(Fig. 2). Retinal analogues (all-*trans*-retinal, *n*-hexenal, β-apo-12′-carotenal, and 2*E*,4*E*-octadienal) could all be incorporated to restore phototactic sensitivity of the bleached zoospores, showing that the loss of sensitivity was due to the loss of native chromophore.

Many retinal analogues[10] have been bound *in vitro* to bovine opsin and bacterio-opsin, and *in vivo* to the opsin of the green alga, *Chlamydomonas reinhardtii*. These studies have revealed the chemistry of binding, the electric field and charge distribution within the binding site, and the shape of the site. Most retinal analogues shift the action spectrum away from that of the native pigment and also from the absorption spectrum of the unbound analogues, demonstrating their incorporation and function.

We compared retinal analogues bound in zoospores with the native chromophore that peaks at 536 nm. The analogue *n*-hexenal[7] blueshifts to 339±32 nm, β-apo-12′-carotenal[8] redshifts to 626±18 nm, and 2*E*,4*E*-octadienal blueshifts to 439±11 nm (Fig. 2). The sensitivities and spectral shifts are comparable with their incorporation in *C. reinhardtii*, suggesting similarities in the binding sites and the interaction of the chromophores with their protein environments. The activity of these analogues and the corresponding shifts of their action spectra show that these zoospores use a rhodopsin to track light for phototaxis.

The use of rhodopsins in phototaxis by both green algae and fungal zoospores suggests that vision may have evolved from the phototaxis of a unicellular ancestor. Because they use the same type of photoreceptor, it is not surprising that some phototactic chytridiomycetes can gather in the same places as the green algae that they parasitize[11]. Most important, a unicellular non-photosynthetic model system for a rhodopsin photoreceptor is now available.

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Uncertain turtle relationships

Turtles have generally been regarded as basal reptiles because they apparently retain the primitive anapsid skull pattern (no temporal fenestrae) unlike other extant reptiles which are diapsid (two temporal fenestrae on each side). However, the phylogenetic relationships between turtles and other amniotes is not well understood. Despite some disagreement over the details, recent phylogenetic analyses[1–3] seemed to be reaching a consensus that the turtles are the only surviving members of the Parareptilia, and thus only very distantly related to other extant reptiles. In contrast, by considering more morphological characters and more taxa than in previous studies, Rieppel and deBraga[4] propose that turtles are crown-group diapsids. We have tested their claim that this parsimony analysis "robustly supports the diapsid affinities of turtles".

We reanalysed the data from ref. 4 with the PAUP[5] software. Our unconstrained analysis replicated the results of ref. 4, yielding two most parsimonious trees with lengths of 770 steps in which the turtles are the sister group of *Placodus* and Eosauropterygia (position 1, Fig. 1). Remarkably, however, the shortest trees produced using topological constraints to force the turtles to lie within the Parareptilia required only three additional steps.

That such a small increase in tree length (< 0.4%) is needed to include turtles within the Parareptilia is not consistent with robust diapsid affinities of turtles. Furthermore, pairwise statistical comparisons of constrained and unconstrained trees reinforce this lack of robustness. We compared the fit of the characters to the alternative trees using the null hypothesis that each character is equally likely to support either tree. Using the Templeton–Felsenstein test[6,7], as implemented in PHYLIP[8], the most parsimonious trees in which turtles are parareptiles are not significantly less likely than those in which turtles are diapsids ($P > 0.05$). Application of the Wilcoxon signed ranks test[6] supported this conclusion ($P > 0.74$, two tailed).

It is interesting that relationships between the other taxa are unaffected by the widely divergent positions of the turtles in the unconstrained and constrained trees, indicating that the turtles are a particularly problematic taxon to classify. Without doubt, the highly distinctive and divergent morphology of turtles is one major obstacle to inferring their phylogenetic relationships and evolutionary origins with much certainty. Another obstacle is the limited morphological data available for extinct putative relatives such as pareiasaurs and placodonts, at least some of which must have extensive convergent similarities to turtles. Thus, barring the discovery of new and intermediate fossil forms, it seems unlikely that the available morphological data will prove sufficient to resolve the relationships of turtles convincingly.

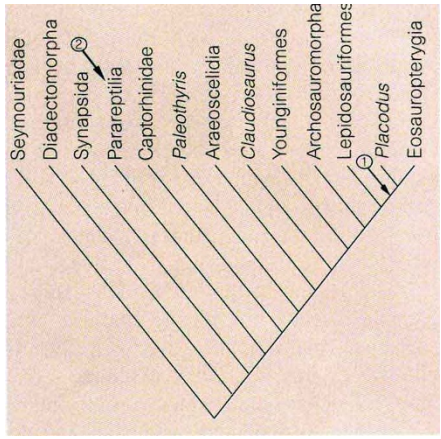

Figure 1 Amniote inter-relationships based on the data of ref. 4 showing the uncertain position of turtles. 1, Turtles as diapsids in unconstrained most parsimonious trees, requiring 770 steps[4]. 2, Turtles as parareptiles as posited in other studies[1–3], requiring only three extra steps.

The alternative hypotheses considered here (Fig. 1) differ in the relationships posited among the extant taxa, with either turtles more closely related to lepidosaurs (lizards) than to archosaurs (crocodilians) or archosaurs and lepidosaurs more closely related to each other than to turtles. They are thus amenable to testing using molecular and other neontological data. Molecular studies have yet to provide a well supported resolution of amniote relationships[9–11], but, simply by virtue of the potential wealth of sequence data, may yet provide our best chance of distinguishing between the alternative phylogenetic placements of turtles. Until such a time, phylogeneticists must be circumspect about the precise affinities of these enigmatic reptiles.

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