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A CATEGORICAL REVIEW OF THE GLASS FROG NICHE

A Close Analysis of the Role Glass Frogs Play in their Ecosystem and the Fascinating Adaptations that Selective Pressures have Created

ABSTRACT

The *Centrolenidae* family, nicknamed "glass frogs," are a small but charismatic tree frog species native to Central American rainforests that are best known for their fascinating transparent skin. They have a variety of remarkable adaptations such as obligate male parental care, humeral spines used in combat, dry ovaposition sites, and their skin which exhibits "clutch mimicry." They play a critical role as mesopredators, feeding on small insects and providing a food source for larger reptiles, arthropods, birds, and bats. Unfortunately, like many other frog species, a combination of climate change, habitat fragmentation, and chytrid fungus threatens the survival of this family.

INTRODUCTION TO GLASS FROGS

The family *Centrolenidae*, colloquially known as "glass frogs" due to their transparent abdominal skin, are part of the Anura order; this means that they are tailless vertebrates with compact bodies that experience complex metamorphic life cycles. They are part of the suborder Neobatrachia, ("new frogs"), the largest suborder of frogs. This suborder also contains the most derived features from the last common ancestor of all frog lineages (Rowley, 2014). The

Centrolinids are all nocturnal and neotropical, and the family contains an estimated 152 species. They are quite a small bodied lineage of frogs, with an average length of about 2 centimeters. Within the *Centrolenidae*, there're three genera: (1) the *centrolene*, known for its humeral spines; (2) the *hyalinobatrachium,* known for their bulbous white liver; and (3) the *cochronella*, who lack hand webbing, humeral spines, and a bulbous liver.

The glass frogs are a type of tree frog, which means that they are adapted to arboreality. Compared to their relatives who are more semiaquatic in adult form, tree frogs have slender bodies for easily locomoting in a sprawled position across thin branches and barks (Rowley, 2014). Because all frogs have highly permeable skin and respirate through it, tree frog species have the added difficulty of staying moist when they are high in the tree canopy of their habitats. To cope, most tree frogs will secrete waxes and lipids from their skin to stop water loss. Tree frogs exhibit two kinds of mating patterns: explosive, where mating happens over a short window of a few days or weeks; and prolonged, where mating happens over longer than a month. In species with explosive breeding, females have limited mate choice due to scramble mating and high amounts of sperm competition (Mangold et al.). In species with prolonged breeding, like in most glass frogs, females have more selectivity over mate choice leading to novel breeding and parental care patterns evolving to combat skews in male reproductive success.

RESULTS AND DISCUSSION BY CATEGORY

MORPHOLOGY

Glass frogs have a variety of morphological features that reflect both primitive tree frog ancestry *and* derived traits that give them their name. Like most arboreal frogs, they have lateral bender legs that allow them to move forward and leap laterally while still being sprawled. This form of locomotion releases far less energy than crunching side to side. Their limbs are thin so as to reduce air resistance when leaping across branches (Federle et al.). Attached to those limbs are enlarged toe pads that provide a firm grip on smooth surfaces, ideal for climbing but not for swimming. These adhering pads are permanently moistened with mucus secreted from glands near the base of their appendages; this keeps their skin pores open and creates a suction like effect.

Unlike other arboreal frogs, glass frogs have forward facing eyes rather than side-facing eyes ("Glass Frogs"). This is a curious feature because in other vertebrates, side facing eyes mean superior peripheral vision, typically reserved for herbivore prey, while forward facing binocular vision is more prevalent in apex predators. Perhaps this indicates that other morphological predator-avoidance features make up for a lack of visual predator detection capacity.

The most distinct feature of glass frogs is of course their varying levels of transparent skin. In fact, some species like the recently discovered *Hyalinobatrachium yaku* are almost completely transparent—one can witness the flow of their circulatory system and the movement of internal organs (Kluger 2017). However, for most species, the rest of their bodies are covered in lime green skin for blending in with their lush, green, tropical rainforest environments. Additionally, males in certain glass frog species possess what is known as humeral spines, bony ventrolateral extensions of their humerus (Hutter et al.). These are used in combat over territory (discussed in more detail under BEHAVIOR).

LIFE CYCLE

Like all anurans, glass frogs have a complex metamorphic lifestyle. This involves development of tadpoles from eggs, which then go on to absorb their tails and develop a spiracle over their external gills. Then, they make the transition to a tetrapod adult body form and become reproductively mature, ready to lay eggs of their own in a jelly-like clutch through dieicious external fertilization. On average, they can live for up to 14 years in the wild ("Glass Frogs").

Like all amphibian eggs, glass frog eggs lack a protective hard coating, meaning that they still rely on aquatic environments to provide structure. This also means that they're delicate, and particularly prone to environmental risk (Hampton and Otto). This has led to the practice of parental care and greater selective pressure to have the ability to identify safe oviposition sites. Glass frog females lay their egg clutches on top of leaves, where they will eventually develop and roll off into the nearby stream to grow into tadpoles. Typical clutch size is 20-30 eggs. This development can be as quick as 9 days, or as long as 16. In many glass frog species, it's actually the fathers who are guarding the egg clutches (discussed in further detail below). In species of glass frogs where there's no parental care, eggs are typically deposited on the underside of leaves to make them less visible to predators.

Plasticity in embryo hatching provides an opportunity for glass frogs to respond to changes in environmental risks. For example, when exposed to a flooding experimental condition, glass frog eggs hatch sooner because of the change in respiratory needs—gasses dissolve more slowly in water than in air, so hatching sooner would mean they could get oxygen more efficiently than being trapped in a membrane surrounded by water. Additionally, clutches hatch sooner when not attended by their fathers in response to only specific threatening stimuli like vibrations of wasps (Lehtinen and Green). Plasticity in hatching times could be very beneficial in light of the rise in environmental variation due to anthropogenic activity. However, hatching too early confers fitness tradeoffs like decreased strength and higher intrinsic mortality from speeding up or stopping development prematurely.

BEHAVIOR

Many frog species, Centrolinids being no different, rely on a complex set of vocalizations in mating, territory defense, or in communicating distress. These calls can vary in duration, shape, and pulse rate to differentiate meaning. Often times, the forest will be loud and alive with choruses of multiple different species of frogs at once (Hutter et al.). Variation in glass frog calls is a reliable predictor of species type. Furthermore, diversity in mating calls can be a critical element in terms of speciation. Genetic testing has shown that there's at least five sympatric species of glass frogs living in Colombian rainforests, all of which mate during the rainy season. In order to maintain reproductive isolation, they utilize distinct species-specific mating calls. Interestingly, males with lower pitched calls have higher reproductive success, perhaps because a lower pitch is indicative of a larger bodies that can better defend the clutches.

The courtship and breeding behaviors of the glass frogs are equally complex. Females chose males based on call frequency, and females must be the choosey sex in order to create an environment for obligate male care and increased survivorship of clutches. The start of courtship includes acoustic signals and visual displays. When a female hops onto a males leaf perch, the male will call to her and then to display strength; he will do body pushups and rapid leg and arm movements. The relative importance of these visual displays has been under scrutiny, but researchers believe that they could be necessary when auditory signals might be inhibited by noisy stream habitats. Also, there is peculiar and routine interruption of amplexus by the male.

They will often halt amplexus and begin mate calling again; it is hypothesized that this is perhaps to attract even more mates to their leaf (Vargas-Salinas et al.). The most understudied aspect of courtship is the pervasive ultrasonic communication utilized by a wide variety of frogs. Though not confirmed, it is predicted that glass frogs have the capability of using this communication system.

The most fascinating aspect of glass frog behavior is by far the male parental care. Researchers have found that males are obligated to guard clutches of eggs, especially in the genera *Centrolene* and *Hyalinobatrachium*. In fact, the courtship ritual itself concludes with the female and male synching up behavior to imply the start of egg attendance (Mangold et al.). This feature has evolved many times within the family *Centrolenidae*, implying that it's an adaptation. Male glass frogs are tasked with guarding multiple clutches per leaf, each with different mothers, implying higher levels of polygyny and fewer benefits conferred by individual females.

Males are known to be highly territorial. This is because males who have higher leaf perch territories having higher reproductive success; these perches are better suited for projecting their calls. Therefore, combat is mostly over territory (prime areas for calling), and rarely results in actual injury. The goal of combat is to keep the rival male away from their leaf (Rios-Soto et al.). Typically, this involves resident males shaking their leaf and making territorial calls during encounters with rivals. Things might even progress to wrestling in a reverse amplexus like form. These fights can last for multiple days, especially when the rival male gets confused and thinks *they* have claim to the leaf. In this case, both the resident male and the rival male will make territorial calls. However, in some species, fighting can become more dangerous. Resident males will grasp rival males with their humeral spines, and let them dangle from their perch for hours, with the victims finally being released when they give out a distress call.

Members of *Centrolenidae* have predator defense behaviors that are common in other tree frog species. This includes a release of cloacal fluids, pungent secretions that deter arthropod predators like tarantulas. Additionally, they will stretch out their limbs and puff up their bodies to make themselves look larger and therefore less mechanically digestible to predators like snakes and bats (Escobar-Lasso and Rojas-Morales). Glass frogs do not have highly specialized behavioral adaptations because of the variety of predators they must deal with—instead, like with their poison dart neighbors, where natural selection really took the reins was in morphological adaptations (detailed further in ADAPTATIONS).

ECOLOGY

The *Centrolenidae* family is indigenous to Central America with its temperate and tropical forests. The rainy season runs from April through October. While temperatures rarely dip below 20° Celsius, which is good for ectothermic frogs, they can get as high as 35° which can be dangerous for anurans who can easily lose water through their skin (Moen, 2016). That's probably why glass frogs spend the majority of their time relatively high in the tree canopy, shaded from the sun's UV rays and also hidden from their stealth hunter predators. When it does come time to breed, they will come down to find suitable leaf perches overlooking streams to project calls and deposit clutches. They cannot lay their eggs submerged in the water because of the tradeoff of slender legs, not at all suited for swimming.

Similar to other frogs, glass frogs are insectivores. The high quality diet of ants, crickets, and flies is necessitated by the high metabolic costs of their compact bodies. Glass frogs feed on insects not by sticking out a long and retractable tongue, but by pouncing on the bug (Lehtinen and Green). If the size of the prey means the frog cannot swallow it whole, then it uses its mouth muscles to squeeze the prey's body until stomach enzymes have effectively immobilized it. However impressive a hunter, glass frogs face competition from other tree frog species, small birds, and reptiles for this popular resource.

Glass frogs support a wide variety of higher *and* lower trophic level predators, during all stages of their life. There's a species of fly that targets tree frog clutches, laying its own eggs on the embryos so that when they hatch into larvae, they will have a nutritious first meal (Ortega-Andrade et al.). Bigger arthropod predators like wasps will also target clutches. Once they have rolled of the leaf into the stream and developed into tadpoles, they face predation from aquatic bugs, turtles, and fish. When they develop into their adult tetrapod form, they become especially enticing to snakes, tarantulas, birds, and fringed-lipped bats. The threat from these bats is so high that glass frogs will assemble in higher chorus density leks when breeding to reduce the extrinsic mortality risk per individual—their calls are loud and distinct, which unfortunately attracts fringed lipped bats. Researchers have found that when males are in larger chorus groups, they are less likely to terminate calls after they perceive bats to be present and more likely to resume faster when they do. Male chorusing frogs might be attracted to one another's calls in order to create the dilution effect: increasing the amount of individuals likely to detect a predator while also lowering the individual's chance of getting eaten (Jennions and Backwell). This is an excellent example of ecology interacting with behavior.

Unfortunately, a combination of anthropogenic land development and the spread of the chytrid fungus has deeply impacted the population sizes and conservation status of glass frogs. These species are especially sensitive to habitat destruction because of the specific locations they use for mating and laying their eggs, and even areas that are allegedly protected zones in Central American forests have experienced illegal destruction and disturbances (Ortega-Andrade et al.). The general lack of population density data and insufficient biodiversity indexing of these difficult to research areas make designing efficient conservation models all the more necessary. Researchers have predicted one can expect to see a 50% reduction in populations the glass frog species within the next ten years.

ADAPTATIONS

The only behavioral adaptation that the *Centrolenidae* family demonstrates is the prevalence of male parental care. Because these species lay their eggs on land, they are especially vulnerable to small terrestrial carnivores, and the low offspring investment by the females makes males obligate guards of their clutches (Mangold et al.). High male territoriality demonstrates to females that they are capable of protecting the clutches from predation, which undoubtedly guided selection for distinct territory calls within the family.

As mentioned above, predation is a huge adaptive pressure for glass frogs. What they lack in behavioral adaptations, they more than make up for in crypto-morphological adaptations. On their backs, they have lime green skin to blend in with the foliage. More interestingly, certain glass frogs' skin has infrared reflectance. This is hypothesized to confer an adaptive advantage both in thermoregulation and cryptic coloration. Photons in infrared waves will lose energy as heat if they are absorbed, so the ability to reflect them can help thermoregulate by preventing excessive heat gain. Also, snakes and birds are known to be capable of perceiving infrared light, so if these frogs can reflect it instead of absorbing it, they would be much harder to detect on leaves (*Infrared Reflectance in Leaf-Sitting Neotropical Frogs, 2016*). Glass frogs that have this adaptation would have a selective advantage over even their most impressively camouflaged amphibian cohorts because they would not be giving off a detectable heat signal.

A sexually dimorphic adaptation only seen in male glass frogs is the humeral spines. While previously thought to be an adaptation used for amplexus, now it seems as though the glass frogs' humeral spines are used to grip during combat; a fighting male can squeeze its arm muscles and drive humeral spines into the opponent male (Hutter et al.). The selective pressure to be capable of defending a high-quality perch and the egg clutches that come along with it have played a critical role in the emergence of this trait.

The most iconic glass frog adaptation is definitely its namesake—the transparent skin on their abdomen. On the surfaces, the benefit of having skin that acts as a window into their internal organs can seem foggy. The most recent hypothesis states that their clear stomachs resemble the clear jelly surrounding their egg clutches. This can make it difficult to distinguish the adult frog from the clutch. As a result, egg predators, typically small arthropods, might mistake the frog for a clutch (providing a free snack for the frog), and frog predators might mistake a frog for a clutch and continue on their way (Guayasamin et al.). One can imagine an adaptive landscape with a wide variety of predators where frogs with this type of "clutch mimicry" would have much greater survival rates and therefore higher reproductive success.

CONCLUSIONS

By analyzing results from the aforementioned studies, it becomes clear that glass frogs are not only a physically fascinating family but also crucial mesopredators in their ecosystem. Their life spent in the canopy alongside streams means that the Centrolinid niche plays an important part monitoring insect population densities and in nutrient cycling where the soil in tropical forests is often shallow and unproductive (Guayasamin et al.). Additionally, maintaining a high biomass of all tree frogs is critical for supporting higher trophic level and keystone species. Unfortunately, the decimating effects of the chytrid fungus means that declining glass frog populations represent only a fraction of the total extinction rate of Anurans. To combat this, more research in these tropical rainforest ecologies is needed to accurately assess biodiversity loss and the impacts of habitat fragmentation. These ecosystems are often the most fragile and least resistant to climate change, so the ability to determine extinction rates and biotic interactions as a whole is critical for designing successful conservation programs for species like the glass frogs. There is still so much to learn from studying these animals that protecting them and other species they interact with as an "umbrella species" should be a priority.

As far as future research that should be conducted, there is a plethora of factors in the glass frog niche that could benefit from further exploration. For example, as mentioned above under BEHAVIOR, little is known about the mechanism and function of ultrasonic communication among frog species, and the role it could play in mating systems for glass frogs. Additionally, there is a potential for it to contribute to allopatric speciation within the family. As with most tropical species, more research should be conducted on intraspecific associations glass frogs might hold in their environment. With predation being such a large adaptive pressure, it is possible that multiple tree frog species' courtship rituals in leks contribute to the dilution effect. Lastly, more detailed ecological studies should be conducted on dietary overlap between frog species and those outside the clade. Understanding the role of direct resource competition versus habitat partitioning in this landscape will help researchers and conservationists alike produce accurate niche partitioning models.

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