if Gregory's specific conception of Marsupionta is true (figure 3), and live-bearing must have evolved independently in placentals even if the relationship of monotremes to marsupials is one of a more general sister-group.

(v) *Summary*

Thus, there are some striking, non-habitus similarities between monotremes and marsupials (in reduction or complete elimination of dental-replacement series, possibly derived reproductive commonalities and molecular distances), and few among those characters considered by Gregory contradict a marsupial–monotreme special relationship other than the relatively pauciösteose yet ornate pectoral girdle shared by marsupials and placentals. Reproductive anatomical differences between marsupials and placentals represent true dichotomies that cannot serve to unite these taxa, and live-bearing can only verbally (and not convincingly, given its recurrence in squamates) be used to combine them as against monotremes.

Therefore, if monotremes are conceived of as the sister-taxon to all marsupials, neither their reproductive apparatus, nor habit of egg-laying, nor apparent retention of pre-mammalian skeletal features, offer serious impediments to recognizing Marsupionta so defined as a valid phylogenetic group. But Gregory's specific derivation of monotremes from a common ancestor shared with diprotodontians does require either a mechanism such as developmental arrest, or else extreme convergence in reproductive physiology and anatomy between diprotodontian and polyprotodontian marsupials, minimally implying a dual origin of marsupialism. A problem with accepting ontogenetic truncation is that such arrest could have happened at any point in mammalian history, or before: it is a hypothesis that permits monotremes to 'slide' up and down the phylogenetic tree to wherever it is convenient to place them. On the other hand, and to repeat, no such heterochronic mechanism (i.e. one permitting completely separate vaginae and 'reinvention' of the egg) is required to reconcile the reproductive or anatomical differences with a general marsupial–monotreme clade, if live-bearing is conceded to have evolved at least twice among 'therians'. The developmental mechanism is only necessary if monotremes are derived from within a fetiparous Marsupialia, a specific affiliation decisively falsified by newer evidence from the fossil record as well as by the molecular data.

Nonetheless, we expect that advocates for the primacy of craniodental and skeletal characters will object that truncational hand-waving (needed or not) is no answer to the witness of additional apparent synapomorphies of marsupials and placentals which we have not considered here, in particular in the shared details of the (otherwise simplified) pectoral girdle and cranium. There is, especially in the German developmental and anatomical literature, information about a vast array of potential therian synapomorphies (e.g. Klima 1987; Maier 1993; Zeller 1993; Starck 1995). However, the existence of these additional features only emphasizes that there remains a clear incongruence between anatomical and molecular data that must be confronted. Whether the eventual resolution of this conflict comes from reassessment of putative homologies or from a 'total evidence' analysis (Eernisse & Kluge 1993), at the very least some alternative explanation for the molecular findings should be entertained, as we do in § 4d.

(c) *Turtles*

Along with the monotreme–marsupial association, our most surprising result is the placement of the turtles within the Diapsida as sister-group to the alligator. It is surprising not just because of the position of the turtles in the 'best-fit' trees, but because the relationship holds up under bootstrapping (890/1000 replicates) and in the average- and range-consensus weighted jackknife trees based on table 1, and also in such trees derived from the 22-taxon sutured matrix.

The case of the turtles, in fact, presents curious parallels to that of the monotremes: having once been generally considered to occupy the most distant position in the phylogeny of their respective groups (monotremes sister to the remaining extant mammals, turtles sister to all other extant amniotes), recent evidence has supported moving turtles from that position to one well within the group. And just as Gregory early championed a view akin to that now resurrected by Janke *et al.* (1996, 1997) and our data, so too did the notion of turtle affinity with diapsids, revived by Rieppel & deBraga (1996) and our data, have an early proponent in Goodrich (1916). Broom (1924, p. 48), in agreeing at least in a general way with Goodrich's ascription of diapsid affinities to the Chelonia, even likened the analysis of chelonian characters to 'the reading of a difficult palimpsest'. (Broom's and Gregory's uses of the palimpsest analogy are remarkably similar. Broom specifically invokes it in discussing 'ancestral characters' (=palaeotelic or heritage features) and 'recent specializations' (=caenotelic or habitus features). Given Broom's residence at the American Museum in 1913 and 1914, this similarity may be homologous.) The chief difference is that, whereas the supposed sister relationship of monotremes and marsupials discussed here is less revolutionary than that proposed by Gregory, the placement of turtles deep within diapsids (albeit in different places according to Rieppel & deBraga (1996) and ourselves) is more radical than the view of Goodrich or the emerging consensus that turtles are not sister to the other amniotes.

In the system of reptilian classification epitomized by Williston (1917, 1925), the position and number of the temporal fenestrae were the key features. This system, although with other characters considered and fenestration groups sometimes divided, was the basis of reptile classification for decades, and remains influential to this day (Romer 1956, 1966, 1968; Kuhn-Schnyder 1980; Carroll 1988). The absence of fenestrae (the anapsid condition), because it is the state in amphibians and the earliest reptiles, is considered the most primitive one; turtles, as the only extant anapsids among the amniotes, were long thought of as the most plesiomorphic Amniota. This could be taken to mean that chelonians are sister to all other amniotes. As reasoning about phylogenetic matters became more refined, it was realized that, as a primitive condition, the anapsid skulls of turtles did not preclude them from a more recent common ancestry with some particular other amniote group. These other groups can, for extant taxa, be divided into synapsids (construed as represented now by the mammals) and diapsids (squamates (which, for simplicity of discussion, and paucity of relevant data, may be considered for our purposes to include the tuatara, which of course is not a squamate), crocodilians and birds).

Goodrich (1916), as mentioned earlier, had noted resemblances among diapsids and turtles, in particular in the shape of the fifth metatarsal and the structure of the aortic arches. The question thus became whether turtles are sister to diapsids, as Goodrich's characters would indicate, or to amniotes as a whole, as the fenestral theory would have them. (For discussion of Goodrich's characters and others, see Broom (1924), Holmes (1975), and Hopson (1991).) In one of the first studies to apply cladistic methodology to the problem, Gaffney (1980; figure 4*a* herein) concluded that the traditional view of turtles as outermost was correct. Gauthier *et al.* (1988*a*) noted that, before the Gardinerian bombshell endorsing Owen's Haematotherma, the question of whether the synapsids or the turtles are the first branch on the amniote tree was the chief issue in amniote systematics. Unlike the case of Gardiner's suggestion of bird–mammal affinities, where further work on Haematotherma has led consensus back to the traditional view (see § 4a), in the case of turtles further work has supported the at least mildly heterodox placement of the turtles as sister to the Diapsida among extant taxa, rather than the Willistonian view espoused by Gaffney (1980). Gauthier *et al.* (1988*a*,*b*), for example, positioned turtles next to diapsids, and Gaffney & Meylan (1988) and Hopson (1991) supported Goodrich's notion of a Sauropsida consisting of all extant non-synapsid amniotes. Benton (1991) regarded this arrangement as the 'standard morphological' view of turtle relationships (figure 4*b*; see also Eernisse & Kluge 1993; Laurin & Reisz 1997; Lee & Spencer 1997). Further morphological work (Laurin & Reisz 1995; Lee 1995,

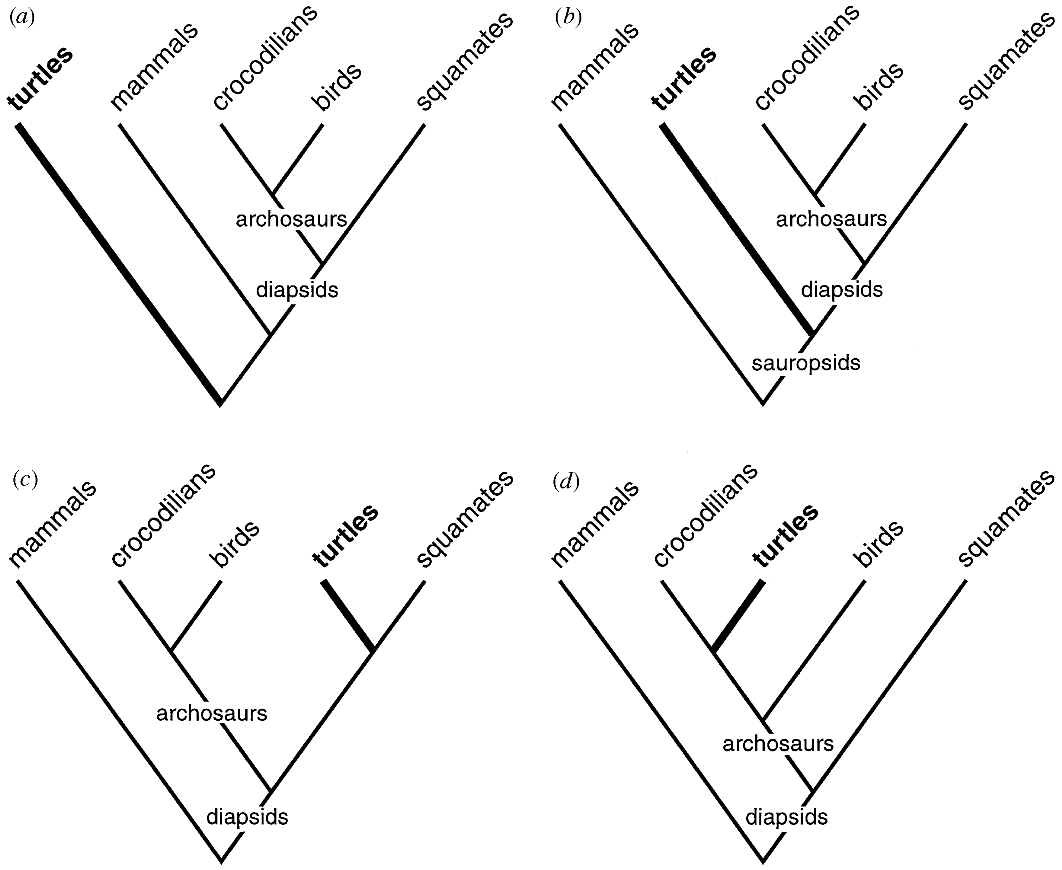

Figure 4. Four hypotheses on the relationships of turtles: (*a*) the traditional, Willistonian view espoused by Gaffney (1980), and still sometimes used, that turtles are basal with respect to all other amniotes; (*b*) the 'consensus' modern view (see Gauthier *et al.* 1988*a*,*b*; Gaffney & Meylan 1988; Benton 1990, 1991; Hopson 1991; Laurin & Reisz 1995; Lee 1995, 1996) that mammals are sister to turtles and all remaining amniotes; (*c*) the view of Rieppel & deBraga (1996), supported by some molecular data (Goodman *et al.* 1987; Bishop & Friday 1988; Hedges *et al.* 1990; Hedges 1994), that turtles are diapsids which are close to squamates; and (*d*) turtles as diapsids which are close to crocodilians, supported by the present study and some other molecular data (Bishop & Friday 1987; Goodman *et al.* 1987; Hedges *et al.* 1990; Mannen *et al.* 1997). Note that in (*c*) and (*d*), sauropsids and diapsids become coextensive for extant taxa.

1996), indicating a relationship of turtles with 'parareptiles' (a group of anapsid reptiles including procolophonids and pareiasaurs), solidified the position of turtles as basal within the Sauropsida.

Our results, placing turtles sister to the alligator, are thus contrary to this consensus, and represent a further departure from the Willistonian tradition. But they are not alone in suggesting that even more radical revision of tradition is needed. First, for several years there have been sequence data available indicating a turtle–diapsid relationship (Bishop & Friday 1987, 1988; Goodman *et al.* 1987; Hedges *et al.* 1990; Hedges 1994; and especially Mannen *et al.* 1997). Second, Rieppel & deBraga (1996), in a morphological analysis using a diverse array of fossil taxa, conclude as well that turtles lie within the diapsids, and are specifically the sister of squamates (among extant taxa). These authors invoke the importance of including fossils of diverse taxa, the lesson learned from the Gardiner affair, as a key contributor to their reassessment of the relationships of turtles. Thus, three sorts of evidence—sequences, DNA hybridization and morphology—point to a position of the turtles within the Diapsida. They differ on whether this position is near the squamates (morphology, some sequences: figure 4*c*) or with the Crocodilia (DNA hybridization, some sequences: figure 4*d*).

The position of the lizard *Crotaphytus* in our trees might also be considered surprising, but in this case not for what it shows, but for what it doesn't. The placement of *Crotaphytus* changes between the 13- and 22-taxon trees, and in both cases collapses to unresolved with respect to nearest relatives in the jackknife range-consensus of each. Given the probable time-depths of the other divergences that collapse in that consensus, it is surprising that the snake–lizard divergence should be among them, as this divergence is likely to be (only) Upper Jurassic in age (Estes 1983; Rage 1984). This irresolution may be due to *Crotaphytus* being closest to *Python* as a tracer, but, unexpectedly, to *Tachyglossus* as a driver (see table 1). Thus, torn between two different clades, it resolves into neither. However, lack of resolution is not resolution, and the positive evidence for the position of turtles amongst the Diapsida is clearly the more difficult to reconcile with traditional views.

(d) *Molecular and algorithmic explanations*

We are left, then, with strong indications from the molecular evidence, including both mitochondrial gene-sequences and single-copy (largely nuclear) DNA-hybridization distances, that Gregory may have been 'right', at least in the general phylogenetic sense that marsupials and monotremes are closer to each other than either is to placentals; and for a surprising affinity of turtles with the crocodilians. In addition, for just the reason that nuclear and mitochondrial studies give the same result regarding the mammals, one cannot claim that the conclusion with regard to monotremes is subject to peculiarities of the mitochondrial genome (e.g. that it is inherited matrilineally and essentially as a single, non-recombining gene). Still, it remains possible that the molecular trees may have been obtained for algorithmic or base-compositional reasons. Regarding the first, the 'attraction of long branches' is a well-known problem of all trees, whereby singletons tend to group either with an out-group or with some other, relatively long-branched clade found among in-group taxa (Felsenstein 1978). Even when multiple but highly divergent representatives of a taxon are included, they may evince peculiar relationships, as must surely be (at least in part) the cause of the glirine scandal that suggested the title of this paper (Graur *et al*. 1991; D'Erchia *et al*. 1996). As Sullivan & Swofford (1997) point out, the apparent non-monophyly of rodents in molecular trees may also be due to randomization of the opossum out-group sequences relative to those of placentals, leading to misplacement of the root among the latter, or to the inappropriate use of an evolutionary model that assumes equal rates of change at all sites.

The long-branch problem may particularly affect the turtles in our trees: as with the monotremes with respect to marsupials, the association of turtles with the alligator seems to be the clear evidential meaning of our data (Sober 1988, after Birnbaum 1962), and it is an association that is not without other supporting data (e.g. from lactic dehydrogenase gene-sequences (Mannen *et al*. 1997)). Nonetheless, we are reluctant to adopt it wholeheartedly. In particular, we note that the alligator branch is undivided, and the turtle branch, although consisting of two taxa, is necessarily subdivided near its terminus. To divide the turtle lineage properly would require pleurodires to be included. Thus, although our own data and that of others (Goodrich 1916; Broom 1924; Bishop & Friday 1987, 1988; Goodman *et al*. 1987; Hedges *et al*. 1990; Hedges 1994; Rieppel & deBraga 1996; Mannen *et al*. 1997) provides grounds for accepting a position of turtles somewhere within the Diapsida, the possibility of an algorithmic artefact owing to the attraction of long branches or another cause cannot be excluded as the reason for our and some others' results.

Similarly, our figure 1 and Janke *et al*.'s (1996) first tree including the platypus and a marsupial might suggest that the single representatives of Marsupialia and Monotremata in each, again necessarily terminating long branches, are associated for that reason. Janke *et al*. (1997) have only partly addressed long-branch attraction in their more recent paper, simply joining one lengthy branch (the opossum lineage) with another (the kangaroo's). However, our figure 2 DNA-hybridization tree, with its inclusion of several other marsupial lineages, still supports a marsupial–monotreme clade. Although this part of our study should obviously be repeated with a more complete matrix, it seems unlikely that the association of monotremes and marsupials observed by Janke *et al*. and ourselves could be due solely to long-branch attraction.

Conversely, the terminal segment bearing *Tachyglossus* in figure 1 appears to be somewhat short compared with others of presumably similar time-depth, implying a slower rate of molecular change. Short monotreme branches have been observed in analyses of gene and protein sequences as well (Gemmell & Westerman 1994; Springer *et al*. 1994; Janke *et al*. 1996, 1997; Messer *et al*. 1998). We suspect that an 'attraction of short branches' is as common as that of long ones (Pettigrew & Kirsch 1997), and may be part of the reason why monotremes tend to link with marsupials. But rather than indicating truly retarded rates of change and thus a joining of branches which are truncated for this reason, a more likely explanation for the shortness and placement of the echidna or platypus branch may be some base-compositional bias, such as that proposed by Pettigrew (1994) to explain the persistent (but, in his view, false) association of bat suborders on molecular trees. Of course, Pettigrew's advocacy of a dual origin for bats is contested, but there is little disagreement that the bat suborders are each monophyletic. However, some gene sequences and DNA hybridization support a special affinity of megachiropterans and the rhinolophoid microchiropterans (Porter *et al*. 1996; Hutcheon *et al*. 1998), an association that we are hard-pressed to explain except (possibly) on the basis of their shared, high AT:GC ratios.

Could the apparent marsupial–monotreme clade have a similar basis? Little is known about base-compositional biases amongst marsupials, although marsupials do seem to have 'normal' compositions for commonly studied genes such as cytochrome *b* (e.g. Patton *et al*. 1996); but G. Bernardi (personal communication to J. D. Pettigrew) finds an overall, unexpected GC bias in the monotreme *Ornithorhynchus*. Although we initially included only the short-nosed echidna (*Tachyglossus aculeatus*) in our DNA-hybridization studies, our and others' information that echidnas and the platypus are quite similar molecularly (Westerman & Edwards 1992; Gemmell & Westerman 1994) suggests that either taxon may serve as a proxy for the other in examination of higher-level relationships: any base-compositional bias present in the platypus is therefore likely to be shared with echidnas.

If this putative bias is further shared, even to a small extent, on a genome-wide level with marsupials, it could help to explain the association of marsupials with monotremes on Janke *et al*.'s and our own trees. As both monotremes and marsupials tend to have lower basal metabolic rates than placentals (Griffiths 1978; Hume 1982), and have correspondingly lower body temperatures, the ultimate explanation for the putative bias could be physiological, because higher-metabolism mammals (placentals generally and shrews and many bats in particular) do incline toward marked overall AT biases (Sabeur *et al*. 1993; Pettigrew 1994). Accordingly, lower-metabolism mammals should have relatively higher GC contents. We note again that short branches for at least monotremes have been observed in several studies (e.g.

Springer *et al*. 1994; Janke *et al*. 1996, 1997; Messer *et al*. 1998); such apparent rate-slowdowns might be expected as a result of base-compositional biases (Pettigrew & Kirsch 1998), and if sufficiently extreme could affect the topology of a FITCH tree (Kirsch & Pettigrew 1998; Hutcheon *et al*. 1998).

Thus, we conclude that although the association of marsupials with monotremes found by Janke *et al*. and ourselves may be 'real', if it is not phylogenetically authentic then it might be a product of a 'short-branch attraction' exacerbated by a possible shared bias toward high GC content.

(e) *Conclusions*

Our results, and those of Janke *et al*. (1996, 1997), suggest a picture of mammalian sub- and infraclass relationships very different from that which has been accepted by most authors for many years. We have shown above that the anatomical, reproductive and palaeontological evidence may not be so contradictory of the molecular findings as first appears, even if the molecular trees could be somewhat influenced by algorithmic or biochemical artefacts. Although our estimate of the position of turtles is less certain, even as a technically reliable result, in common with the monotreme finding it underlines the point that that there may still be enormous surprises to be found in the phylogenies of supposedly well-studied groups such as the Amniota.

We are grateful to J. D. Pettigrew for the invitation to write this paper, and to J. M. Hutcheon for suggesting its title and for comments on the manuscript; M. Sánchez-Villagra also offered comments and provided additional bibliographic references. We thank L.-L. Chang Chien, T. Garland, R. Gundersen, C. Luedtke, C. A. Ross, K. Rusecki, and P. Weldon for tissue samples. The work reported here was initiated while G.C.M. was a Guyer Fellow in Zoology at the University of Wisconsin-Madison. Financial support for the laboratory work was provided by the School of Science & Technology and the Committee on Research and Creative Activity, University of Wisconsin-Parkside, and by private donations. Figures 1 and 2 were originally drawn using Page's (1996) TreeView program, and all figures were completed by William Feeny. This is Contribution No. 47 from the University of Wisconsin Zoological Museum Molecular Systematics Laboratory.

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