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Parental care drives the evolution of male reproductive accessory glands across ray-finned fishes

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Abstract

Reproductive accessory glands are organs involved in reproduction that do not directly produce or release gametes but can play crucial roles in securing reproductive success. In fishes, the 2 leading hypotheses about why accessory glands evolved are (1) in response to sperm competition, or (2) to facilitate parental care activities. Here, we investigate the evolutionary history of accessory glands and test these hypotheses by estimating quantitative differences in evolutionary rates. We found that accessory glands are present in 116 of the 607 sampled species of ray-finned fishes, representing 26/267 families. We estimated that accessory glands have arisen independently ~20 times and that these glands were gained 5.8 times faster in lineages with male parental care, compared to those without male care, supporting the hypothesis that they evolved to facilitate care. In contrast, group spawning, used as a proxy for sperm competition risk, seemed to select against the evolution of accessory glands, as lineages exhibiting group spawning gained accessory glands 3.9 times slower than those with pair spawning (though this failed to reach statistical significance). This study provides new insights into the evolutionary history of accessory glands in fishes and highlights the importance of parental care in shaping reproductive anatomy.

Keywords: accessory glands, parental care, sperm competition, fishes, phylogenetics

Introduction

Sexually reproducing animals must mate to reproduce, often competing with conspecifics for fertilization opportunities (Andersson & Simmons, 2006; Shuker & Kvarnemo, 2021). In addition to traits used to compete with rivals and attract mates before mating takes place (i.e., pre-mating sexual selection), competition among males can continue after gamete release (i.e., post-mating sexual selection). Notably, this competitive interaction occurs in the form of sperm competition, which can affect male reproductive success and shape reproductive anatomy, physiology, and behavior (Birkhead & Pizzari, 2002; Simmons & Fitzpatrick, 2012). However, successful fertilization may only be the first step to secure fitness, as in many species individuals must also provide parental care to increase the chances of offspring survival (Klug & Bonsall, 2010; Royle et al., 2012). Traits that confer fitness benefits during either mating or parental care are therefore likely to experience strong selection (Barson et al., 2015; Simmons et al., 2017).

Male reproductive accessory glands have been associated with both sperm competition and parental care (Pepler et al., 2021; Ramm et al., 2005) and are thought to directly influence reproductive success (Chowdhury & Joy, 2007; Leopold, 1976; Mann & Lutwak-Mann, 1951; Ramm, 2020). A reproductive accessory gland is any organ or gland involved in the reproductive system that does not directly produce gametes (e.g., prostates and seminal vesicles in humans); such structures are known to exist in mammals (Mann & Lutwak-Mann, 1951; Ramm et al., 2005), insects (Gillott, 1996; Leopold, 1976), and fishes (Chowdhury & Joy, 2007; Richtarski & Patzner, 2000). Both males and females can possess accessory glands; however, in this study we focused on male accessory glands. Across taxa, these organs can be quite complex and variable, secreting products involved in multiple reproductive functions. These glands can aid in sperm storage and performance, enhancing sperm competitive abilities (Chapman et al., 2000; Chowdhury & Joy, 2007; Ramm, 2020; Ramm et al., 2005). For example, the seminal fluid produced by accessory glands in mice contains proteins that affect fertilization ability (Lardy, 2003) and enhance sperm motility (Luo et al., 2001). Accessory glands also influence reproductive success through other post-mating mechanisms, such as the production of mating plugs in many insects and rodents (Gillott, 1996; Voss, 1979) and through effects that modify the behavior and physiology of mated females, such as an elevation in egg-laying rates, reduced mating receptivity,

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and conformational changes in the female reproductive tract in fruit flies (Chapman et al., 1995; Wigby et al., 2020; Wolfner, 1997). In some externally fertilizing fish species, these glands play a role in parental care, producing proteins that aid in egg adhesion to substrate or that protect the developing embryo against microbial infection (Giacomello et al., 2006, 2008; Pepler et al., 2021; Pizzolon et al., 2010). Despite the variation in function and the diversity of taxa that possess accessory glands, research on their evolution has mostly been restricted to mammals and insects (though Mazzoldi et al. (2005) provide an exception in an elegant study on goby fishes). Moreover, most previous studies have only considered how sperm competition shapes the evolution of accessory gland products (Boomsma et al., 2005; Mazzoldi et al., 2005, 2007; Ramm et al., 2005); most likely because internally fertilizing mammals and insects rarely exhibit male-only care (Clutton-Brock, 1991). Consequently, the potential role of parental care in selecting for and shaping the evolution of accessory glands has been largely overlooked.

Here, we explore the evolution of accessory glands across ray-finned fishes (Actinopterygii) in the context of both sperm competition and parental care. Ray-finned fishes are an ideal system to study the evolution of reproductive traits due to their remarkable diversity of reproductive life histories (Breder & Rosen, 1966; Wootton & Smith, 2014). Several clades of fishes are known to possess accessory glands (Mazzoldi et al., 2005, 2007; Miller et al., 2019; Richtarski & Patzner, 2000), and fishes vary widely in both sperm competition risk and parental care activities (Balshine, 2012; Fitzpatrick, 2020; Gross, 2005; Mank et al., 2005). The diversity of mating systems and spawning behaviors across fishes results in variable risk of sperm competition (Fitzpatrick, 2020; Stockley et al., 1997; Taborsky, 1998). Various forms of parental care are prevalent in many groups of fishes, with paternal care being the dominant form, while maternal and biparental care are less common (Blumer, 1979; Gross & Sargent, 1985). The variation in these traits and behaviors makes fishes a useful group to study the interactions between sperm competition, parental care, and the evolution of accessory glands. Here, we contrast evolutionary rates, estimated from a model that includes data from over 600 species of ray-finned fishes, to test the hypotheses that sperm competition and/or parental care have influenced accessory gland evolution. Concurrently, we reconstruct the evolutionary history of accessory glands, estimating the number of times this trait has evolved independently across fishes.

If the risk of sperm competition selected for the evolution of these glands, we would expect accessory glands to be more prevalent in lineages with spawning behaviors associated with greater risk of sperm competition. Specifically, we would expect accessory glands to be more likely in species where multiple males are present at the point of spawning, and thus sperm from multiple males typically compete for fertilization. If parental care favored the evolution of these male glands, then we would expect accessory glands to be more prevalent in lineages that exhibit parental care, specifically where the care is provided by the male. It is important to note that these hypotheses are not mutually exclusive; both may have influenced the evolution of these glands. Evaluating whether accessory gland evolution is correlated with sperm competition risk and/or with parental care across fishes will help clarify the key selective forces in the evolution of these specialized and understudied glands.

Methods

Data collection

Accessory glands

The phylogenies used were taken from Rabosky et al. (2018) and were accessed using the *fishtree* package (Chang et al., 2019) in R (R Core Team, 2021). We searched the published literature on Actinopterygiian fishes to retrieve information on the presence or absence of male reproductive accessory glands as well as the state of parental care and spawning behaviors, collecting information for 607 species representing 267 families of fishes. To achieve taxonomic coverage, we attempted to collect information from two or more species from each family in the phylogeny. However, information on additional species was sometimes also added when multiple types of accessory glands (as determined by their structures) were present within a single family (Fishelson, 1991; Rasotto, 1995). We considered male reproductive accessory glands to be any non-spermatogenic organ or gland associated with or connected to the male testes or sperm duct, such as a seminal vesicle or testicular gland (Chowdhury & Joy, 2007). A species was considered *not* to have accessory glands if (1) the absence of the gland was explicitly stated in a paper, or (2) given the aims and contents of the paper (i.e., a description of the reproductive anatomy of a species), we were confident that the authors would have provided a description of an accessory gland had it been present. Google Scholar was used as the primary search tool to locate all published sources of all accessory gland information, and searches for source material were conducted between September 2018 to June 2021. Google Scholar was chosen as it enables researchers to search for target keywords (e.g., accessory gland) through the entire text of an article, in contrast to most other databases that restrict the search for keywords to the title, keywords, and abstract. A full-text search was particularly relevant for our study, as information about the presence or absence of accessory glands was rarely the main question in the papers examined, often being mentioned only as part of the general description of a species' reproductive anatomy. A full list of the keywords used in our search can be found in the Supplementary Material.

Parental care

At the same time, we also collected all available species-specific information on parental care and spawning mode using Google Scholar, FishBase (Froese & Pauly, 2019), and information from Breder and Rosen (1966). We recorded the reported presence or absence of parental care, and if a species provided care, we recorded the sex of the caregiver (i.e., male, female, or biparental). Following Benun Sutton and Wilson, (2019), we considered parental care to be any postfertilization parental investment in offspring, including both internal and external care (such as nest guarding and mouthbrooding). Parental care was determined to be absent if it was explicitly stated, or if the behavior of the parents or development of the eggs was described in enough detail to be sure that care was not present. Because we were interested in how care may have selected for the evolution of *male* accessory glands, for all subsequent analyses, we focused on male care only, treating it as a binary variable describing whether males provided care (as in species with paternal care or biparental care) or not (as in species with female care or no care). Making parental care a binary variable was necessary to reduce model complexity, though we acknowledge that different forms of care could indeed have distinct effects on accessory gland evolution.

Spawning mode

We also collected and categorized spawning mode information, classifying each species as either pair spawning or group spawning, again following Benun Sutton and Wilson (2019). A species was categorized as pair spawning if a single female and male are typically present at the time of gamete release, whereas a species was considered group spawning whenever there are often more than two individuals present when eggs are released. Using this definition, group spawning included species with alternative reproductive tactics (ARTs), such as those with satellite or sneaker morphs that release sperm into the nests of other males (Taborsky, 1998). We used this binary spawning mode variable as a proxy for sperm competition risk, with pair spawning being associated with a lower risk of sperm competition and group spawning having higher risk (Stockley et al., 1997). All the information collated on the presence versus absence of accessory glands, parental care, and spawning mode can be found in the archived data.

Fertilization mode

Although it was not the target of our search, information on fertilization mode (external vs. internal) was also recorded when available, again following the definition of Benun Sutton and Wilson (2019). While fertilization mode can shape the evolution of reproductive traits (Kahrl et al., 2021, 2022), since it was not directly linked with either of the hypotheses we aimed to test, we did not analyze the effect of fertilization mode on the evolution of accessory glands. However, we did note that accessory glands were found in roughly equal proportions of species with external fertilization (21% or 82 of 382 species) versus those with internal fertilization (23% or 22 of 95 species). Species that possessed both accessory glands and internal fertilization included some species of blennies, catfishes, and the salamanderfish.

Correlated evolution of accessory glands, parental care, and spawning mode

The evolutionary model

We used the trait data and phylogeny to test whether the states of male care and spawning mode influenced the rates of accessory gland gain and loss. We built on existing software (the *corHMM* package, Beaulieu et al., 2017) that uses a continuous-time, discrete-state model of evolution; for this analysis we assumed that the current trait values of a lineage completely determine the transition rates, and that (conditional on trait values) these rates are constant across the tree. Since the evolutionary process runs in continuous time, only one character can change at any given moment. As we did not retrieve data on all traits for each species, missing data were treated as ambiguous, with each state having an equal probability. See the Supplementary Material for more detail on trait combinations and missing data.

The transition rates are the rates of change from one state (i.e., a particular combination of spawning mode, parental care, and presence/absence of accessory glands) to another in an infinitesimally short time interval (Pagel, 1994); mathematically, these rates are *hazards* (i.e., the limit of Prob(change from state i to state j)/Δt as $\Delta t \rightarrow 0$). The model rates were defined in terms of (natural) logarithms of transition rates to ensure that estimated values of rates were always positive. Differences in transition rates were measured as differences in log rates or, by exponentiating these differences, as ratios of rates (i.e., how many times faster/slower a transition occurs based on the traits present in a lineage).

We limited model complexity by reducing the number of parameters we estimated (by constraining multiple possible transitions to be equal), focusing on those most relevant to testing our hypotheses. We further constrained the model by using neutral Bayesian priors (described below) that limited the range of transition rates within plausible ranges, while not biasing us toward accepting any particular biological hypothesis (as shown in the sensitivity analyses in the Supplementary Material).

To estimate the full, unconstrained model of transitions among three binary states, we would need to specify 24 separate parameters (given eight possible combinations of spawning mode, male care, and accessory glands, this is the number of transitions that allow exactly one trait at a time to change—the total number of arrows in Figure 1). To focus on the evolution of accessory glands, we assumed the rates of change of spawning mode and male care were independent of each other's current state and of the presence or absence of accessory glands—this assumption reduces the number of estimated parameters from 24 to 12. In reality, spawning mode and parental care are correlated, with pair spawning being more prevalent in species with parental care (Benun Sutton & Wilson, 2019), and thus may influence the evolution of each other, but this assumption was necessary to reduce complexity of the model (which we address in the sensitivity analyses in the Supplementary Material). To determine how spawning mode and male care affect transitions of accessory glands, we computed *linear contrasts* that describe the differences in evolutionary rates between conditions (log-transition-rate differences or rate ratios), comprising six of the 12 log-rate parameters. For example, rather than presenting separate log-rate parameters describing the rate of gain of accessory glands when male care is absent or present, we estimated the baseline rate of gain when male care is absent and the difference between this baseline average and the average rate when male care is present; we call this change the "effect of male care on the gain of accessory glands." We defined similar contrasts for the effect of male care on loss of accessory glands, the effect of group spawning (vs. pair spawning) on gain and loss of accessory glands, and the effects of male care × spawning mode interactions on gain and loss. The interactions represent the difference in the rate of gain/loss of accessory glands when both male care and group spawning are present relative to what would be expected if these traits had independent effects. Beyond these six contrast parameters, the remaining six parameters were the baseline rates for the gain and loss of accessory glands, as well as a gain rate and loss rate for each of the non-focal traits, spawning mode, and male care (since we assumed that changes in these states occur at a constant rate, independent of other traits). See the Supplementary Material for further details on how the contrasts were computed.

Our approach of computing linear contrasts in evolutionary rates, with confidence intervals, differs from a more purely hypothesis-testing (or model-comparison) framework. In the hypothesis-testing framework, researchers fit multiple evolutionary models that differ in the presence or absence of certain transitions, or in the equality or difference of particular rates; they then compare the overall fit of the models (e.g., using likelihood ratio tests or Bayes factors) to determine

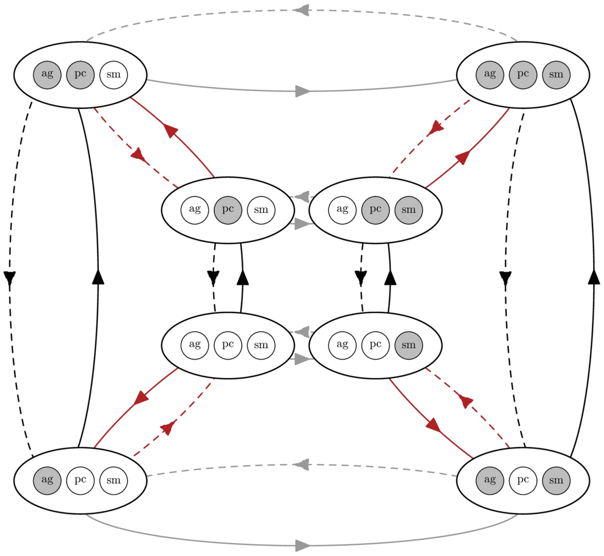

Figure 1. Diagram of allowed transitions among states of accessory glands (ag), parental care (pc), and spawning mode (sm). A filled gray circle represents the presence of accessory glands (vs. the absence), the presence of male care (vs. no male care), and group spawning (vs. pair spawning). Sample sizes of each state can be found in Figure S1 in the Supplementary Material. Arrows represent evolutionary transitions between states, with solid arrows generally representing gains and dashed arrows representing losses. Red arrows represent gains and losses of accessory glands, which are all computed independently. Black and gray arrows represent the background rates on the gains and losses of male care and group spawning, respectively.

whether particular rates are statistically significantly different. However, the hypothesis-testing framework typically only tells us whether one hypothesis is significantly better supported than another; we argue that when testing specific biological hypotheses, researchers will often gain more insight by quantifying the magnitude of effects, which can be achieved by estimating contrasts from the transition rates, as we do here.

Prior distributions

To determine prior distributions for the parameters, we specified a plausible central range for the transition rates or rate ratios and created log-Normal distributions where central ranges represented the mean ± 3 *SD*s on the log scale. To improve the interpretability of the transition rates, we scaled the sum of all branch lengths in the phylogeny to 1.0. We used Normal priors on the log-rate scale. We defined a lower "bound" on all gain/loss rates (i.e., the lower end of the central range: the -3σ level, or 0.1% critical value) of 1.0, corresponding to an expected minimum of only one evolutionary transition of each type across the entire tree. We set the upper bound (the $+3\sigma$ level, or 99.9% critical value) to 10 times the number of species in the tree, corresponding to an expectation of 10 transitions *per species* across the tree, resulting in a very wide range. In addition to these overall priors on the rates, we set log-Normal priors on the gain/loss ratio for each trait. All transitions were biased toward losses (expected gain/loss ratio less than 1), based on the idea that it is easier to lose than to gain a complex trait or behavior during evolution (Johnson et al., 2012), but were still very wide. For transitions in male care, we set the lower end (again, the -3σ level) of the gain/loss ratio distribution to a gain/loss ratio of 1/10, and the upper end ($+3\sigma$ level) to a gain/loss ratio of 5. The range for transitions in spawning mode was from ⅕ to 10, and for accessory glands from 1/1,000 to 10. In contrast to behavioral traits, where rates of gains and losses may be more even, we considered a morphological trait such as an accessory gland to be potentially more prone to losses compared to gains (Blomberg et al., 2003; Johnson et al., 2012). In any case, all of these ranges are very wide and should not rule out any reasonable gain/loss ratio values. To set the value at the root of the phylogenetic tree, following Mank et al. (2005), we assumed that the ancestral fish species was a group spawner without male care and without accessory glands (Baylis, 1981; Choat, 2012; Erisman et al., 2009; Gittleman, 1981; Mank et al., 2005).

MCMC sampling

In order to sample from the posterior distribution, we used a robust adaptive MCMC sampling algorithm (Vihola, 2012), as implemented in the *ramcmc* package (Helske, 2021). To accommodate uncertainty in the phylogeny, each evaluation of the posterior distribution used a phylogenetic tree randomly sampled from the tree block, which is the set of 100 phylogenies from Rabosky et al. (2018), representing different random imputations of the position and branch lengths for species with missing genetic data. We ran eight chains with a burn-in/adaptation time of 4,000 steps and 80,000 subsequent steps, thinning the results by a factor of 10 to 8,000 samples. Using the improved Gelman–Rubin statistic (R-hat) as recommended by Vehtari et al. (2021), we assessed the chains as having converged adequately to the posterior distribution.

Results of the sampling are reported as the univariate posterior median for each contrast, along with the 95% credible interval based on the 2.5% and 97.5% quantiles of the marginal posterior distribution. A Bayesian analog of a p-value, p_{MCMC}, was computed for each contrast estimate as twice the posterior probability that the estimate is negative or positive, whichever probability was smallest (Hadfield et al., 2013; Shi & Yin, 2021).

Sensitivity analyses

In addition to our main analysis of the simplified (12-parameter) model described above, we tried several variations to assess the sensitivity of our results to various assumptions (see Supplementary Material). To test the importance of using the imputed phylogenetic branches from Rabosky et al. (2018), we fitted the model using only those taxa for which full genetic information was available, reducing the size of the phylogeny from 607 to 478 species. To explore the effect of using ambiguous states for missing data, we fitted the model on just the species for which we had complete trait and phylogenetic data (234 species). To evaluate the effect of our priors on relative gain and loss rates, we fitted a separate model without these priors. To assess our ability to estimate parameters without constraining our model, we also fitted the full (24-parameter) model that avoids the assumption that spawning mode and male care evolve independently. Finally, to provide a comparison with more traditional (i.e., frequentist) methods, we also fitted a series of simplified (nested) submodels by maximum likelihood and used likelihood ratio comparisons to test specific hypotheses of independence. Detailed methods and results from all sensitivity analyses are reported in the Supplementary Material.

Reconstructing the evolutionary history

To estimate the number of gains and losses of accessory glands and where these transitions occurred, the estimated transition rates from our model were used to simulate the evolutionary history of accessory glands, as well as male care and spawning mode, using stochastic character mapping (Bollback, 2006; Revell, 2012). We ran 100 simulations per phylogeny in the tree block and summarized the results to visualize the evolutionary history of each trait, estimate the number of transitions between states, and determine the posterior probability of each state at each node.

Results

Accessory glands were present in 116 of 607 sampled species of ray-finned fishes (Figure 2), representing 26 of 267 sampled families in five different orders. Most species with accessory glands belong to three clades: blennies (Blenniiformes, 55/57 with accessory glands), gobies (Gobidae, 28/37 with accessory glands), and catfishes (Siluriformes, 23/57 with accessory glands). Accessory glands were also reported to be present in three toadfishes (Batrachoididae), two cardinalfishes (Apogonidae), two threadfin breams (Nemipteridae), one wrasse (*Cheilinus undulatus*), one sculpin (*Radulinopsis taranetzi*), and one salamanderfish (*Lepidogalaxias salamandroides*).

In our stochastic character mapping simulations (Figure 2), accessory glands were gained a median of 20 times (95% CI = 17–24). Each of the aforementioned clades usually represented a single independent gain of accessory glands, with the exception of catfishes, where accessory glands were gained on average 11 times independently. Though cardinalfishes are closely related to gobies, accessory glands evolved independently for both groups. Accessory glands were lost a median of 9 (95% CI = 6–14) times across the phylogeny, with losses in each of the three major clades where accessory glands were present (gobies, blennies, and catfishes).

Accessory glands were gained 5.8 times faster in lineages with male care compared to lineages without (Figure 3; median = 5.8, 95% CI = 1.4–23.5, $p_{\mathrm{MCMC}} = .019$). In extant taxa, accessory glands were much more prevalent in species with male care, with 50% (58/116) of those male-caring species possessing accessory glands, while accessory glands were only present in 4% (10/256) of species without male care.

Accessory glands were gained 3.9 times slower in lineages with group spawning, compared to those with pair spawning (Figure 3; median = 0.254, 95% CI = 0.06–1.06); however, we cannot be sure if the effect of group spawning is negative as the sign of the effect was not statistically clear due to wide CIs ($p_{\mathrm{MCMC}} = .061$). Accessory glands were present in 29% (57/194) of species that exhibited pair spawning, but only 16% (18/112) of species with group spawning. Furthermore, all of the 18 group-spawning species that possessed accessory glands were classified as exhibiting ARTs—no species that exhibited simple group spawning possessed accessory glands. Because both accessory glands and ARTs are relatively rare in our data set, we did not have the statistical power necessary to further explore the relationship between the two.

The effects of male care or spawning mode on the rates of loss of accessory glands had wide CIs, making the sign of the effect unclear (Figure 3; male care: median = 0.966, 95% CI = 0.133–5.667, $p_{\mathrm{MCMC}} = .97$; spawning mode: median = 0.687, 95% CI = 0.117–4.698, $p_{\mathrm{MCMC}} = .68$). Because accessory glands are absent throughout most of the ray-finned fishes' phylogeny, there is very little opportunity for them to be lost, and hence little statistical power to estimate differences in loss rates precisely. The estimated effects of interactions between male care and spawning mode on either accessory gland gain or loss were similarly unclear (Figure 3; gain: median = 0.511, 95% CI = 0.119–2.118, $p_{\mathrm{MCMC}} = .36$; loss, median = 0.496, 95% CI = 0.074–2.832, $p_{\mathrm{MCMC}} = .44$), meaning we were unable to tell whether parental care and spawning mode had combined effects that differed from what would be expected from their independent effects.

Of the variety of models that we fit as sensitivity tests, the only models that gave qualitatively different answers were the full 24-parameter model, for which none of the contrasts showed a clear effect ($p_{\mathrm{MCMC}} > .05$ for all contrasts), and the 12-parameter maximum likelihood model, for which all CIs were extremely wide (without our neutral priors constraining the range of biologically plausible rates, the data do not provide enough information to constrain the model). The model restricted to species with complete data ($n = 234$ species) still found a positive effect of male care on accessory glands, but the estimated effect was smaller and the 95% CIs included zero (unsurprising given that the data set was reduced to 39% of its original size). All other models gave similar results, indicating that our decisions to (1) impose neutral priors on all rates and (2) assume independence of spawning mode and male care evolution were important, with other decisions, such as using the tree block with imputed branches or assuming differential rates of gain and loss, having less impact (see Supplementary Material for full descriptions of sensitivity analyses).

Discussion

Contrary to the prevailing view in fishes and other taxa, we show that parental care—not sperm competition—is the main driver of reproductive accessory gland evolution in fishes, as accessory glands were gained faster in lineages with male parental care, compared to those without. In contrast, the presence of group spawning did not increase the rate at which accessory glands were gained; in fact, accessory glands were gained almost four times slower on average in lineages with group spawning, though this result did not reach statistical significance. These results support the hypothesis that parental care has driven the acquisition of these glands in fishes (Miller et al., 2019; Pepler et al., 2021) and suggest that increased sperm competition risk may actually oppose their evolution.

The association between accessory glands and male care implies that parental care could select for the evolution of these glands (Miller et al., 2019; Pepler et al., 2021). At the level of extant taxa, this association is clear when examining the blennies, gobies, and toadfishes, where accessory glands are exclusive to species with male parental care. However, in the catfishes the association between accessory glands and male care is less straightforward, with only four species having both accessory glands and male care, whereas six catfish species with accessory glands are known to have no care at all. Unfortunately, little is known about parental care habits of many species of catfishes in their natural habitats; we were only able to find information on parental care for fewer than half of the catfish species in this study. Catfishes are also the only group in this study where accessory glands

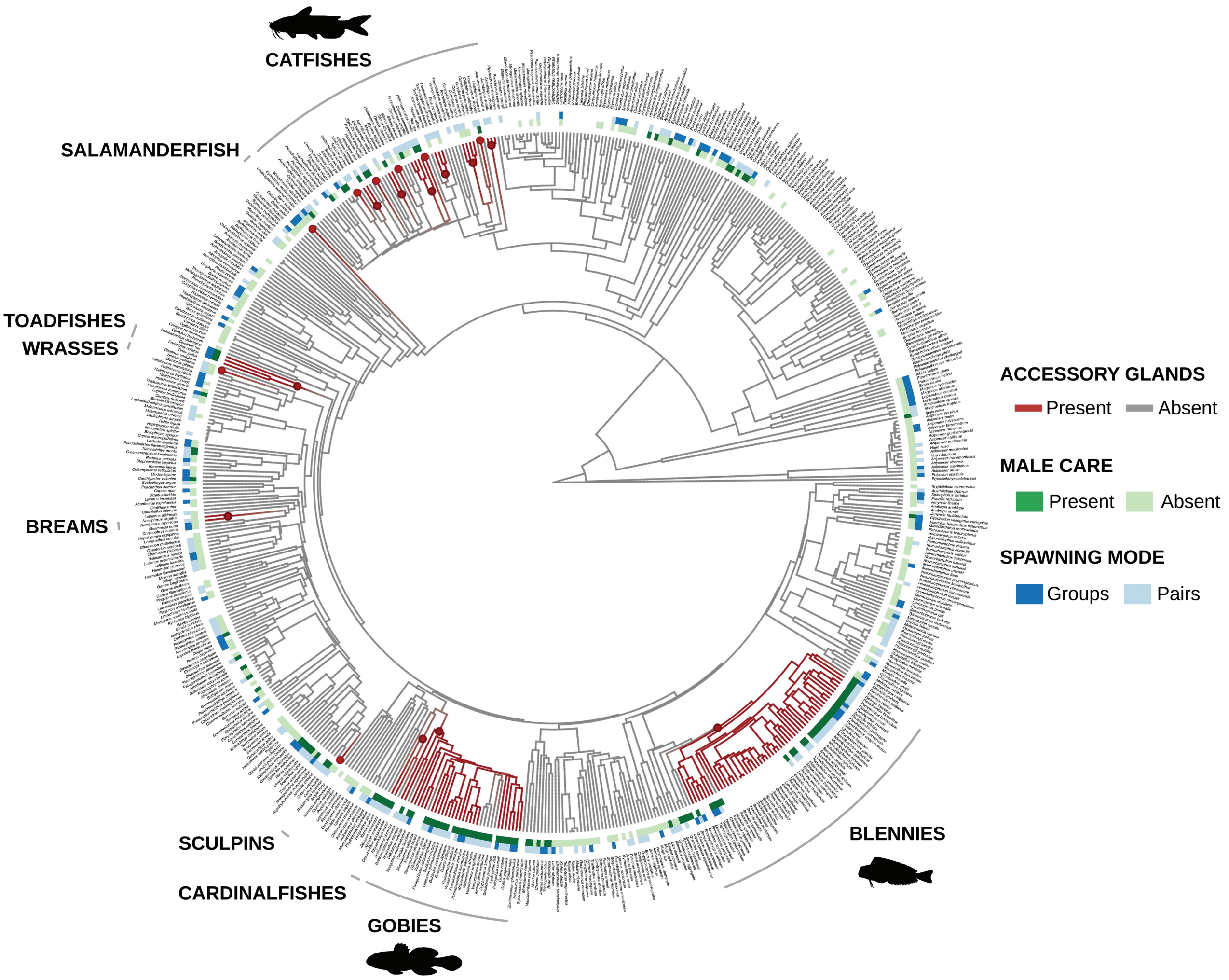

Figure 2. Evolution of accessory glands across fishes. Branches are colored with a gradient according to the posterior probability of possessing accessory glands, with red indicating the presence and gray the absence. The most likely transitions to accessory glands are indicated with red circles. The nine clades that possess accessory glands are labeled. Green and blue rings around the outside indicate the states of male care and spawning mode for each species. White spaces in the rings indicate missing data. Note that this figure presents only one of the 100 phylogenies that comprise the tree block used for the simulations and analysis; this particular phylogeny was chosen for illustration as it reflects the average number of gains and losses of accessory glands across all simulations (see Supplementary Material).

were estimated to have arisen independently more than once. However, it is important to note that the number of gains and losses are estimates based upon the data currently available. While we made every effort to avoid false absences in our data collection, the presence of accessory glands in more catfish species could substantially alter the number of estimated gains (e.g., a single gain of accessory glands might become most likely, as seen in the other clades). Regardless, catfishes make a promising group for further investigation.

Several specific functions of accessory glands can help explain why the presence of male care may have selected for these glands. Accessory glands can produce mucins, glycoproteins that increase the viscosity of seminal fluid (Lahnsteiner et al., 1992). Because this viscous seminal fluid slowly dissolves in the water, it defers the activation of sperm and potentially allows males to multitask, so they can provide parental care by defending the nest and tending to any older eggs even while a new female is spawning (Marconato et al., 1996; Ota et al., 1996; Rasotto & Mazzoldi, 2002; Scaggiante et al., 1999). Such adaptations that facilitate care are especially important if female egg-laying is extended and intermittent (Marconato et al., 1996). A second care-related function of accessory glands is the production of antimicrobial compounds that protect the eggs from harmful bacteria and enhance egg survival (Giacomello et al., 2006, 2008; Pepler et al., 2021; Pizzolon et al., 2010). Indeed, Pizzolon et al. (2010) showed that accessory gland secretions may double egg survival rates in a species of blenny (*Salaria pavo*), conferring large potential benefits in male fitness. Such effects explain why accessory glands would be under strong positive selection in species that exhibit male parental care.

Lineages with group spawning, used in our study as a proxy for high sperm competition risk, gained accessory glands at slower rates than those with pair spawning—though we acknowledge that spawning mode may not perfectly capture the degree of sperm competition experienced.

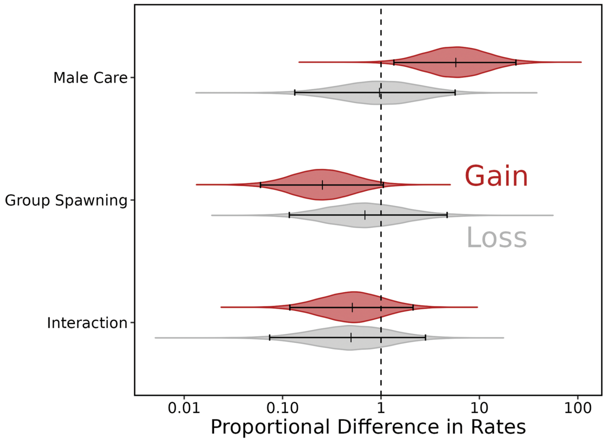

Figure 3. The effect of male care and spawning mode (group vs. pair spawning) on the gain (in red) and loss (in gray) of accessory glands. The violins show posterior distributions, the ticks show posterior medians, and the error bars show 95% credible intervals. The dashed line indicates no difference in rates (no effect).

Still, these results are surprising given the documented association between accessory glands and sperm competition in some fishes as well as in insects and mammals (Gillott, 1996; Ramm et al., 2005). In a study focused on 12 species of gobies, Mazzoldi et al. (2005) found that accessory glands were associated with higher degrees of polygyny; with absent or rudimentary accessory glands in monogamous species, and well-developed glands in polygynous species. The products of accessory glands in those goby species have also been shown to enhance sperm performance (Marconato et al., 1996), suggesting that the increased accessory gland investment in polygynous species may be due to selection by sperm competition. However, the influence of parental care on accessory gland presence/absence was not investigated in the Mazzoldi et al. (2005) study and all of the species included in their study exhibited parental care. Perhaps the role of accessory glands in parental care has been overlooked in past studies, only now being revealed by examining the evolution of these organs across fish species with and without parental care. It is also possible that the drivers of accessory gland evolution differ depending on the scale of the analysis; the broad scale of this study elucidates the role of parental care in the initial development of these glands, but sperm competition may become more important in the retention of accessory glands, which could be elucidated in analyses at smaller scales (within clades).

Further evidence for accessory glands being associated with parental care rather than sperm competition comes from species with ARTs. In species with both accessory glands and ARTs, including species of gobies (Marentette et al., 2009; Rasotto & Mazzoldi, 2002; Scaggiante et al., 1999; Utne-Palm et al., 2015), blennies (Neat, 2001; Neat et al., 2003; Oliveira et al., 2001), and toadfishes (Miller et al., 2019), it is always the care-providing male morph, not the sneaker morph, that invests more in the accessory gland, while the sneaker morph generally invests more in sperm production and the testes (Kustra & Alonzo, 2020). In some species, specific functions of the accessory glands differ between morphs, with the sneaker morph exhibiting higher concentrations of stored sperm in the accessory gland, whereas the accessory glands of caring male morphs store little to no sperm (Neat et al., 2003; Rasotto & Mazzoldi, 2002; Utne-Palm et al., 2015; Zarini et al., 2023). Furthermore, in the plainfin midshipman toadfish (*Porichthys notatus*), the antimicrobial activity of the accessory gland fluid was found to be much more effective in preventing bacterial infections when it came from the caring morph rather than from sneaker males (Pepler et al., 2021). While differential investment in reproductive traits in species with ARTs has typically been theorized and interpreted in the light of sperm competition theory (Cameron et al., 2007; Dougherty et al., 2022; Kustra & Alonzo, 2020), our study again highlights the importance of also considering parental care activities.

Male accessory glands are an often-overlooked part of the reproductive system. In the species that possess them, these glands play diverse and crucial roles in ensuring reproductive success and offspring survival. Historically, research into accessory glands has focused mainly on competitive benefits conferred on sperm by seminal fluid, and other aspects of sperm competition (Gillott, 1996; Ramm et al., 2005). Examining the distribution of these glands among fish species reveals that parental care has also been a major selective factor in their evolution, challenging the role of sperm competition in accessory gland evolution in this taxonomic group, and underscoring the importance of parental care in shaping reproductive anatomy.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Data/code are on Zenodo (https://doi.org/10.5281/zenodo.14968237).

Author contributions

S.B. and J.S.M. conceived the idea for the manuscript which was refined with help from all authors. L.E. and J.S.M. conducted the literature search. L.E. and B.M.B. conducted the analysis and made the figures. L.E. wrote the first draft of the manuscript with input from all authors. Funding was provided by S.B. and B.M.B.

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