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## Spotlight

### Testes size seen through the glass of amphibian care

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**Despite the great diversity of parental care types found in amphibians, studies linking them to post-copulatory sexually selected traits are scarce, presumably due to a lack of data. Valencia-Aguilar *et al.* used fieldwork and museum collections to show that paternal care appears to trade-off with testes size in glass frogs.**

When asked to think about parental care in non-human animals, our mind inevitably directs towards the textbook examples learnt at school: birds and mammals. With nearly 100% of the known species in these groups devotedly caring for their offspring, this bias in our thoughts seems warranted. Thinking about amphibians in this context, however, is uncommon, even though ~10–20% of the species provide parental care to their offspring [1]. To date, over 30 different forms of parental care are recognised in this clade composed of frogs and toads, newts and salamanders, and caecilians [2]. Amphibian parental care varies in relation to reproductive modes, which are also highly diverse in this group (>40 modes described to date [3]), and their mode of fertilisation, which is mostly external in frogs and toads and internal in salamanders and newts. Male care, as opposed to female care, is highly correlated with external fertilisation, as providing some post-mating care also helps ensure paternity. For similar reasons, male care often goes hand in hand with territoriality [4], which is in turn often associated with the possession of weaponry.

Birds, in which the burden of parental care is predominantly shared between males and females, and mammals, where female care is obligatory (male-only care is not known to occur), have been the subject of multiple studies linking testes size and sperm competition to parental care [5,6]. In contrast, despite their remarkable diversity in forms of parental care, studies on amphibians exploring said link have been scarce. In a recent study, Vági *et al.* [4] investigated how the climate, social environment, and mating system might influence parental care in frogs. The authors found that in species where females participate in nest building, males have larger testes, but no other forms of care were found to relate to testes size. However, Vági *et al.*'s analyses were limited due to missing data on testes size for groups where a large proportion of species display extended paternal care (e.g., Centrolenidae, Aromobatidae, Dendrobatidae).

This specific gap was addressed by Valencia-Aguilar *et al.* [7], who focused on glass frogs (Centrolenidae), a group in which short-term clutch attendance (hydrating the eggs for several hours) by females is widespread, whereas male care seems to have evolved independently two to three times [8]. The authors took advantage of fieldwork and museum collections to gather data on male and testes size in 37 out of the 150 known glass frog species. With these data at hand, they tested whether relative testes size was associated with post-mating sexual selection processes [the occurrence of paternal care (proxy for sperm competition risk) and interspecific variation in clutch size (proxy for sperm depletion risk)], or reflected an investment trade-off in features shaped by pre-mating sexual selection (i.e., sexual size dimorphism or weaponry, presumably for mate guarding). Their study is the first known comparison of its kind in an amphibian group with extended paternal care.

### How are testes size, parental care, and territoriality related?

Testes size is considered a proxy for the type of mating system, level of sperm competition, and sperm demand rate of a species [9]. Large testes are associated with a promiscuous mating system, while smaller testes are associated with monogamy or sequential mating. Clutch attendance reduces the risk of predation and increases egg hydration, thus increasing embryo survival [2]. Clutches from species with paternal care have lower amounts of egg-jelly and most are deposited in sheltered locations, which reduces the amount of rainwater reaching the eggs, thus increasing the risk of dehydration [10]. Territoriality, however, allows males to care for multiple clutches within their own territory, ultimately leading to increased fitness. Territory defence also increases males' certainty about paternity, as other males are prevented from fertilising the clutches therein. Therefore, males of territorial species have a lower risk of sperm competition and, in turn, are expected to have smaller testes than males of species where sperm competition is high [9]. However, in most cases, caring for more clutches also implies having fertilised them, which could involve a high risk of sperm depletion, particularly if clutches are large. Testing the intricate relationships between territoriality and paternal care with testes size thus requires a study system in which both territorial and non-territorial species, as well as species with and without paternal care, exist. Glass frogs are one such group.

Valencia-Aguilar *et al.* [7] show that glass frog species exhibiting paternal care have smaller testes relative to their body size, but found no correlation between paternal care or relative testes size and clutch size. These results indicate that the evolution of testes size in glass frogs is influenced by a reduction of sperm competition risk, as seen in other taxa [9], specifically by clutch

guarding and sequential mating. Thus, as the authors state, glass frog males have traded-off testes size with parental care [7].

### Frogs' diverse reproductive modes and parental care types call for more diverse questions

Given their enormous diversity of reproductive modes and parental care types, and the high prevalence of male-only care (~20%), frogs are inarguably an excellent, yet overlooked system on which to test specific hypotheses linking aspects of post-copulatory sexual selection and paternal care and to identify cases of pattern convergence across families. The study by Valencia-Aguilar *et al.* is a great starting point, but we suspect that not all parental care types are equal when it comes to trade-offs with testes size (Figure 1). Also, to date, most studies have used presence/absence data to measure the influence of parental care. However, a further leap would be measuring the duration of care. For example, clutch attendance requires the father to stay in the same area until the embryos are developed enough to hatch; however, time differs between

species with free-living larvae (e.g., Centrolenidae, Mantellidae, Rhacophoridae) and species with direct development (e.g., Eleutherodactylidae, Craugastoridae). Also, the costs of caring for a clutch in a single location may differ from those of transporting the offspring from place A to B (e.g., Dendrobatidae, Aromobatidae, Dicroglossidae), which requires the male's displacement: displacing males could incur potential territory loss and increased predation risk. Are the patterns found by Valencia-Aguilar *et al.* present in other frog clades with male clutch attendance? Are these patterns inverted in species with other types of male care? How high are the risks of sperm competition versus sperm depletion in relation to the type and duration of care?

### Closing knowledge gaps requires addressing data gaps first

Getting to ask more detailed questions on the evolution of life-history and ecological patterns demands high amounts of reliable raw data. This requires collecting, curating, and publishing trait databases [11], which involves a considerable time, effort,

and resource investment and entails high responsibility, as even the smallest mistakes can contaminate the scientific record. Previous attempts to compile a database of amphibian ecological traits reported a frustrating 30% of completeness [12]. We therefore advocate for the collection of more natural history (broadly speaking) data and to make them publicly available. Likewise, we underscore the value of museum collections as a source of, for example, morphological data, as demonstrated by Valencia-Aguilar *et al.* [7]. Obtaining additional information on relevant traits will improve our understanding of ecological and evolutionary processes and could continue to challenge our perception of amphibians as 'lower vertebrates'.

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### Declaration of interests

The authors declare no competing interests.

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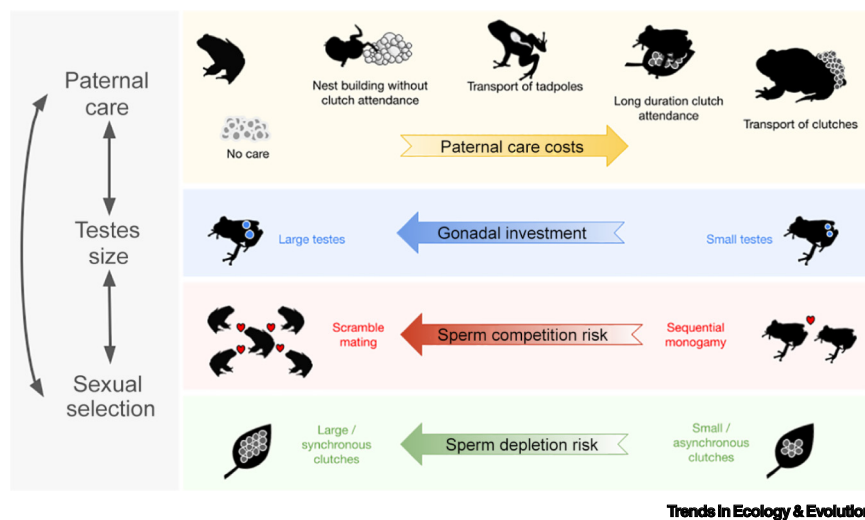


Figure 1. Paternal care diversity in anurans, with hypothesised interspecific trade-offs between cost of care and postcopulatory sexual selection processes (investment in testes and sperm competition and sperm depletion risks). This is a simplified view that ignores the more well-established patterns between paternal care and precopulatory traits (such as territoriality and different aspects of sexual dimorphism).

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