

## PHYLOGENETIC EFFECTS, THE LOSS OF COMPLEX CHARACTERS, AND THE EVOLUTION OF DEVELOPMENT IN CALYPTRAEID GASTROPODS

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**Abstract.**—Despite considerable theoretical and empirical work on the population genetic effects of mode of development in benthic marine invertebrates, it is unclear what factors generate and maintain interspecific variation in mode of development and few studies have examined such variation in a phylogenetic context. Here I combine data on mode of development with a molecular phylogeny of 72 calyptraeid species to test the following hypotheses about the evolution of mode of development: (1) Is the loss of feeding larvae irreversible? (2) Is there a phylogenetic effect on the evolution of mode of development? (3) Do embryos of direct-developing species lose the structures necessary for larval feeding and swimming and, if so, is the degree of embryonic modification correlated with the genetic distance between species? The results of these analyses suggest that mode of development evolves rapidly and with little phylogenetic inertia. There are three cases of the possible regain of feeding larvae, in all cases from direct development with nurse eggs. It appears that species with planktotrophic, lecithotrophic, or direct development with nurse eggs all have equal evolutionary potential and retain the possibility of subsequent evolution of a different mode of development. However, species with direct development from large yolky eggs appear to be subject to phylogenetic constraints and may not be able to subsequently evolve a different mode of development. Finally, species that have more recently evolved direct development have less highly modified embryos than older direct-developing species. Since species with nurse eggs generally have fewer embryonic modifications than those from large yolky eggs, this embryological difference may be the underlying cause of the difference in evolutionary potential.

**Key words.**—Comparative biology, *Crepidula*, *Crucibulum*, mode of development, nurse eggs.

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The evolutionary history of mode of development in marine invertebrates provides an excellent system for examining the loss and possible regain of complex, adaptive, and evolutionarily important traits. In most groups of marine invertebrates there are species in which small eggs develop into morphologically complex, free-living larvae that swim and feed in the water column (planktotrophic larvae). These species with planktotrophic larvae often appear to be closely related to species that produce larger eggs that either develop directly into juveniles or produce swimming, nonfeeding larvae. The complex characters used for planktotrophic larval feeding and swimming are often reduced or lost in nonfeeding or direct development. The rate and frequency of changes in mode of development, and the losses and possible regain of complex morphological features associated with larval swimming and feeding have been examined in the context of a species-level phylogeny for few groups. Here I use a molecular phylogeny of calyptraeid gastropods to investigate the evolutionary dynamics of mode of development.

Most groups of marine invertebrates display an enormous diversity in mode of development. Many genera or families include species with planktotrophic (planktonic feeding) development and species with nonfeeding development (e.g., calyptraeid gastropods: Hoagland 1986; Collin 2003a; *Conus* gastropods: Kohn and Perron 1994; asterinid starfish: Hart et al. 1997; *Ophionereis* brittlestars: Selvakumaraswamy and Byrne 2000). In many cases there are multiple kinds of non-feeding development (e.g., Huber et al. 2000; Collin 2003a). Interspecific correlative data show that mode of development is associated with egg size, clutch size, and duration of development in most groups. Mode of development has also

been demonstrated to vary with latitude (Thorson 1946, 1950; Ockelmann 1965; Mileikovsky 1971; Collin 2003a) and with depth in some groups, and might be associated with female body size (Strathmann and Strathmann 1982).

One class of potentially important factors in the evolution of mode of development that has not received widespread attention is phylogenetic effects (but see Strathmann 1978a; Wray 1996; Cunningham 1999; Duda and Palumbi 1999). Here I use the term phylogenetic effect to refer to the tendency of closely related species to be more similar to each other than they are to a randomly selected species (phylogenetic inertia of Blomberg and Garland 2002). Unfortunately, the few studies that have been framed in an explicit phylogenetic context include insufficient numbers of species or focus on groups with too little variation to adequately address the importance of phylogenetic effects (reviewed in Hart 2000; see Duda and Palumbi 1999 for an exception). Studies examining patterns of mode of development in marine invertebrates, from studies of egg-size distributions to studies of latitudinal patterns have therefore usually treated the data from closely related species as independent. These analyses have been conducted without accounting for the possibility of phylogenetic effects or constraints and therefore assume that each species' developmental characteristics are purely a reflection of the current selective factors that are acting on them. Because there are so many groups that display large variation in mode of development, as well as in developmental features such as egg size and hatching size, it may be reasonable to expect that these characters are evolutionarily labile and that phylogenetic effects may not be strong.

However, there are also good reasons to think that phylogenetic effects may be important in the evolution of mode

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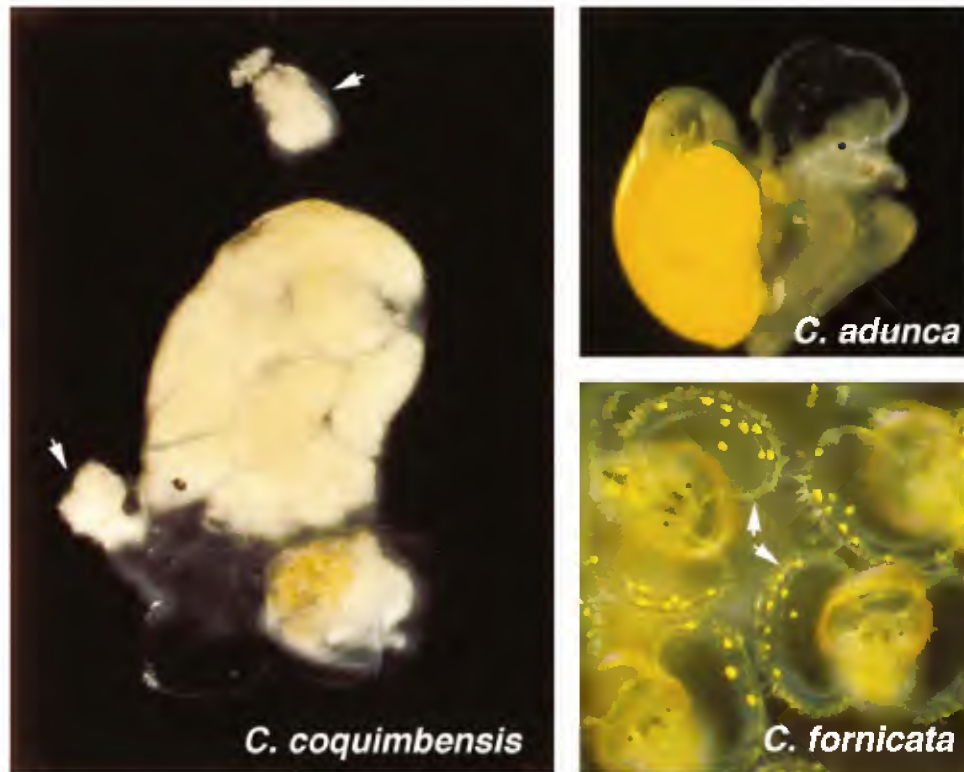


FIG. 1. Photomicrographs of different modes of development in calyptraeids. The typical planktotrophic larvae of *Crepidula fornicata* have yellow spots on the velum (arrows). Endogenous yolk supplies are visible in the embryo of the direct developing *C. adunca*. Embryos of *C. coquimbensis* ingest nurse eggs (arrows), which are stored in the embryo's head (above) prior to being transferred to the viscera (lower right).

of development. There are several groups in which there is no variation in mode of development. For example, there are no species of articulate brachiopods, crinoids, fissurellid gastropods, or patellogastropods with feeding larvae (Strathmann 1978a). The most convincing argument that there may be phylogenetic effects on the evolution of mode of development is based on ideas about the evolution of complex characters. In most groups of marine invertebrates the planktotrophic larvae have a number of complex morphological features used for swimming and feeding in the water column. These features are often (but not always) reduced or lost in direct-developing species. The evolutionary loss of complex morphological characters is often considered irreversible (Simpson 1953) and has been used to justify the idea that the loss of feeding larvae is irreversible. Such a constraint on the evolution of mode of development would be a special case of a phylogenetic effect and fits McKittrick's definition of phylogenetic constraint ("any result or component of the phylogenetic history of a lineage that prevents an anticipated course of evolution in that lineage" McKittrick 1993, p. 309).

Here, I combine data on the development of 78 species of calyptraeid gastropods (Collin 2003a) with a phylogeny for the family based on molecular data (Collin 2002, 2003b) to examine how mode of development evolves and how many times it changes, and to answer the following questions: (1) Is the loss of feeding larvae irreversible? (2) What is the extent of the phylogenetic effect on the evolution of mode of development? (3) Do embryos of direct-developing species

lose the structures necessary for larval feeding and swimming and, if so, is the degree of embryonic modification correlated with the genetic distance between species?

## MATERIALS AND METHODS

### *Calyptraeid Development*

Numerous modes of development are present in calyptraeids: there are species with planktotrophic larvae, species with lecithotrophic larvae, species with direct development from large eggs, and species with direct development from small eggs in which the embryos consume other eggs within the same capsule (reviewed in Hoagland 1986 and Collin 2003a; Fig 1; Table 1). Here I use the data from Collin (2003a), which reviews and documents aspects of the development of 78 species of calyptraeids, in combination with a molecular phylogeny of the group (Collin 2002, 2003b). Most of the observations of development were from the same populations as the material used for the phylogenetic analysis (Collin 2002, 2003b).

### *Calyptraeid Phylogeny*

Two best estimates of the phylogeny of calyptraeid gastropods were obtained from Bayesian and matrix-weighted parsimony analyses of a combined dataset of COI, 16S, and 28S DNA sequence data (Collin 2002, 2003b). The taxa for which mode of development is unknown were pruned from

TABLE 1. Character coding for models for development in calyptreaeids.

Five-state coding	Abbreviation	Three binary coded characters		
		Swimming	Feeding	Nurse eggs
Planktotrophic	pt	Yes	Yes	No
Lecithotrophic	lt	Yes	No	No
Lecithotrophic with nurse eggs	ltne	Yes	No	Yes
Direct development	d	No	No	No
Direct development with nurse eggs	dne	No	No	Yes

both phylogenies. The phylogenies present in Collin (2002, 2003b) include more than a single individual from broadly distributed species. Because mode of development is not thought to vary within most caenogastropod species (Hoagland and Robertson 1988; Bouchet 1989), and there is no evidence that poecilogony (i.e., such within species variation in mode of development) occurs in any calyptreaeids, the inclusion of more than one individual from a single species would create a phylogenetic effect at the level of the present analysis. Therefore, the individual representing the population for which most developmental data are available was retained and additional individuals believed to belong to the same species were removed from the phylogeny. Phylogenies of the ingroup alone as well as the ingroup plus a small outgroup (trichotropids and capulids) were analyzed. Because the results of these two analyses did not generally differ (Collin 2002) the analyses for the ingroup alone are presented here.

#### *Test for Irreversibility of Character Loss*

To test for irreversibility of the loss of feeding larvae, I reconstructed the evolution of mode of development on the best-estimate trees using parsimony and maximum likelihood. Mode of development is a complex character that can be broken down into several components. I first coded mode of development as an unordered five-state character (0, planktotrophic; 1, lecithotrophic larvae from large eggs; 2, lecithotrophic larvae from nurse eggs; 3, direct development from large eggs; 4, direct development with nurse eggs; Table 1). This five-state character was coded as unordered since there is no evidence the lecithotrophic development has to occur as an intermediate between planktotrophy and direct development or that direct development with nurse eggs precedes the evolution of direct development from large eggs in this group. I also divided mode of development into three binary characters: presence/absence of (1) larval feeding, (2) larval swimming, and (3) nurse eggs. Parsimony was used to reconstruct state changes for these characters on the tree (MacClade; Maddison and Maddison 1992), by varying the relative weights of the transitions between states and their reversals from 1:1 to 1:4 (changes and reversals equally likely, and changes four times as likely as reversals, respectively) and by counting the number of character-state changes and reversals observed on the tree for each transition weighting. Areas with equivocal reconstructions were examined with transformations both accelerated and delayed along the branches of the phylogeny (ACCTRAN and DELTRAN options in MacClade).

I also used maximum likelihood as implemented in the

program Discrete (Pagel 1999a) to reconstruct the pattern of binary character state evolution on the Bayesian estimate of calyptreaeid phylogeny. I used a likelihood-ratio test to determine whether a model with different frequencies of character-state gains and losses (alpha and beta in Discrete) is significantly better than a model in which the frequencies of gains and losses are equal (alpha = beta in Discrete). For each character, I used the most likely model to reconstruct the likelihood of each character state at each internal node using the local reconstruction option in the graphics menu in Discrete. Those nodes in which the likelihoods of the two states differed by more than two log units were considered to provide significant support for one state at that node in preference to the other state (Pagel 1999a).

#### *Examination of Phylogenetic Effects*

I employed three different approaches to test for phylogenetic effects on the evolution of mode of development. First, if the evolution of a character is under some type of phylogenetic constraint it might evolve more slowly than expected otherwise. Therefore the length of the character (i.e., the number of changes) on the phylogeny should be shorter than expected for a random character. In addition, if a character state changes less frequently than expected at random there should be less homoplasy and the retention index (RI; Farris 1989) values should be greater than expected at random. To test these predictions for mode of development, I examined character length and RI of the developmental characters on the best-estimate trees and compared them to two different types of random distributions. First I calculated the character length and RI for the same characters on 10,000 random trees created in PAUP\* (Swofford 1998). This gives the distribution of expected values if the species relationships and tree topology are random. Because realized values of RI are somewhat dependent on tree topology, I also asked whether the distribution of developmental characters on the known topology is different from a random distribution of states across the tips of the same topology. Each character was randomly shuffled 5000 times using the shuffle command in MacClade to create a random distribution of characters on the fixed "best topology," and the character length and RI were calculated for each of the 5000 replicates. Again, the values observed on the best-estimate trees were compared to the random distribution.

For the second test for phylogenetic effects, I examined the relationship between sister taxa. If there is no phylogenetic effect, pairs of sister species should share the same mode of development as frequently as expected at random. If there is a phylogenetic effect that causes close relatives to

be similar due to shared history (phylogenetic inertia), then sister species should share the same mode of development more frequently than expected at random. If there is a phylogenetic effect that causes close relatives to have different characters (for example, due to reproductive niche partitioning or character displacement among close relatives), this phylogenetic repulsion would result in sister species sharing the same mode of development less frequently than expected at random.

A chi-square test was used to find deviations from the expected numbers of pairs with similar and different development. Because it is not clear what factors might be driving the possible patterns, this analysis was conducted by coding development in two ways. First, I coded each species as having either direct or planktotrophic development. Direct development with and without nurse eggs and lecithotrophic development probably do not differ with respect to dispersal, hatchling size, and maternal investment per embryo and were therefore all coded as "direct." Second, I coded all the different observed modes of development as different states (planktotrophic, direct development with big eggs, direct development with nurse eggs, and lecithotrophic). The number of species expected for each cell in the chi-squared contingency table was obtained by calculating the observed frequency of each mode of development among all the species that have a single sister. The probability of randomly obtaining pairs with either the same or different development was then calculated from these frequencies (see Tables 2 and 3).

Finally, I used a phylogenetic generalized least-squares (PGLS) approach to examine the variance and covariance of characters explained by the relationships of the taxa (i.e., phylogenetic variance; Martins and Hansen 1997; Pagel 1997). The implementation of PGLS in Continuous (Pagel 1997) uses a parameter,  $\lambda$ , for the phylogenetic variance. I implemented the likelihood-ratio tests as outlined by Pagel (1999a,b) to determine whether there is significant phylogenetic variance. The overall likelihood of the PGLS model was calculated when  $\lambda = 0$  (no phylogenetic effect) and using the maximum likelihood estimate of  $\lambda$ . A likelihood-ratio test was used to determine whether the model including  $\lambda$  was significantly more likely than the model that does not account for phylogenetic variance. I examined  $\lambda$  for egg size and hatching size, the two continuous characters I have measured for the most taxa (see Collin 2003a).

*Correlation of Genetic and Embryological Divergence*

The idea that the loss of feeding larvae is irreversible is based on the assumption that species that lack feeding larvae lose the embryological structures used by planktotrophic species for feeding and swimming. It is a common observation that direct-developing embryos can be highly modified, but it has also been observed that some species with direct development show little modification (littorinid gastropods: Moran 1999; *Crepidula dilatata*: Chaparro et al. 2002). It is reasonable to think that the loss of structures used for planktotrophy would occur gradually after the evolution of direct development and that those direct-developing species that retain larval morphologies have not yet had time to lose them.

TABLE 2. Chi-square test of association of mode of development in sister species for the weighted parsimony analysis of ingroup only. pt, planktotrophic; d, direct; dne, direct with nurse eggs; o, other; n, sample size; p, proportion.

	Observed			Expected		
	Planktotrophic	Direct	Other	Planktotrophic	Direct	Other
Same	12	16	0	$10.58 \text{ pt} \times \text{pt} \times n$	$14.58 (1 - \text{pt}) \times (1 - \text{pt}) \times n$	$0.5$
Different	11	11	5	$12.42 \text{ pt} \times (1 - \text{pt}) \times n$	$12.42 \text{ pt} \times (1 - \text{pt}) \times n$	$0 \times o \times n$
$n = 50; \text{pt} = 0.46; 1 - \text{pt} = 0.54; \text{df} = 1; \chi^2 = 0.652; P > 0.1$						
	Observed			Expected		
	Planktotrophic	Direct	Other	Planktotrophic	Direct	Other
Same	12	12	0	$\text{pt} \times \text{pt} \times n$	$d \times d \times n$	$0.98$
Different	11	3	7	$12.42 ( \text{pt} \times d + \text{pt} \times \text{dne} + \text{pt} \times o ) n$	$10.5 ( \text{pt} \times d + d \times \text{dne} + d \times o ) n$	$6.02 ( \text{dne} \times \text{dne} + d \times \text{dne} + \text{dne} \times o ) n$
$n = 50; \text{p}(\text{pt}) = 0.46; \text{p}(d) = 0.3; \text{p}(\text{dne}) = 0.14; \text{p}(o) = 0.1; \text{df} = 3; \chi^2 = 19.905; P = 0.006$						

TABLE 3. Chi-square test of association of mode of development in sister species for the Bayesian ingroup only.

A	Observed		Expected					
	Planktotrophic	Direct	Planktotrophic	Direct				
Same	10	16	9.19	15.19				
Different	11	11	11.81	11.81				
$n = 48$ ; $p(\text{pt}) = 0.4375$ ; $1 - p = 0.5625$ ; $df = 1$ ; $\chi^2 = 0.04$ ; $P > 0.9$								
B	Observed				Expected			
	Planktotrophic	Direct	Direct with nurse eggs	Other	Planktotrophic	Direct	Direct with nurse eggs	Other
Same	10	12	0	0	9.19	4.69	1.02	0.52
Different	11	3	7	5	11.81	10.31	5.98	4.48
$n = 48$ ; $p(\text{pt}) = 0.4375$ ; $p(\text{d}) = 0.3125$ ; $p(\text{dne}) = 0.1458$ ; $p(\text{o}) = 0.1042$ ; $df = 3$ ; $\chi^2 = 18.497$ ; $P < 0.001$								

If this is the case, there may be a time after the evolution of direct development, before the larval structures are lost, during which planktotrophy could re-evolve. This scenario would receive support if direct-developing species that had diverged recently from a sister showed less embryological modifications than direct developers that are more distant from their planktotrophic sisters.

To examine this idea I scored the degree of embryological modification of each direct-developing species of calyptroid. I scored each species for modifications of the following characters: operculum, embryonic kidneys, shell coiling, distinct velum, food groove, swimming, feeding, and modified head vesicle (Appendix 1). Genetic distance (a proxy for evolutionary time) between the species pairs was calculated as the number of changes in COI reconstructed by parsimony along the branches separating the pair on the most parsimonious topology and by the sum of the Bayesian estimates of COI branch lengths separating the pair on the Bayesian estimate of phylogeny. Distances were limited to COI because it was the most complete molecular dataset, and 16S and 28S showed very little variation between some of the closely related taxa examined here. The differences in the scores of developmental modification were plotted against the COI distances. Species pairs in which both species are planktotrophic were excluded from the analysis because there were no embryological modifications in these pairs.

## RESULTS

### *Irreversibility of Character Loss*

Parsimony reconstruction of the mode of development on the most parsimonious tree showed that loss of feeding larvae occurs frequently but that reversals may have occurred in three places on the tree (Fig. 2). An equal-weighted unordered character state reconstruction between the five states of mode of development with ACCTRAN found planktotrophy as the ancestral condition (Fig. 2) regardless of whether the two planktotrophic outgroups were included. According to this reconstruction, direct development from large eggs arose 11 times, direct development with nurse eggs arose eight times, and there were five independent origins of lecithotrophic development. In addition, there were three cases in which planktotrophic development was regained: two cases from ances-

tral direct development with nurse eggs (the *C. onyx* clade and the *C. dilatata* clade), and a single case in which it is equivocal whether planktotrophy re-evolved from direct development with or without nurse eggs (the *C. aculeata* clade). When DELTRAN reconstructions were used, there were 12 independent origins of direct development, nine origins of direct development with nurse eggs, and only a single re-evolution of planktotrophy (from the *C. dilatata* clade). When the loss of planktotrophy was given half the weight of the regain of planktotrophy, there is a single possible regain of planktotrophy, in the *C. dilatata* group, but this is sensitive to the choice of ACCTRAN or DELTRAN reconstructions. When reversals to planktotrophy were weighted more highly, the re-evolution of planktotrophy was not reconstructed.

When this complex character mode of development is broken down into its component parts the pattern is less clear (Fig. 3). With equal-weighted transitions and reversals, the ancestral reconstruction of the binary character of larval swimming is ambiguous, with six independent origins of swimming and 10 losses. The ancestral reconstruction of larval feeding suggests that nonfeeding development is ancestral, with 15 independent origins of planktotrophy and five subsequent losses. This apparently disagrees with the results of the reconstruction of the characters as a single complex character. However, a 1:2 transition weighting gives results more similar to the multistate reconstructions. With a 1:2 weighting, the ancestral calyptroid condition is reconstructed as swimming planktotrophic larvae. There are 19 reconstructed losses of larval feeding and 17 losses of larval swimming. For both characters there are two clear and one ambiguous reversals. With a 1:3 weighting, larval swimming and feeding are never regained.

When the evolution of nurse eggs is reconstructed using equally weighted gains and losses, there are nine or 10 independent origins and one or two losses. When the weighting is increased to 1:2 there is possibly a single loss, and with a weighting of 1:3, there are 12 independent origins and no losses of nurse eggs.

The results of likelihood reconstructions of two binary characters using Discrete (Fig. 4) show that character state gains and losses do not occur at significantly different frequencies in swimming or feeding (i.e.,  $\alpha = \beta$ ,  $LR < 0.1$ ,  $df = 1$ ,  $P > 0.5$ ;  $LR < 0.3$ ,  $df = 1$ ,  $P > 0.5$ ; respectively).

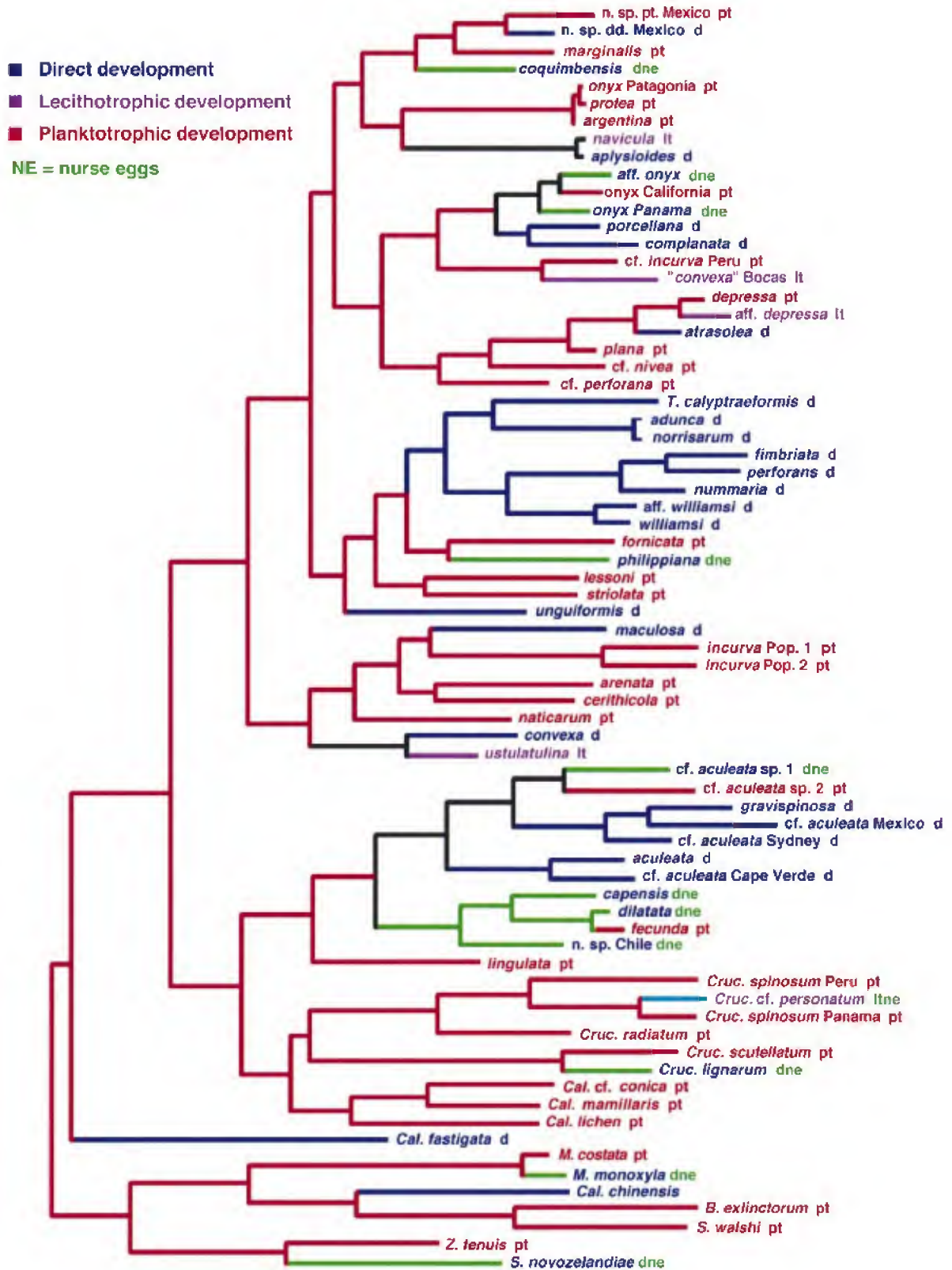


FIG. 2. Equal-weighted parsimony reconstruction of the five-state character mode of development on the parsimony tree of calyptraeids (a pruned version of the tree reported in Collin 2002).

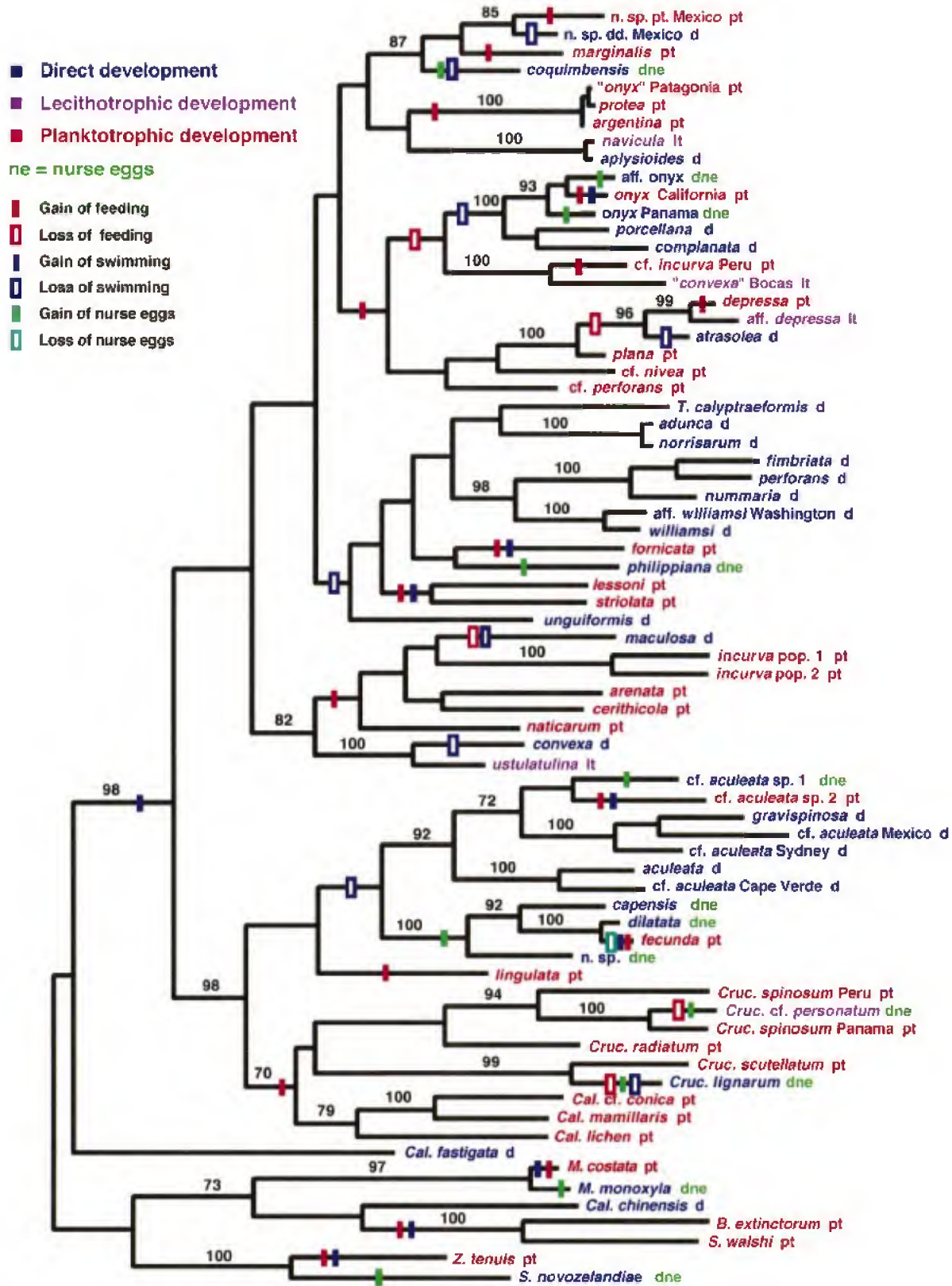


FIG. 3. Equal-weighted parsimony reconstructions of the binary characters larval feeding, larval swimming, and nurse eggs on the most parsimonious tree (a pruned version of the tree reported in Collin 2002). The character state changes are indicated on the branches where they are reconstructed by ACCTRAN. Numbers above branches are bootstrap support for the branches that occur in the unpruned tree.

Local reconstruction of the character states at individual nodes shows that both states of the swimming and feeding characters are more or less equally likely at almost every node in the tree, but there are a few nodes on short branches where one state receives significant support (Fig. 4). The nurse eggs character does show asymmetric evolutionary transitions ( $\beta = 4.7 \times \alpha$  LR 10.97,  $df = 1$ ,  $P < 0.001$ ) with loss of nurse eggs almost five times more likely than gaining them. The presence or absence of nurse eggs could not be reconstructed at most nodes of the tree with any statistical confidence (Fig. 3).

#### Examination of Phylogenetic Effects

Comparisons of the observed character length and RI for developmental characters with distributions generated by both 10,000 random trees and 5000 shuffled characters give no support for phylogenetic effects on the presence/absence of nurse eggs or presence/absence of feeding larvae ( $P > 0.05$ ; Fig. 5). The observed character lengths of mode of development and presence/absence of larval swimming were significantly shorter than expected ( $P < 0.05$ ; Fig. 5) and the RI for both characters were significantly greater than expected ( $P < 0.05$ ; Fig. 5). This supports the idea that there is some phylogenetic inertia in larval swimming and in the five-state mode of development character.

Chi-square analysis of the frequency of sister species with similar or different modes of development also gave some support for the presence of phylogenetic effects. When development was scored as a binary (planktrophic/direct) character the frequency with which sister species had the same or different mode of development did not differ from the random expectation ( $P > 0.9$ ; Tables 2 and 3). However, when the mode of development was divided into four states (planktrophic, direct development, direct development with nurse eggs, and lecithotrophic) there were significant deviations from the random expectation ( $P < 0.01$ ; Tables 2 and 3). The frequency with which direct-developing species without nurse eggs were paired with sisters with the same mode of development was much higher than expected, whereas the frequency with which they were paired with species with different development was lower than expected (Tables 2 and 3). The frequency with which species with the three other types of development were paired with similar or different species was not different from random. This suggests that there is phylogenetic inertia among the species with direct development without nurse eggs, but that there is little support for phylogenetic effects for species with other types of development.

Examination of the character reconstruction on the phylogeny suggests that the significant results of the chi-square analysis and of the comparisons of character length and RI to random distributions is the result of a single large clade of direct-developing species (including *C. adunca*, *Trochita calyptraeformis*, and the flat white species from the northern Pacific). When this single clade is removed from the analysis, the randomization tests show that the observed character lengths and RIs are no longer different from random. Removal of the three species pairs from this clade in the chi-square analysis also results in a loss of statistical significance.

Removal of two other clades with a preponderance of the same mode of development (the *C. dilatata* group and the *C. aculeata* group) does not have this effect. This pattern could be caused by asymmetric rates of character transitions to direct development from other forms of development and from direct development to other types of development. It could also be due to a higher rate of speciation in direct developers with large eggs than in species with other modes of development. This could lead to a greater number of sister species with direct development.

Likelihood-ratio tests fail to reject the null hypothesis that  $\lambda = 0$  (LR = 0;  $P = 1$ ; Fig. 6) for both egg size and hatching size independently as well as for the combination of egg size and hatching size for the 42 taxa for which both variables are available (Collin 2003a). This shows there are no detectable phylogenetic effects on these continuous variables.

#### Correlation of Genetic and Embryological Divergence

Comparisons of embryonic modifications and genetic divergence for the sister-species pairs for which developmental data were available (Appendices 1 and 2), and for which at least one species was not planktrophic, showed that the degree of developmental modification may be correlated with genetic differentiation (Fig. 7). The developmental divergence between sister species increased with both the parsimony and Bayesian divergence in COI ( $n = 12$ ;  $r^2 = 0.36$ ;  $P = 0.041$ ;  $n = 12$ ;  $r^2 = 0.46$ ;  $P < 0.02$ , respectively). When the outlier from the analysis of the Bayesian distance data is removed, the regression is still marginally significant ( $n = 11$ ;  $r^2 = 0.36$ ;  $P = 0.05$ ). This suggests that the amount of embryological divergence correlates somewhat with time since divergence.

#### DISCUSSION

Among marine invertebrate biologists there is a rich tradition of research focused on patterns and processes in development. However, the present study is one of the first to examine the evolutionary patterns of mode of development in an explicit phylogenetic context that includes a large proportion of the extant species. The results presented here reinforce those of previous more limited studies (reviewed in Hart 2000) in documenting pervasive changes in mode of development throughout a clade.

#### Phylogenetic Patterns of Mode of Development

The diversity of modes of development found among calyptraeid species makes them an ideal group in which to examine possible ecological factors influencing the evolution of life histories and development. However, this rich diversity also complicates studies of the phylogenetic patterns of development. The high frequency of three of the five modes of development in calyptraeids mean that a high number of sister-species pairs differing in mode of development are expected if mode of development is distributed at random. A cursory examination of the distribution of development and the high number of sister-species pairs that differ in mode of development makes it appear superficially that divergence in developmental features may be somehow causally asso-



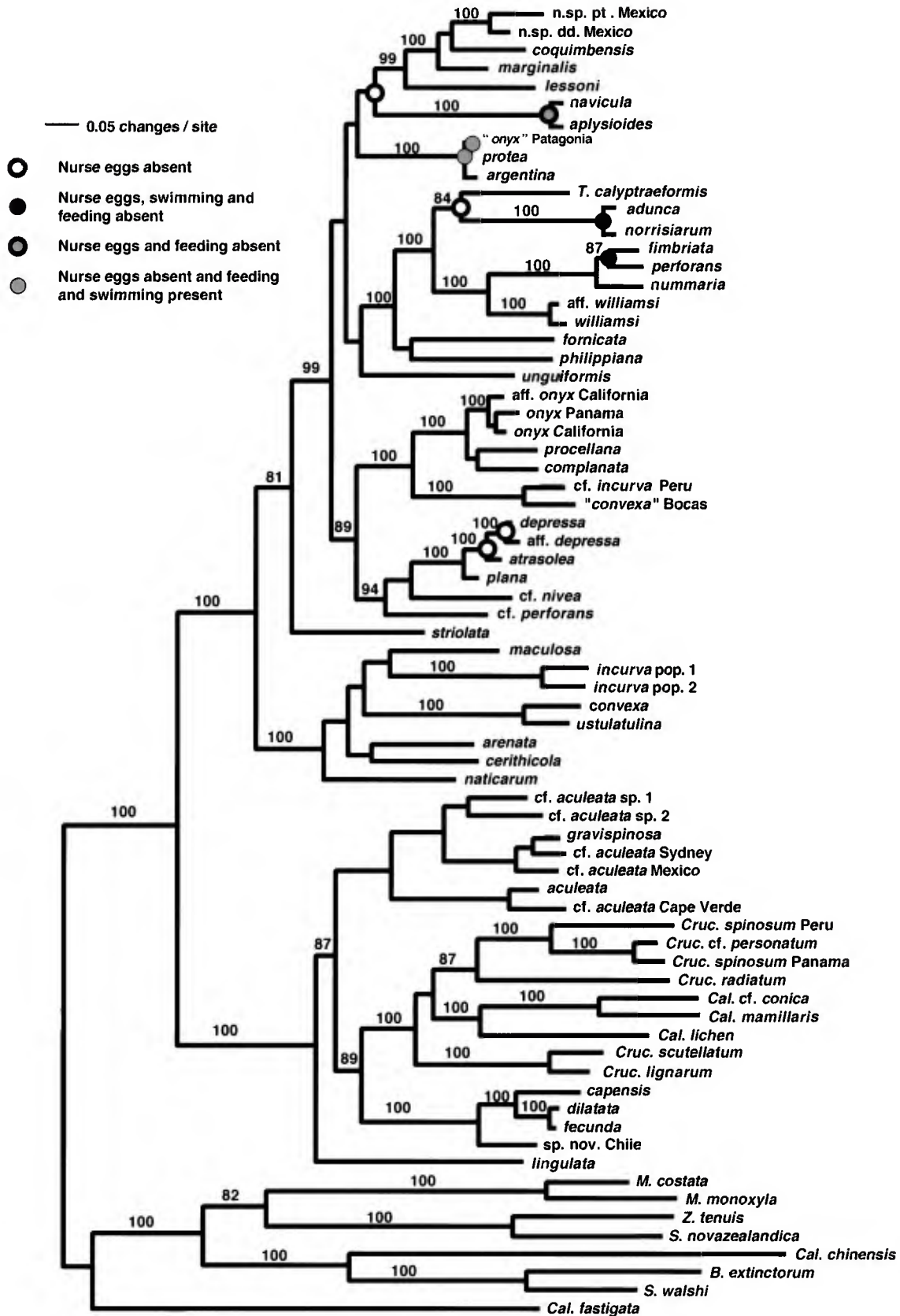


FIG. 4. Likelihood reconstructions of the three different binary characters from the local option in the graphics menu in Discrete (Pagel 1999a,b), on the Bayesian estimate of calyptraeid phylogeny (a pruned version of the tree reported in Collin 2002). The reconstructions of larval swimming and feeding are more or less equally frequent at all internal nodes, whereas nurse eggs are reconstructed as absent

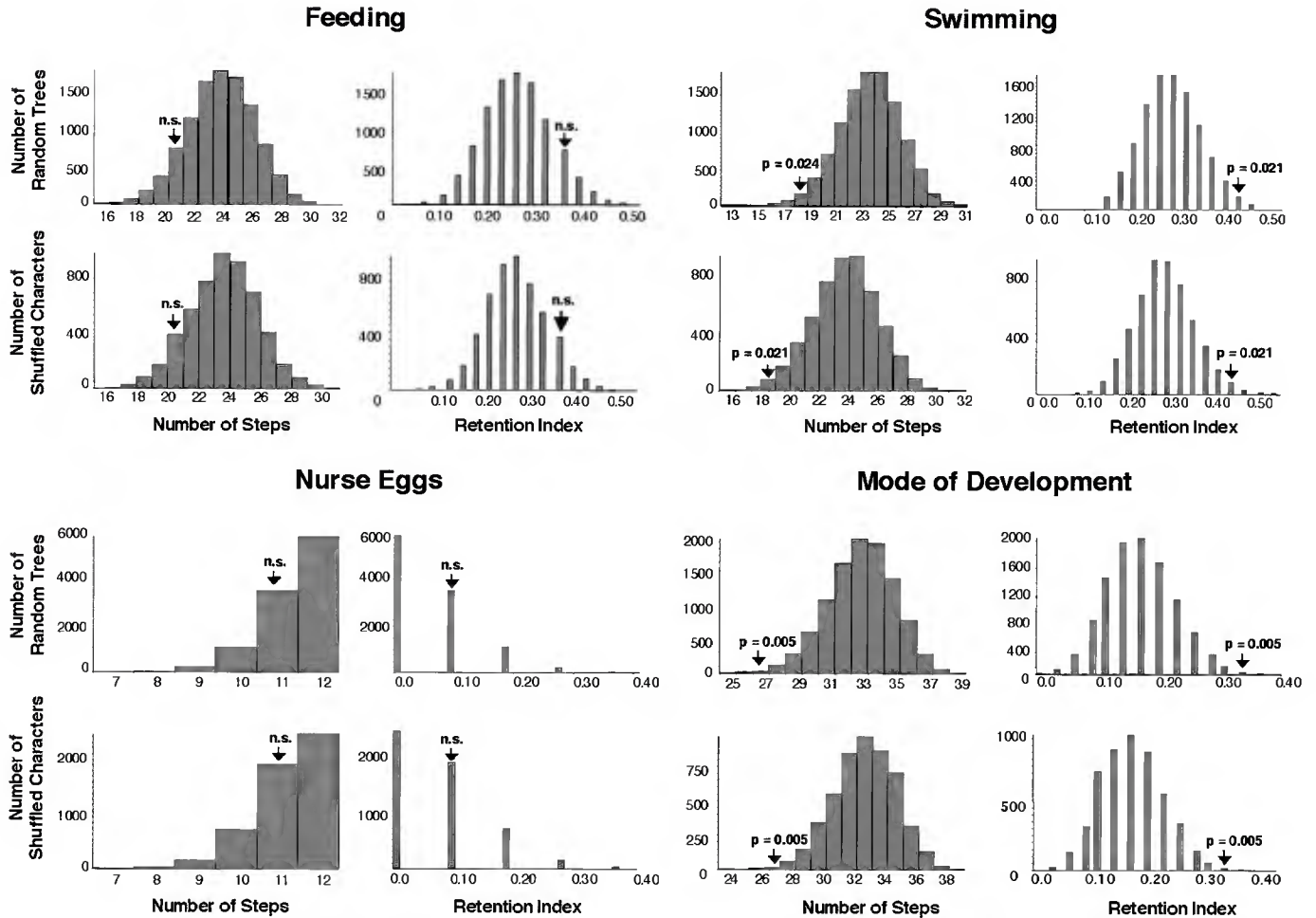


FIG. 5. Histograms of the distribution of character length and retention index for larval feeding, larval swimming, nurse eggs, and mode of development, from 10,000 random trees and 5000 shuffled character sets. The values observed on the best-estimate parsimony tree are indicated on each distribution with an arrow. n.s., nonsignificant.

ciated with speciation in this group (e.g., via character displacement). The results reported here show that sister species do not differ in mode of development more often than expected at random. This result could change if more sister-species pairs with differing mode of development are discovered. As it stands, however, it appears that mode of development is randomly distributed across the calyptraeid phylogeny, with the exception of a single clade of eight direct-developing species. These results demonstrate that there are no phylogenetic effects or constraints that operate across the tree, but at least one clade shows that there are phylogenetic effects within smaller groups.

Very different phylogenetic patterns of mode of development are found in echinoid echinoderms and polychaete worms. In echinoids, planktotrophy is significantly more common than direct development, and direct development appears to occur in isolated twigs on the phylogeny (Smith

1997). The isolated occurrence of direct development in single species in several genera of predominantly planktotrophic species has been used to suggest (in combination with the loss of complex larval characters in direct developers) that the loss of feeding larvae is irreversible (Strathmann 1978b; Wray 1996). In polychaete worms, there is also a high diversity of mode of development. It appears that most of the variation in mode of development occurs among families rather than within families and could therefore result from phylogenetic effects (Rouse 2000). Unfortunately, development has been documented for few species in each polychaete family. The observed phylogenetic distributions of echinoid and polychaete mode of development have not been compared to random expectations, and therefore these results may be due to the random distribution of a rare character (in echinoids) and sparse sampling (in polychaetes). Future studies seeking to understand patterns in the evolution of mode

←

twice as often as they are reconstructed as present. However, only at the nodes indicated with circles is there statistically significant support for the reconstructions. The legend indicates which reconstructions are supported. Numbers above the branches indicate Bayesian support for those nodes in the unpruned tree.

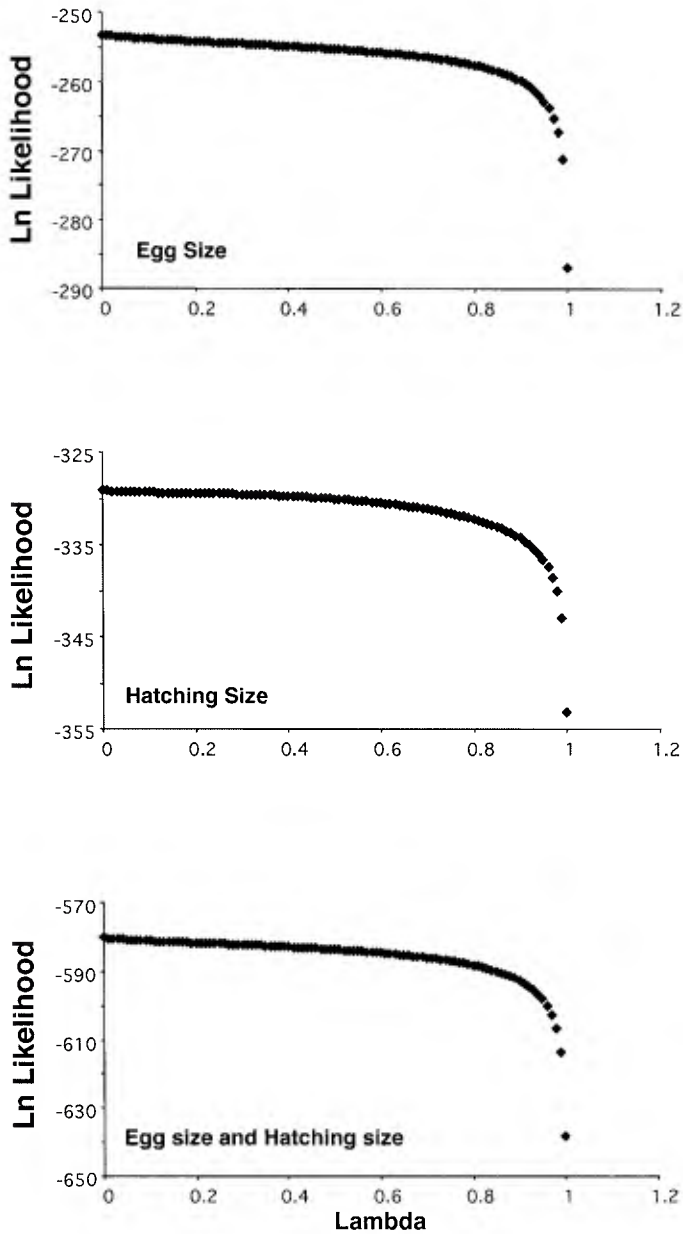


FIG. 6. Likelihood function of  $\lambda$  for comparative data on egg size, hatching size, and egg size and hatching size together, as estimated by Continuous.

of development should compare the observed distributions of different types of mode of development with random expectations before drawing conclusions about evolutionary constraints.

The high levels of variation in mode of development in calyptraeids also compromise the confidence with which ancestral states can be reconstructed. Because very closely related species differ in mode of development, the likelihood estimates of transition rates are high. This results in the reconstruction of either state as possible at most of the internal nodes because they are separated by relatively long branches. The only nodes for which likelihood strongly favors one reconstruction over the other occur in areas of the tree where

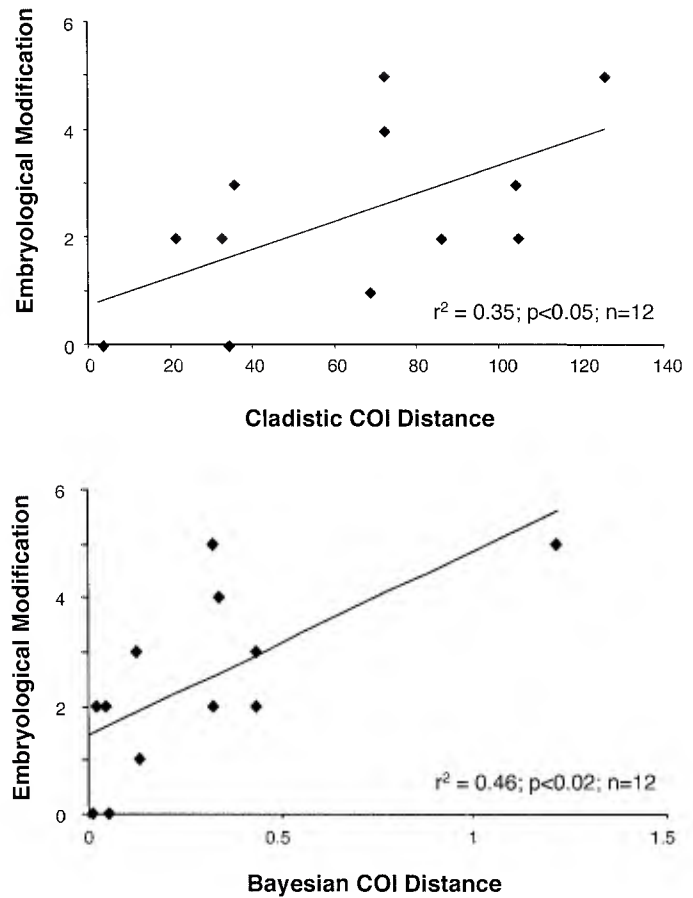


FIG. 7. Scatter plot and regressions of developmental versus genetic COI distances for sister species pairs that differ in development. (Top) Parsimony topology distances ( $n = 12$ ;  $r^2 = 0.36$ ;  $P = 0.041$ ) and (Bottom) Bayesian topology and distances ( $n = 12$ ;  $r^2 = 0.46$ ;  $P < 0.02$  and  $n = 11$ ;  $r^2 = 0.36$ ;  $P = 0.05$  when the high outlier is excluded).

there are several very closely related species with the same mode of development. At the deeper nodes the reconstructions appear to reflect the overall proportion of the states among the operational taxonomic units. This pattern of more or less equal support for either state deep in the tree was also obtained in the likelihood analyses of mode of development in echinoid and asterinid echinoderms (Cunningham 1999). Although the taxon sampling in the asterinid phylogeny is on a level with the sampling of calyptraeids reported here, most of the nodes near the tips of the tree are reconstructed with high confidence. This suggests that the rate of evolution of development is slower in this group of starfish. Analysis of the echinoid tree results in little confidence in the reconstructions at any of the nodes. This is most likely the result of the peculiar taxon sampling. In this case an assortment of echinoid genera are scored for the presence or absence of nonfeeding development in such a way that genera in which there are species with both feeding and nonfeeding development are sometimes scored as nonfeeding (e.g., *Heliocidaris*) and sometimes as planktotrophs. And in yet another case two nonfeeding species from a single genus are coded separately. This artificially inflates the proportion of direct

developers in the analysis and therefore increases the likelihood of reconstructions of direct development at internal nodes. If each of the numerous species with feeding larvae in the genera coded as feeding were included in the analysis as well as the species with feeding larvae from genera coded as nonfeeding, the states could probably be reconstructed with greater statistical confidence.

Characterization of calyptraeid development as feeding versus nonfeeding or swimming versus nonswimming is an obvious oversimplification of a complex multistate character. Because other studies of marine invertebrate development often characterize development in this way (e.g., Leiberman et al. 1993; Cunningham 1999) it is useful to compare the evolutionary patterns observed in the complex character with those observed in the simplified binary characters. The parsimony reconstructions of the binary and complex characters give roughly concordant results toward the tips of the tree. For all codings, possible re-evolution of feeding larvae is reconstructed in the *C. dilatata*, *C. aculeata*, and *C. onyx* clades. The major difference occurs in the reconstructions deeper in the tree, where the multistate coding supports planktotrophy as the ancestral condition but the reconstruction of the binary characters varies with weighting scheme and the inclusion of outgroups. The parsimony reconstructions are entirely consistent with the results of the likelihood reconstructions.

#### *Re-evolution of Planktotrophy*

The mapping of mode of development on the tree suggests there are three clades in which planktotrophy may have evolved from direct development (the *C. aculeata*, *C. onyx*, and *C. dilatata* groups). Interestingly, in all three cases the sister species to the putative secondary planktotroph is a species with direct development with nurse eggs. This is consistent with the result from the chi-square and the randomization tests, which show that species with direct development from large eggs appear to be subject to some kind of phylogenetic constraint, whereas mode of development evolves randomly in species with planktotrophic larvae, lecithotrophic larvae, and direct development with nurse eggs. This suggests that direct development with nurse eggs in some way retains the evolutionary potential for regaining planktotrophy, whereas direct development from large eggs does not. Observations of embryonic development of larval features (Appendix 1) show that it is more common for direct developers with nurse eggs to retain a distinct velum with a food groove than it is for direct developers without nurse eggs to retain these structures. In only two cases, *C. monoxyla* and *C. philippiana*, do direct developers with nurse eggs lose all velar characters. In both of these species only a single embryo develops per capsule, and the juveniles are unusually large. The embryological characters for *C. philippiana*, figured in Gallardo (1977), are strikingly similar to those of *C. monoxyla* (R. Collin, pers. obs.).

An idea commonly cited in the literature is that lecithotrophic or facultative feeding larvae are transitional, intermediate forms between planktotrophy and direct development (Emlet 1986; Hart 1996; Wray 1996). Wray (1996) argues that this is the pattern observed in echinoid devel-

opment. In the calyptraeids examined here, species with lecithotrophic larvae were equally likely to be sister to planktotrophs or to direct developers but do not consistently appear in a sequence between the two. It has also been suggested that direct development with nurse eggs is an intermediate state between planktotrophy and direct development without nurse eggs. However, among the calyptraeids, direct developers with nurse eggs are usually more closely related to planktotrophs than they are to species with direct development from large eggs. They do not occur in an intermediate position between planktotrophs and direct developers with large eggs anywhere on the tree. Such sequences of evolutionary intermediates could easily have been obscured by extinction, but the data presented here are insufficient to address the probability of such events.

#### *Conclusions*

Evolution of mode of development is not subject to strong phylogenetic effects within the Calyptraeidae and therefore mode of development appears to reflect ecological factors rather than historical constraints. As would be expected for such an adaptive character, mode of development evolves rapidly among the sampled species. Patterns of mode of development on the phylogeny and of embryonic modification in direct-developing species both support the idea that direct developers with and without nurse eggs have different evolutionary potentials. Species with direct development with nurse eggs appear in clades with species that are possibly secondarily planktotrophic, they do not usually lose embryonic larval features, and they do not appear to be subject to phylogenetic constraint. Species with direct development from large eggs produce more highly modified embryos, appear in at least one unusually large clade of exclusively direct developers, and are responsible for the detected phylogenetic effects. The rapid, diverse evolution of mode of development in calyptraeids makes it difficult to reconstruct the ancient patterns of evolution in their development. However, the speed and frequency with which changes in mode of development evolve in this group make them ideal for studies of the details and processes of divergence in development.

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APPENDIX 1.  
Coding for modifications of development. Question marks indicate character modifications unknown.

Species	Operculum	Embryonic kidneys	Shell coiling	Distinct velum	Food groove	Swimming	Feeding	Head vesicle	Score
Codings	0 = present 1 = absent	0 = simple 1 = multiple 1 = very long	0 = coiled 1 = uncoiled	0 = present 1 = small 2 = absent	0 = visible 1 = not visible	0 = swimming 1 = not swimming	0 = feeding 1 = not feeding	0 = small empty 1 = large 1 = large yolky	
All planktotrophs									
<i>Crepidula</i> aff. <i>depressa</i>	1	0	0	0	0	0	0	0	0
<i>C. atrasolea</i>	1	0	0	0	0	0	1	0	2
<i>C. complanta</i>	1	1	1	2	1	1	1	0	4
<i>C. procellana</i>	?	?	?	?	?	?	?	?	9
<i>C. aff. onyx</i> Panama	0	0	0	1	0	1	1	?	?
<i>C. aff. onyx</i> California	0	0	0	1	0	1	1	0	3
<i>C. "convexa"</i> Bocas	0	0	1	0	0	0	1	0	3
<i>C. aplysioides</i> <sup>1</sup>	?	?	?	?	?	?	1	0	2
<i>C. navicula</i> <sup>2</sup>	?	?	?	2	1	1	1	?	?
<i>C. n. sp. dd.</i> Mexico	1	0	0	1	0	1	1	?	?
<i>C. coquimbensis</i>	1	absent	1	2	1	1	1	1	5
<i>C. nummaria</i>	?	?	?	?	?	?	1	1	8
<i>C. perforans</i>	?	?	?	?	?	?	1	?	?
<i>C. fimbriata</i>	1	1	1	1	1	1	1	?	?
<i>C. aff. williamsi</i>	1	1	0	2	1	1	1	0	7
<i>C. williamsi</i>	1	1	0	2	1	1	1	1	8
<i>C. norrisarum</i>	1	1	1	2	1	1	1	1	9
<i>C. adunca</i>	1	1	1	2	1	1	1	1	9
<i>Trochita calyptraeformis</i>	0	1	0	1	1	1	1	0	5
<i>C. plitippiana</i> <sup>3</sup>	1	?	0	1	1	1	1	0	5
<i>C. unguiformis</i>	?	?	?	?	?	?	1	?	?
<i>C. ustulatulina</i>	1	0	1	0	0	0	1	0	3
<i>C. convexa</i>	1	0	1	2	1	1	1	0	7
<i>C. maculosa</i>	?	?	?	?	?	?	1	?	?
<i>C. cf. aculeata</i> Cape Verde	?	?	0	?	?	?	1	?	?
<i>C. aculeata</i>	0	1	0	1	1	1	1	0	5
<i>C. cf. aculeata</i> Sydney	1	1	0	2	1	1	1	0	7
<i>C. gravispinosa</i>	?	?	0	?	?	?	1	?	?
<i>C. cf. aculeata</i> Mexico	0	0	0	1	0	1	1	0	3
<i>C. cf. aculeata</i> sp. 1	0	0	0	1	0	1	1	0	3
<i>C. dilatata</i>	0	0	0	0	0	1	1	0	2
<i>C. capensis</i>	1	0	1	2	1	1	1	1	9
<i>C. n. sp.</i> Chile	0	0	0	0	0	1	1	0	2
<i>Crucibulum lignarum</i>	0	0	0	0	0	1	1	0	2
<i>Crucibulum</i> cf. <i>personatum</i>	0	0	0	0	0	0	1	0	1
<i>Maoricrypta monoxyla</i>	1	?	0	2	1	1	1	0	6
<i>Calyptraea chinensis</i> <sup>4</sup>	?	?	0	0	0	1	1	?	?
<i>Calyptraea fastigata</i>	1	1	0	2	1	1	1	1	8
<i>Sigapatella novozelandiae</i>	0	?	0	0	0	1	1	?	?

<sup>1</sup> Miloslavich and Penchaszadeh 2001.

<sup>2</sup> Miloslavich and Penchaszadeh 1997.

<sup>3</sup> Gallardo 1977.

<sup>4</sup> Troncoso et al. 1988.

APPENDIX 2.  
Developmental and genetic distance between sister species pairs.

Species pair	Distances		
	Developmental	Parsimony COI	Bayesian COI
<i>Crepidula depressa</i> × aff. <i>depressa</i>	2	32.32	.059
<i>C. onyx</i> × cf. aff. <i>onyx</i> California	3	35.33	.133
<i>C. "convexa"</i> Bocas × cf. <i>incurva</i> Peru	2	85.6	.332
<i>C. n. sp. dd</i> Mexico × <i>n. sp. pt.</i> Mexico	5	71.92	.335
<i>C. aff. williamsi</i> × <i>williamsi</i>	0	33.95	.066
<i>C. norrisarum</i> × <i>adunca</i>	0	3.29	.008
<i>C. philippiana</i> × <i>fornicata</i>	5	125.61	1.227
<i>C. ustulatulina</i> × <i>convexa</i>	4	72.05	.348
<i>C. aculeata</i> sp. 2 × <i>aculeata</i> sp. 1	3	103.94	.446
<i>C. fecunda</i> × <i>dilatata</i>	2	20.87	.030
<i>Crucibulum scutellatum</i> × <i>Cruc. lignarum</i>	2	104.33	.450
<i>Crucibulum</i> cf. <i>personatum</i> × <i>spinosum</i> Panama	1	68.28	.141