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The evolution of exceptional diversity in parental care and fertilization modes in ray-finned fishes

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Abstract

Among vertebrates, ray-finned fishes (Actinopterygii) display the highest diversity in parental care, and their diversification has been hypothesized to be related to phylogenetic changes in fertilization modes. Using the most comprehensive, sex-specific data from 7,600 species of 62 extant orders of ray-finned fishes, we inferred ancestral states and transitions among care types and caring episodes (i.e., the stage of offspring development). Our work has uncovered 3 novel findings. First, transitions among different care types (i.e., male-only care, female-only care, biparental care, and no care) are common, and the frequencies of these transitions show unusually diverse patterns concerning fertilization modes (external, or internal via oviduct, mouth, or brood pouch). Second, both oviduct and mouth fertilization are selected for female-biased care, whereas fertilization in a brood pouch is selected for male-biased care. Importantly, internal fertilization without parental care is extremely unstable phylogenetically. Third, we show that egg care in both sexes is associated with nest building (which is male-biased) and fry care (which is female-biased). Taken together, the aquatic environment, which supports considerable flexibility in care, facilitated the diversification of parenting behavior, creating the evolutionary bases for more comprehensive parenting to protect offspring in semiterrestrial or terrestrial environments.

Keywords: phylogenetic comparative methods, life history evolution, ray-finned fishes, teleost, fertilization, parental care

Introduction

Parental care is highly variable among vertebrates, with considerable diversity in terms of the care-provider sex, in functional forms, and in complexity (Furness & Capellini, 2019; Mank et al., 2005; Reynolds et al., 2002; Royle et al., 2012). Because parental care has played a key role in the colonization of terrestrial habitats by vertebrates (Gomez-Mestre et al., 2012; Vági et al., 2019, 2022), it is more common among terrestrially reproducing lineages. Care is ubiquitous in birds and mammals (Cockburn, 2006; Gonzalez-Voyer et al., 2022; Gubernick & Klopher, 1981; Remeš et al., 2015), as well as in terrestrially reproducing amphibians (Gomez-Mestre et al., 2012; Vági et al., 2019, 2022). Only non-avian reptiles represent an exception as they mostly provide passive protection through the thick shells of their amniotic eggs (Shine, 1987; Somma, 2003). Despite parental care being much less frequent in aquatic vertebrates (Martin & Carter, 2013; Vági et al., 2019), the most speciose lineage, the ray-finned fishes (Actinopterygii) show the most diverse patterns of parenting (Balshine & Sloman, 2013; Mank et al., 2005). Given that

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the aquatic environment in general is not as hostile to the gametes (Martin & Carter, 2013) as a terrestrial environment where anamniotic eggs always face desiccation (Ishimatsu et al., 2018), the factors that drive the emergence of care in ray-finned fishes, and its subsequent radiation into diverse forms, are less well understood.

Both uniparental care, by either the male or female parent, and biparental care occur in ray-finned fishes (Blumer, 1979; Mank et al., 2005). However, the causes of the frequent evolutionary transitions between these care strategies remain unclear. Gross and Sargent's (1985) "stepping stone" model proposed a sequential evolutionary process: male care initially emerges from territorial defense, followed by the evolution of biparental care from male care. Subsequently, female care evolves from biparental care by male desertion. The "stepping stone" model would primarily explain care emergence in external fertilizers since it requires male territoriality to initiate the entire evolutionary sequence of transitions in parental care types. The advantages of territoriality in external fertilizers are at least twofold: to protect the clutch from fertilization by sneakers or other males and to protect the progeny from predators. Protecting a clutch in an established territory is usually not substantially more costly than defending the territory itself (however, protection of the clutch from predators may involve extra energy and increased predation risk for the parent). In addition, males can defend multiple clutches in the same territory, by which the males can substantially increase their reproductive success (Lindström, 1992; Reynolds et al., 2002; but see Benun Sutton & Wilson, 2019). Moreover, males can further increase their reproductive success by building a nest for the eggs, which can both provide protection and enhance their attractiveness to the females (Lindström et al., 2006; Mank et al., 2005; Svensson & Kvarnemo, 2023). However, the stepping-stone model has been criticized, as several studies have found that female parental care more likely evolved directly from no care (Benun Sutton & Wilson, 2019; Mank, 2005).

Recent studies suggest that the fertilization mode played an important role in the evolution of parental care in ray-finned fishes. Specifically, internal fertilization in the oviduct selects for female care, which creates an opportunity to retain the eggs inside the female's body. In contrast, external fertilization is associated with male care (Benun Sutton & Wilson, 2019; Mank, 2005), although most ray-finned fishes are external fertilizers and the vast majority do not provide any parental care (Balshine, 2012; Reynolds et al., 2002). Therefore, external fertilization, which was likely the ancestral condition in ray-finned fishes (Mank et al., 2005), does not seem sufficient alone to promote parental care by males. Benun Sutton and Wilson (2019) argued that external fertilization moved the control of the process out of the female's reproductive tract, giving more confidence in paternity to the males. However, paternity in external fertilizers is often uncertain, as group spawning and sneaker males frequently cause shared paternity (Taborsky & Neat, 2010). In fact, species that enhance spatial separation for the breeding pair by nest building and/or pair spawning provide more paternal care than species with group spawning (Benun Sutton & Wilson, 2019; Mank, 2005).

In addition, ray-finned fishes also evolved alternatives to oviduct fertilization, such as fertilization in a brooding pouch or in the mouth. Unfortunately, earlier studies (Benun Sutton & Wilson, 2019; Mank et al., 2005) did not consider these forms or classified them as external, even though they have much in common with oviduct fertilization: i.e., moving fertilization from the outside, unsafe environment into the body of one of the parents and thereby providing considerably more control over the process. Given the availability of extended phylogenetic coverage and state-of-art comparative methodologies (Boyko & Beaulieu, 2020; Rabosky et al., 2018), it is timely to revisit the associations between fertilization modes and parental care, and mapping the total diversity in both.

In the present study, we investigate the distribution and ancestral states of parental care type in approximately 7,600 ray-finned fish species. We have four main objectives. First, to infer the ancestral care state and then map the transitions between main care types to test the validity of the "stepping stone" model and alternative evolutionary pathways (Benun Sutton & Wilson, 2019; Gross & Sargent, 1985; Mank et al., 2005). Second, to map the distribution and reconstruct evolutionary transitions of diverse episodes of parental care (such as nest building, egg care, and fry care) exhibited by both males and females. We hypothesize that early care episodes (nest and egg care—Furness & Capellini, 2019) are more prevalent and evolutionarily more stable in males (Reynolds et al., 2002), while late care episodes (juvenile care) occur among females, as male care associates with territoriality and nest building, and female care with oviduct fertilization with egg retention (Benun Sutton & Wilson, 2019; Mank et al., 2005). Third, to infer the ancestral state and the transitions in the mode of fertilization by using fine-scale analyses that include all three different modes of internal fertilization: pouch, oviduct, and mouth. We hypothesize that all internal fertilization modes evolved from external fertilization (Benun Sutton & Wilson, 2019; Mank et al., 2005; Vági et al., 2022), and transitions between internal modes are missing or rare. Finally, we evaluate the relationship between types of parental care and mode of fertilization, and test whether fertilization modes predict the occurrence of each parental care type. We hypothesize that the sex that gains control over fertilization (i.e., which retains gametes or releases them after the other sex: females in oviduct fertilization and mouth fertilization, males in pouch fertilization) will provide more parental care and that transitions from external to internal fertilization triggered the evolution of parental care (Balshine & Abate, 2021; Wilson et al., 2003).

Methods

Data collection

Data on parental care was collected from Fishbase (Froese & Pauli, 2021; Supplementary Material S1). Our database includes parental care information for 7,601 ray-finned fish species representing 62 out of 65 recently recognized orders of ray-finned fishes (Supplementary Figure S1). We coded the type of parental care as follows: no care; male-only care; female-only care; and biparental care. We define no care as parents providing no care to the offspring. Female-only care and male-only care were assigned when either the female or the male solely provided offspring care. Care type was classified as biparental care if both the male and female parents provide care for the offspring and in cases of cooperative care.

Next, we classified care episodes according to the life stages of the progeny in separate variables as follows: (i) nest building (present/absent): pre-fertilization care, when a nest is constructed for the eggs based on a reproductive guild category on Fishbase (nesters) and/or when description of parental care behavior mentioned that one parent or both manipulate the substrate or make any construction prior to egg-laying; (ii) egg care (present/absent): if any form of care is provided to the eggs (guarding, fanning, cleaning, transportation/brooding). Live-bearing was considered as a specialized form of egg care. (iii) Fry care (present/absent): if care (guarding, transportation/brooding, feeding) is provided for the offspring that already have hatched. Nest building, egg care, and fry care are coded both in a non-sex-specific way and also separately for males and females. All of these variables are binary (i.e., as either present or absent). Next, based on these binary variables, we calculated a four-level "duration of care," calculated both in non-sex-specific way and separately for males and for females. Duration of care was coded as (0) no care when the males and/or the females lack any form of care provision; (1) nest building, when the nest is constructed for the offspring but no subsequent care is provided by the focal parent (in the sex-specific coding) or by any parent; (2) egg care, when the males or the females provide care for the eggs, but none for the fry; and (3) fry care if any form of parental care is provided for the fry. Duration of care tells how long one or both parents stay with the nest, the eggs, and/or the offspring. Finally, from the sex-specific duration of care variables we calculated care bias as (duration of $\text{care}_{\text{males}}$) minus (duration of $\text{care}_{\text{females}}$). This index is positive where males provide longer care than the females, 0 when the two sexes have care contribution of equal length (including non-caring species), and negative when the females provide longer care than the males, while its value (from −3 to + 3) is related to the difference in care length between the sexes, according to the developmental stages of the progeny.

FishBase has often been used in broad-scale studies on the evolution and ecology of fishes (e.g., Barneche et al., 2018; Kasimatis & Riginos, 2016; Vanadzina et al., 2021). To test whether Fishbase provides reliable data for parental care, we compared our parental care data with previous work (Benun Sutton & Wilson, 2019) that used other literature sources (in addition to Fishbase) for mapping parental care in fishes with broad phylogenetic coverage. Of the 808 species common to the two datasets, 793 species (98.14%) were consistently assigned to the categories no care, male-only care, female-only care, and biparental care. Most of differences between the two coding systems (1.23 %) arose from different parental care definitions in the two studies (we included nest building among care forms, in contrast to Benun-Sutton & Wilson, 2019), and our coding was in line with the information found in Fishbase. Therefore, we consider Fishbase to be a reliable source of parental care information.

Data on the mode of fertilization was collected from Fishbase for 7,302 species for which we had parental care information. Mode of fertilization refers to the location where fertilization takes place. Fishbase discerns four fertilization modes: external fertilization, where eggs are fertilized outside the parent's body; oviduct fertilization, where eggs are fertilized inside the mother's oviduct; mouth (or buccal) fertilization, where eggs are fertilized inside the mouth of a parent and pouch fertilization, where eggs are fertilized in a brood pouch (i.e., sea horses) or similar structure (e.g., in a protected area of the body—for example, between the fins). However, we revised the original classification of fertilization modes in Fishbase, as sometimes we found that the separation of the categories is confusing. Namely, some South American armored catfishes (Callichthyidae: Corydorinae) fertilize their eggs (after the female drinks the sperm and passing it through the intestines) between the basket-like pelvic fins of the females (Burgess, 1989; Kohda et al., 1995), while labyrinth fishes (Anabantiformes: gouramis, bettas, snakeheads, etc.) fertilize their eggs on the wide anal fin of the male when the pair coils around each other. In some species within these two groups, the location where fertilization occurs was classified as external, in other species, it was classified as in a pouch or similar structure in Fishbase. To account for this inconsistency, we categorized the fertilization mode of corydorine catfishes and labyrinth fishes both ways in two alternative systems (pouch fertilization in a broad sense—including these two groups; or in a narrow sense—excluding these two groups from pouch fertilizers, considering them as external fertilizers). We also excluded a hermaphroditic fish with ovarian self-fertilization, *Kryptolebias marmoratus* from oviduct fertilizers.

Phylogeny and taxonomy

We used the comprehensive actinopterygian phylogenetic tree from Rabosky et al. (2018). Any species missing from this tree and for which parental care information was available was added ($N = 3{,}443$) to its respective genus or family (depending on the resolution of the original tree) using the R package "ape" (Paradis & Schliep, 2019). In these cases, we collapsed the genus or family-level nodes to which new species were added to polytomies. For assigning species into orders in Supplementary Tables S1 and S2, we followed the taxonomy of the Fishbase. To ensure the robustness of the phylogenetic analysis to the editing that we made on the tree, we repeated the analysis using the original phylogeny presented in Rabosky et al. (2018) (see Supplementary Material S2 for details).

Statistical analysis

We carried out stochastic character reconstruction for parental care types, episodes, and mode of fertilization, using the "make.simmap" function in the "phytools" package (Revell, 2012) in R. We present here "all rates different" (ARD) models where different transition rates are allowed between the states as this type of model had the highest support compared to "equal rates" ("ER") and "symmetric" models ("SYM"); see Supplementary Material S2 for details. We calculated transition rates as the relative frequency of transitions: the number of transitions divided by the mean number of occurrences of the initial state.

The "corHMM" package (Boyko & Beaulieu, 2020) in R was used to calculate the evolutionary transition rates between character combinations of parental care and the mode of fertilization. This package fits hidden Markov models on discrete character evolution which allow different transition rate classes on different portions of the phylogeny. Applying this approach, we excluded the simultaneous transitions of the two given character states when including parental care and the mode of fertilization in one model. First, we built a simple model using binary coding for both variables (parental care: present/absent; fertilization: external /internal, where "internal" merged all three modes: mouth, oviduct, and pouch). Next, we built a finer-resolution model using a three-level coding for parental care bias (male-bias, no bias, female-bias, where "no bias" also included non-caring species) and all four modes of fertilization. To test phylogenetic robustness of our results, we repeated "corHMM" runs using a different phylogenetic tree (see Supplementary Material S2).

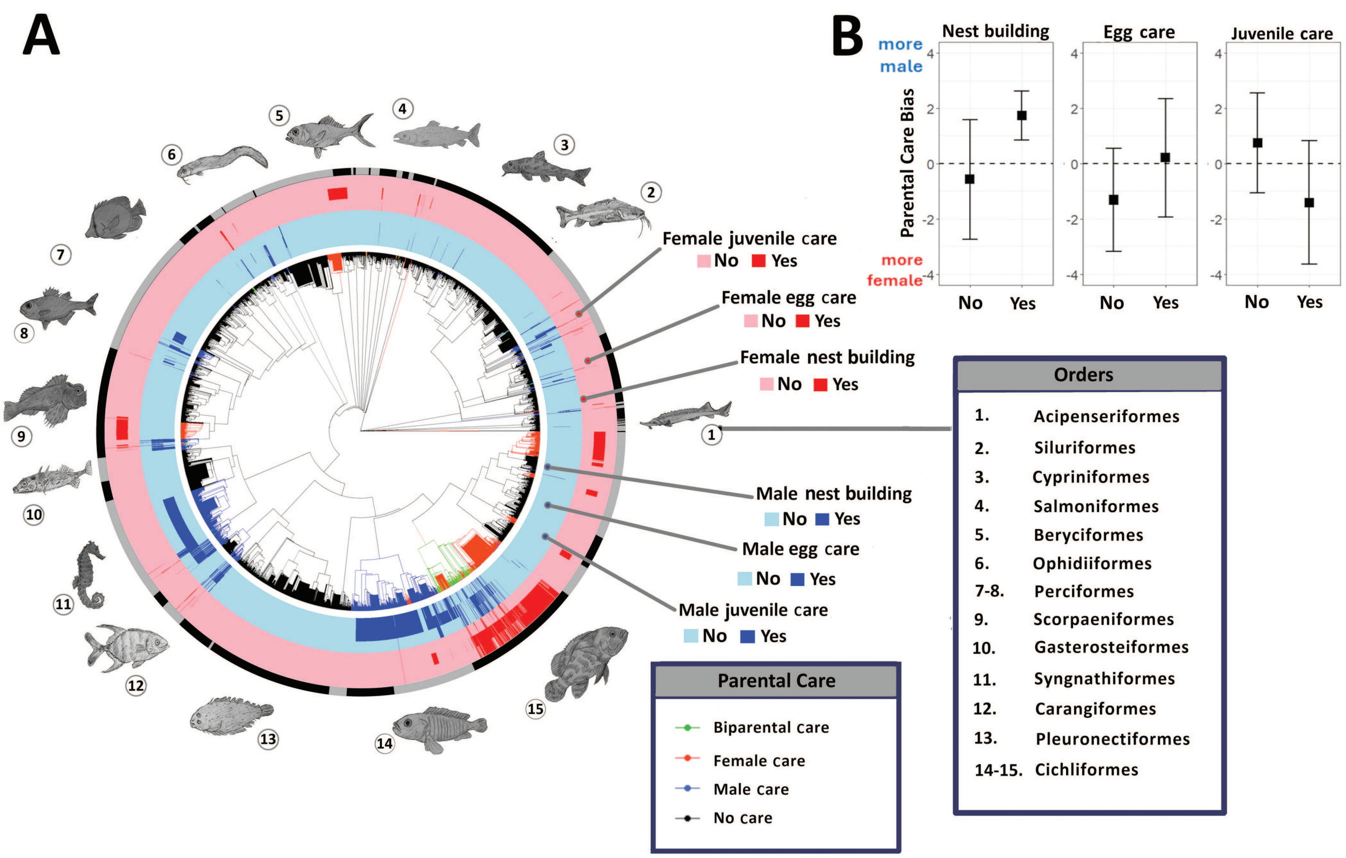

Figure 1. (A) Phylogenetic distribution of care types (branch colors) and care episodes in male (inner three rings) and female (outer three rings) in ray-finned fishes ($n = 7{,}581$ species). Ancient character states were reconstructed using an "all rates different" model by the "make.simmap" function in R package "phytools." Contemporary character states were visualized using the "gheatmap" function in the R package "ggtree." Fish images are original artwork by M. G. Mándi. (B) Parental care bias in the presence/absence of three episodes of care. For statistics, see Table 2.

We used phylogenetic linear models (Martins & Hansen, 1997) to investigate the associations among care types, care bias, and their relation to fertilization modes. First, we tested the associations between care episodes (nest building, egg care, and fry care) within sexes using phylogenetic generalized linear models (Ives & Garland, 2010) in the R package "phylolm" (Ho & Ane, 2014). These models are appropriate when the response variables are binary. We used egg care in males as the dependent variable and male nest building and male fry care as predictors. Then we designed models with egg care in females as the dependent variable and female nest building and female fry care as predictors. Next, we investigated whether care is male- or female-biased in each episode using phylogenetic linear models in the package "phylolm," using care bias as the dependent variable and the presence/absence of non-sex-specific nest building, egg care, or fry care as the predictors in three separate models. These tests were repeated after excluding non-caring species. In the next step, we tested if fertilization mode predicts bias in parental care. We used parental care bias as the response variable, and fertilization mode as the predictor in the phylogenetic linear model. We conducted these analyses using both the broad sense and narrow sense definitions for pouch fertilization. To test phylogenetic robustness and sensitivity to the phylogenetic method, we also tested the same associations using a different phylogeny, and a different comparative model (phylogenetic least squares, see Supplementary Material S2 for details).

All models and visualizations were made in the R statistical environment (R Core Team, 2023).

Results

Evolutionary patterns in parental care

The most diverse orders with regard to parental care are the Anabantiformes, Cichliformes, Gobiiformes, Scorpaeniformes, Siluriformes, and Tetraodontiformes, where all three types of care (male care, female care, and biparental care) occur (Supplementary Figure S1). Our analyses confirm that the absence of care was the most supported ancestral state (Figure 1).

Transition rates and numbers between types of care varied considerably. Specifically, the transition from no care to male care was more than twice as likely as from no care to female care (Figure 2). Transition rates and numbers between female and male uniparental care occurred with a similar, low rate in both directions. We found a low incidence of biparental care evolving from no care; however, biparental care evolved both from male or female care with an order of magnitude higher rates and 2–3 times more than from no care (Figure 2). Evolutionary transitions from biparental care to female care were 2.5–7 times more likely than from no care or from male care, respectively (Figure 2). All parental care types in ray-finned fishes prove to be unstable, as male-only care and female-only care are lost with a higher rate to no care, while biparental care more frequently evolved back to uniparental

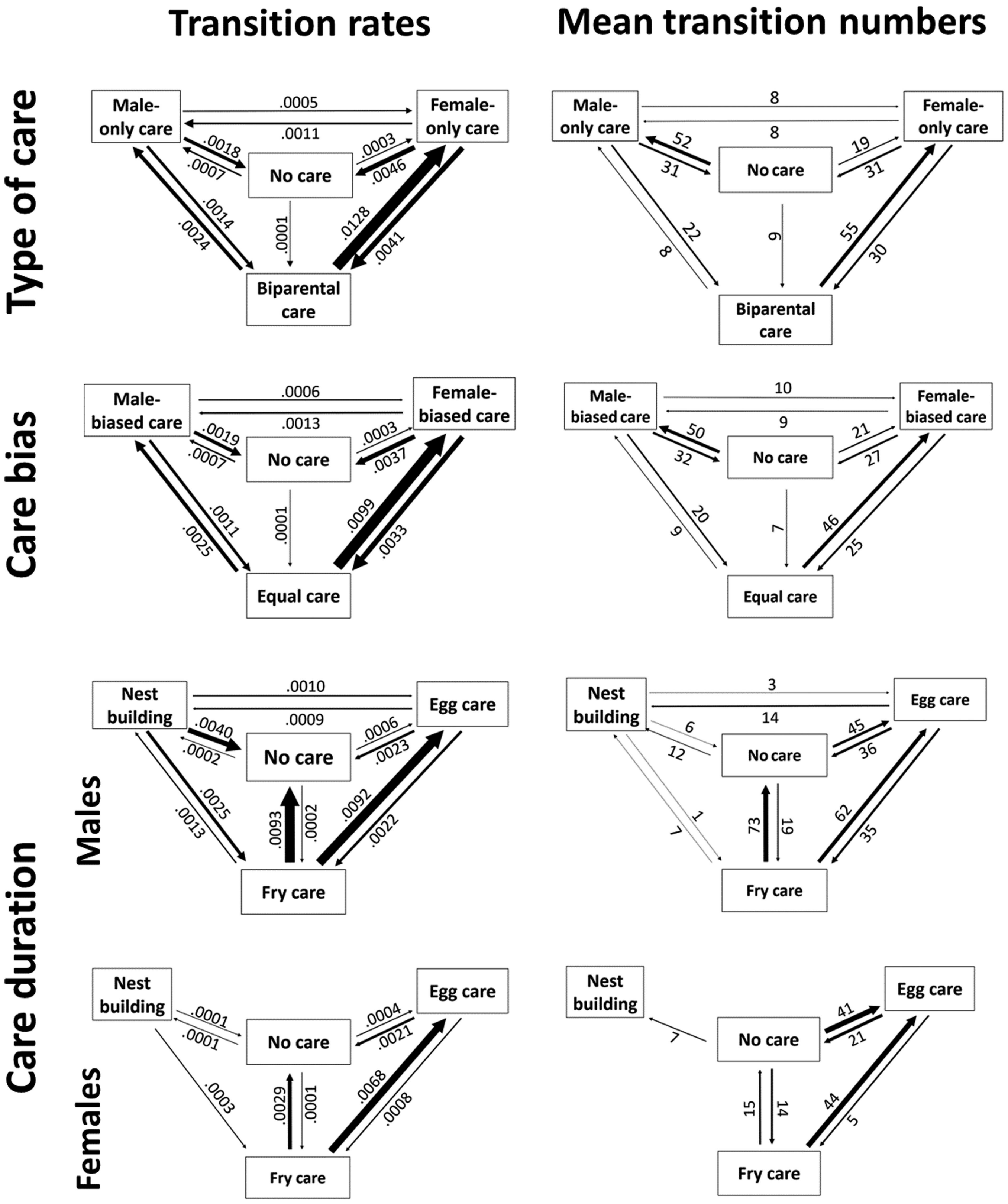

Figure 2. Transition rates and mean transition numbers in type of care, care bias, and care duration in male and female ray-finned fishes were calculated with an "all rates different" model in the R package "phytools." Mean transition numbers were rounded to integers.

care, more commonly to female-only care. However, in extant lineages, biparental care was never completely lost by one evolutionary step to no care. Transitions in care bias yielded very similar results to transitions in the type of care (Figure 2).

Sex-specific analyses of care duration confirm the instability of care forms in ray-finned fishes, as both nest building and fry care tend to evolve back to no care or to egg care, while egg care itself is relatively stable in both sexes (Figure

2). Sensitivity analyses of transition rates yielded qualitatively very similar results (Supplementary Figures S3–S6). In our phylogenetic comparative analyses we found that parental care episodes provided by one parent in different life stages of the progeny are highly correlated both in males and in females (Table 1; Supplementary Table S1). However, in general, males provide more nest building and egg care, while females provide more fry care (Table 2, Figure 1; Supplementary Table S2).

Evolutionary patterns in fertilization modes and associations with parental care

Internal fertilization evolved in only c. 20% of the orders (12 out of 62 orders), with the single-species order Lepidogalaxiformes showing only internal fertilization and Siluriformes standing out as the only one displaying multiple modes of internal fertilization (Supplementary Figure S2). The ancestral state was external fertilization from which multiple independent evolutionary pathways of divergent internal fertilization arose (Figure 3). The gain of internal fertilization modes is relatively rare with extremely low rates for pouch fertilization. Oviduct and pouch fertilization seem relatively more stable states than mouth fertilization, but each mode has high rates to evolve back to external fertilization. In this analysis, we found no evidence of a direct transition between different types of internal fertilization (Figure 3). Analysis with an alternative phylogeny detected more stable oviduct fertilization with a lower reversion rate to external fertilization than *vice versa*, in other terms it showed similar patterns to the original analysis (Supplementary Figure S7).

Species with oviduct and mouth fertilization have more female-biased parental care than species with external or pouch fertilization even when phylogenetic signal is accounted for (Table 3; Figure 3; Supplementary Table S3). Analysis of the transition rates between character pair presence/absence of care and external/internal fertilization shows that parental care tends to evolve without internal fertilization, but this remains an unstable state combination: it tends to either revert to external fertilization or gain parental care (Figure 4, Supplementary Figure S8). In the presence of parental care, internal fertilization tends to evolve, but its loss occurs with a higher rate than its gain. On the other hand, internal fertilization without parental care is very unstable and unlikely to evolve, but the already evolved internal fertilization likely gives rise to parental care (Figure 4). In the analyses with a different phylogeny, we did not detect a reversal from internal to external fertilization, but other transition patterns were similar to our original model (Supplementary Figure S8).

Analysis of transition rates between fertilization modes and care bias shows that in external fertilizers, no care bias is the most stable state, followed by male-bias, while female-bias is the least stable (Figure 5A). Internal fertilization tends to revert back to external, or be stabilized by the emergence of sex-biased parenting. We also find that oviduct fertilization associates with stable female-biased care, and pouch fertilization associates with stable male-biased care (Figure 5B). In contrast, mouth fertilization is dominated by female-biased care, but this is more likely to revert to external fertilization than the other two internal fertilization modes. In contrast to the previous four-state trait combination analysis, this 12-state analysis detected transitions between internal fertilization modes. Analysis with a different phylogeny yielded very similar results, except even more dynamic transition patterns in care bias in mouth fertilizers (Supplementary Figure S9).

Discussion

Using the most extensive care and fertilization mode dataset to date, our research confirms many key findings of the

Table 1. Association between episodes of care (i.e., egg care, nest building, and fry care) in each sex in ray-finned fishes. Phylogenetic generalized linear models with male egg care or female egg care as the response variable, and male nest building and male fry care, or female nest building and female fry care as predictors.

Response	Predictor	*N* species	Estimate	St. err	*t*	*p*	R^2
Male egg care	Male nest building	7,580	0.261	0.012	21.462	**<0.0001**	**0.057**
	Male fry care		0.597	0.013	44.462	**<0.0001**	**0.207**
Female egg care	Female nest building	7,581	0.153	0.018	8.401	**<0.0001**	**0.009**
	Female fry care		0.583	0.011	51.939	**<0.0001**	**0.263**

Bold values indicate significant associations.

Table 2. Association between parental care bias and the presence of non-sex-specific care episodes (nest building, egg care, and fry care). Phylogenetic linear regression models with care bias as the response and the presence of nest building, egg care of fry care as the predictors. We repeated each model run after excluding noncaring species. A positive estimate represents male bias, while a negative one represent female bias.

Response	Predictor	*N* species	Estimate	St. err.	*t*	*p*	R^2
Care bias (all species)	Nest building	7,581	1.284	0.043	30.13	**<0.0001**	**0.026**
Care bias (caring species only)	Nest building	2,363	1.179	0.077	15.27	**<0.0001**	**0.007**
Care bias (all species)	Egg care	7,580	0.170	0.048	3.537	**<0.001**	**0.008**
Care bias (caring species only)	Egg care	2,362	–0.189	0.120	1.569	0.117	<0.001
Care bias (all species)	Fry care	7,580	–1.975	0.044	44.87	**<0.0001**	**0.079**
Care bias (caring species only)	Fry care	2,362	–2.241	0.071	31.57	**<0.0001**	**0.166**

Bold values indicate significant associations.

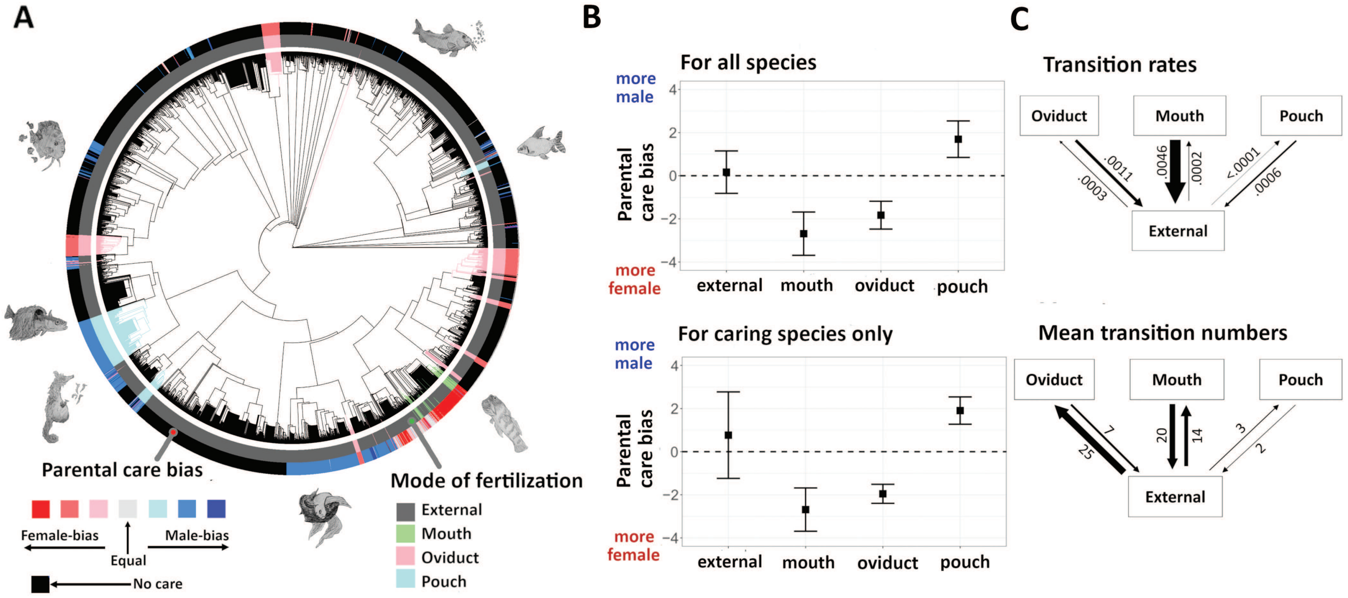

Figure 3. The evolution of fertilization modes in ray-finned fishes and their association with parental care bias. (A) The evolution (depicted by branch colors) and current distribution (depicted by the inner color ring) of fertilization modes, along with the distribution of parental care bias (shown in the outer color ring) across 7,289 species of ray-finned fishes. Ancient character states were reconstructed using the "ARD" model by the "make.simmap" function in R package "phytools." Contemporary character states were visualized using the "gheatmap" function in the R package "ggtree." Fish images are original artwork by M. G. Mándi. (B) The extent of parental care bias (mean ± *SD*) in different fertilization modes. For statistics, see Table 3. (C) Transition rates and mean transition numbers between fertilization modes by "ARD" model in "phytools."

Table 3. Association between parental care bias and fertilization modes. Phylogenetic linear regression models with care bias as the response and the fertilization mode as the predictor. We repeated each model run using the "restricted" definition for pouch fertilization (see *Methods* section). Positive association represent male bias, while negative association represent female bias.

Response	Predictor	*N* species	Estimate	St. err.	*t*	*p*	R^2
Care bias (pouch definition extended)	Fertilization mode	7,289					
	External-mouth		−1.271	0.057	22.28	<0.0001	0.082
	External-oviduct		−1.442	0.100	14.41	<0.0001	
	External-pouch		−0.064	0.290	0.219	0.826	
Care bias (pouch definition restricted)	Fertilization mode	7,289					
	External-mouth		−1.271	0.057	22.28	<0.0001	0.082
	External-oviduct		−1.441	0.100	14.41	<0.0001	
	External-pouch		1.831	1.522	1.203	0.229	

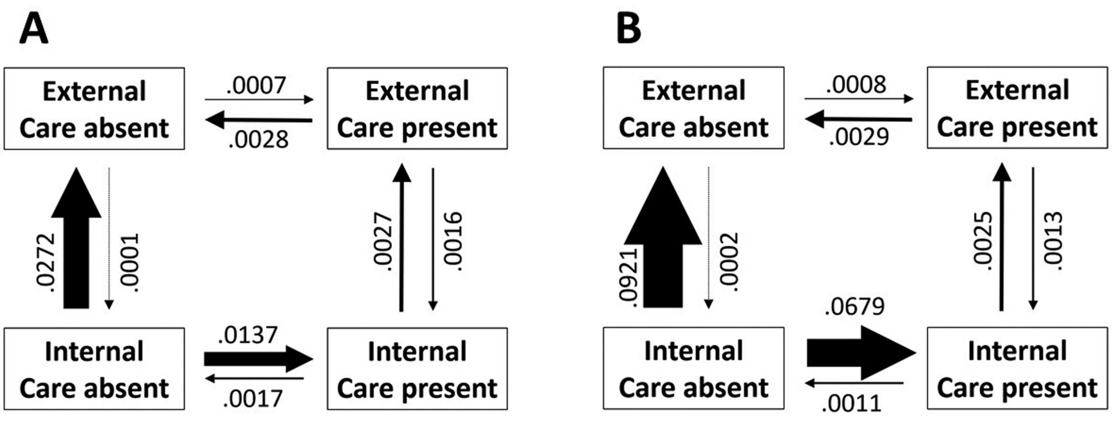

Figure 4. Coevolution of parental care presence and the mode of fertilization, displaying the transition rates between different character combinations. (A) With an extended definition for pouch fertilization including armored catfishes and labyrinth fishes (see *Methods* section). (B) A restricted definition for pouch fertilization is applied, with armored catfishes and labyrinth fishes considered as external fertilizers.

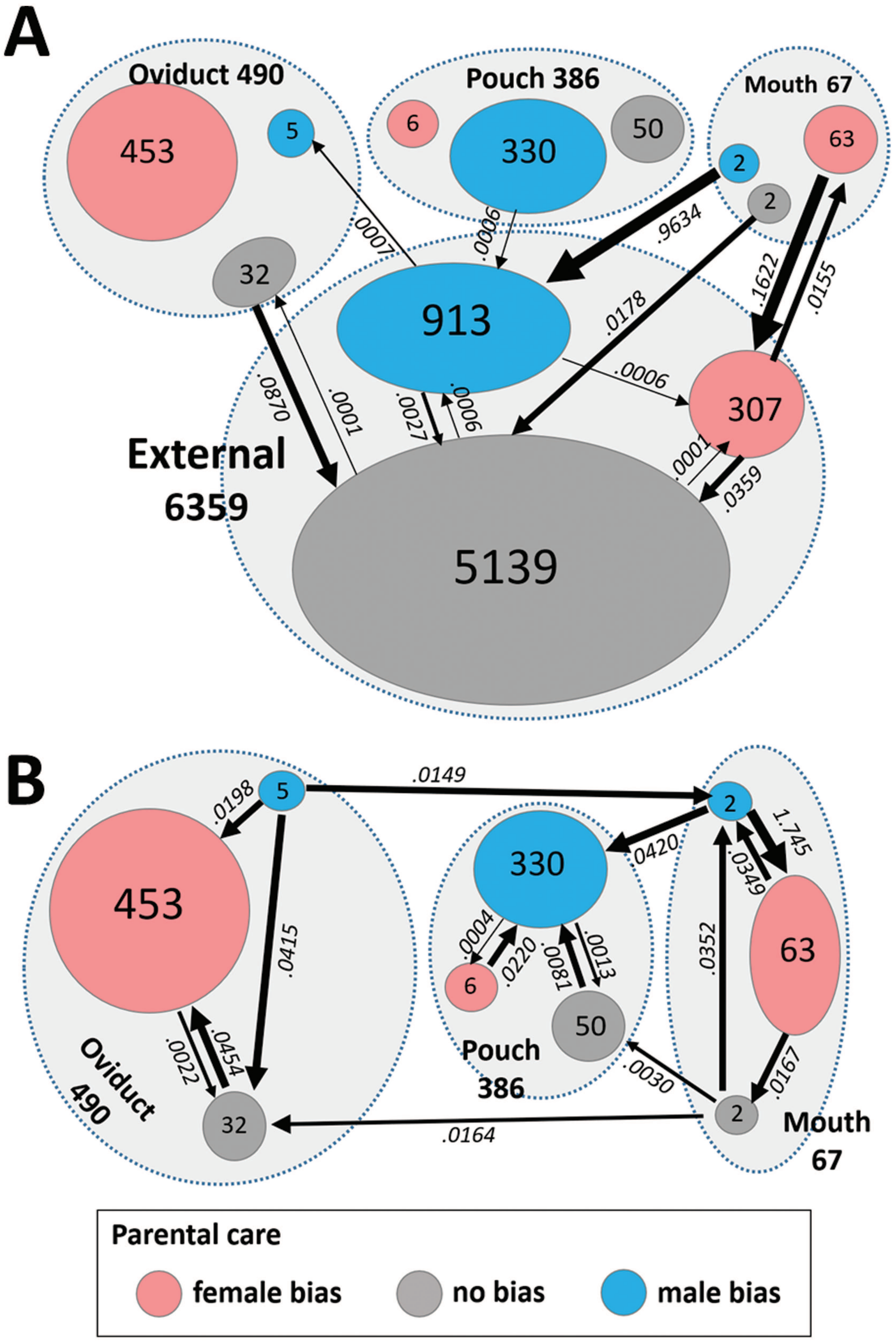

Figure 5. Coevolution of parental care types and fertilization modes in 7,302 species of ray-finned fishes. (A) Transitions between external fertilization and oviduct, pouch, or mouth fertilization and transitions between external fertilizers with different types of parental care. (B) Transition rates between oviduct, egg, and mouth fertilization, and within these types between different types of parental care bias. Note that all transition rates are obtained from the same model; it is divided into two subfigures only to have a better overview. Numbers in the ovals indicate contemporary species numbers in our data, while numbers on the arrows show transition rates. See *Methods* section for details.

influential paper of Mank et al. (2005), but also extends our understanding of the evolution of fish reproduction. In line with earlier studies (Benun Sutton & Wilson, 2019; Mank et al., 2005), we detected parallel evolution of male and female uniparental care, and refuted that female-only care originated predominantly from biparental care. We confirm male-biased early care (nest-building and egg care) as reported by Mank et al. (2005), but we are the first to demonstrate female-biased care at the fry stage. We also confirm the findings of Mank et al. (2005) that external fertilization was the ancestral state and internal fertilization evolved multiple times independently. Overall, our findings are congruent with Mank et al. (2005) that parental care is a labile trait in fishes with many gains and losses. However, we substantially extend prior knowledge by recognizing that internal fertilization forms can stabilize care. Moreover, each form of internal fertilization associates with either male-biased or female-biased parenting.

The distribution of parental care episodes in males and in females and care bias in each episode demonstrates that male care is mostly restricted to pre-fertilization care and egg care, while females mostly provide later care forms, i.e., care for juvenile offspring. In males, nest building can secure a higher (but not exclusive—Svensson & Kvarnemo, 2023) share in paternity. Nests, along with their effects increasing egg and offspring survival, may be provided for the clutches of multiple females simultaneously (Lindström & Seppä, 1996; Reynolds et al., 2002), and may be subjected to sexual selection (Kvarnemo, 2006; Lindström et al., 2006; Svensson & Kvarnemo, 2023). In addition, male fish can potentially replenish their gamete reserves faster than females. Therefore, early desertion of parental duties and the pursuit of new mating opportunities are efficient male strategies for maximizing reproductive success. In contrast, females are less capable of achieving the same outcome through the same approach (the Bateman gradient—Janicke et al., 2016; Mokos et al., 2021). Consequently, the females more likely to provide care for the juveniles—probably in accordance with the different cost-benefit balance of early desertion. It is also possible that viviparity and other forms of egg incubation create a contact between the mother and the offspring, and this can promote social interactions in ectotherm vertebrates (Halliwell et al., 2017). Another potential cause of female-biased fry care is that nourishment of the offspring is typically the females' task in ectotherms (Katona et al., 2023; Kupfer et al., 2016; Vági et al., 2019); however, in fishes multiple cases are known when males solely (or together with the female) also provide nutrients or other secretions for the fry (Ahnesjö, 1996; Chong et al., 2006; Giacomello et al., 2006).

Despite the different offspring life stages involved and a potential division of labor among sexes, care is uniparental in most fishes. This and the general instability of parental care indicate that in environments where care provision is not essential for ensuring offspring survival, its evolutionary loss or simplification is common, in contrast to harsh and unpredictable environments, where care is necessary to ensure the survival of the next generation (AlRashidi et al., 2011; Balshine & Sloman, 2013; Kasimatis & Riginos, 2016; Vincze et al., 2013). In fishes, biparental care often leads to uniparental care, most frequently to female care by male desertion, and in turn, uniparental care may also revert to no care at high rates. The common loss and regain of parental care may be a source of its unparalleled diversity among fishes, in line with Dollo's law (Dollo, 1893), which states that complex traits when lost are unlikely to re-evolve along the same evolutionary and ontogenetic pathway. However, more stability for parenting can be provided by complex anatomical and physiological adaptations, such as pouch or oviduct fertilization. In addition, mouth fertilization is a process that is controlled by both parents and thus has a potential to strengthen pair bonds and social monogamy, which converges to stable biparental care with specialized parental roles (usually mouth-brooding by the female and territory defense by the male; Balshine & Abate, 2021). However, as mouth fertilization does not need as many special adaptations as the other two internal types, its evolution is more dynamic.

Our results confirm that when females gain control over the mode of fertilization, they will provide more parental care (Benun Sutton & Wilson, 2019). In species with oviduct and mouth fertilization, females may take control of the reproductive process when they can choose among potential sires based on external sexual characters, e.g., the length of the gonopodium in guppies or other fin attributes in cichlids (Haesler et al., 2011; Taborsky & Neat, 2010). In oviduct-fertilizing live-bearers or mouth-fertilizing cichlids behavioral observations confirm that females can gather sperm from multiple males (Haesler et al., 2011; Immler & Taborsky, 2009). However, the males of some poecilid species, such as guppies may force copulation with females, which can circumvent female choice and also produce multiple paternity (Head & Brooks, 2006). Species with pouch fertilization do not provide substantially more male-biased care than external fertilizers, though brooding pouches require considerably less sperm to successfully fertilize all eggs compared to external fertilizers (Van Look et al., 2007), which benefits males. While sperm is usually considered inexpensive to produce in comparison to eggs, ejaculate production may have substantial costs and sperm should be often carefully economized in external fertilizers (Dewsbury, 1982; Hettyey et al., 2009; Shapiro et al., 1994). Thus, male investment in sperm production is proportionately lower than in other fishes, while they invest much more into parental care. In addition, the pouch fertilization observed in pipefishes and seahorses also creates the opportunity for cryptic mate choice (Ahnesjö, 1996).

While pouch fertilization in the pipefish family evolved from external male brooding of the eggs (Whittington & Friesen, 2020), internal fertilization in the oviduct likely preceded the evolution of female viviparity. Therefore, it would be interesting to investigate what drove the evolution of oviduct fertilization without parental care or with external egg guarding (some examples: Phallostetidae, Sebastidae, stervardiine characins, auchenopterine catfishes). As this would not provide benefit for the developing eggs, we hypothesize that sexual selection might be a factor. However, after the switch to internal fertilization, internal gestation likely evolves (Mank et al., 2005). As the eggs of ray-finned fishes are small and their embryonic development is relatively fast compared to sharks, batoids, and chimeras (Chondrichthyes), egg retention in the oviduct would provide protection for a short period of the development. Nevertheless, in the eelpout *Zoarces viviparus*, internal gestation replaces the two to three months of egg guarding found in congeners. In chondrichthyans, large embryos with longer development constituted the ancestral state, and internal fertilization is exclusive in this group, which could explain the frequent transitions to viviparity among them (Blackburn & Hughes, 2024; Katona et al., 2023).

While pouch and oviduct fertilization seem to form stable trait combinations with male-biased and female-biased care, respectively, female-biased care with mouth fertilization remains a less stable trait combination. Therefore, the three internal fertilization modes are different in their evolutionary dynamics. Moreover, our results suggest that even the four-level coding of fertilization modes employed here is insufficient to describe its total diversity in fishes, especially the forms categorized as "pouch or similar structure" seem to evolve in a different way. Fertilization on basket-like fins are controlled more by the male in labyrinth fishes (Anabantiformes), where the male coils around the female during the mating process. In contrast, it is controlled more by the female in armored catfishes (Corydorinae), when it occurs between the pelvic fins of the female, after she passes the sperm through its digestive system. We found male care (nest building and guarding or mouth-brooding) in the first case, and no further parental care in the second case. These forms are considered transitional between external and oviduct fertilization regarding the control over the process and anatomical/physiological complexity (Mank et al., 2005). On the other hand, pouch fertilization of seahorses and pipefishes seems to be the male equivalent of oviduct fertilization and live-bearing, where the gametes do not leave the parent's body, their number is limited, and the incubating parent also provisions the offspring with nutrients. This mode of fertilization has evolved very infrequently and should thus be considered a low-probability event.

Although the care-providing sex is strongly determined by the mode of fertilization, other causes potentially also contributed to the emergence of parental care in either sex, which is similar to recent findings in salamanders (Vági et al., 2022). The absence and presence of parental care in males and in females are often driven by the abiotic environment (Ishimatsu et al., 2018; Martin & Carter, 2013), life history traits (Benun Sutton & Wilson, 2019; Vági et al., 2019; Vanadzina et al., 2021), or the social context and mating system (Liker et al., 2013, 2014; Vági et al., 2020), or the interplay between these factors (Azad et al, 2022; Fresneau et al., 2024). For example, highly fluctuating (e.g., temporal or seasonal) environments like intertidal zones might favor the evolution of parental care (Almada & Serrao Santos, 1995; Balshine & Sloman, 2013; Horn, 1998). In addition, environmental conditions not only act on the egg but also on the sperm and ejaculates (Balshine et al., 2001; Liao et al., 2018; Taborsky & Neat, 2010) and this can substantially influence sex-specific reproductive investment and anisogamy. And the complex relations with the abiotic environment are in turn connected to the inter- and intraspecific diversity of mating systems—i.e., reproductive investment of territorial, "bourgeois" males, satellites, and sneakers are different (Cogliati et al., 2013; Taborsky & Neat, 2010), and the presence of these strategies in a species likely shapes the evolution of fertilization mode and parental care. Future studies will need to consider how these traits affect the evolution of reproductive modes and parental roles, along with more detailed investigations of the associations found between parental care and dichromatism (Mank et al., 2005) and parental investments into the gametes (Benun Sutton & Wilson, 2019).

Conclusion

While we confirm the association between fertilization modes and parental care in fishes, we do not find support that changes in fertilization mode alone give rise to parental care. Instead, control over the mode of fertilization may involve not only behavioral, but also anatomical and physiological adaptations to stabilize the otherwise unstable parental care systems, and vice versa, in an aquatic environment. Sex roles in parenting and the episodes of offspring ontogeny when care is typically provided by either sex are influenced by the different costs and benefits for males and females and in line with the Darwin–Bateman paradigm. We encourage follow-up studies to test the impacts of ecological, social, and life-history variables on the evolution of parental care by exploiting the unusual diversity of breeding systems, parenting, and reproductive modes in ray-finned fishes.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All relevant data and analyses within the paper and its electronic supplementary material are available in the following public GitHub repository: https://github.com/mihalymandi/ray-finned-fish-paper

Author contributions

B.V., T.S., and Z.V. conceived the study; B.V., T.S., Z.V., R.P.F., and A.L. designed the analyses; G.K. and B.V. collected data; B.V., G.K., O.G.M., M.G.M., and Z.V. conducted analyses; M.G.M., B.V., and O.G.M. visualized the results; and B.V. and G.K. wrote the original draft, which was reviewed and edited by B.V., G.K., O.G.M., M.G.M., H.A.H., E.P., Z.V., A.L., R.P.F., and T.S.

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