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Supertree analyses of the roles of viviparity and habitat in the evolution of atherinomorph fishes

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Abstract

Using supertree phylogenetic reconstructions, we investigate how livebearing and freshwater adaptations may have shaped evolutionary patterns in the Atherinomorpha, a large clade ($\approx$1500 extant species) of ray-finned fishes. Based on maximum parsimony reconstructions, livebearing appears to have evolved at least four times independently in this group, and no reversions to the ancestral state of oviparity were evident. With respect to habitat, at least five evolutionary transitions apparently occurred from freshwater to marine environments, at least two transitions in the opposite direction, and no clear ancestral state was identifiable. All viviparous clades exhibited more extant species than their oviparous sister taxa, suggesting that transitions to viviparity may be associated with cladogenetic diversification. Transitions to freshwater were usually, but not invariably associated with increased species richness, but the trend was, overall, not significant among sister clades. Additionally, we investigated whether livebearing and freshwater adaptations are currently associated with elevated risks of extinction as implied by species' presence on the 2004 IUCN Red List. Despite being correlated with decreased brood size, livebearing has not significantly increased extinction risk in the Atherinomorpha. However, freshwater species were significantly more likely than marine species to be listed as endangered.

Introduction

Atherinomorphs are unusual among the ray-finned (actinopterygiian) fishes in that they have repeatedly evolved both livebearing (Lydeard, 1993; Reznick *et al.*, 2002; Mank *et al.*, 2005) and freshwater adaptations (Helfman *et al.*, 1997). Both of these traits have profound effects on dispersal, reproductive rate and life history, characters that have been implicated in diversification and extinction in a variety of organisms (MacArthur & Wilson, 1967; Vrba, 1983; Diamond, 1984; Lydeard, 1993; Trewick, 1997; Crooks & Soule, 1999; Purvis *et al.*, 2000; Winker, 2000; Waters & Wallis, 2001).

Two forms of livebearing – lecithotrophy (wherein the embryo is nourished by egg yolk that was provisioned prior to fertilization) and matrotrophy (where nutrient transfer occurs directly from mother to embryo) – have

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each evolved numerous times in fishes (Gross & Shine, 1981; Wourms, 1981; Gross & Sargent, 1985; Meyer & Lydeard, 1993; Reznick *et al.*, 2002; Mank *et al.*, 2005). Livebearing (as contrasted with external embryonic development) profoundly affects both maternal–fetal relationships and the mating behaviours by both sexes. In particular, each evolutionary transition to viviparity from oviparity requires the establishment of complex suites of physiological, anatomical and behavioural adaptations for internal brooding (Amoroso, 1968; Wourms, 1981; Wourms *et al.*, 1988; Guillette, 1989; Schindler & Hamlett, 1993; Reznick *et al.*, 2002) as well as the evolution of intromittant organs and behavioural modifications for internal fertilization (Rosen & Gordon, 1953; Zauner *et al.*, 2003).

Being presumably intricate, these physiological and anatomical changes might also act as an evolutionary ratchet for livebearing, perhaps inhibiting the loss of viviparity even in lineages that may no longer benefit from it. Indeed, the precocity of live-born progeny presents a reproductive tradeoff: viviparous females

normally produce fewer progeny but these offspring may experience a higher survival rate than oviparous progeny (Wourms, 1981; Wourms *et al.*, 1988; Wourms & Lombardi, 1992). In other animals, higher extinction risks have sometimes been associated with smaller litter sizes (Bennett & Owens, 1997; Jones *et al.*, 2003; Saether *et al.*, 2005) and higher trophic levels (Diamond, 1984; Crooks & Soule, 1999).

Many atherinomorph fishes also spend all or most of their lives in freshwater (Helfman *et al.*, 1997). Due to the discontinuous physical nature of such habitats, stream and lake-dwelling fishes often experience lower dispersal and inter-population gene flow than comparable marine species (DeWoody & Avise, 2000), and these factors can promote vicariant speciations (Vrba, 1983; Winker, 2000; Waters & Wallis, 2001). But disjunct freshwater habitats can also reduce local population sizes and perhaps thereby increase inbreeding, two classic correlates of increased extinction risk (Lande, 1999).

Our aims in this study are threefold: to test whether important evolutionary adaptations have cladogenetic effects; to ascertain whether an elevated extinction risk is associated with these adaptations; and to test the feasibility of supertree construction (given current computational capabilities) for an exceptionally large clade with more than 1500 terminal taxa. The Atherinomorpha, comprised of Cyprinodontiformes (ca. 1000 species of guppies, platyfish and allies), Beloniformes (nearly 250 species of needlefish, ricefish and allies), and Atheriniformes (about 300 species of silversides, rainbowfish and allies), is phylogenetically one of the best-characterized clades of ray-finned fishes, making it an ideal group for these goals. To that end, we have constructed a species-level supertree for Atherinomorpha and used its topology to identify sister clades with alternate character states for both livebearing and freshwater adaptations. By definition, sister taxa are equally old (Cracraft, 1981), so independent contrasts between many such pairs permit tests of possible lifestyle associations with other evolutionary features such as speciation rate and extinction risk.

Materials and methods

Supertree construction

We constructed a formal matrix representation with parsimony (MRP) supertree (Ragan, 1992) from available phylogenetic literature for all currently recognized species (Eshmeyer, 1990) of Atherinomorpha, being careful to omit phylogenetic inferences based on livebearing or its associated anatomies *per se* (to avoid circular reasoning). The data matrix underlying our supertree reconstruction consisted of cladogenetic information from 58 published source phylogenies (listed in supplemental references), each generated from molecular or morphological data for overlapping subsets of atherinomorph

species. We reconciled all nomenclature in these source phylogenies according to recent taxonomic work (Eshmeyer, 1990; Nelson, 1994). In our matrix, which was coded in standard binary format (Baum, 1992; Ragan, 1992), each column represents a provisional monophyletic clade as identified in a given source phylogeny, with the information from all 58 source phylogenies concatenated into a supermatrix that consisted of 1355 columns (putative clades) and 1544 rows (species).

Because recent phylogenetic appraisals have shown good support for the monophyly of each atherinomorph order (Parenti, 1981; Miya *et al.*, 2003), and because datasets with > 200 taxa create massive computational complexity, we performed the following two parsimony-based analyses on each taxonomic order independently before reassembling the ordinal-level topologies into an atherinomorph supertree. First, using PAUP* 4.0B (Swofford, 2003), we conducted 100 heuristic searches of the data matrix using random-order addition. Each search starts in a different area of tree space, so multiple searches help ensure that a local optimum in not mistaken for the global optimum. We computed a 90% consensus tree from 100 000 trees with the best tree score, and used its topology for our subsequent comparative phylogenetic analyses. Second, to confirm our best tree score, we preformed 1000 iterations (five sets of 200 iterations each) of the parsimony ratchet (Nixon, 1999) as implemented by PAUPRAT (Sikes & Lewis, 2001). Agreement between the heuristic searches and the parsimony ratchet does not guarantee the best possible tree, but it does suggest a good provisional tree. For Atheriniformes and Cyprinodontiformes, the parsimony ratchet did not result a lower tree score than did the heuristic PAUP* searches with random order addition. In Beloniformes, however, the parsimony ratchet returned a lower tree score than our standard PAUP* searches, so in this case we used the ratchet to generate 10 000 trees with this lower score and then used these equally parsimonious trees to generate a 90% consensus phylogeny. Current molecular (Miya *et al.*, 2003), morphological (Parenti, 1981), and supertree meta-analysis (Mank *et al.*, 2005) concordantly indicate that Atheriniformes is outgroup to Beloniformes and Cyprinodontiformes, so we assembled the ordinal phylogenies accordingly.

Species diversity and extinction risk

We assembled from several sources a database on fertilization mode and category of embryonic development for all recognized species of Atherinomorpha (Breder, 1922; Breder & Rosen, 1966; Constanz, 1989; Ghedotti, 2000; Froese & Pauly, 2004). Then, using the supertree topology and maximum parsimony reconstruction as implemented in MACCLADE 4 (Maddison & Maddison, 2000), we examined the evolutionary histories of livebearing. We assumed that the ancestral state for Atherinomorpha was oviparity with external

fertilization, as viviparity has previously been shown to be a derived trait in ray-finned fishes (Lydeard, 1993; Mank *et al.*, 2005). We identified sister clades with alternate reproductive modes, and then evaluated whether viviparous clades tend overall to be more diverse than oviparous clades in terms of numbers of extant species, using 1000 iterations in a randomization test as recommended by Nee *et al.* (1996) and Vamosi & Vamosi (2005). Such randomization tests evaluate in this case whether a particular adaptation is correlated with increased diversity across (not within) all analyzed sister-clades. Only non-nested sister clades that were exclusively viviparous or oviparous were considered in these comparisons.

We also examined whether transitions to freshwater habitat may have promoted species diversity. We assembled a database on numbers of extant freshwater and marine species for all the atherinomorphs (Froese & Pauly, 2004), and then performed the same types of comparative phylogenetic analyses as described above for livebearing. As with the above-described analysis, all comparisons were non-nested.

Finally, we mapped extinction risk as determined by the IUCN 2004 Red List (Baillie *et al.*, 2004) onto the supertree phylogeny. Species with the following IUCN designations were treated as those 'at elevated risk' for extinction: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened and conservation dependent. Many sister clades contained few taxa and thus provided very low statistical power, so we examined possible associations of elevated extinction risk with livebearing and freshwater adaptations across the entire Atherinomorpha. According to the IUCN Red List, 9.85% of atherinomorph species are now in jeopardy. Accordingly, we used this figure to calculate expected numbers of at-risk species given the observed numbers of taxa with viviparous vs. oviparous and with freshwater vs. marine lifestyles, assuming random associations between the variables. For each pair of reproductive or habitat states, we compared the random distribution to the observed distribution using one-tailed Fisher's exact test.

Results

The full supertree for Atherinomorpha, available in supplemental materials, is presented in condensed form in Fig. 1. Although the published data were insufficient to resolve the phylogenetic placement of all 1500+ species, nearly all relationships among genera were resolved in the 90% consensus tree.

Based on our phylogenetic analyses, viviparity in Atherinomorpha apparently evolved on at least four separate occasions: once in Beloniformes and thrice in Cyprinodontiformes (Fig. 1). We found no evidence that livebearing, once gained, was ever subsequently lost from any atherinomorph lineage. In sister-clade analyses,

each of the viviparous clades identified proved to be significantly more species-rich than its oviparous sister-lineage (Table 1), a trend that overall was marginally nonsignificant (randomization test, $P = 0.0625$). Furthermore, viviparity proved not to be significantly associated with elevated extinction risk at the present time (Fisher's exact test, $P = 0.09$; Table 2).

Cyprinodontiform fishes inhabit freshwaters primarily, but inter-conversions between marine and freshwater habitats clearly have occurred on multiple occasions in Beloniformes and Atheriniformes. These inter-conversions (at least five transitions from freshwater to marine, two from marine to freshwater) were primarily at the congeneric or confamilial level and therefore are not shown in Fig. 1. Compared to marine lineages, freshwater lineages do not appear to have experienced a higher rate of diversification (randomization test, $P = 0.125$), but they do appear to suffer a higher current risk of species extinction (Fisher's exact test, $P < 0.0001$; Tables 3 and 4). Because lifestyle transitions between freshwater and marine environments have been rather frequent and scattered across Atherinomorpha, no ancestral state for this trait could be identified unequivocally.

Discussion

Supertree reconstruction is the only approach currently available for combining phylogenies from diverse and otherwise incompatible data matrices (Ragan, 1992). Supertree methods have made possible the construction of cohesive phylogenies from disparate data sets for several major taxonomic groups, such as angiosperms (Davies *et al.*, 2004) and bats (Jones *et al.*, 2002). The phylogeny for Atherinomorpha presented here, with 1544 included species, is to our knowledge the largest supertree yet reported for any animal assemblage (Bininda-Emonds, 2004). Most of the source phylogenies for this supertree were based on molecular data (notably mtDNA sequences), but a sizable portion also utilized morphological evidence.

Supertrees with large taxon samples present enormous challenges for maximum parsimony searches. We tried to minimize this complexity in two ways. First, we divided the sample according to taxonomic order. Restricting the supertree searches to monophyletic clades reduced complexity of the supertree reconstruction. Second, we also employed the parsimony ratchet, which has been shown to outperform standard heuristic parsimony searches in some cases (Nixon, 1999), including supertrees (Price *et al.*, 2005). Despite these shortcuts, the analyses presented here represent the equivalent of two+ years of computational time for one computer (to greatly reduce the search time, we actually used 14 dual processor G4 MacIntosh machines). Without far more powerful search methods, it seems doubtful that supertrees with much larger numbers of taxa could be recovered. For example,
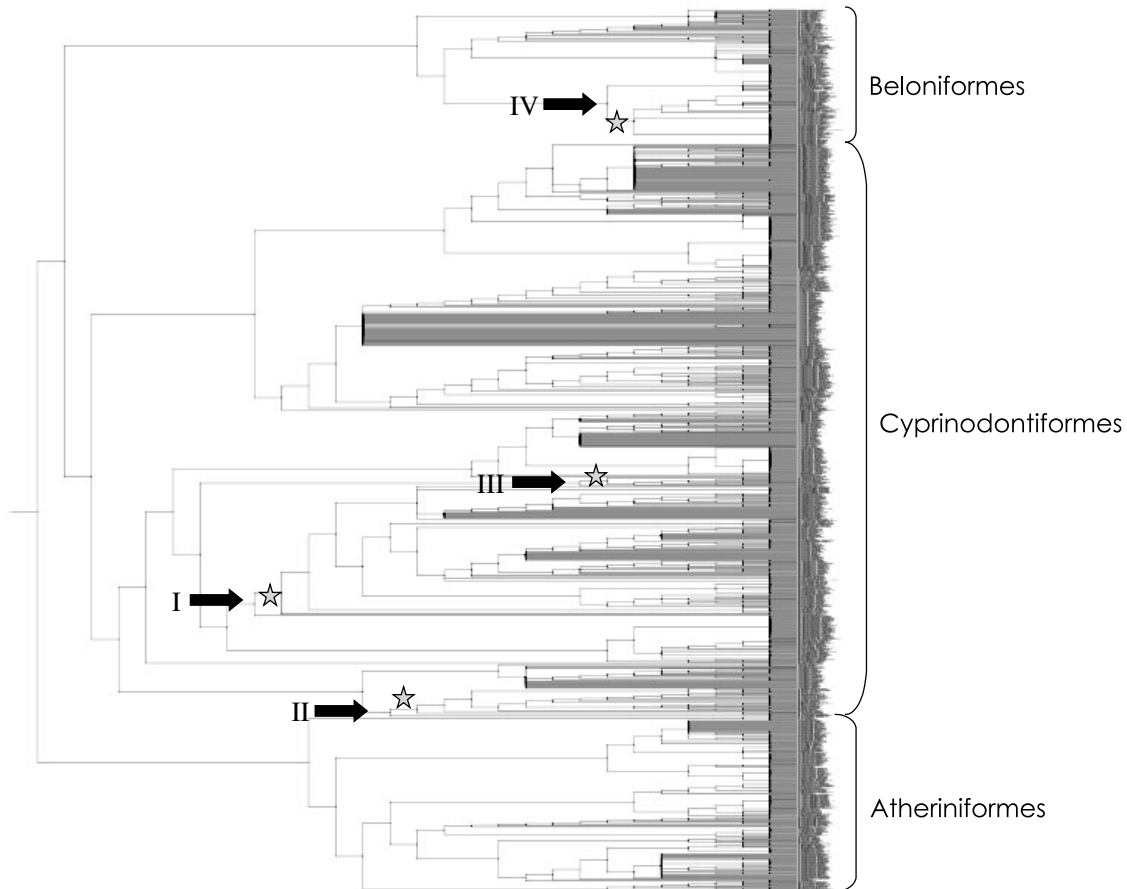
Fig. 1 Supertree topology for Atherinomorpha. Shown is the condensed, 90% consensus of 100 000 equally parsimonious trees. Taxonomic orders within the Atherinomorpha are indicated to the right of the topology. Stars indicate independent origins of livebearing in this group, and arrows show the root of sister-clade comparisons. Roman numerals adjacent to sister clades correspond to the statistical comparisons in Table 1.

a species-level supertree for all Actinopterygii, with >20 000 extant species, would require either enormous computational capacity or much cleverer search methods than those available at present.

The supertree presented here is of course preliminary and potentially subject to topological revision at internal nodes (especially at genus and species levels) as additional phylogenetic information becomes available. The same applies to supertrees constructed for other taxa. Although biological conclusions from supertree approaches are inherently provisional, we expect that similar analysis methods will soon become increasingly feasible and popular for comparative phylogenetic applications in many taxonomic groups and biological settings.

Implications of viviparity

In agreement with previous appraisals (Lydeard, 1993; Mank *et al.*, 2005), we estimate at least four separate origins of livebearing in Atherinomorpha. Our results show that each of these four viviparous clades was much more species-rich than its respective oviparous sister-lineage (Table 1), but also that the overall trend *across* these clades was only marginally significant ($P = 0.0625$) in randomization tests (due to the inevitably poor power of this test statistic when only a few sister-clades are available for analysis). Whether or not viviparity increases diversification, it does not appear to be associated with an increased risk of extinction at the present time (Table 2). Both of these findings suggest that any benefits of livebearing, including larger offspring born to a higher trophic level, may generally outweigh potential disadvantages of smaller clutch sizes (Wourms & Lombardi, 1992).

These findings raise questions as to why viviparity is relatively uncommon in Atherinomorpha (only about 25% of species are livebearers) and even rarer across the entire Actinopterygii. This probably reflects, at least in part, the difficulty of evolving sophisticated suites of physiological, anatomical, and behavioural features asso-

Table 1 Sister-clade comparisons of species richness in relation to livebearing.

Comparison	Clade	Number of viviparous species	Number of oviparous species
I	Poeciliinae	230	1
II	Goodeidae/Profundulidae	46	5
III	Anablepinae	14	1
IV	Hemiramphidae	95	21

Overall trend *across* clades in a randomization matched pairs test: $P = 0.0625$.

Table 2 Extinction risk in relation to reproductive mode.

Reproductive Mode*	Observed (and expected‡) number of threatened taxa	Significantly *higher* risk of extinction than expected?
Viviparous (4)	34 (37)	no
Oviparous (1)†	135 (114)	no§

Viviparity and oviparity were evaluated, using Fisher's exact test, for a possible significant difference in the current number of threatened species.
*Estimated number of unambiguous origins under maximum parsimony.
†Assumed ancestral state, and no reversions from livebearing.
‡Expected under random association with lifestyle.
§$P = 0.09$, one-tailed Fisher's exact test.

ciated with livebearing. Several key biological modifications are entailed in any evolutionary transition from oviparity to viviparity, and these evolutionary hurdles may be difficult to overcome. On the other hand, as judged by recurrent evolutionary origins of viviparity in Atherinomorpha, at least some lineages in this clade appear predisposed for transitions to livebearing, possibly due to the high incidence of internal fertilization in this group (Breder & Rosen, 1966).

Implications of freshwater occupancy

Any evolutionary transition from marine to freshwater lifestyles could have both positive and negative impacts on a lineage's prospects for cladogenesis. Freshwater habitats to most fishes are like land islands to many terrestrial organisms (MacArthur & Wilson, 1967), physically subdividing a species and limiting gene flow

Table 4 Extinction risk in relation to aquatic habitat.

Habitat (number of independent origins)*	Observed (and expected†) number of threatened taxa	Significantly *higher* risk of extinction than expected?
Freshwater (2)	151 (132)	Yes‡
Marine (5)	1 (20)	No

Marine and freshwater adaptations were evaluated, using Fisher's exact test, for a possible significant difference in the current number of threatened species.
*Estimated number of unambiguous origins under maximum parsimony.
†Expected under random association with habitat.
‡$P < 0.001$, one-tailed Fisher's exact test.

in ways that can promote local diversification. On the other hand, these same fragmenting processes can produce small and specialized populations that individually may be subject to higher risks of extinction (Lande, 1999). Our comparative phylogenetic analyses for the Atherinomorpha suggest that evolutionary transitions from marine to freshwater environments have not always been associated with significant bursts of cladogenesis. Although four of the five analyzed sister-clades showed higher species richness in freshwater lineages, the low power of the randomization test prevented demonstration of a significant trend overall (Table 3). Despite the lack of a consistent correlation between freshwater habitat and higher species diversity, freshwater occupancy does appear to significantly increase current risks of species' extinction (Table 4).

Physical barriers to dispersal tend to be stronger for freshwater fish populations than their marine counterparts occupying otherwise comparable geographic ranges. Among the possible ramifications of this physical partitioning are the following: decreases in local effective population sizes, perhaps leading to occasional difficulties of inbreeding and mutational load; tendencies for specialized adaptations due to gene flow restrictions in conjunction with selection for local habitat conditions; and predisposition for restricted spatial distributions. In principle, any or all of these factors might make freshwater fishes especially vulnerable to extinction risks. Although our current findings are consistent with the notion that freshwater species are more likely than marine species to be considered in jeopardy at the present

Table 3 Sister-clade comparisons of extant species richness in relation to occupancy of freshwater or marine habitats.

Taxonomic order (family)	Freshwater clade (number of extant taxa)	Marine clade (number of extant taxa)
Beloniformes (Hemiramphidae)	*Nomorhamphus* and *Dermogenys* (31)	*Hemirhamphodon* (6)
Beloniformes (Belonidae)	*Xenentodon* (2)	*Stronglyura, Tylosurus,* and *Ablennes* (21)
Atheriniformes (Atherinidae)	*Craterocephalus* (24)	*Atherinion* (3)
Atheriniformes (Atherinopsidae)	*Basilichthys* and *Odontesthes* (24)	*Atherinops, Atherinopsis, Colpichthys,* and *Leuresthes* (5)
Atheriniformes (Telmatherinidae)	All other telmatherinids (16)	*Kalyptatherina* (1)

Overall trend *across* orders in a randomization matched pairs test: $P = 0.156$.

time, more detailed analyses will be needed to identify the separate or interactive effects of various factors involved in each situation.

Regardless of the cladogenetic implications, the biological alterations required for each successful evolutionary transition between marine and freshwater environments would seem to be fewer and less complex than those involved in the evolutionary transitions between oviparity and viviparity. Freshwater adaptations primarily involve osmo-regulatory adjustments (Helfman *et al.*, 1997), and many freshwater (and diadromous) lineages clearly retain a tolerance for high salinity regimes (Froese & Pauly, 2004). The suspected relative ease with which many fish lineages can make evolutionary transitions between freshwater and marine habitats is consistent with the rapid and recent inter-conversions (many at the level of taxonomic genus) between these lifestyles that are evident in our current phylogenetic appraisals.

Acknowledgments

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Supplementary Material

The following supplementary material is available for this article online:

Supplemental References

Source phylogenies used in constructing atherinomorph supertree.

This material is available as part of the online article from http://www.blackwell-synergy.com