THE JOURNAL OF EXPERIMENTAL ZOOLOGY 266:394–413 (1993)

Evolution of Oviductal Gestation in Amphibians

MARVALEE H. WAKE
Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, California 94720

ABSTRACT Oviductal retention of developing embryos, with provision for maternal nutrition after yolk is exhausted (viviparity) and maintenance through metamorphosis, has evolved independently in each of the three living orders of amphibians, the Anura (frogs and toads), the Urodela (salamanders and newts), and the Gymnophiona (caecilians). In anurans and urodeles obligate viviparity is very rare (less than 1% of species); a few additional species retain the developing young, but nutrition is yolk-dependent (ovoviviparity) and, at least in salamanders, the young may be born before metamorphosis is complete. However, in caecilians probably the majority of the approximately 170 species are viviparous, and none are ovoviviparous. All of the amphibians that retain their young oviductally practice internal fertilization; the mechanism is cloacal apposition in frogs, spermatophore reception in salamanders, and intromission in caecilians. Internal fertilization is a necessary but not sufficient exaptation (sensu Gould and Vrba: *Paleobiology* 8:4–15, '82) for viviparity. The salamanders and all but one of the frogs that are oviductal developers live at high altitudes and are subject to rigorous climatic variables; hence, it has been suggested that cold might be a "selection pressure" for the evolution of egg retention. However, one frog and all the live-bearing caecilians are tropical low to middle elevation inhabitants, so factors other than cold are implicated in the evolution of live-bearing. Viviparity might facilitate life in a rigorous environment, but likely is not "caused" by such an existence.

Oviductal retention of developing embryos has evolved independently several times in members of the Class Amphibia—twice in Anura (frogs and toads), once in Urodela (salamanders and newts), and at least twice, perhaps three times, in Gymnophiona (caecilians). Each group has effected retention in rather different ways, and none have developed placentas (with the possible exception of a pseudoplacenta in one group of caecilians). In all species of amphibian oviductal embryo retainers for which there are data, either little to no nutrition is supplied in addition to yolk, though there may be gaseous exchange across vascularized membranes (ovoviviparity), or cells of the oviductal epithelium secrete a highly nutritious material that is orally ingested by the developing fetuses after yolk is resorbed (viviparity) (Amoroso, '52, '68). Amphibians may present a special case in that viviparity and ovoviviparity can be rather clearly delineated in terms of presence or absence of significant maternal nutrition (a protein, carbohydrate and lipid-rich material, depending on the stage of development) following yolk resorption. I therefore will use the restrictive definitions mentioned above for viviparity and ovoviviparity in this discussion, since the "grey areas" of nutrient uptake found in placental reptiles, and in fishes, seem not to obtain in amphibians.

Of nearly 4,000 species of anurans, only five retain the developing young in the oviducts—four are members of the African genus *Nectophrynoides* in the family Bufonidae, and one is a member of the New World tropical family Leptodactylidae. However, frogs have performed numerous "experiments" in reproductive biology, and oviductal retention is only one. All but a handful of frogs practice external fertilization, and that in water, no matter how terrestrial the rest of their lives. Yet, once the eggs are fertilized, many means for their care have been effected. Frogs practice many kinds of parental care. They include bringing food to the developing tadpoles (Weygolt, '80), foam nests, parents carrying developing tadpoles on their backs to water, etc. (Duellman and Trueb, '86). It has been suggested that parental care is a precursor for the evolution of more complex modes of reproduction, and that direct development (the laying of few, large, yolky eggs on land, with development through metamorphosis before hatching from the egg membrane, thus obviating the aquatic larval period), is an additional "step" toward the evolution of egg retention mechanisms (Salthe and Mecham, '74; Duellman and Trueb, '86). The implications of these ideas are discussed below.

Several different "embryo retention" mechanisms have also evolved in frogs. For example, some frogs have evolved means of retaining developing embryos in the skins of their backs at least twice, and in distantly related groups (pipids and hylids); evidence suggests that the same sort of endocrine control that mediates pregnancy facilitates this form of maintenance (Jones et al., '73).

Another mode of "retention" is that of the two species that compose the family Rhinadermatidae of southern Chile and Argentina. Following amplexus and egg-laying and external fertilization, the eggs develop for approximately 20 days in moist soil, then the male, which had remained in the vicinity of the egg clutch, noses about the eggs, and picks up by mouth the fertilized ova from their degenerating egg jelly. *Rhinaderma rufrum* merely carries the ova in its mouth to water; *R. darwini* males deposit the ova in their vocal sacs, where the developing embryos are maintained through metamorphosis (Busse, '70). Juvenile froglets emerge from their father's mouth after a 52 day developmental period (Jorquera et al., '72). There is indirect evidence for possible nutrient transfer from the father to the developing young (uptake by embryos of horseradish peroxidase injected in the paternal endolymphatic sac and transported vascularly; Goicoechea et al., '86).

Perhaps the most extreme example of developmental retention in frogs is that of *Rheobatrachus silus,* a myobatrachid frog of Australia. The females ingest their externally fertilized egg clutch, and brood the developing young in the stomach for some eight weeks. Fully metamorphosed froglets are "regurgitated" by the female (Corben et al., '74; Tyler and Carter, '81). The tadpoles secrete prostaglandin E_2, which serves to inhibit gastric acid secretion in the stomach of the female (Tyler et al., '82). The tadpoles are apparently dependent on their yolk supply for nutrition.

However, none of the above-cited frogs are closely related to those that have evolved oviductal retention of their young. As mentioned above, four species in the African bufonid genus *Nectophrynoides,* which also includes a direct-developing species and one with "typical" toad development, retain their developing young in their oviducts. The species all live at high altitudes in the mountains of West (Liberia, Ivory Coast, Guinea) and East (Tanzania, Ethiopia) Africa. Two of these species apparently do not provide maternal nutrition in addition to the yolk reserve; one is suspected to provide nutrients, but there is no direct evidence for this; the fourth is perhaps the best understood example of viviparity in amphibians (see below). A fifth species, *Eleutherodactylus jasperi,* a leptodactylid frog from Puerto Rico, also retains its embryos in its oviducts until they are fully metamorphosed. They appear to be fully yolk-dependent, without additional maternal nutrition (Wake, '78a). The genus *Eleutherodactylus* includes more than 300 species, all direct developers except for the one ovoviviparous taxon. Internal fertilization seems to be a prerequisite for oviductal retention of embryos; the above-mentioned frogs, and a very few others, are the only ones that have cloacal apposition as a means of directly transferring sperm from male to female. (The direct-developing *Nectophrynoides,* a few other bufonids, and a few *Eleutherodactylus* also have cloacal apposition; the primitive frog *Ascaphus* and the bufonid *Mertensophryne* use modified cloacal and tail structures as intromittent organs, but though they have internal fertilization, they lay their fertilized eggs rather than retaining them.)

Salamanders, in general, have attempted fewer "natural experiments" in reproductive mode than have frogs, but all but the most primitive salamanders practice internal fertilization, and many have direct development. The means of fertilization in all salamanders except members of the families Cryptobranchidae and Hynobiidae is via deposition of a spermatophore (a gel-like pedicel secreted by cloacal glands capped by a packet of sperm). During courtship, the male attracts the female, and lures her in a complex path during which he deposits the spermatophore, and she picks it up with the lips of her cloaca. Females of some species can store sperm for some time in specialized cloacal structures, the spermathecae. With few exceptions, the female salamanders lay the internally fertilized clutch either in water or on land. Direct development has developed in several lineages, especially in the family Plethodontidae. The developmental period can be many months, especially in the high altitude tropics. As with frogs, however, it is not the salamanders that have evolved direct development that are related to those that have oviductal retention, but another group that otherwise is a "typical" aquatic breeder with a free-living larval period. Only one species of salamander of the approximately 400 in the order, *Salamandra atra,* has long been known to be obligately viviparous. Another member of the genus, *S. salamandra,* and members of the closely related genus *Mertensiella,* include the five or six ovoviviparous species. Recently, a subspecies of *S. salamandra, S. s. bernardezi* from Oviedo in northwestern Spain, was described to be obligately viviparous, in that females normally give birth to fully metamorphosed young, though some are in late metamorphosis (Fachbach, '69; Thiesmeier and

Haker, '90). There is no demonstration that females provide nutrition to the developing young after yolk is exhausted (and Fachbach indicated that a large amount of yolk is available to late "larvae"), so I suspect that the subspecies is ovoviviparous, and simply retains its young through metamorphosis most of the time, as members of several subspecies of *S. salamandra* are known to do occasionally. Fully metamorphosed young are born of *S. atra* after a lengthy gestation period; larvae are born at diverse stages in most of the ovoviviparous species. Retention has therefore evolved only in "end taxa" of one lineage of salamanders. The live-bearing salamanders live in the Alps and adjacent mountain ranges.

Caecilians, too, have performed relatively few reproductive "experiments," but the few are very successful, and are the predominant reproductive modes. All caecilians practice a unique mode of internal fertilization. Males evert the rear part of the cloaca, which effects an intromittent organ that is inserted into the vent of the female so that sperm is transported directly, without being strewn into water. Of the six families of caecilians, the three most primitive (one in northwestern South America, two in southeast Asia) lay eggs on land, the female guards them until they hatch, and the larvae wriggle into streams. The larvae spend perhaps a year in their aquatic phase, then metamorphose abruptly (Wake, '89), spending the rest of their lives on land. One family, the paraphyletic, world-wide Caeciliaidae, includes species with free-living larval stages, others with direct development, and yet others that are obligately oviductal egg retainers that provide maternal nutrition after the yolk supply is exhausted (viviparity). All members of the two remaining families, the aquatic Typhlonectidae of northern and central South America and the Scolecomorphidae of east and west Africa, apparently are obligately viviparous. There are no species that appear to be ovoviviparous, or retained but dependent only on yolk for nutrition. Viviparity has evolved at least twice, once in the Old World and once in the New World, and there may have been additional events. I can only speculate about this until the systematics of caecilians in general and caeciliads in particular are better understood.

OVIDUCTAL GESTATION IN FROGS

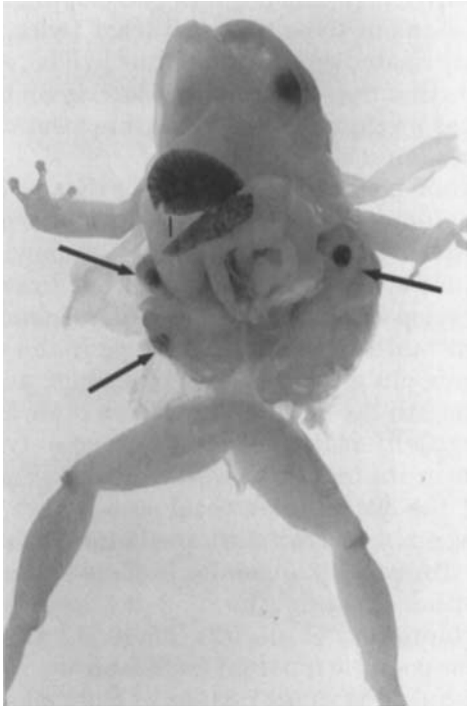

Fig. 1. Female *Eleutherodactylus jasperi* (20.5 mm snout-vent length [SVL]) with its clutch of three near-birth froglets (arrows; 6.2–7.0 mm SVL) in the oviducts.

Eleutherodactylus jasperi (Fig. 1) is an ovoviviparous frog that gives birth to 3–5 metamorphosed froglets following brooding by the female in a chamber formed of the fused posterior parts of the oviducts (Drewry and Jones, '76; Wake, '78a). It is a member of the highly speciose genus *Eleutherodactylus,* all of whose approximately 300 members are direct developers, except *E. jasperi,* the ovoviviparous taxon. The species may well be extinct, for it has not been collected in its restricted habitat on Puerto Rico for several years (D. S. Townsend, personal communication).

The gestation period is approximately 33 days, for frogs gave birth 33 days after they were found in amplexus (Drewry and Jones, '76). Internal fertilization is inferred, but has not been observed. (Other *Eleutherodactylus* are demonstrated to have internal fertilization, though they are direct developers [Townsend et al., '81]). Wake ('78a) described the morphology of the reproductive organs and the intra-oviductal development of the tadpoles of *E. jasperi*. Corpora lutea were not observed in the ovaries of pregnant females. The oviducts of the females are modified for retention of the developing embryos by having the posterior 5 mm of the 12 mm oviducts fused as a "uterus." When the female is pregnant, this part of the oviducts is highly distended, and the epithelial lining is lower in height than that of the anterior oviduct (Fig. 2). The "uterine" epithelial cells also lack cilia and microvilli present on more anterior cells. The cells of the anterior duct are secretory; there is an abrupt transition to nonsecretory columnar epithelium low in the

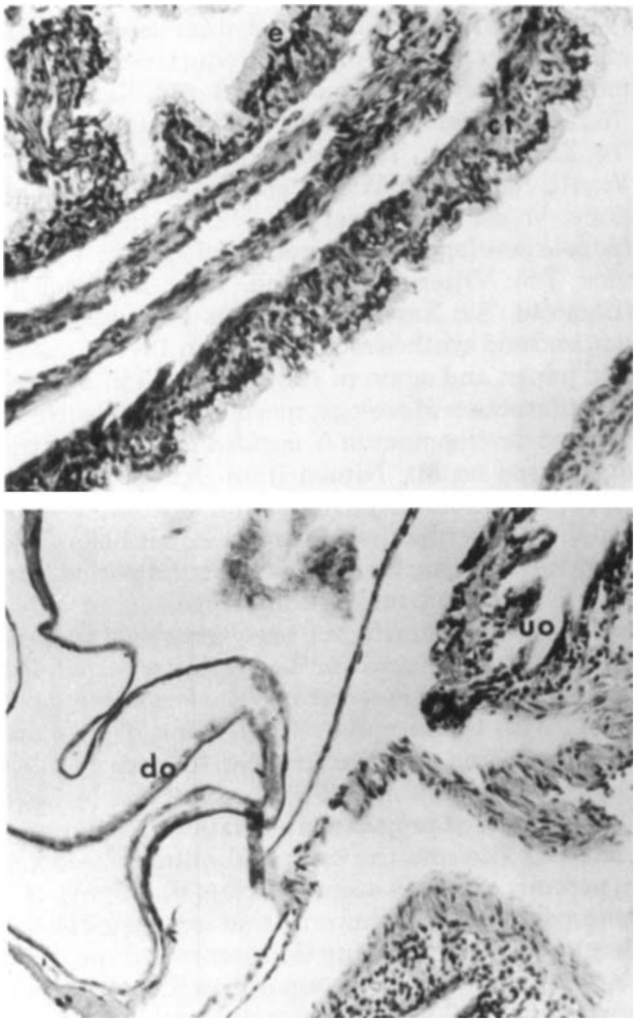

Fig. 2. *Eleutherodactylus jasperi* pregnant maternal oviduct lining. **Top:** Anterior part of duct with thick wall and high epithelial cells. **Bottom:** Dilated posterior portion of the oviduct that houses the embryos. Note that the oviductal epithelium is stretched and thin, but not secretory in the region of the embryos; its cells are lower and lack cilia and microvilli (from Wake, '78a). Bar = 0.1 mm. c = capillary; ct = connective tissue; e = epithelium; do = dilated posterior oviduct; uo = upper thick oviduct.

convoluted part of the duct, and then to the monolayer of cuboidal cells of the "uterus." Capillaries lie just below the epithelial monolayer of the "uterus," so there is the potential for gaseous exchange with the developing embryos.

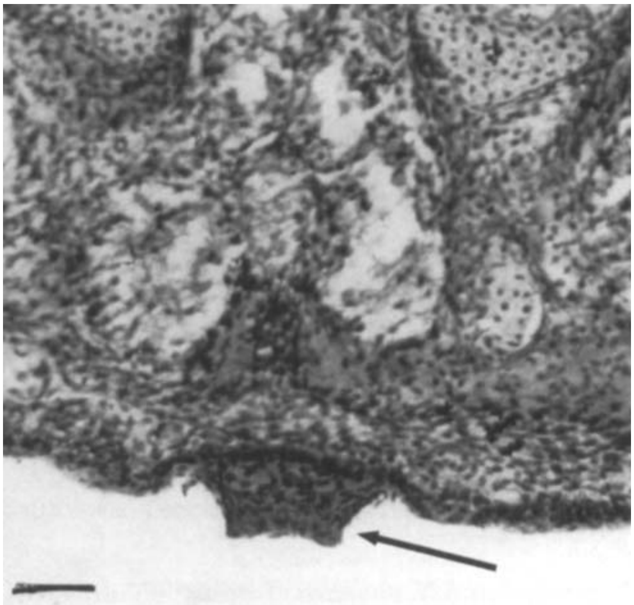

Fig. 3. Egg tooth (arrow) of oviductal embryo of *Eleutherodactylus jasperi* (after Wake, '78a). Bar = 40 μm.

The development of the tadpoles is similar to that of other *Eleutherodactylus* reported in the literature. Wake ('78a) noted that *E. jasperi* tadpoles have an egg tooth (Fig. 3) similar to that of direct-developing *Eleutherodactylus*. The egg tooth is used to penetrate the tough egg membrane in many species of *Eleutherodactylus* in order for the direct-developing froglets to "hatch", and may be so used intraoviductally by *E. jasperi,* though the tooth is small, not heavily keratinized, and lacks an outer layer. The tail is thin, broad, and highly vascularized. In most of the intraoviductal tadpoles or froglets examined, the tail was appressed to the oviductal epithelium and the dorsum of the young. Further, the tail is retained throughout virtually all of the oviductal developmental period, with metamorphosis being abrupt and very near the time of birth. It is plausible that the tail might function for gaseous exchange between tadpole/froglet and the adult. The tail has been suggested to be a respiratory structure in direct developers as well (Lynn, '42).

Ovoviviparity is suggested for *E. jasperi* because the abdominal cavity of newborns is dominated by unresorbed yolk. This is characteristic of some of the direct developing species as well. With a large volume of yolk available, and with no apparent oviductal or embryonic modifications for other kinds of maternal nutrition, it is unlikely that nutrition other than the yolk is provided (Wake, '78a). Wake ('78a) has discussed the scenarios for the evolution of ovoviviparity from direct development in the genus *Eleutherodacylus*.

The African bufonid genus *Nectophrynoides* includes species that reflect the evolution of live-bearing reproductive modes in frogs. *Nectophrynoides osgoodi* is a "typical" bufonid, with external fertilization and aquatic tadpoles and *N. malcolmi* (Fig. 4) is a direct developer (Grandison, '78; Wake, '80a);

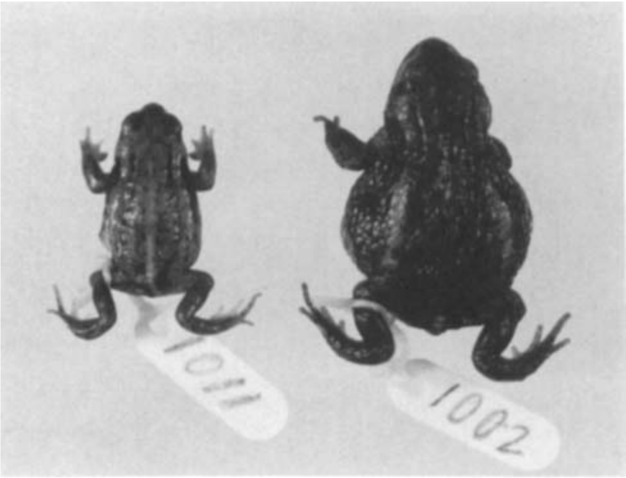

Fig. 4. Male (16.0 SVL) and female (25.9 mm SVL) of *Nectophrynoides malcolmi*.

N. tornieri and *N. vivipara* (Tornier, '05) are ovoviviparous and *N. occidentalis* is viviparous (Angel, '43; Angel and Lamotte, '44, '48); *N. liberiensis* is viviparous as well (Xavier, '78). Wake ('80a) summarized new information and that from the literature on egg size, clutch number, and features of tadpole development in all of these species save *N. liberiensis* (Table 1). The "typical" bufonid, *osgoodi*, has a large clutch size for the genus at approximately 300; the ova are 2.5–3.0 mm dia, and the tadpoles are free living, with beaks, labial teeth a coiled gut, and a moderately large tail. The direct developer, *malcolmi*, has eggs of the same size as *osgoodi*, but a clutch of 11–31 ($\bar{x} = 18$). The tadpoles have a closed spiracle, lack beaks and labial teeth, have short guts and narrow tails. The two ovoviviparous species, *tornieri* (see Orton, '49) and *vivipara*, have similarly reduced tadpoles, fairly large, yolky eggs (3.0–4.0 mm dia), but vary in clutch size, *tornieri* having a small clutch of 9–37, and *vivipara* a clutch of 114–135. The viviparous species, *occidentalis* and *liberiensis*, have small ova (0.5–0.6 mm dia) and small clutches (4–35 and 6–24, respectively. The tadpoles of *liberiensis* are not described, but those of *occidentalis* are similar to those of the ovoviviparous species, but they also have several rows of papillae around their mouths; these are thought to be involved in the ingestion of the maternal nutrient secretions, perhaps acting as "sponges."

The mechanism of viviparity, if not its evolution, is best known in *N. occidentalis* of any amphibian. In a series of careful, detailed, and informative papers, Lamotte and Xavier and their colleagues reported on the development, endocrinology, and ecology of viviparity in the species. They studied oogenesis, including corpora lutea development (Vilter and Lugand, '59b), and other aspects of the morphology of the female reproductive cycle (Lamotte and Rey, '54; Lamotte et al., '64; Xavier, '73, '75a), the endocrinology of the system (Xavier, '70a,b, '74; Xavier et al., '70; Xavier and Ozon, '71; Zuber-Vogeli, '68; Zuber-Vogeli and Doerr-Schott, '76; Zuber-Vogeli and Xavier, '72, '73), intra-oviductal tadpole development (Lamotte and Xavier, '72; Xavier, '75b; Vilter and Lugand, '59a), and ecology (Lamotte, '59; Xavier, '75a). This work was summarized and synthesized by Xavier in 1977 in a seminal paper, and again in 1986. Figure 5 illustrates the interaction of ecology, morphology, endocrinology and development in *N. occidentalis*. During the dry season on Mt. Nimba from October through April, the female frogs retreat underground. Ovulation and fertilization take place just before the frogs retreat. During the underground period, corpora lutea are active, secreting progesterone which inhibits oocyte growth and slows growth of the embryos. During this part of the gestation period, the oviductal mucosa is secretory. The frogs emerge in April, with the inception of the rains. They move about and forage. With emergence, the corpora lutea begin to degenerate, and this is complete by May. With decreased progesterone secretion, oocytes increase in size and the oviductal mucosa becomes hyperemic and very secretory (Fig. 6), largely of a mucopolysaccharide material that is ingested by the developing young. Parturition occurs in June, with the birth of 4–35 fully metamorphosed froglets, each approximately 7.5 mm snout-vent length and weighing 45 mg. Most of the oviductal epithelium dies and exfoliates, then it regenerates. With progesterone levels diminished, the ovary enters a follicular phase, and oocytes mature and vitellogenesis takes place as estrogen titers rise. In early October, ovulation occurs, and the cycle begins again. The entire gestation period is 9 months, and for approximately the last two months, the fetuses are nourished by secretions from the oviductal epithelium. The fetuses have large numbers of papillae around their mouths, and it has been conjectured that these facilitate ingestion of the nutritive secretions. *N. occidentalis* is clearly the most derived species in the genus, with its reduced ovum size, hormonal mediation of pregnancy correlated with environmental conditions, maternal nutrition by oviductal epithelial secretions, and the modifications of the developing tadpoles for lack of a free-living larval stage and for ingestion of the nutritive secretions. The two ovoviviparous species are modified similar to direct developers, largely involving mod-

TABLE 1. *Developmental features of species of* Nectophrynoides *(after Wake, '80a)*

Species and source of information	Egg size (dia in mm)	Clutch size	Tadpoles spiracle	Beak	Labial teeth	Papillae	Gut	Tail
occidentalis (Lamotte and Xavier, '72a)	0.6	4–35	Closed	None	None	Several rows surrounding mouth	Short, curved	SV, fins 1/3 tail muscle depth
tornieri (Orton, '49; Lammote and Xavier, '72b)	3.0–4.0	9–37	Sinistral	None	None	None	—	SV, fins 1/3 tail muscle depth
vivipara (Tornier '05)	3.0–4.0	114–135	—	None	None	None	Short	SV, fins 1/3 tail muscle depth
malcolmi (Grandison, '78; Wake '80a)	2.7–3.0	11–31 (x 18)	Closed	None	None	None	Short	SV, fins 1/3 tail muscle depth
osgoodi (Grandison, '78; Wake, '80a)	2.5–3.0	307	Sinistral	Present, not heavily cornified	I:I + I/III	Single rows at corners of mouth	Coiled	Apparently SV, fins 1/3 tail muscle depth but =

ification of the tadpoles for the absence of an aquatic phase, with none of the more extensive modifications that appear to facilitate maternal nutrition.

OVIDUCTAL GESTATION IN SALAMANDERS

There has been surprisingly little research on the viviparous *Salamandra atra,* especially in contrast to work on its more abundant ovoviviparous congener, *S. salamandra.* Vilter (1986) summarized research on *S. atra.* The gestation period in *S. atra* is reported to be 2–5 years, depending on the rigor of the alpine climate (Browning, '73; Fachbach, '69; Freytag, '55; Hafeli, '71; Schwalbe, '96; Vilter and Vilter, '60; Wiedersheim, 1890; Wunderer, '09). Only two young are born, fully metamorphosed. They develop one in each oviduct (Fig. 7). Unfertilized ova and ova fertilized apparently later than a first ovum, as well as the yolk reserve, are consumed by the developing embryos during the first year of gestation. The ova are not much reduced (3.0 mm dia), and the clutch is relatively large (up to 30 per ovary) (Fachbach, '69). Vilter and Vilter ('64) described the presence of actively secreting corpora lutea virtually throughout gestation, and Browning ('73) concluded that the progesterone secreted both maintained the pregnancy and slowed development of the tadpoles. Little, other than that, is known of the endocrinology of *S. atra.* Since yolk is exhausted in the first year of gestation, it has long been assumed that maintenance of the tadpoles up to the point of their metamorphosis and birth is a consequence of uptake of nutrient material secreted by the oviductal epithelium of the female. Fachbach ('65), '69), Greven ('77), Niederl ('81), Vilter ('67), and Vilter and Vilter ('62) have studied the histology of the female oviduct in order to assess its potential for secreting nutrients (Fig. 8). Fachbach, Greven, and Vilter all emphasize that only a small anterior region of the oviduct epithelium that they called a "zona trophica" undergoes significant changes and becomes highly secretory (Niederl indicates that the modification might be more extensive). The increase in numbers of secretory cell types is correlated with an increase in corpus luteum activity in the second year of gestation, according to all authors. The histology of the oviducts at different stages in gestation has been characterized by these workers. Greven ('84) notes that the "zona trophica" proliferates extensively. The developing fetuses ingest the cells of the zona, and there is a high rate of turnover of the cells. Greven has found zona cells in the mouths and intestines of fetuses. He described a "fetal dentition" on the premaxil-

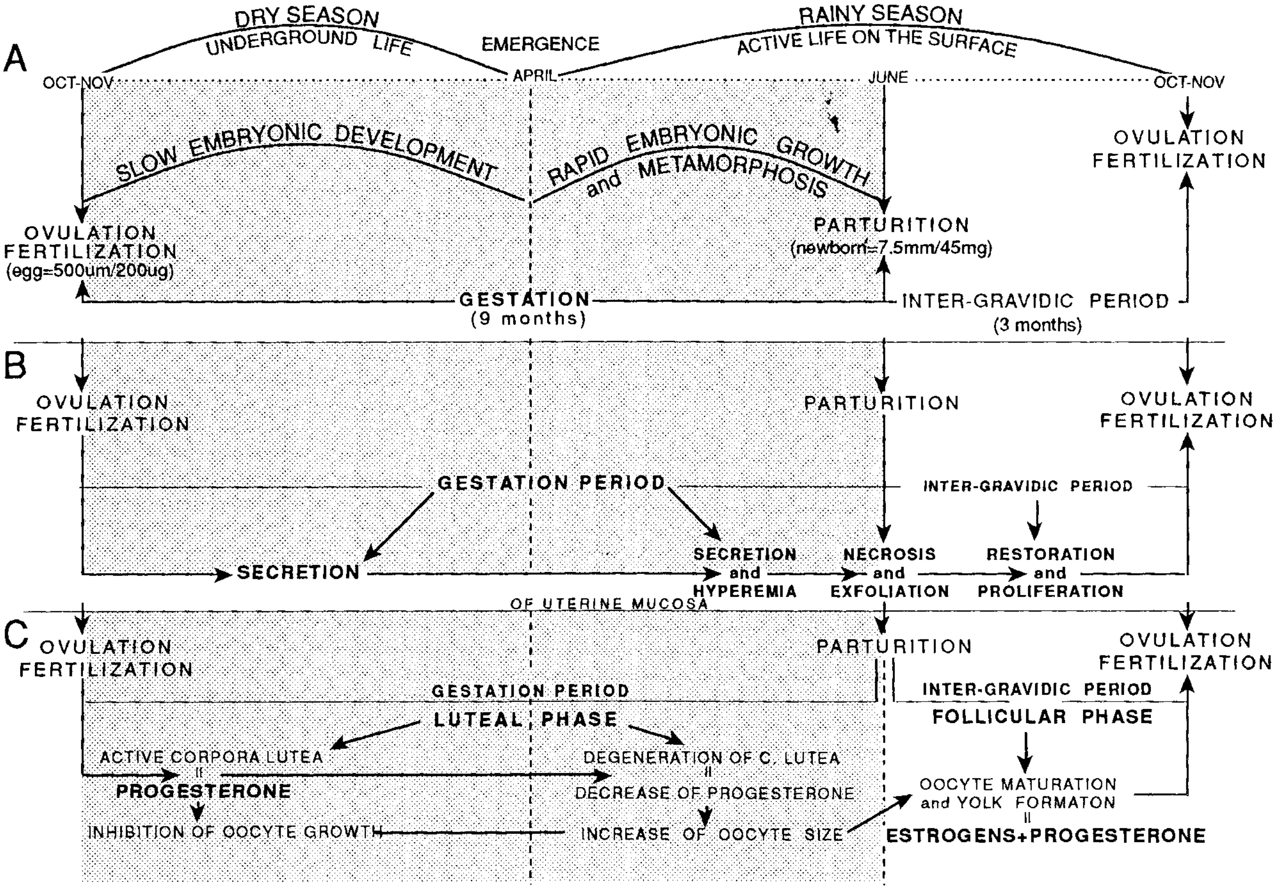

Fig. 5. Ecological and reproductive cycles in *Nectophrynoides occidentalis*. **A:** Ecological cycle and reproductive activity. **B:** Oviductal cycle. **C:** Ovarian cycle. From Xavier, '77, with permission.

lary bones of the fetuses that he considers functional to scrape the cells and their secretions into the mouth. *S. atra* is now protected through much of its range, but since it is apparently becoming scarce, I strongly recommend that careful but thorough research programs be undertaken to more closely examine the ecology, endocrinology, morphology, development, and evolution of viviparity in this fascinating animal.

Fortunately, more is known of the reproductive biology of the ovoviviparous *S. salamandra* (summarized by Joly, '86). Most subspecies of *S. salamandra* give birth to larvae in water, though they have developed extensively in the oviducts ("uteri" of some workers) (Greven, '76). However, as noted above, at least one subspecies, *bernardezi*, obviates the free-living larval period by retaining its young through metamorphosis at least most of the time (Fachbach, '69; Thiesmeier and Haker, '90). Intraoviductal development in this subspecies deserves more attention, for retention of young through metamorphosis, as in the frog *E. jasperi*, is a logical step in an evolutionary scenario of the evolution of viviparity through reduction in ovum size, acquisition of maternal nutrition, and further modification of embryos (Wake, '82, '89).

Joly ('60, '61, '68) and Joly and Boisseau ('73) have studied the ecology and reproductive biology of French subspecies, including films of birth of late larvae in water. Joly and Picheral ('72) studied the corpus luteum as well. Greven and his colleagues have extensively examined the ultrastructure, innervation, and biochemistry of the oviduct of *S. salamandra* (Greven, '77, '80a, '81; Greven and Baldus, '84; Greven and Robenek, '80, '82; Greven and Ruterbories, '84; Greven et al., '75, '83, '86). Greven ('80b) found that there is active transport of solute *out* of the uterine lumen; he concludes that this may be involved in the female regulating the intrauteine milieu during embryonic development. Greven et al. ('83) found a dense, particularly adrenergic, innervation of the uterus, and thought that it might

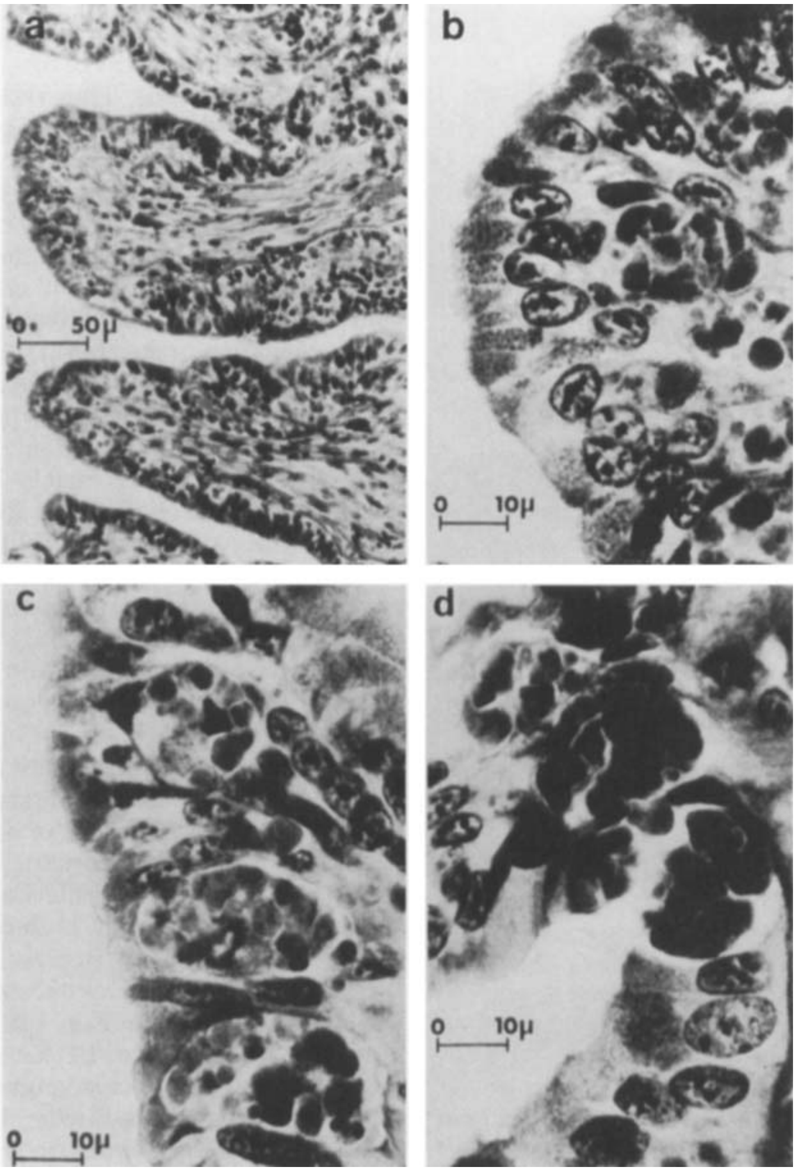

Fig. 6. Epithelium of the "common uterus" of *Nectophrynoides occidentalis* 8 hours after birth of the fully metamorphosed young. Secretions of the epithelium are the nutrient material for the developing young for much of the gestation period. **a:** Low magnification photomicrograph of the proliferated epithelium (compare to Fig. 13C). **b–d:** Macrophages invading the epithelium: b, superficial; c, migrating; d, luminal. Reprinted from Xavier, '73, with permission.

be involved in regulating uterine muscle activity at birth. Greven ('80c) also examined the ultrastructure of the epidermis and gill epithelia of intrauterine larvae, and found no morphological evidence for any uptake of material by these surfaces, which is further evidence that *S. salamandra* does not provide any nutrient material to the yolk-dependent developing young. Gasche ('39) also examined the development of the oviductal embryos in *S. salamandra.*

Several species and subspecies of *Mertensiella,* which is closely related to *Salamandra,* are reported to retain developing embryos in their oviducts, and to give birth in water to advanced larvae (Duellman and Trueb, '86), but little is known of the biology of their live-bearing.

OVIDUCTAL GESTATION IN CAECILIANS

Oviductal gestation has been very successfully exploited in the limbless, tropical, fossorial or swimming caecilians (Fig. 9A,B). It is likely that more

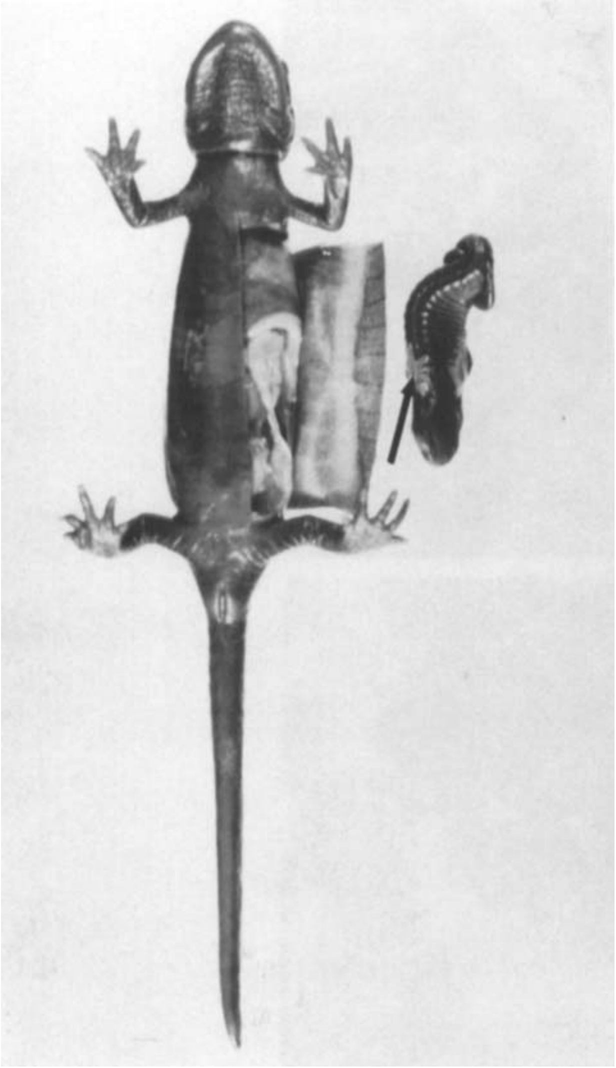

Fig. 7. *Salamandra atra* female (68.0 mm SVL) and near-birth young excised from the oviduct. Note the triramous gills still present.

than half of the known species are obligately viviparous (Wake, '77a,b, '82, '89, '92). These species are found in three of the six families of caecilians, and it is apparent that viviparity has evolved *at least* twice, once in the Old World (caeciliads and scolecomorphids) and once in the New World (caeciliads and typhlonectids). It is possible that viviparity arose independently in scolecomorphids and in typhlonectids, but until relationships within and among these groups is better understood, this remains speculative. Several caeciliaids are direct developers, and members of that family and the more primitive Rhinatrematidae, Ichthyophiidae, and Uraeotyphlidae are oviparous. In apparently all of the egg layers, direct developers or not, the female guards the clutch, at least for a time. In contrast to other amphibian groups, in which viviparous species are terminal taxa in genera that also include ovoviviparous forms, there are apparently no ovoviviparous caecilians. They either provide maternal nutrition, or they lay their clutch shortly after it is fertilized internally.

All caecilians are presumed to use internal fertilization. The mechanism differs from that of frogs and salamanders. Male caecilians evert the rear part of the cloaca as a phallodeum and insert it into the vent of the female. (Effectively nothing is known of mate location or courtship in caecilians, though copulation has been observed in *Chthonerpeton* and *Typhlonectes* [Barrio, '69; Billo et al., '85; Murphy et al., '77; Exbrayat and Laurent, '83; Exbrayat et al., '83]). Birth was first reported by Heinroth ('15). The morphology of the phallodeum apparently is species-specific, with patterns of longitudinal and transverse connective tissue ridges in all species and paired, posteriorly-opening sacs in many (Tonutti, '31, '33; Wake, '72). Contraction of body wall musculature, filling of vascular sinuses, and hydrodynamics of the phallodeal sacs have all been implicated as involved in the mechanism of eversion (Taylor, '68), but none of these have been demonstrated.

Males have an additional adaptation for internal fertilization that Wake ('81) considered correlated with terrestriality of reproduction. The posterior 10–30 mm of the paired Mullerian ducts (which early in embryology develop in both males and females, but which regress early in most males, and become the oviducts of all females except teleosts) becomes a glandular structure (Fig. 10) in male caecilians (Tonutti, '31, '33; Wake, '81; Exbrayat, '85). It is secretory during the active spermatogenic phase of the testis, and regressed when the testis is, so the Mullerian gland presumably is responding to the same hormonal regime. Wake ('81) suggested that the Mullerian gland is the homologue of the turtle and mammal prostate gland (the medial prostate of mammals is of Mullerian duct origin; references in Wake, '81), and she demonstrated that the content of the glandular secretion is similar to that of mammalian ejaculate in sugars, ions, etc. She also postulated that provision of fluid for transport and nutrients for sperm is a concomitant of terrestrial reproduction.

Both ovum size and ovum number are reduced in viviparous caecilians, as they are in other viviparous amphibians. However, in contrast to the situation in frogs and salamanders in which direct developers have the largest, yolkiest eggs, oviparous caecilians with free-living larvae have the largest ova (8 × 10 mm), direct-developers moderate-sized

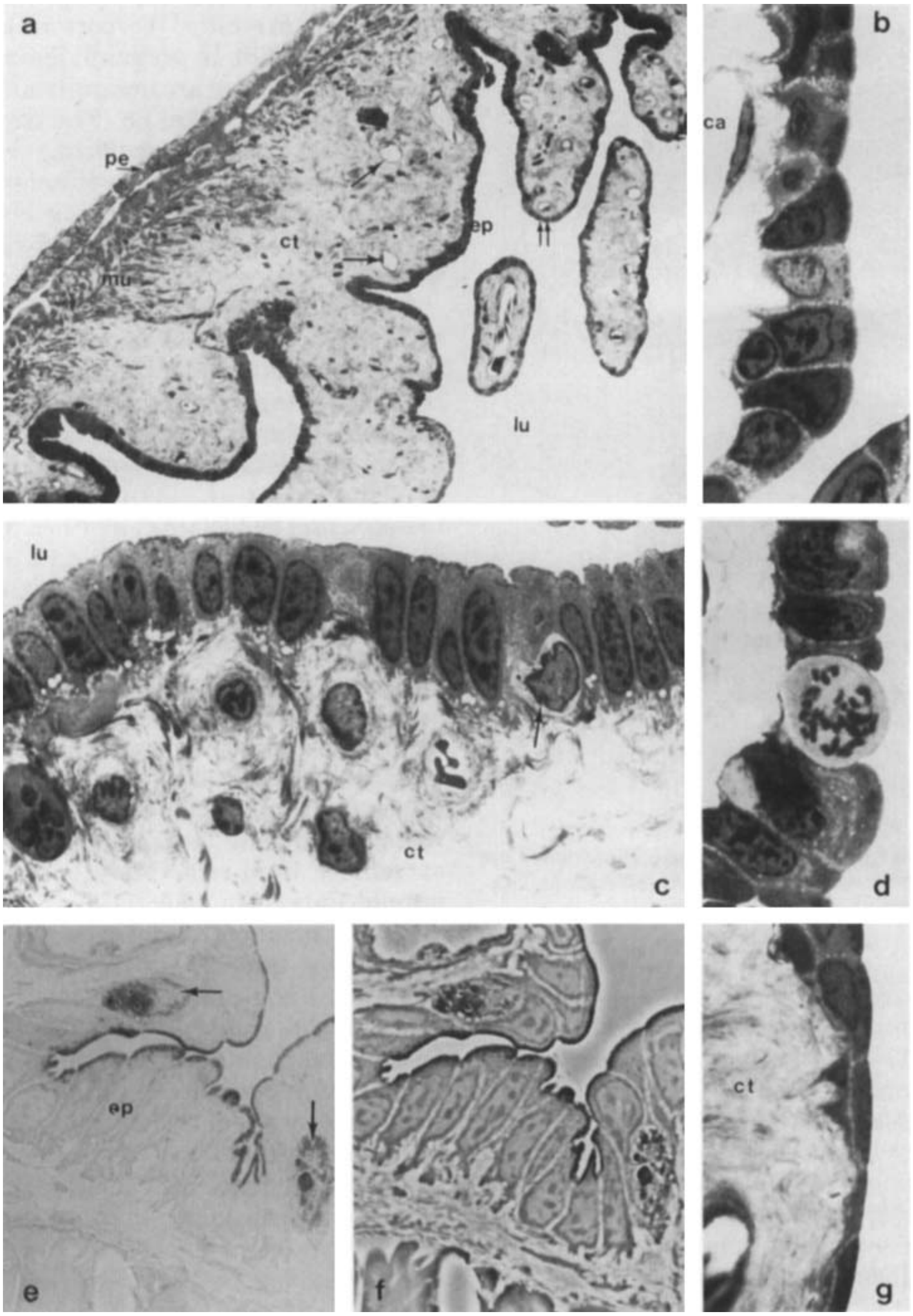

Fig. 8. Oviductal epithelium of *Salamandra atra*. **a–b:** Cross sections of the posterior oviductal epithelium of an ovulating *S. atra* (a, × 75; b, × 756). **c:** Epithelium of a pregnant female (× 756). **d:** Epithelium of a late-pregnant female with fully metamorphosed young (× 756). **e–f:** PAS stained section of epithelium of female in D (× 75). **g:** Epithelium of non-pregnant female (× 756). ca = capillary; ct = connective tissue; ep = epithelium; lu = lumen of uterus; mu = muscle layer; pe = peritoneal epithelium. Reprinted from Greven, '77, with permission.

ova (3–6 mm dia), as have some oviparous species, and viviparous species have the smallest ova at 1–2 mm dia (Wake, '77a, and unpubl.). Egg number is reduced in caecilians in general (approximately 50 in oviparous species with the greatest numbers; Wake, pers. obs.), and ranges from 10 to 50 in viviparous species (Wake, pers. obs.; data are for fully yolked, ovarian ova). However, the number of fetuses carried by viviparous females is considerably smaller than the number of ovarian ova, 4–12 ($\bar{x}$ = 7) in

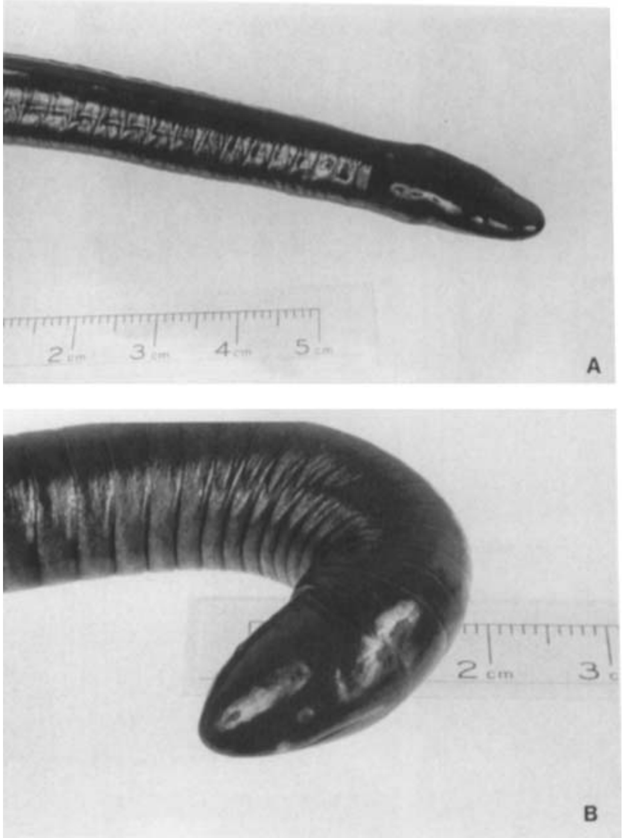

Fig. 9. Viviparous caecilians. **A:** *Dermophis mexicanus*, Family Caeciliaidae (Central America). **B:** *Typhlonectes natans*, Family Typhlonectidae (northwestern South America).

Dermophis mexicanus (Wake, '80a) and 2–11 in *Typhlonectes compressicaudus* (Exbrayat, '83; Exbrayat et al., '81, '82). In contrast to the situation in most amphbians (and reptiles), clutch size is *not* correlated with body size (Fig. 11) (Wake, '80b).

The ovarian cycle has been described only for *D. mexicanus, Gymnopis multiplicata* (Wake, '68, '80b), and *Typhlonectes compressicaudus* (Exbrayat, '83). Three classes of ova are normally present in the ovary: small, pale pre-vitellogenic ova, vitellogenic ova, and nearly to fully yolked ova. Vitellogenesis apparently takes at least a full year, for it appears that viviparous caecilians reproduce biennially (Wake, '80a; Exbrayat et al., '81, '82). The breeding cycle is seasonal. In *Dermophis mexicanus,* fertilization occurs in June–July and birth in May–June, after an 11-month gestation period (Wake, '80a). The gestation period is 6–7 months in *Typhlonectes compressicaudus* (Delsol et al., '81 '83; Exbrayat et al., '81, '82, '83). Fertilization and birth may be less rigidly synchronous in *Typhlonectes* than in *Dermophis.*

Following ovulation in all species (oviparous or viviparous) examined, corpora lutea (Fig. 12) develop (Wake, '68). In pregnant females the corpora lutea are large, and are maintained throughout the gestation period (Wake, '68, '77a, '82; Exbrayat, '83). Preliminary data on circulating progesterone levels indicate an elevated titer throughout pregnancy (Wake, unpubl. data). Nothing else is known of the endocrinology of caecilians, viviparous or otherwise.

Oviduct morphology of non-pregnant and pregnant females has been examined in *D. mexicanus and G. multiplicata* for the entire reproductive cycle, and for stages of pregnancy and non-pregnancy in several other species by Wake ('70, '72, '80b, '85, and unpubl. data), in *Typhlonectes compressicaudus* by Exbrayat et al. ('83), and in *Chthonerpeton indistinctum* by Welsch et al. ('77). In non-pregnant females the oviductal epithelium is a low monolayer. Early in pregnancy the oviduct wall hypertrophies and develops deep folds, covered by a proliferated and hyperemic epithelium. Proliferation and secretion begin at 2–3 months into the 11 month pregnancy in *D. mexicanus* (Wake, '80a). The epithelial cell contents early in pregnancy are rich in free amino acids and carbohydrates; in mid-pregnancy conjugated amino acids and carbohydrates predominate; and during late pregnancy, the secretion is extremely lipid-rich (Welsch et al., '77; Wake, unpubl. data) (Fig. 13A,B,C).

Development of the embryos is dependent on the yolk supply; that of fetuses on maternal nutrition. I define the beginning of the "fetal" period as that at which yolk is fully resorbed and development is dependent on maternal nutrition. At the time yolk is resorbed and oviductal epithelial proliferation occurs, the fetuses mineralize their fetal dentition and the components of the jaw articulation (Fig. 14) (Wake and Hanken, '82). The fetal dentition is characterized by tooth crown shape and distribution that differs markedly from that of the adult, and that changes during fetal ontogeny (Fig. 15) (Wake, '76, '77a, '77b, '78b, '80b) (free-living larvae have teeth of the adult form, though fewer of them). The fetal dentition is shed at birth, and replaced by the adult configuration. Wake ('77a, '80b) has conjectured that the teeth are used to stimulate secretion by mechanically abrading the oviductal epithelium; the secreted material as well as epithelial cells often fill the mouth and pharynx of fetuses (Wake, unpubl. data).

Typhlonectid embryos and fetuses differ markedly in gill structure from those of other viviparous (and non-viviparous) species (Fig. 16ab). The gills of terrestrial species are triramous, with long or short

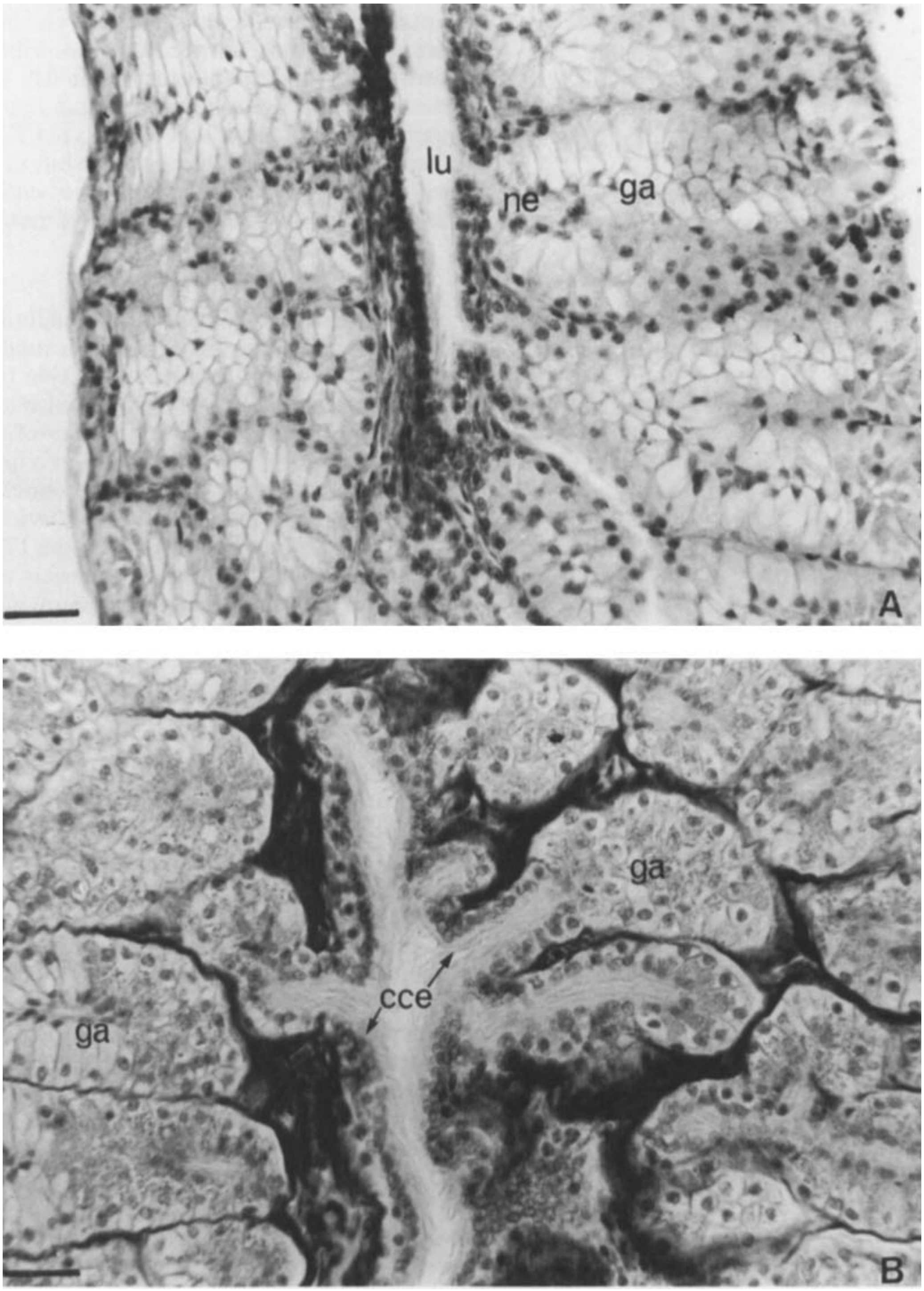

Fig. 10. Frontal sections through secretory Mullerian glands of **A:** *Herpele squalostoma*. **B:** *Scolecomorphus vittatus*. Bar = 0.25 mm. cce = ciliated columnar epithelium; ga = gland; lu = lumen; ne = neck.

fimbriae on each gill; the gills are lost in a prolonged metamorphosis well before birth (Wake, '67, '77a). Typhlonectids, however, have a single pair of large, sac-like gills. These gills are highly vascular, and the supply is from the same three aortic arches as serve the triramous gills of terrestrial taxa (Wake, unpublished data). Delsol et al. ('81, '83, '86) contend that typhlonectid gills function as pseudo-

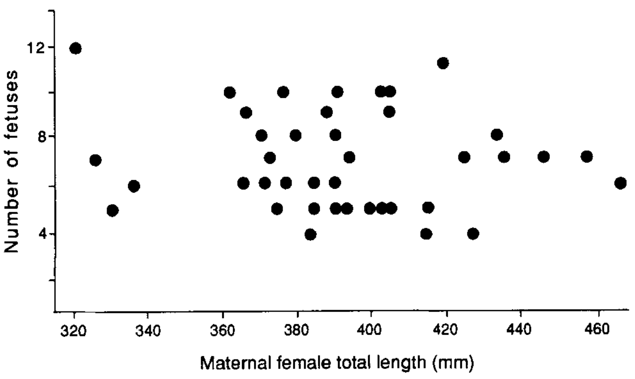

Fig. 11. Female body size versus oviductal clutch size in *Dermophis mexicanus* (after Wake, '80b). Note that maternal body size is *not* correlated with clutch size in this viviparous species, in contrast to most amphibians.

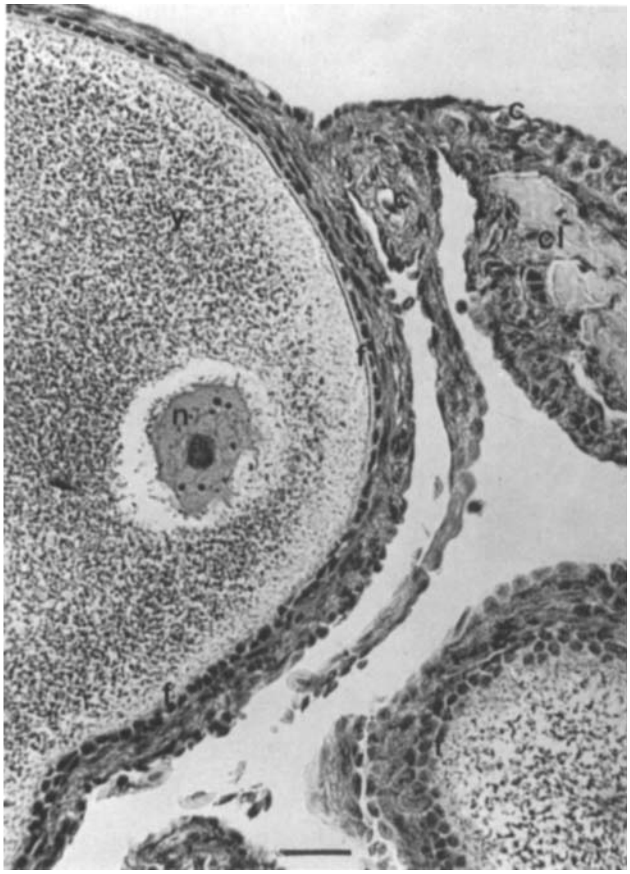

Fig. 12. Section through ovary, showing vitellogenic ovum and corpus luteum in *Scolecomorphus vittatus*, a viviparous east African scolecomorphid caecilian (after Wake, '68). Bar = 0.2 mm. c = capillary; cl = corpus luteum; f = follicle; n = nucleus; y = yolk.

placentae and that gaseous exchange and perhaps nutrient uptake occurs across the epithelial membrane into the circulation. They describe the structure of the gills as an ectotrophoblast. There have been no experimental tests of this hypothesis reported to date. Toews and Macintyre ('77) described the fetal-maternal O_2-hemoglobin shift in *Typhlonectes*. High O_2 saturation of fetal blood can take place despite only "moderate" saturation of maternal blood.

CONCLUSIONS

Members of the three orders of the living Amphia have converged on viviparity as a mode of reproduction. There are some similarities in the ways they have achieved viviparity, but also many differences. Only two species of frog and one of salamander are viviparous (a few others are ovoviviparous); but viviparity is a major mode of reproduction among caecilians, having evolved at least twice and characterizing perhaps half or more of the 170 + species in the order, and there are no known ovoviviparous caecilians. Features of amphibian viviparity—maintenance of developing young in the oviducts of the female with maternal nutrition provided after the yolk supply is exhausted—can be generalized and summarized as follows:

1. All viviparous amphibians have internal fertilization; this is a necessary but not sufficient component of viviparity. However, the means of internal fertilization differs among members of the three orders. Frogs use cloacal apposition; salamanders use spermatophore transfer via the substrate; caecilians insert the male phallodeum into the vent of the female.
2. All viviparous amphibians have corpora lutea that appear to be involved in maintenance of the pregnancy. The endocrinology of the system is known only for the frog *Nectophrynoides*.
3. All viviparous amphibians have relatively long gestation periods. Those of frogs apparently are rigidly controlled by an interaction of internal and environmental factors; the gestation period of *Salamandra atra* is apparently very plastic (2–5 years), but thought to be correlated with the rigor of the cold season; the gestation period of caecilians, based on only a few species, seems to be strongly tied to environmental factors, especially birth at the inception of the rains, but is synchronous within a population.
4. Viviparous amphibians characteristically have small ova and small clutches. The young are born fully metamorphosed. Ovoviviparous species, which

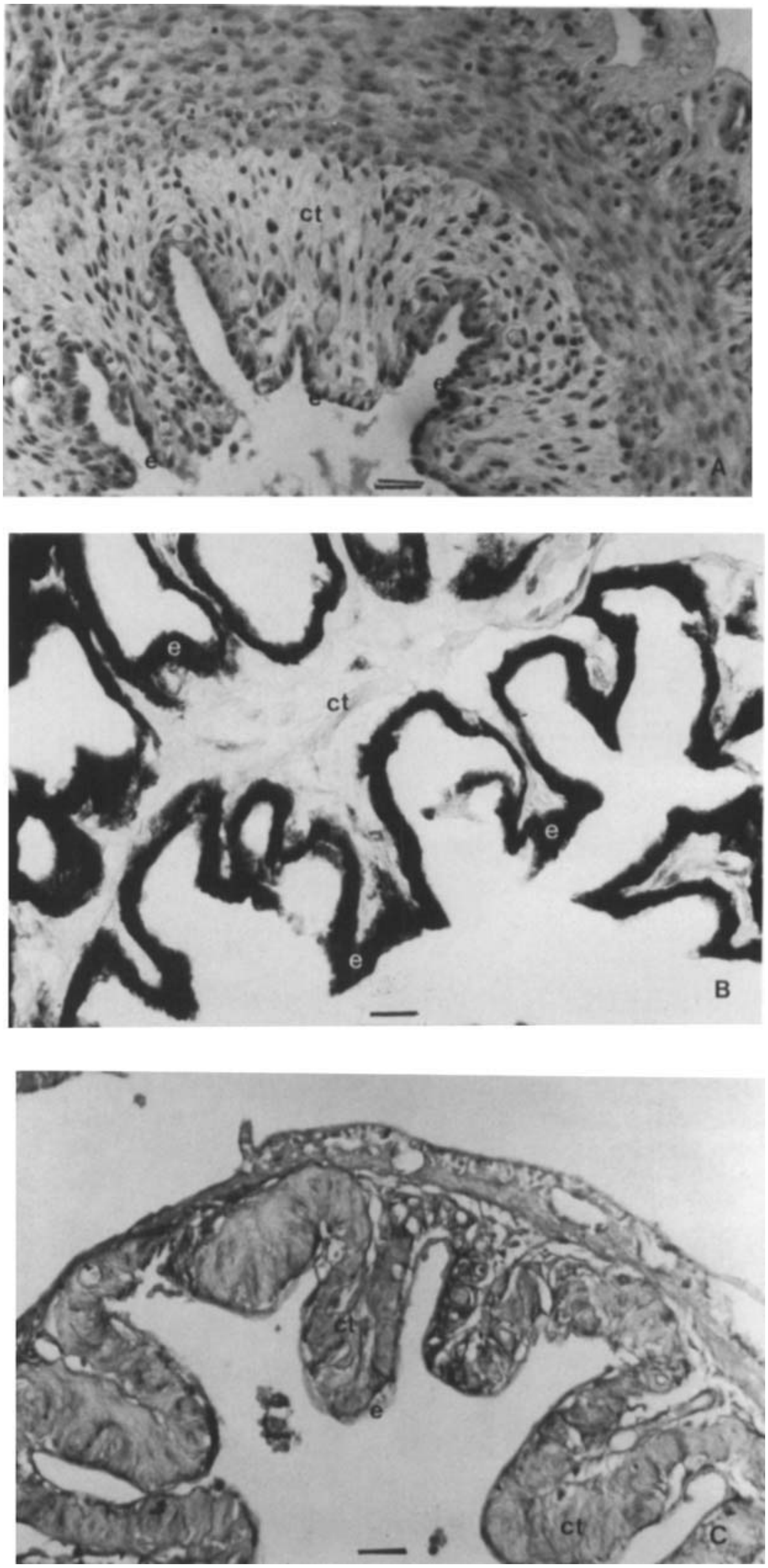

Fig. 13. Oviductal epithelial cycle in *Dermophis mexicanus*. **A:** Nonpregnant oviduct; epithelium is thin and nonsecretory (hematoxylin and eosin stain). **B:** Mid-pregnant oviduct; connective tissue and epithelium are extended into deep folds, and the epithelium is proliferated, hyperemic, and secretory (stained with Sudan black B). **C:** Post-pregnant oviduct; folds are reduced, and epithelium is much eroded and a monolayer where present (hematoxylin and eosin). Bars = 10 μm. ct = connective tissue; e = epithelium.

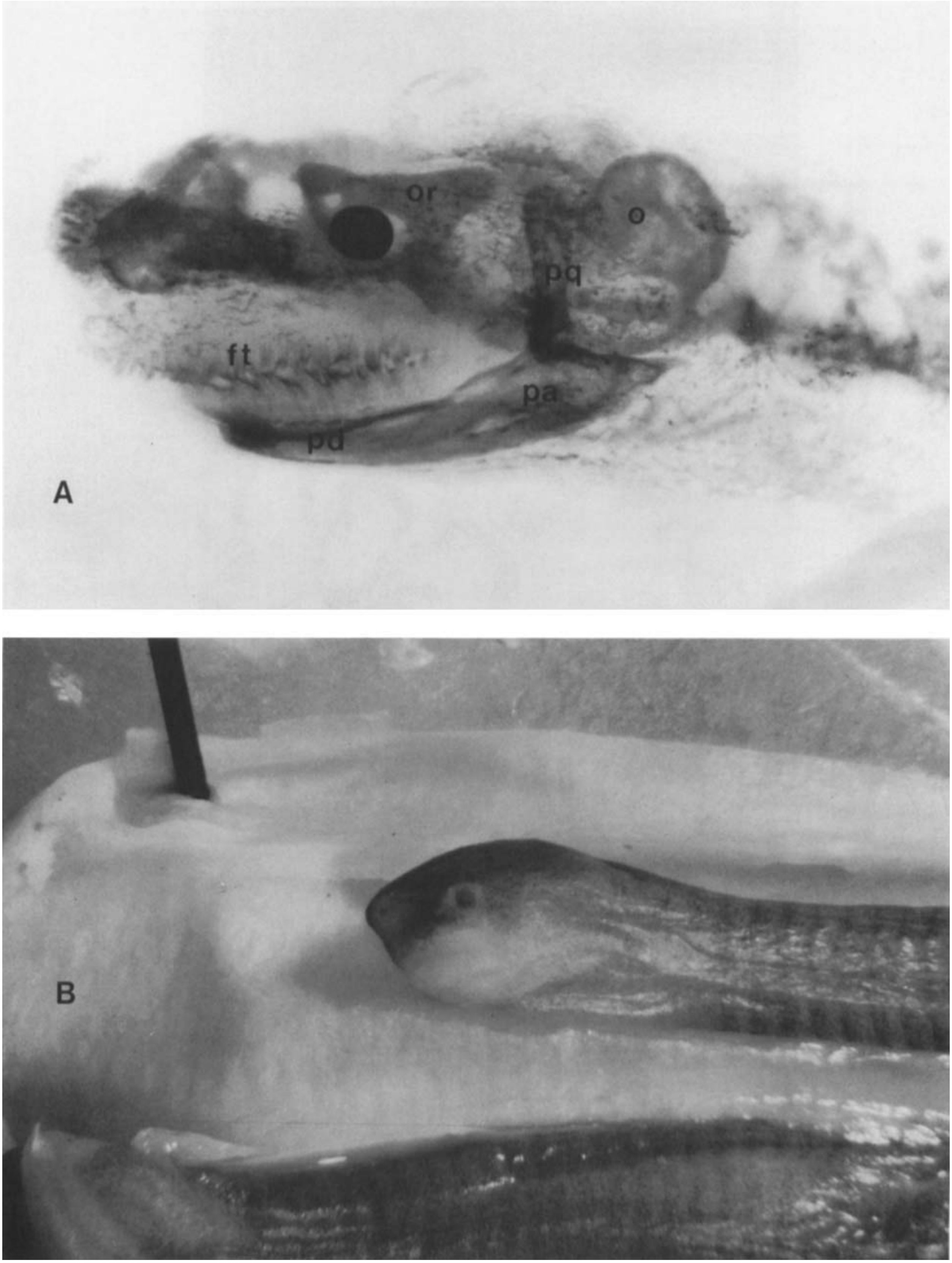

Fig. 14. Fetuses of *Dermophis mexicanus*. **A:** At 37 mm total length, the tooth crowns and the jaw articulartion elements are mineralized (after Wake and Hanken, '82). ft = fetal teeth; o = otic capsule; or = orbital cartilage; pa = pseudarticular bone (dermal bone forming posterior lower jaw); pd = pseudodentary (dermal bone forming anterior lower jaw); pq = palatoquadrate (lower part ossifying; incipient quadrate element of the adult articulation). **B:** 75 mm TL fetuses of *D. mexicanus* in situ in the oviduct.

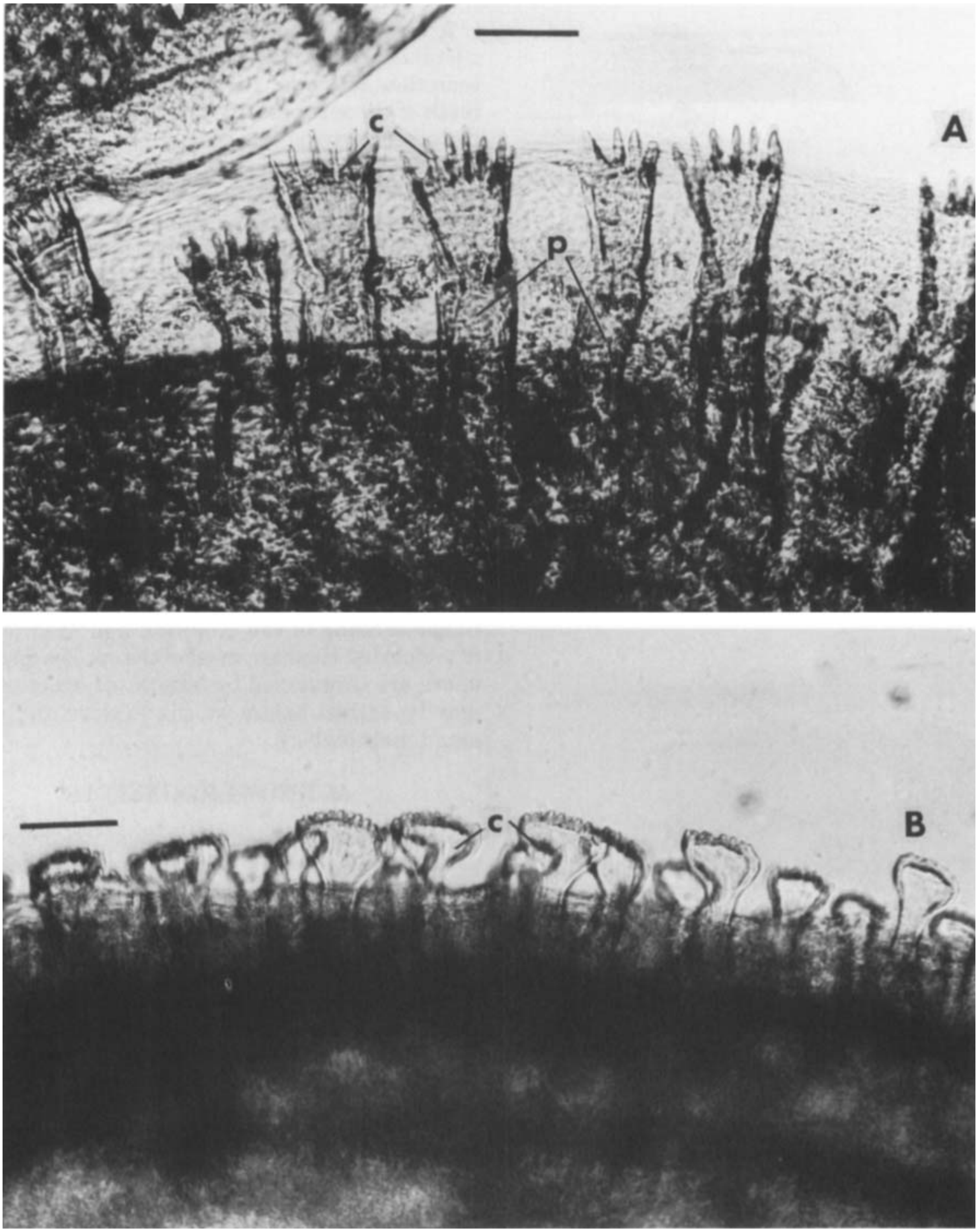

Fig. 15. Fetal teeth of **A)** *Gymnopis multiplicata* (57 mm TL), **B)** *Typhlonectes natans* (75 mm TL) (from Wake, '77b). c = tooth crowns; p = tooth pedicels. Note differences in crown shapes in the two species.

do not supply significant nutrition in addition to the yolk, give birth either to metamorphosed juveniles (*Eleutherodacylus jasperi*), or variably to young at premetamorphic as well as metamorphosed states (*S. salamandra, Mertensiella*).

5. In viviparous frogs and caecilians, virtually the entire oviductal epithelium proliferates and secretes nutrient material. In the salamander, only an anterior region, the zona trophica, produces the nutrient secretion.

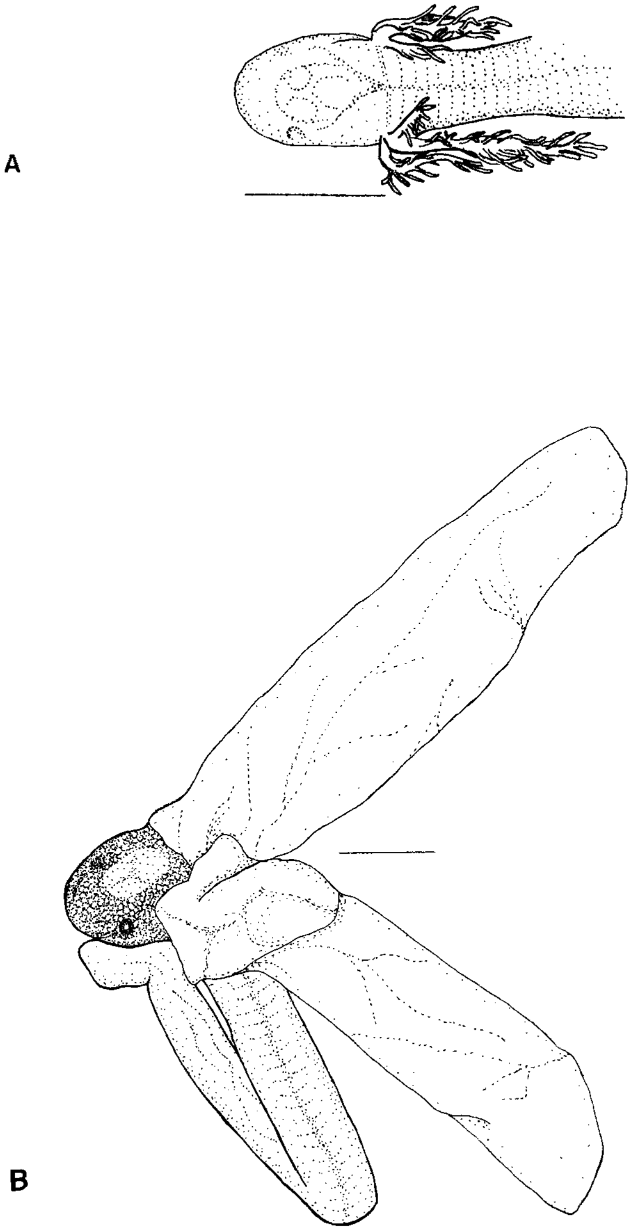

Fig. 16. Fetal gills of viviparous caecilians. **A:** A caeciliaid, *Dermophis mexicanus* (40 mm TL; note triramous, fimbriated gills). **B:** *Typhlonectes natans* (36 mm TL; note enlarged, saclike gills (from Wake, '77b). Bars = 5 mm.

6. Viviparity in amphibians involves means of oral ingestion of the maternal nutrient material; placentae or pseudoplacentae are not developed in either the frog or the salamander, or in caecilians with the possible exception of the typhlonectids. This is in marked contrast to the situation in many viviparous fishes, in which many embryonic epithelia serve as pseudoplacentae, and in viviparous reptiles in which the yolk sac and even the chorionic and allantoic extraembryonic membranes function as placentae.

7. Viviparous caecilians and the salamander have a fetal dentition involved in ingesting the nutrient secretion. However, the salamander uses "larval" teeth of the sort present in larvae of many species; the several viviparous species of caecilians have a fetal dentition that is unique in tooth crown shape and distribution among species and relative to the adult condition. The developing frog may make use of the fimbriae around its mouth as a "sponge" to gather the nutrient secretion for ingestion.

The endocrinology of viviparity in amphibians remains to be understood (Gallien, '59; Amoroso et al., '79), save for the elegant work of Xavier and her colleagues on the frog *Nectophrynoides*. In the same vein, the interplay of environmental factors with the internal maintenance of pregnancy must be better understood. So little is known of the details of reproductive biology of these amphibians that research agendas might well be mounted. These animals could be "model systems" amenable to experimental manipulation in order to increase our understanding of the evolution and maintenance of viviparity. However, most of the species discussed above are threatened by habitat destruction, and may be extinct before we can explore and understand their biology.

ACKNOWLEDGMENTS

I thank the organizers of the Symposium on Comparative Gestation and Placentation in Vertebrates, July 1–6, 1991, for inviting me to participate. I appreciate the stimulation that my graduate students who have worked on the evolution of viviparity (Lorrie Klosterman and Wendy Marlor) have given me. I wish to acknowledge the support of the National Science Foundation for my research on the evolutionary biology of amphibians.

LITERATURE CITED

Amoroso, E.C. (1952) Placentation. In: Marshall's Physiology of Reproduction. A.S. Parkes, ed. Longmans Green, New York, pp. 127–311.

Amoroso, E.C. (1968) The evolution of viviparity. Proc. Roy. Soc. Med., *61:*1188–1200.

Amoroso, E.C., R.B. Heap, and M.B. Renfree (1979) Hormones and the evolution of viviparity. In: Hormones and Evolution. Vol. 2. E.J.W. Barrington, ed. Academic Press, New York, pp. 925–929.

Angel, F. (1943) Description d'un nouvel Amphibien anoure, ovovivipare, de la Haute-Guinée françoise. Bull. Mus. Paris, *15:*167–169.

Angel, F., and M. Lamotte (1944) Un crapaud vivipare d'Afrique occidentale *Nectophrynoides occidentalis* Angel. Ann. Sci. Nat. Zool., *6:*63–89.

Angel, F., and M. Lamotte (1948) Nouvelles observations sur

Nectophrynoides occidentalis Angel. Remarques sur le genre *Nectophrynoides*. Ann. Sci. Nat. Zool., *10:*115–147.

Barrio, A. (1969) Observaciones sobre *Chthonerpeton indistinctum* (Gymnophiona, Caecilidae) y su reproducción. Physis *28:*449–503.

Billo, R.R., J.O. Straub, and D.G. Senn (1985) Vivipare Apoda (Amphibia: Gymnophiona), *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841): Kopulation, Tragzeit, und Geburt. Amphibia-Retpilia, *6:*1–9.

Browning, H.C. (1973) The evolutionary history of the corpus luteum. Biol. of Repro., *8:*128–157.

Busse, K. (1970) Care of the young by male *Rhinaderma darwini*. Copeia, *1970:*395.

Corben, C.J., G.J. Ingram, and M.J. Tyler (1974) Gastric brooding: unique form of parental care in an Australian frog. Science, *186:*946–947.

Delsol, M., J. Flatin, J.-M. Extrayat, and M. Bons (1981) Développement de *Typhlonectes compressicaudus*. C.R. Acad. Sci. (Paris) *293:*281–285.

Delsol, M., J. Flatin, J.-M. Extrayat, and J. Bons (1983) Développement embryonnaire de *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841). Constitution d'un ectotrophoblaste. Bull. Soc. Zool., France *108:*680–681.

Delsol, M., J.-M. Extrayat, J. Flatin, and M. Gueydan-Baconnier (1986) Nutrition embryonnaire chez *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841), Amphibien Apode vivipare. Mem. Soc. Zool., France *43:*39–54.

Drewry, G.E., and K.L. Jones (1976) A new ovoviviparous frog, *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), from Puerto Rico. J. Herpet., *10:*161–165.

Duellman, W.E., and L. Trueb (1986) Biology of Amphibians. McGraw-Hill, New York.

Exbrayat, J.-M. (1983) Premières observations sur le cycle annuel de l'ovaire de *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841), Batracien Apode vivipare. C.R. Acad. Sci. (Paris) *296:*493–498.

Exbrayat, J.-M.(1985) Cycle des canaux de Muller chez le mâle adulte de *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841), Amphibien Apode. C.R. Acad. Sci. (Paris) *301:*507–512.

Exbrayat, J.-M., M. Delsol, and J. Flatin (1981) Premières remarques sur la gestation chez *Typhlonectes compressicaudus* (Dumeril et Bibron, 1841), Amphibien Apode vivipare. C.R. Acad. Sci. (Paris) *292:*417–420.

Exbrayat, J.-M., M. Delsol, and J. Flatin (1982) Observations concernant la gestation de *Typhlonectes compressicaudus* (Dumeril et Bribron, 1841), Amphibien Apode vivipare. Bull. Soc. Zool., France *107:*486.

Exbrayat, J.-M., M. Delsol, and J. Lescure (1983) La viviparité chez *Typhlonectes compressicaudus*, amphibien apode. Bull. Soc. Herpet., France *26:*23–24.

Exbrayat, J.-M., and M.-T. Laurent (1983) Quelques observations concernant le maintien en élevage de deux amphibiens apodes: *Typhlonectes compressicaudus* et *Ichthyophis*. Reproduction de *Typhlonectes compressicaudus*. Bull. Sol. Herpet., France *26:*25–26.

Fachbach, G. (1965) Stimulation des Seitenliniensystems bei dem Uterus entnommen Alpensalamander-Larven (*Salamandra atra*). Zool. Anz. Suppl., *29:*97–106.

Fachbach, B. (1969) Zur Evolution der Embryonal-bzw. Larvalentwicklung bei Salamandra. Zeit. f. Syst. u. Evol., *7:*128–145.

Freytag, G.F. (1955) Feuersalamander und Alpensalamander. Die Neue Brehm-Bucherei, Berlin.

Gallien, L. (1959) Endocrine basis for reproductive adaptations in Amphibia. In: Comparative Endocrinology. A. Gorbman, ed. John Wiley, New York, pp. 479–487.

Gasche, P. (1939) Beitrag zur Kenntnis der Entwicklung von *Salamandra salamandra* L. mit besonderer Berucksichtigung der Winterphase, Metamorphose und des Verhaltens der Schilddruse (Glandula thyroidea). Rev. Suisse Zool., *46:*403–548.

Goicoechea, O., Garrido, O., and B. Jorquera (1986) Evidence for a trophic paternal-larval relationship in the frog *Rhinaderma darwinii*. J. Herpet., *20:*168–178.

Gould, S.J., and E.X. Vrba (1982) Exaptation—a missing term in the science of form. Paleobiology, *8:*4–15.

Grandison, A.G.C. (1978) The occurrence of *Nectophrynoides* (Anura: Bufonidae) in Ethiopia. A new concept of the genus with a description of a new species. Mon. Zool. Ital., *6:*119–172.

Greven, H. (1976) Notizen zum Geburtsvorgang beim Feuersalamander, *Salamandra salamandra* (L.). Salamandra, *12:*87–93.

Greven, H. (1977) Comparative ultrastructural investigations of the uterine epithelium in the viviparous *Salamandra atra* Laur. and the ovoviviparous *Salamandra salamandra* (L.) (Amphibia, Urodela). Cell Tiss. Res., *181:*215–237.

Greven, H. (1980a) Licht und elektronenmikroskopische Untersuchungen zur Struktur und Histochemie des Oviduktepithels. Z. Microsk.-Anat. Forsch., *94:*387–429.

Greven, H. (1980b) Ultrastructural investigations of the epidermis and the gill epithelium in the intrauterine larvae of *Salamandra salamandra* (L.) (Amphibia, Urodela). Z. Mikrosk. -Anat. Forsch., *94:*196–208.

Greven, H. (1980c) Ultrahistochemical and autoradiographic evidence for epithelial transport in the uterus of the ovoviviparous salamander, *Salamandra salamandra* (L.) (Amphibia, Urodela). Cell Tissue Res., *212:*147–162.

Greven, H. (1981) Na-pump sites in the oviduct of *Salamandra salamandra* (L.) (Amphibia, Urodela). Experientia, *37:* 771–772.

Greven, H. (1984) Zona trophica and larval dentition in *Salamandra atra* Laur. (Amphibia, Urodela): adaptations to intrauterine nutrition. Verh. Dtsch. Zool. Ges., *77:*184.

Greven, H., and B.B. Baldus (1984) The distribution of monosaccharides and hexosamines in the oviduct of *Salamandra salamandra* (L.) (Amphibia, Urodela). Comp. Biochem. Physiol., *79B:*229–232.

Greven, H., D. Kuhlmann, and U. Reineck (1975) Anatomie und Histologie des Oviductes von *Salamandra salamandra* (Amphibia: Urodela). Zool. Beitr. N.F, *21:*325–345.

Greven, H., D. Passia, S.g. Haider, and U. Mockenhaupt (1986) Enzymhistochemie am Uterus des Feuersalamanders. Fort. Fertilitatsf., *13:*71–74.

Greven, H., and H. Robenek (1980) On the lumenal plasmalemma and associated structures of the uterine epithelium in pregnant and non-oregnant females of *Salamandra salamandra* (L.) (Amphibia, urodela). Electr. Microsc., *2:*34.

Greven, H., and H. Robenek (1982) Further freeze-fracture studies on the uterine epithelium of *Salamandra salamandra* (L.) (Amphibia, Urodela) using the antibiotic filipin. Cell Tissue Res., *226:*441–447.

Greven, H., and H.-J. Ruterbories (1984) Scanning electron microsocpy of the oviduct of *Salamandra salamandra* (L.) (Amphibia, Urodela). Z. microsk-anat. Forsch., *98:*49–62.

Greven, H., J. Schindelmeiser, and H. Straub (1983) The innervation of the uterus in *Salamandra salamandra* (L.) (Amphibia, Urodela) A morphological and biochemical study. Cell Tissue Res., *232:*421–431.

Hafeli, H.P. (1971) Zur Fortpflanzungsbiologie des Alpensalamanders (*Salamandra atra* Laur.). Rev. Suisse Zool., *78:*235–293.

Heinroth, O. (1915) Geburt von *Typhlonectes natans* (Blindwuhle) im Aquarium. Blatt. f. Aqu. u. Terr., *26:*34–35.

Joly, J. (1960) Le cycle sexuel de la salamandre tachetée, *Salamandra salamandra quadri-virgata,* dans l'ouest de la France. C.R. Acad. Sci. (Paris) *251:*2594–2596.

Joly, J. (1961) Le cycle sexuel biennial chez la femelle de *Salamandra salamandra quadri-virgata* dans les Hautes-Pyrénées. C.R. Acad. Sci. (Paris) *252:*3145–3147.

Joly, J. (1968) Donnés écologiques sur la salamandre tachetée *Salamandra salamandra* (L.). Ann. Sci. Nat. Zool., *10:*301–366.

Joly, J. (1986) La reproduction de la salamandre terrestre (*Salamandra salamandra* L.). In: Traité de Zoologie Amphibiens. Tome 14. P.P. Grasse, and M. Delsol, eds. Masson, Paris, pp. 471–486.

Joly, J., and C. Boisseau (1973) Localisation des spermatozoïdes dans l'oviducte de la salamandre terrestre, *Salamandra salamandra* (L.), (amphibien urodèle) au moment de la fécondation. C. R. Acad. Sci. (Paris) *277:*2537–2540.

Joly, J., and B. Picheral (1972) Ultrastructure, histochimie, et physiologie du follicule pré-ovulatoire et du corps jaune de l'urodèle ovo-vivipare *Salamandra salamandra* (L.). Gen. Comp. Endocrinol., *18:*235–259.

Jones, R.E., A.M. Gerrard, and J.J. Roth (1973) Estrogen and brood pouch formation in the marsupial frog, *Gastrotheca riobambae.* J. Exp. Zool., *184:*177–184.

Jorquera, B., E. Pugin, and O. Goicoechea (1972) Tabla de desarrollo normal de *Rhinaderma darwinii.* Arch. Med. Vet., *4:*1–15.

Lamotte, M. (1959) Observations écologiques sur les populations naturelles de *Nectophrynoides occidentalis* (Fam. Bufonidees). Bull. Biol. Fr. et. Belg., *93:*355–413.

Lamotte, M., and P. Rey (1954) Existence de corpora lutea chez un batracien anoure vivipare, *Nectophrynoides occidentials* Angel; leur evolution morphologique. C. R. Acad. Sci. (Paris) *238:*393–395.

Lamotte, M., P. Rey, and M. vogeli (1964) Recherches sur l'ovaire de *Nectophrynoides occidentalis,* batracien anoure vivipare. Arch. Anat. Mic. Morphol. Esp., *53:*179–244.

Lamotte, M., and F. Xavier (1972a) Recherches sur le développement embryonnaire de *Nectophrynoides occidentalis* Angel, amphibien anoure vivipare. I. Les principaux traits morphologiques et biométriques du développement. Ann. Emb. Morphol., *5:*315–340.

Lamotte, M., and F. Xavier (1972b) Les Amphibiens Anoures à développement direct d'Afrique. Observations sur la biologie de *Nectophrynoides tornieri* (Roux). Bull. Soc. Zool. France *97:*413–418.

Lynn, W.G. (1942) The embryology of *Eleutherodactylus nubicola,* an anuran which has no tadpole stage. Carn. Inst. Wash. Publ., *541:*27–62.

Murphy, J.B., H. Quinn, and J.A. Campbell (1977) Observations on the breeding habits of the aquatic caecilian *Typhlonectes compressicaudus.* Copeia, *1977:*66–69.

Niederl, H. (1981) Anatomical and histological studies of the female and male genital cycle of *Salamandra atra* Laur. Zool. Jb. Anat., *106:*46–63.

Orton, G.L. (1949) Larval development of *Nectophrynoides tornieri* (Roux), with comments on direct development in frogs. Ann. Carnegie Mus., *31:*257–278.

Salthe, S.N., and J.S. Mecham (1974) Reproductive and courtship patterns. In: Physiology of the Amphibia. Vol. II. B. Lofts, ed. Academic Press, New York, pp. 309–521.

Schwalbe, G. (1896) Zur Biologie und Entwicklungsgeschichte von *Salamandra atra* und *maculosa.* Z. Biol., N.F., *16:*340–396.

Taylor, E.H. (1968) The Caecilians of the World: A Taxonomic Review. Univ. Kansas Press, Lawrence, KA.

Thiesmeier, B., and K. Haker (1990) *Salamandra salamandra bernardezi* Wolterstorff, 1928 aus Oviedo, Spanien, nebst Bemerkungen zur Viviparie in der Gattung *Salamandra.* Salamandra, *26:*140–154.

Tonutti, E. (1931) Beitrag zur Kenntnis der Gymnophionen. XV. Das Genitalsystem. Morphol. Jahrb., *70:*101–130.

Tonutti, E. (1933) Beitrag zur Kenntnis der Gymnophionen. XIX. Kopulationsorgane bis weiteren Gymnophionen. Morphol. Jahrb., *72:*155–211.

Toews, D., and D. Macintyre (1977) Blood respiratory properties of a viviparous amphibian. Nature, *266:*464–465.

Tornier, G. (1905) *Pseudophryne vivipara,* sp. n., ein lebendig gebarender Frosch. Sitzb. Ak. Berlin, *2:*855–857.

Townsend, D.S., M.M. Stewart, F.H. Pough, and P.F. Brussard (1981) Internal fertilization in an oviparous frog. Science, *212:*469–471.

Tyler, M.J. and D.B. Carter (1981) Oral birth of the young of the gastric brooding frog *Rheobatrachus silus.* Anim. Beh., *29:*280–282.

Tyler, M.J., D.J.C. Shearman, R. Franco, P. O'Brien, R.F. Seamark, and R. Kelly (1982) Inhibition of gastric acid secretion in the gastric brooding frog, *Rheobatrachus silus.* Science, *216:*609–610.

Vilter, A., and V. Vilter (1962) Rôle de l'oviducte dans l'uniparité utérine de la salamandre noire des Alpes orientales (*Salamandra atra* Laur.), C. R. Soc. Biol. (Paris) *156:*49–51.

Vilter, V. (1967) Histologie de l'oviducte chez *Salamandra atra* Mature, urodèle totalement vivipare de hautre montagne. C. R. Soc. Biol. (Paris) *161:*260–264.

Vilter, V. (1986) La réproduction de la salamandre noire (Salamandra atra). In: Traité de Zoologie Amphibiens. Tome 14. P.P. Grasse and M. Delsol, eds. Masson, Paris, pp. 487–495.

Vilter, V., and A. Vilter (1960) Sur la gestation de la Salamandre noire des Alpes, *Salamandra atra* Laur. Cr. R. Soc. Biol. (Paris) *154:*290–294.

Vilter, V., and A. Vilter (1964) Sur l'évolution des corps jaunes ovariens chez *Salamandra atra* Laur. des Alpes vaudoises. C. R. Soc. Biol. (Paris) *158:*457–461.

Vilter, V., and A. Lugand (1959a) Trophisme intra-utérin et croissance embryonnaire chez *Nectophrynoides occidentalis* Angel crapaud totalement vivipare du Mont Nimba (Haute-Guinée). C. R. Soc. Biol. (Paris) *153:*29–32.

Vilter, V., and A. Lugand (1959b) Recherches sur le déterminisme interne et externe du corps jaune gestatif chez le crapaud vivipare du Mont Nimba, le *Nectophrynoides occidentalis* de la Haute Guinée. C. R. Soc. Biol. (Paris) *153:* 294–297.

Wake, M.H. (1967) Gill structure in the caecilian genus *Gymnopis.* Bull. So. Calif. Acad. Sci., *66:*109–116.

Wake, M.H. (1968) Evolutionary morphology of the caecilian urogenital system. Part I. The gonads and fat bodies. J. Morphol., *126:*291–332.

Wake, M.H. (1970) Evolutionary morphology of the caecilian urogenital system. Part II. The kidneys are urogenital ducts. Acta Anat., *75:*321–358.

Wake, M.H. (1972) Evolutionary morphology of the caecilian urogenital system. Part IV. The cloaca. J. Morphol., *136:* 353–366.

Wake, M.H. (1976) The development and replacement of teeth in viviparous caecilians. J. Morphol., *148:*33–64.

Wake, M.H. (1977a) Fetal maintenance and its evolutionary significance in the Amphibia: Gymnophiona. J. Herpetol., *11:*379–386.

Wake, M.H. (1977b) The reproductive biology of caecilians: an evolutionary perspective. In: The Reproductive Biology of Amphibians. D.H. Taylor and S.I. Guttman, eds. Plenum, New York, pp. 73–101.

Wake, M.H. (1978a) The reproductive biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with comments on the evolution of live-bearing systems. J. Herpetol., *12:*121–133.

Wake, M.H. (1978b) Ontogeny of *Typhlonectes obesus,* with emphasis on dentition and feeding. Papeis Avulsos, Zool., *12:*1–13.

Wake, M.H. (1980a) The reproductive biology of *Nectophrynoides malcomi* (Amphibia: Bufonidae), with comments on the evolution of reproductive modes in the genus *Nectophrynoides.* Copeia, *1980:*193–209.

Wake, M.H. (1980b) Reproduction, growth, and population structure of *Dermophis mexicanus* (Amphibia: Gymnophiona). Herpetologica, *36:*244–256.

Wake, M.H. (1981) Structure and function of the male Mullerian gland in caecilians, with comments on its evolutionary significance. J. Herpetol., *15:*17–22.

Wake, M.H. (1982) Diversity within a framework of constraints. Amphibian reproductive modes. In: Environmental Adaptation and Evolution. D. Mossakowski and G. Roth, eds. Gustav Fischer, Stuttgart, pp. 87–106.

Wake, M.H. (1985) Oviduct structure and function in nonmammalian vertebrates. Fortsch. Zool., *30:*427–435.

Wake, M.H. (1989) Phylogenesis of direct development and viviparity in vertebrates. In: Complex Organismal Functions: Integration and Evolution in Vertebrates. D.B. Wake and G. Roth, eds. pp. 235–250.

Wake, M.H. (1992) Reproduction in caecilians. In: Reproductive Biology of South American Vertebrates. W.C. Hamlett, ed. Acad. Press, New York, pp. 112–120.

Wake, M.H. and J. Hanken (1982) The development of the skull of *Dermophis mexicanus* (Amphibia: Gymnophiona), with comments on skull kinesis and amphibian relationships. J. Morphol., *173:*203–223.

Welsch, U., W. Muller, and C. Schubert (1977) Electron-microscopical and histochemical observations on the reproductive biology of viviparous caecilians (*Chthonerpeton indistinctum*). Zool. Jahrb. Anat., *97:*532–549.

Weygoldt, P. (1980) Complex brood care and reproductive behavior in captive poison-arrow frogs, *Dendrobrates pumilio* O. Schmidt. Behav. Ecol. Sociobiol., *7:*329–332.

Wiedersheim, R. (1890) Beitrage zur Entwicklungsgeschichte von *Salamandra atra.* Arch. mikr. Anat., *36:*469–482.

Wunderer, H. (1909) Beitrage zur Biologie und Entwicklungsgeschichte des Alpensalamanders (*Salamandra atra* Laur.) Zool. Jb. Syst., *28:*23–80.

Xavier, F. (1970a) Analyse du rôle des corpora lutea dans le maintien de la gestation chez *Nectophrynoides occidentalis* Angel. C. R. Acad. Sci., (Paris) *270:*2018–2020.

Xavier, F. (1970b) Action modératrice de la progestérone sur la croissance des embryons chez *Nectophrynoides occidentalis* Angel. C. R. Acad. Sci. (Paris) *270:*2115–2117.

Xavier, F. (1973) Le cycle des voies génitales femelles de *Nectophrynoides occidentalis* Angel, amphibien anoure vivipare. Zeit. Zellforsch., *140:*509–534.

Xavier, F. (1974) La pseudogestation chez *Nectophrynoides occidentalis* Angel. Gen. Comp. Endocrinol., *22:*98–116.

Xavier, F. (1975a) Étude expérimentale du rôle des facteurs externes sur l'évolution de la gestation chez *Nectophrynoides occidentalis* Angel (Amphibien anoure vivipare). Publ. Lab. Zool. E.N.S., *6:*101–132.

Xavier, F. (1975b) Rôle de l'éclairement dans le mécanisme de la parturition, la croissance ovocytaire et l'évolution de la vitellogenèse chez *Nectophrynoides occidentalis* Angel (Amphibien anoure vivipare). Publ. Lab. Zool. E.N.S., *6:* 133–164.

Xavier, F. (1977) An exceptional reproductive strategy in Anura: *Nectophrynoides occidentalis* Angel (Bufonidae), an example of adaptation to terrestrial life by viviparity. In: Major Patterns in Vertebrate Evolution. M.K. Hecht, P.C. Goody, and B.M. Hecht. Plenum, New York, pp. 545–552.

Xavier, F. (1978) *Nectophrynoides liberiensis,* a new species (Anura: Bufonidae) from the Nimba ridge, Liberia: 1. Description of the species. Bull. Soc. Zool., France *103:*431–442.

Xavier, F. (1986) La réproduction des *Nectophrynoides.* In: Traité de Zoologie Amphibiens. Vol. 14. P.P. Grasse and M. Delsol, eds. Masson, Paris, pp. 497–513.

Xavier, F., and R. Ozon (1971) Recherches sur l'activité de l'ovaire de *Nectophrynoides occidentalis* Angel (Amphibien Anoure vivipare) II. Synthèse in vitro des steroïdes. Gen. Comp. Endocrinol., *16:*30–40.

Xavier, F., M. Zuber-Vogeli, and Y. Le Quang Trong (1970) Recherches sur l'activité endocrine de l'ovaire de *Nectophrynoides occidentalis* Angel (Amphibien Anoure vivipare). I. Étude histochimique. Gen. Comp. Endocrinol., *15:* 425–431.

Zuber-Vogeli, M. (1968) Les variations cytologiques de l'hypophyse distale des femelles de *Nectophrynoides occidentialis.* Gen. Comp. Endocrinol., *11:*495–514.

Zuber-Vogeli, M., and J. Doerr-Schott (1976) L'ultrastructure de quatre catégories cellulaires de la pars distalis de *Nectophrynoides occidentalis* Angel (Amphibien Anoure vivipare). Gen. Comp. Endocrinol., *28:*299–312.

Zuber-Vogeli, M., and F. Xavier (1972) Les modifications cytologiques de l'hypophyse distale des femelles de *Nectophrynoides occidentalis* (Angel) castrées en début de gestation. C. R. Acad. Sci. (Paris) *274:*1361–1364.

Zuber-Vogeli, M., and F. Xavier (1973) Les modifications cytologiques de l'hypophyse distale des femelles de *Nectophrynoides occidentalis* Angel après ovariectomie. Gen. Comp. Endocrinol., *20:*199–213.