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Poison frogs as a model system for studying the neurobiology of parental care

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Parental care is essential for the survival and well-being of offspring in many species. Understanding the mechanisms underlying parental involvement can lend insight into general and conserved principles governing the neural basis of a major life history stage, parenthood. While many animal models of parental behavior display maternal care, studying male involvement is challenging. Male parents are usually involved in a strong relationship with their partner, and this makes separating reproductive and parental mechanisms difficult. This separation is possible in poison frogs, where different species provide biparental care and male or female uniparental care without pair bonding. Poison frogs provide a great comparative framework to study parental care within a relatively simple neural architecture easily amenable in the field and laboratory.

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Becoming a parent is a considerable life transition and in many species requires precious time and energy from invested parents to raise their offspring. Such transition to parental care behavior requires transformative changes in physiology and neurochemistry to facilitate a switch from apathy or aggression toward offspring to the powerful bond that most human parents experience. Across the animal kingdom, various parental care strategies (biparental and male or female uniparental care) have evolved numerous times in every major lineage [1], exemplifying the adaptive significance of parental behavior in diverse ecological contexts. Whether one or both parents will be involved in offspring care is influenced by many factors including certainty of parentage, the population operational sex ratio, or the reproductive mode [2–6]. For example, maternal care is more common in genera with internal fertilization or when females are the sole food source as with lactation in mammals. On the other hand, paternal care is more common in genera with external fertilization in the form of egg guarding as a way to guarantee paternity. Understanding why and how either one or both parent(s) care for offspring requires integrating complementary approaches to answer the proximate and ultimate facets of behavior, best put forth by Niko Tinbergen's 'Four Questions' over 50 years ago [7]:

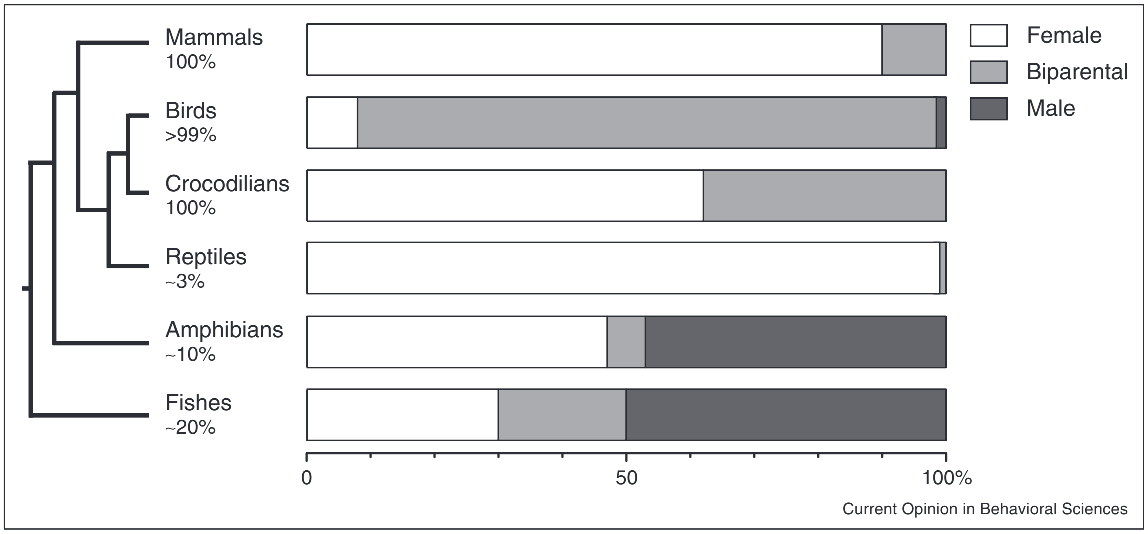
How does parental behavior work? (Mechanism/causation)
How does this behavior develop during the lifetime of an individual? (Ontogeny)
What is the function of parental behavior? (Adaptive/survival value)
Why did this behavior evolve over the history of a species? (Evolution/Phylogeny)

While many animal models have attempted to address the causes and consequences of variation in parental care, most have fallen short of their utility in answering all four of Tinbergen's questions. Thus, our understanding of parental care, especially care of offspring by males, is still relatively poor compared to other social behaviors. Exploring the physiological and neural mechanisms underlying different parental stages within a comparative framework could highlight conserved mechanisms promoting parental care across many species.

Parental care in animal models

Specialized parental care strategies have evolved across the animal kingdom, yet the underlying mechanisms promoting the convergent evolution of these behavioral phenotypes are poorly understood [1,8]. Among vertebrate species that show parental care, the diversity of strategies is unequally distributed (Figure 1). The physiological and neural mechanisms that promote parental care are best understood in rodents, especially in the context of maternal care [9] which occurs in roughly 90% of mammalian species [10]. However, less is known about the biological basis of paternal care, which is mainly informed by monogamous and biparental animal models, including *Microtus* voles, *Peromyscus* mice, Estrildid finches and cichlid fish [11–16]. In these contexts, males usually form a pair bond with their partner before engaging in parental care, likely modifying neural connectivity and gene expression through multiple behavioral transitions [14,17,18]. Thus, these intermingled behaviors

Figure 1



Estimated representation of parental care strategies among the major vertebrate lineages. Percentages under each vertebrate lineage are estimated proportions of the number of species with parental care. Bars represent an estimated proportion of each type of parental care system according to species, genera or families displaying parental care within the lineage. Male uniparental care is absent in mammalian and reptilian linages. In birds, about 9% display cooperative breeding and less than 1% does not display parental care through either geothermal heat incubation or brood parasitism. Most fish, amphibians and reptiles (except crocodilians), do not display parental care.
Source: Data from Refs. [3,10,23,66].

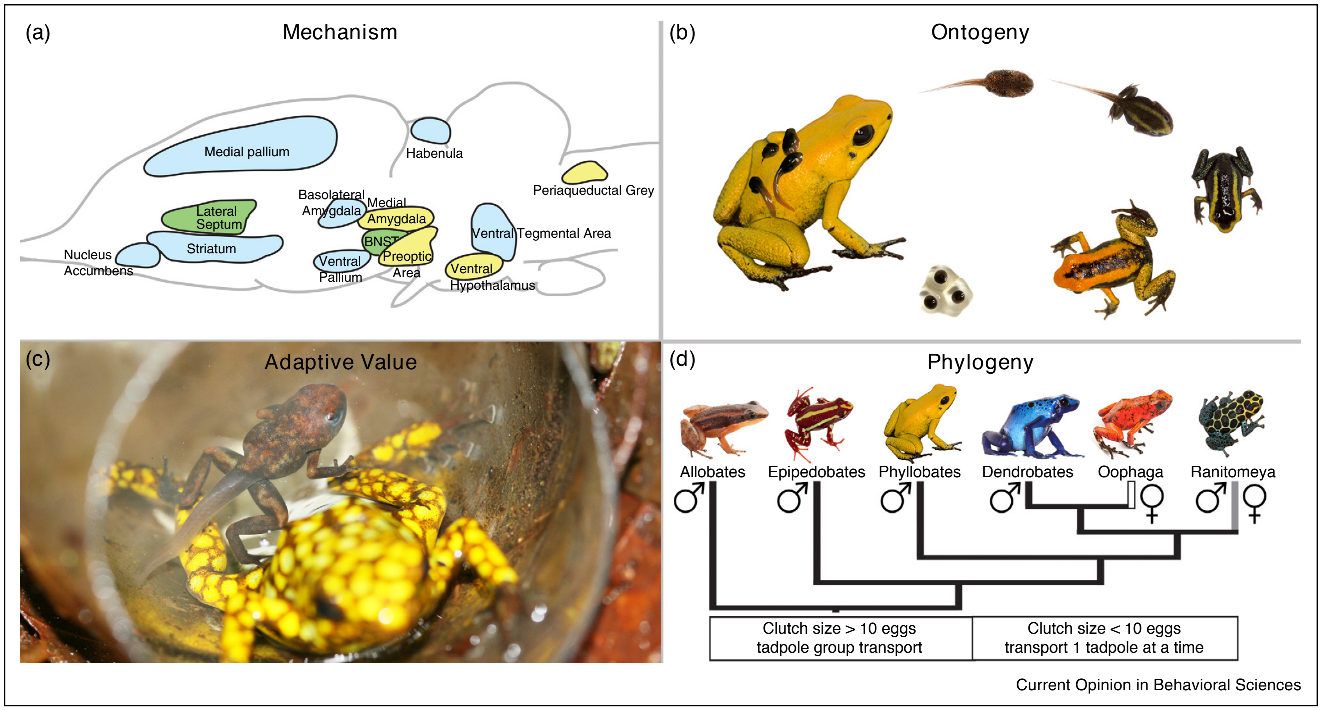
make the neural bases of parental care difficult to disassociate from partner affiliation in animals with monogamous mating systems. Some inbred laboratory rodents demonstrate 'facultative' male parental care without pair-bonding [19,20•], where within a specific timeframe after mating, infanticide is suppressed and males sometimes lick and groom pups in a coordinated care effort with the mother [21]. However, even in these species, female involvement in offspring care is obligate and how neural mechanisms promote care for offspring in a single father without maternal involvement is unknown.

A good strategy for identifying conserved and causal mechanisms governing parental care is to perform a comparative analysis across closely related species that vary in parental care strategies in a manner that is independent of pair-bonding. Involvement in offspring care is generally correlated with the natural history of the species and the reproductive benefit for each sex. Most established animal model systems of parental care have internal fertilization, which favors female or biparental care presumably due to lack of paternity assurance [22]. External fertilization in amphibians and fishes provides a better substrate for diversification of parental care behavior. Although most species in these clades do not care for offspring, fish and amphibians offer the greatest diversity in parental care strategies (Figure 1), including repeated and independent transitions from no offspring care to biparental care and female or male uniparental care [4,10,23]. Male fish displaying parental care fan and guard eggs, carry their brood in their mouth and even feed their own skin mucus to offspring [10,24]. The most striking example comes from seahorses and pipefishes, where males possess a brood-pouch that maintains a placenta-like connection to supply offspring with nutrients [25]. Similarly, amphibian parental care involves egg defense, egg incubation (in dorsal pouch, vocal bag or stomach), and tadpole transport and feeding [4,26,27]. Two clades that show extreme diversity in parental care within closely related species are cichlid fish (Family Cichlidae [28]) and poison frogs (Family Dendrobatidae [26]), making them excellent animal models to ask how the brain promotes parental behavior within a comparative context.

What poison frogs can teach us about the parenting brain

Poison frogs show substantial variation in parental care tactics, including male uniparental care, female uniparental care, and biparental care [26] (Figure 2d, Phylogeny). Importantly, both paternal and maternal care occurs with and without pair bonding across different poison frog species [29]. Most investigations into poison frog behavior have focused on field observations and manipulations to study ultimate questions, such as the adaptive value of parenting behavior or the evolution of behavioral diversity within the clade [30,31]. However, studies into proximate mechanisms of parental care are sparse even though behavioral diversity of poison frogs nominates these species as a promising model system in comparative neuroscience. Current technological advances, such as high throughput sequencing and genome editing methods, allow researchers to address mechanistic questions in

Figure 2



Poison frogs as a single system to answer Tinbergen's four questions. Poison frogs are useful for integrating all four levels of analysis to address outstanding questions in animal behavior. **(a)** *Mechanism or causation*. Frog brains offer a relatively simple system in which to investigate the neural basis of behavior. Conserved brain regions important for social decision-making in vertebrates, including the social behavior network (yellow) and mesolimbic reward system (blue; green represents the overlap of these two networks) are shown. **(b)** *Ontogeny or development*. Investigation of how behavior is learned or influenced by life history in poison frogs is tractable given their easy rearing in laboratory conditions. **(c)** *Adaptive value or function*. The current utility of many poison frog behaviors, such as parental behavior or predator avoidance, has been a topic of field research for many decades. **(d)**. *Phylogeny or evolution*. The diverse behaviors within closely related species and coupled with a robust phylogeny makes poison frogs an exceptional clade for comparative approaches to understanding animal behavior and its evolution. *Source*: Photograph in (c) by Elicio E. Tapia and used with his permission; photograph of *Allobates kingsburyi* in (d) by Dr. Luis A. Coloma and used with his permission.

non-traditional model systems and move away from purely correlative studies toward causal evidence. As the poison frog clade is amenable to behavioral and genetic manipulations in the field and laboratory, they provide an outstanding framework in which to study the evolution of ecologically relevant traits by addressing all four of Niko Tinbergen's questions, and would increase our knowledge of both the causes and consequences of adaptive behaviors.

Mechanism

Anurans offer relatively simple brains in which to understand the neural basis of behaviors and have a rich history as models in animal behavior and sensory processing [32,33]. Vertebrate neural networks important for social behavior, including the Social Behavior Network and the Mesolimbic Reward System [34], have been delineated in the amphibian brain, which allows for direct comparisons with other vertebrate taxa (Figure 2a). Despite the rich diversity of poison frog parental behavior, no studies have investigated the underlying neural mechanisms. Tools are available for research into the neural mechanisms of behavior in non-traditional model systems, such as immunohistochemistry and ribosome capture to measure gene expression specifically in behaviorally relevant neurons [35•]. More recent advances in genome editing techniques enable functional testing of neurons in specific behaviors, tractable across a wide variety of species. Using the crispr-Cas9 system to modulate gene expression [36•], or using DREADD channels [37] to manipulate neurons in freely behaving animals would allow researchers to functionally test neural circuits underlying behavior in real time. Applying these techniques to studies of poison frog behavior will reveal general mechanisms governing the neural basis of parental care.

Ontogeny

Life history of an individual has a large impact on behavior (Figure 2b). In many species, variation in parental quality alters offspring behavior in adulthood, including aggression [38], parental behavior [39], cognition [40], and other personality traits (boldness or anxiety [41]). For example, variation in parental quality (high vs. low contact parents) in the biparental and monogamous *Microtus ochrogastor* alters offspring behavior in adolescence and adulthood [42]. Other research in songbirds, which are mostly biparental species, show that nutritional stress during the early song-learning phase effects male song quality in adulthood [43]. Studies in poison frogs could further add to the ontogeny field by examining how variation in parental care quality across a range of parental strategies alters behavior and fitness in adulthood. Moreover, the unique variation in parental care strategies allows one to examine how variation in paternal behavior alters offspring development independent of maternal influence.

Parent–progeny relationships can also alter offspring mating choices and reproductive behavior later in life. This pattern is best understood in birds, where offspring imprint on their parents' morphology [44] or vocalizations [45], which permanently impacts their adult behavior. Adult behavior in poison frogs could also be influenced by the morphology of a parent. Literature on poison frog mating preferences based on color is conflicting, where some populations homogeneous for color patterning seem to prefer a mate of their own color [46,47•], while other studies do not find any correlation [48]. Most of these studies focus on the Strawberry poison frog (*Oophaga pumilio*) where mothers provide nutritive eggs to her tadpoles. In such a system with an intimate parent–progeny relationship, offspring could imprint on the mother's morphology which later would influence the offspring's own mate choice behavior toward its mother's phenotypic model. Cross-fostering studies would reveal if parental morphology influences offspring mate choice later in life.

Although parental care systems in poison frogs have historically been considered rigid, a recent study shows plasticity in parental behavior using a male uniparental care poison frog species [49••]. In this study, females that are normally non-parental can be induced to care for offspring whenever the male is removed. Comparative analyses of behavioral plasticity using different parental models in poison frogs will likely highlight conserved mechanisms shared across this clade and in other vertebrates. Moreover, the relative ease of rearing and breeding these frogs in the laboratory allows further tests into how life history impacts an individual's behavior.

Adaptive value

Offspring survival is dependent of parental care in poison frogs, as parents must transport their larvae from leaf litter to a pool of water, where tadpoles will live until completing metamorphosis. Some poison frog species deposit their offspring into small individual pools of water, where predation risk is reduced at the cost of low food resources. However, this ecological transition in tadpole deposition, from large pools to small water accumulations in plants (phytotelmata), requires an associated change in parental behavior and led to biparental care in *Ranitomeya* and female-biased care in *Oophaga* [29]. In *Ranitomeya*, the cost of time and resources dedicated to rearing offspring by both parents likely reduces their ability to find new partners, leading some species to monogamy [50]. Changes in parental behavior are also associated with trophic specialization of the larvae, particularly in *Oophaga*, where tadpoles are dependent on the unfertilized eggs deposited by their mothers (Figure 2c). This tadpole feeding mechanism also provides a predator deterrent, as the trophic eggs contain alkaloid toxins and provide chemical defenses to the tadpoles [51••].

Phylogeny

The evolution of parental care in poison frogs has been established through decades of field observations and analysis of genetic markers [26,29,52]. Male uniparental care is the ancestral state in poison frogs (Figure 1d), and presumably evolved as a method of ensuring paternity [30,53]. The associated cost in time and resources by males caring for offspring likely also favored clutch size reduction and an increase in egg size [54]. In most poison frog species, the female is not involved in offspring care after eggs are laid and males do not form a pair bond with their mates [29,30]. However, some species have transitioned to more specialized tadpole rearing environments that are nutrient-deficient, necessitating the involvement of nursing females, which in some species has led to monogamy/pair-bonding [50,55•]. Studying parental care in this clade with such extreme diversity in behavioral strategies allows the elucidation of the neuroendocrine contributions to parental care without the confounding effects of pair bonding or maternal care, which is not possible in mammalian species. Furthermore, poison frogs represent an independent transition of parental strategies among vertebrates, from ancestral uniparental male, to uniparental female or biparental care, allowing us to determine if mechanisms are analogous across poison frogs, amphibians species and other vertebrates.

Conclusions

We have presented here the utility of using poison frogs as a model system to study outstanding questions in animal behavior through addressing all four levels of Tinbergen's questions. Although we have focused on parental care and mating strategies here, poison frogs are an excellent model clade to study the neural basis of many other adaptive behaviors to environmental opportunities and challenges [56], such as boldness [57], spatial memory [58,59], predator avoidance [60–63], and foraging specializations [64,65]. Joining of laboratory experiments and fieldwork with poison frogs will broaden our knowledge of the proximate and ultimate mechanisms of parental care and other adaptive behaviors.

Conflict of interest

None declared.

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