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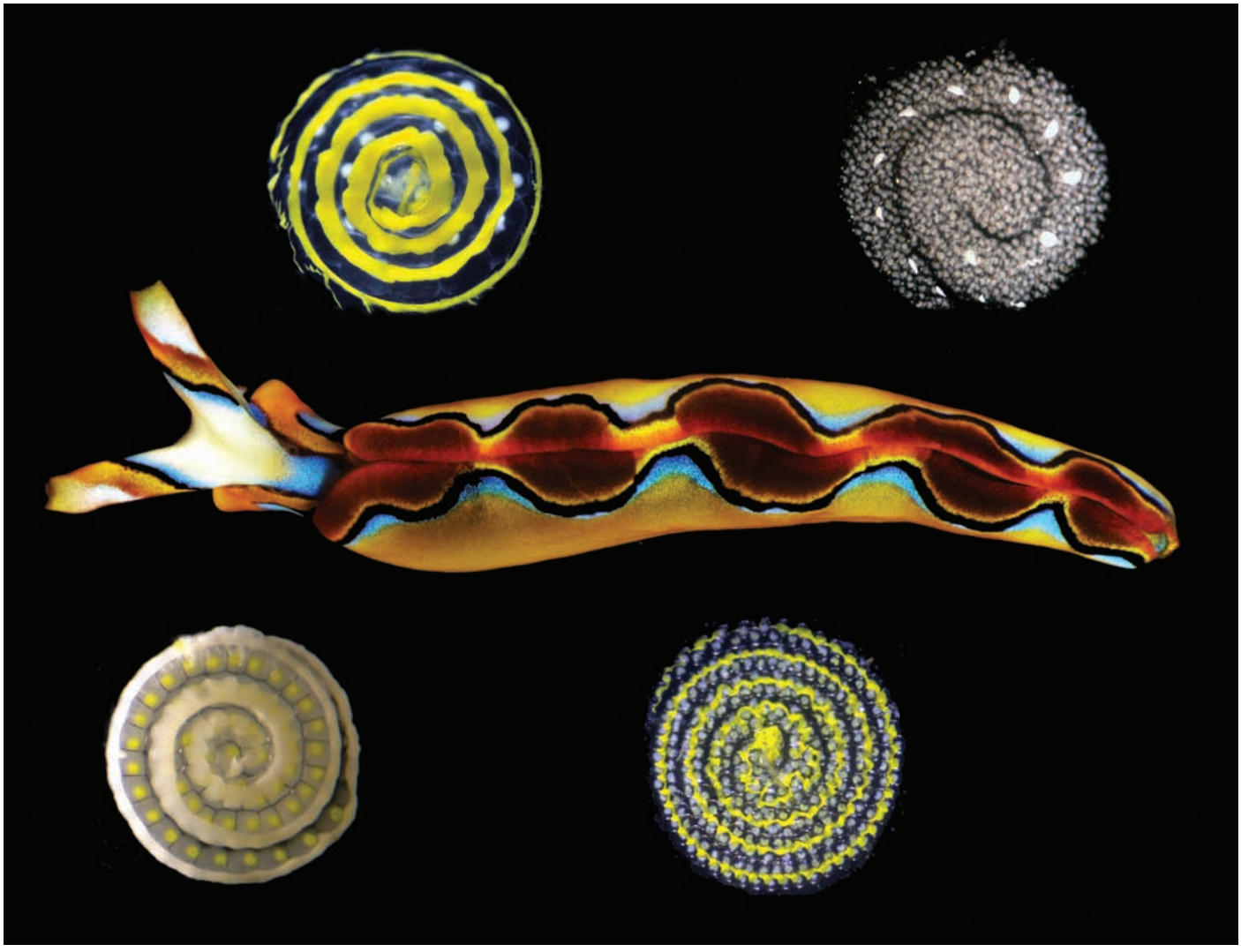

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Species Selection Favors Dispersive Life Histories in Sea Slugs, but Higher Per-Offspring Investment Drives Shifts to Short-Lived Larvae

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Abstract.—For 40 years, paleontological studies of marine gastropods have suggested that species selection favors lineages with short-lived (lecithotrophic) larvae, which are less dispersive than long-lived (planktotrophic) larvae. Although lecithotrophs appeared to speciate more often and accumulate over time in some groups, lecithotrophy also increased extinction rates, and tests for state-dependent diversification were never performed. Molecular phylogenies of diverse groups instead suggested lecithotrophs accumulate without diversifying due to frequent, unidirectional character change. Although lecithotrophy has repeatedly originated in most phyla, no adult trait has been correlated with shifts in larval type. Thus, both the evolutionary origins of lecithotrophy and its consequences for patterns of species richness remain poorly understood. Here, we test hypothesized links between development mode and evolutionary rates using likelihood-based methods and a phylogeny of 202 species of gastropod molluscs in Sacoglossa, a clade of herbivorous sea slugs. Evolutionary quantitative genetics modeling and stochastic character mapping supported 27 origins of lecithotrophy. Tests for correlated evolution revealed lecithotrophy evolved more often in lineages investing in extra-embryonic yolk, the first adult trait associated with shifts in development mode across a group. However, contrary to predictions from paleontological studies, species selection actually favored planktotrophy; most extant lecithotrophs originated through recent character change, and did not subsequently diversify. Increased offspring provisioning in planktotrophs thus favored shifts to short-lived larvae, which led to short-lived lineages over macroevolutionary time scales. These findings challenge long-standing assumptions about the effects of alternative life histories in the sea. Species selection can explain the long-term persistence of planktotrophy, the ancestral state in most clades, despite frequent transitions to lecithotrophy. [Development mode; gastropod; lecithotrophy; macroevolution; planktotrophy; Sacoglossa; species selection.]

Species selection results when the diversification rate of a lineage is character-state dependent (Stanley 1975; Jablonski 2008). Despite resurgent interest in comparative studies of species selection, it remains challenging to identify traits linked with shifts in diversification rate (Rabosky and McCune 2009; Rabosky and Goldberg 2015). Ideally, candidate traits that may trigger species selection should be mechanistically linked to speciation and extinction, and change state often enough to provide the evolutionary replication needed for statistical analysis (Jablonski 2008; Goldberg et al. 2010).

In marine invertebrates, larval development mode is a binary trait long thought to affect the evolutionary success of a lineage (Shuto 1974; Strathmann 1985). Larvae must either feed in the plankton to complete development (planktotrophy), or have enough yolk to complete metamorphosis without feeding (lecithotrophy) (Levin and Bridges 1995). Planktotrophic larvae may be carried long distances by ocean currents while feeding, maintaining gene flow among populations of benthic animals (Pechenik 1999; Bradbury et al. 2008). Lecithotrophic taxa have an abbreviated larval period that reduces dispersal, often resulting in genetically subdivided and locally adapted populations (Vermeij 1982; Selkoe and Toonen 2011). As lecithotrophic eggs are more energetically expensive to produce, selection on dispersal is inherently correlated with adult per-offspring investment (Marshall and Morgan 2011). However, we still understand little about the selective regimes that favor, or the long-term results of, changes in larval type.

Marine life-history evolution tends to be unidirectional, from planktotrophy to lecithotrophy (Gould 1982; but see Rouse 2000; Collin et al. 2007). Developmental and phylogenetic studies suggest reversals are rare due to constraints on re-evolving complex feeding structures that are often reduced in transitions to lecithotrophic development (Strathmann 1978; Wray 1995). Despite involving substantial and largely irreversible changes, shifts to lecithotrophy have occurred frequently in most clades, creating a naturally replicated experiment with which to evaluate the evolutionary origins and consequences of reduced dispersal.

Theory has long held that planktotrophy should impede speciation by slowing divergence among demes while buffering against local extinction (Scheltema 1971, 1978). For 40 years, studies of the gastropod fossil record reported lecithotrophs had elevated rates of both speciation and extinction, and tended to accumulate faster over time in some neogastropod clades

(Shuto 1974; Hansen 1978, 1980, 1982; Jablonski 1982; Jablonski and Lutz 1983; Jablonski 1986a, 1986b). Gastropod larval type became a textbook example of selection at the species level due to the increased speciation rate of lecithotrophs (Jablonski and Lutz 1983; Jablonski 1986a; Ridley 2004; Bergstrom and Dugatkin 2012). However, evidence remains lacking that lecithotrophs actually diversify more due to species selection. Highly cited paleontological studies never calculated diversification (the net difference between speciation and extinction), the measure of species selection (Hansen 1980; Jablonski and Lutz 1983; Jablonski 1986a). Traits that increase speciation rate, but that increase extinction rate proportionately *more*, will lower net diversification and be disfavored by species selection (e.g., self-compatible pollen in angiosperms; Goldberg et al. 2010). In fact, estimated speciation and extinction rates suggest diversification was not appreciably higher for lecithotrophic gastropods (Jablonski 1982, 1986a), and may have been lower after correcting for character change (see below).

Early phylogenetic studies challenged long-standing interpretations of the fossil record, revealing that independent gains of lecithotrophy were more common than expected, and rarely followed by bursts of cladogenesis (Lieberman et al. 1993; Hart et al. 1997; Hart 2000; Jeffery et al. 2003; Meyer 2003; Collin 2004; Hart and Podolsky 2005; Krug 2011). High rates of forward character change could produce a steady accumulation of distantly related lecithotrophs, mimicking state-dependent diversification in the fossil record (Duda and Palumbi 1999). Planktotrophs disappear from the fossil record in two ways: extinction, and by undergoing character change to lecithotrophy; similarly, lecithotrophs arise in two ways: speciation and character change in a planktotrophic ancestor. Failure to correct for rates of developmental evolution when modeling diversification would yield overestimated rates of extinction for planktotrophs, and of speciation for lecithotrophs, and could thus lead to false inferences regarding species selection.

Progress in understanding the evolutionary effects of larval type requires formal analyses using likelihood-based tests for detecting state-dependent diversification. Although no study has yet compared planktotrophs to lecithotrophs, in ascidians (all lecithotrophic), diversification was positively correlated with larval swimming ability, contrary to the prediction that less dispersive taxa should diversify faster (Maliska et al. 2013). The hypothesis that species selection actually favors planktotrophs therefore warrants testing in a phylogenetic framework. Other hypotheses regarding the evolutionary role of developmental shifts also await comparative tests. Incompatible genetic programs controlling early development may create reproductive isolation between planktotrophic and lecithotrophic populations, yielding incipient sister taxa (Raff et al. 2003). Transitions in larval type may thus contribute to speciation, but no study has yet tested whether such shifts are temporally coincident with divergence. Lecithotrophy has also been hypothesized to increase rates of molecular evolution by intensifying genetic drift, but comparative tests are needed (Foltz 2003; Foltz et al. 2004; Lee and Boulding 2009).

Although lecithotrophy has repeatedly evolved in most groups, we understand little about adult traits correlated with such transitions. Discrete-state models have provided scant insight into life-history evolution due to uncertainty of ancestral development mode at deep nodes (Collin 2004; Keever and Hart 2008; Waeschenbach et al. 2012). Evolutionary quantitative genetic models may better reflect the underlying complexity of developmental changes, but have not been used to explore the evolution of larval type (Hadfield and Nakagawa 2010; Revell 2013). Remarkably, no adult trait has been correlated with gains of lecithotrophy across a clade. Identifying a correlated reproductive trait would facilitate intraspecific studies of how selection acts on development mode. Although predicted (Oliphant and Thatje 2013), no prior study has tested whether a relative increase in per-offspring investment by a planktotrophic lineage also increases the rate at which lecithotrophy evolves.

New insight into the evolutionary dynamics of marine life histories requires phylogenies and developmental data for taxa in which transitions have been frequent enough to detect correlated traits or rate shifts. Sacoglossa (Heterobranchia: Panpulmonata) is a clade of sea slugs noted for the photosynthetic ability of some taxa (Akimoto et al. 2014; Christa et al. 2014). Sacoglossa contains five of the eight animal species known to express intraspecific dimorphism in egg size (poecilogony), suggesting evolutionary lability in development mode (Vendetti et al. 2012; Cooke et al. 2014; McDonald et al. 2014). Offspring provisioning strategies also vary markedly among sacoglossans; some species deposit extra-capsular yolk (ECY) that larvae ingest or absorb prior to hatching from egg masses (Fig. 1). Analogous to nurse eggs in many groups, ECY increases per-offspring investment and permits the development of larger larvae from eggs of a given size (Allen et al. 2009; Krug 2009). Although well studied within species, extra-zygotic investment has yet to be linked to phylogenetic patterns in life-history evolution (Collin 2004).

Using a four-gene molecular phylogeny of 202 species, we reconstruct the evolution of development in Sacoglossa, and test four hypotheses regarding the causes and consequences of transitions to lecithotrophy: (i) Does lecithotrophy evolve more often from planktotrophic lineages investing in ECY? (ii) Are rates of diversification higher for lecithotrophs, as predicted by paleontological studies? (iii) Does development mode show cladogenetic change (at nodes), consistent with a role in speciation? (iv) Do lecithotrophs have higher rates of molecular evolution? Our results challenge long-standing assumptions about how species selection acts in the sea, and provide compelling new insights into the selective forces that shape marine life histories.

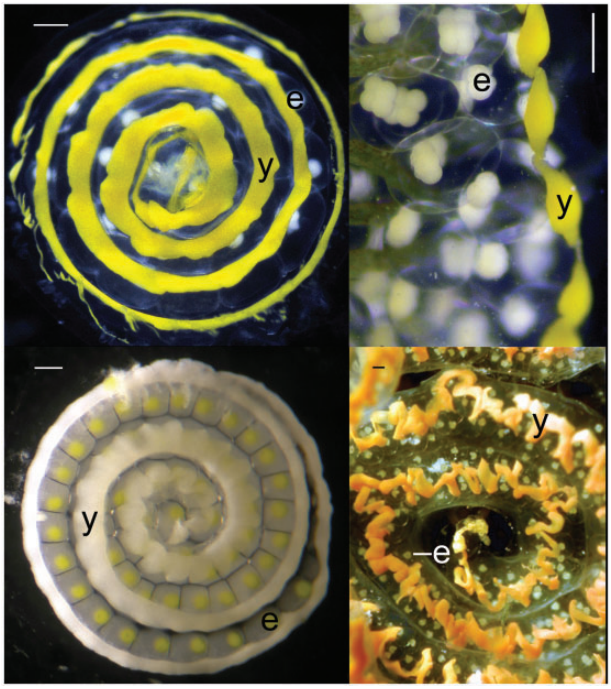

FIGURE 1. Patterns of ECY deposition in egg masses of *Elysia* spp. Images clockwise from top left: *E. patina*, *E. furvacauda*, *E. subornata*, *E.* sp. 25. Scale bars = 200μm. e = encapsulated embryo; y = extracapsular yolk.

MATERIALS AND METHODS

Collection of Specimens, Character and Molecular Data

Specimens representing all widely accepted genera except the monotypic *Roburnella* were collected with host country or state permission and provisionally identified by the authors, or were obtained from donors or museum collections (Supplementary Table S1 available on Dryad at http://dx.doi.org/10.5061/dryad.88mv3). Live slugs were held in aquaria to obtain egg masses; egg diameters were measured from calibrated digital images, and patterns of ECY recorded. Lecithotrophy, or competence to metamorphose without feeding (Krug 2009), was confirmed by inducing metamorphosis using the adult host alga or 20 mM excess K^+. Larval shell width across the aperture was measured for replicate larvae from one or more clutches. Original data on egg and larval sizes are reported for 58 spp., either as the mean diameter ± SD (standard deviation) for one clutch, or as a grand mean-of-means calculated from the mean values for replicate clutches; literature data were summarized for 64 spp., yielding character data for a total of 113 taxa (Supplementary Table S2).

As a high proportion of sacoglossan diversity is undescribed or difficult to match to older names, we performed an initial screen for candidate species (CS) from material collected over a decade of intensive sampling. A portion of the mitochondrial cytochrome *c* oxidase I (COI) gene was sequenced from two specimens of most ingroup taxa, and up to 30 specimens of 58 taxa sampled from multiple populations. Following Krug et al. (2013), a COI threshold distance of 8% was used to classify unidentified taxa as (i) confirmed CS if they were morphologically distinctive or (ii) unconfirmed CS, if known only from NCBI sequences. We numbered CS within a genus as "sp. #" in order of discovery, except that members of species complexes were provisionally named "cf." to the best described appropriate name, plus a number denoting order of collection. New sequence data were generated for 180 ingroup taxa, and public data were obtained for a further 22 species (Supplementary Table S3). Our final data set comprised 128 identified spp., plus 70 confirmed CS (21 from Krug et al. (2013), 49 identified herein), and four unconfirmed CS from public sequences, for a total of 202 ingroup species. Phylogenetic analyses included six siphonarioidean taxa as outgroups (Kocot et al. 2013).

For one to two exemplars per taxon, portions of four loci were sequenced: (i) COI; (ii) mitochondrial large ribosomal subunit rRNA (16S); (iii) nuclear histone III (H3); and (iv) nuclear large ribosomal subunit rRNA (28S). Amplifications and sequencing followed published protocols, with 28S amplified as three overlapping fragments and assembled prior to alignment (Krug et al. 2008; Händeler et al. 2009). Data matrix completeness was 94% (840 cells, locus × taxon). Initial alignments of all loci were done using MUSCLE with default settings in Geneious v6.1.6. Based on published models for rRNA genes (Lydeard et al. 2000; Medina and Walsh 2000; Mallatt et al. 2010), we developed secondary structure models to refine alignments of 16S (Supplementary Figs. S1 and S2) and 28S (Supplementary Fig. S3). Adjustments were made by eye to maintain predicted base pairing interactions in stem regions conserved across Mollusca. Loop regions of ambiguous alignment were removed, as were sequence blocks masked by the least stringent criteria in Gblocks v.0.91b (Castresana 2000). Final aligned sequence partitions were 658 bp (COI), 404 bp (16S), 1392 bp (28S), and 328 bp (H3); NCBI accession numbers are given in Supplementary Table S3. Individual gene trees were built for all loci using Bayesian Inference (BI) and maximum likelihood (ML) as detailed below, to ensure no rogue sequences or unstable taxa were included; topologies were consistent among gene trees except in unresolved regions (Supplementary Fig. S4a–d).

Phylogenetic Analyses

A concatenated alignment of all four loci was analyzed using Markov-chain Monte Carlo (MCMC) methods, implementing mixture models in *BayesPhylogenies* to capture heterogeneity in mutation rates and base frequencies without *a priori* partitioning (Pagel and Meade 2004). Per-site rate estimates of the concatenated dataset revealed only three positions at the COI locus with atypically high substitution rates (Ellingson et al. 2014), and excluding these sites had no effect on phylogenetic analyses. Four chains were run for 10^8 generations, each using 3 GTR +Γ models from which

the best-fit model was assigned to each position; a fourth model did not improve L scores. Trees were saved every 5000 generations; L scores and parameter estimates were inspected to confirm all runs reached stationarity. The final 400 trees from each run were pooled into a posterior sample and a 50% consensus tree generated (Supplementary Fig. S4e). Posterior probabilities (PP) $\geqslant 0.9$ were considered significant (Huelsenbeck and Rannala 2004).

ML analyses were run with RAxML v7.6.6 (Stamatakis 2006) through the CIPRES Science Gateway v3.3 (Miller et al. 2010), using one GTR $+\Gamma$ model with four rate multipliers. Adding a second data partition (for 16S + 1st position of COI) was recommended by PartitionFinder (Lanfear et al. 2012) but yielded no meaningful change in topology or support values (Supplementary Fig. S4f) compared to the less parameterized, unpartitioned analysis. Nodal support was assessed from 250 bootstrap pseudoreplicates, taking values $\geqslant 70\%$ as significant (Hillis and Bull 1993). The ML tree was made ultrametric by transforming branch lengths via penalized likelihood using the chronos function in "ape" v.3.0.11 in R (Paradis et al. 2004; Paradis 2013). Trees and alignments were deposited in TreeBASE (www.treebase.org).

Drivers of Evolutionary Shifts in Larval Development Mode

Ancestral character states at key nodes were reconstructed for two binary traits: larval development mode (planktotrophic/lecithotrophic) and presence/absence of ECY. The posterior distribution of states at each node was estimated in a Bayesian framework using an evolutionary quantitative genetics model (Revell 2013). Binary characters were modeled as threshold traits evolving under Brownian motion, with expression dependent on the value of an underlying, continuously distributed trait to which many loci contribute. In contrast, discrete-state models allow instantaneous transitions regardless of time spent in a given state, which is biologically less realistic. Uniform prior distributions were used for taxa of unknown state. Using the ancThresh function in phytools (Revell 2012), MCMC analyses were run for 10^7 generations, discarding the first 50% as burn-in. Stochastic trait mappings of development mode (binary) and egg diameter (continuous) were also performed using densityMap and contMap functions in phytools, after pruning taxa that lacked trait data. The above analyses used the topology and branch lengths from the ML phylogenetic analysis.

Discrete-state Markov models were also fit to data on development mode and ECY production, and posterior distributions of transition rates between states jointly estimated, to perform statistical tests of whether the preferred ancestral state was significantly more likely at key nodes. A reversible-jump (RJ) procedure was implemented in BayesTraits using exponential hyperpriors (Pagel and Meade 2006), drawing trees from the combined posterior distribution of BI analyses ($n = 1600$ trees) to accommodate phylogenetic uncertainty. Chains were run for 2×10^7 generations, discarding the first 50% as burn-in, and sampling rate coefficients with their associated tree every 500 generations to minimize autocorrelation. The "most recent common ancestor" technique was used to estimate the ancestral state for select clades, side-stepping the low confidence limits imposed by poorly supported nodes by comparing L values after fixing alternative states at a given node. Median L values from five runs of each alternative state were compared by log-Bayes Factor (BF) tests, by calculating twice the difference in the harmonic mean of L scores between alternative parameterizations.

Correlated trait evolution.—We predicted that gains of lecithotrophy would occur more often in planktotrophic lineages *with* ECY than *without*. We compared the fit of independent models (Fig. 2a) to correlated models of trait evolution (Fig. 2b), using the *RJ Discrete* option in BayesTraits (Pagel and Meade 2006). Chains were run for 2×10^7 generations, burning in the first 50%; rate parameters were sampled every 500 iterations as chains visited models in proportion to their PP. Means of exponential priors were seeded from a uniform

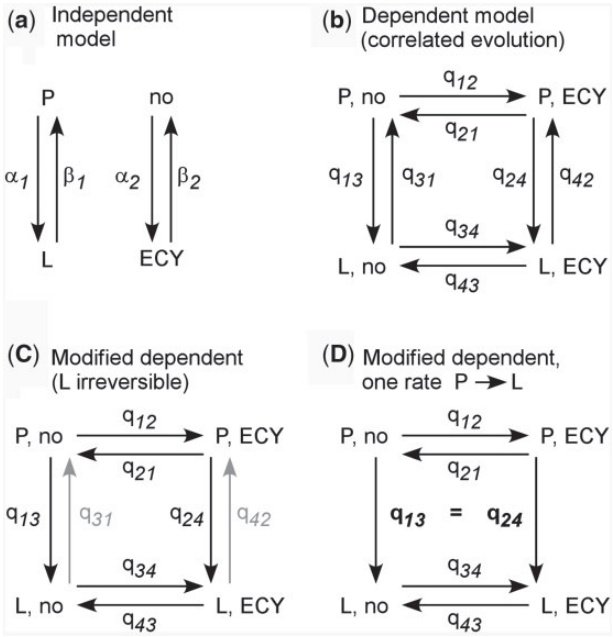

FIGURE 2. Alternative discrete-state models of correlated versus independent evolution for two binary traits: presence/absence of ECY, and planktotrophy/lecithotrophy. a) Independent model of trait evolution, with four possible transition rates. b) Dependent model, in which the rate of transitions between larval types varies between lineages with versus without ECY (eight rate parameters). c) A restricted version of the dependent model, in which reversals to planktotrophy were prohibited by setting the shaded transition rates (q31, q42) to zero. Rates of gain of lecithotrophy differed for lineages with or without ECY. d) Restricted independent model, with one transition rates from planktotrophy to lecithotrophy (q13 = q24); shifts in larval type were thus forced to be independent of investment in ECY.

distribution (0-30) using an RJ hyperprior approach; results using gamma-distributed priors (0-10, 0-30) were indistinguishable. Ratedev was set to 0.8 for suitable acceptance rates (31 ± 8% SD, median-dependent model; 19 ± 5% SD, median independent model). Analyses were repeated five times, and the median *L* score used to test whether dependent models were supported (BF > 2) or strongly supported (BF > 5) over independent models (Rafftery 1996). For the median-scoring run, posterior distributions of rate parameters were plotted and the percentage of time each was assigned to the zero bin was calculated.

Rate coefficients in the unrestricted model of correlated trait evolution suggested frequent reversals from lecithotrophy to planktotrophy (see section "Results"). However, developmental and embryological evidence suggests evolutionary reversions to planktotrophy are rare even among spiralian clades where feeding structures may be retained for swimming in lecithotrophs (Collin et al. 2007). We therefore also parameterized dependent models that prohibited reversals ($q_{31} = q_{42} = 0$), and used BF tests to compare model fit if gains of lecithotrophy were (i) correlated with ECY (two rates of gain, $q_{24} > q_{13}$; Fig. 2c) or (ii) independent of ECY (one rate, $q_{13} = q_{24}$; Fig. 2d). Five runs of each restricted model (two-rate vs. one-rate) were performed as described, setting ratedev to 0.1 (acceptance rates of 20 ± 5%). As hyperpriors cannot be implemented for restricted models in *BayesDiscrete*, a uniform prior from 0-50 was used for rate parameters based on ML analyses.

Tempo and mode of developmental evolution.—Evolution of egg diameter, a continuous trait, was modeled in the *Continuous* program of *BayesTraits*. A generalized least squares approach was used to estimate Pagel's scaling parameters to detect phylogenetic correlation (λ), punctuational change (κ), or adaptive radiation (δ) from the distribution of egg sizes among extant taxa (Pagel 1999). To test for phylogenetic signal in egg size, BF tests compared model fit when $\lambda = 0$ versus when λ took its ML value. We then compared models setting $\kappa = 0$ (punctuational change), $\kappa = 1$ (gradualism), or κ taking its ML value (scaled gradualism). Finally, we compared models when $\delta = 1$ versus when δ took its ML value, with $\delta < 1$ indicating adaptive radiation, and $\delta > 1$ indicating recent adaptation. Markov chains were run for 2×10^7 generations discarding the first 25% as burnin, sampling models and rate parameters every 500 iterations with default uniform priors, and setting ratedev to achieve 20–40% acceptance rates. Runs were repeated three times, all converging on similar ML estimates and *L* scores; the median harmonic mean of *L* scores was then compared between nested models by BF test. Reported egg sizes for *Berthelinia limax* (250 μm) and *Limapontia senestra* (200 μm) were outliers, but results were unaffected by recoding these values at 130 μm, the upper limit of egg size we measured.

Macroevolutionary Consequences of Developmental Shifts

Diversification rate.—The classic species-selection hypothesis predicts a net increase in speciation rate relative to extinction rate for lecithotrophs. The BiSSE model (Binary-State Speciation and Extinction) tests whether an excess of species with one character state results from asymmetric rates of (i) trait evolution, (ii) speciation, or (iii) extinction (Maddison et al. 2007). We compared goodness-of-fit of alternative parameterizations of the BiSSE model using "diversitree" in R, using two different approaches to accommodate missing trait data and unsampled taxa (FitzJohn 2012). First, we estimated species richness in each monophyletic genus or new genus-level clade recovered from phylogenetic analyses, based on (i) valid, described species listed by Jensen (2007), (ii) species described since 2007, and (iii) 74 CS. To account for generic paraphyly, we lumped (i) *Soghenia* with *Cyerce*, (ii) *Roburnella* with *Lobiger*, and (iii) unsampled *Stiliger* spp. with *Placida*, which was paraphyletic with respect to the type species *Stiliger ornatus*. Two '*Stiliger*' spp. (*Stiliger smaragdinus*, *S.* sp. 6) formed their own genus-level clade, and *Stiliger fuscovittatus* belongs in *Hermaea*. Known species not sampled in this study were assigned to their traditional genus. Divergent clades falling outside of recognized genera were treated as genus-level, but no unsampled taxa were assigned to them. For each genus-level clade, we then determined the number of species with planktotrophic, lecithotrophic, or unknown development, using original data and literature values (Supplementary Table S1). The ML tree was pruned to include one exemplar (the most basal taxon) for each clade with unsampled diversity or incomplete trait data. All terminals were retained for fully sampled clades. Outgroups were removed prior to analyses. The pruned tree had 41 terminal taxa, including representatives of 20 incompletely sampled clades. For each tip on the pruned tree, we specified the total number of species known from the represented clade (N_c), and of those, the number with planktotrophic (n_0) and lecithotrophic (n_1) development (Supplementary Fig. S5). For the second approach, sampled taxa missing trait data were pruned from the tree, leaving 113 species (out of 363 known and CS in Sacoglossa); a global estimate of unsampled species diversity (69%) was then included in the model to correct for missing data.

As BiSSE assumes uniform diversification rates across a phylogeny, we first used MEDUSA in R to test for rate shifts independent of larval type (Alfaro et al. 2009). Two increases in background diversification rate were detected: (i) near the root of Plakobranchacea, excluding two "*Costasiella*" spp. that fell outside of *Costasiella* s.s.; and 2) in the lineage leading to family Plakobranchidae. To accommodate these rate shifts in BiSSE analyses, the pruned tree was "split" (using the "make.bisse.split" function) at the node following each increase in diversification. Rates of speciation (λ), extinction (μ), and character change (*q*) were then estimated independently

for each of three resulting partitions: Oxynoidea + two "*Costasiella*" spp.; the remaining members of traditional superfamily Limapontioidea plus *Bosellia*; and family Plakobranchidae (Supplementary Fig. S5). These splits also received high PP support in BAMM (Robosky 2014).

Using BiSSE, rates of character change, speciation and extinction were modeled across Sacoglossa using different combinations of parameters. Models with two rates of character change were never favored over nested models with the same forward and reverse rate in preliminary runs; we therefore compared model fit using Akaike Information Criterion (AIC) scores when allowing rates of speciation (λ) and/or extinction (μ) to vary with larval type versus a nested model assuming no correlation between evolutionary rate and development mode. To compare evolutionary scenarios under the *a priori* expectation that reversals to planktotrophy are rare, we also modeled diversification with reversals in larval type constrained to a low rate ($q_{10} = 0.01$, $< 1\%$ of ML estimated forward rates); prohibiting reversals ($q_{10} = 0$) yielded nearly identical results.

Role in speciation.—If shifts in development contribute to reproductive isolation, transitions should occur at nodes (cladogenetic change) as well as along branches (anagenetic change). We tested this hypothesis using the BiSSEness model (Magnuson-Ford and Otto 2012), which includes two parameters allowing cladogenesis. The unpruned phylogeny was used for greater power to detect an association between character evolution and splits. Models allowing or excluding cladogenetic change were compared by AIC scores, using two speciation rates and one rate each for extinction and character change, based on BiSSE analyses (see section "Results").

Rate of molecular evolution.—We also tested the hypothesis that lecithotrophy is associated with higher rates of DNA substitution using "traitRate" to model sequence evolution with or without a state-dependent rate multiplier (Mayrose and Otto 2011). Evolution of sequences and larval type were modeled with 100 iterative searches using the ultrametric ML tree, and a GTR +Γ model with 8 multipliers. A likelihood ratio test (LRT) compared model fit when substitution rate varied with larval type.

Results

Phylogeny of Sacoglossa and Drivers of Developmental Evolution

We sampled extensively to include 128 out of 289 described species, plus 74 unidentified CS (Supplementary Table S1). Topology and branch length estimates were comparable between the BI consensus tree (Supplementary Fig. S4e) and ML tree (Fig. 3). In both analyses, traditional suborders Oxynoacea (shelled taxa) and Plakobranchacea (shell-less slugs) were reciprocally monophyletic (Fig. 3). *Cylindrobulla* was an ingroup taxon with unresolved affinity to other shelled taxa. Within Plakobranchacea, superfamily Limapontioidea was paraphyletic with respect to superfamily Plakobranchoidea. Two of the three traditional families (Limapontiidae, Polybranchiidae) were non-monophyletic, as were 9 of 22 genera (Fig. 3, Supplementary Fig. S5). Four divergent lineages fell outside of traditional genera, and were treated as genus-rank clades in subsequent diversification analyses.

Development mode was determined for 16 species in Oxynoacea (44% lecithotrophic) and 97 species in Plakobranchacea (25% lecithotrophic) (Supplementary Table S2). Egg diameter was bimodal, with no overlap for planktotrophs (46–82 μm) and lecithotrophs (91–126 μm, plus two outliers $\geqslant$200 μm). Of 31 lecithotrophic species, most laid egg masses from which some or all larvae hatched as short lived, swimming veligers. Only seven species had "direct" development (non-planktonic lecithotrophy), with all larvae metamorphosing before hatching: *Elysia subornata*, *E. pratensis*, lecithotrophic morphs of the poecilogonous species *E. chlorotica* and *Costasiella ocellifera*, and three ametamorphic species (*Limapontia senestra*, *E. furvacauda*, *E.* sp. 25) in which embryos never developed larval features (velum, shell).

Ancestral character-state reconstructions using an evolutionary quantitative genetics model supported 27 origins of lecithotrophy and no losses, or 26 origins plus one or more reversals (Fig. 3). Equivalent results were obtained using stochastic character mapping for development mode as a discrete character (Fig. 4a) or egg size as a continuous trait (Fig. 4b). At the root of Oxynoacea, neither larval type was supported by discrete-state or quantitative genetic models. Lecithotrophy was favored as the ancestral state of *Berthelinia* and *Cylindrobulla* (quantitative genetics model) but was not significantly supported (discrete-state model, BF < 1). If the last common ancestor of Oxynoacea was planktotrophic, there were at least five origins of lecithotrophy in this clade; if lecithotrophic, at least one reversal to planktotrophy and three regains of lecithotrophy occurred. Despite occurring at a high frequency in Oxynoacea, lecithotrophy was fixed in only one clade of three species.

In Plakobranchacea, the quantitative genetics model (Fig. 3) and stochastic character mapping (Fig. 4) overwhelmingly supported planktotrophy as plesiomorphic. Discrete-state models returned equivocal support for planktotrophy at the root of Plakobranchacea (BF $= 0.6$). The quantitative genetics model indicated 22 independent origins of lecithotrophy among the shell-less taxa (including five poecilogonous species), and no reversals (Fig. 3b). Notably, shifts to lecithotrophy were disproportionately concentrated in Plakobranchoidea (17 origins; Fig. 3b), with only five origins among the traditional limapontioidean taxa (Fig. 3a). Most lecithotrophic species had a planktotrophic sister taxon.

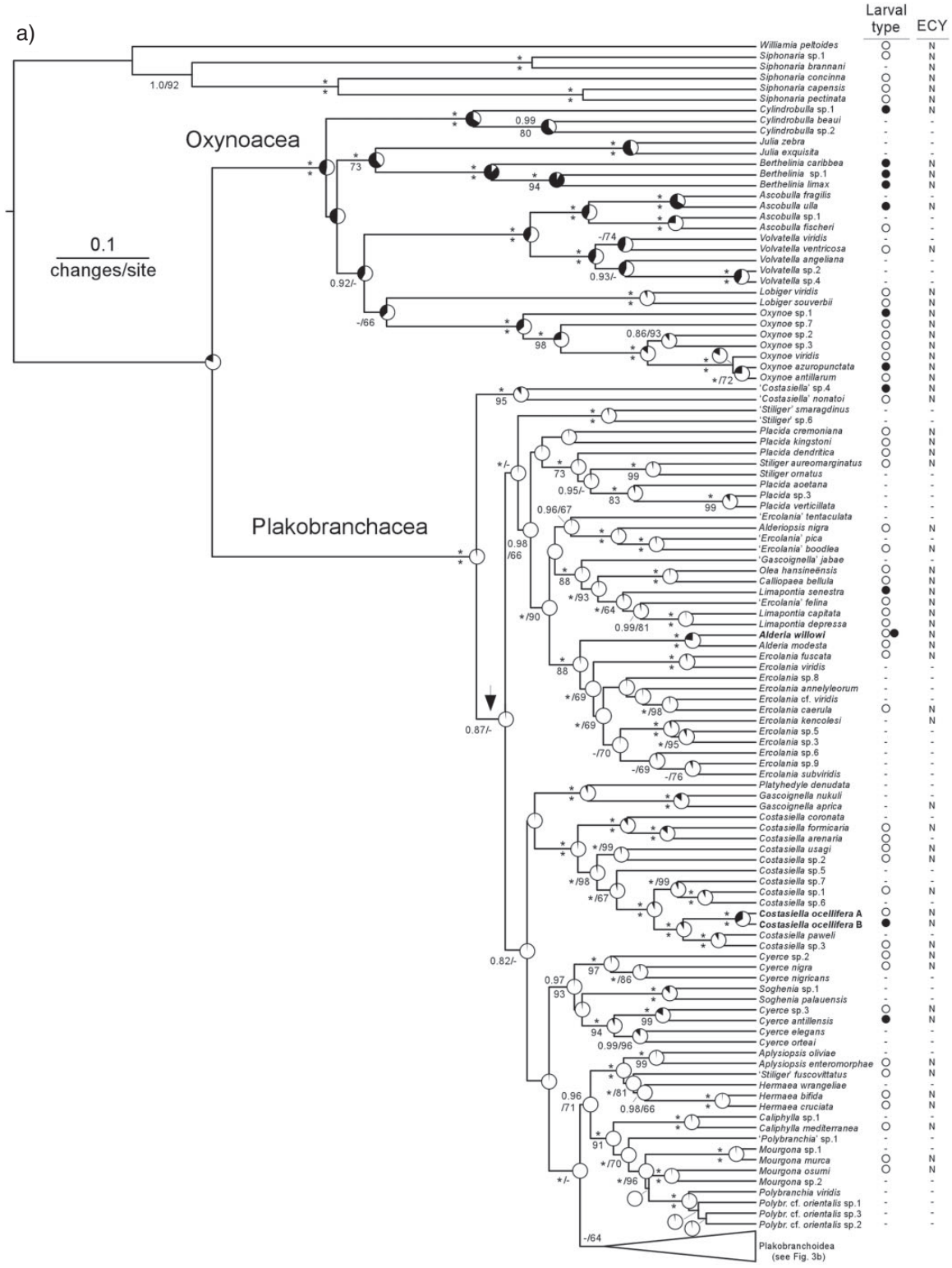

FIGURE 3. Phylogenetic hypothesis for Sacoglossa based on analyses of 2782 bp of DNA from four genetic loci. Topology and branch lengths are given from the ML analysis, with significant or borderline support values given as PP (above branch, or before slash) and bootstrap % (below branch, or after slash); asterisk = 1.0 or 100% support. Trait distributions among extant taxa and ancestral character state reconstructions are given for development mode (white, planktotrophic; black, lecithotrophic) and ECY (Y, present; N, absent); dash indicates missing data. Poecilogonous species are bolded. Pie charts show PP of ancestral states from an evolutionary quantitative genetics model. a) Relationships in suborder Oxynoacea (shelled taxa; monophyletic) and traditional superfamily Limapontioidea (unshelled, paraphyletic). b) Evolutionary relationships in superfamily Plakobranchoidea, with mirrored trees showing PP of ancestral development mode (left) and ECY (right; blue or Y, present; red or N, absent). Icons depict color and pattern of ECY in extant taxa as blobs or ribbons, with white ECY drawn against a grey background. See online version for references to colors.

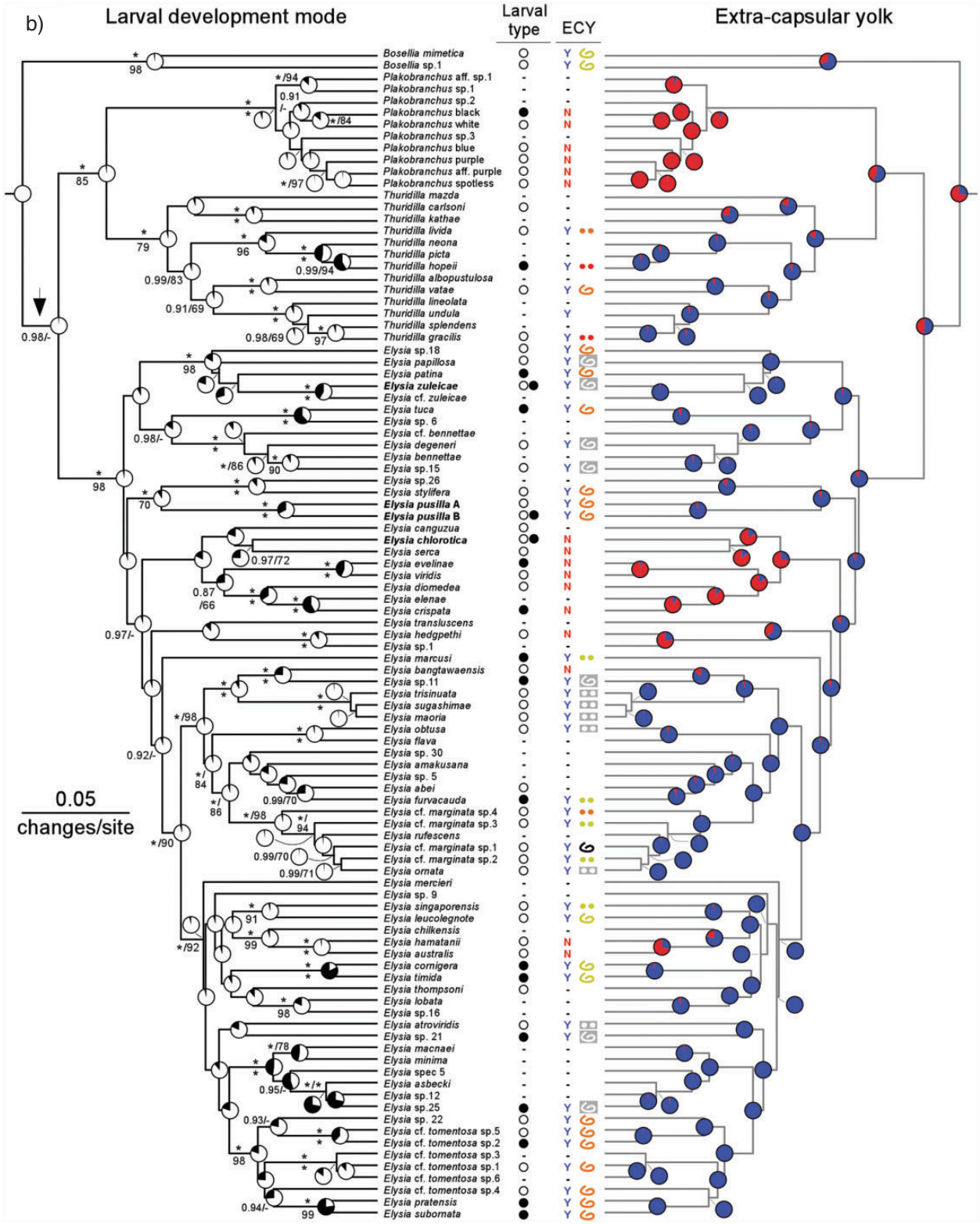

FIGURE 3. Continued.

Production of ECY was restricted to several lineages within Plakobranchoidea. Posterior distributions from the quantitative genetics model favored two to three origins of ECY (Fig. 3b), whereas discrete-state models weakly supported ECY production as the ancestral condition in the family (BF = 2.2). Bayesian analyses supported five losses of ECY, in two large-bodied tropical taxa (*Plakobranchus*, *Elysia bangtawaensis*) and

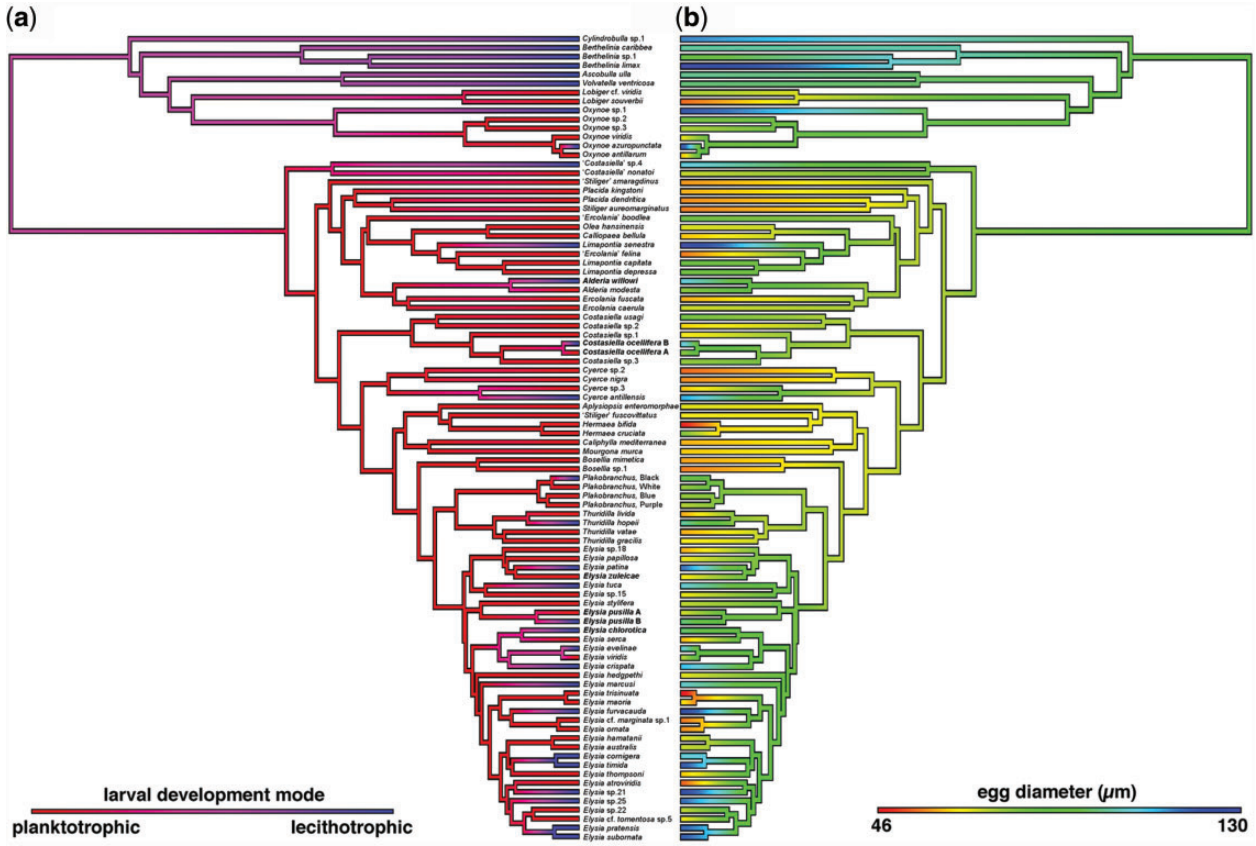

FIGURE 4. Stochastic character mapping of reproductive trait evolution across Sacoglossa. a) Evolutionary shifts in larval development mode, modeled as a binary trait. Color gradients represent the history of character transitions across the ML phylogeny; red = planktotrophy, blue = lecithotrophy. b) Evolutionary change in egg diameter, a continuous trait, represented by gradients from warmer colors (small eggs) to cooler colors (larger eggs). See online version for references to colors.

three temperate lineages of *Elysia*: (i) a North Atlantic clade (e.g., *E. canguzua*); (2) sister species *E. hedgpethi* and *E.* sp. 1, from upwelling coasts; and (3) sister species *E. hamatanii* and *E. australis*, from the temperate western Pacific.

Discrete-state models supported correlated evolution of ECY and lecithotrophy across Sacoglossa; models allowing covariance of ECY and lecithotrophy were highly preferred over models of independent evolution (Table 1, Fig. 5). Lineages with ECY evolved lecithotrophy almost twice as often as those lacking ECY (Fig. 5a). In unconstrained analyses, estimated rates of reversal to planktotrophy were high, but reversals were less likely for ECY-producing taxa (Fig. 5, Supplementary Fig. S6). When reversals were prohibited, lecithotrophy evolved three times as often with ECY, and log-BF tests again supported correlated evolution (Fig. 5b, Table 1).

Consequences of Shifts in Larval Type

Tests of egg diameter as a continuous variable detected no phylogenetic signal (λ) across Sacoglossa (Table 1). Egg size evolved by scaled gradualism, with models in which κ took its ML value (0.50±0.13) supported over

TABLE 1. Comparison of nested models describing the evolution of development mode and ECY production (a, b) via RJ exploration of discrete-state Markov models, or egg diameter (c–e) using evolutionary generalized least squares methods

Model	*N*	Median *L*	log-BF[a]
a) ECY & lecithotrophy, unrestricted	5		
correlated		−106.40	**5.04**
uncorrelated		−108.92	
b) ECY & lecithotrophy, no reversals	5		
correlated ($q_{13} \neq q_{24}$)		−112.62	**4.01**
uncorrelated ($q_{13} = q_{24}$)		−114.63	
c) Phylogenetic effects on egg size[b]	3		
λ = 0		−428.14	
λ = ML value (0.38 ± 0.18)		−428.01	0.25
d) Punctuational vs. gradual change	3		
κ = 0 (punctuational)		−446.96	**−9.05**[c]
κ = ML value (0.50 ± 0.13)		−442.44	
κ = 1 (gradualism)		−448.66	**−12.43**[c]
e) Slow-down vs. accelerated change	3		
δ = 1		−448.42	
δ = ML value (2.77 ± 0.19)		−435.46	**25.92**

[a]Bolded values indicate support (BF > 2) or strong support (BF > 5) for a model based on the median *L* score of *N* replicate analyses.
[b]λ = Pagel's correction for phylogenetic correlation.
[c]Negative BF values indicate support for the ML estimate of κ.

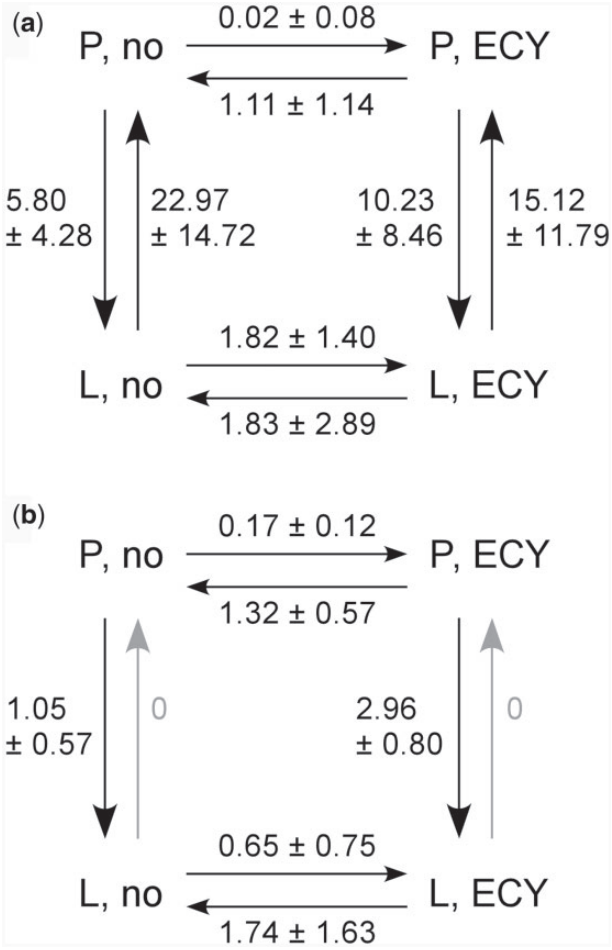

FIGURE 5. Posterior distributions of rate parameters estimated by discrete-state Markov models of correlated evolution between yolk production (none vs. ECY) and development mode (P, planktotrophic vs. L, lecithotrophic). Z denotes the proportion of time a rate was in the zero bin. The distribution for each rate is summarized as the mean ± SD from the run with the median *L*score, out of five replicate runs. a) Estimates from the full dependent model, with unconstrained rates of gain and loss of lecithotrophy. b) Rate estimates from the restricted model, prohibiting reversals to planktotrophy.

punctuational change ($\kappa = 0$) or pure gradualism ($\kappa = 1$). A model of accelerated evolution over time ($\delta = 2.77 \pm 0.19$) better described change in egg size than a constant-rate model ($\delta = 1$) or adaptive radiation scenario ($\delta < 1$); most change in egg size thus occurred in the recent history of Sacoglossa, likely reflecting species-specific adaptations.

Despite the many shifts to less dispersive larvae, lecithotrophy did not trigger apparent cladogenesis. Only one surviving species was associated with each of 24 shifts to lecithotrophy (89% of origins), while the three lecithotrophic clades comprised only two ($n=2$) or three ($n=1$) extant species. This pattern contrasts markedly with the hypothesis that lecithotrophs diversify at a higher rate. Based on preliminary analysis using MEDUSA, Sacoglossa was divided into three partitions with separate background rates of diversification (Fig. 3). The distribution

TABLE 2. ML tests for state-dependent diversification in three phylogenetic partitions of Sacoglossa, with (a) one rate of character change, or (b) rates of reversal to planktotrophy (q_{10}) constrained to be rare (<1%) relative to estimated forward rates (q_{01})

	df	ln(L)	AIC[a]	χ^2[b]	*P*
a) Unrestricted BiSSE[b]					
λ (1), μ (1), *q* (1)	9	−68.73	155.46	n/a	n/a
λ (1), μ (2), *q* (1)	12	−66.06	156.12	5.33	0.149
λ (2), μ(1), *q* (1)	**12**	**−61.90**	**147.79**	**13.67**	**0.003**
λ (2), μ (2), *q* (1)	15	−61.20	152.40	15.06	0.020
b) Restricted BiSSE					
λ (1), μ (1), *q* (1)	9	−70.33	158.66	n/a	n/a
λ (1), μ (2), *q* (1)	12	−66.87	157.75	6.91	0.075
λ (2), μ (1), *q* (1)	**12**	**−63.78**	**151.55**	**13.10**	**0.004**
λ (2), μ (2), *q* (1)	15	−63.29	156.58	14.08	0.029

[a]Goodness of fit of alternative parameterizations assessed by AIC scores and chi-square tests of nested models; values for the preferred model are bolded.
[b](#) denotes one versus two rates of speciation (λ) or extinction (μ).

of species and development modes was therefore modeled across 31 genus-level clades in BiSSE, with parameters estimated separately for each partition, to test for state-dependent diversification driven by larval type.

Diversity was best explained by a BiSSE model in which speciation rate varied by development mode, whether reversals to planktotrophy occurred at the same rate as gains of lecithotrophy (Table 2A) or were constrained to be rare (Table 2B). However, contrary to predictions from the fossil record, state-dependent diversification favored planktotrophy. Parameter estimates for the preferred model showed higher net diversification for planktotrophs across all phylogenetic partitions. In Oxynoacea, planktotrophs had a slightly higher speciation rate than lecithotrophs if forward and reverse rates of character evolution were equal (Table 3A). When reversals were constrained to be rare, planktotrophs diversified ($\lambda_0 > \mu_0$), but lecithotrophs did not ($\lambda_1 < \mu_1$) (Table 3B). In the second partition (Limapontioidea + *Bosellia*), speciation rate was much higher than extinction rate for planktotrophs (Table 3). In contrast, speciation rate was negligible for lecithotrophs, which did not diversify regardless of whether reversals were estimated by the model or constrained to be rare. Thus, in Limapontioidea, each lecithotroph arose independently via character change from a planktotrophic ancestor.

The third partition, clade Plakobranchidae, contains over a third of sacoglossan species richness (134 out of 363 spp.), and over half the known lecithotrophs (19 out of 35 spp.). BiSSE estimated that in Plakobranchidae, speciation rates were about twice as high for planktotrophs as for lecithotrophs, whether reversals were unconstrained or rare (Table 3). However, speciation outpaced extinction for both development modes, explaining the overall increase in diversification in Plakobranchidae detected by MEDUSA. Rates of character change were higher in Plakobranchidae

TABLE 3. ML parameter estimates for state-dependent models of diversification in three phylogenetic partitions of Sacoglossa, with (a) one rate of character change, or (b) rates of reversal to planktotrophy (q_{10}) constrained to be rare (<1%) relative to forward rates (q_{01}); estimates from the preferred model are bolded, with alternatives shown in descending order of AIC scores

	Oxynoacea					Limapontioidea + Bosellidae					Plakobranchidae				
a) Unrestricted BiSSE model	λ_0	λ_1	μ_0	μ_1	q	λ_0	λ_1	μ_0	μ_1	q	λ_0	λ_1	μ_0	μ_1	q
$\lambda(2)$, $\mu(1)$, $q(1)$	**17.95**	**16.54**	**14.77**		**4.27**	**10.35**	**$<10^{-9}$**	**4.29×10^{-8}**		**1.06**	**26.10**	**10.08**	**$<10^{-7}$**		**9.79**
$\lambda(2)$, $\mu(2)$, $q(1)$	12.42	34.24	8.66	33.98	5.64	10.36	$<10^{-9}$	10^{-9}	$<10^{-8}$	1.07	27.97	21.44	$<10^{-8}$	17.96	14.10
$\lambda(1)$, $\mu(2)$, $q(1)$	16.91		13.05	16.43	3.99	9.83		$<10^{-5}$	11.15	1.16	27.78		$<10^{-6}$	24.39	14.05
b) Constrained ($q10=0.01$)	λ_0	λ_1	μ_0	μ_1	q_{01}	λ_0	λ_1	μ_0	μ_1	q_{01}	λ_0	λ_1	μ_0	μ_1	q_{01}
$\lambda(2)$, $\mu(1)$, q_{01}	**18.67**	**11.94**	**14.58**		**2.46**	**9.94**	**$<10^{-6}$**	**$<10^{-5}$**		**0.78**	**23.69**	**10.09**	**$<10^{-6}$**		**4.87**
$\lambda(2)$, $\mu(2)$, q_{01}	12.54	21.32	5.36	26.63	4.84	9.97	$<10^{-4}$	$<10^{-5}$	0.59	0.83	28.09	15.25	$<10^{-4}$	21.75	10.08
$\lambda(1)$, $\mu(2)$, q_{01}	14.38		8.58	18.42	3.65	9.88		$<10^{-5}$	0.59	0.83	28.03		$<10^{-6}$	34.05	9.99

than in the two basal partitions, consistent with the correlated evolution of lecithotrophy and ECY, produced by a subset of lineages in Plakobranchidae.

Allowing estimated extinction rate to covary with larval type did not significantly improve model fit, although the five-parameter model (state-dependent speciation and extinction rates) was still significantly better than the state-independent model (Table 2). ML estimates of state-dependent extinction rates were consistently higher for lecithotrophs than planktotrophs ($\mu_1 \gg \mu_0$) across all three partitions. Diversification was higher for planktotrophs across all partitions regardless of reversal rate, and lecithotrophs did not diversify in most partitions if reversals were constrained to be rare (Table 3). Thus, planktotrophy conferred a higher diversification rate regardless of whether extinction was state-dependent, but estimated extinction rates for lecithotrophs were higher than the corresponding rates for planktotrophs. Equivalent results were obtained with an alternative parameterization of BiSSE using a pruned tree and a proportion of unsampled taxa (Supplementary Tables S4 and S5); thus, our findings were robust to different methods of correcting for missing data.

If shifts in development contributed to the speciation process, then some character change should occur close to the point of lineage divergence–that is, at nodes on the tree. We compared alternative parameterizations of the BiSSEness model allowing only anagenetic character change (along branches), or also allowing cladogenetic change (at nodes). Model fit was not improved by allowing cladogenetic change in larval type (df = 8, $\ln(L) = -77.3$, AIC = 170.6), compared to a nested model that constrained change to occur along branches (df = 6, $\ln(L) = -77.8$, AIC = 167.6). Thus, there was no evidence for change in development coincident with speciation.

To test the hypothesis that substitution rates are higher for lecithotrophs, we compared the fit of a model with state-dependent rate matrices to a nested model where mutation rates were the same for planktotrophs and lecithotrophs. Allowing DNA substitution rates to vary by development mode ($L = -90{,}820.7$) was highly favored over a model in which rates were independent of larval type ($L = -91{,}528.6$) (LRT = 1415.8; $P < 0.00001$). Substitution rates were estimated to be nearly twice as high for lecithotrophs as for planktotrophs.

DISCUSSION

Evolutionary Consequences of Larval Type

This study is the first to show that, contrary to long-standing interpretations of the fossil record, planktotrophs diversify more than lecithotrophs and are favored by species selection. We sampled only one surviving species from 24 out of 27 origins of lecithotrophy in Sacoglossa, and no lecithotrophic clade contained more than three taxa. Lecithotrophy appears to evolve often, but by increasing extinction rate more than speciation rate, lowers net diversification. This can explain the long-term maintenance of planktotrophy in most clades despite a high rate of character change to lecithotrophy. In angiosperms, self-compatible (SC) pollen similarly evolves often from self-incompatible (SI) lineages, elevating both speciation and extinction rates; however, extinction rate rises disproportionately more, leading to species selection favoring the plesiomorphic condition (SI) (Goldberg et al. 2010). Our findings are consistent with phylogenetic studies of diverse groups, showing that cladogenesis rarely follows gains of lecithotrophy, and with previous observations that among and within animal phyla, clades containing both development modes are usually more speciose than lecithotrophic clades (Duda and Palumbi 1999; Collin 2004; Krug 2011; Nützel 2014).

Larval type can be inferred from the protoconch on adult shells of fossil gastropods, facilitating 40 years of study on the macroevolutionary role of development (Shuto 1974; Hansen 1978, 1980, 1982; Jablonski 1982; Jablonski and Lutz 1983; Jablonski 1986a, 1986b). However, early paleontological studies never tested the hypothesis that species selection favored lecithotrophs, because none estimated diversification rates. Instead, species selection was inferred to favor lecithotrophs due to their increased rates of speciation, and accumulation over time in some neogastropod groups. Although acknowledging that lecithotrophy

also raised extinction rates, classic studies failed to recognize that if extinction rates increased *more* than speciation rates, species selection would favor planktotrophy. Subtracting estimated rates of extinction (Jablonski 1982) from speciation (Jablonski 1986a) for late Cretaceous gastropods yields similar diversification rates for planktotrophs (0.06/MA) and lecithotrophs (0.09/MA); however, those estimates ignored character change. If lecithotrophy evolved at even 10% of the speciation rate estimated for planktotrophs (0.23/MA), then actual diversification was higher for planktotrophs ($0.06+0.023=0.083$) than lecithotrophs ($0.09-0.023=0.067$). Thus, our results are consistent with evidence from (but not interpretations of) the fossil record for other gastropod groups.

Early phylogenetic studies suggested lecithotrophs could accumulate without diversifying through frequent, irreversible character change, showing that asymmetric transition rates could produce the appearance of species selection favoring lecithotrophy (Lieberman et al. 1993; Duda and Palumbi 1999). We extend this argument by showing that lecithotrophy not only evolves often, but decreases diversification rates relative to the ancestral condition of planktotrophy. Thus, the loss of a highly dispersive larval stage is often favored and fixed within a species, but is usually an evolutionary dead end for a lineage. Ours is the second study to reject the hypothesis that species selection favors life histories that reduce dispersal (Maliska et al. 2013). Widely held assumptions about the macroevolutionary role of larval development mode should thus be reconsidered, and further tests performed to assess whether dispersal ability broadly promotes evolutionary success in the sea.

Paleontological studies could not distinguish between lecithotrophs with a short larval swimming period, and those with no swimming period (aplanktonic development). Phylogenetic studies suggest endemic radiations can follow shifts to aplanktonic development in peripheral isolates, such as *Conus* from the Cape Verde islands (Cunha et al. 2005; Duda and Rolan 2005), cypraeids from southern Australia or South Africa (Meyer 2003), or Antarctic dorid nudibranchs (Wilson et al. 2009; 2013). Most lecithotrophic sacoglossans produce larvae that swim for at least a few hours or days; aplanktonic development is rare, and not associated with recent diversification. Groups in which aplanktonic lecithotrophy is more common that pelagic (free-swimming) lecithotrophy may be more likely to experience species selection favoring lecithotrophy, unless endemic clades are also more vulnerable to extinction (Jablonski 2008).

Ecological attributes or key characters may explain different background diversification rates among phylogenetic partitions within Sacoglossa, and may interact with the state-dependent benefits of planktotrophy. Most oxynoaceans feed on one host genus (*Caulerpa*), whereas diverse genera are consumed by the unshelled plakobranchaceans (Christa et al. 2014). Overall diversification rate was low in Oxynoacea but increased dramatically for planktotrophs near the root of Plakobranchacea, concordant with a niche expansion allowing unshelled taxa to exploit a wider range of host algae. Despite a concentration of both biodiversity and lecithotrophy in Plakobranchidae, species selection still favored planktotrophy in this clade: planktotrophs diversified at twice the rate of lecithotrophs, although lineages in both states diversified at elevated rates. Increased diversification in Plakobranchidae may be linked to key innovations such as kleptoplasty (retention of diet-derived chloroplasts) and/or parapodial side flaps that cover the dorsum and protect plastid light-harvesting complexes from burnout. Ecological caps on diversity, and/or trait-mediated photosynthetic capabilities, likely contribute to variable evolutionary success across this group of specialized consumers.

The overall bias toward planktotrophy in Sacoglossa is typical for marine heterobranchs, and many caenogastropod clades in which the distribution of development modes is reasonably well known (Table 4). In contrast, planktotrophy is rare or unreported among extant species in some neogastropod families (e.g., Volutidae, Buccinidae; Radwin and Chamberlin 1973) that were the focus of paleontological studies. Thus, our results are not unusual due to the preponderance of planktotrophy in Sacoglossa; rather, the lack of planktotrophy in some neogastropods is atypical for gastropods, and most higher-order invertebrate clades. Patterns inferred from the fossil record of families with few if any surviving planktotrophs may not generalize to groups in which planktotrophy is common, including highly diverse caenogastropod families (e.g., Calyptraeidae, Conidae, Cypraeidae). Indeed, whether lecithotrophy became fixed in some clades by species-level drift or selection would be difficult to ascertain from fossil evidence (Hansen 1982). Moreover, even in groups like Volutidae, lecithotrophs underwent repeated bursts of speciation yet showed no greater net accumulation over time than planktotrophs (e.g., Fig. 1 in Hansen 1978;

TABLE 4. Distribution of developmental modes across select clades in Heterobranchia and Caenogastropoda; P = planktotrophic, L= lecithotrophic (including direct development)

	# P	# L	% P	References[a]
Heterobranchia[b]				
Anaspidea	17	2	89.5	1
Cephalaspidea	47	13	78.3	1–4
Notaspidea	7	3	70.0	5
Nudibranchia	171	60	74.0	1, 6–16
Sacoglossa	108	35	75.5	Present study
Caenogastropoda				
Calyptraeidae	39	39	50.0	17
Conus	56	35	61.5	18
Littorininae	139	13	91.4	19
Fasciolariidae	9	25	26.5	20
Muricidae	36	46	43.9	21–23

[a]References given in Supplementary Information.
[b]Taxa that were systematically transferred into other clades since the relevant reference was published were excluded from calculations, as warranted.

net loss of two planktotrophs and two lecithotrophs over ~30 MA).

Several processes could result in the low phylogenetic signal for egg size noted here for Sacoglossa, and previously in Calyptraeidae (Collin 2004). Rapid, reversible change in larval type would reduce phylogenetic signal, but reversals to planktotrophy are rare (Collin et al. 2007). Dimorphisms in larval type occur in Sacoglossa (Vendetti et al. 2012) and Calyptraeidae (McDonald et al. 2014), and might obscure phylogenetic patterns if evolutionarily stable in the long term; however, all origins of poecilogony in Sacoglossa appear to be recent. Our results are most consistent with frequent character change producing many origins of lecithotrophy near the tips of the tree, and a lack of old lecithotrophic clades due to their reduced diversification rate. Notably, this pattern may pose problems for discrete-state models of trait evolution; in our analyses, unconstrained models estimated high rates of reversal, a biologically implausible result. The total evidence suggests instead that lecithotrophy evolves often but dooms most lineages to extinction, a result supported by BiSSE analyses when reversal rates were constrained to be equal to, or much less than, forward rates of change. State-dependent extinction may thus pose challenges for existing models of trait evolution, causing an upward bias in estimated reversal rates to accommodate the lack of old lineages with the derived character state.

Our findings should be robust to recent evidence that BiSSE is prone to Type I errors (Rabosky and Goldberg 2015). First, phylogenetic pseudoreplication is not a concern given 27 origins of lecithotrophy. Second, we performed partitioned BiSSE analyses to accommodate background shifts in diversification rate. Third, our data show the opposite pattern to that expected under the paradigm that lecithotrophs benefit from species selection. Fourth, we found speciation rate dropped for the derived, rarer larval type (lecithotrophy), whereas simulations generally showed false-positive associations between lower speciation and (i) root states and (ii) the more common state (Rabosky and Goldberg 2015). Fifth, the highest proportion of lecithotrophy occurred in the clade with the highest background rate of diversification, yet lecithotrophy was associated with lower diversification, consistent with the scarcity of lecithotrophic clades. However, multi-clade meta-analyses of state-dependent diversification in other invertebrate groups are warranted to confirm the generality of our findings that link dispersal with evolutionary success in the sea.

Finally, we also tested whether gains of lecithotrophy were temporally associated with speciation events, or increased rates of molecular evolution. Development mode did not appear to change at nodes, and thus no evidence supports the hypothesis that population-level shifts in larval type contribute to reproductive isolation. However, our analyses did support the hypothesis that lecithotrophs have accelerated rates of molecular sequence evolution, which was not previously tested with comparative methods that correct for phylogenetic relatedness. Elevated rates of molecular evolution are consistent with smaller effective population sizes in lecithotrophs, and more rapid fixation of mutations by drift (Foltz 2003; Foltz et al. 2004). Accelerated substitution rates could also reflect increased local selection for lecithotrophs, with neutral substitutions hitchhiking to fixation through linkage to polymorphism under selection.

Inferring the Evolutionary History of Shifts in Development Mode

Efforts to model lifehistory evolution using discrete-state models have yielded ambiguous reconstructions at deep nodes in most groups (Collin 2004; Keever and Hart 2008; Waeschenbach et al. 2012; Pappalardo et al. 2014). Evolutionary quantitative genetic models may outperform discrete-state models for life-history traits, because reversals become less likely the longer a lineage drifts away from the threshold for character-state transitions (Revell 2013). This is more biologically realistic for development mode than a fixed rate for instantaneous transitions, as larval traits needed for swimming and feeding tend to be reduced or lost over time in lecithotrophs (Strathmann 1978, 1985). Retention of facultative feeding ability in some lecithotrophs suggests reversals to planktotrophy may be transiently possible (Botello and Krug 2006), but other recent gains of lecithotrophy have produced highly modified larvae, indicating some shifts in larval type may quickly become irreversible.

Reconstructions were unambiguous at most nodes in Plakobranchacea using an evolutionary quantitative genetics model, but ancestral states were not well resolved by either quantitative genetic or discrete-state models in Oxynoacea. The most likely scenario was 27 independent origins of lecithotrophy and no reversals, but the directionality of developmental change did not drive our results. Diversification analyses and tests for correlated trait evolution yielded equivalent results whether reversals to planktotrophy occurred at the same rate of forward change, or were constrained to be rare or prohibited. Changes in development were not unusually frequent in Sacoglossa. Roughly estimated as the number of shifts divided by number of branches, transition rates in Sacoglossa (0.067) were comparable to rates in *Conus* (0.068, Duda and Palumbi 1999), and lower than in Calyptraeidae (0.176, given ~25 shifts on 142 branches including one reversal; Collin 2004, Collin et al. 2007).

We present the first evidence that higher per-offspring investment (in the form of ECY) is correlated with gains of lecithotrophy. Although predicted (Oliphant and Thatje 2013), no prior study had showed that increased investment in planktotrophic offspring would accelerate evolutionary shifts to lecithotrophy. Having identified a reproductive character associated with changes in larval type, future studies can focus on how selection acts intraspecifically on offspring provisioning

(e.g., Allen et al. 2009). Such studies are needed to identify selective regimes that favor evolutionary transitions away from dispersive life-history strategies. Mechanisms that increase per-offspring investment are varied and phylogenetically widespread, including extra-zygotic yolk, capsular fluid, polar bodies, nurse eggs, egg energetic content, and brooding structures; future studies should test whether any such traits are broadly correlated with gains of lecithotrophy across other lineages.

In lecithotrophs, ECY may be analogous to nurse eggs: mothers sacrifice fecundity to produce larger offspring at hatching. Indeed, post-metamorphic ingestion of ECY by juvenile slugs can greatly affect size at hatching (Krug 2009). However, ECY in planktotrophs is harder to explain. Planktotrophy raises adult fecundity while lowering per-offspring costs and survival rates; however, most planktotrophs produce larger eggs than necessary for development (Levitan 2000). ECY allows mothers to produce larger larvae at hatching without adjusting egg size (Allen et al. 2009). Shifting yolk into ECY may thus reflect selection favoring smaller eggs to accelerate cleavage during the encapsulated benthic phase, when mortality rates can be higher than in the plankton (Strathmann et al. 2002; Allen and McAlister 2007). Alternatively, ECY consumed before hatching could buffer planktotrophic larvae against starvation in oligotrophic tropical waters, consistent with repeated losses of ECY from lineages in high-productivity temperate regions. A third possibility is that, since all embryos do not have equal access due to physical proximity in the egg mass, ECY increases intra-clutch variance in offspring size for planktotrophs as predicted by bet-hedging theory (Marshall et al. 2008). Comparative studies are needed to distinguish among hypotheses regarding the value of ECY production by planktotrophs, and to identify the selective regimes that drive its repeated loss.

Whatever the advantage, planktotrophic lineages investing in ECY appear to reach an evolutionary tipping point that favors transitions to lecithotrophy. Such developmental shifts further increase per-offspring costs, due to the larger egg sizes associated with non-feeding development, and have far-reaching consequences for the evolutionary success of a lineage. Correlated trait evolution thus links processes acting on individual fitness at the population level with macroevolutionary trends, since development mode (i) is dependent on another trait that varies intraspecifically (e.g., ECY production), and (ii) produces emergent species-level properties that influence clade diversification.

Reconstructions favored two to three independent origins of ECY in Plakobranchoidea, as well as five losses. Independent origins in closely related lineages raise the possibility of phylogenetic pseudoreplication (Maddison and Fitzjohn 2015). The distribution of ECY is akin to the scenario depicted in Figure 3C of Maddison and Fitzjohn (2015), which the authors deem a gray area for concerns over false positives due to coinheritance of two traits. However, multiple losses of ECY make our test for correlated evolution more conservative. Moreover, the biological relationship between ECY and egg size argues against meaningless coinheritance. The two traits are (i) mechanistically linked, as yolk is either packaged into eggs or granules of ECY, and (ii) functionally linked, through effects on per-offspring investment and larval size at hatching (Allen et al. 2009). In contrast, synapomorphies of Plakobranchoidea (e.g., kleptoplasty) or Plakobranchidae (e.g., parapodia) have no mechanistic or functional links to development, arguing against the possibility of an unidentified, ancestral trait that drives gains of lecithotrophy and happens to correlate with ECY.

The oceanic environment in which larvae mature is also expected to influence marine life-history evolution. Thorson (1950) proposed that planktotrophy predominates in tropical waters, supported by a recent meta-analysis of over 1000 invertebrates (Marshall et al. 2012). However, it was also suggested that Sacoglossa violates "Thorson's Rule" (Clark and Goetzfried 1978; Clark and Jensen 1981). Indeed, we found nearly a third of tropical sacoglossans were lecithotrophic (28 spp.), versus only three temperate lecithotrophs (two of which were poecilogonous: *Alderia willowi*, *Elysia chlorotica*; Vendetti et al. 2012). Greater trophic stability may make availability of algal hosts more predictable in the tropics, favoring nondispersive larvae in Sacoglossa due to adult specialization (Jensen 1997). Biogeography of development mode likely reflects complex interactions between selection on larval stages, driven by circulation patterns (Pringle et al. 2014) or water temperature (Marshall et al. 2012), as well as adult ecology and correlated reproductive traits. More sophisticated models are needed to dissect the contributions of these various potential drivers of developmental change.

Phylogenetic Implications

This study advanced our understanding of evolutionary relationships in Sacoglossa, by sampling more species than any prior work and including representatives of all widely accepted genera except the monotypic *Roburnella*. *Cylindrobulla* has contentiously been placed either sister to Sacoglossa (Jensen 1996), or within Oxynoacea (Mikkelsen 1998). Our work supports inclusion of *Cylindrobulla* in Sacoglossa, but its phylogenetic affinities remain unresolved. Traditional families and genera were monophyletic in Oxynoacea and Plakobranchoidea but systematic revision is needed for Limapontioidea, a paraphyletic grade in which two of three families and nine genera were non-monophyletic. Consistent with their history of taxonomic instability, *Stiliger* and *Ercolania* were polyphyletic, each including members of three clades. Both *Placida* and *Limapontia* were paraphyletic. Several divergent clades may warrant elevation to new genera, including "*Costasiella*" *nonatoi* + sp. 4, "*Stiliger*" *smaragdinus* + sp.6, "*Gascoignella*"

jabae, and a clade comprising three "*Ercolania*" spp. and *Alderiopsis nigra*.

Conclusions

Our findings challenge long-standing assumptions about the effects of alternative life histories in the sea, and provide novel insight into how per-offspring investment can bias the evolutionary fate of a lineage. Lecithotrophy evolved at least 27 times in Sacoglossa, with gains disproportionately concentrated in ECY-producing lineages; increased offspring provisioning in planktotrophs may thus promote shifts to non-feeding larvae. Lecithotrophy facilitates local adaptation and may be selectively favored over ecological time scales, but is usually an evolutionary dead end, as evidenced by the absence of old or diverse lecithotrophic lineages. Contrary to classical interpretations of the gastropod fossil record, models of state-dependent diversification strongly supported species selection favoring planktotrophy. Lecithotrophic lineages arose often, but rarely persisted long enough to diversify. Our findings may thus explain the long-term persistence of planktotrophy in most clades, despite high rates of character change to lecithotrophy. Smaller effective population sizes for lecithotrophs may drive higher rates of both DNA substitution and extinction. Selection on offspring provisioning can thus promote life-history shifts with cascading effects on evolutionary rates, with frequent origins of short-lived larvae resulting in short-lived lineages, rather than bursts of diversification as long envisioned.

Supplementary Material

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.88mv3.

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