MORPHOLOGICAL DIVERSITY AND EVOLUTION OF EGG AND CLUTCH STRUCTURE IN AMPHIBIANS

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ABSTRACT: The first part of this synthesis summarizes the morphology of the jelly layers surrounding an amphibian ovum. We propose a standard terminology and discuss the evolution of jelly layers. The second part reviews the morphological diversity and arrangement of deposited eggs—the ovipositional mode; we recognize 5 morphological classes including 14 modes. We discuss some of the oviductal, ovipositional, and postovipositional events that contribute to these morphologies. We have incorporated data from taxa from throughout the world but recognize that other types will be discovered that may modify understanding of these modes. Finally, we discuss the evolutionary context of the diversity of clutch structure and present a first estimate of its evolution.

Key words: Amphibia; Clutch; Eggs; Evolution; Jelly layers; Oviposition; Ovulation.

AMPHIBIANS surely have the most varied and least known life histories of all terrestrial vertebrates. Typically, terrestrial adults return to aquatic sites where courtship, egg deposition, and fertilization occur. Eggs hatch into larvae that are mobile feeding forms. After a period of growth and development, these larvae or tadpoles usually undergo some form of metamorphosis and move onto land where they grow into reproductive adults. Variations on this pattern most often represent evolutionary changes in developmental or life history traits (e.g., paedomorphy and pedotypy, egg placement, tadpole development) expressed during the ontogeny of an individual prior to its becoming an adult. McDiarmid and Altig (1999) summarized the biology of tadpoles, and we know even more about the adult stage (e.g., Duellman and Trueb, 1986). Although the research efforts of embryologists and cell biologists have provided extensive information on ovum production and early development, even if involving a relative few taxa, nearly every facet of the field biology of amphibian eggs is poorly documented. In this paper we focus on egg structure, particularly the jelly layers, and patterns of egg deposition. We also consider the characteristics of the physical and biological environments in which the immobile eggs are laid that likely account for the diversity of egg jellies and ovipositional modes.

Field identification of amphibian eggs is difficult at best, in part because authors of published descriptions of eggs have used inconsistent and inexact terminology and misinterpreted certain structures. For example, the vitelline membrane *sensu lato* actually takes on three successive states with different biochemical and physical properties and functions: coelomic, vitelline, and fertilization membranes (Carotenuto, 2001; Gerton and Hedrick, 1986; Takamune et al., 1987), but most authors fail to distinguish among them. In addition, different observational techniques can lead to different results, so that researchers often disagree on traits as seemingly easy to observe as the number of jelly layers around an ovum.

While writing a key to the eggs of North American amphibians, we realized that egg identifications based on properly described ovipositional modes were likely to be more accurate than those based on the more labile and poorly documented features of individual eggs. Clutch morphology is easier to see, more readily definable, and usually less variable within species. By combining these definitions with careful field observations (e.g., Pombal and Haddad, 2005) made under well-documented environmental conditions (e.g., Wright and Wright, 1924), a good observer should be able to identify the eggs of North American amphibian taxa at least to genus with reason-

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able confidence. We suspect that the same could be done with other faunas as well.

Our initial goal in this review is to develop a generalized framework for observed diversity of amphibian eggs and ovipositional modes that will contribute to a broad understanding of the evolution of amphibian reproduction. We believe that the ovipositional mode is a factor that can profitably augment our notions of breeding or reproductive modes (e.g., Crump, 1974; Duellman and Trueb, 1986; Haddad and Prado, 2005). In the process, we present a standardized terminology for egg jellies and clutch structures and discuss the evolution of these features. Although data acquired by cell biologists, developmental geneticists, and particularly histochemists will enhance the eventual understanding of the biology of ova and egg jellies, we do not delve into this extensive literature.

Also, one should be constantly aware of the integration of the data discussed herein with the concept of breeding mode that is being constantly revised and expanded (e.g., Crump, 1974; Duellman and Trueb, 1986). What we present is a subset of the larger concept, although this integration is left for a future synthesis when egg biology is better understood.

The morphological surveys of egg structure by Salthe (1963) and Salthe and Duellman (1973) suggested that intriguing morphological and ecological patterns exist within amphibians. Variations in the surface morphologies of ovum coverings and the nature of the arrangement and attachment of eggs in the environment as reported in other organisms (e.g., Mooi, 1990; Stiassny and Mezey, 1993; Strathmann and Chaffee, 1984) surely exist in amphibians, but certain factors obfuscate their interpretation in this group. For example, the number of jelly layers surrounding an ovum and the number of secretory areas in an oviduct may vary independently (Greven, 2003). In part, these variations can be explained by oviductal (e.g., changes in secretory regions in the oviduct; preovipositional changes in jelly; D. M. Hardy and Hedrick, 1992), postovulatory (e.g., dissolution of some jelly layers) and postovipositional (e.g., layers melding together; formation of the

capsular chamber in salamanders and some frogs) events. Of greater import in essentially all cases is the lack of data or the lack of integration of those data across disparate fields of research. Diagrams of eggs are schematic and usually drawn in lateral view taken at the equator with the animal pole uppermost. Concentric circles drawn around the ovum (e.g., continuous, dashed, or stippled lines or zones; Hoyt, 1960; McDiarmid and Worthington, 1970) represent visually perceived jelly layers. Even so, no adequate description of the arrangement of jelly layers around freshly oviposited eggs based on observations using standardized techniques (i.e., proper illumination, dissection, sectioning, staining) has been published.

We summarize the literature on amphibian egg morphology, especially that addressing the production and evolution of the jelly layers surrounding the amphibian ovum. We follow with a synthetic treatment of the morphology, diversity, and evolution of the egg clutch. We also propose a standardized terminology for describing eggs and egg clutches that we believe facilitates communication and advances the study of this important but neglected stage of the amphibian life cycle.

Egg Morphology and its Variations
Definitions

We define 'egg' as an ovum (= gamete through gastrulation, stages 1–12; Gosner, 1960; other versions in Duellman and Trueb, 1986 and McDiarmid and Altig, 1999, Chapter 2) and its vitelline membrane of ovarian origin surrounded by one to several oviductally-produced jelly layer(s). The internal layer is deposited first by an anterior region of the oviduct, and as the egg moves down the oviduct more layers are deposited sequentially by more posterior oviductal regions.

Various terms have been used to refer to the individual jelly layers, and they and the entire complement of layers as a unit generally lack discrete definitions; some of the terms used in different research fields are inaccurate (e.g., capsule, envelope). We suggest that the entire assemblage of oviductal materials deposited around the ovum be referred to as 'jelly' or 'jelly layers' regardless of variations in

their apparent physical structure (e.g., toughness, density, thickness, water content). Layer, zone, and membrane refer to units within this assemblage. A 'layer' (often referred to in the cell biology literature by the letter 'J' with a numerical subscript to denote position) is a morphologically discrete, easily recognizable region of jelly surrounding the ovum; it has a discernible thickness along a radius and typically has a uniform and visually apparent optical density. Layers are numbered from interior to exterior (e.g., Daniel, 1937) in the order in which they are formed. The term 'layer' does not encompass any specific function or physical characteristic.

Daniel (1937) and others pointed out that some layers have discernible but less discrete subdivisions. We designate these subdivisions as 'zones' to suggest areas that are less easily visualized than layers (see Steinke and Benson, 1970), but we do not imply that all zones of nonstained eggs are visible.

Finally, we use 'membrane' as a functional term to describe what appears to be a delimiting boundary between layers, exclusive of the vitelline membrane. Data are currently insufficient to determine if membranes are actual structures or if they are merely an optical representation of the plane along which two layers of different densities abut. In some instances two layers apparently abut without a visible membrane, thereby suggesting that the densities of the two layers must reach some threshold before a visual manifestation of the transition appears. If the 'membranes' between layers are discrete structures, we do not know if they are independent of adjacent layers and produced by a specific oviductal secretory region, the result of some postovipositional reaction of the jelly layers, or an outer or inner boundary of a particular layer.

Structure

Clear jelly layers certainly are not as structurally uniform (e.g., Carroll et al., 1991) throughout their thickness as they appear. The egg of *Rana pipiens*, for example, has two, clear layers of jelly (Wright and Wright, 1949:35). Studies (Steinke and Benson, 1970) of the taxon of the same name with immunological and histochemical techniques revealed 5–6 layers, whereas dissections (Salthe, 1963) and other techniques (Shaver, 1966; Shivers and James, 1970) indicated 3–5 layers. Descriptions of the eggs of *Dicamptodon* sp., probably based on visual examination of intact eggs (Stebbins, 2003), indicated 2 jelly layers, whereas visual and tactile detection of density differences noted during dissection (Nussbaum, 1969) showed 5 layers.

More structure certainly exists in the jelly layers than can be seen with simple microscopy. Other observational techniques (e.g., differential interference contrast [DIC], phase-contrast and fluorescence microscopy and laser illumination) do not produce better images. Laser confocal imaging and some techniques of electron microscopy provide better results but are costly and specimen preparation is laborious (e.g., Bonnell and Chandler, 1998; Larabell and Chandler, 2005). More refined zones, which can be detected with histological and histochemical techniques, likely will reveal patterns of biological, ecological, or phylogenetic interest (e.g., Hedrick and Katagiri, 1988; Smith et al., 2002). Biochemical analyses show that egg jellies consist of "a fibrous glycoprotein superstructure that acts as a scaffold to which globular glycoproteins are bound" (Fig. 5E; Bonnell and Chandler, 1998); which of these groups of molecules is structural and which is biologically active is not known, but both are expected to have species-specific qualities (Maes et al., 1995). The three jelly layers of the eggs of *Xenopus laevis* are composed of at least nine glycoproteins (Yurewicz et al., 1975).

These kinds of data, while interesting, are of little use to field biologists who usually rely on visual impressions of structure viewed under incident, white light. Some data suggest that it may not be possible to formulate a descriptive model useful to all researchers. Accordingly, field biologists, histochemists, and biologists in other fields may find it expedient to use their own sets of terms to describe observed variations in jelly traits.

In summary, jelly occurs in layers and a layer may be subdivided into less easily discernible zones. Visually perceived layers typically would be the morphological trait
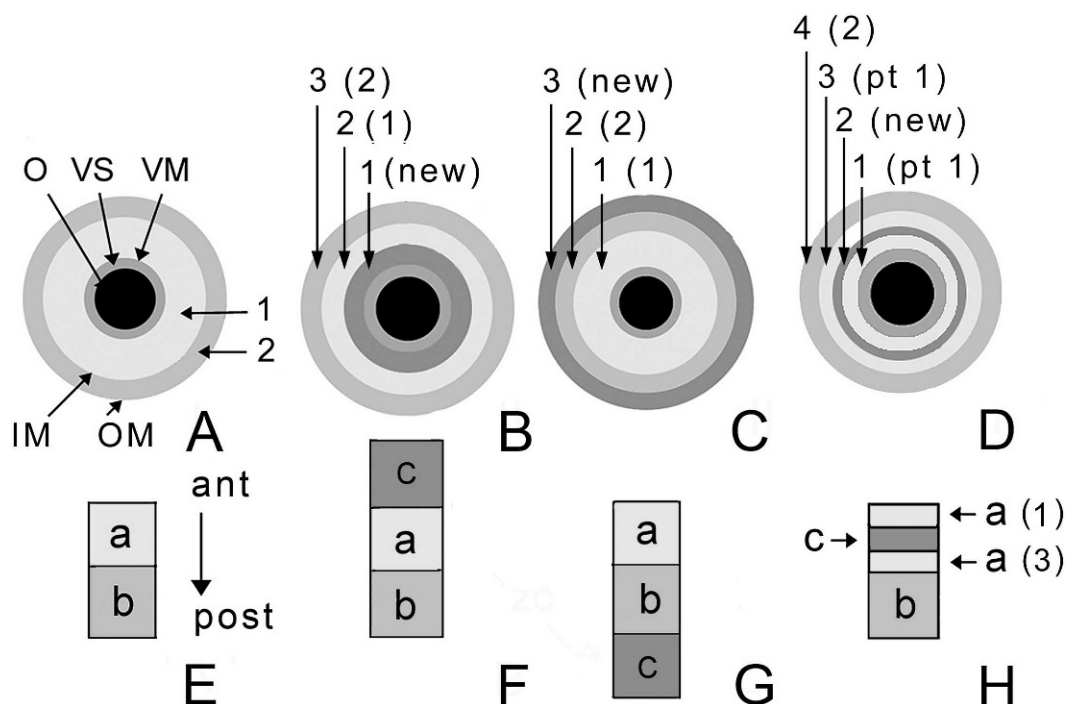
FIG. 1.—Schematic drawings of an amphibian egg with its component parts, the oviductal regions a–c that produce the jelly layers, and three hypothetical paths for adding a jelly layer. (A) An ovum with two membranes (see text) and two jelly layers produced in sequence by oviductal sections a and b arranged anteriorly to posteriorly. (B) A hypothetical case where a new layer is added directly external to the vitelline membrane; new numbering of the jelly layers and the arrangement of the oviductal regions are shown. (C) A case where a new layer is added externally. (D) A case where a new layer is added within the original first layer. Abbreviations: 1–4 = jelly layers numbered from ovum to surface, ant = anterior, IM = inner membrane, O = ovum, OM = outer membrane, post = posterior, VM = vitelline membrane, and VS = vitelline space.

most useful for identification. For example, the basic egg structure consists of an ovum with a vitelline membrane surrounded by jelly layers 1 and 2 (Fig. 1A, E) of oviductally-produced materials. Inner and outer membranes are labeled, although oviductal secretory zones for their production are not, and no zones are shown.

EVOLUTION OF EGG JELLY

The evolution of additional layers of jelly does not appear to result only from the subdivision of ancestral layers. Thus, egg diameter often is greater in species with more layers rather than in those with fewer layers, even though the thickness of each layer, or at least of some, often decreases as the number of layers increases. Although the thickness of jelly layers must be mediated by the general trade-offs between the requirements for gas ex-change and need for mechanical support, other factors (e.g., homospecific sperm attraction [Al-Anzi and Chandler, 1998], heterospecific sperm avoidance, heat conservation, and predator defense) likely have played roles in the evolution of the number, thickness, and physical characteristics of the layers. To appreciate layer homologies one must ultimately understand the morphology of the secretory regions of the oviduct and presumably have some notion of evolutionary relationships among taxa. Presumably, selection for differences in the number and characteristics of the jelly layer has influenced oviductal morphology rather than the reverse, although other factors that seemingly are not related to egg survival (e.g., Anderson et al., 2006) must be considered. Evidence suggests that environmental demands and phylogenetic constraints have influenced the evolution of egg morphology, although little is known about the

selective factors involved, and details and patterns of the morphology in most taxa are still lacking (Salthe, 1963; Greven, 2003). Hypothetical scenarios diagramed (Fig. 1B–D, F–H; also Greven, 2002:fig. 11) illustrate potential modifications in oviductal secretory regions that would produce given changes in jelly layers; the review by Wake and Dickie (1998) provides informative discussions of oviductal anatomy relative to breeding mode. If we assume for heuristic purposes that combinations of the following alternatives did not occur, then a third jelly layer might be added (1) between the vitelline membrane and the first jelly layer; Fig. 1B, F), (2) between the two original jelly layers), (3) external to the second layer of the original egg; Fig. 1C, G), or within an existing layer; Fig. 1D, H). If a posterior (i.e., new layer added externally) or anterior (i.e., new layer internally) region of the oviduct were involved and a phylogenetic scheme were known, one potentially could track the products and thus hypothesize homologies. Identification of other types of additions may require histochemical techniques, and examination of changes in oviductal regions. Which evolutionary pathway is most likely can be debated and will depend largely on the ease with which homologies can be assigned. Layer thickness also is likely influenced either by the length of the oviductal region, passage rate of the ovum, rate of jelly production, or differences in hygroscopic qualities of the jelly. Although these parameters are probably important, we have ignored them because of the lack of data.

Salthe (1963) presented the only suggestions of homologies of jelly layers in caudates. His interpretations, based on 15 caudate genera in 8 families suggest that (1) 8 jelly layers (as in *Hynobius lichenatus*) is the primitive condition; (2) changes in the number of layers occur only through (a) loss of the most external layers (e.g., ambystomatids) or (b) loss of more internal layers (i.e., elimination of layers somewhere within the series; particularly plethodontids); and (3) eggs with 3 layers of undetermined homologies (as in of *Cryptobranchus*) is the simplest condition. All of these ideas are based on the assumption that layers in discernible positions and of similar construction are homologs.

We know of no proposed scheme of jelly layer homologies for anurans, and phylogenetic patterns and possible selective factors contributing to their evolution are often discordant. For example, among North American frogs (Moore, 1940), many cool-water breeders deposit clumps of eggs with either 2 or 4 jelly layers, whereas warm-water breeders lay either clumps or films of eggs with 2 layers. 'Wood frogs' (West Coast endemics plus *Rana sylvatica*) produce clumps of eggs with 2–3 jelly layers in cool water. Members of the *Rana catesbeiana* group breed in warm weather and deposit eggs with 1–2 layers as clumps or surface films. Members of the *Rana pipiens* complex lay eggs with 2 jelly layers in clumps and usually in cool water. Considering the four options for layer homologies (Fig. 1B–D), one can ask if the outer two or the inner two layers of the wood frogs are homologous to the two layers of the other groups of North American ranids and which of the layers might reflect responses to temperature or ovipositional mode and which to phylogenetic constraint.

A complete histochemical data set for an amphibian egg might agree with Salthe's (1963) morphological data. One still would not be sure of layer homologies, but his hypotheses based on position and construction would have stronger support. Mapping histochemical data onto appropriate cladograms would likely provide some insight about the evolution of jelly layers, and information on the ecological functions of egg jellies should reveal correlations useful to interpreting their evolution. Salthe (1963) suggested that layer losses in plethodontids that lay terrestrial eggs involve changes of internal layers and that the tough outer layer is retained for protection. Evaluations of the hypotheses about jelly layer homologies will have to wait additional data.

ANCILLARY SUBJECTS ON EGGS

Characteristics of Jelly Layers

Anyone who has handled eggs and especially those who have manually dejellied them are familiar with features such as elasticity, stickiness, toughness, turgidity, and wateri-

ness. Aquatic eggs usually are spherical when submerged but sag when placed on a surface in air. The jellies of most terrestrial forms that do not lay suspended eggs have jelly with sufficient tensile strength and turgidity to remain spherical in air. The outer jelly of eggs of *Ambystoma opacum* and other terrestrial salamanders and frogs with direct development is tough relative to that of aquatic eggs. Tougher membranes and increased turgidity of the enclosed fluids help to maintain the spherical shape of these large eggs in air and thereby allow proper development, oxygenation, and protection from trampling by an attendant parent. These terrestrial eggs can be grasped with minimal distortion and will bounce if dropped. If the outer layer is removed, the remaining jellies spread out, and the ovum usually ruptures. In some aquatic eggs the most external visible layer is surrounded by a transparent, watery gel that appears to lack a defining exterior surface but is crucial for flotation (see below).

Asymmetries of jellies caused by tensile differences, such as the drooping of egg jellies of terrestrial plethodontid eggs or the pentagonal appearance of jellies of eggs tightly spaced in a film, are common. The observation by Wright and Wright (1949) that the inner jelly layer of the eggs of *Rana clamitans*, which is not under any tensile forces, may be elliptical or pear-shaped needs verification. We assume that the conical ova that Wright and Wright (1949) observed in *Bufo alvarius* resulted from tension on the jelly string.

There are cases of egg jelly asymmetries in the absence of tension. The outer jellies of some salamandrid eggs (*Notophthalmus viridescens*; Bishop, 1943) are oval and attached individually or in small groups to plants. Parts of the outer jelly layers of the eggs of some Old World microhylids (e.g., *Kaloula rugifera* and *K. macroptica*, Fig. 2A, B, and Liu, 1950; *Kaloula borealis* in Li, 1934; *Kalophrynus pleurostigma* in Taylor, 1922; and *Paradoxophyla palmata* in Glaw and Vences, 1992; RA, personal observation) are asymmetrical and form a flange or rim which allows the eggs to float. Asymmetry in jelly layers seems at odds with their mode of formation in the oviduct, but the asymmetry results from differences in hygroscopic properties of specific portions of

the jelly immediately after oviposition rather than structural differences per se. The jelly that forms the flange surely surrounds the entire ovum, in contrast to being deposited as a band, and is likely very watery. The pressure of the water surrounding the egg as it sinks partway through the water surface likely pushes this flimsy jelly to the air-water interface and forms the flange. Different flange positions (e.g., equatorial in *Kaloula* spp. and near the vegetal pole in *Paradoxophyla palmata*) may reflect interspecific differences in the density of the egg-jelly complex or in the hygroscopic qualities of the jelly. Li (1934) noted that the rim in *Kaloula borealis* did not appear until about 1 min after oviposition, and Taylor (1922) described the gelatinous flange in *Kalophrynus pleurostigma* as gradually widening to about 6 mm diameter after extrusion.

Inclusions in Jelly

The structure, origin, and function of various crystalline inclusions in the jelly of salamanders (e.g., *Ambystoma maculatum*, Salthe, 1963; Ruth et al., 1993; *Siren lacertina* and *Hynobius lichenatus*, Salthe, 1963) need further examination. For example, the egg jelly of *Ambystoma maculatum* can be clear or opaque white (L. M. Hardy and Lucas, 1991). White jelly gets its color from glycoprotein crystals that are produced with the jelly in cells in the oviductal wall. Populations with clear-jellied, white-jellied, or both types of egg masses have been reported from northwestern Louisiana and adjacent areas of Texas and Arkansas. The function of the crystals is unknown, but they may reflect light and thereby offer some protection to the developing embryos or concealment from predators (L. M. Hardy, personal communication). Similarly, the jelly of the eggs of the frog *Mantidactylus depressiceps* (Mantellidae) is milky white (RA, personal observation) although the cause of the color is unknown. What appear as striae, furrows, or corrugations in the outer layer of jelly of some salamanders (e.g., *Hynobius lichenatus*, Salthe, 1963) and frog egg masses (e.g., *Cochranella pulverata* [RWM, personal observation]) also deserve attention.

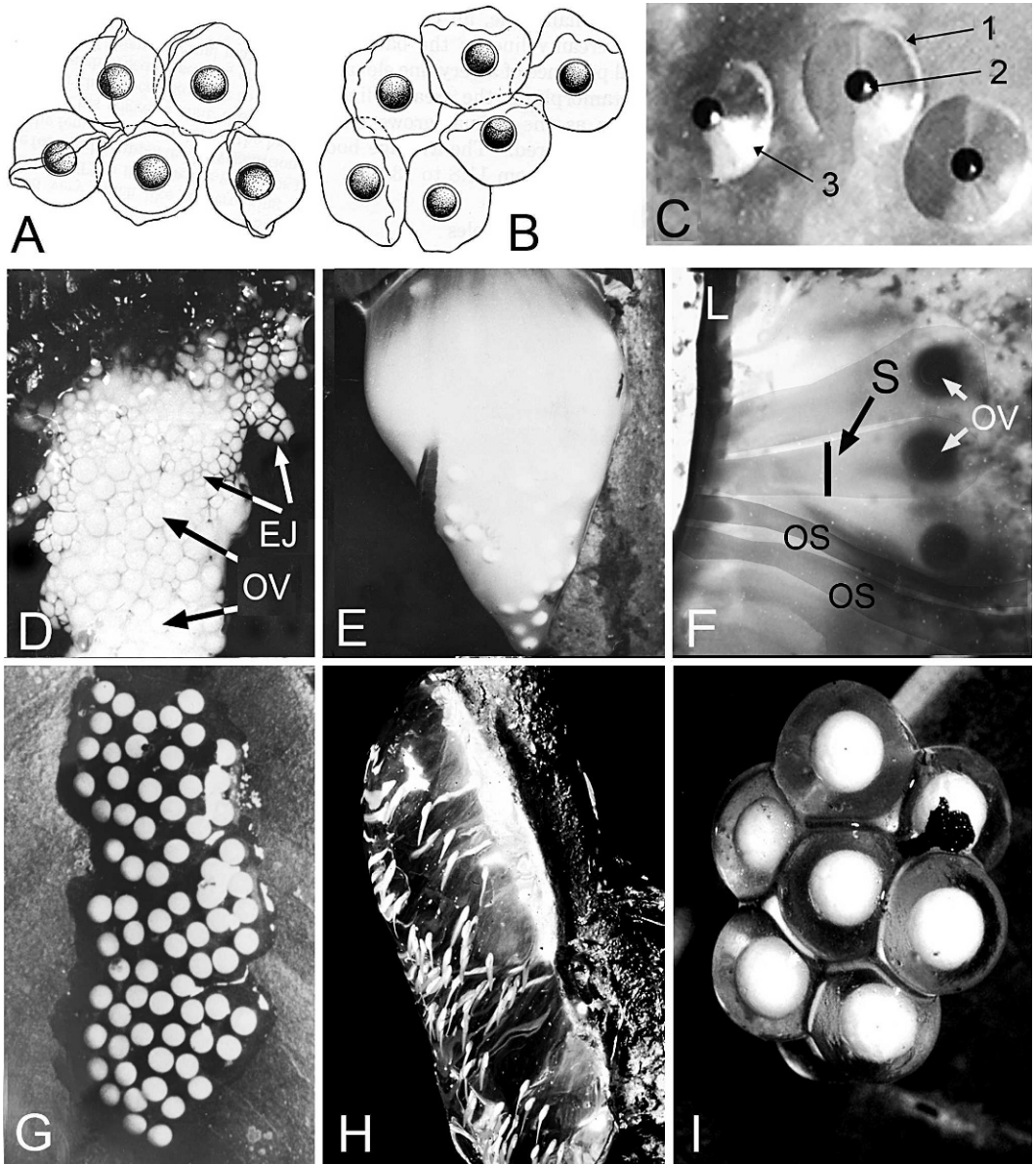Some ambystomatid eggs, notably those of *Ambystoma maculatum* and *A. gracile* with

FIG. 2.—(A–C) Microhylid, (D–H) mantellid, and (I) arthroleptid frog eggs. Asymmetrical egg jellies of (A) *Kaloula rugifera* (modified from Liu, 1950:fig. 58) and (B) *K. macroptica* (modified from Liu, 1950:fig. 60) caused by differential hydration of specific parts of the jelly. (C) Part of a coherent film of *Gastrophryne carolinensis* eggs soon after oviposition showing the upper hemisphere of jelly projecting above the water surface (1 = downward flexed meniscus at jelly margin, 2 = glint on surface of ovum or vitelline membrane, and 3 = trapezoidal reflection on curved, upper hemisphere of outer egg jelly). Laminar array of a clutch of *Guibemantis depressiceps* eggs on a leaf (D) prior to hydration (OV = ova; EJ = empty jellies), (E) the same clutch showing a large increase in volume after hydration, and (F) the same clutch cut longitudinally after hydration, stained with Toluidine Blue, and viewed with transmitted light (L = cut leaf; OS = ovumless stalk, OV = ova, and S = limits of one stalk with an ovum; stalks slightly darkened electronically for better visibility). (G) Recently laid clutch of '*Mantidactylus*' sp. on a leaf showing structure when hydration is normally less extensive, and (H) older clutch of '*Mantidactylus*' sp. attached to the bark of a tree with clear jelly and a large increase in jelly with hydration (embryos that appear deep in the jelly are actually on the sides of the jelly). (I) Terrestrial clump of *Arthroleptis schubotzi* found in leaf litter (photo by R. C. Drewes).

exceptionally dense jellies, have a symbiotic green alga (*Oophila amblystomatis*) within the inner jelly layers of the egg (e.g., Bachmann et al., 1986; Hammen, 1962).

Capsular Chamber

In salamanders and a few primitive frogs (Salthe, 1963:165), the jelly layer abutting the vitelline membrane dissolves soon after oviposition to form the capsular chamber; remnant debris, termed 'white plac', puddles at the bottom of the capsular chamber (Daniel, 1937). Eggs with a capsular chamber are less confined than those without such a chamber so that the ova lie slightly below center in the remaining jelly; if the egg mass is inverted, the ova immediately turn so that the animal pole is uppermost. In eggs without a capsular chamber the ova are constrained by the jelly layers and take several minutes to right themselves (Salthe, 1963). Embryos usually exit the vitelline membrane long before they hatch from the jelly (Salthe, 1963), and one can often see the vitelline membrane crumpled up at the bottom of the inner jelly layer in advanced embryos.

Functions of Egg Jelly

Suggested functions of egg jellies (Greven, 2002, 2003) include mechanical support for the ovum, attachment of eggs to each other or a structure in the environment, enhancement or prevention of entry by conspecific and heterospecific sperm respectively (e.g., Barbieri and Del Pino, 1970), prevention of polyspermy, sperm capacitation, differential protection from water molds *Saprolegnia* and *Achlys* (Gomez-Mestre et al., 2006), protection from contaminants (Marquis et al., 2006), and protection from predators, pathogens, and environmental stressors such as temperature and UV light (Hunter and Vogel, 1986; Itoh et al., 2002; McLaughlin and Humphries, 1978; Ward and Sexton, 1981). Incubation in birds helps to inhibit growth of bacteria and fungi on egg shells (Cook et al., 2005); jelly layers may be a prepackaged way to protect ova from these perils after they exit from the relatively sterile oviduct. The influence of jelly on light refraction is thought to be insignificant (Cornman and Grier, 1941)

but possibly needs further study (Bragg, 1964).

Hatching

Hatching occurs when a developmental threshold is reached in concert with various biotic and environmental cues. Hypoxia (e.g., Petranka et al., 1982) likely is the proximal trigger, but factors such as pathogens and predators (e.g., Chivers et al., 2001; Touchon et al., 2006; Warkentin, 1995) or low pH (Dunson and Connell, 1982) can modify timing. Thumm and Mahony (2002) demonstrated that the stage and degree of development at hatching in *Pseudophryne australis* (Myobatrachidae) is variable. Hatching mechanisms seemingly are universal (Noble, 1926; Duellman and Trueb, 1986) and fall into two groups. In most amphibians enzymes from the frontal (= hatching) glands on the head and snout cause at least partial chemical degradation of the jelly (e.g., Carroll and Hedrick, 1974; Urch and Hedrick, 1981), which may often be augmented by simple writhing and pushing by the embryo to break through the layers (also Bragg, 1940; Gollman and Gollman, 1993; Lutz, 1944) or by rain (Noble, 1926). In some direct developing frogs an egg tooth on the snout or other part of the body mechanically ruptures the membranes and jelly layers to allow hatching (e.g., J. D. Hardy, 1984; Noble, 1926).

Ovum Pigmentation

Even though the pigments in most ova are melanic and not contained in cellular organelles, data on this pigmentation of maternal origin are confusing. In large part this is because there are no standards for describing color, intensity (e.g., dark, diffuse, pale), pattern, location, or the gradation from dorsal darkness to ventral paleness. In addition, apparent changes in pigmentation with development or after preservation have rarely been documented. Potential influences on ovum coloration include age of female, stage of oogenesis, changes during development (i.e., when and how maternally-provided pigment is supplanted during oogenesis by that from the developing embryo), and egg-laying site (i.e., geographical and elevational variation). Likewise, color and pattern and

their development in hatchlings (e.g., Altig, 1972) that are useful for species identifications (e.g., hylid hatchlings commonly are strikingly patterned, ranids usually unicolored) are poorly documented.

Nevertheless, some general correlates of ovum pigmentation are apparent (Salthe, 1963). Eggs laid in open, exposed areas, regardless of the specific site, taxon, or ovipositional mode, usually have melanic pigment at the animal pole (e.g., *Ambystoma*, *Bufo*, *Hyla*, and *Rana* of North America). As the number of embryonic cells increases and the cells move during gastrulation, the fertilized ova become more uniformly pigmented and paler because the ovum pigment is dispersed among more cells until embryonic pigment appears. Eggs laid in secluded sites (e.g., *Amphiuma* in burrows near lentic sites, *Cryptobranchus*, *Dicamptodon*, numerous plethodontids, and *Ascaphus* hidden among rocks in streams, some dendrobatid and microhylid frogs among forest debris and phytotelmata, some centrolenids on leaves) tend to be pale to nonpigmented regardless of ovipositional mode. Biologists working in temperate areas often assume that amphibian eggs are pigmented because those the taxa most commonly encountered are pigmented, but in fact, many taxa in tropical regions have nonpigmented ova.

Several functions of egg pigmentation and adult behaviors associated with that trait have been proposed. Communal oviposition of masses of dark eggs apparently enhances absorption of heat thereby increasing developmental rate in early breeding species of some North American ranids (Hassinger, 1970). Pigmentation in eggs can also provide protection from deleterious effects of specific wavelengths of light or heat (Barrio, 1965; Jones, 1967). Biliverdin and lutein can produce greenish to bluish ova in some phyllomedusines (Pyburn, 1963; Marinetti and Bagnara, 1983), some hyperoliids (Wager, 1965), some rhacophorids (Liu, 1950), and certain centrolenids (RWM, personal observation). These pigments are housed in the yolk platelets (Barrio, 1965) and are from a different source than the melanic pigments. The greenish color can enhance concealment of frog eggs from some predators (e.g., greenish eggs deposited on the tops or undersides of leaves) or provide infrared camouflage (Schwalm et al., 1977; also see Saito, 2001). Falchuk et al. (2002) have shown that biliverdin IXα functions as a cytoplasmic determinant that is essential to normal embryogenesis in early stages of *Xenopus laevis*. The destruction of biliverdin via some wavelengths of UV light may provide a mechanism for understanding their detrimental effects (e.g., Blaustein et al., 1994). The omission of melanic pigment in ova surely conserves some reproductive energy, and embryos developing from nonpigmented eggs sometimes do not develop pigment until well after hatching.

CLUTCH MORPHOLOGY

Definitions

'Clutch' describes the total number of eggs deposited per ovulation event independent of the number or presence of a male(s), reproductive or ovipositional mode, ovipositional behavior of parents, or number of 'groups' (= aggregate of eggs produced in a single ovipositional bout; this term is useful when one does not know the taxon or ovipositional mode or number of bouts [i.e., single egg laying event]) that occurred. The number of ova ovulated sometimes exceeds the number of eggs oviposited. For example, if a female oviposits her ovulated complement in multiple bouts, the total number of eggs deposited makes up the clutch (i.e., a group in this case is not the clutch). A pair of *Smilisca phaeota* may deposit eggs among one to several puddles in a single night (RWM, personal observation); the eggs in each puddle comprise a group, are produced during a single bout, and are only part of the clutch. Female *Ambystoma tigrinum* may partition their clutches into multiple discreet groups which may be sired by one or more males; distances between these groups in a single pond may exceed 40 m (Gopurenko et al., 2006). Thus, the organization of the total clutch, and not the number or manner in which eggs emerge from the female in a single bout, is important. If a female ovulates and oviposits more than once a year or in multiple years, she has produced multiple clutches.

'Ovipositional mode' describes the morphology of an aggregation of deposited eggs (i.e., the clutch structure). Explanations of how egg aggregates are produced and postulates about the evolution of different structures are largely lacking. It seems apparent that oviductal (i.e., jelly formation), ovipositional (i.e., parental behavior), and postovipositional (e.g., jelly dissolution) processes are involved. Differences in the rate or pattern of release of eggs from a female also can influence clutch structure. Oviposition site, ovum number and size (Bernardo, 1996; Pombal and Haddad, 2005; Salthe and Duellman, 1973; Summers et al., 2007), energy content (e.g., Komoroski and Congdon, 2001; Komoroski et al., 1998), pigmentation, subsequent characteristics of development, larval ecomorphology, and other such factors are not directly considered in this review. In part, this is because the functional roles of oviductal (e.g., Greven, 2002, 2003) and especially ovipositional (e.g., Aronson, 1943) factors are not understood in sufficient detail. Likewise, inaccurate, nonstandard terminology is a constantly confounding problem. Livezey and Wright (1947) and Wright and Wright (1949) presented at least 14 terms, many of which were ill-defined, to describe some arrangement of a group of eggs. Several other workers (e.g., Stebbins, 1951, 2003; Corkran and Thoms, 1996) have used similar terms in the same or different ways. A notable recent exception is Anstis (2002) who clearly defined the terms she used to describe the types of egg aggregations of frogs from southeastern Australia. In the interest of clarity and with no implication of discrete organization, we use 'tier(s)' to describe a two- or three-dimensional arrangement of eggs and restrict 'layer' to descriptions of jelly morphology.

Other Considerations

Accurate observations and descriptions of ovipositional events and the appearance of deposited groups of eggs are lacking for most amphibians but deemed essential for understanding amphibian reproduction in a broader context. Difficulties stem from a general lack of knowledge of the natural history of amphibians, problems with egg and species identifications, comparisons of eggs of differ-

ent ages, and inadequate observational techniques. The mere presence of multiple, confusing reflections and refractions from various water and egg surfaces and the extreme transparency of egg jellies can interfere with accurate observations. For example, Alcala and Brown (1956) reported that *Rana microdisca* lays eggs on moist surfaces on stream banks in a "twice-coiled string." This note, presuming correct identifications of the adult, the eggs, and the ovipositional mode, is particularly vexing for the following reasons: a moist surface on a stream bank is a rare site for ranid egg deposition, to our knowledge a string is unknown among ranid frogs, and 'twice-coiled' is difficult to envision (i.e., single string coiled upon itself versus two strings with simple coils that are intertwined) and possibly the result of disturbance.

The notion that each species oviposits in one mode is usually sound (see Williams and Tyler, 1994 for an interesting exception; Marsh and Borrell, 2001), but some ovipositional modes seem to blend from one into another. This apparent blending frequently is the result of variations in the ovipositional behavior of the adults, disturbance at the deposition sites, comparisons of eggs of different ages, or a misinterpretation of the mode. For example, an investigator who observes single eggs deposited close together or on top of each other, but with the presence of single, disjunct eggs, might conclude (inaccurately in our opinion, see below) that the eggs are a clump. Eggs deposited by different taxa as surface films float by different means and must be observed closely to determine whether the outer jellies are adherent or coherent. Pairs of frogs that produce surface films must have adequate room to maneuver; movements in confined spaces sometimes cause the eggs to sink. Species that deposit egg masses and those depositing single eggs must have suitable substrates to which to attach their eggs. Eggs and clutches deposited under artificial conditions (e.g., laboratory containers, terrariums, plastic bags) may differ from those deposited in the wild. As embryos in clumps and masses approach hatching and jelly layers begin to break down, a group of eggs may rise to the water surface. Any environmental condition that produces lots

of bubbles (e.g., increase in temperature, bottom fermentation) can also raise eggs to the surface where they may appear as a film. Eggs in films often sink even during light rain, strings and particularly rosaries often are broken, and egg clusters commonly become detached from their suspension points by activities of attendant parents. All such contingencies must be taken into account. Additionally, more observations on the actual ovipositional behavior of adults (e.g., Aronson, 1943, 1944; Norris and Hosie, 2005; Pyburn, 1967, 1970, 1971) would add immeasurably to our collective knowledge and facilitate a better understanding of ovipositional modes.

Alytid (*Alytes* and *Discoglossus*) and pipid (*Pipa*) frogs have a capsular chamber, and the direct-developing '*Eleutherodactylus*' may also have such a chamber (Salthe, 1963). It may be imperative that large eggs turn over quickly when inverted to keep the embryo from being damaged; the morphology of the jellies of more direct developing eggs need to be examined.

Information on the eggs of caecilians is particularly scarce, but large eggs laid as a rosary seem to prevail (Exbrayat, 2006; M. Wake and M. Wilkinson, personal communications): *Caecilia*, Funk et al., 2004; *Epicrionops niger*, A. Lathrop, personal communication; *Gegeneophis carnosus*, Exbrayat and Delsol, 1988; Seshachar, 1942; *Hypogeophis*, RA, unpublished data); *Ichthyophis* "*glutinosus/malabarensis*," Balakrishna et al., 1983; Breckenridge and de Silva, 1973; Breckenridge and Jayasinghe, 1979; Breckenridge et al., 1987; Seshachar et al., 1982; *I.* cf. *kohtaoensis*, Kupfer et al., 2004; *Idiocranium russeli*, Sanderson, 1937; *Siphonops annulatus*, Cei, 1980; Göldi, 1899; *S. paulensis*, Gans, 1961; Montero et al., 2005; and *Typhlonectes compressicaudus*, Sammouri et al., 1990). These descriptions lack certain details, and some are surely inaccurate. For example, certain drawings of eggs of *Ichthyophis* spp. (Himstedt, 1996:fig. 57; Sarasin and Sarasin, 1887–90, as reprinted in Angel, 1947:fig. 107) suggest a cluster, but others (e.g., Himstedt, 1996:fig. 47) seemingly show a rosary. Considering that the preponderance of recent data countering the likelihood of a cluster morphology, we suggest that the earlier interpretations are in error or the result of artistic license.

Some aquatic (e.g., *Dicamptodon*, plethodontines) and terrestrial (e.g., *Aneides*) salamanders 'suspend' (i.e., usually attach them to the lower surfaces of substrates) their eggs. The eggs may be 'pendant' (i.e., ovum is not centered in the drooping outer jelly layer; Fig. 3A) or not (i.e., ova centered within outer jelly; Fig. 3B). As defined here, a ball with the turgidity to maintain its spherical shape that is stuck directly to a ceiling is suspended but not pendant. A small area termed the 'pedicel' attaches a suspended egg to a substrate, and 1–2 jelly layers may be involved in the supporting part of the jelly.

Deposition Sites

Similar ovipositional modes are scattered across amphibian groups and breeding sites, and egg deposition site often has little to do with clutch structure (e.g., foam nests are placed in aquatic, arboreal, subterranean, and terrestrial microhabitats; clumps are laid in many different sites). Even so, the site of egg deposition is a useful adjunct to egg identification and species biology and has been incorporated in the definitions of breeding modes (Crump, 1974; Haddad and Prado, 2005). No available classification easily accommodates all variations of oviposition sites without being excessively complex. We suggest that the environmental conditions to which eggs are exposed would be a more informative feature than specifically where the eggs are deposited. Because eggs and embryos associated with a mobile parent's body (i.e., in or on a parent's body for all or part of development, whether terrestrial or aquatic; all endotrophic categories of Altig and Johnston, 1989), especially those with a physiological association with the parent, are subjected to a different set of environmental variables than eggs and embryos in the environment. We place them in a separate general category and note that their jellies, development, and morphology are different from other types. We thus recognize four major categories of oviposition sites (parent-associated, terrestrial, semiterrestrial, and aquatic) based on the suite of biological (i.e., predators) and physical (i.e., temperature and oxygen as mediated by
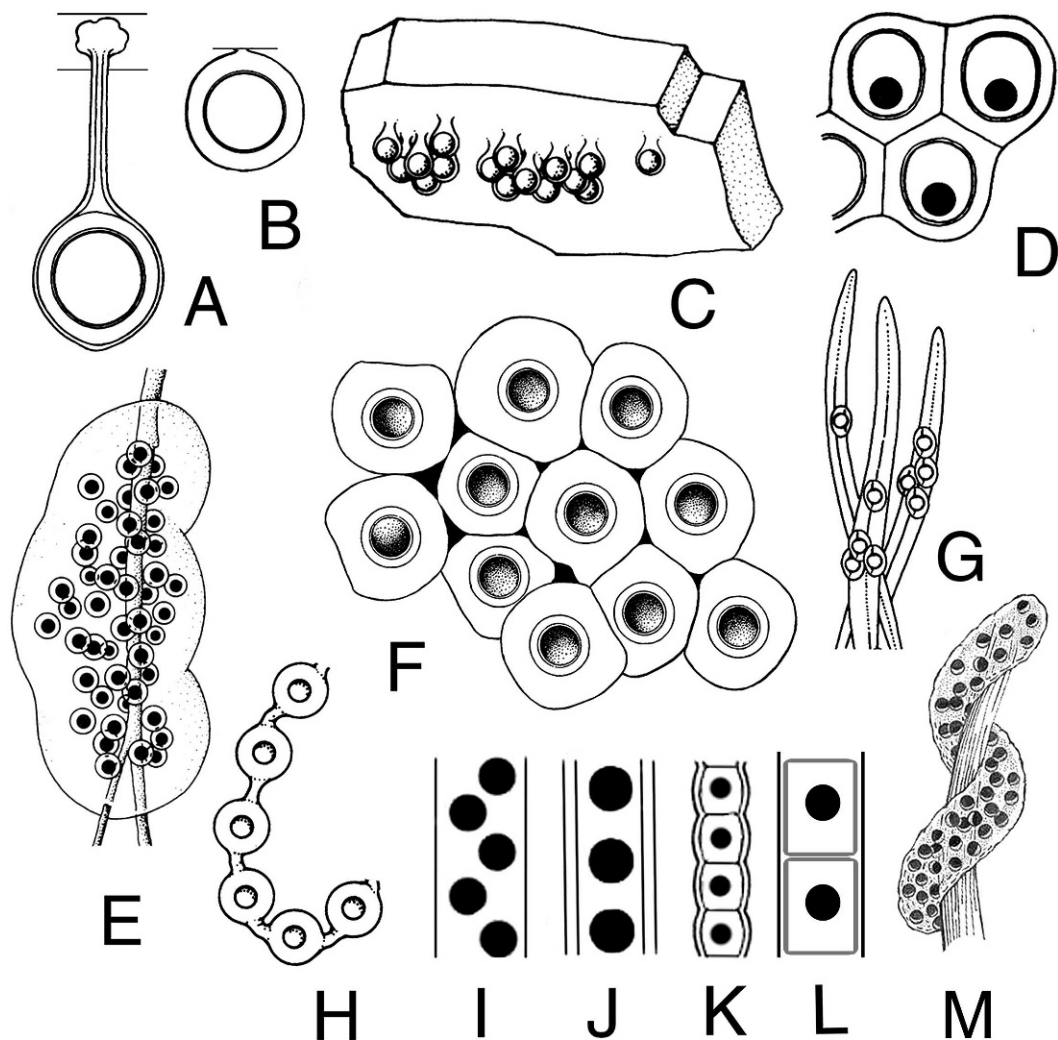
F<small>IG</small>. 3.—Ovipositional modes. (A) A suspended, pendant egg, a group of which would form an array or cluster depending on their arrangement, (B) a suspended, nonpendant egg, (C) an array, (D) a melded clump, (E) a mass, (F) part of an open clump with interstices blackened for emphasis, (G) single eggs attached to submerged vegetation, (H) a rosary, (I–K) egg strings that are (I) staggered in a unilayered tube, (J) uniserial in a bilayered tube, (K) uniserial in a bilayered tube with partitions and scalloped margins, a (L) schematic of two adjacent eggs in a string showing how jelly layers around each egg form partitions within the outer tube, and (M) a strand. A–B, D: modified from Stebbins, 2003, C, E, G, H: modified from Pfingsten and Downs, 1989; F: modified from Liu, 1950; I–K: modified from Wright and Wright, 1949, and M: redrawn by P. C. Ustach from Arnold and Burton, 1978.

water and light; Cohen and Strathmann, 1996) conditions that the eggs experience. Obviously each category can be divided into subcategories based on biological and physical factors.

Eggs in the parent-associated category experience a unique and species-specific range of physical conditions whether they are exposed to the air (e.g., *Alytes*: wound around legs of terrestrial male parent; *Hemi-*

phractus: attached openly on back of terrestrial female parent; Fig. 4A) or not (e.g., *Gastrotheca*: dorsal pouch of terrestrial female; *Pipa*: embedded in back of aquatic female). These conditions influence development more than whether the eggs are considered terrestrial or aquatic.

Terrestrial eggs, including those in foam nests and in arboreal, fossorial, and sub-
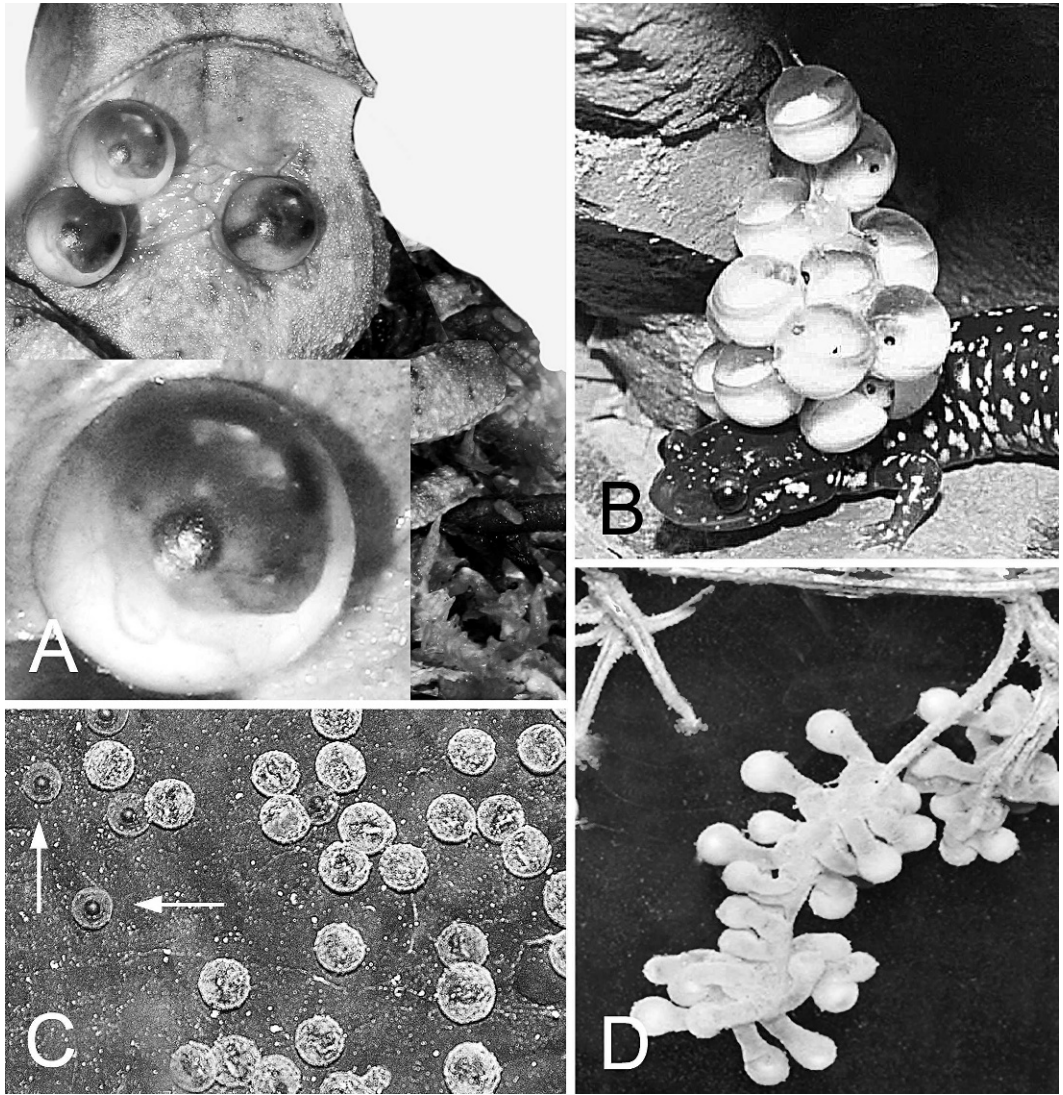
FIG. 4.—Ovipositional modes. (A) Eggs attached to the back of a female *Hemiphractus fasciatus* (Hemiphractidae: photo by E. Griffith), inset, most anterior embryo at larger size, (B) a cluster of *Plethodon albagula* (Plethodontidae; photo by S. E. Trauth), (C) an array of *Ambystoma barbouri* (Ambystomatidae) on the underside of a stone in flowing water (arrows = egg laid most recently by same or different female have less silt attached), and an (D) array of *Spea multiplicata* (Scaphiopodidae) placed haphazardly on vegetation in a desert pool.

terranean sites, are exposed to little or no free water at least at the time of oviposition. Both endotrophic and exotrophic forms deposit eggs in the terrestrial environment. The deposition sites often are near or above water, but they are not part of an aquatic habitat. As a result, predators on terrestrial eggs are usually very different from those in aquatic systems.

Semiterrestrial eggs are usually placed adjacent to some source of free water but are not submerged (e.g., in natural crevices or constructed burrows in a stream bank, natural or constructed depressions or chambers next to or in an area where a pond will form, among moss at the edge of a bog, in a seepy talus). The nest site is sometimes temporary in that it may become part of an aquatic system

TABLE 1.—Summary of the various ovipositional modes (i.e., clutch structure) discussed in the text.

Independent Eggs

Single (Fig. 3G)—eggs free or attached to substrates, singly or haphazardly grouped; arrangements governed by movement of parents during oviposition

Array (Figs. 3C, 4C, D)—singly placed eggs in some order, usually on a flat substrate; arrays on vegetation are more haphazardly arranged; eggs on lower surfaces of submerged object usually suspended and pendant

Laminar array (Figs. 2D–H, 5D)—outer jellies of most, closely-spaced eggs independently contact a substrate (i.e., one tier); various degrees of jelly hydration cause eventual shape of group.

Cluster (Figs. 3A, 4B)—small number of independently oviposited, suspended, pendant eggs with adjacent or overlapping pedicels; usually terrestrial

Three-dimensional Arrangements

Clump (Fig. 2I, 3D, F)—a multitiered stack of aquatic or terrestrial eggs that lack a common, surrounding surface or matrix; interstices among eggs common at least early in development; adjacent jellies remain distinct even if melded; adjacent eggs adherent or not

Mass (Fig. 3E)—each egg has an individual jelly layer(s) plus an outer jelly layer that melds imperceptibly with its neighbors so that ova appear embedded in a jelly matrix (i.e., the eggs and their jelly layers are like marbles embedded in a volume of gelatin, the matrix); interstices between adjacent eggs absent

Sac (Fig. 5F–G)—a turgid, sausage-shaped covering formed by the basal portion of the oviduct that enclose all ova once the jelly layers around individual eggs are formed by more anterior zones of the oviduct

Floating

Film (Fig. 2A–C)—adherent or coherent, usually single-tiered, group of eggs that float at or on the surface

Froth Nests

Foam (Fig. 5B–C)—each ovum with an independent jelly layer(s) embedded in a foam formed by many, small bubbles being trapped throughout more fluid parts of the jelly by leg motions of the parents

Bubble (Fig. 5A)—a film or multitiered group of eggs supported by relatively few, larger bubbles captured by the undersurface of the jelly; hind limbs of parents not involved in bubble production; limnodynastid females paddle with the front limbs and pass bubbles beneath their bellies that float upwards under the eggs; one or both parents of some microhylids expel bubbles from the nares beneath a film

Linear Arrangements

Bar—a short string, sometimes multiple short strings of eggs attached at their bases; may result at times when the outer jelly layers of adjacent single eggs adhere to each other temporarily

String (Fig. 3I–L)—lengthy, uni- or bilayered outer jelly tube encasing a uniserial or biserial series of ova; each ovum with independent jelly layer(s)

Strand (Fig. 3M)—lengthy, multiserial group of eggs in a large diameter, flimsy tube

Rosary (Fig. 3H)—lengthy, uniserial group of eggs in a string with jelly constricted between successive ova

either through flooding or some form of adult behavior.

Aquatic eggs that are placed in a lentic or lotic site, in bromeliads or other phytotelms, and in tree holes are submerged or nearly so and experience distinct conditions particularly associated with oxygenation. Within this context, the differences between a stream and a temporary puddle are obvious, but some cases can be subjective. Water at lentic sites may flow temporarily after heavy rains, and the flow rate of some lotic sites is so slow that they effectively represent lentic habitats. Consideration of how an aquatic system functions as a unit through time (e.g., bank configuration, basin shape, vegetation patterns, and debris distributions) and the bi-

ology of the amphibians that breed in them usually will resolve conflicts in microhabitat classification.

OVIPOSITIONAL MODES

In the following pages we describe five categories of ovipositional modes: various arrangements of independent eggs, three-dimensional arrangements, floating arrangements, froth nests, and linear arrangements. Among these categories, the constructs of the 14 ovipositional modes are summarized in Table 1. Although the specific events that occur during oviposition were not instrumental in defining the modes, knowing that information often enhances one's understanding of the how the final clutch structure was formed.

Independent Eggs

We identified four arrangements of independent eggs that are most often oviposited singly or a few at a time and do not produce an organized clutch structure. The arrangement of the eggs most often results from the behavior of the breeding pair.

Single eggs.—Single eggs can be scattered free, attached to substrates (Fig. 3G) in the open or wrapped in aquatic leaves (Orizaola and Braña, 2003) but usually are not pendant. These eggs may be positioned singly or in haphazard groups (i.e., as individuals placed onto substrates); eggs deposited close to or on top of each other usually have associated outliers (e.g., *Ambystoma mavortium*, *Pseudacris crucifer*; Fig. 3G) that signal that such a group is not a clump.

Array.—An array is a group of eggs suspended individually from the lower (under surface of rocks - some plethodontine salamanders [Fig. 3C] and *Ambystoma barbouri* [Fig. 4C]) or attached to the upper side of rocks or tree roots (e.g., some cycloramphid and phrynobatrachid frogs). Each egg that is oviposited and attached independently may be pendant or not and often has a distinct pedicel composed of various jelly layers. The eggs may be placed in an area with a diameter as large or larger than the length of the adult, and individual eggs may or may not contact neighboring eggs. Arrays attached to a single surface are orderly, but the group appears more haphazard if it is deposited on multiple stones or vegetation. The orderliness of the egg arrangement is also likely a function of the female's behavior (e.g., Giaretta and Facure, 2004). Eggs of some *Spea* form a haphazard array on sprigs of vegetation (Fig. 4D), and differential hydration of the jelly around each ovum forms a stalk without the ovum being suspended or confined by other eggs.

Laminar array.—A laminar array is defined by the method of egg attachment to a substrate—all eggs are attached individually to a substrate, usually in a single tier—like on a leaf overhanging a water body. The shape of the array may change dramatically depending on whether the top or underside of a leaf is used and on the subsequent pattern of hydration. Eggs on the upper surface of leaves frequently absorb water from rain and swell considerably. Those on the undersides of leaves (e.g., Lima et al., 2007) have less direct contact with rain water, may not swell appreciably, and are relatively spherical or planar. Jelly layers of adjacent hydrated eggs often meld imperceptibly to produce what appears to be a melded clump or even a mass, but eggs in clumps and masses are laid in multiple tiers. Ova in laminar arrays may be pigmented or not.

A hydrated clutch of *Guibemantis depressiceps* (Mantellidae) looks like a clump, but in fact, all the ova are usually in a single tier (Fig. 2D) and a 'stalk' from each egg (Fig. 2F) partially supports their attachment to the upper surface of a vertically-oriented leaf overhanging a pool (Fig. 2E). Only by observing oviposition and subsequent effects of hydration is the real structure revealed. Based on pictures of hydrated clutches from other species, we assume that all species of '*Mantidactylus*' (Fig. 2G–H) have stalked eggs and produce this sort of laminar array. Other species that produce similar arrays include phrynobatrachids (e.g., *Phrynodon sandersoni*, Amiet, 1981), many centrolenids (Kubicki, 2005; RA and RWM, personal observations; Fig. 5D), some microhylids (e.g., *Oreophryne* spp., Johnston and Richards, 1993), and many hylids (e.g., species in the *leucophyllatus*, *microcephalus*, and *parviceps* groups of *Dendropsophus*, Faivovich et. al., 2005, RA and RWM, personal observations). The closeness of the eggs, consistency of the jelly, and amount of hydration may influence clutch shape. If the jelly is relatively stiff (Figs. 2F, H, 5D) and becomes significantly hydrated, the clutch may be thick and variously shaped; in contrast, a clutch with watery jelly with rather little hydration may be quite thin (Fig. 2G). In all cases, the jelly becomes more watery as embryos approach hatching. The entire jelly volume of some centrolenid and hylid eggs with this ovipositional mode droops well beyond the original boundary of the attached array and forms a "drip-tip" (Schlüter, 2005:figs. 64 and 65); tadpoles frequently hatch from the eggs during rainstorms and slide downwards off the tip of the leaf or exit the drip tip into water below.

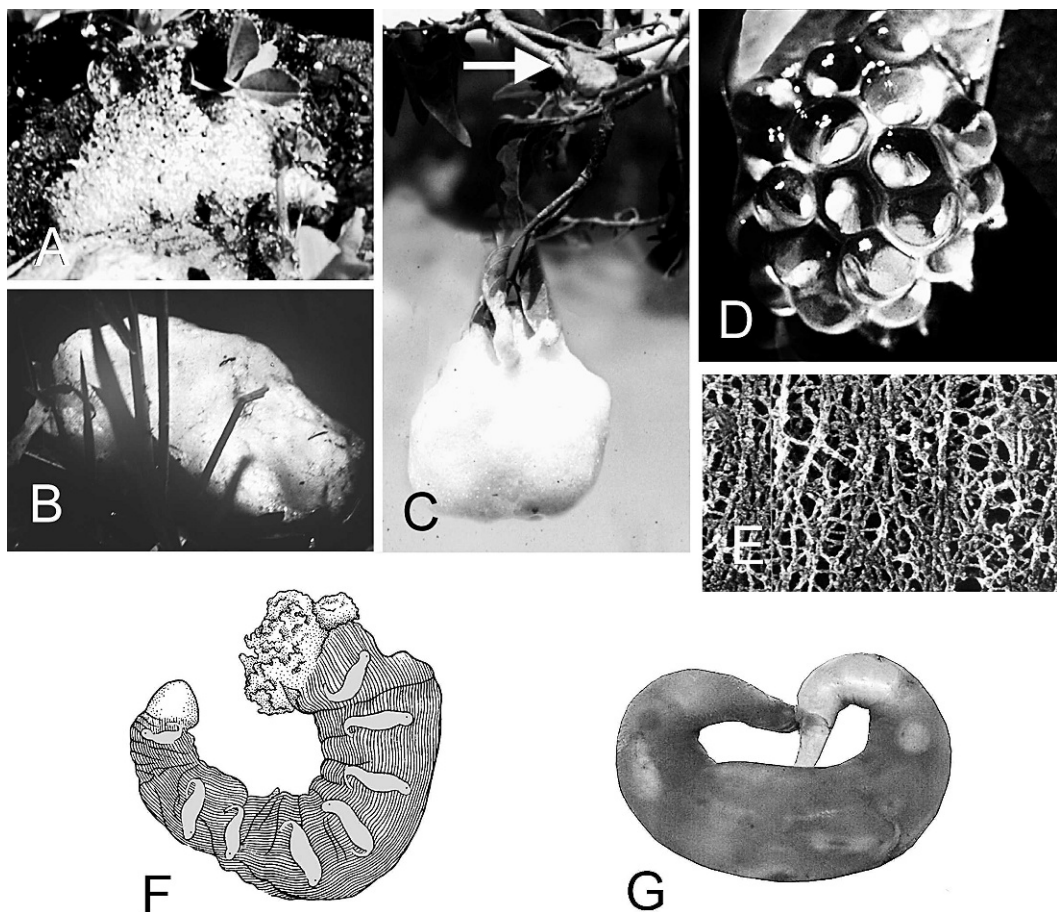*Cluster.*—A cluster (Figs. 3A, 4B) is a group of a few, individually suspended, pendant eggs

Fɪɢ. 5.—Ovipositional modes. (A) Bubble nest of *Limnodynastes* sp. (Limnodynastidae) (B) foam nest of *Physalaemus pustulosus* (Leiuperidae), (C) foam nest of *Chiromantis xerampelina* (Rhacophoridae) with attendant parent (arrow), (D) arboreal clump of *Hyalinobatrachium fleischmanni* (Centrolenidae) on the underside of a leaf, (E) electron micrograph of outer jelly layer of *Xenopus laevis* (modified from Bonnell and Chandler, 1998), (F) sac of *Batrachuperus karlschmidti* (Hynobiidae) with embryos shaded for emphasis (modified from Liu, 1950), and (G) sac of *Hynobius kimurae* (Hynobiidae; Southern Illinois University H-07590).

in one tier (dependent on the substrate) that are typically laid by terrestrial salamanders. The pedicels of the few large eggs lie close to or on top of each other, and adjacent eggs usually contact their neighbors (e.g., Paine, 2005).

Three-dimensional Arrangements

Eggs are arranged in some sort of three-dimensional arrangement in three ovipositional modes.

Clump.—A clump (Fig. 3D, F) is a multi-tiered stack of aquatic or terrestrial (Fig. 2I) eggs, that lack a common, surrounding surface or matrix; adjacent jellies remain distinct even if melded and may be adherent or not. A clump is analogous to a stack of marbles, although in terrestrial settings, the stack sometimes slides into a single tier (e.g., plethodontids). The surfaces of adjacent eggs may be turgid enough to form interstices (i.e., 'open clump'; Fig. 3F) or sag or fit together without interstices (i.e., 'melded clump'; Fig. 3D) either at the time of oviposition or later. One can pour colored water over an open clump and see the water percolating through the interstices. A similar morphology results when single eggs are haphazardly placed close to or on top of each other; however, clumps do not have outlier eggs.

Thus, one must examine clumps closely to determine their actual morphologies.

Most clumps involve relatively few eggs. In contrast, many ranids lay eggs in large, aquatic clumps that are open at least at oviposition and deposited as a single unit. Adjacent jellies are adherent. Even after the jellies swell or sag enough to obliterate the interstices, a regularly lobate surface is different from the smooth to irregular surface of a mass (see below). Some aquatic and terrestrial clumps commonly have one surface attached to a substrate or surrounded by a folded leaf (e.g., *Phyllomedusa hypochondrialis*, Pyburn, 1971) and may result from multiple oviposition bouts (e.g., *Pachymedusa dacnicolor*, Bagnara et al., 1986). Other aquatic clumps are typically rounded in top view, horizontally oblong, and commonly surround a piece of aquatic vegetation. Old, aquatic clumps with embryos nearing hatching may break free from their supports and float to the surface, often with many bubbles trapped in the jelly; the bubbles and multitiered structure distinguish them from surface films.

Mass.—In a mass of eggs (Fig. 3E), each egg has an individual jelly layer(s) that appears to be embedded in a jelly matrix (i.e., the eggs and their jelly layers are like marbles embedded in a volume of gelatin, the matrix; see below). Colored water poured over a mass flows only over the surface, which may be irregular but is never regularly lobed like that of a melded clump. The matrix may be moderately sparse or voluminous relative to the volume of the ova, which gives the impression of the ova either being uniformly distributed within the mass (e.g., *Pseudacris*) or absent from the periphery of the mass (e.g., *Ambystoma*). The material forming the matrix is not extruded separately from the outer layers of jelly surrounding the ova (see below). The egg jelly of most species of *Ambystoma* or *Pseudacris* is watery and usually falls apart if handled. In contrast, masses of *Ambystoma gracile* and *A. maculatum* have a voluminous, dense matrix and retain their shape when removed from the water. Most masses start to disintegrate at or immediately after hatching, but the dense masses of these two *Ambystoma* often persist for more than a month after the embryos hatch (Regester et al., 2005; Regester

and Whiles, 2006). Most masses are vertically oblong and usually attached to vegetation, and females of *Ambystoma tigrinum* and others may deposit a clutch among several, widely-spaced masses (Gopurenko et al., 2006).

The fact that a mass may involve an entire or a partial clutch reveals something about its formation. The inner jelly layer(s) around each ovum is distinct, but the more voluminous outer layer is indistinct, seemingly homogeneous, lacks a defining outer membrane, and melds imperceptibly with neighboring jellies. Thus, the outer egg jellies form the seemingly homogeneous matrix of the mass. The widths of the hydrated outer layers determine the distribution and spacing of the ova within a mass. If the width of the outer jelly layer is thin, eggs appear uniformly distributed throughout the mass (e.g., *Pseudacris*); if the layer is thick, peripheral eggs appear more widely spaced and particularly isolated from the margin of the mass (e.g., *Ambystoma*).

Sac.—Sacs (Fig. 5F, G) are unique to hynobiid salamanders. They are turgid sacs that enclose all ova once the layers around individual eggs are formed by the penultimate zones of the oviduct (Greven, 2002, 2003). Females usually extrude two unfused sacs per ovulation event, one from the ovisac of each oviduct. The sac is usually shaped like a curved or spiraled sausage with a pointed distal end, which emerges first and serves as the pedicel. According to Liu (1950; also Sato, 1992) the wall of the sac of *Batrachuperus karlschmidti* is striated longitudinally, the embryos lie at 90° to the axis of the sac, and hatchlings escape by forcing off the distal cap. Hasumi (1996) noted that it took about 18 h for the sac to form in *Hynobius nigrescens* after all eggs arrived in the ovisac. The secretory mechanics that form a sac around a group of eggs needs further study.

Floating Arrangement

A single mode of floating eggs is described.

Film.—A film is usually a single sheet (i.e., a two-dimensional or planar tier) of either 'adherent'(i.e., outer jelly surface sticky so that adjacent eggs adhere to each other; some hyline and pelodryadinine hylids and ranid frogs) or 'coherent' (i.e., outer jelly surface not

sticky so that adjacent eggs merely cohere and can be pushed apart easily; many microhylid frogs) dark or black eggs that float at or above the water surface of a puddle or pond. Some pelodryadinines deposit what appear to be multi-tiered surface films (RA, personal observation). The statement by Schlüter and Salas (1991) that the "entire clutches [of *Chiasmocleis ventrimaculatus* and *Ctenophryne geayi*] are held together by a thin layer of viscous jelly" needs verification; the "layer of viscous jelly" they observed may actually be the meniscus distorted by the egg jellies. Even light rain causes surface films to sink, and eggs wetted by rain usually do not float again. Films also may sink when the embryos approach hatching. In either case, a sunken film appears as a sheet draped over vegetation or lying on the bottom. A film may consist of part or all of the eggs in a clutch. Films appear as single groups of eggs in species (e.g., some microhylids) whose amplectant pairs do not move around much between ovipositional bouts. In other species (e.g., some hylids and other microhylids) the amplectant parents may move around the pond or even between puddles with each egg-laying bout, splitting the clutch among multiple ovipositional groups of eggs (= 'rafts'). In most cases, egg films remain afloat until after the embryos hatch, but they may sink at an earlier stage if they are physically disturbed or rained upon.

Outer layers of jelly deposited in water are somewhat hydrophobic at the moment of oviposition. If eggs that typically are deposited below the water happen to be extruded above the water surface, part of the outer jelly surface may remain unwetted, causing some of the eggs to float (e.g., *Scaphiopus holbrookii*; RA, personal observation). Although these are not films, one must conclude that species that produce films must have some behavior whereby the vents of the parents are temporarily lifted above the water surface. In hylids and microhylids, this is accomplished by the amplectant pair tipping head-downward for a few seconds while eggs are extruded (e.g., Pyburn, 1967; Webb, 1971). In at least some North American ranids, the female arches her back downwards, briefly placing her vent above the water surface (Aronson, 1943). In either case, extruded eggs, presumably surrounded with either hydrophobic fluids or flimsy jelly layers, spread outwards as they fall upon the water surface. Eggs in such films may rest entirely on the water surface (some microhylids), be partially submerged (other microhylids), or be submerged so that the upper surface of the outer jelly lies at the water surface (some hylids and ranids). Eggs that rest on the surface have asymmetrical egg jellies that likely facilitate floatation (e.g., species of *Kaloula*, Liu, 1950).

Eggs of *Hyla chrysoscelis* are laid as multiple rafts of single-tiered, adherent films. The rafts may drift together and adhere, and if a breeze occurs, all films may end up on one side of a pond and not at the actual ovipositional sites. In freshly laid eggs, the top part of the upper hemisphere of the outer jelly layer is at the water surface but not flattened; the ovum is centered at the radius of the outer layer. An amorphous, very watery gel that is not visible except by careful examination in the laboratory lies external to what appears as the outer jelly layer. This adherent gel is more or less equidimensional around the more discernible next inner layer in submerged eggs. Observations through a microscope suggest that pressure caused by the weight of the egg sinking through the surface distorts this flimsy gel toward the top of the outer jelly layer to form a flat, hydrophobic, surface that affords flotation for the egg.

Eggs of *Gastrophryne carolinensis* are oviposited as coherent films and lack the rim-like protrusion of the outer jelly that is found in several Old World microhylids. The eggs float with about the upper fifth of the outer jelly hemisphere above the water surface (Fig. 2C). The jellies apparently lose turgidity after a few hours, and the top of the outer jelly then lies flattened and nonwetted at the surface. The ovum remains centered within the jelly so that its upper surface lies slightly below the plane of the water surface. Wright and Wright's (1949; also Noble, 1927) notations that the jellies of *G. carolinensis* and *Hypopachus variolosus* are flat-topped spheres apparently was based on observations of eggs a number of hours after oviposition. This nonstructural configuration apparently is

a function of surface tensions and hydrophobic characteristics of the jelly. Jellies of submerged eggs are spherical.

Froth Nests

Froth nests are mixtures of oviductal secretions (Bhaduri, 1932; Kabisch et al., 1998) and air that have groups of eggs dispersed through them. Such nests are produced by certain hylids (Haddad et al., 1990), hyperoliids (Amiet, 1974), leptodactylids (e.g., Hödl, 1990; Schlüter, 1990; Shepard and Caldwell, 2005), limnodynastids (e.g., Littlejohn, 1963; Tyler and Davies, 1979), microhylids (e.g., Glaw and Vences, 1992; Haddad and Hödl, 1997), and rhacophorids (e.g., Coe, 1974; Fukuyama, 1991; Liu, 1950); and see Duellman and Trueb (1986). Suggested functions for froth nests include: escape from the aquatic environment (Heyer, 1969), protection of eggs and embryos from desiccation and thermal damage (Gorzula, 1977; Heyer, 1969; Hödl, 1986), floatation for aquatic eggs (Haddad and Hödl, 1997), protection from aquatic predators and cannibals (Hödl, 1990; but see Drewes and Altig, 1996 and Menin and Giaretta, 2003), and enhanced oxygenation of the eggs and embryos either from being held near the meniscus (Haddad and Hödl, 1997) or from the air trapped in the bubbles per se. There are two types of froth nests: foam and bubble.

Foam nests.—Foam nests typically are formed when one or both parents whip oviductal secretions with the hind legs to trap numerous small air bubbles. The foam of *Scinax rizibilis* is produced by the pair jumping onto the jelly. The elaborate sequence of kicking and wiping behavior (e.g., Heyer and Rand, 1977; Hödl, 1990), which enhances the mixing of sperm and eggs, varies among species. This behavior likely evolved from general movements used by the parents to stabilize or maintain their positions in the water (e.g., Rabb, 1973; *Pipa pipa*). Hödl (1990; *Physalaemus ephippifer*), Liu (1950; *Polypedates leucomystax*, and Coe (1974; *Chiromantis rufescens*) indicated that the foam-forming material is distinct from and released prior to egg extrusion. In contrast, Heyer and Rand (1977, *Leptodactylus pentadactylus*, *Physalaemus pustulosus*), Ryan

(1985, *Physalaemus pustulosus*, Fig. 5B), and seemingly Schlüter (1990, *Edalorhina perezi*) pointed out that it is a jelly-ova mixture that is whipped into foam. Further detailed observations are needed in all cases. Perhaps the ova with their jelly layers and an oviductal secretion(s) that is whipped are released at the same time. Simultaneous release of eggs and secretions has been reported for *Polypedates bambusicola* by Liu (1950). Coe (1974) described and illustrated a discrete structure called a 'foam gland' that formed from three large oviductal folds along the posterior sections of the oviduct in *Chiromantis rufescens*; secretory cells lining the lumen reportedly contained 'foam parent material.' Similar structures present in foam-nesting *Leptodactylus* spp. (RWM, personal observation) will be reported elsewhere.

Foam nests are formed in aquatic, arboreal, subterranean, and terrestrial sites, and are generally the result of a single reproductive bout. Schlüter (1990) reported up to three nestings by single pairs of *Edalorhina perezi* (Leiuperidae) in a single night in captivity. Whether this happens in other species is not known. Males of *E. perezi* also are reported to smooth the surface of the foam between egg laying bouts (Schlüter, 1990). In most species, a foam platform is built before the ova are released. As eggs are extruded, the male catches them in a 'basket' formed by his hind feet, presumably fertilizes and moves them dorsally onto his back, and then pushes them into the foam with his hind legs (e.g., *Physalaemus ephippifer*, Hödl, 1990). In those species studied, eggs are released in several bouts and scattered throughout the mass of foam, and trophic eggs are sometimes deposited in foam nests (Prado et al., 2005). In arboreal cases the outer portion of the foam dries to a crust (Fig. 5C; Coe, 1974). Bossuyt and Milinkovitch (2000) suggested that arboreal foam nests of Asian *Polypedates* spp. could be an initial step toward direct development on land or trees (see Altig and Crother, 2006). Actions of hatchlings and enzymes eventually degrade the inner portion of the foam, and the hatchlings stream out into the water or drop into the water in arboreal species of *Chiromantis* spp. and *Polypedates* spp. (Rhacophoridae; Liu, 1950; Coe, 1975).

Brizzi et al. (2003), Duellman and Trueb (1986) and Hödl (1990) discussed variations in foam nests and provided pertinent citations. The 'foam' in nests of the hylid frog *Scinax* cf. *rizibilis* was white, fragile, and full of large air bubbles (Haddad et al., 1990); these authors were able to produce foam by manually beating the mucus present in the spawn of *Scinax heimalis*, a close relative of *S. rizibilis*, although a foam nest is not known in this species. Perhaps foam can be formed from the jelly of any species that has watery jelly if parental behaviors were modified. Haddad and Hödl (1997) pointed out that the foam nests of *S. rizibilis* seem intermediate between a typical foam nest and the bubble nest of *Chiasmocleis leucosticta* (see below), and commented further that *Scinax* foam is produced by "a few leaps of the female on the eggs and jelly." If true, we predict the absence of 'foam glands' in these species of *Scinax*.

Small groups of tadpoles of *Leptodactylus fuscus* and related species can produce froth from skin secretions and active wiggling well after the original foam nest has disappeared (Caldwell and Lopez, 1989; Downie and Smith, 2003). The larval froth is clear with larger bubbles than the white foam produced by adults and allows larvae to survive for longer periods out of water. The results of this behavior appear adaptive, but whether the actions are part of the tadpole's behavioral repertoire or byproducts of wiggling and air gulping in a mucin-rich and probably oxygen-poor environment is not known.

Bubble nests.—Bubble nests resemble foam nests, but differ in having relatively few, large bubbles. The resulting nest is typically flat to dome-shaped in aquatic or subterranean sites. The bubbles are formed in two different ways. Some female limnodynastids (Tyler and Davies, 1979; Williams and Tyler, 1994), with flanges on some fingers, place their hands successively above the water and then paddle down and back to propel bubbles along their bellies; the bubbles float upwards under the surface film of oviposited eggs (Fig. 5A). This may be another case in which escape or stabilization maneuvers have been co-opted for breeding functions. In contrast, pairs of *Chiasmocleis leucosticta* (Microhylidae) deposit a surface film typical of most aquatic-

breeding microhylids. After oviposition is complete, either the amplectant pair or the male alone swims below the floating eggs and expels air bubbles from the nostrils (Haddad and Hödl, 1997). Presumably, eggs in bubble nests develop faster in the better oxygenated surface water and derive additional respiratory benefit from the bubbles themselves.

Linear Arrangements

There are four morphological types of linear arrangements.

Bar.—A bar is a short, linear arrangement of eggs formed when the outer jelly layers of adjacent eggs adhere to each other. A bar is reminiscent of a short string of bufonid eggs, but without the outer tube(s); several bars can attach to each other at a common point to form a rosette (Wright and Wright, 1949:plate 6). Whether a bar is real or a temporary happenstance of single eggs remains unclear. The outer tubes enclosing the eggs of *Bufo debilis* and *B. quercicus* (Volpe and Dobie, 1959) are so diaphanous that they may not be noticed, and these strings may look as if they are arranged in a bar. The single eggs of *Bufo punctatus* sometimes temporarily cohere to form a bar or small groups of eggs one tier thick (Stebbins, 1951; Miller and Stebbins, 1964:fig. 107).

String.—Strings (Fig. 3I–L) consist of a single or double series of ova aligned linearly inside either a uni- or bilayered jelly tube; how the one or two tubes are formed around a linear group of eggs is not known. An entire clutch usually is laid as one, continuous, and usually lengthy cord. The outer tube collects silt but usually does not stick to itself; uncommonly it is so diaphanous as to be nearly invisible, and it is variably fragile (Sweet, 1993; notably more fragile in *Bufo californicus* than in *B. boreas*). One or commonly two strings are extruded simultaneously, and an unknown tensile quality of the jelly often causes the strings to lie initially in a loose spiral. Slight constrictions between successive ova may cause the outer surface of the tube to appear scalloped (e.g., *Bufo cognatus*, Bragg, 1936, 1950), and partitions form by the juxtaposition of jelly layers surrounding successive ova (Fig. 3L). Movement of the amplectant adults during ovipo-

sition causes the string to be wound around stems and leaves of aquatic plants or haphazardly about the bottom of the breeding site.

Rosary.—A rosary (Fig. 3H) is a string of usually large and widely-separated ova in which the jelly between successive eggs is distinctly constricted and sometimes twisted; whether the jelly is twisted during or after oviposition is not clear. All components of the tube appear to be continuous, but each ovum may also have its own associated layers. A rosary commonly becomes twisted upon itself and may appear as a clump in terrestrially breeding species (e.g., caecilians). The common inclusion of large ova is a striking feature of this clutch form whether it occurs in caecilians, salamanders that breed aquatically (e.g., cryptobranchids), semiterrestrially (e.g., amphiumids), or terrestrially (e.g., various plethodontines), or frogs (e.g., leiopelmatids, alytids, some scaphiopodids).

Strand.—A strand (Fig. 3M; *Pelobates*) is a large diameter tube with flimsy, sometimes indistinct walls that contain multiple rows of eggs that may meld together to form what appears to be a mass. The rows of ova are not arranged in lines as orderly as those in strings. Strands typically are wound around aquatic vegetation, often from bottom to top, or short strands may be attached to vegetation or to the bottom.

EVOLUTION OF CLUTCH STRUCTURE
Selective Factors

Amphibian eggs are rather fragile structures that are subject to a variety of selective factors in both the physical and biological environments in which they develop. Primary among these are the abiotic factors of moisture, temperature, and oxygen and the biological factor of predation (e.g., Holomuzski, 1995; Magnusson and Hero, 1991; Malone, 2006; Mills et al., 2001; Woods, 1999). We contend that the complex interaction among these ecological factors and their temporal and spatial patterns in those habitats in which amphibians evolved have resulted in the clutch structures characteristic of amphibians. For purposes of this discussion, we assume that the ancestral amphibian laid single eggs (Mamay et al., 1998) in an aquatic environ-

ment. One can imagine that the dispersion of eggs (highly clumped to widely scattered) would pose different challenges for egg-eating predators and, depending on the types of predators and their ability to locate and consume eggs, might favor the evolution of different ovipositional modes. One set of conditions might select for dispersed eggs (e.g., single eggs scattered over a large area), whereas another would favor groups of eggs placed in areas that were difficult to locate.

At the same time, differences in moisture availability (ephemeral puddles versus permanent ponds), temperature (shallow and exposed versus deep and shaded pools; temperate versus tropical habitats; lowland versus montane environments), and oxygen concentration (surface versus bottom) would have been important environmental variables that may have favored one type of egg or ovipositional mode over another. While these environmental factors are easy to measure today, their evolutionary impacts are more difficult to demonstrate. Nevertheless we suggest that predation on single eggs by bottom feeding organisms (e.g., fish, other amphibians, crustaceans, insects, leeches) had a major selective impact on the placement of eggs in ancestral environments. One solution to the predation problem would be to hide eggs beneath structures (e.g., under rocks in streams) which would generally decrease, if not eliminate, exposure to some bottom feeding predators. Other solutions might involve placement of eggs on aquatic vegetation above the bottom or into the surface film. Such shifts could potentially facilitate development by exposing eggs to higher oxygen concentrations and warmer temperatures (e.g., surface film) that result in faster development and thereby a temporal escape from predation. Avoidance of predators and exposure to better developmental conditions could also serve as the primary selective factors resulting in shifts from one aquatic ovipositional mode to another. Placing the eggs completely out of the water on overhanging leaves or in burrows adjacent to the stream or pond would eliminate exposure to all aquatic predators. Such shifts would, however, expose eggs to a different suite of environmental problems (e.g., desiccation,

terrestrial predators) and favor the evolution of elaborate behavioral traits (e.g., froth nests, egg guarding, tadpole transport) and direct development. The latter mode is often accompanied by changes in the outer jelly layers and parental attendance.

Evolution of Ovipositional Modes

Observations of actual oviposition are uncommon, and hypotheses about the evolution of ovipositional modes are almost completely lacking. Primitive fishes (i.e., some lungfishes, gars, paddlefish, and sturgeon) either scatter single eggs on the bottom or among vegetation, and a few reports of eggs of primitive amphibians exist. Mamay et al. (1998) described groups of eggs assumed to be those of a dissorophoid temnospondyl (Lower Permian, Wichita Group, Leonardian Series; Baylor County, Texas). Based on terminology presented in this paper, those eggs were likely oviposited as grouped singles or clumps. The diameters of the ova and eggs (~0.5 mm) are comparable to those of small, extant frogs. A vitelline membrane and one jelly layer also appears around each ovum. Witzmann and Pfretzschner (2003) discussed the ontogeny, morphology, and biology of an animal that may have been similar to one that developed from those eggs. Špinar (1972) reported ovarian or abdominal eggs (up to 0.6 mm diameter) in *Palaeobatrachus* from the Tertiary of central Europe but provided no information on egg or clutch structure. With no evidence to the contrary, we consider single eggs as an appropriate starting point for discussing the evolution of amphibian oviposition modes. Based on observations of oviposition in a few species of temperate *Rana*, Aronson (1943) posited that a surface film type of oviposition likely was derived from some sort of a submerged egg group oviposition. Given the behavioral coordination required of both parents to produce a film, we agree.

The known diversity of ovipositional modes in amphibians is not high, and with few exceptions (e.g., sacs in hynobiid salamanders), modes that appear structurally similar are scattered throughout the Order. That is, there seem to be relatively few ways to package a series of spheres, and no clear

TABLE 2.—Changes in egg and clutch morphology that may represent evolutionary modifications during the processes of egg production and deposition. Oviductal processes = physiological and chemical changes in secretory sites and their products that affect the ova as they pass down the oviduct. Ovipositional modifications = morphological changes to eggs and clutches effected by behavioral changes of parents at the time of egg deposition. Postovipositional changes = changes in the physical structure and chemical composition of the jelly.

Oviductal Processes
 Increase or decrease in the number of jelly layers/zones
 Change in stickiness so eggs are coherent or adherent
 Shift from single eggs to string
 Shift from single eggs to rosary
 Change in hydrophobicity of outer jellies
 Change in hygroscopic capacity of jelly components

Ovipositional Processes
 Shift from single eggs to clump
 Shift from single eggs to surface film
 Shift from egg mass to surface film
 Shift from single eggs to array
 Shift from single eggs to cluster
 Shift from single eggs to froth nests

Postovipositional Processes
 Development of a capsular chamber
 Shift from clump to a mass
 Shift from string to strand

patterns of relationships between ovipositional mode and systematic position are known. These observations suggest that the evolutionary transition from one mode to another is not a difficult matter, and that the resulting morphological diversity likely is a consequence of shifts in three aspects of amphibian biology: changes in oviductal function, changes in ovipositional behavior of the parents, and postovipositional changes in the jelly layers (e.g., formation of capsular chamber; also see Lindsay and Hedrick, 2004). Variations in the morphological and physiological components of the female reproductive tract provide the material on which selection can operate and likely involve shifts (Table 2) in the following components: timing of reproduction, rate and synchrony of ovum maturation, frequency of ovulation within and among clutches, rate of egg passage down the oviduct, and changes in the number, position, and function of various secretory sections of the oviduct. Any changes in the reproductive biology of females must also be accompanied by concomitant changes in the physiology and behavior of males, and both are subject to natural selection from

abiotic and biotic components of the habitats in which amphibians breed.

A. Kupfer (personal communication) noted that 1–2 h may pass between successive expulsion events of the few eggs in a rosary clutch of *Ichthyophis* cf. *kohtaoensis*. An interaction between the rates of ovulation and jelly formation likely explains this slow rate of oviposition. It also seems likely that the rate of jelly formation is the primary controlling factor in most cases, although in the narrow confines of the vermiform body of a caecilian, the ovulation rate of the rather large eggs may also be constrained. In contrast, the ovipositional periods (time between first to last egg) of an hour or less in North American *Hyla* and *Rana* indicate that all eggs have been ovulated and jellies produced before oviposition begins. An intermediate situation may occur in *Bufo* that lay long strings of eggs. In these species the clutch is laid over several hours with periodic expulsions of sections of the string; this pattern suggests that jelly formation and perhaps even ovulation have not been completed prior to the start of oviposition. These responses may be a result of large clutch sizes that cannot be accommodated within the oviducts. Different reproductive strategies associated with the timing of ovulation relative to amplexus in frogs is another issue. If ovulation is stimulated by amplexus, as apparently occurs in some species of *Hyla*, the amplectant pair must wait some period for ovulation and jelly formation to occur before depositing eggs (Scarliata and Murphy, 2003; RA, personal observations). In contrast, ovulation and formation of jelly layers have already occurred at some point prior to amplexus in female *Pseudacris crucifer* (Oplinger, 1966) and *Acris* spp. (RA, personal observation), as females of these species begin to lay shortly after entering amplexus and females ready to oviposit can hardly be handled without eggs being extruded.

In summary, the reproductive patterns characteristic of living amphibians are products of a variety of selective regimes encountered during the course of their evolution. The diversity of modes reflects a long, complex evolutionary history and the morphological, physiological, and behavioral changes that have been refined by various ecological and energetic constraints (e.g., see Broomhall, 2004; Byrne et al., 2003; Kaplan, 1989; Lüddecke, 2002; Ogielska and Kotusz, 2004; Parichy and Kaplan, 1992, 1995; Seymour et al., 2000; Semlitsch and Gibbons, 1990; Smith et al., 2005). The eggs of *Hyla chrysoscelis* laid late in the season often accumulate gas bubbles released from bottom sediments and suffer total mortality by daybreak presumably from high temperatures and low oxygen concentrations (RA, personal observation), even though they are laid as a film. A review and synthesis of the timing and influences of factors such as the environment, adult morphology, endocrinology, behavior, phylogeny, and adult condition (i.e., all factors included in the definitions of breeding mode) that modulate vitellogenesis, ovulation, jelly formation, and breeding across taxa would provide additional information useful in understanding the evolution of these modes.

Changes in Oviductal Function

Changes in oviductal function across lineages likely are manifest in structural changes in the jelly layers and in clutch morphology (e.g., singles to strings; singles to sac; clump to mass; and perhaps strand-string-rosary transitions). Strings versus rosaries likely exemplify the influences of changes in ovulatory and oviductal functions (i.e., string: many small ova ovulated rapidly and passing quickly in succession down the oviduct; rosary: few, larger eggs ovulated less frequently and passing more slowly). The suspended, pendant eggs of some plethodontids could be derived from another mode if the few, large, infrequently ovulated eggs that typically form a rosary, were oviposited singly. These ideas would demand that secretory sequences of certain oviductal regions be modulated (e.g., continuous production of the outer jelly tube in bufonids; intermittent production in pendant eggs noted here). We presume that all areas of the egg surface are competent to form a pedicel at least for some short period immediately after oviposition.

Changes in Adult Behavior

Courtship behaviors differ appreciably between species with internal (most salamanders) versus external (most frogs) fertilization and those that deposit eggs in terrestrial

versus aquatic habitats (Duellman and Trueb, 1986). It seems likely that many changes in ovipositional mode are a result of changes in the behavior of adults, especially in frogs (see Hödl, 1990) where the presence of both parents could influence ovipositional behaviors. The different roles that males and females play during oviposition have been described in detail for only a few species (e.g., Miller, 1909: *Bufo americanus*; Aronson, 1943: *Rana clamitans* and *R. catesbeiana*; Aronson, 1944: *B. americanus*, *B. fowleri*, and *B. terrestris*; Aronson and Noble, 1945; Noble and Aronson, 1942: *R. pipiens*; Gosner and Rossman, 1959: *Pseudacris triseriata*; Hödl, 1992: *Pleurodema diplolistris*; and Williams and Tyler, 1994: *Limnodynastes tasmaniensis*). Male hynobiids sometimes pull the egg sacs from the female but this behavior does not modify the ovipositional mode. The parents, especially in those species with external fertilization, often engage in elaborate and coordinated behavior (e.g., frogs that build froth nests). In frogs, the position and behavior of the male relative to the female during external fertilization of eggs is especially variable. Knowing if and how the activities of each partner influence clutch structure, the timing of ovulation relative to oviposition, the patterns of oviposition (e.g., partial or entire clutch extruded/bout), and the co-ordination of behavioral signals between sexes would be valuable adjuncts to understanding frog breeding biology, as would insights into the presumed selective advantages of the various modes of oviposition (e.g., Moore, 1940). Future consideration of data on clutch structure from different lineages, examination of phylogenetic influences (e.g., Gomes and de Carvalho, 1995 and citations therein), and an understanding of the evolution of complicated behaviors and how such behaviors might influence clutch structure surely will improve the resolution of our preliminary ideas relative to the evolution of clutch structure within Amphibia.

For example, surface films usually are oviposited by the pair making temporary forward dips to place the vents of both individuals above the water surface, and gametes of both sexes are released onto the water surface. This behavior may have arisen as an aborted surface dive initiated primarily by the female and perhaps mediated by the male. Wright's (1914) description of oviposition and fertilization in *Hyla versicolor* seems untenable; he stated that "… fertilization took place beneath the surface of the water…" and "After each fertilization, the female would raise her vent above the water and lay 18 to 25 eggs." Fertilization surely had to accompany the release of the eggs. The oviposition of *Microhyla* sp. was closely observed during a frenzy of many pairs ovipositing at daybreak in southern Vietnam (R. Altig, personal observation). Pairs repeatedly made ovipositional dips without moving, and a single, conglomerate film of eggs accumulated directly behind each pair. One frightened female made a presumed escape dive in a similar manner and angle as an ovipositional dip; at that point, some fluid, presumably semen but it could have been urine, escaped the vent of the male. Without knowing what or if signals were being passed between the partners to coordinate oviposition and fertilization, one could interpret this observation as the male mistaking the escape movement for the next ovipositional dip. An analogous argument for the ovipositional initiation and completion of the loop-and-roll movement off the bottom in *Pipa pipa* (Rabb, 1973; Rabb and Rabb, 1960) could be made. Perhaps the process is initiated by the female (i.e., the loop, perhaps an aborted trip to the surface to breath) but modified or guided (i.e., the roll, attempting to intercept her progress) by the male. Detailed observations and analyses of other species are needed to clarify the origins of these behaviors. The question concerning the influences of males on ovipositing females remains open.

Another example of how ovipositional behavior influences clutch structure involves the egg rafts produced by some hylid frogs (e.g., *Hyla chrysoscelis*) that oviposit their clutches in multiple rafts. These packets likely are homologous to those parts of a clutch deposited as separate masses by other hylids (e.g., *Pseudacris* spp.). Stated otherwise, the surface rafts of some *Hyla* would form a mass if the eggs were oviposited underwater, and eggs of *Pseudacris* that normally are laid as a mass likely would produce a film if

oviposited onto the water surface. If an amplectant pair that lays a clump or mass moves sufficiently during oviposition, then a group could be distributed as singles, as in *P. crucifer*.

Building of froth nests is accomplished by some amazing behaviors by the parent frogs. Hödl (1990) presented a detailed description of the formation of a foam nest in *Physalaemus ephippifer*. Some movements of the pair are similar to those of other frogs and may have been derived from the two-legged frog kick commonly used for swimming (Abourachid and Green, 1999), stabilization, or escape. The froth beating movements of the male, on the other hand, seem different and may have been associated with vent cleaning or some other activity that facilitates fertilization (basket formation, Miller, 1909). In some ways the hind limb movements of males are reminiscent of the peculiar 'wiping' behavior of some phyllomedusine hylids (e.g., Blaylock et al., 1976) and likely are derived from the generalized cleaning and grooming behaviors used by most frogs to remove debris or shed skin. The limb churning is not rapid, but sufficient air is mixed with the oviductal secretions from the 'foam gland' to form a surprisingly dense foam that is sometimes voluminous. Similar behaviors have been reported for nest construction in arboreal rhacophorids but in these species the females do most of the construction (Liu, 1950; Coe, 1975). Bubble nests are less organized structures than foam nests and are generated by arm movements of the amplectant female (limnodynastids) or bubble blowing by the pair (microhylids). In bubble nests, egg jellies typically remain visible (i.e., jelly not very foamy) and adherent, and the whole clutch floats on the surface (Fig. 5A).

Examples from North American Amphibians

Two examples from North American frogs illustrate patterns of evolutionary shifts between ovipositional modes. For this discussion, we distinguish three major groups of North American ranids: (1) seven species of 'wood frogs' (*R. sylvatica* plus *R. boylii* group; Hillis and Wilcox, 2005; Frost et al., 2006) which have eggs with 2–3 jelly layers and oviposit clumps (Wright and Wright, 1949),
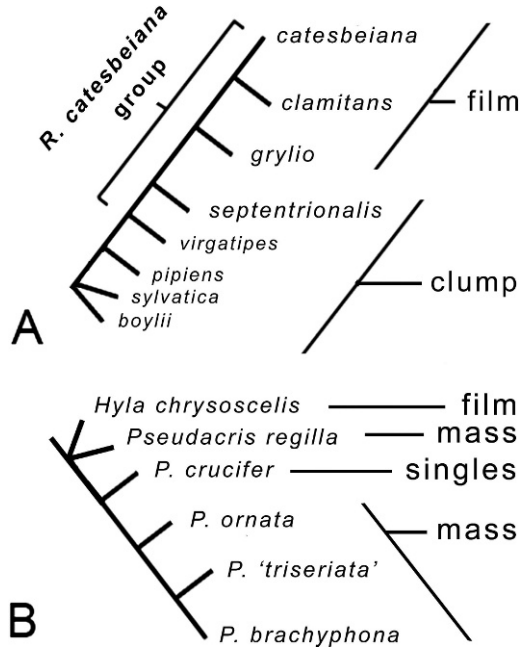

FIG. 6.—Two cladograms showing the distributions of oviposition modes in groups of (A) ranids (modified from Wolf, 1996:fig. 23) and (B) hylids (modified from Cocroft and Ryan, 1995:fig. 2).

(2) 13 species in the *R. pipiens* complex, which have 2 jelly layers and oviposit clumps, and (3) seven species of the *R. catesbeiana* group, which have eggs with 1–2 jelly layers and oviposit clumps (*Rana septentrionalis*, a boreal species, and *R. virgatipes*, a southeastern Coastal Plain inhabitant) or surface films (5 species). A simplified hypothesis illustrates the distribution of clutch structures on a cladogram (Fig. 6A; modified from Wolf, 1996:fig. 23). Species that oviposit clumps include members of the *R. pipiens* group, 'wood frogs,' and two species of the *R. catesbeiana* group. The remainder of the *R. catesbeiana* group oviposits films. The previously proposed origin of a floating surface arrangement from a submerged group seems to be supported (see Evolution of Ovipositional Modes and Aronson, 1943).

Another scheme (Fig. 6B; modified from Cocroft and Ryan, 1995:fig. 2) for North American hylids shows a different pattern. Some hylids (e.g., *Hyla chrysoscelis*) oviposit surface films while others (e.g., *Pseudacris* spp.) deposit masses attached to vegetation or

scattered singles (e.g., *P. crucifer*). *P. crucifer* lies among other mass-producing taxa. This case is likely an example of adult behavior modifying ovipositional mode. If amplectant *P. crucifer* did not move as much during oviposition, they would likely produce masses. Patterns such as these suggest that certain ovipositional modes are relatively labile and easily changed by the behavior of amplectant adults.

CONCLUDING COMMENTS

In summary, we have identified 14 ovipositional patterns within the Amphibia. These modes reflect egg morphology, breeding ecology, and postovipositional events, biotic and abiotic conditions (i.e., ovipositional site), and parental behavior (i.e., actions of males and females during oviposition and fertilization). We argue that the appearance of some of these patterns is best understood from a phylogenetic perspective but that others may more closely reflect ecological influences (e.g., Moore, 1940). More information on ovipositional behavior is needed, as are experimentally-based evaluations of the presumed ecological benefits of the various modes. For example, when the temperature is high, films seemingly are most effective because they place the eggs in the most highly oxygenated water at the surface; likewise, because of their structure and position below the surface, masses and clumps presumably avoid freezing in cold weather. We suspect that certain modes and their described variations are not always advantageous, and strategies that are successful in one situation may fail in another (margins of a species range; modified or fragmented habitats; regional changes in climate).

Clearly, information about amphibians eggs, accounts of egg laying behavior, and basic descriptions of the resulting clutch morphology are limited, even for most of the common members of the North American fauna. We believe that considerable research on descriptive natural history is needed. We especially encourage colleagues to extend our treatment across groups and into different faunas. We are convinced that more detailed examinations of ovipositional behaviors and clutch structures will lead to the description of additional ovipositional modes and the expansion of our organizational framework. We are aware that thorough examination of the details of oviposition in some modes may show that some of our interpretations are incorrect and that some modes may better be treated as a subsets of another. The extreme transparency of egg jellies and the multiple reflections from the jellies and water surfaces present challenges to those studying eggs. Nevertheless, we encourage field biologists to collect and properly preserve identified groups of amphibian eggs, recognizing that appropriate fixatives are not generally available. The standard fixative of 10% buffered formalin is clearly inadequate as it seldom maintains the integrity of egg jellies over time. Streck Tissue Fixative (STF) suggested as an egg preservative by McDiarmid and Altig (1999, Chapter 2) is no longer produced; we are testing other nonaldehyde fixatives, and new methods of preserving eggs and clutches should be found. In addition, a relatively simple method of sectioning eggs that keeps the jelly layers intact would be helpful.

Amphibian biologists need to determine if consistent and predictable patterns linking ovum characteristics, habitat features, ovipositional modes, and deposition sites exist, and if so, how they vary geographically and taxonomically. The concept of reproductive mode (e.g., Crump, 1974; Haddad and Sawaya, 2000) attempts to distinguish some of these patterns, but we suggest that the undue attention given to features of deposition site and to egg number and size has masked any phylogenetic signal that may be revealed by the details of egg and clutch structure. After improved observational techniques have been devised and ovipositional modes have been refined and described more completely, a comprehensive and coordinated research effort focused on the structure of egg jellies and clutches should be undertaken. We believe that the incorporation of egg and clutch morphology into phylogenetic and ecological analyses is long overdue and anticipate the myriad positive contributions that such information will make to our understanding of amphibian diversity.

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