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Evolutionary biology

Developmental mode influences diversification in ascidians

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Ascidian species (Tunicata: Ascidiacea) usually have tailed, hatching tadpole larvae. In several lineages, species have evolved larvae that completely lack any tail tissues and are unable to disperse actively. Some tailless species hatch, but some do not hatch before going through metamorphosis. We show here that ascidian species with the highest speciation rates are those with the largest range sizes and tailed hatching larval development. We use methods for examining diversification in binary characters across a posterior distribution of trees, and show that mode of larval development predicts geographical range sizes. Conversely, we find that species with the least dispersive larval development (tailless, non-hatching) have the lowest speciation rates and smallest geographical ranges. Our speciation rate results are contrary to findings from sea urchins and snails examined in the fossil record, and further work is necessary to reconcile these disparate results.

1. Introduction

Marine species have evolved modes of larval development that differ in dispersal potential. Thorson asserted that the primary advantage of swimming larvae for sessile or sedentary marine invertebrates is increased dispersal capabilities [1]. However, an alternative hypothesis suggests that planktonic larval development may have evolved in some lineages as an adaptation for escaping from benthic predators [2]. Nevertheless, studies of sister species in divergent metazoan phyla have shown that greater dispersal potential in feeding larvae often positively correlates with higher rates of gene flow between populations [3].

Studies explicitly examining the relationship between larval development and geographical range in extant marine metazoan species have found a positive correlation between larval dispersal potential and geographical range [4,5]. Marine invertebrates species with higher dispersal potential positively correlate with longer species durations and larger geographical ranges [6–9]. Fossil snails and urchins with the derived non-planktonic or less dispersive larval phase show higher speciation rates than planktonic species [6–10]. In fossil studies, examining the evolution of larval development has often not been done in a phylogenetic framework [6–8], which may overestimate trait effects.

Most of the 3000 described ascidians (Tunicata: Ascidiacea) develop as nonfeeding tadpole larvae that swim for a short period (hours to days), then settle and metamorphose into sessile, filter-feeding adults [11]. The Styelidae and Molgulidae show at least five independent origins of tailless larval development [12]. Some tailless species develop indirectly by hatching from the chorion before metamorphosis, and some hatch from the chorion only during metamorphosis (see the electronic supplementary material, tables S1 and S2).

Likelihood models have been developed recently for examining how discrete characters influence diversification rates inferred from incompletely sampled phylogenies [13–15]. Using a phylogeny of 45 ascidian species in the Molgulidae and Styelidae, and current distribution records of these species to estimate geographical range sizes, we find that species with less dispersive larval development (tailless non-hatching species) have lower speciation rates and smaller geographical ranges.

2. Material and methods

Trees were generated from 1840 bp of 18S rDNA for 45 ingroup taxa of Styelidae and Molgulidae from Genbank and from the phlebobranch outgroup, *Ciona intestinalis*. Sequences were aligned using MAFFT v. 6 [16]. We used a GTR $+ \Gamma +$ I substitution model selected using the Akaike Information Criterion (AIC; [17]) in MRMODELTEST v. 2.3 [18]. Because ascidians have a scanty fossil record [19], we used an uncorrelated, relaxed-clock phylogenetic estimation in BEAST [20] for 100 million generations.

Data on tailed and tailless larval development, and hatching and non-hatching tailless larvae (see the electronic supplementary material, tables S1 and S2) were used to examine how these categorical traits influence speciation rates using a binary-state speciation and extinction (BiSSE) and multi-state speciation and extinction model (MuSSE) [13,14] in the R package diversitree [15]. The BiSSE framework compared models where diversification differed between tailed and tailless larval development, hatching and non-hatching as well as the combination of these characters in the MuSSE framework.

AIC [17] was used to test between different models on the consensus tree in BEAST and 100 posterior trees. We compared only speciation rates in the BiSSE and MuSSE analyses because simulation studies have shown extinction rates are difficult to estimate assuming a birth–death model of speciation, and extinction as in BiSSE and MuSSE [21]. The 'skeletal trees' incomplete sampling method was used to account for the missing taxa in our tree when estimating rates using BiSSE and MuSSE [14]. Extinction rates were set to be the same rates, given the potential for mis-estimation and parameter correlation. However, it is possible that if extinction rates were widely divergent between species with different larvae, then our model would not be an adequate description of the process, and our inferences may be misled. While we acknowledge this possibility, given how notoriously unreliable estimates of extinction rates are [21], and that estimates derived from molecular phylogenies tend to be very low (often close to 0), we do not think this is a likely scenario.

Geographical range sizes were estimated using current species distribution records (see the electronic supplementary material for more information). In brief, we estimated range sizes by removing outlier points, then used a longest straight line distance (rhumbline) or ellipsoidal area. We then tested to see if either binary or additively larval traits were a predictor of geographical range size using Bayesian phylogenetic mixed models in the R package MCMCglmm [22]. The posterior probability of different models were estimated using a MCMC approach, running the chains for 10 million iterations with a one million iteration burn-in.

3. Results

All 100 trees show higher speciation rates in tailed species compared with tailless species (figure 1*a*), and 99/100 trees show higher speciation rates in hatching species compared with non-hatching species (figure 1*b*).

We tested to see if a combination of our two sets of binary larval traits (tailed and taillessness, hatching and non-hatching) is better for assessing speciation rates using a recently developed test [15]. All 100 trees show a better AIC fit for a model where speciation is estimated as a combination of these binary traits (table 1). 94/100 trees and 100/100 trees show higher speciation rate estimates in the interaction model when compared with speciation rate estimates for tailed and tailless alone, or for hatching and non-hatching alone, respectively. 100/100 posterior trees show the highest speciation rates in tailed hatching species when compared with tailless hatching and tailless non-hatching species. Tailless hatching species show higher speciation rates when compared with tailless non-hatching species in 65/100 posterior trees.

Mean rhumbline range sizes for all tailed, hatching species used in the study were $2425 \pm$ a standard error (s.e.) of 1201 km. Range sizes were $2164 \pm$ s.e. of 1038 km, and $201.4 \pm$ 239.9 km for tailless, hatching, and tailless, non-hatching species, respectively (see the electronic supplementary material). For rhumbline range sizes (see the electronic supplementary material, figure S2), we found species with hatching larval development to have larger range sizes than species with non-hatching larval development ($\beta = 2.51 \pm$ s.e. 2.12; $p = 0.0212$). We found similar results for total range sizes comparing species with hatching and non-hatching larvae ($\beta = 5.066 \pm 2.56$; $p < 0.001$). We found mean range sizes for species with tailed hatching larval development to be the largest, mean range sizes for species with tailless hatching larval development to be intermediate and mean range sizes for species with tailless non-hatching larval development to be the smallest ($\beta = 4.80 \pm 3.41$; $p = 0.0067$). Results were consistent with analyses on ellipsoid ranges (see the electronic supplementary material for more details).

4. Discussion

Our results demonstrate that species with tailed, hatching larval development have higher speciation rates than ascidian species with tailless hatching, and tailless non-hatching larval development (figure 1), based on the available phylogeny (figure 2). Data for 45 of an estimated 762 described species in the Molgulidae and Styelidae were used, but we did use an incomplete sampling method to take this missing data into account [14]. Nevertheless, the small sample of species could bias our results. We also have to be cautious that the phylogeny used for this study may not be the true species tree owing to the coalescent process [23]. However, most clades are reflective of taxonomic and morphological relationships [24], and are in agreement with a phylogeny we inferred on a subset of taxa at 18S and 28S rRNA genes. We accounted for low support for species relationships in our phylogeny by comparing BiSSE and MuSSE analyses across 100 posterior trees.

A simulation study examining the ability to detect differences in rates of speciation, extinction and character transitions found there to be a decrease in the ability to detect the true simulated differences using BiSSE when phylogenies were moderately sized [25]. However, low power should tend to reduce our ability to detect differences between parameters, rather than exacerbate them. We have found that when we simulate trees with a 2.5 times difference
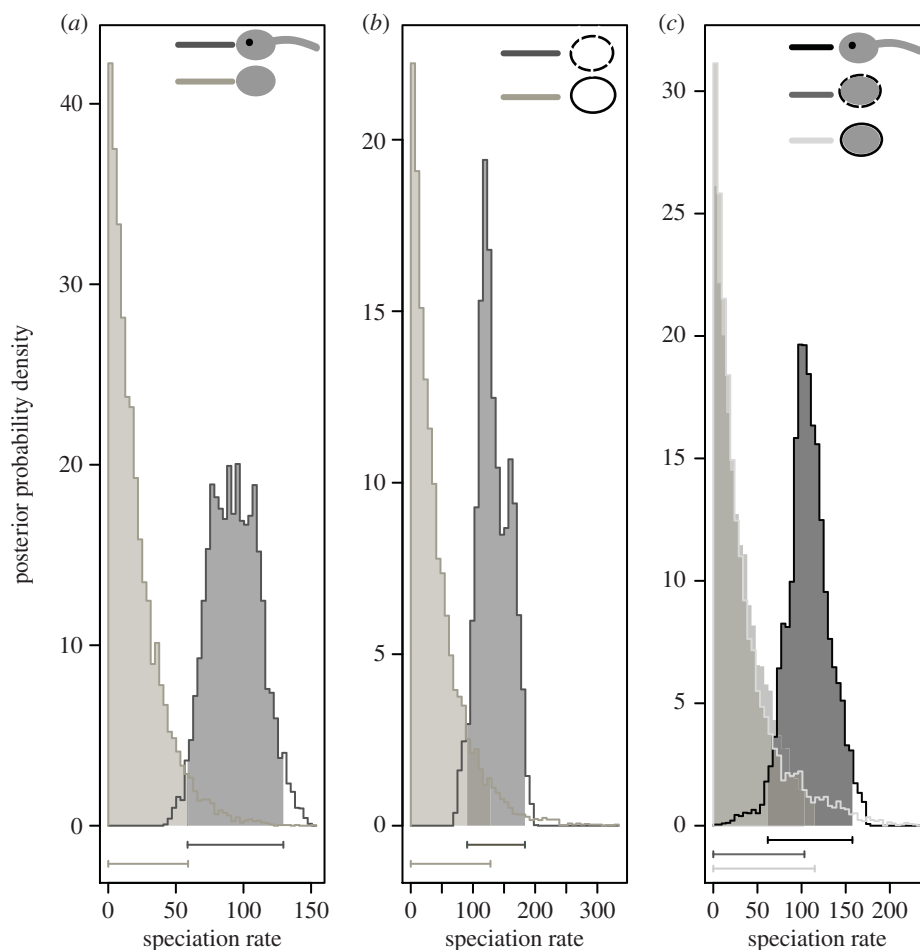
Figure 1. Speciation rates (in speciation events per substitution per site) for different larval character states using BiSSE and MuSSE. (*a*) Speciation rates from BiSSE analyses of tailed and tailless species; (*b*) speciation rates from BiSSE analyses of hatching and non-hatching species; (*c*) MuSSE model for species with tailed hatching, tailless hatching and tailless non-hatching larval development. The line below each speciation rate distribution is the 95% credible interval. Species with tailed larvae have a tail and eyespot, tailless species that hatch have a dotted circle and tailless species that do not hatch have a solid circle.

Table 1. Model comparison using AIC for BiSSE and MuSSE analyses. The best-fit model from the BEAST consensus tree is in italic, and the numbers of 100 posterior trees that agree with the best-fit model from the consensus tree are given. Td, tailed; Tl, tailless; H, hatching; NH, non-hatching; SI, state independent; SD, state dependent; q, transition.

BiSSE Td versus Tl		BiSSE H versus NH		MuSSE interaction		MuSSE Td + H versus Tl + H versus Tl + NH	
model	**AIC**	**model**	**AIC**	**model**	**AIC**	**model**	**AIC**
SD	−212.08	SD	−223.82	SI	−174.58	SD	−183.72
SD-qTl-Td	−209.38	*SD-qNH-H*	−226.12	SD	−145.94	SD-q21	−186.84
		SD-qH-NH	−225.58	*interaction*	−191.26	SD-q31	−188.30
						SD-q32	−184.95
						SD-q21, q31	−190.43
						SD-q21, q32	−192.38
no. 100 trees agree	80/100	no. 100 trees agree	61/100	no. 100 trees agree	100/100	no. 100 trees agree	54/100

in speciation rates between the ancestral and the derived state, the power to detect different rates decreases in trees of 23 species (77/100 trees). However, our tree size of 45 tips is enough to detect similar results in large trees (95/100 trees with 45 tips show higher speciation rates in the ancestral

character compared to 98/100 trees with 450 tips; see electronic supplementary materials for more details).

We believe that tailed, hatching species cannot evolve from a tailless ancestor because there is molecular evidence to show that pseudogenes are formed in proteins critical for
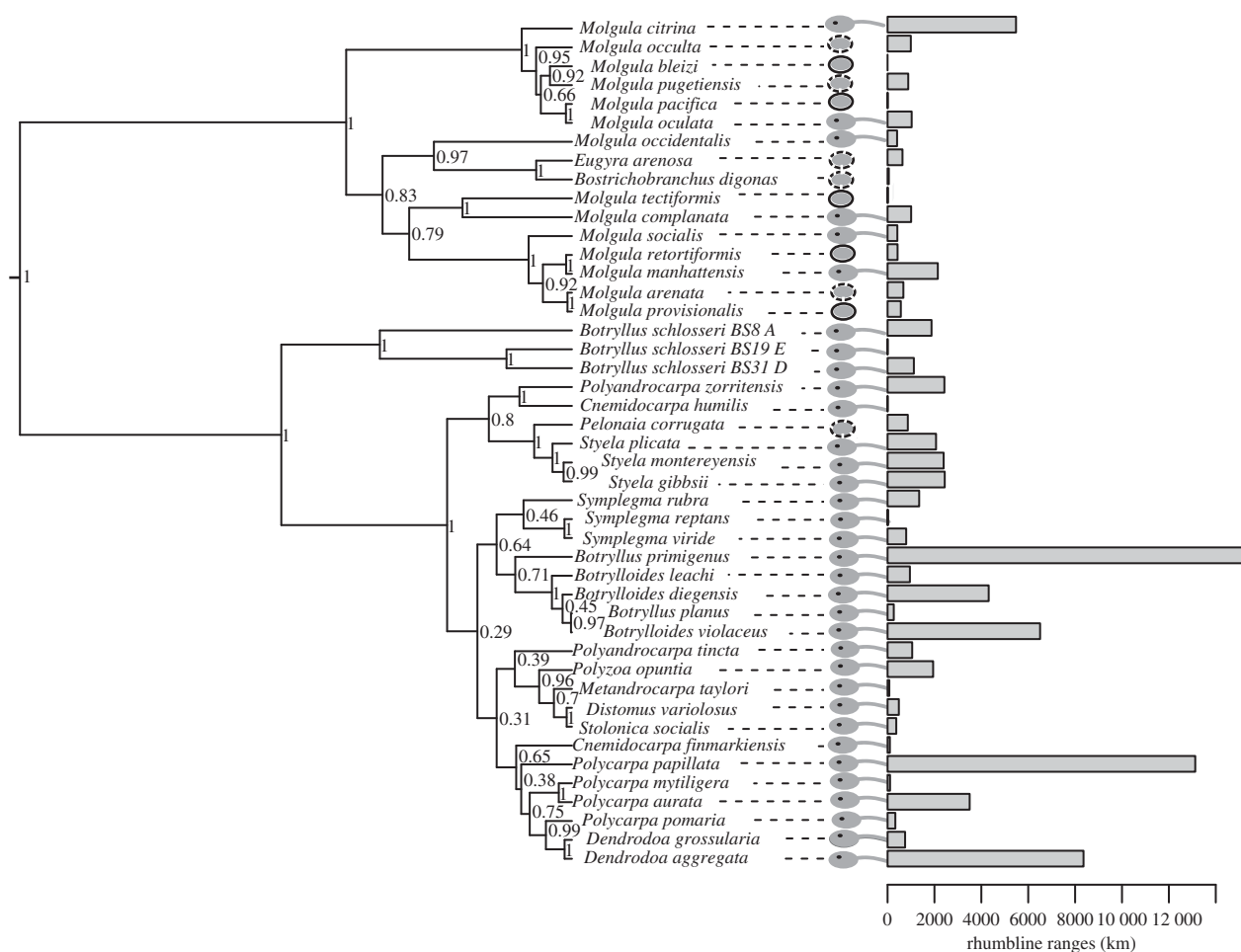
Figure 2. Ultrametric phylogeny of molgulid and styelid ascidians from a consensus BEAST tree. The lower axis bar graph shows range size for each species in km. Species with tailed larvae have a tail and eyespot, tailless species that hatch have a dotted circle and tailless species that do not hatch have a solid circle.

swimming in multiple tailless species [26]. It is still unclear, however, why these derived modes of larval development have evolved multiple times (figure 2). Species selection may be acting to maintain tailed, hatching larval development in molgulid and styelid ascidians; this argument was made to explain higher diversification rates in plant species with ancestral self-incompatibile reproduction than in species with derived self-compatible reproduction [27].

Our results showing lower speciation rates in the derived tailless and non-hatching species, which also have significantly smaller geographical ranges, is contrary to the findings of snails and urchins in the fossil record where derived species with smaller geographical ranges have higher speciation rates [6–9]. These contrary results may also be due to unforeseen issues with comparing speciation rates estimated from fossil data and molecular phylogenies. It is also possible that these different results could be due to comparing speciation rates for groups with alternative larval development that are fundamentally different. The transition from feeding to non-feeding larval development in fossil snails and sea urchins may fundamentally affect dispersal and diversification dynamics in different ways than do the loss of the tail and hatching in ascidians.

While these transitions to a less dispersive larval mode decrease range sizes in all of the groups compared, range size may not predict speciation dynamics entirely. The findings of lower speciation rates in derived tailless and non-hatching styelid and molgulid ascidian species could also be influenced by the population dynamics of these species with alternative modes of larval development. Some simulation studies support lower speciation rates in species with ecological and geographically patchy distributions [28,29]. Species with non-hatching tailless larval development typically have patchy distributions and are found in very specific locations. For example, *Molgula pacifica* is found in high wave action 'blow holes' [30].

Stanley's work on burrowing bivalves predicted a log-normal relationship between mean population size and speciation rate [31]. This curve would predict small population sizes at very low speciation rates, and we suggest that this may apply for tailless non-hatching molgulids [31]. This rationale was used to describe species with small average population sizes that are going through a higher rate of extinction than speciation, so 'many entire species are dying out, then few small populations representing incipient species will be able to blossom into full-fledged species' [32]. While we are unable to reliably estimate extinction rates using our methods [21], more work will be needed to understand the population dynamics of species with different modes of development in ascidians and other marine invertebrates.

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