Anuran Reproductive Modes: Evolving Perspectives

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Abstract.—Although most of the unique ways that frogs reproduce were described in the 1800s and 1900s, additional modes are still being discovered in the 21st Century. The concept of reproductive mode has evolved from descriptive natural history to an integration of developmental biology, genetics, systematics, evolution, ecology, behavior, and physiology that frames our thinking about the transition of vertebrates from water to land and about anuran reproductive adaptations to diverse environments today. We have classified reproductive modes, examined quantitative parameters, searched for geographical and ecological patterns, and described variability. We have speculated about selective pressures guiding the evolution of terrestrial reproduction and argued about the usefulness of reproductive mode as a character to construct phylogenies. In the past, researchers assumed that the reproductive modes exhibited by living frogs represented stages in an incomplete, linear sequence of steps toward greater independence from open water, with direct development at the end of the spectrum. Newly proposed phylogenies based on molecular data allow us to re-think the evolution of anuran reproductive modes. On another level, we are increasingly realizing the value of incorporating life history information (aquatic larvae or terrestrial development) in setting priorities to formulate more effective and ecologically relevant conservation strategies. The next decade is certain to witness significant advances in our understanding of anuran reproductive modes.

Three-hundred and fifty-million years ago, lobe-finned fishes gave rise to amphibians. Descendants of these early amphibians have radiated into most habitats on Earth. They have acquired physiological, morphological, behavioral, and ecological attributes that have afforded them greater independence from a purely aquatic existence. As part of this transition from water to land, amphibians have evolved the greatest reproductive diversity of all tetrapod vertebrates, ranging from aquatic eggs and larvae to viviparity and direct development (Goin, 1960; Duellman, 1985; Pough et al., 2009).

For the last 46 of these 350 million years, I have been intrigued with "the many ways to beget a frog" (Crump, 1977). It all began in 1968 when, as a newly graduated zoology major, I participated in a herpetofaunal survey, led by Bill Duellman, of the area around Santa Cecilia in the Upper Amazon Basin of Ecuador. Within my first week I had seen 35 species of frogs, including direct-developing *Pristimantis*, poison frogs carrying tadpoles, *Phyllomedusa* eggs hanging over water, and leptodactylid foam nests. I knew I would return to Santa Cecilia someday and learn more about these unusual frogs. Return I did, less than 3 yr later.

In this essay, I will offer my perspective on how the topic of anuran reproductive modes has changed. From Boulenger's (1886) division of anurans into 10 groups based on how they deposit or protect their young (Table 1), to our current recognition of 39 modes of egg deposition and development (Haddad and Prado, 2005), herpetologists have long classified the ways frogs reproduce (Fig. 1). In the late 1960s we began to think about the various forms of frog reproduction as reproductive modes, a multi-faceted combination of ovipositional and developmental factors including oviposition site, ovum and clutch characteristics (e.g., size and number of eggs), rate and duration of development, stage and size of hatchling, and type of parental care, if any (Salthe, 1969). The concept of reproductive modes frames our thinking about the evolutionary transition of vertebrates from water to land and allows us to formulate questions and test predictions to understand how extant anurans breed in semi-terrestrial and terrestrial environments. We have passed from a largely descriptive phase to an integrative phase that uses developmental biology, genetics, systematics, evolution, ecology, behavior, and physiology to interpret and understand reproduction in frogs. Especially exciting areas of current research involve reinterpretation of the evolution of reproductive modes and use of information on reproductive mode in formulating conservation strategies. Another generation of biologists, armed with new technologies, is asking intellectually challenging questions that will provide clearer insights into how and why so many semi-terrestrial, terrestrial, and arboreal modes of reproduction have evolved within the Anura.

Natural History of Anuran Reproduction: The "Early" Years to 1960

Let us begin at the beginning—fertilization. In the 1730s, René-Antoine Ferchault de Réaumur, a member of the Paris Academy of Sciences, wondered how frog eggs were fertilized (Terrall, 2011). He considered that perhaps the male's fingers digging into the female's chest squeeze open an aperture. If there is no aperture, perhaps seminal fluid is transported up the male's body to his thumbs and absorbed into the female's skin. Or, perhaps sperm exuded from the male's chest glands fertilize the eggs. Réaumur dressed male frogs in pants made of waxed taffeta, secured with suspenders, to seal off their hind ends. Although two females deposited eggs while mating with males that kept their pants on, the results were inconclusive and Réaumur could not determine if the pants had prevented fertilization. He thought about putting taffeta gloves on males' hands, but eventually lost interest in frog sex, frustrated that he could never "see" fertilization. Thirty years later, Italian naturalist Lazzaro Spallanzani revisited the question. In one experiment, he repeated Réaumur's protocol of outfitting males in pants. Spallanzani's experiments were successful, and in 1768 he reported that frogs have external fertilization (Terrall, 2011). I begin with this story to point out that researchers of frog reproduction have long combined observations with experiments.

Imagine being a biologist a century ago. You observe something never seen before and have little or no context for interpretation. Your first thought: *That can't be! No frog does that!* This combination of amazement and skepticism must have been

TABLE 1. Boulenger (1886) classification system of anuran reproductive modes. I have included one of Boulenger's examples, using his taxonomy, for each mode.

I. Ovum small, larva hatches in comparatively early embryonic condition
  A. Eggs deposited in water ("Probably the majority of Batrachians")
  B. Eggs deposited out of water
     1. In holes on banks of pools; tadpoles washed into pool after heavy rain (*Leptodactylus ocellatus*)
     2. On leaves above water; tadpoles fall into water below (*Chiromantis rufescens*)
II. Ovum large, young undergoes all or part of metamorphosis within egg
  A. Eggs deposited in damp situations or on leaves; young leaves egg in the "perfect" air-breathing form (*Rana opisthodon*)
  B. Eggs carried by parent
     1. By male
        a. Around the legs (*Alytes*)
        b. In the vocal sac (*Rhinoderma*)
     2. By female
        a. Attached to the belly (*Rhacophorus reticulatus*)
        b. Attached to the back (*Pipa*)
        c. In a dorsal pouch
           (1) Young leave pouch in tadpole state (*Nototrema marsupiatum*)
           (2) Young leave pouch in "perfect" state (*Nototrema oviferum*)

experienced by the biologists during the 1700s to early 1900s who discovered female *Pipa pipa* carrying eggs embedded in their backs (Fermin, 1765), male *Rhinoderma darwinii* (Darwin's Frog) brooding tadpoles in their vocal sacs (Jiménez de la Espada, 1872), amplectant *Phyllomedusa iheringii* laying and fertilizing eggs on leaves above water (Ihering, 1886), adult dendrobatids transporting tadpoles on their backs (Smith, 1887; Boulenger, 1895), female *Fritziana goeldi* carrying eggs in dorsal pouches (Boulenger, 1895; Goeldi, 1895), and viviparity in *Nectophrynoides tornieri* (Krefft, 1911).

As I think back on my first reproductive mode-related discovery, I can relate to the amazement and skepticism those biologists must have felt. In 1971, while a student in an Organization for Tropical Studies course in Costa Rica, I watched a female Granular Poison Frog, *Oophaga granulifera*, approach a calling male. Eventually the pair hopped into a partially curled-up leaf. The female rubbed her head against the male's head, then turned and stuck her rear end in his face. After five repetitions of this sequence, the male turned and touched his rear end to hers. While in this vent-to-vent position, she laid three eggs. The male wriggled, presumably releasing sperm onto the eggs. Both frogs sat next to the eggs. After 4 min, the male hopped away. Thirty minutes later, the female left. I could hardly wait to share my observation with the course coordinator, Roy McDiarmid. Were the eggs really fertilized without amplexus? I scooped the eggs into a plastic bag and took them to the field laboratory. Later examination under a microscope revealed fertilized eggs (Crump, 1972). I learned early that the thrill of discovery fuels a scientist's passion.

But I digress. Let us return to the earlier natural history of anuran reproduction. Sampson (1900) reviewed the unusual anuran modes of breeding reported until that time and wrote that she did so as a "service" to fellow amphibian biologists because she found these accounts to be fragmentary and widely scattered. I suspect another reason was that she was awed by
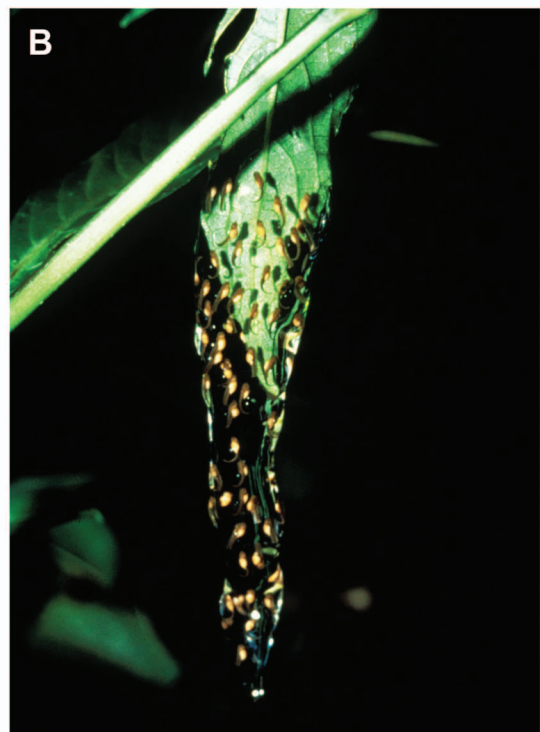

FIG. 1. Eggs of representatives of the three main groups of reproductive modes: A = *Dermatonotus muelleri* (eggs deposited in water, aquatic larvae); B = *Dendropsophus bokermanni* (eggs deposited out of water, aquatic larvae); C = *Craugastor* sp. (eggs deposited out of water, no aquatic larval stage—in *Craugastor*, direct development). Photos by Martha Crump.

the amazing reproductive behaviors of frogs and wanted to share them with the broader scientific community.

Between 1900 and 1960, in addition to describing unique reproductive behaviors, biologists addressed the evolutionary relationships of anuran life histories. Noble (1925, 1927, 1931) pointed out that in many groups of genera in which morphological characteristics suggest close relationships, "mode of life history" (roughly what we now refer to as reproductive mode) is the same. Lutz (1947, 1948) reviewed anuran trends toward non-aquatic and direct development and suggested that increased yolk reserve was the most-important morphological change in the progression toward greater terrestriality. Orton (1949, 1951) noted that direct development occurs in 10 of the 13 families of frogs then considered taxonomically valid. Jameson (1955) pointed out that there were (at that time) too many gaps in our knowledge to trace a complete phylogenetic sequence of courtship and mating behavior, but he noted that the various stages of terrestrial reproduction occur independently of phyletic lineages. Goin (1960) wrote that although previous researchers had addressed morphological and ecological adaptations of vertebrates' invasion of land, not much had been said about development of "reproductive devices." He reiterated Jameson's observation (contrary to Noble) of the lack of correlation between amphibian life histories and their evolutionary relationships.

Quantitative Analyses, Reviews, and Categorization of Reproductive Modes

Observations of frog life histories up to the early 1960s provided background for quantitative analysis of reproductive patterns, preceded by work with salamanders (Salthe 1969). Salthe and Duellman (1973) examined relationships between quantitative parameters including female body size, egg size, and clutch size (number of eggs) within, between, and among reproductive modes. Their conclusions have been tested and generally supported for many species and larger clades. Salthe and Duellman (1973) hypothesized that selection favors increased clutch size in larger species of frogs and increased ovum size in smaller species. Because only small to mid-sized species seem to evolve fully terrestrial reproductive modes, they suggested that small body size in frogs is a "preadaptation for reproductive experimentation."

In the 1970s and 1980s, reviews and syntheses of descriptive and quantitative aspects of reproductive behavior provided a framework for interpreting anuran reproductive modes. Salthe and Mecham (1974) summarized numerous aspects of reproduction in amphibians, including adaptations that allow reproduction away from open water. Lamotte and Lescure (1977) reviewed anuran reproductive modes, McDiarmid (1978) reviewed anuran parental care, and Wake (1982) discussed evolution of viviparity in amphibians. Duellman (1985) recognized 29 reproductive modes in frogs. Elinson (1987) reviewed changes that have occurred in anuran embryonic developmental patterns, reflecting the shift from aquatic to non-aquatic eggs.

Altig and Johnston (1989) divided anuran larvae into two developmental modes based on how they obtain nutrients. Exotrophic ("feeding") tadpoles obtain developmental energy from the environment. Endotrophic ("non-feeding") tadpoles or froglets obtain developmental energy entirely from their own yolk or from other material produced by a parent. The authors identified six developmental guilds of endotrophs: (1) vivipa-

rous, (2) ovoviviparous, (3) direct developing, (4) paraviviparous, (5) exoviviparous, and (6) nidicolous. A decade later, Thibaudeau and Altig (1999) reviewed endotrophic tadpoles and provided a detailed synthesis of development in these forms and discussed evolution of endotrophy.

Altig and McDiarmid (2007) developed a generalized framework of amphibian eggs and ovipositional modes. They defined ovipositional mode as clutch structure, including morphological diversity and arrangement of deposited eggs, and they recognized five morphological classes (14 modes). The authors called for additional research on various aspects of amphibian ovipositional patterns including egg-laying behavior, clutch morphology, and ovum characteristics.

Classification systems are human constructs and, as such, any classification of anuran reproductive modes is subjective. Some modes, such as oviposition in still water, encompass a wide range of microhabitats that differ in depth, temperature, and permanence. Other modes, such as tadpoles brooded in the male's vocal sac or eggs embedded in the dorsum of the aquatic female, are represented by one or only a few species. Whereas some modes differ greatly from all others, some overlap widely in one or more aspects. Curiously, although type of parental care, if any, is one component of reproductive mode, it is not used to differentiate modes. For example, in the currently accepted system of 39 modes (Haddad and Prado, 2005), species in Mode 20 have eggs that hatch into exotrophic tadpoles carried to water by either the male or female parent, and species in Mode 23 have direct development of terrestrial eggs, either with or without parental attendance. Wells (2007) pointed out that if we were to fine-tune differentiation among types of oviposition sites, larval development, and parental care, we could easily devise a classification system that would include 50 or 60 distinct reproductive modes.

Despite subjectivity in the classification of reproductive modes, the current system (Haddad and Prado, 2005) frames our thinking about questions such as: Does diversity of reproductive modes influence assemblage-level interactions and patterns? Can we identify geographical and ecological patterns of reproductive modes related to environmental variables? What selective pressures favored the evolution of non-aquatic reproductive modes? How much variability in reproductive parameters do species exhibit, and how does variation affect fitness? Attempts to answer these questions, and others, have taken the study of anuran reproductive modes to new levels.

Assemblage Analyses

The 1960s and 1970s witnessed a proliferation of hypotheses and predictions relating to species diversity, resource partitioning, and community structure and organization. As a graduate student in the early 1970s, I jumped on the bandwagon, but I also seized my chance to combine theoretical ecology with fieldwork at Santa Cecilia by focusing on anuran reproductive modes. At the time, Santa Cecilia held the record for highest anuran species richness anywhere in the world—81 species in a 3-km^2 area. I wondered if diversity of reproductive modes might facilitate coexistence through partitioning of breeding sites. During my year of fieldwork, I identified 10 reproductive modes within the assemblage (Table 2). Thirty-four species lay exposed eggs in open water. Six species produce foam nests in or near water. Two species lay eggs in basins or tree cavities. Nineteen species deposit eggs out of water but have aquatic

TABLE 2. Reproductive modes of 78 of the 81 species of frogs at Santa Cecilia, Ecuador (Crump, 1974). At the time, the mode of reproduction was unknown for the remaining three species—*Edalorhina perezi*, *Oreobates quixensis*, and *Lithodytes lineatus*. We now know that *E. perezi* and *L. lineatus* produce foam nests; *O. quixensis* deposits terrestrial, direct-developing eggs.

Mode	Description	N spp.	Example
1	Eggs deposited in open water, tadpoles develop in open water	34	*Rhinella marina*
2	Eggs deposited in tree cavities, tadpoles develop therein	1	*Nyctimantis rugiceps*
3	Eggs deposited in constructed basin, tadpoles develop therein	1	*Hypsiboas boans*
4	Eggs deposited on vegetation above water, tadpoles develop in water	14	*Dendropsophus leucophyllatus*
5	Eggs suspended in foam nest on or near water, tadpoles develop in water	6	*Leptodactylus pentadactylus*
6	Eggs deposited terrestrially, larvae carried to water on dorsum of adult	5	*Ameerega parvula*
7	Eggs deposited in foam nest on land, tadpoles develop within foam nest	1	*Adenomera andreae*
8	Eggs deposited terrestrially, direct development	14	*Pristimantis variabilis*
9	Eggs and young buried in skin on dorsum of aquatic female; direct development	1	*Pipa pipa*
10	Eggs and young attached to dorsum of terrestrial female; direct development	1	*Hemiphractus proboscideus*

larvae. By the very nature of the diverse oviposition sites associated with the range of reproductive modes, partitioning of aquatic breeding sites facilitates coexistence of this species-rich assemblage (Crump, 1974). The diverse breeding phenologies of species, from opportunistic to continuous, allow temporal partitioning of aquatic breeding sites (Crump, 1974). Seventeen species neither deposit eggs in water nor have aquatic larvae; thus, they do not overlap in oviposition or larval developmental sites (or both) with the other 61 species. (At the time, the reproductive mode of three species was unknown. We now know that *Oreobates quixensis* has direct development, and both *Edalorhina perezi* and *Lithodytes lineatus* produce foam nests.) I also measured reproductive parameters to test predictions from Salthe and Duellman (1973). Among other conclusions, hylids that deposit eggs in water had a significantly higher ovarian size factor [clutch size (mean ovum diameter)/snout–vent length] (Duellman and Crump, 1974) than did hylids that deposit eggs on vegetation above water. Clutch size, time until hatching, and hatchling size did not differ significantly between species that deposit eggs in temporary vs. permanent bodies of water.

Several other studies have focused on the assemblage level, using reproductive modes as a framework. Working in rain forest near Manaus, Brazil, Magnusson and Hero (1991) examined desiccation, predation, competition, and water quality as possible selective pressures maintaining and leading to the evolution of terrestrial oviposition. Donnelly and Guyer (1994) described patterns of reproduction and habitat use within an assemblage of pond-breeding frogs at La Selva, Costa Rica. Perotti (1997) examined quantitative reproductive variables of an anuran assemblage from Chaco habitat of northern Argentina, and Hartmann et al. (2010) analyzed body size–fecundity relationships for anurans representing 13 modes of reproduction at a site in the Atlantic rain forest of Brazil.

During a sabbatical in northern Argentina, I used reproductive modes as a framework to examine the coexistence of predaceous and prey tadpoles at a site near Joaquin V. Gonzalez (Salta Province) in semi-arid Chaco habitat. I briefly summarize some results of this unpublished study in the hope of stimulating interest in using reproductive modes to frame additional questions relating to anuran assemblages.

My field site in northern Argentina experiences pronounced seasonality, with more than 75% of annual rain occurring between November and March. After this rainy period, ponds dry and little precipitation occurs until the following November. Frogs at the site breed only during the rainy season. The anuran assemblage includes several species with predaceous tadpoles that eat other tadpoles (Cei, 1980). How do frogs with prey tadpoles coexist with species that have predaceous tadpoles? I addressed five predictions: (1) prey species time breeding to minimize overlap with predator species, (2) prey species choose oviposition sites lacking predator tadpoles, (3) prey species are toxic or distasteful to predator tadpoles, (4) prey species have large clutch sizes relative to predator species, and (5) reproductive modes of prey species involve protection of early, vulnerable stages.

In 1989, rains began in mid-November. Eleven species of frogs reproduced during the rainy season. I surveyed occurrence and relative abundance of tadpoles of each species in each of five breeding ponds weekly for 6 wk, from 6 December 1989 to 15 January 1990 (seven sampling periods). The ponds were located on both sides of a 200-m stretch of dirt road, all within 20 m of the road edge. Ponds A, B, and C held water throughout the study period ("permanent"); ponds D and E each dried and re-filled at least twice during the 6 wk ("temporary") (Tables 3 and 4).

Three of the 11 species—*Ceratophrys cranwelli*, *Lepidobatrachus laevis*, and *Lepidobatrachus llanensis*—have predaceous tadpoles that prey on anuran eggs and tadpoles (Table 3). *Lepidobatrachus laevis* laid eggs only once at the study site, following the first heavy rain in November, and only at the largest permanent pond (A) (Tables 3 and 4); the tadpoles metamorphosed by the third sampling week. Most oviposition in *C. cranwelli* and *L. llanensis* occurred early in the breeding season; *C. cranwelli* oviposited at least twice and *L. llanensis* at least four times. *Lepidobatrachus llanensis* laid eggs in all ponds except for the medium-sized permanent pond (B); *C. cranwelli* oviposited only in the large and small permanent ponds (A, C) (Table 4). Of the five ponds, only B was not used by a predator species. Although B was the only pond where I found turtles and snakes, both of which eat tadpoles, and it was frequented most often by goats, cattle, and pigs, seven of the eight species with prey tadpoles laid eggs in this pond (Table 4).

One might expect strong selection for prey species to time breeding to minimize overlap with predator species or to choose oviposition sites lacking predaceous tadpoles (or both). Neither behavior was possible, however, because the three predaceous species were the first to oviposit. By the time prey species laid eggs, tadpoles of predaceous species were large enough to eat prey tadpoles. On each night following a hard rain, both species with predator and prey tadpoles laid eggs in the same ponds.

Contrary to my prediction, prey tadpoles were not toxic or unpalatable. Feeding experiments and analysis of intestinal contents revealed that predaceous tadpoles readily consumed all eight species of prey tadpoles. Bufonid tadpoles were the most abundant prey item in the intestines of wild-caught

TABLE 3. Anuran assemblage at study site near Joaquin V. Gonzalez, northern Argentina. All 11 species have tadpoles that develop in the water. Temp = temporary; perm = permanent. Clutch size: 1 = 501–1,000 eggs; 2 = 1,001–1,500; 3 = 1,501–2,000; 4 = 2,001–7,000; 5 = >7,000.

Species	Oviposition	Breeding sites[a]	Breeding season	Clutch size	Tadpoles
Bufonidae					
Rhinella granulosa	Eggs in strings in water	Temp and perm	Early only	5	Prey
Rhinella schneideri	Eggs in strings in water	Temp and perm	Throughout	5	Prey
Hylidae					
Phyllomedusa sauvagii	Eggs on vegetation over water	Perm only	Throughout (mostly late)	1	Prey
Scinax x-signatus	Eggs in clumps in water	Temp and perm	Mid to late	3	Prey
Leptodactylidae					
Ceratophrys cranwelli	Eggs in water, deposited singly, on substrate	Perm only	Early to mid (mostly early)	4	Predator
Lepidobatrachus laevis	Eggs in water, deposited singly, on substrate	Perm only	Early only	2	Predator
Lepidobatrachus llanensis	Eggs in water, deposited singly, on substrate	Temp and perm (mostly temp)	Throughout (mostly early)	1	Predator
Leptodactylus bufonius	Foam nest within mud nest on land, next to depression	Temp and perm	Throughout	1	Prey
Physalaemus biligonigerus	Foam nest on water surface	Temp and perm	Throughout	5	Prey
Pleurodema tucumanum	Foam nest on water surface	Perm only	Throughout	2	Prey
Microhylidae					
Dermatonotus muelleri	Eggs in water, surface film	Temp and perm	Throughout	5	Prey

[a] The permanent sites held water throughout the study period but dried after the rainy season ended; the temporary sites dried and re-filled during the study period.

predator tadpoles and were preferred in experiments, perhaps because they were more active than other tadpoles offered.

My results suggest that two aspects of reproductive mode might facilitate coexistence of prey and predator species. First, half of the prey species have large clutch sizes relative to the predator species (Table 3). Both species of *Lepidobatrachus* deposit fewer than 1,500 eggs, and *C. cranwelli* deposits fewer than 4,000 eggs. In contrast, four prey species deposit over 7,000 eggs (both *Rhinella schneideri* and *Dermatonotus muelleri* deposit over 10,000 eggs). The sudden appearance of many thousands of eggs and later prey tadpoles might overwhelm consumptive capacity of predaceous tadpoles.

Second, oviposition characteristics of four species protect the egg stage from predator tadpoles, which eat frog eggs. *Phyllomedusa sauvagii* deposits eggs on vegetation above water, and advanced tadpoles drop into the water where they complete development. *Leptodactylus bufonius* constructs mud nests at pond edges and produces a foam nest within the mud nest. Once nests flood, tadpoles are washed into the newly formed pond. *Physalaemus biligonigerus* and *Pleurodema tucumanum* produce foam nests on the water surface (Table 3). Presumably the foam deters predator tadpoles. *Physalaemus*

TABLE 4. Assemblage composition at the five breeding ponds. The number is the total number of species. The species identifications, in parentheses, are: a = *Lepidobatrachus laevis*; b = *Lepidobatrachus llanensis*; c = *Ceratophrys cranwelli*; d = *Rhinella granulosa*; e = *Rhinella schneideri*; f = *Leptodactylus bufonius*; g = *Scinax x-signatus*; h = *Pleurodema tucumanum*; i = *Physalaemus biligonigerus*; j = *Phyllomedusa sauvagii*; k = *Dermatonotus muelleri*.

Pond	N Predator species	N Prey species
A. Large permanent pond	3 (a, b, c)	8 (d, e, f, g, h, i, j, k)
B. Medium permanent pond	0	7 (d, e, f, h, i, j, k)
C. Small permanent pond	2 (b, c)	4 (d, h, j, k)
D. Temporary pond	1 (b)	4 (e, f, i, k)
E. Temporary water-filled ditch pond	1 (b)	5 (e, f, g, i, k)

biligonigerus is also one of four species with a large clutch size (>7,000 eggs).

Scinax x-signatus is the only prey species that has neither large clutch size nor a reproductive mode that protects the egg stage. Of the eight prey species, *S. x-signatus* was the least common in my samples. I found few breeding adults or tadpoles in the five ponds, and few tadpoles were consumed by predaceous tadpoles.

As I worked with this assemblage of frogs in semiarid Chaco habitat, so different from the species at Santa Cecilia, I often thought about how reproductive modes reflect environmental conditions such as temperature, humidity, rainfall, physiognomy of the vegetation, and topography. Following are a few studies that have compared reproductive modes between and among geographical regions.

REGIONAL COMPARISONS OF REPRODUCTIVE MODES

Duellman (1985) noted that reproductive modes involving aquatic eggs and tadpoles are the only ones found in all major environments where frogs occur. Modes involving exposed terrestrial and arboreal eggs are restricted to humid environments. Many intermediate modes, such as foam nests, are found in dry areas or seasonally wet environments with high temperatures and fluctuating water levels (Fig. 2).

Hödl (1990) compared reproductive modes of 130 Amazonian lowland frog species from Belém (Brazil), Manaus (Brazil), Panguana (Peru), and Santa Cecilia (Ecuador). More than 56% of species from the wetter sites (Santa Cecilia and Panguana) deposit eggs out of water as compared to less than 44% of species from the sites with pronounced dry seasons (Manaus and Belém). Percentages of species with semi-terrestrial modes are similar within assemblages throughout the Amazonian lowlands, but the proportion of specific reproductive modes differs. Humidity-sensitive dendrobatids and leaf-breeding frogs make up 66% of the semi-terrestrial species in the Upper Amazon Basin; the more dry-resistant foam-nesting breeders represent 50% of the semi-terrestrial species at Belém and 48% at Manaus—a difference predicted by differences in environmental humidity.
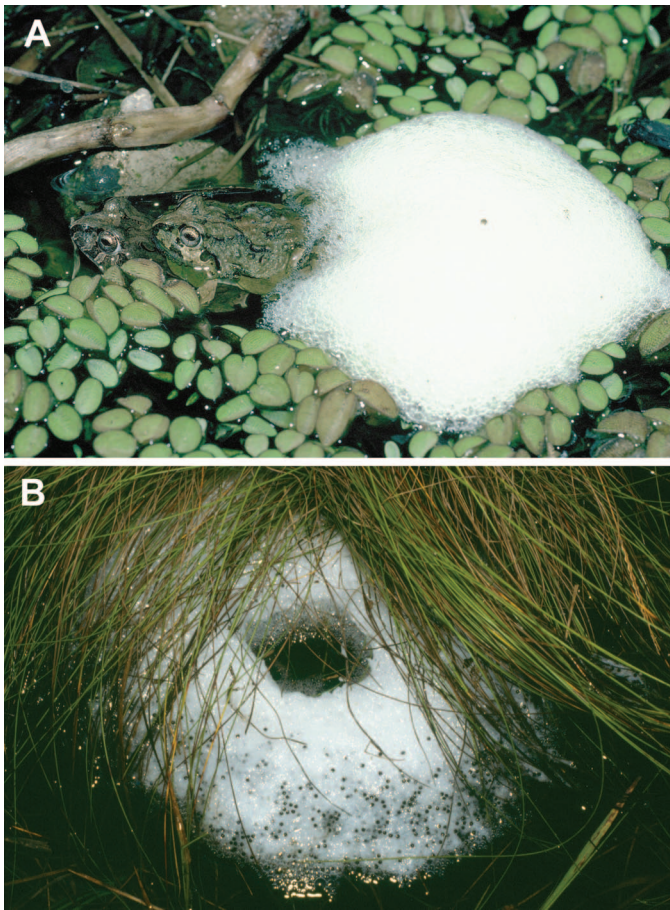
FIG. 2.   Nests of two foam nest-breeding species: A = *Pleurodema borelli* (Rufous Four-eyed Frog; eggs abandoned after deposition); B = *Leptodactylus latrans* (Criolla Frog; female stays in center of foam nest and guards clutch against potential predators). Photo A by Martha Crump; B by Célio Haddad.

Haddad and Prado (2005) compared number of species, number of reproductive modes, ratio of number of reproductive modes to number of species, and number of species depositing eggs out of water for nine Neotropical sites in Brazilian Atlantic and Amazonian forests. Of 39 modes recognized by Haddad and Prado worldwide (Table 5), 31 have been recorded in these Neotropical sites; 27 of those 31 are exhibited by Brazilian Atlantic forest species. Although Amazonian forest has five times the area of Atlantic forest and more species of frogs, only 22 modes have been reported within the Amazonian anuran fauna. The assemblages from the Upper Amazon Basin (Panguana and Santa Cecilia) have more species that deposit terrestrial or arboreal eggs compared to Atlantic forest assemblages. Of the five sites from the Atlantic forest, Boracéia has the highest number of species: 68, represented by 16 reproductive modes; number reproductive modes/number species = 0.24. In contrast, of the four Amazonian forest sites, Parque Nacional da Serra do Divisor has the most species: 124, represented by 12 reproductive modes; number reproductive modes/number species = 0.10. Haddad and Prado (2005) concluded that high diversity of reproductive modes in the Atlantic forest reflects radiation into the complex topography and diverse humid microhabitats of the local environment more than it does phylogenetic relationships of families.

da Silva et al. (2012) analyzed 27 sites in Brazilian Atlantic forest to evaluate effects of humidity on the number of reproductive modes exhibited by the respective anuran fauna. They hypothesized that sites with high humidity levels would support more species and more reproductive modes than would drier sites. The data supported their hypothesis. da Silva et al. (2012) concluded: "Given that reproductive modes are associated with susceptibility to desiccation, their incorporation into explanatory models may trigger a significant advance in the understanding of the mechanisms regulating the species richness and composition of amphibian communities."

Gomez-Mestre et al. (2012) likewise found a strong association between climatic variables and anuran reproductive modes. Although there are exceptions, terrestrial breeders (direct-developing species in particular) generally occur in areas with higher annual rainfall and warmer mean temperatures compared to species with aquatic larvae. The authors noted: "Intriguingly, the same adaptive features that allowed direct developing embryos independence from large water bodies may have largely constrained them to moist tropical and subtropical areas with high humidity."

VARIABILITY ASSOCIATED WITH REPRODUCTIVE MODES

The longer we study reproductive modes, the more we realize that many components, including egg size, growth and developmental rates, hatching time, and parental care, are highly variable. Such variability makes sense given diverse and changing environmental conditions. Throughout my research concerning anuran reproductive modes I have gravitated to questions involving variability. What is the advantage of a large vs. a small egg; of metamorphosing quickly but starting terrestrial life small; of ingesting food rather than simply absorbing yolk; of selecting oviposition sites free of predators or competitors?

I carried out much of my research on reproductive mode variability while caring for my own two altricial young (ages 6 wk to 6 yr) during the 1980s. Experiments with Meadow Treefrog (*Isthmohyla pseudopuma*) tadpoles housed in cups and bowls in my spare bedroom in Costa Rica allowed me to involve the kids in my work and share with them the "magic" of metamorphosis. I designed one experiment after we had watched Meadow Treefrog tadpoles flopping about in desiccating puddles. I wondered if the tadpoles can speed development as their habitat dries. After a false start when my 18-mo-old son evened out the water levels in tadpole-occupied containers with his juice cup, experimental results revealed that tadpoles exposed to a rapidly evaporating environment developed significantly faster than siblings exposed to a constant high or low water level (Crump, 1989). Tadpoles of other species can speed development under deteriorating conditions. Spadefoot toad tadpoles (*Scaphiopus*, *Spea*) are exceptional in this regard, able to reduce the length of the larval period by as much as 50% in response to drying conditions (Newman, 1988; Denver et al., 1998).

Many studies have documented egg size variability among and within populations and within individual clutches (e.g., Crump and Kaplan 1979; Crump, 1981, 1984; Kaplan, 1989; Williamson and Bull, 1989, 1995; Kaplan and King, 1997; Dzimnski and Alford, 2005). A range of egg sizes within a clutch may be advantageous for many reasons. In a favorable environment, small eggs might survive; if so, a female could increase her fitness by producing many small eggs (Crump, 1981). In an unpredictable environment, however, a female might "hedge her bets" if a range of egg sizes increases the

TABLE 5. Anuran modes of reproduction (after Haddad and Prado 2005, which was modified from Duellman and Trueb, 1986). Representative examples are indicated in parentheses.

Aquatic eggs
  Eggs deposited in water
    Mode 1: Eggs and exotrophic tadpoles in lentic water (*Lithobates*)
    Mode 2: Eggs and exotrophic tadpoles in lotic water (*Atelopus*)
    Mode 3: Eggs and early larval stages in constructed subaquatic chambers; exotrophic tadpoles in streams (*Crossodactylus*)
    Mode 4: Eggs and early larval stages in natural or constructed basins; after flooding, exotrophic tadpoles in ponds or steams (some *Hypsiboas*)
    Mode 5: Eggs and early larval stages in subterranean constructed nests; after flooding, exotrophic tadpoles in ponds or streams (*Aplastodiscus leucopygius*)
    Mode 6: Eggs and exotrophic tadpoles in water in tree holes or aerial plants (*Phyllodytes*)
    Mode 7: Eggs and endotrophic tadpoles in water-filled depressions (*Eupsophus roseus*)
    Mode 8: Eggs and endotrophic tadpoles in tree holes or aerial plants (*Dendrophryniscus*)
    Mode 9: Eggs deposited in lotic water, then swallowed by female; egg and larval development in female's stomach (*Rheobatrachus*)
  Eggs in bubble nest
    Mode 10: Bubble nest floating on pond; exotrophic tadpoles in pond (*Chiasmocleis leucosticta*)
  Eggs in foam nest (aquatic)
    Mode 11: Foam nest floating on pond; exotrophic tadpoles in pond (*Physalaemus*)
    Mode 12: Foam nest floating on still water; exotrophic tadpoles in still or slowly-moving water (*Limnodynastes interioris*)
    Mode 13: Foam nest floating on water accumulated in constructed basins; exotrophic tadpoles in ponds (*Leptodactylus podicipinus*)
    Mode 14: Foam nest floating on water accumulated on axils of terrestrial bromeliads; exotrophic tadpoles in ponds (*Physalaemus spiniger*)
  Eggs embedded in dorsum of aquatic female
    Mode 15: Eggs hatch into exotrophic tadpoles (*Pipa carvalhoi*)
    Mode 16: Eggs hatch into froglets (*Pipa pipa*)
Terrestrial or arboreal eggs (not in water)
  Eggs on ground, on rocks, or in burrows
    Mode 17: Eggs and early larval stages in excavated nests; after flooding, exotrophic tadpoles get washed into ponds or streams (*Pseudophryne*)
    Mode 18: Eggs on ground or rock above water; exotrophic tadpoles move to water (*Phrynomedusa appendiculata*)
    Mode 19: Eggs on humid rocks, in rock crevices, or on tree roots above water; exotrophic tadpoles living on rocks in water film or in water-land interface (some *Cycloramphus*)
    Mode 20: Eggs hatching into exotrophic tadpoles carried to water by parent (*Allobates*)
    Mode 21: Eggs hatching into endotrophic tadpoles that complete development in nest (*Zachaenus parvulus*)
    Mode 22: Eggs hatching into endotrophic tadpoles that complete development on dorsum or in pouches of adult (*Rhinoderma darwinii*)
    Mode 23: Direct development of terrestrial eggs (*Pristimantis*)
  Arboreal eggs
    Mode 24: Eggs hatching into exotrophic tadpoles that drop into still water (*Phyllomedusa*)
    Mode 25: Eggs hatching into exotrophic tadpoles that drop into running water (*Hyalinobatrachium*)
    Mode 26: Eggs hatching into exotrophic tadpoles that develop in water-filled cavities in trees (*Nyctimantis rugiceps*)
    Mode 27: Eggs hatching into froglets (*Ischnocnema nasuta*)
  Eggs in foam nest (terrestrial or arboreal)
    Mode 28: Foam nest on forest floor; after flooding, exotrophic tadpoles get washed into ponds (members of *Physalaemus signifer* group)
    Mode 29: Foam nest with eggs and early larval stages in open basins; after flooding, exotrophic tadpoles get washed into ponds or streams (some *Leptodactylus*)
    Mode 30: Foam nest with eggs and early larval stages in subterranean constructed nests; after flooding, exotrophic tadpoles get washed into ponds (*Leptodactylus fuscus*)
    Mode 31: Foam nest with eggs and early larval stages in subterranean constructed nests; after flooding, exotrophic tadpoles get washed into streams (*Leptodactylus cunicularius*)
    Mode 32: Foam nest in subterranean constructed chambers; endotrophic tadpoles in chamber (some *Adenomera*)
    Mode 33: Arboreal foam nest; tadpoles drop into still or running water (*Rhacophorus*)
  Eggs carried by adult
    Mode 34: Eggs carried on legs of male; exotrophic tadpoles in ponds (*Alytes*)
    Mode 35: Eggs carried in dorsal pouch of female; exotrophic tadpoles in ponds (some *Gastrotheca*)
    Mode 36: Eggs carried on dorsum or in dorsal pouch of female; endotrophic tadpoles in bromeliads or bamboo cavities (*Flectonotus*)
    Mode 37: Eggs carried on dorsum or in dorsal pouch of female; direct development into froglets (*Hemiphractus*)
  Eggs retained in oviducts
    Mode 38: Ovoviviparity (nutrition provided by yolk) (*Eleutherodactylus jasperi*)
    Mode 39: Viviparity (nutrition provided by oviductal secretions) (*Nimbaphrynoides occidentalis*)

chance that some offspring will survive (Crump, 1981). Because larger eggs result in larger hatchlings, these offspring might be better competitors and able to leave the pond earlier than could hatchlings from smaller eggs. Thus, larger eggs might have an advantage in unpredictable environments. The disadvantage of producing all large eggs is a reduced clutch size. A range of egg sizes might be optimal. Long-term field and laboratory studies of *Bombina orientalis* reveal that when less food is available, females produce smaller eggs (Kaplan, 1987; Kaplan and King, 1997). Researchers continue to examine and interpret egg size variability (e.g., Yeager and Gibbons, 2013).

Fig. 3.   Male *Hypsiboas faber* (Blacksmith Treefrog) guarding eggs floating on water surface in constructed nest. Photo by Célio Haddad.

Parental care occurs in at least 10% of all anuran species (Crump, 1995, 1996; Lehtinen and Nussbaum, 2003). Like other aspects of reproductive mode, study of parental care has expanded from descriptive natural history to a focus on broader questions. The more we observe frogs in their natural environments, the more we document variability in timing, extent, and specific behaviors involved in parental care. For example, many Neotropical centrolenids care for their eggs deposited on vegetation above water (McDiarmid, 1978). Egg attendance by male *Hyalinobatrachium fleischmanni* reduces embryo dehydration (Hayes, 1991), and recent observations from Oaxaca, Mexico, reveal that males exhibit higher levels of attendance in drier years than in wetter years (Delia et al., 2013). Parental care to prevent desiccation is so critical in this species that embryos hatch earlier than normal if males abandon their clutches (Delia et al., 2014). Another aspect of variability is reflected in parental care behavior of *Hyalinobatrachium orientale* on Tobago, West Indies. Egg attendance by male *H. orientale* significantly increases offspring survivorship during both wet and dry seasons but benefits vary with season (Lehtinen et al., 2014). During the wet season, paternal care decreases risk of predation by arthropods, whereas during the dry season egg attendance decreases risk of desiccation.

We used to assume that a given species of frog exhibited one reproductive mode. The more we study frogs, however, the more we document that some species exhibit alternative reproductive modes depending on environmental conditions. In Costa Rica, some *Smilisca sordida* bury eggs beneath the substrate of basins they construct; others construct open basins and deposit eggs floating on the surface or attached to rocks at the bottom of the basin (Malone, 2004). Basin construction is facultative, however, as some pairs deposit eggs in streams or attached to vegetation in streams. *Dendropsophus ebraccatus* deposits eggs on vegetation above water or in water, either on the surface or submerged (Touchon and Warkentin, 2008). Light level, temperature, and humidity likely influence whether eggs are deposited in or out of water (Touchon and Warkentin, 2008). This plasticity seems to be maintained by the balance of mortality risks: desiccation of non-aquatic eggs and high predation pressure on aquatic eggs (Touchon, 2012).

Likewise, some species of *Hypsiboas* are flexible in oviposition behavior. In Rondônia, Brazil, male *Hypsiboas boans* construct nests in sand along small river tributaries, whereas in granitic rock outcrops pairs deposit eggs in small, semi-isolated pools among the rocks or in dense leaf litter (Caldwell, 1992). In Guatopo National Park, Venezuela, female *Hypsiboas crepitans* oviposit in constructed nests and in small pools along the stream edge (Caldwell, 1992). In the Atlantic forest of Brazil, female *Hypsiboas faber* typically deposit eggs as a surface film in water-filled nests constructed by males (Fig. 3). When the water level rises high enough that males cannot construct nests, females lay eggs as a surface film in ponds (Martins, 1993). Höbel (1999) reported facultative nest construction in *Hypsiboas rosenbergi* in southeastern Costa Rica. Males use existing water-filled basins whenever available and construct basins only when no suitable alternatives are available.

Within one population of *Physalaemus spiniger* from southeastern Brazil, individuals exhibit three reproductive modes (Haddad and Pombal, 1998). This species breeds during the rainy season in areas subject to flooding. When ponds contain water, males typically call from pond edges. Pairs produce foam nests either on the water surface at pond margins or on damp leaf litter near ponds; flooding later washes tadpoles into the water. In contrast, after a breeding pond dries, males call from terrestrial bromeliads, and pairs produce foam nests on water accumulated in bromeliad axils. Once rains flood the bromeliads, tadpoles wash to the ground and develop in the newly-formed pond.

Toledo et al. (2012) revealed how little we know about alternative reproductive modes when they reported that all six anuran species studied at four sites in the Brazilian Atlantic forest exhibited greater intraspecific variability in reproductive modes than was previously known. The authors suggested that because of such variability, the evolution of reproductive modes might better be interpreted by expanding the definitions of reproductive modes by considering continuous rather than categorical variables for some characters.

I have mentioned only a few of the many studies focused on variability of reproductive modes. Continued documentation of costs and benefits of alternative strategies and fitness consequences of variation will shed light on selective pressures that favor particular reproductive modes under given ecological scenarios.

New Modes of Reproduction and Reproductive Behaviors Discovered Within the Past 25 Years

One would think that after all these years of focus on anuran reproduction, we would have a clear picture of the many ways to beget a frog, but discoveries of unique reproductive modes and behaviors, including parental care, continue to be made—modes and behaviors that would have amazed Boulenger, Sampson, Noble, Lutz, Orton, and Goin. New species are discovered every year—2,980 species have been added within the past 27 yr (the difference between Duellman and Trueb, 1986 and Frost, 2014). Some of these newly discovered species exhibit previously undescribed behaviors.

Novel reproductive modes have been described from the Brazilian Atlantic forest within the past 25 yr. Male *Crossodactylus gaudichaudii* (Weygoldt and Carvalho e Silva, 1992), *Hylodes asper* (Haddad and Giaretta, 1999), and *Hylodes dactylocinus* (Narvaes and Rodrigues 2005) excavate underwater oviposition chambers, a mode previously known only in some
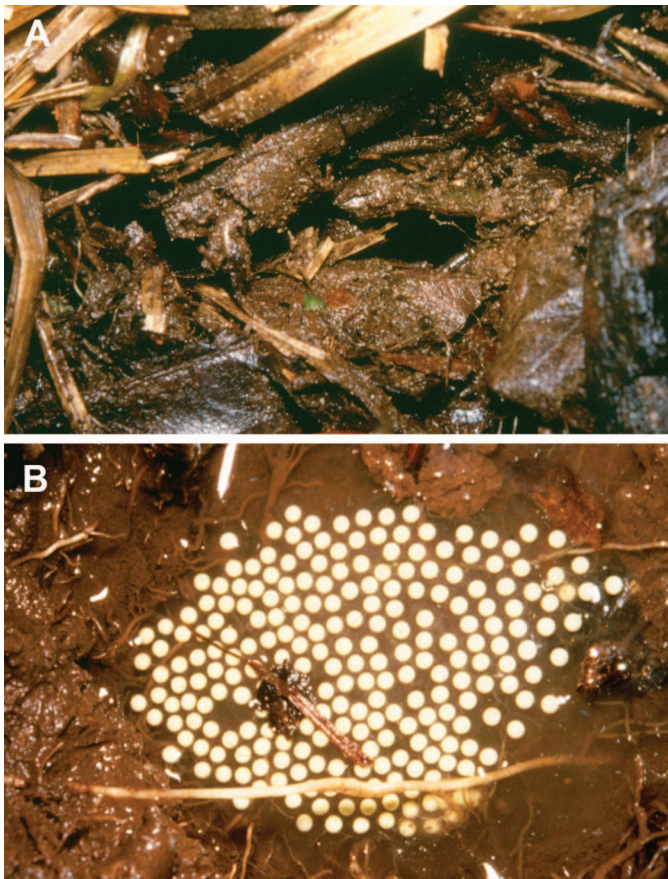
FIG. 4. In *Aplastodiscus leucopygius* (Guinle Treefrog) eggs are deposited in a constructed subterranean chamber: A = entrance to subterranean chamber; B = unpigmented eggs floating on water in chamber. Photos by Célio Haddad.

FIG. 5. Eggs of *Chiasmocleis leucosticta* (Santa Catarina Humming Frog) floating on water surface in bubble nest. Photo by Célio Haddad.

fishes. These chambers might protect against predators and prevent eggs from drifting downstream in the fast-flowing streams where the frogs deposit their eggs. In both *C. gaudichaudii* and *H. dactylocinus*, the male closes the nest entrance with sand and pebbles; oviposition has not yet been observed in *H. asper*. Male *Aplastodiscus leucopygius* construct subterranean nests, where females lay unpigmented eggs that float on the water surface (Haddad and Sawaya, 2000) (Fig. 4). After rainfall the nest floods, the roof collapses, and exotrophic tadpoles are washed into ponds or streams, where they develop until metamorphosis. *Chiasmocleis leucosticta* deposits eggs in a bubble nest floating on water (Haddad and Hödl, 1997). After the eggs are fertilized at the water surface, they adhere to one another by viscous mucus presumably secreted by the oviduct. The pair of frogs dives under the clutch and both individuals compress their throats to release air through their nostrils, which forms bubbles that attach to mucus around the eggs (Fig. 5). The function of a bubble nest may be to suspend the eggs as a surface film where the water is better oxygenated. This nest is similar to bubble nests or rafts produced by air-breathing anabantoid fishes of Asia, except that the fish produce bubbles through their mouths (Breder and Rosen, 1966).

Novel reproductive behaviors have been reported recently from areas of the world where there has been little fieldwork until the past 2 decades. For example, Bickford (2002) reported that males of two microhylids from highland Papua New Guinea, *Liophryne schlaginhaufeni* and *Sphenophryne cornuta*
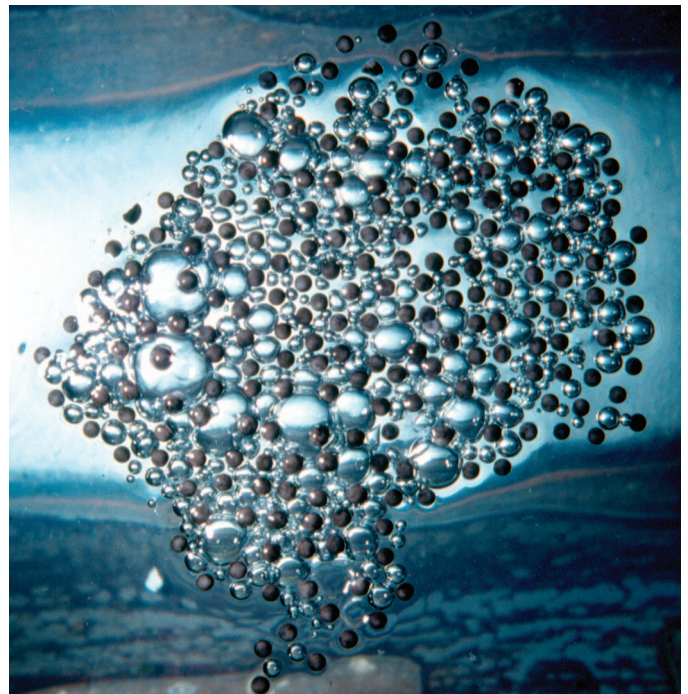
transport froglets on their backs. Observations revealed that after the direct-developing eggs hatched, males transported froglets during 3–9 nights and covered distances from 34–55 m. Bickford (2002) speculated that because the froglets drop off at different points, the young may benefit from reduced competition for food, lower predation pressure, and fewer opportunities for inbreeding.

Three novel reproductive behaviors have been reported recently from the Western Ghats. Gururaja (2010) reported that female *Micrixalus saxicola* (Micrixalidae) dig aquatic subterranean cavities in streams, where they oviposit; females cover the eggs with pebbles and gravel, and there is no further parental care. This behavior is similar to Mode 3 (Table 5), except that in *Crossodactylus* and *Hylodes* the male digs the underwater chamber and, if the entrance is closed, it is the male that performs the behavior. Gururaja et al. (2014) reported "mud-packing" by *Nyctibatrachus kumbara* (Nyctibatrachidae). Breeding occurs along stream edges, where the female lays 4–6 eggs out of water. Following fertilization, the male spreads mud collected from the streambed onto the eggs. Gururaja et al. (2014) speculated that mud might minimize dehydration of the eggs, camouflage them from predators, or both. Seshadri et al. (2014) reported that males of two species of rhacophorids— *Raorchestes chalazodes* and *Raorchestes ochlandrae*—provide parental care for direct-developing eggs laid inside hollow bamboo internodes.

EVOLUTION OF REPRODUCTIVE MODES

For most of the last century, researchers assumed that the anuran reproductive modes we see today represent stages in an incomplete linear sequence of steps toward greater independence from water, with direct development at the spectrum's end. A classic example of this thinking is reflected in Lutz (1948): "The modified anuran life histories already known, when placed in their proper sequence, form a number of linear
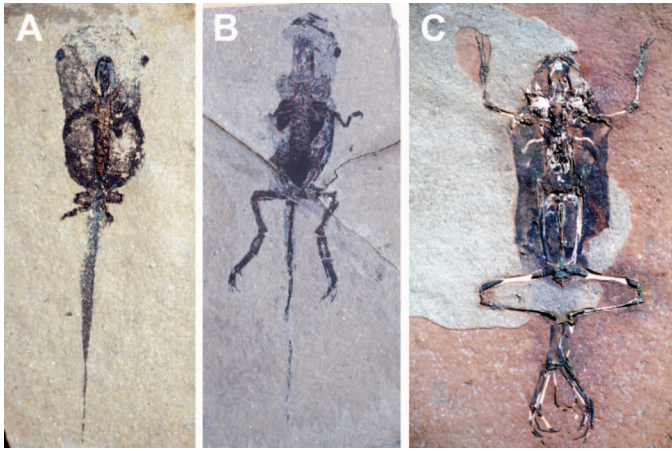
FIG. 6.   The ancestral reproductive mode is presumed to be eggs and exotrophic tadpoles that develop in open water. Pictured here is a new genus and species of pipid from Minas Gerais, Brazil, around 30 million years old, which likely exhibited this reproductive mode. A = tadpole; B = metamorphosing young; C = adult. Photos by Célio Haddad, who is currently describing the fossils.

The ancestral anuran reproductive mode is presumed to be oviposition in open, still water, with exotrophic tadpoles (Duellman, 1985) (Fig. 6). Not coincidentally, the two most-phylogenetically widespread modes involve eggs deposited in open water, either standing or flowing, and an aquatic larval stage. This biphasic life history is thought to be maintained because of the benefit accrued from tadpoles' ability to exploit high levels of aquatic primary productivity (Wassersug, 1975; Wilbur, 1980). Correlated with the trend toward greater terrestriality is a trend toward larger eggs (greater yolk reserves) and fewer eggs per clutch. Duellman and Trueb (1986) suggested that life histories involving eggs with sufficient yolk to provide nourishment for tadpoles after they hatch from aquatic eggs represent independent pre-adaptations for direct development. An estimated 24% of anuran species have direct development (Duellman, 2007). The move from reproduction in large bodies of water to reproduction on land exposed anurans to harsh environmental conditions, especially to increased risk of desiccation. Evolution of parental care (Fig. 7) ameliorated this problem, whether eggs were deposited on land or in small bodies of water such as within bromeliad axils.

Biologists have long speculated about selective pressures that favored evolution of terrestrial reproduction in frogs. The most common explanation is that oviposition out of water allows the most vulnerable life history stages—eggs and larvae—to avoid heavy predation from aquatic invertebrates and vertebrates (Lutz, 1948; Crump, 1974; Magnusson and Hero, 1991; Haddad
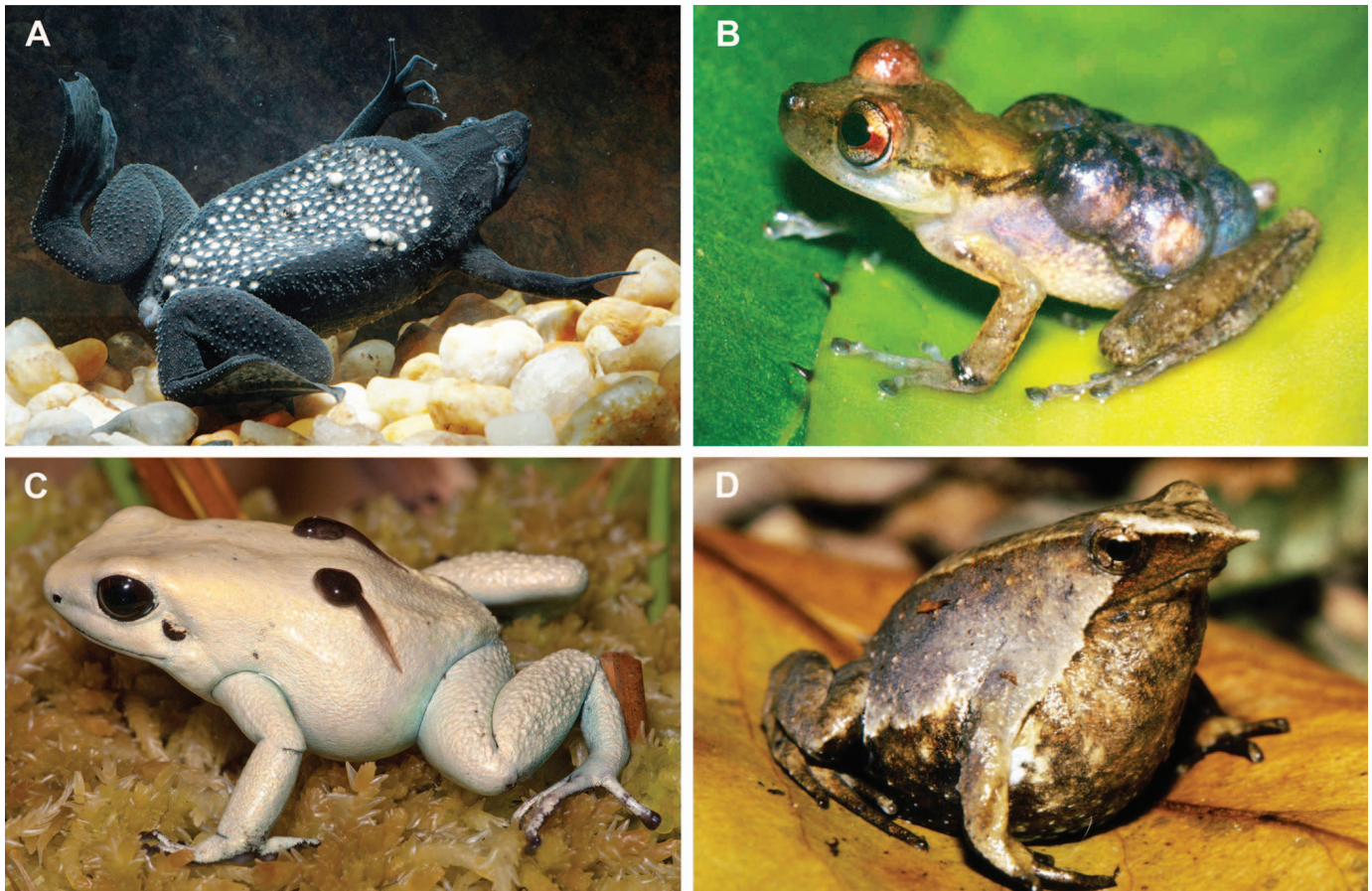
series of ontogenetic evolution, leading from special aquatic to terrestrial environments and from abridged free swimming larval life to direct development." Lutz noted that some steps are missing but suggested that once we discover the life histories of more species, the gaps might be filled.

FIG. 7.   Examples of parental care: A = female *Pipa carvalhoi* (Carvalho's Surinam Toad) with eggs embedded in dorsum; B = female *Fritziana* sp. (undescribed species) carrying eggs in dorsal pouch; C = male *Phyllobates terribilis* (Golden Poison Frog) transporting tadpoles; D = male *Rhinoderma darwinii* (Darwin's Frog) brooding tadpoles in vocal sac. Photos A and B by Célio Haddad; C by Danté Fenolio; D by Martha Crump.

and Prado, 2005). Terrestrial reproduction is not without associated predators, however. Eggs deposited out of water are subject to predation by vertebrates such as snakes and various invertebrates, including crabs and spiders, and terrestrial and arboreal eggs are attacked by fungi. Unpredictability of aquatic larval environments might also have favored terrestrial modes of reproduction (Crump, 1974; Magnusson and Hero, 1991; Haddad and Pombal, 1998). In montane environments, heavy loss of eggs and larvae from swift currents might have been a selective pressure favoring terrestrial oviposition (Alcala, 1962; Goin and Goin, 1962; Weygoldt and Carvalho e Silva, 1992). Other advantages of non-aquatic oviposition include reduced larval competition (Lutz, 1948; Crump, 1974) and avoidance of low oxygen concentration in tropical ponds (Van Dijk, 1971).

Parasites have been suggested as another selective pressure favoring non-aquatic reproductive modes (Todd, 2007). The rationale is threefold. First, most known amphibian parasites rely on aquatic infective stages for reproduction and transmission. Avoidance of open water should reduce parasite load. Second, terrestrial adults typically congregate at aquatic breeding sites, resulting in high host contact rates that promote parasite transmission and facilitate persistence. Terrestrial reproduction typically frees adults from congregating to breed. Third, larval and metamorphosing anurans have lower immunities than do adults. Metamorphosis is a period of immune system vulnerability (Rollins-Smith, 1998), and many frogs are not protected by antimicrobial peptides in their integumentary mucus until after metamorphosis (Clark et al., 1994; Reilly et al., 1994).

Altig and Crother (2006) suggested an alternative to selection for the evolution of endotrophy and focused on three reproductive grades—arboreal eggs, nidicolous larvae, and direct development. They proposed that these grades have arisen independently by various genetic mechanisms, usually multiple times. For example, they proposed that: "The presumed switch from exotrophic tadpoles to direct development most likely occurred via major regulatory gene(s) deep within the developmental cascade, and the wide-ranging occurrences of direct development among families suggests that the genomes of all frogs contain the basic components of this developmental program. The origin of this novel embryogenesis must have been associated with a release from the developmental bias or constraint that governed the biphasic life cycle." Instead of gradual change via small multiple steps, a single, radical developmental change could have happened that bypassed the aquatic tadpole stage. The idea is that selection probably has maintained lineages of direct developers but that the required embryological changes did not result from selective forces, per se.

Our views of the evolution of anuran reproductive modes have changed drastically since the days when we presumed a linear sequence from aquatic to terrestrial reproduction. Analyses of evolutionary relationships based on molecular data have revealed some surprises in terms of life histories nested within the new phylogenies. For example, a reversal resulting in re-evolution of the aquatic larval stage from direct development was proposed but supported only weakly in hemiphractid frogs (Duellman and Hillis, 1987; Duellman et al., 1988) until recently. In 2007, John Wiens and colleagues constructed a phylogeny for the Hemiphractidae based on nuclear and mitochondrial DNA sequences and then used the resulting tree to reconstruct the evolution of life history within the group. Species having a
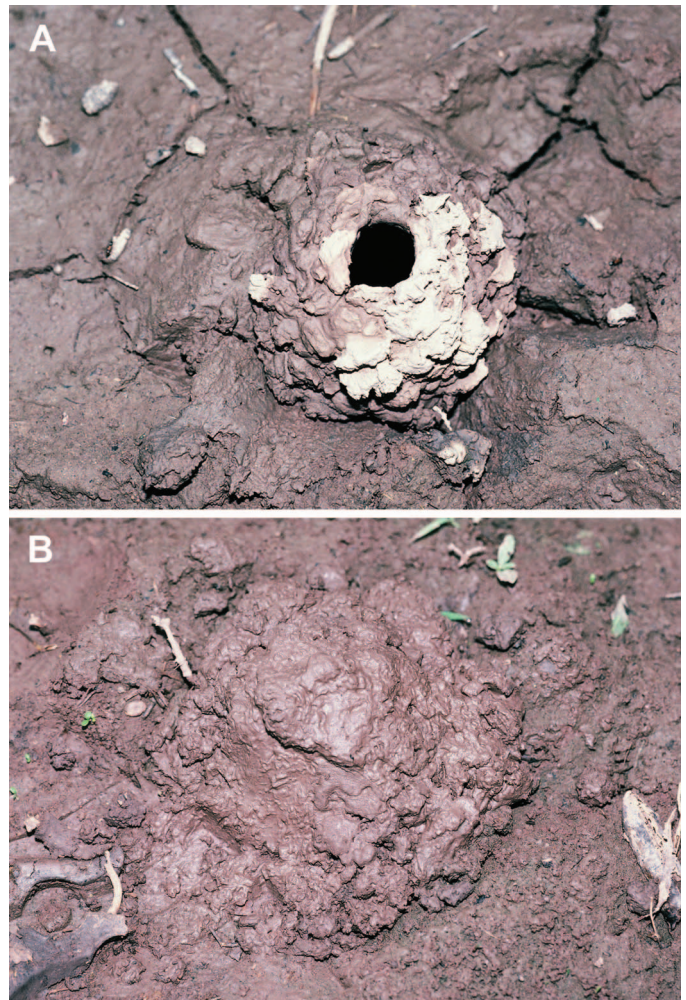

Fig. 8. *Leptodactylus bufonius* (Mud-nesting Frog) an example of a reproductive mode with terrestrial eggs and aquatic larvae. Males construct mud nests on the ground in low-lying areas and call from inside the nests. Amplectant pairs produce foam nests inside the mud nests, and the females cap the nest openings with mud. Following heavy rains, the nests break open and exotrophic tadpoles are washed out into the newly-formed ponds where they complete development. A = newly-constructed mud nest; B = capped mud nest with foam nest inside. Photos by Martha Crump.

tadpole stage were nested among species and genera with direct development (Wiens et al., 2007). Reconstruction of the evolution of reproductive modes on the phylogenetic tree differed depending on the method of trait-reconstruction used. The method assuming a single rate for both gains and losses of reproductive mode suggested that the free-living tadpole stage was lost early in the evolutionary history of the group and then reappeared within *Gastrotheca*. In the model that allowed gains and losses of direct development to evolve under different rates, the free-living tadpole stage was retained in *Flectonotus* and some *Gastrotheca* and lost repeatedly in all other hemiphractid lineages. Several lines of evidence support the first hypothesis: the tadpole stage was lost and then reappeared within *Gastrotheca* (Wiens et al., 2007).

On a broader scale, Gomez-Mestre et al. (2012) addressed the question of which anuran reproductive modes have given rise to direct development. The authors used comparative methods on a phylogeny based primarily on Pyron and Wiens (2011) and a matched database of life history characteristics of 720 species
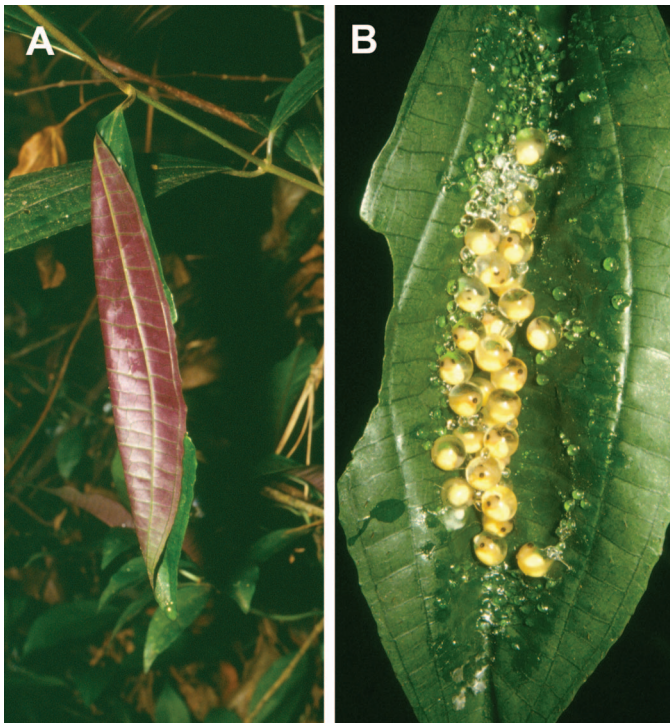
FIG. 9. *Phasmahyla cochranae* (Chocolatefoot Leaf Frog) an example of a reproductive mode with terrestrial eggs and aquatic larvae. Eggs are deposited on leaves above fast-flowing streams. After hatching, the exotrophic tadpoles fall into the water below where they complete development. Empty capsules among the eggs provide moisture, and the folded leaf further protects the eggs from desiccation. A = folded leaf containing egg clutch; B = opened leaf exposing eggs. Photos by Célio Haddad.

(12% of all anurans) that emphasized groups with a diversity of reproductive modes. The analysis documented multiple origins for terrestrial reproduction (about 48) and for direct development (about 19) and revealed some unexpected patterns. One surprising outcome was that direct development seems to have evolved directly from aquatic breeding nearly as often as from modes with terrestrial eggs and aquatic larvae (Figs. 8, 9). Reproductive modes with exotrophic larvae frequently gave rise to direct development, whereas modes with endotrophic larvae did not. Furthermore, although reproductive modes involving eggs and larvae unprotected from aquatic predators have often given rise to modes with protected eggs and unprotected larvae, these modes have rarely given rise to modes involving protected eggs and protected larvae. Especially surprising is that the ancestral mode (unprotected eggs and larvae) has given rise to direct development more often than have modes with protected eggs or larvae or both.

The Gomez-Mestre et al. (2012) analysis indicated that direct development has never reverted directly to aquatic eggs and larvae and only rarely to terrestrial eggs with larval development. The overall picture challenges the conventional view that direct development has arisen as the endpoint in a long sequence of changes leading to greater terrestriality (Lutz, 1948; Salthe and Duellman, 1973; Duellman and Trueb, 1986; Wells, 2007) and suggests that frogs have frequently bypassed many seemingly intermediate stages in the evolution of direct development. These results are consistent with the ideas proposed by Altig and Crother (2006). I predict, and hope, that the combination of ideas offered by Altig and Crother (2006) and Gomez-Mestre et al. (2012) will spark additional research.

What do these new ideas suggest regarding use of reproductive mode as a valid character in interpreting anuran evolutionary relationships? For nearly a century, researchers have questioned such use (e.g., Noble, 1927; Jameson, 1957; Goin, 1960; Duellman, 1985, 2007). Because many modes have evolved independently in two or more families, unrelated species share similar reproductive behaviors not derived from a common ancestor. Such convergence might have evolved because natural selection favored similar reproductive behaviors as solutions to problems encountered by anurans breeding in similar environments. For reproductive mode to be useful as a phylogenetic character, it would need to be stable within a species. Clearly it is not, as is reflected in extensive intraspecific variability. Neither Frost et al. (2006) nor Pyron and Wiens (2011) used life history data in their phylogenetic analyses. We might have seen the end of reproductive mode as a character in constructing anuran phylogenies.

The concept of reproductive mode has other uses, though. In addition to using it to frame questions involving mechanisms, processes, and patterns of ecological interactions at the assemblage level, we are using reproductive modes to address issues related to amphibian declines.

REPRODUCTIVE MODES, AMPHIBIAN DECLINES, AND CONSERVATION

The framework of reproductive modes has been used to search for patterns between declining species and breeding habitat, but the data are varied and inconsistent (Collins and Crump, 2009). Stuart et al. (2004) found that worldwide, amphibians associated with running water are declining significantly more than expected. In contrast, based on a data set for 103 reported Neotropical anuran declines, I concluded that declines were independent of the three broad categories of reproduction: (1) eggs deposited in water, aquatic larvae; (2) eggs deposited out of water, aquatic larvae; (3) neither eggs nor exotrophic larvae in water (Crump, 2003). I found that numbers of declines were almost identical to expected values from a chi-square test of heterogeneity. On a more local scale, in Costa Rica, species that oviposit in running water seem more likely to have declined than the terrestrially breeding species within the same community (Pounds et al., 1997; Lips, 1998). Likewise, population declines from eastern Australia have been associated with stream habitats (Laurance et al., 1996; Williams and Hero, 1998; McDonald and Alford, 1999). In contrast, in southeastern Brazil, species exhibiting more-terrestrial modes of reproduction were more likely to decline relative to aquatic-breeding species in association with severe weather conditions (Heyer et al., 1988; Weygoldt, 1989). Clearly, additional analyses are needed to identify meaningful patterns.

We have searched for possible relationships between clutch parameters and anuran declines, but again, no clear global patterns have emerged. Within Australian rainforest frogs, low clutch size (fewer than 200 eggs) was associated with declining species (Williams and Hero, 1998; McDonald and Alford, 1999). Elsewhere in the world, however, many ranids with large clutch sizes (thousands of eggs) have declined. In another study of Australian frogs, neither clutch size nor egg size was significantly related to declines (Murray and Hose, 2005). Morrison and Hero (2003) suggested that populations of amphibians living at high altitudes and latitudes might be more likely to decline or go extinct because of the influence of altitude on life

history characteristics. Amphibians at higher altitudes and latitudes tend to have shorter breeding seasons, fewer clutches per year, smaller clutches relative to body size, larger eggs, and longer larval periods compared to lower latitude and lower altitude populations. Within eastern Australia, a higher proportion of frogs at high elevations are listed as vulnerable, endangered, or extinct under International Union for Conservation of Nature (IUCN) criteria compared to populations at low elevations (Morrison and Hero, 2003), but whether this trend will hold up worldwide remains to be seen.

I have long thought that there must be a profound difference in reproductive mode-related vulnerability to population change in response to habitat fragmentation. All 11 species within the Argentine Chaco assemblage I studied rely on standing water for reproduction. When roads are built in that scrub habitat, adult anurans and newly metamorphosed young are exposed to potential dehydration, predation, and increased pollutants as they move to and from aquatic breeding sites. In contrast, of the 81 species breeding in the rain forest at Santa Cecilia, more than 50% have semi-terrestrial or terrestrial development; most of these do not migrate to breeding sites. I am delighted that researchers have argued recently for the need to consider life history traits, especially developmental mode (aquatic vs. terrestrial development), in conservation planning.

Studies have shown that integrating information about landscape configuration and anuran developmental mode can produce more ecologically relevant conservation strategies. For example, efforts to enrich breeding sites in fragmented habitat need to address the range of reproductive modes. In areas where species oviposit in arboreal and terrestrial sites, it is not sufficient to dig ponds and drainage ditches. Bickford et al. (2010; Bickford, pers. comm.) found in Singapore that placing water-filled basins and bamboo or PVC cylinders in forest fragments increased abundance of *Kalophrynus* and *Pelophryne* species. Loyola et al. (2008) found that when developmental mode was not considered, priority-setting scenarios for potential reserves in the Neotropics tended to favor species with terrestrial development and to underrepresent those with aquatic larvae.

Becker et al. (2007) suggested that habitat split (human-induced disconnection between habitats used by different life history stages of a species) is likely a major cause of anuran declines at 12 sites studied in the Brazilian Atlantic forest. The authors found that sites more strongly modified by separation of terrestrial and aquatic habitat housed a lower proportion of biphasic anurans. In contrast, habitat split had no effect on species richness of anurans with terrestrial development. Habitat split also contributes to local amphibian population declines (Becker et al., 2009). Becker et al. (2010) evaluated scenarios for amphibian conservation in southeastern Brazil based on how life history traits interact with deforestation patterns. When mode of development was incorporated, the protected areas selected by the conservation planning scenarios encompassed reduced habitat split and habitat loss. Conversely, habitat split and loss were not reduced in the absence of life history information. For all these reasons, reproductive modes of species in fragmented areas should be considered in conservation planning.

Where are We and What is Next?

The study of anuran reproductive modes has advanced in significant ways since its early descriptive phase thanks to researchers who, like Réaumur, have kept an open mind while asking questions, observing animals, testing predictions, and performing experiments. Although we have taken study of reproductive modes to new levels, natural history observations of reproductive biology, as in other areas of biology (Greene, 2005; Schmidly, 2005; Schwenk et al., 2009), are just as relevant and as critical now as ever. As pointed out by Vitt (2013), natural history data form the basis to test emerging ideas about phylogeny and other issues. In his words, "Interpretation of the evolution of behavioral, ecological, physiological, reproductive, and morphological traits differs radically depending upon which phylogeny one believes to be best supported. However, the natural-history data remain the same: they consist of facts about species—they describe reality."

Natural history has always provided the core for framing my questions concerning anuran reproductive modes. Although it is difficult to secure funding to carry out observational fieldwork, and difficult to publish natural history observations, we must continue to describe reality. The mode of reproduction is still unknown for many anurans. No doubt many species are yet to be described, and some likely have reproductive modes as yet unimagined. Exciting opportunities await field biologists. Advances in technology within the past several decades have allowed field researchers to obtain data and answer questions in ways that were previously unimaginable. In the 1970s, I thought Rite-in-the-Rain paper was the end-all, but now we have GPS, powerful miniature radio transmitters, wireless data loggers, sophisticated software for analyzing data in the field, and portable field techniques for extracting DNA.

We need to investigate further the selective pressures that drive evolution of terrestrial reproduction. Such studies ideally would combine field observations and experiments and might involve laboratory experiments. Although biologists speculate about what led to terrestrial reproduction, we need data on survivorship to reproductive maturity for species exhibiting a variety of reproductive modes to test these hypotheses. We should continue to reinterpret evolutionary patterns of reproductive modes based on new phylogenies. Patterns that made sense at one time no longer do because our understanding of evolutionary relationships has changed.

We need more research to understand how reproductive characters vary with environmental conditions and how flexibility will affect species' abilities to survive climate change. Donnelly and Crump (1998) suggested that increased temperature, increased length of dry season, decreased soil moisture, and increased inter-annual rainfall variability will affect frogs in different ways based on their reproductive modes. Species that lay eggs in open water might experience truncated breeding seasons with shorter hydroperiods and be at risk of ponds drying before the tadpoles can metamorphose; species that oviposit on land might be at risk of their eggs desiccating if soil moisture is reduced (Donnelly and Crump, 1998). Predictions need to be formulated regarding how climate change will affect diverse reproductive modes and then experiments performed to gain insight. If we can identify the most-vulnerable species, we can attempt to reduce or prevent losses.

One of the most productive ways to use our expanding understanding of anuran reproductive modes is to incorporate life history information in priority-setting assessments and related actions to produce more ecologically relevant conservation strategies. I encourage everyone interested in anuran reproductive modes to think about how our knowledge of the animals' life histories might help to ensure that these animals

are still around for centuries to come—and then to apply those ideas to anuran conservation.

We have come a long way from the 1730s when Réaumur dressed male frogs in taffeta pants to discover how frog eggs are fertilized (Terrall, 2011) to our current ability to isolate genomic DNA from frog embryos and perform genetic analyses to determine paternity (e.g., Ringler et al., 2012). Nonetheless, there is much left to understand about the many ways to beget a frog. By asking comparative questions and using methods ranging from field observations to molecular analysis, we all can feel like the pioneers of past centuries who attempted to tease order out of the chaos of frog reproduction. We, too, can experience the thrill of discovery.

POST-REVIEW COMMENT

As if to underscore the dynamic nature of our knowledge of anuran reproductive modes, after I read proofs on this paper, Djoko Iskandar and colleagues (Iskandar et al. 2014) reported on a novel reproductive mode that involves internal fertilization and birth of tadpoles. The frog, *Limnonectes larvaepartus* (Dicroglossidae), is endemic to Sulawesi Island, Indonesia. The authors observed females' abdominal walls "quivering" with tadpoles in their oviducts; one female gave birth to tadpoles in a collector's hand; and four females gave birth to tadpoles while in collecting bags. There is still much to learn about this fanged frog. How does internal fertilization take place? Do females always give birth to tadpoles, or can they retain the tadpoles in the oviducts through metamorphosis and give birth to froglets? The authors strongly suspect the former: that tadpoles are born after they exhaust their yolk supply, as evidenced by free-living tadpoles of this species found in small pools of water on the margins of streams.

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