Notes and Comments

Parasites Lost? An Overlooked Hypothesis for the Evolution of Alternative Reproductive Strategies in Amphibians

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abstract: Amphibians exhibit the greatest diversity of reproductive strategies of all tetrapod vertebrates. While authors have traditionally attributed the evolution of these strategies to factors such as complex topography, unpredictable larval environments, and predation on larvae and eggs, support for any of these hypotheses has been limited. Importantly, most authors have ignored parasites, including unicellular pathogens and multicellular parasites, as selective agents capable of influencing amphibian evolution. Insights in disease transmission, amphibian immunity, and their interaction with various life histories require that we consider parasites to be selective pressures in our exploration of the evolution of amphibian reproductive strategies. I review recent findings and describe how these principles converge to form a novel conceptual hypothesis for the evolution of alternative reproductive strategies in amphibians. I offer some specific predictions and recommend that parasites be considered with other selective pressures when constructing formal, falsifiable hypotheses during evaluative studies of amphibian reproductive behavior.

Keywords: anurans, chytrid, disease, iridovirus, life history, salamanders.

The ancestral life history in recent amphibians (i.e., Lissamphibia) is hypothesized to have been biphasic, with an aquatic larval period and a terrestrial adult stage (Duellman and Trueb 1986; Milner 1993; Hanken et al. 1997), a strategy that remains widespread in extant amphibians (table 1). However, amphibians exhibit the greatest diversity in reproductive strategies of all tetrapod vertebrates (Haddad and Prado 2005), with alternative reproductive strategies that deviate from the ancestral biphasic mode and become increasingly terrestrial, including numerous independently derived forms of terrestrial oviposition, nest brooding, egg carrying, and direct development that bypasses the aquatic larval stage altogether (table 1). Traditionally, pressures such as complex topography, unpredictable larval environments, and predation on larvae and eggs have been suggested as promoting the radiation of amphibian reproductive strategies into terrestrial environments. Importantly, parasites as selective forces in amphibian evolution have been largely ignored (but see Forester 1979 and citations therein for discussion of terrestrial nest brooding in plethodontid salamanders), leading to an incomplete evaluation of multiple competing hypotheses (Platt 1964). Here, I define parasites to include unicellular disease-causing pathogens and multicellular endo- and ectoparasites. I argue that parasites must be considered in the evolution of amphibian reproductive behaviors for at least three key reasons. First, the vast majority of known amphibian parasites rely on aquatic infective stages for transmission and reproduction. Second, the ancestral biphasic life history of amphibians that requires aquatic reproduction increases host contact rates and promotes parasite persistence in otherwise dispersed terrestrial populations. Third, larval and metamorphosing amphibians have diminished immunities compared to adults, making these stages more susceptible to parasite-driven mortality. The combination of these factors creates predictable patterns of mortality that correspond with amphibian reproductive strategies and life-history traits, and it suggests that we more carefully consider parasites as potential selective agents in amphibian evolution.

Common Amphibian Parasites

Previous authors have summarized the role of parasites in amphibian mortalities and declines (Carey et al. 1999, 2003; Daszak et al. 1999, 2003), but it is important here to highlight aspects that relate to parasite transmission. Most amphibian parasites that cause high rates of mortality

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Table 1: Distribution of reproductive strategies in amphibian families of the orders Anura and Caudata

	Anura																																Caudata									
Reproductive strategy	Al	Ar	Ac	At	Bo	Br	Bu	Ce	De	Di	He	Hm	Hl	Hp	Le	Lp	Ma	Me	Mi	My	Na	Pb	Pd	Pi	Ra	Rh	Rd	Rp	So	Ab	Ap	Cr	Dc	Hn	Pl	Pr	Ry	Sa	Si
Egg deposition strategy:																																										
Ponds and streams[a]	x	x	x	x	x		x			x	x		x	x		x	x	x	x	x	?	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x
Terrestrial pools							x		x							x	x																							
Terrestrial on ground		x				x	x					x		x	x	x	x		x	x					x	x	x		x	x	x				x				x
Arboreal pools							x		x				x	x		x	x		x							x									x					
Attached arboreally								x			x		x	x			x		x						x	x									x					
Carried by parent until hatched										x			x							x				x																
Larval feeding strategy:																																									
Exotrophic larvae[a]	x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	?	x	x	x	x	x		x		x	x	x	x	x	x	x	x	x	x	x
Endotrophic larvae		x				x	x		x				x	x	x	x			x	x				x	x	x	x		x											
Larval habitat:																																									
Aquatic[a]	x	x	x	x	x		x	x	x	x	x	x	x	x		x	x	x	x	x	?	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x
Confined to nests							x		x				x	x		x	x		x	x					x	x														
Carried by parent through metamorphosis													x		x					x				x			x		x											
Direct development (no larvae)		x				x	x						x		x	x	x		x	x					x	x			x						x					
Ovoviviparity							x									x																						x		
Viviparity							x																																x		
Paedomorphosis:																																									
Facultative																													x			x	x	x			x		
Obligate																														x	x			x	x				x

Note: The taxonomy used here follows Frost (1985). Recent studies have suggested a revised taxonomy based on molecular evidence, and these newer changes are noted by Frost et al. (2006). Categories are not mutually exclusive. The life history of the recently described monophyletic Nasikabatrachidae is largely unknown but is presumed to be similar to that of other burrowing frogs that follow an ancestral biphasic life-history strategy (Biju and Bossuyt 2003). Al = Allophrynidae, Ar = Arthroleptidae, Ac = Ascaphidae, At = Astylosternidae, Bo = Bombinatoridae, Br = Brachycephalidae, Bu = Bufonidae, Ce = Centrolenidae, De = Dendrobatidae, Di = Discoglossidae, He = Heleophrynidae, Hm = Hemisotidae, Hl = Hylidae, Hp = Hyperoliidae, Le = Leiopelmatidae, Lp = Leptodactylidae, Ma = Mantellidae, Me = Megophryidae, Mi = Microhylidae, My = Myobatrachidae, Na = Nasikabatrachidae, Pb = Pelobatidae, Pd = Pelodytidae, Pi = Pipidae, Ra = Ranidae, Rh = Rhacophoridae, Rd = Rhinodermatidae, Rp = Rhinophrynidae, So = Sooglossidae, Ab = Ambystomatidae, Ap = Amphiumidae, Cr = Cryptobranchidae, Dc = Dicamptodontidae, Hn = Hynobiidae, Pl = Plethodontidae, Pr = Proteidae, Ry = Rhyacotritonidae, Sa = Salamandridae, Si = Sirenidae.

[a] Ancestral life-history mode.

use an aquatic stage for transmission or are constituted of viruses that contaminate aquatic environments and are readily transmissible from infected host animals (Davidson et al. 2003; Brunner et al. 2004). For example, amphibian chytridiomycosis is caused by the recently described chytrid fungus *Batrachochytrium dendrobatidis*, and the infective stage is an aquatic, flagellated zoospore (Longcore et al. 1999). Viruses of the family Iridoviridae are another major amphibian parasite, and transmission of iridoviruses occurs aggressively through exposure to infected animals or water, resulting from a high rate of aquatic transmissibility (Jancovich et al. 2001). Both the chytrid fungus and the iridoviruses cause high levels of morbidity and are particularly lethal to metamorphosing animals and larvae, respectively (Collins et al. 2003; Brunner et al. 2004). Additionally, endoparasitic helminths that afflict anurans typically require aquatic environments for the development and transmission of infective stages or to reach primary or secondary hosts (Johnson and Sutherland 2003; Brooks et al. 2006). Thus, the increased risk of infection and mortality that aquatic environments pose could select for reduced dependency on aquatic environments, reducing the duration of aquatic exposure in favor of increased terrestriality.

Aquatic-Driven Parasite Dynamics in Amphibian Populations

For many amphibians with generalized biphasic life histories, juvenile and adult animals lead solitary lives dispersed in terrestrial landscapes and may reach high densities only during breeding aggregations at aquatic sites (as with many bufonid, ranid, and hylid frogs). In general, whereas parasites may intermittently afflict populations and cause individual mortalities, parasites cannot become widely established in host populations unless host contact rates are high (Anderson and May 1978, 1979; May and Anderson 1978, 1979; McCallum et al. 2001), a scenario that typically arises during aquatic reproduction and in larval amphibian stages. Daszak et al. (1999) and Brunner et al. (2004) have also determined that the division of an amphibian life history into two distinct stages, aquatic and terrestrial, sustains parasites by creating intraspecific host reservoirs that reinfect naive life-history stages, providing an exception to typical host contact–driven dynamics by allowing parasite persistence even in small populations. Therefore, a biphasic life history with aquatic reproduction promotes increased parasite transmission and can support a persistent host-parasite dynamic, whereas terrestrial life histories that lack aquatic reproduction may not, although parasite dynamics in terrestrial amphibians have received less attention. Iridovirus epidemics have been shown to cause local declines of aquatic-breeding amphibians (Brunner et al. 2004) but have not been reported to cause declines in species with alternative modes of reproduction (see Green et al. 2002 for list of affected species). Clearly, changes from an aquatic reproductive strategy to alternative strategies that liberate animals from aquatic-terrestrial linkages could limit mortality incurred from aquatic parasites. Alternatively, an increase in larval immunity could reduce host mortality and limit parasite dynamics, but this may be unattainable for many species because of the physiological constraints imposed by extensive development of new tissues during metamorphosis (see below).

The Ontogeny of Amphibian Immunity

Amphibians exhibit complex immunity with forms of both innate and adaptive immunity. However, there is a distinct ontogenetic shift in the robustness of their immune defenses (Du Pasquier 1973; Du Pasquier et al. 1987), and metamorphosis is a period of particular immune system vulnerability (reviewed in Rollins-Smith 1998). For example, natural killer (NK) cells provide a method of innate immunity by destroying virus-infected cells and by detecting and destroying tumor cells, but tadpole NK cells are capable of detecting or attacking tumor cells from allogenic animals only weakly, compared to the aggressive response in adults (Horton et al. 1996). There is also growing experimental evidence that many amphibians produce antimicrobial peptides in their dermal mucous that limit infection from environmental contact with pathogens (Chinchar et al. 2001; Rollins-Smith et al. 2002*a*, 2002*b*, 2003). These peptides are composed of 20–46 amino acids, and their effectiveness and potency vary significantly with small substitutions in the amino acid sequence (Carey et al. 1999; Rollins-Smith et al. 2003). Importantly, the ability of many frogs to synthesize these antimicrobial peptides does not appear to develop until after metamorphosis (Clark et al. 1994; Reilly et al. 1994), indicating that the larval stage may be a period of reduced antimicrobial defense. Additionally, major histocompatibility complex (MHC) antigens are normally absent or weak in developing amphibian larvae compared to those of adults (Flajnik et al. 1986, 1987; Salter-Cid et al. 1998). The ontogenetic changes in immunity that amphibians experience is further exemplified by the dramatic loss of lymphocytes that occurs during metamorphic climax and in tadpoles undergoing hormone-induced precocious metamorphosis (Cohen et al. 1985; Flajnik et al. 1987; Rollins-Smith 1998).

Evidence of the ontogenetic change in anuran immunity is widely reported in laboratory studies of the African clawed frog *Xenopus laevis* (e.g., Cohen et al. 1985; Flajnik et al. 1986, 1987), but this pattern has also been shown in studies of the ontogeny of allograft rejection in the bullfrog *Rana catesbeiana* (Hildemann and Haas 1959;

Baculi and Cooper 1970), the northern leopard frog *Rana pipiens* (Bovbjerg 1966), and the European common frog *Rana temporaria* (Plytycz 1981). The diminished immunity of larval and metamorphosing amphibians compared to adult immunity has also been demonstrated among caudates in controlled studies of the ontogeny of allograft rejection in tiger salamanders *Ambystoma tigrinum* (Cohen 1969) and alpine newts *Triturus alpestris* (Plytycz et al. 1982), as well as in ontogenetic comparisons of mitogen activity in the axolotl *Ambystoma mexicanum* (Salvadori and Tournefier 1996), providing additional evidence that the observed pattern is widespread in the Amphibia. Presumably, a strong larval immunity would result in autoimmune attacks on newly developing somatic tissues that form during amphibian metamorphosis, thus necessitating the observed immune suppression that occurs before and during metamorphosis (Cohen et al. 1985; Flajnik et al. 1987).

Numerous field studies confirm that larval and metamorphosing amphibians are particularly susceptible to parasites because parasites often cause greater infection and mortality in these stages than in adults. For example, Berger et al. (1998) found that newly metamorphosed frogs are highly susceptible to chytridiomycosis, and in the Kaibab Plateau, Arizona, 90% of recently metamorphosed salamanders were infected with the *A. tigrinum* virus, or ATV (Collins et al. 2003). Additionally, the analysis of 64 amphibian mortality events in the United States from 1996 to 2001 indicated that iridoviruses and the chytrid fungus preferentially affected late-stage larvae and recently metamorphosed animals, respectively (Green et al. 2002). In another study, researchers experimentally infected *Xenopus* frogs with the iridovirus FV3 and discovered that tadpoles exhibited high susceptibility to the virus, in contrast to adults (Gantress et al. 2003). Infected tadpoles experienced 80% morbidity over 2 months, and the researchers attributed the high larval morbidity to a lack of MHC class I expression as determined by a poor ability to detect viral antigens in the tadpoles.

Evolution of Alternative Reproductive Strategies

Amphibians with exotrophic aquatic larvae are able to exploit high levels of productivity in aquatic systems, which enables rapid growth (Wilbur 1980). However, optimal environments are only those that maximize growth while minimizing mortality (Werner 1986). When the ratio of mortality to growth in the aquatic stage exceeds that of the terrestrial stage, there is selective pressure to shorten the larval period (Callery et al. 2001). Over time, continuous reductions of the larval period have been suggested to lead to direct development (Callery et al. 2001). Alternatively, intraspecific variation in oviposition sites with failures of clutches deposited in parasite-rich aquatic sites may favor the development of alternative oviposition strategies, such as those seen in many mantellid and dendrobatid frogs that use increasingly isolated and terrestrial pools for larval development (table 1). Waterborne parasites and reduced immunity in larval and metamorphosing amphibians provide ample opportunity for escalating mortality in the aquatic stage, which should favor increased terrestriality. Nevertheless, researchers often fail to consider parasites as selective forces capable of contributing to the evolution of the great diversity of reproductive strategies used by amphibians.

Predictions and Suggested Studies

There are several lines of inquiry that can expand our understanding of the role of parasites in the evolution of alternative reproductive strategies in amphibians. For example, it is not known whether direct-developing amphibians that forgo metamorphosis have immunities similar to those of their metamorphic counterparts and simply benefit from an alternative life history that makes them less ecologically susceptible to parasitism, or whether their immunities differ from those of biphasic counterparts (Altig and Crother 2006). Additionally, under the premise that parasites promote increasingly terrestrial life histories, amphibians that have close contact with parasites yet maintain aquatic paedomorphic life histories should have robust MHC I and II expression and competent innate and adaptive immune responses across their ontogeny, in contrast to species with biphasic life histories. Much could be gained from comparative studies of the parasitology and immunology of paedomorphic salamanders such as sirenids, cryptobranchids, and amphiumids (table 1). One might also predict that aquatic anurans, such as adult African clawed frogs *Xenopus* sp., have more robust immune systems than their biphasic counterparts, which live life as terrestrial adults. Interestingly, *Xenopus laevis* is believed to be the source host of the emergent chytrid fungus, and wild *X. laevis* rarely suffer clinical effects from chytrid infection or experience die-offs (Weldon et al. 2004). Comparative immunological studies such as the ones proposed above have not been conducted, leaving incredible opportunities for study.

We must also clarify the phylogenetic distribution and abundance of parasites in relation to the distribution of alternative life histories among amphibians. To this end, there is a critical need to conglomerate reports and distributions of verified parasites in amphibian populations. Investigators should ensure that they examine field-collected study animals when possible or construct a standing arrangement with facilities or researchers who might be willing to assist in such endeavors, such as the USGS National

Wildlife Health Center, San Diego Zoo's Wildlife Disease Laboratory, or the Consortium for Conservation Medicine. Optimally, the discovery of novel parasites or expansions to new host populations should be published and made publicly available. A database of known amphibian parasites and their distributions would aid both conservation scientists concerned about global amphibian declines and biologists interested in ecological and evolutionary questions. Ideally, future work could elaborate on the prevalence and distribution of parasites in closely related species that differ in reproductive modes and life histories, allowing a properly controlled phylogenetic analysis that is not currently possible. Specifically, one may predict that parasite occurrences within phylogenies are greater in species with biphasic life histories than in close relatives that use alternative reproductive modes. Recently, a study by Brooks et al. (2006) demonstrated that parasite richness is greater in host frog species that spend more time in aquatic habitats than in those that do not, a pattern consistent with such predictions.

A final suggested topic for additional study includes the observation of reproductive behaviors and community structure before and after unintentional exposure to new or emergent parasites. The introduced chytrid fungus has spread through Central America, leaving behind a wake of decimated amphibian populations (Lips et al. 2006). In nearly all reports of chytrid-related declines, amphibians with aquatic larvae and those that inhabit riparian habitats have declined. For example, Lips (1999) reported the decline of two riparian frog species, *Eleutherodactylus emcelae* and *Eleutherodactylus rugulosus*, in Las Tablas, Costa Rica, whereas two sympatric congeners with terrestrial egg deposition and direct development remained unaffected (*Eleutherodactylus caryophyllaceus* and *Eleutherodactylus gollmeri*). Such changes in community structure that result from interspecific variation in reproductive strategies and the presence of an aquatic parasite may signal the possible evolutionary outcomes expected for species that exhibit intraspecific variation in reproductive behaviors, with terrestrial or arboreal breeding being favored in the presence of aquatically transmitted parasites. More studies that document changes in community structure or intraspecific reproductive behaviors following the progression of chytrid through Central and South America could add significantly to our understanding of parasites as selective forces.

Conclusions

Evolutionary fitness is determined as the successful production of viable offspring, and any behaviors, morphologies, or physiologies that maximize an individual's production of viable offspring compared to that of its conspecifics should be favored. However, evolution is often a conservative process, and the development of elaborate or costly behaviors will not occur if the current reproductive methods are sufficient for maintaining relative fitness (Lehtinen and Nussbaum 2003). The deposition of a single clutch of numerous small eggs into ponds where aquatic larvae feed and develop is hypothesized to have been the ancestral life-history strategy for amphibians (Duellman and Trueb 1986). The ability to exploit high levels of aquatic productivity through the preservation of aquatic larvae presents a strong counterselective force likely to be responsible for the maintenance of biphasic life histories still common in many amphibians (table 1). Nonetheless, adaptive radiation in amphibians has produced reproductive strategies that are incredibly diverse and phylogenetically widespread (Lehtinen and Nussbaum 2003; Haddad and Prado 2005). The prevalence of parasites in aquatic habitats, the host-parasite dynamics supported by biphasic life histories, and the ontogeny of amphibian immunity collectively indicate that parasites may play a prominent role in amphibian life-history evolution, despite having been largely ignored in previous treatments of amphibian evolution. While it is unlikely that parasites are solely responsible for the evolution of alternative reproductive strategies in amphibians, the role of parasites in amphibian evolution must be considered along with other hypotheses when constructing formal, falsifiable predictions during evaluative studies of amphibian behavior.

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A marsupial frog, *Gastrotheca cornuta*, carries its eggs in a dorsal pouch. Photograph by Scott Connelly, University of Georgia.