shown). This supports the link between turgor and glycerol accumulation but also implies that the melanized wall is largely impermeable to glycerol.

Appressoria of *M. grisea* are heavily melanized[9,10] and it has been shown genetically[11] that non-melanized appressoria fail to generate turgor and are non-pathogenic[5]. We found much lower levels of intracellular glycerol in appressoria from non-melanized strains carrying single gene mutations at *ALB1* and *RSY1* (Fig. 2), genes encoding enzymes required for dihydroxynaphthalene–melanin biosynthesis[10]. We found a similar reduction in glycerol accumulation after treatment of *M. grisea* with tricyclazole, a melanin biosynthesis inhibitor[12,13] (Fig. 2a). Thus, melanin biosynthesis is required for efficient glycerol accumulation.

In cytorrhysis experiments we found that *alb1*$^-$ mutant appressoria collapsed in hyperosmotic solutions of glycerol but quickly recovered (in under 1 min) and instead became plasmolysed[5]. This indicates that the non-melanized wall is permeable to glycerol and after initially causing cytorrhysis, glycerol diffuses through the cell wall and induces plasmolysis of the appressorial protoplast. This is in marked contrast to wild-type melanized appressoria which showed only limited recovery from cytorrhysis even after 48 h incubation in hyperosmotic glycerol (Fig. 2b). Maintenance of the enormous glycerol concentrations within appressoria is likely to be a consequence of the reduced permeability of melanized cell walls to glycerol preventing rapid leakage of the solute.

Several important plant pathogens form appressoria[1,2] and although secretion of enzymes may aid cuticular degradation[2], mechanical infection of plant tissues is probably widespread. The infinite solubility and metabolic compatibility of glycerol therefore provides a simple and durable mechanism for plant infection which may be widely applied by pathogenic fungi.

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Reptile relationships turn turtle...

Turtles are so anatomically bizarre that their affinities with other reptiles remain contentious. Wilkinson *et al.*[1] showed how even the extensive morphological information amassed by Rieppel and deBraga[2,3] provides only weak support for their view that turtles are advanced diapsid reptiles, rather than descendants of primitive anapsid reptiles, as conventionally thought. But there seem to be errors in Rieppel and deBraga's data matrix, many involving turtles or their putative 'anapsid' relatives[4,5]. I have corrected and reanalysed the data (see 'Incorrectly coded characters', overleaf), and find that Rieppel and deBraga's data actually support, rather than challenge, the traditional view.

The modified data yield a tree where diapsid monophyly is restored and turtles are related to anapsid pareiasaurs (Fig. 1a). This result is consistent with other recent analyses[4,5]. In particular, the tree is almost identical to that proposed in another detailed phylogenetic analysis of the entire Reptilia[6] (Fig. 1b). The large impact of these apparently minor corrections to Rieppel and deBraga's data set is not surprising.

In their phylogeny (Fig. 1 in ref. 2) almost all of the characters interpreted as supporting turtle–diapsid affinities (those diagnosing clades 3 to 7) also occur in all or many anapsid 'parareptiles', or are absent (presumed reversed) in turtles. Thus, only a slight modification to the data caused turtles to shift from deep within diapsids (as lepidosaur relatives) to deep within parareptiles (as pareiasaur relatives).

My results, together with those of Wilkinson *et al.*[1], emphasize the importance of evaluating just how strongly a preferred tree is supported over alternatives. Rieppel and deBraga have identified a surprisingly strong phylogenetic signal linking turtles and advanced diapsids. However, the conventional views regarding

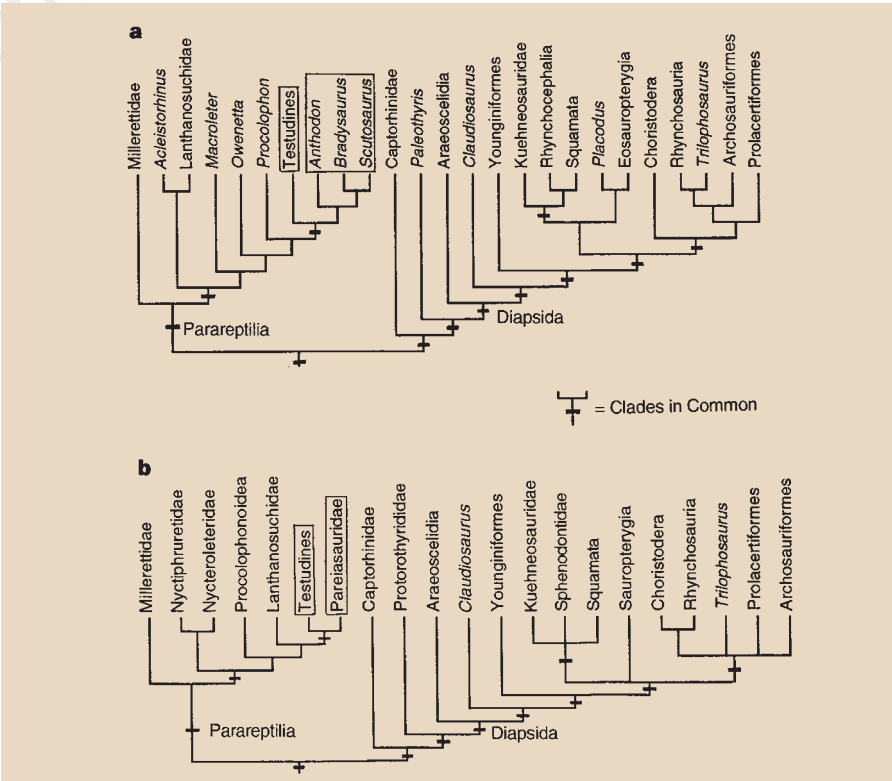

Figure 1 Alternative reptilian phylogenies. **a,** The single most parsimonious tree (length 763 steps, consistency index 0.511, retention index 0.695), based on an analysis of the data matrix of Rieppel and deBraga[2,3], after corrections detailed in footnote. Turtles (Testudines) are nested within 'anapsid' parareptiles, as the nearest relatives of pareiasaurs (*Anthodon*, *Scutosaurus*, *Bradysaurus*), and diapsid monophyly is restored. **b,** Phylogeny obtained from an independent study of higher-level reptile phylogeny[6]. Note the close similarity to **a**. Clades in common are indicated by horizontal bars. In both trees, diapsids are monophyletic and turtles are nested within parareptiles.

the anapsid derivation of turtles and the monophyly of diapsid reptiles need not yet be abandoned.

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Incorrectly coded characters. I have provisionally accepted codings for other disputed characters (for example the acromion process[2–5]) where there is disagreement over the interpretation of homologies but room for debate. The data set was analysed using the same methods and outgroup rooting as refs 2 and 3. Characters are numbered as in ref. 3. **44–46,** Stapes unknown in *Bradysaurus* and *Anthodon.* **65,** Basal tubera on basioccipital and basisphenoid present in all pareiasaurs[5]. **70,** Rieppel and deBraga interpret the anterior braincase ossification in the primitive turtle *Proganochelys* as unequivocally a sphenethmoid, but then code turtles as lacking the element, instead of as polymorphic. **73,** Interpterygoid vacuity closed in *Bradysaurus* and slit-like in *Anthodon*[7]. **77, 82,** Transverse flange of pterygoid oriented anterolaterally, and jaw joint located anterior to occiput, in turtles (primitively)[8,9]. **87,** Lateral shelf of surangular present in all pareiasaurs (for example, Figs 5-viii, 6 and Plate 33 of ref. 10). **103,** Cervical centra keeled in all pareiasaurs (for example, *Bradysaurus* BMNH R1971, *Scutosaurus* PIN 2005/1532-8, *Anthodon* BPI 1/548). **120, 121,** Two coracoids present in synapsids[11]. Rieppel and deBraga suggest that the acromion in turtles might represent a modified anterior coracoid. If so, the coracoid foramen in turtles lies totally within the coracoid homologues. Under their interpretation, turtles must be coded as equivocal for both coracoid number and coracoid foramen position. **124, 126, 127,** Humeral shaft short, supinator process absent, and ectepicondylar foramen present (primitively) in turtles[8]. **140,** Fourth trochanter absent in all pareiasaurs[5]. **150,** Well-defined articulation between astragalus and fourth distal tarsal present in *Scutosaurus* (for example PIN 2005/1877), condition unknown in *Bradysaurus* and *Anthodon.* **152,** First distal tarsal present (primitively) in turtles[8,12]. **160,** Metapodials overlapping proximally in *Bradysaurus* (for example, plate 37 of ref. 13) and *Scutosaurus* (for example PIN 2005/1532), condition unknown in *Anthodon.* **164,** Long unguals primitively present in turtles[8,9].

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...and turn back again

The conventional view of evolutionary relationships within the living reptiles is that turtles are basal to the other groups. Their placement is based largely on the absence in turtles of temporal fenestrae, openings on either side of the skull involved in jaw muscle attachment, which all other groups of living reptiles and their descendants have. Rieppel and deBraga's recent analyses[1,2] of fossil and living groups of reptiles challenged this long-held conclusion, although their results have since been questioned[3]. We present a molecular analysis in support of Rieppel and deBraga's original conclusions.

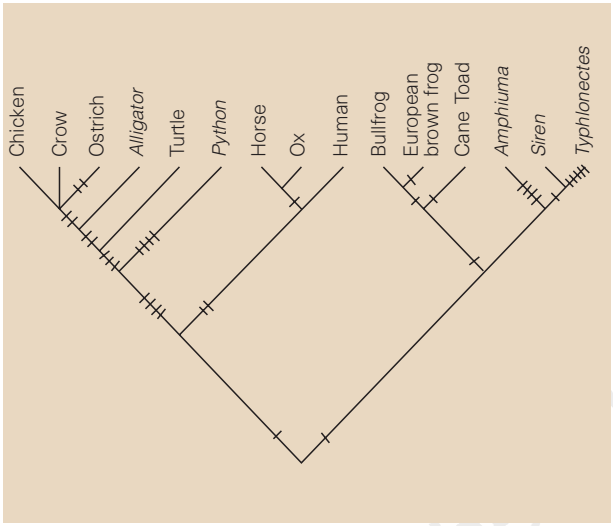

Figure 1 Strict consensus cladogram of the three shortest phylogenetic trees obtained by analysing tetrapod interrelationships on the basis of pancreatic polypeptide amino-acid sequences. Hash marks represent derived states for various amino acids. Those lying below a branch containing more than one taxon represent synapomorphies, each supporting the members of that branch. Similar marks on single terminal taxa are autapomorphies. The consistency index for each tree was 0.738.

Rieppel and deBraga[1] used 168 morphological characters, of which 13 (10 cranial and 3 postcranial traits) supported the placement of turtles within the ranks of reptiles that have twin temporal fenestrae on each side of the skull (the diapsid condition). On the basis of these 13 derived characters they concluded that there was robust support for considering turtles to be members of the crown group Diapsida. More recently, Wilkinson *et al.*[3] reanalysed Rieppel and deBraga's data[1] and found that by using different topological constraints it required only three additional steps (773 as opposed to 770) to produce a tree which supports the original consensus of basal status for turtles. Wilkinson *et al.*[3] acknowledged the difficulty of discerning the proper phylogenetic placement of turtles and suggested that molecular approaches would aid in determining the placement of turtles among the living reptiles.

Pancreatic polypeptide is a protein composed of 36 amino-acid residues and is a tetrapod product produced primarily in the islets of Langerhans. We determined the amino-acid sequence of pancreatic polypeptide for a representative of the turtles and compared it with published sequences for 14 additional tetrapod taxa. For phylogenetic analysis we used PAUP[4]. We examined representatives of five living taxa which have diapsid skulls or represent a derived condition from this ancestry (alligator, snake, and three species of birds). Nine additional taxa provided representatives of all other major groups of living tetrapods and include three orders of mammals and all three orders of living amphibians. The six amphibians represent tetrapods, a group that evolved before reptiles and were used in this study as the outgroup for rooting phylogenetic trees.

The 'branch and bound' method[4] of analysis resulted in three equally parsimonious trees of 84 steps each. Of the 36 residues in pancreatic polypeptide, 24 were informative in establishing the branching pattern in Fig. 1. Of these residues, 15 were useful in defining the two main elements (mammals and reptiles) within the ingroup taxa that separate into two major clades shown to the left in Fig. 1.

All three trees produced the same two assemblages (mammals and reptiles) with the same nodal memberships within each group (consensus indices, 100%). The only node not resolved was that of the birds. The reptile clade was supported by four shared derived characters (Fig. 1). Based on the conventional view of turtles as anapsid reptiles, we had initially expected them to be basal to the other reptiles and birds, but this was not the case in any of the three trees. The branch order determining the placement of turtles was always immediately after that of snakes, which are clearly diapsid reptiles.

Our results were the same regardless of the topology constraints invoked during analysis. This branch of the tree is supported by three shared derived characters adding a molecular perspective congruent with the analysis by Rieppel and deBraga based on morphological grounds. Our results are therefore consistent with the recognition of turtles as diapsids and provide a focus for additional molecular considerations, which Rieppel and deBraga[1] and Wilkinson *et al.*[3] encouraged.

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