

# Comparing patterns of evolution: larval and adult life history stages and ribosomal RNA of post-Palaeozoic echinoids

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## SUMMARY

We have taken a total-evidence approach to the phylogeny of 29 extant echinoids, combining data from larval morphology, adult morphology, small subunit rRNA complete gene sequence and large subunit rRNA partial gene sequence: a total of 176 morphological and 121 molecular phylogenetically informative characters. Also included are 13 extinct taxa for which we know only adult morphology. Parsimony analysis of the combined data generated 28 equally parsimonious solutions, differing primarily in the positioning of a few fossil taxa. We reduced these to a single working hypothesis of echinoid relationships by pruning fossil taxa and one extant species.

Patterns of morphological evolution of larval and adult stages were compared by optimizing character sets onto the total evidence tree and assigning each character transformation to a branch. Branch nodes were dated by reference to the first appearance of one or other sister taxon in the fossil record. From this we demonstrate that larval and adult morphological evolution has proceeded in a mosaic-like fashion over the last 250 Ma. A similar comparison between morphological and molecular data finds equally weak correlation between rates of ribosomal RNA evolution and rates of morphological evolution, implying that morphology and ribosomal genes have also evolved largely independently. Larval characters appear to be more prone to homoplasy than adult characters, even when comparison is restricted to adult organs of similar size and structural complexity as the larvae. As morphological and molecular apomorphies accrue over time, there is a general correspondence between the duration of a particular branch and the number of apomorphies assigned to that branch. However, we found no evidence that overall molecular rates of evolution were any more strictly clock-like than morphological character transformations, although mapping transversions only improved the fit to a clock-like model for molecular data.

## 1. INTRODUCTION

The Echinoidea are an excellent group for evolutionary studies because of their skeletal complexity and relatively good fossil record. A direct reading of their phylogenetic history, as deduced from the fossil record, indicates a complex history that includes periods of rapid morphological radiation as well as periods of extreme conservatism. However, all that we glean from the fossil record is the history of adult morphology. There is no record of how larval morphology has changed (other than our ability to distinguish fossils with planktotrophic from non-planktotrophic life-histories on the basis of adult apical disk crystallography; see Emler 1985, 1989) and no fossil biomolecules have yet been recovered from geologically ancient echinoids. Thus we have only a partial and highly biased understanding of how evolution has proceeded in the Echinoidea.

Like most marine invertebrates, echinoids pass through a complex life history cycle composed of two phases which, in morphological terms, contrast highly: the adult benthic phase and the larval planktonic

phase (echinopluteus) (see figure 1). Furthermore, most of the complex morphological features of the larval stage are resorbed at metamorphosis, and adult structures form *de novo* from a rudiment on the larval body, so there is little obvious connection between larval and adult morphological characters.

We knew virtually nothing about the evolutionary history of echinoid larval forms until one of us (Wray 1992) compiled a 75 character data matrix for the echinoplutei of extant echinoids, optimizing character changes onto a cladogram constructed from adult morphology. Using this Wray investigated several aspects of the evolution of echinopluteus morphology concluding, amongst other things, that a number of important characters probably evolved very early on during the echinoid crown-group radiation. He argued that the persistence of major traits over time was evidence for strong selective pressure, and provided several examples of parallel evolutionary transformations that he interpreted as having strong functional importance. Finally, Wray observed that larval and adult characters probably displayed mosaic evolution, noting that larval morphology was very

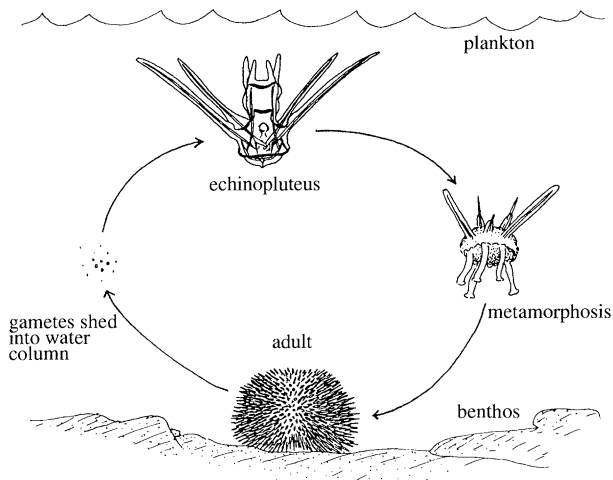


Figure 1. Schematic life-history cycle of an echinoid, with benthic adult phase alternating with pelagic planktonic phase. (Not to scale.)

highly conserved within Clypeasteroidea but highly varied in Echinoida, whereas the opposite was true for adult morphologies.

We wish to explore comparative patterns of evolution in a more rigorous way, to test whether larval and adult morphologies are evolving in tandem or independently, and to explore the relation between morphological and molecular evolution. The earlier study (Wray 1992) was largely anecdotal and flawed by having optimized larval characters onto a phylogenetic tree constructed solely from adult morphology. Both larval characters and adult characters provide phylogenetic information about the relationships of echinoid taxa, and there is no *a priori* reason for believing one to be more correct than the other. In particular, if we wish to ask whether larval characters show more convergence than adult characters, then it is important that we have a phylogeny that is not based solely on just one set of data, as this will minimize the homoplasy in that data set at the expense of the other.

We use a total evidence approach, combining all data before constructing a phylogenetic hypothesis. This approach identifies the best supported topology (and hence provides our best estimate of the correct phylogeny), and allows us to compare evolutionary patterns from diverse data sets directly (De Queiroz 1993; Eernisse & Kluge 1993; Kluge & Wolf 1993; Larson 1994). Subsequent mapping of groups of characters used to construct the tree implies no circularity, and is preferable to optimizing one subset of characters onto a tree constructed from another, independent, subset of characters (Deleporte 1993).

The questions that we wish to tackle in this paper are as follows.

1. Have larval and adult morphological traits evolved independent of one another or in concert?
2. Are larval characters more prone to homoplasy than adult characters or are homoplasy levels broadly similar?
3. Are rates of change in the ribosomal RNA gene correlated to morphological change? Do they correlate with elapsed time more closely than morphological characters?

## 2. METHODS AND MATERIALS

### (a) *Taxa and characters used*

We began with 29 species of Recent echinoid (see table 1), which we have scored for 163 morphological characters of the adult (136 phylogenetically informative characters, listed in Littlewood & Smith 1995). For 26 of these species we have compiled complementary information on their planktotrophic larval morphology (49 characters, of which 40 are phylogenetically informative). For *Fellaster zelandiae* we have used the larval characters of a closely related species (*Echinarachnius parma*). Of the other two species, one has lecithotrophic development and thus lacks a planktotrophic larva, and larval development of the other has never been reported. Larval characters were compiled from the original 75 characters used previously (Wray 1992), removing characters that were invariant amongst our 28 species, and deleting characters now known to be variable within species. The data matrix is available on request. Complementary to these morphological data we have complete small subunit ribosomal RNA (ssu rRNA) gene sequences for 22 of the species (278 characters, of which 85 are phylogenetically informative), and partial large subunit ribosomal RNA (lsu rRNA) sequences for 12 of these species (91 characters of which 36 are phylogenetically informative) (see data in Littlewood & Smith 1995). Finally, we also included an additional 13 fossil species for which we can score only adult morphological traits, but which belong to basal parts of long branches leading to extant taxa. Details of all taxa and their sources are given in Littlewood & Smith (1995).

Whereas we can objectively quantify the numbers of fixed point mutations that distinguish two or more molecular sequences, the definition of morphological characters and their transformational states is much more subjective. Morphological attributes cannot be codified objectively in the same way that nucleotide bases can, and the numbers and states of the characters defined will vary to some extent from observer to observer. However, we have tried to make our morphological data bases as comprehensive as possible, including all characters that have been used as differential characters by systematists in the past. Our assumption throughout this paper is that our morphological sets of characters, though neither totally objective nor exhaustive, are a reasonable and unbiased subset of all morphological characters that can be defined.

### (b) *Echinoid phylogenetic relationships: the working hypothesis*

Individual data sets (adult morphology, larval morphology, large and small subunit ribosomal RNA gene sequences) each provide independent evidence from which to estimate the phylogenetic relationships of the taxa under study. The true phylogenetic relationships of these taxa are unknowable, but parsimony analysis of each data set alone gives an independent estimate (with error) of this phylogeny.

Table 1. Recent species used in this study and the character sets available

(Full details of source for these echinoids is given in Littlewood &amp; Smith (1995).)

taxon	morphology		gene sequence	
	larva	adult	ssu rRNA	lsu rRNA
<i>Cidaris cidaris</i>	✓	✓	—	✓
<i>Eucidaris tribuloides</i>	✓	✓	✓	—
<i>Asthenosoma owstoni</i>	✓	✓	✓	✓
<i>Centrostephanus coronatus</i>	—	✓	✓	—
<i>Diadema setosum</i>	✓	✓	✓	—
<i>Cassidulus mitis</i>	—	✓	✓	—
<i>Echinolampas crassa</i>	✓	✓	—	—
<i>Fellaster zelandiae/Archanoides placenta</i>	✓	✓	✓	—
<i>Echinocyamus pusillus</i>	✓	✓	—	✓
<i>Encope aberrans</i>	✓	✓	✓	✓
<i>Echinodiscus bisperforatus</i>	✓	✓	✓	—
<i>Echinocardium cordatum</i>	✓	✓	✓	✓
<i>Meoma ventricosa</i>	✓	✓	✓	—
<i>Spatangus purpureus</i>	✓	✓	—	✓
<i>Brissopsis lyrifera</i>	✓	✓	✓	—
<i>Arbacia lixula</i>	✓	✓	✓	✓
<i>Stomopneustes variolaris</i>	✓	✓	✓	—
<i>Glyptocidaris crenularis</i>	✓	✓	—	—
<i>Temnopleurus hardwickii</i>	✓	✓	✓	—
<i>Salmacis sphaeroides</i>	✓	✓	✓	—
<i>Mespilia globulus</i>	✓	✓	✓	—
<i>Echinus echinus</i>	✓	✓	✓	✓
<i>Psammechinus miliaris</i>	✓	✓	✓	✓
<i>Paracentrotus lividus</i>	✓	✓	—	✓
<i>Strongylocentrotus intermedius</i>	✓	✓	✓	—
<i>Colobocentrotus atratus</i>	✓	✓	✓	—
<i>Tripneustes gratilla</i>	✓	✓	✓	—
<i>Sphaerechinus granularis</i>	✓	✓	✓	✓
<i>Lytechinus variegatus</i>	✓	✓	—	✓

These separate analyses produce cladograms that have many branches in common, or represent more or less resolved topologies that are compatible with one another. However, there are some significant differences in the position of a few taxa (notably the placement of *Arbacia* with the spatangoids in larval data and as sister group to Echinacea in ssu rRNA and adult morphology data). *A priori* we have no way of telling which is likely to be closest to the truth, so we have combined all our data into a single large matrix to determine where strong signal is to be found.

We ran a parsimony analysis on the data matrix of all data combined for the 29 Recent and 13 fossil taxa using the computer program PAUP (Swofford 1993: heuristic search with all characters treated as unordered and of equal weight). This found 28 equally parsimonious trees with most of the variation generated by instability of certain fossil taxa for which there was a large amount of missing data. When fossil forms are pruned from this tree only two equally parsimonious solutions for the remaining taxa result. These are not precisely the same as the trees constructed by parsimony analysis using just extant taxa alone; the inclusion of fossil taxa in the parsimony analysis alters relationships for one or two extant taxa compared to analyses from which they are omitted. The inclusion of fossils helps to discriminate homoplasy from homology because it provides a denser sampling of all character

associations that have existed within the clade (Smith 1994; Littlewood & Smith 1995).

The two rival cladograms differ only in the relative placement of *Psammechinus*, *Paracentrotus* and *Strongylocentrotus*. We resolved this conflict by pruning *Psammechinus* from the cladogram, to arrive at a single topology that represents our best working hypothesis of the relationships amongst 28 extant taxa. Bootstrap values, based on 1000 replicates, are greater than 70% for all but two branches of this cladogram (see figure 2a) and we use this topology in all subsequent analyses.

### (c) Character optimization and cladogram calibration

Each set of morphological or molecular data was optimized onto the total evidence cladogram and individual character transformations were assigned to a single branch. For morphological data pertaining to adults, a few trivial corrections to the computer-based optimization were required because there were instances where character convergence between two extant taxa, demonstrable as homoplasy in analyses where all taxa were included, were mistakenly listed as homologies in our 'living taxa only' cladogram. We could not make similar corrections for other data sets, but simply accepted the optimization as derived from character distributions of extant taxa alone. Although

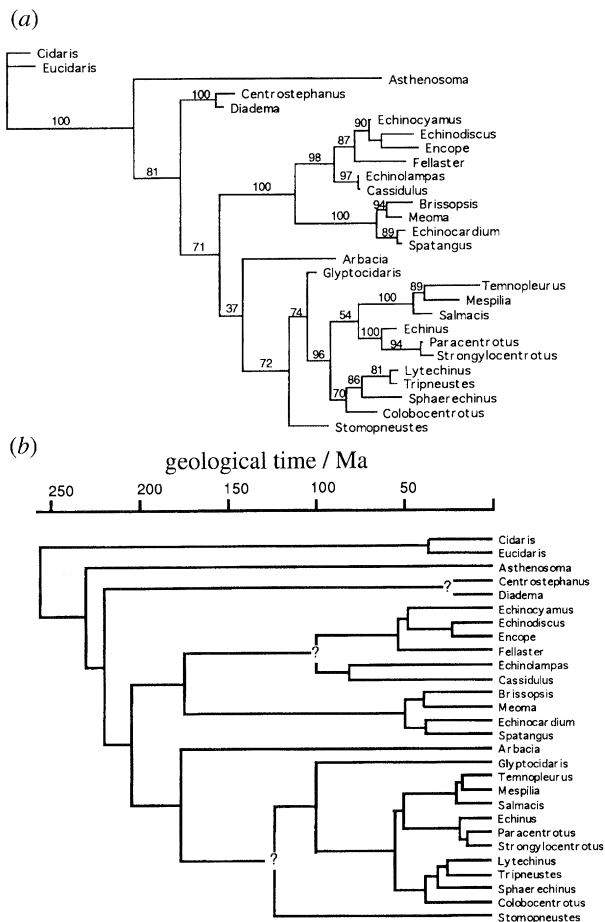


Figure 2. (a) Cladogram of 27 extant taxa used as our best working estimate of echinoid phylogenetic relationships. This was derived by parsimony analysis of the full 46 Recent and fossil taxa, and all 580 morphological and molecular characters, but fossil taxa and *Paracentrotus* have been subsequently deleted by pruning. Bootstrap values are shown for each branch based on 1000 replicates. (b) Calibrated evolutionary tree derived by using the fossil record of first appearances to date nodes identified in the combined data cladogram. Question marks indicate major branch points where there is little or no fossil evidence to date the event.

this will have introduced some error, we do not believe that it is either significant or systematic in its bias and all character reconstructions were checked for consistency. Where doubt existed, characters were partitioned between two or more branches. For each branch of our total evidence cladogram we therefore derived an estimate for the number of character transformations for larval and adult characters and for rRNA fixed point mutations.

Our rRNA data show that there have been major differences in the rate of molecular evolution amongst echinoids, with three clades with much longer branches than the rest. Consequently we did not feel we could use a molecular clock hypothesis to calibrate our cladogram, especially as one of our goals was to investigate how clock-like evolution has been amongst our different character sets. We therefore calibrated the total evidence cladogram by reference to the fossil record of adult morphologies. The first appearance in the stratigraphical record of a fossil with one or more apomorphies of a clade defines the latest divergence

time of that clade. For each pair of sister taxa we took the earlier first appearance to date the time of sister group divergence, and hence date the node on the cladogram. This provides estimates of the absolute time represented by branches on the cladogram. However, not all lineages have equally dense and well-sampled fossil records. Whereas we can be reasonably confident about dates such as the first appearance of irregular echinoids (Sinemurian, Early Jurassic, *ca.* 205 Ma BP), other parts of the cladogram are more problematic. For example, it is impossible to be precise about the dating of the split between *Diadema* and *Centrostephanus* because that clade has a very poor fossil record. Nor can we be certain as to the date at which cassiduloids split from clypeasteroids as the sister group of clypeasteroids amongst cassiduloids remains poorly constrained (Suter 1994). However, removing poorly dated nodes leaves 29 branches for which we have reasonably strong fossil evidence to provide estimates of their duration. A calibrated tree is shown in figure 2b.

### 3. RESULTS AND DISCUSSION

#### (a) *Has larval and adult morphological evolution proceeded independently or in concert?*

Given the very different modes of life that larva and adult echinoid pass through, it is likely that at least some of the selective pressures acting on one phase of the life cycle will be irrelevant to the other phase. Over geological time, therefore, one might expect adult and larval morphological character transformations to have gone on more or less independently. Such mosaic evolution (DeBeer 1958) has often been invoked to explain dissociated patterns of evolution between larval and adult form in echinoids and other groups (see, for example, Jablonski & Lutz 1983; Levinton 1989; Wray 1992). Conversely though, periods when morphological diversification of adult form was proceeding rapidly might also coincide with periods when larval morphology was also diversifying rapidly. Wray (1992) noted that many changes in the larval form appeared early on in the diversification of crown group echinoids, corresponding with the well-established rapid morphological diversification in adult form.

If adult and larval evolution have proceeded in tandem then branches of the evolutionary tree where adult morphology has been evolving rapidly should correspond to branches when larval character evolution has also been proceeding rapidly. Conversely, if larval and adult morphology have been evolving largely independently then we should see no correlation between periods of enhanced morphological evolution in larval and adult forms.

To address this question we compared the number of inferred larval character transformations with the number of inferred adult character transformations for each branch on the total evidence cladogram. Because our estimates of the number of changes are not precise, due to subjectivity of character definition and the problem of occasional homoplasies mistaken for homology, we have used a non-parametric rank correlation statistic which is dependent upon only the relative amount of change. Our total evidence cladogram

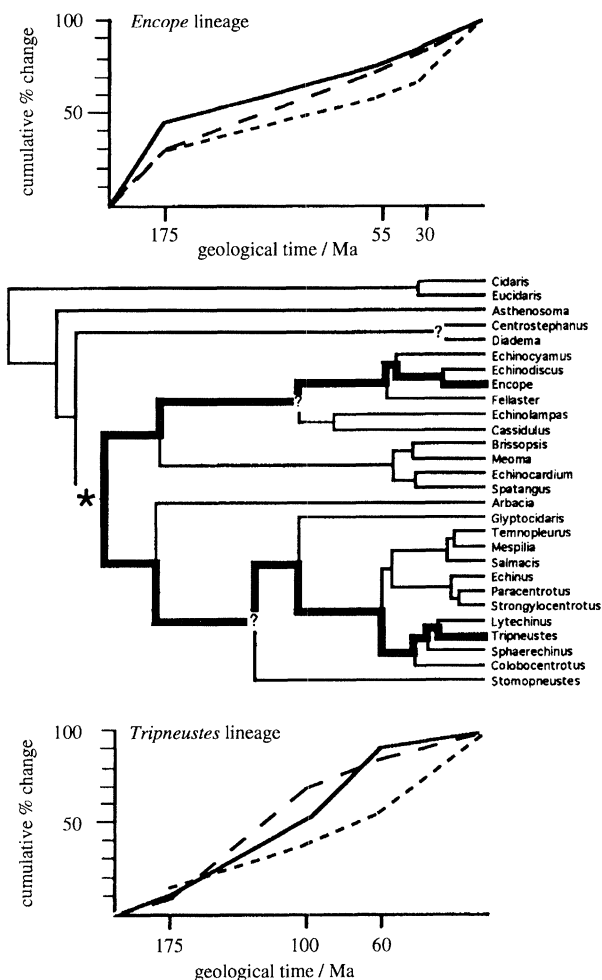


Figure 3. Number of character transformations as a cumulative percentage of the total calculated along two independent branch lineages (diverging from the split between Irregularia and Echinacea 205 Ma BP) for larval (long-dashed line), adult (solid line) and ssu rRNA gene (short-dashed line) data. Absolute dates are estimated from the dating of selected branch points using the first appearance of a fossil member of one or other sister group.

provides 46 branches where the numbers of both larval and adult character transformations can be estimated. Applying Spearman's rank correlation test gives a correlation coefficient of 0.35, showing that there is little evidence for concerted evolution between adult and larval life-history stages (see table 3). Although the largest number of character transformations in both cases occurred along the branch separating cidaroid and non-cidaroid taxa, the second largest change in terms of morphology (the origin of irregular echinoids) was associated with very little larval morphological transformation.

**(b) Are larval characters more prone to homoplasy than adult characters?**

Strathmann (1988) pointed out that amongst echinoderm classes, no matter what phylogenetic relationship was adopted, considerable convergent evolution must have occurred in larval form. At that time he was not sure whether this was unusual or whether the levels of homoplasy in adult characters were comparable. Wray

(1992) also noted a number of parallel transformations in different lineages of echinoplutei, emphasizing the prevalence of homoplasy amongst larvae as evidence of selective evolutionary pressures. Here we wish to address the question of whether larval characters show more homoplasy than adult characters.

The simplest and most direct measure of homoplasy is the Consistency Index (CI) (Swofford 1991) which describes the number of observed character transformations divided by the minimum number of possible character transformations, summed over all characters. A CI of 1 implies no homoplasy and homoplasy levels increase in the data matrix as the CI index approaches 0. However, there are problems with the CI index (Archie 1989; Sanderson & Donoghue 1989; Meier *et al.* 1991) as it is known to be affected by both the numbers of characters and taxa included in the matrix, and by the amount of missing data. In comparing the CIs derived from adult and larval morphological data optimized onto the total evidence tree we have removed all uninformative sites and are comparing cladograms based on the same number of taxa and with similar levels of missing data. However, the numbers of informative characters differ considerably, (136 adult characters as opposed to 41 larval characters) and may bias our result. Meier *et al.* (1991, see figure 3) showed empirically that data sets with fewer characters tend to have higher CIs.

Our 163 adult morphological characters optimized on the total evidence tree imply a total of 247 character transformations, giving a consistency index of 0.70 (excluding uninformative positions), a retention index of 0.88 and a rescaled consistency index of 0.64. By comparison, the 49 larval morphological characters optimized on the total evidence tree imply a total of 99 character transformations, giving a consistency index of 0.50 (excluding uninformative positions), a retention index of 0.75 and a rescaled consistency index of 0.41. Note that the CI is considerably lower for the smaller data set contrary to expectation. As the CI tends to increase not decrease with smaller sample size, the observed difference may even be underestimating the difference between adult and larval characters. Similar results are obtained using other measures of homoplasy such as the Retention Index and the Rescaled Consistency Index (see table 2). Thus at this level of analysis there is *prima facie* evidence for larval characters being more prone to homoplasy than adult characters.

However, a factor that may bias this analysis is the relatively small size and architectural simplicity of many of the larval characters compared to adult characters. Convergence in gross form may be more readily distinguished and differentially scored if there is structural complexity to support or contradict hypotheses of homology. Homoplasy amongst larval characters may therefore simply reflect the small size and simplicity of the elements being compared.

It is important therefore, for a more accurate assessment of homoplasy levels, that larval characters are compared specifically with structures in the adult of comparable size and complexity. Most larval characters refer to the structure and arrangement of

Table 2. *Consistency indices excluding invariant positions (CI), Retention indices (RI) and Rescaled consistency indices (RC) for morphological and molecular characters optimized onto the total evidence tree*

	number of characters	CI	RI	RC
larval characters	49	0.50	0.74	0.40
adult morphology (all)	163	0.70	0.88	0.64
test construction	8	0.78	0.91	0.74
apical disk	20	0.62	0.88	0.58
ambulacral structure	23	0.72	0.88	0.66
interambulacral structure	11	0.60	0.81	0.57
peristomial region	11	0.77	0.94	0.72
spines and tubercles	29	0.62	0.86	0.58
pedicellariae	18	0.58	0.79	0.51
perignathic girdle	4	0.80	0.92	0.76
lantern	29	0.71	0.89	0.66
internal anatomy	8	0.87	0.95	0.86
ssu rRNA, all variable positions	279	0.50	0.54	0.39

the small skeletal rods that together form a basket-like framework of the echinopluteus. The most obvious structures in adult echinoids of comparable size and complexity are the pedicellariae. These are small and relatively simple structures found on all echinoids and which have been given great importance in systematic work. Like larvae most of the characters are derived from the structure of the few simple skeletal elements of which they are composed (the stem and the three beak-like valves). Pedicellariae are appendages on the test and are functionally and structurally independent of other morphological traits.

In table 2 the 163 adult characters are partitioned into a number of subsets representing different skeletal and organ systems. Each subset of characters has been optimized over the total evidence tree to calculate homoplasy levels. Pedicellarial characters have higher homoplasy ( $CI = 0.6$ ) than any of the other subsets of adult morphological characters, but still show considerably lower levels of homoplasy than do larval characters ( $CI$  of 0.5). Indeed the  $CI$  for larval characters alone lies outside two standard deviations from the mean  $CI$  of the ten subsets of adult morphological characters. We therefore conclude that larval characters may indeed be more prone to homoplasy than adult characters of a similar structure and complexity. Note that levels of homoplasy amongst larval characters are comparable with those displayed by sequence data of the ssu rRNA gene: it is the adult morphological characters that display an anomalously low level of homoplasy. This may be because, historically, experienced systematists working with adult morphology have already sorted and rejected a number of potential characters as too homoplasious for consideration.

### (c) *Rates of morphological and molecular evolution over geological time*

Character transformations accumulate in lineages over time and, so long as there is not extensive character reversal, there will be a general correlation between the number of character transformations accumulated and the length of time between nodes. However, we know that this is not clock-like for adult morphological characters, as we have evidence from the fossil record that there have been short periods of geological time when considerable morphological diversification has occurred. The diversification of irregular echinoids between about 205 and 175 Ma BP, for example, was accompanied by a great number of morphological innovations in adult form.

Whereas adult and larval morphology are likely to have responded in an *ad hoc* way to external events, the same is less likely to be true for ssu rRNA gene sequence data. Although the sequence of nucleotide bases for the ssu rRNA gene is also under selective pressure (to maintain the mature molecule's functionality), it is not directly affected by external factors in the way that morphology is thought to be. Consequently there has been an expectation that molecular sequences will evolve in a clock-like manner (see, for example, Li & Graur 1991; Fitch & Ayala 1994). Although few now believe in the reality of a strict molecular clock (Vawter & Brown 1986; Runnegar 1991, p. 392), there is still a generally held belief that molecular sequence evolution proceeds more regularly than does morphological evolution (Olsen & Woese 1993). However, previous work on echinoderm LSU rRNA gene sequences (Smith *et al.* 1992) suggested that this molecule evolved just as irregularly over geological time as did adult morphology. Cumulative plots of number of changes against geological time for single lineages demonstrate that all three data sets (adult and larval morphology, ssu rRNA) show a positive correlation with time (see figure 3), but it is not immediately apparent whether any data set is significantly better correlated than the others.

To test whether molecular evolution of rRNA has occurred more regularly over geological time than morphological evolution we used the fossil record of first appearances to date nodes on the cladogram. This gave us 29 branches in total where we had estimates of duration (in Ma), numbers of adult and larval morphological character transformations, and the numbers of fixed point mutations and transversions for the ssu rRNA molecule.

Using Spearman's rank correlation test we found that statistical correlation between time and morphological character transformation is poor (see table 3). The correlation coefficient for adult morphology is just 0.42 and for larval characters is even less, at 0.29. However, molecular fixed point mutations of ssu rRNA are no better, and show a correlation coefficient of just 0.39. When transversions alone are considered, the correlation improves slightly, the coefficient rising to 0.47. We conclude from this that morphological and molecular rates of evolution are proceeding more or less equally haphazardly, but that the rate at which

Table 3. Spearman Rank Correlation coefficients ( $R_s$ ) obtained from pairwise comparison of numbers of apomorphies at each branch on the total evidence tree

(Estimated branch durations recorded as Ma.)

	$R_s$	number of pairwise comparisons
adult versus larval morphology	0.35	46
adult morphology versus ssu rRNA, all positions	-0.09	41
larval morphology versus ssu rRNA, all positions	-0.17	37
ssu rRNA loop versus stem regions	0.74	40
estimated branch duration versus adult morphology	0.42	29
estimated branch duration versus larval morphology	0.29	28
estimated branch duration versus ssu rRNA, all positions	0.39	29
estimated branch duration versus ssu rRNA, transversions only	0.47	29

transversions become fixed is more regular over time than morphological character transformation. This confirms Vawter & Brown's (1993) findings that, for ssu rRNA, transversions show the lowest rate of variation amongst different branches and are likely to produce the best 'molecular clock'.

We found no evidence for loop or stem differences being important in terms of rates of evolution. When loop and stem fixed point mutations are treated separately, they show a strong correlation of 0.76. Thus when loop regions are evolving rapidly so too are stem regions, and there is no real reason to favour one over the other for phylogenetic analysis.

Finally, we found that the transition/transversion ratio for the entire ssu rRNA molecule is 1.66:1. For branches that have arisen entirely within the last 60 Ma the ratio is 1.98:1, so there may be a tendency for longer or deeper branches to become saturated (and thus underestimate the rate of change along deeper branches). This is most noticeable in the long branches (> 220 Ma) leading to cidaroids and echinothurioids. However, shorter deep branches (before 175 Ma BP) show, if anything, higher transition/transversion ratios than recently diverged clades. Vawter & Brown (1993) have found no consistent transition-transversion bias in ssu rRNA genes and we are currently investigating the structural evolution of the ssu rRNA molecule in echinoids in order to clarify the meaning of this pattern.

#### 4. CONCLUSIONS AND PROSPECTS

The ability to construct robust phylogenies from the combination of several independent data sets allows the evolutionary behaviour of different kinds of characters to be compared over geological time. By including morphological characters as well as molecular characters, not only do we obtain the best possible estimate of a clade's phylogenetic history, but

we also gain the possibility of calibrating the cladogram and transforming it into an evolutionary tree through recourse to the fossil record. Applied to groups with a reasonably well-established fossil record this approach can provide estimates of branching times, allowing the question of rates of evolution through geological time to be tackled more rigorously. This in turn allows general questions that compare and contrast the way in which different segments of life history, or different kinds of molecular data (for example, non-coding versus coding sequences or transitions versus transversions) have evolved in a clade over geological time to be investigated. A great deal has been learned about the structure and evolution of ribosomal RNA genes over geological time (Vawter & Brown 1993; Gutell *et al.* 1994) but we envisage that well-documented phylogenies such as that presented here could help improve our understanding of their structural evolution.

We thank the following individuals for help in locating, providing and transporting echinoid material: S. Amemiya, R. Aronson, A. Baker, A. Bentley, H. Dixon, S. Donovan, R. Emson, C. Freire, P. Gayle, J. Gage, M. Gibbons, M. Hart, H. Hayashi, G. Hendler, W. Hide, T. Kikuchi, J. Korrubel, B. Lafay, D. McKenzie, T. Matsuoka, P. Mikkelsen, B. Morton, T. Motokawa, D. Nichols, S. Palumbi, N. Suzuki, J. Taylor, P. Tyler, T. Uehara, J. Woodley and C. Young. We would also like to thank Robin Gutell for providing us with the secondary structure of *Strongylocentrotus*. This study benefitted from the SEQNET facilities and was directly supported by NERC grant GR3/7960.

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