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The Reproductive Biology of *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), with Comments on the Evolution of Live-bearing Systems

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ABSTRACT—Eleutherodactylus jasperi females retain developing embryos in the oviducts. Three to five fully metamorphosed froglets are born approximately 33 days after the female was in amplexus. Internal fertilization is presumed. Adult morphology of the reproductive organs is not unique. The oviduct has two distinct regions. Clutch size is very small (1-6 ova). The development of the froglets is very similar to that described for several species of direct developing *Eleutherodactylus.* The froglets have a small egg tooth. The tail is thin, highly vascularized, and overlies the dorsum or venter of the embryo. It may function as an organ for gaseous exchange with the oviducal capillaries. Unresorbed yolk is present in froglets even after birth. No other maternal nutrition is suspected. *E. jasperi* reproductive biology is compared with that of *Nectophrynoides* and with trends in reproductive biology within the genus *Eleutherodactylus.*

* * *

INTRODUCTION

Members of the genus *Eleutherodactylus* are terrestrial frogs, all of which are direct developers that lack a free-living larval stage. Most species for which the reproductive biology is known lay clutches of relatively few (10-104) large yolky eggs in protected sites on the ground, on leaves, or in bromeliads; fully metamorphosed froglets hatch from these eggs. However, one species of *Eleutherodactylus,* the recently described *E. jasperi* (Drewry and Jones, 1976), is ovoviviparous. The species lives in bromeliads in the mountains south of Cayey, Puerto Rico, at elevations above 700 m. Females of the species retain the developing eggs in a chamber formed of the fused portions of the oviducts, and fully metamorphosed froglets are subsequently born. This report describes the gross and microscopic morphology of the adult male and female reproductive systems, the anatomy of the later developmental stages of the froglets, and discusses the acquisition of ovoviviparity as a reproductive mode and its evolutionary significance.

MATERIALS AND METHODS

Nine adult specimens of *E. jasperi* were available for study. Eight (four males and four females; three of the latter carrying oviducal froglets) were preserved in formalin in the field and transferred to 70% ethanol. One specimen was preserved in the laboratory in Zenker's formol in order to better control potential shrinkage. In addition, seven newborn and thirteen oviducal froglets were available. Table 1 summarizes information about the specimens and their manner of study.

TABLE 1. Material of *Eleutherodactylus jasperi* Available for Study [Brackets associate members of clutches with the maternal female.]

Specimens	Manner of Study		
Sex	Snout-vent Length (mm)	Dissection	Histological Preparation
♂	17.4	x	Testis, kidney, ducts
♂	18.2	x	
♂	19.1	x	Testis, kidney, ducts
♂	18.8	x	Testis, kidney, fat body
♀	19.4	x	Ovary, kidney, ducts
♀	20.5	x	Ovary, kidney
♀	20.7	x	Ovary, oviduct, cloaca
♀	20.9	x	
♀	21.5	x	Ovary, oviducts
Newborn	6.2		
Newborn	6.8		
Newborn	7.0		
Newborn	7.1	–20.7 ♀	
Newborn	7.2		Entire
Newborn	6.7		
Newborn	7.3		Entire
Oviducal	6.1		
Oviducal	6.4	–20.9 ♀	Entire
Oviducal	Two unmeasured		
Oviducal	6.2		
Oviducal	6.5	–20.5 ♀	Entire
Oviducal	7.0		
Oviducal	7.4		
Oviducal	4.6		
Oviducal	4.8	(with membrane)	Entire
Oviducal	5.0	(with membrane)	Entire
Oviducal	5.1	–19.4 ♀	Entire
Oviducal	5.1		

Reproductive organs and froglets were sectioned at 10 μ and stained with hematoxylin-eosin or picro-ponceau. All measurements were read with a dial caliper and most dissections were done with the aid of a Wild dissecting microscope.

GROSS MORPHOLOGY OF THE FEMALE REPRODUCTIVE TRACT

The ovaries.—The ovaries are the paired sacciform structures typical of most frogs, but they do not fill the abdominal cavity since an ovary contains a maximum of six fully yolked large (to 3.3 mm diameter) eggs. The ovary walls contain large melanophores, and the presence of eggs under the pigmented wall visible through the skin of the venter is used in the field to determine that ovulation has not occurred (Drewry and Jones, 1976). Each ovary contains one to six large fully yolked eggs and many (12-25) small (< 0.6 mm diameter) immature ova. All of five females, collected variously from early April to the end of July, have two visible size classes of eggs in the ovaries. In addition, four of the five carry oviducal froglets in advanced developmental stages.

The fat bodies.—Fat bodies in females carrying developing young are large flat lobes that fan out under the liver and over the oviducts. However, the fat bodies of the female that had fully yolked ovarian eggs but was not carrying young were short, thin finger-like structures.

The oviducts.—The oviducts extend from the region of the heart, where they are held by connective tissue to the pericardium, to the cloaca. The anterior part of the duct, extending from the heart region to below the liver, is narrow and convoluted; the infundibular orifice is not expanded. The posterior part of the duct is a highly dilated thin-walled sac. The diameter of the dilated part of the duct of a female 20.5 mm snout-vent length (SV) carrying developing young is 8.8 mm. The straight-line length of the entire duct is 11.5 mm; the terminal 5 mm of the paired ducts are fused forming a common chamber to contain the developing young. The oviducts join the rectum and the urinary ducts only 2.2 mm anterior to the vent. The cloacal region is therefore very small. In females carrying froglets, only the anteriormost 3 to 5 mm of the 12 mm duct is constricted and convoluted. In the female without froglets but with large ovarian eggs, some 10 mm of the duct is constricted and the left duct particularly is very convoluted. The ducts of all females are highly dilated posteriorly, and fused for some 5 mm, forming a common "uterus."

GROSS MORPHOLOGY OF THE MALE REPRODUCTIVE TRACT

The testes.—Testes are flat and round, 1.2-1.4 mm in diameter (three males examined). The testes of two of the three have punctate melanophores while those of the third appear unpigmented. The testes overlie the kidneys. The testes grossly appear to be comprised of several (10-15) lobes.

The fat bodies.—The fat bodies of the specimen with unpigmented testes are large flat lobes; those of the specimens with pigmented testes are very reduced short, thin finger-like processes. I do not see any correlation of testis and fat body states with reproductive or nutritive condition.

The ducts.—Thin archinephric ducts are visible leading to the cloaca from the testes. Ducts from the testes to the kidneys or the archinephric ducts cannot be discerned with the dissecting microscope, but probably extend from testis to kidney tubules since the testis overlies the kidney and is bound to it by connective tissue.

EXTERNAL MORPHOLOGY OF THE FROGLETS

All of the oviducal froglets examined are in advanced stages of development (Fig. 1). Fore and hind limbs are well developed. The smallest froglet examined (4.6 mm SV) is the developmental equivalent of Lynn's (1942) stage IV; the largest is a 7.3 mm newly born free-living froglet, fully metamorphosed except for the presence of a tail rudiment and an as yet poorly developed tympanum, and the equivalent of Lynn's post-hatching stage V.

The female carrying the oviducal young at the earliest stage of development had three fetuses in the left oviduct, two in the right. The two in the right duct were surrounded by membranes, probably the egg membranes. The others were free in the duct, closely appressed to the duct wall. Pairs of young in this female, and in the others dissected, were usually face-to-face and 'clasping' each other in a characteristic 'fetal' position with limbs drawn up to the body, back arched, and head lowered. This is very similar to the position of froglets of other species of *Eleutherodactylus* in egg membranes previous to hatching (Lutz, 1944; Lynn, 1942; Lynn and Lutz, 1947; Schmidt, 1920, etc.).

4.6 mm SV froglet.—This froglet was least advanced developmentally of those available for study. It still had vestiges of the egg membrane around it. It was, however, nearly fully metamorphosed, with well developed fore and hind limbs, and body proportions similar to those of a newborn. Its broad, flat tail was curved over the left latero-dorsal side of the body and extended to the eye. The tail was thin, translucent, and highly vascularized with extensive branching of vessels. The froglet lay in the oviduct with the tail, overlain by the egg membrane, appressed to the well-vascularized oviduct wall. Fore and hind limbs were folded and held on the abdomen, and

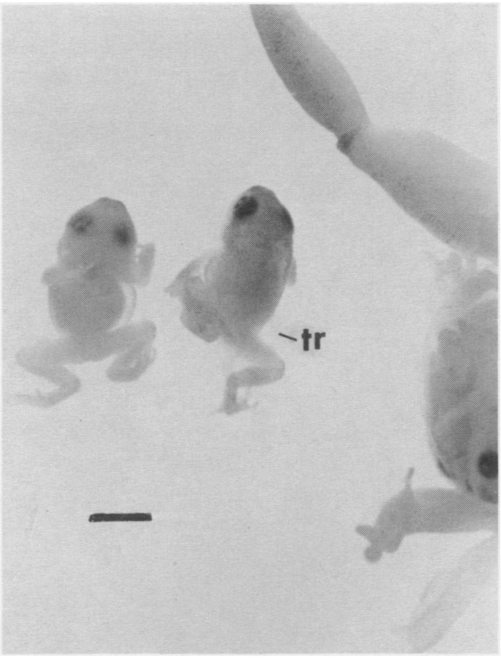

FIGURE 1. Nearly metamorphosed froglets excised from the maternal oviduct. 5.4 (left) and 5.7 (right) mm SV. Bar = 2 mm. Note the tail rudiment curved over the dorsum of the animal. Abbreviations: tr = tail rudiment.

the head and back formed a pronounced curve so that the froglet was nearly spherical. The abdomen contained considerable yolk. The dorsum was covered by scattered melanophores, the greatest concentration occurring on the snout. Melanophores are distributed on the outer surfaces of the limbs, including the digits. The venter lacks melanophores, except for a double row on the margin of the lower jaw; the tail also is unpigmented. The froglet has a tiny bicuspid keratinized egg tooth whose two tips are slightly pigmented.

6.2 mm froglet.—The 6.2 mm SV froglet was posturally very similar to the 4.6 mm froglet as it lay in the oviduct. It did, however, have siblings whose legs were extended. It differed morphologically in having a few scattered melanophores on the venter, as well as on the dorsum, and in having the tail nearly resorbed. The tail was reduced to a fan-like rudiment 3 mm long and 3 mm at its greatest breadth. Its abdomen was swollen with the liver mass high in the cavity and much yolk below it, as seen through the translucent skin of the venter. The egg tooth was smaller than that of the 4.6 mm specimen.

7.3 mm froglet.—The newborn froglet resembled the two described above, except that the tail was resorbed, melanophores were more widely spaced so the coloration more nearly resembled the pattern of the adult, and the egg tooth was sloughing. Body proportions were more nearly adult in that the head was less than one-third of the total length, whereas it exceeded that in smaller specimens. It should be noted that 7.3 mm is not necessarily the normal size at birth; another female contained four oviducal froglets ranging in snout-vent length from 6.2 to 7.4 mm. All of these froglets had a tail rudiment flattened over the dorsum, and all had egg teeth, as did certain other newborns examined.

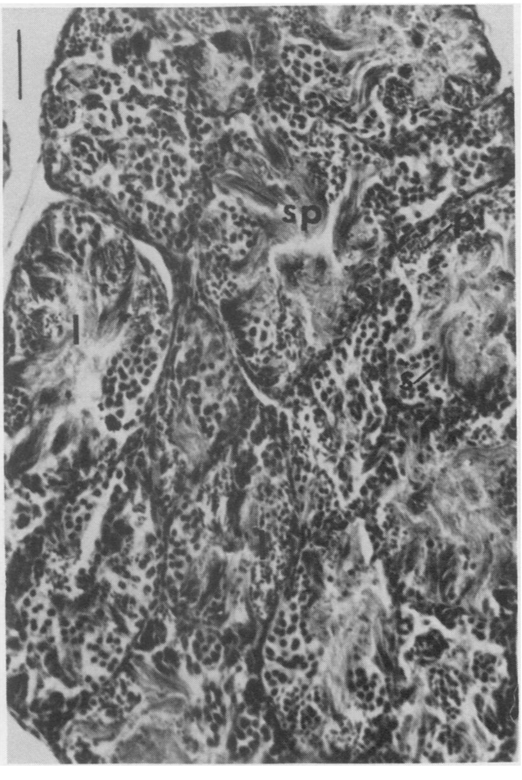

FIGURE 2. Adult male testis. Line = 60 μ. Testes of all males examined were in active spermatogenesis. Abbreviations: l = locule, p = primary spermatocytes, s = secondary spermatocytes, sp = spermatids.

MICROANATOMY OF THE ADULT REPRODUCTIVE SYSTEMS

The ovary-structure of the ovary is typical of that generally found in frogs. The wall of the ovary is highly distended. All of the ovaries examined contain several (1-6) large post-vitellogenic eggs. The wall contains a few widely dispersed capillaries and the ovarian vein and artery are rather small, compared to that of *Rana pipiens.* The ovary wall is pigmented, with large scattered melanocytes. The tissue of the wall contains fibrous connective tissue. Oogonia are embedded in the wall, and as they develop they bulge into the fluid-filled cavity of the ovary. There is no central ovarian stroma such as occurs in mammals. Three classes of potential ova are recognizable microscopically. Nests of cells which will provide future follicles are embedded in the wall and are usually located near a capillary. A second class of ova includes growing vitellogenic follicles which are sequestering yolk precursors. The ovary contains 10 to 25 of these, which is two to three times the number of eggs of the third class, the large post-vitellogenic eggs in which yolk platelets are well formed. Eggs of the latter two classes are surrounded by a layer of flattened follicle cells. These eggs have large nuclei containing numerous peripheral basophilic nucleoli.

A few atretic follicles were seen in the ovaries of the females that were carrying oviducal froglets, but none were observed in the female with fully yolked ovarian eggs but no oviducal young. It was noted above that the vitellogenic eggs are twice as many as the fully yolked eggs. Presumably about fifty per cent of the vitellogenic follicles degenerate, giving rise to atretic follicles. No corpora lutea were observed in any ovaries.

The testis.—Testes are comprised of twelve to 25 lobes. Each lobe is approximately 1.25 mm wide and 1.75 mm long, with anterior- and posteriormost lobes slightly smaller. Testes examined were from animals collected 12 April (2) and 7 July. There was little variation in spermatogenic state among them. Each lobe contained cells in all phases of spermatogenesis. Clusters of primary and secondary spermatocytes were situated randomly in each lobe, with more clusters of secondaries than of primaries apparent (5-10 per lobe vs. 2-6 per lobe). Clusters of spermatids were also present, some central but most at the periphery of the lobe (Fig. 2). The heads of the spermatids are aggregated and rest on a large Sertoli cell. Sperm are relatively long, at least compared to those of *Rana pipiens* and *Hyla regilla.* They are approximately .05 mm long. The head and midbody are 55% of the total length, the tail 45%. That the midbody and tail are proportionately large may be significant if fertilization is internal and sperm must expend energy swimming up the oviducts.

There are only two or three ducts from the testis to the kidney for sperm transport. These are short and tenuous, for the testis is very closely bound to the anterior part of the kidney. The ducts can be traced in serial sections. Some interstitial tissue is present, widely distributed on the peripheries of lobes.

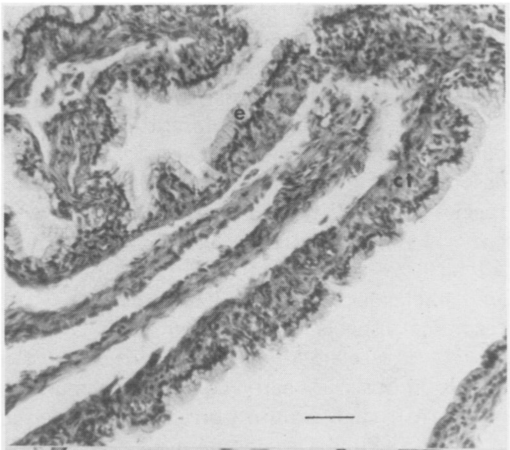

FIGURE 3a. Anterior portion of the pregnant female oviduct. Line = 0.1 mm. Note the thickness of the wall, particularly the height of the epithelial cells. Abbreviations: ct = connective tissue, e = epithelium.

FIGURE 3b. Dilated posterior portion of the pregnant oviduct. Note particularly that distension of the "uterine" portion also involves a lower epithelial height with absence of cilia and microvilli, and capillaries are distributed just below the epithelium. Abbreviations: c = capillary, do = dilated lower "uterine" oviduct, ud = upper oviduct as in figure 3a.

The oviduct.—The oviducts have two distinct histological regions. The upper part of the duct is lined with a ciliated and secretory epithelium (Fig. 3a). There is an abrupt transition to a non-secretory columnar epithelium low in the convoluted part of the duct (Fig. 3b). There connective tissue underlying the epithelium and the epithelium are thrown into folds that pervade the lumen of the anterior part of the duct. In the dilated portion of the duct, the wall and its overlying epithelium are distended and flattened so that they are very thin. The epithelial lining is of a single layer of cuboidal cells. The dilated part of the duct is not particularly highly vascularized though substantial numbers of capillaries are found near the lumens of the ducts. The capillaries are just under the mono-layer of epithelium so that there is potential gaseous exchange between the oviducal capillaries and those of the tails of the froglets in the ducts.

The froglets.—There are in the literature a number of descriptions of development through metamorphosis in several species of direct-developing *Eleutherodactylus* and its relatives, such as *Hylactophryne* (Adamson et al., 1960; Bogert, 1969; Chibon, 1960; Gitlin, 1954; Goin, 1947; Jameson, 1950; Lutz, 1944, 1948; Lynn, 1942, 1948; Lynn and Lutz, 1946, 1947; Sampson, 1904; Valett and Jameson, 1961) of which Lynn (1942) has presented the most complete. Since I lack material representing early and middle stages of development, and since later development does not appear to vary greatly among species of *Eleutherodactylus,* I shall describe the microanatomy of development in *E. jasperi* only if it differs from that of other species or if it is highly significant to the ovoviviparous mode of reproduction.

Skeletal structure: All froglets sectioned had a well developed skeleton that was formed primarily of cartilage. Elements of the skull, particular elements of long bones, carpals, tarsals, and phalanges were cartilagenous. Most froglets showed the beginning of ossification of vertebrae, especially of the centrum. Ossification had also begun in the long bones and certain of the skull elements (see below). It is of interest that ossification does not take place until metamorphosis is nearly complete in many species of *Eleutherodactylus,* including *jasperi.* The newborn froglet is almost entirely cartilagenous.

The skull: Development of the skull is similar to that illustrated by Lynn (1942) for 4 days pre-hatching to 10 days post-hatching *E. nubicola.* A number of tooth buds were present on the maxillary-premaxillary arc. Several contained mineralized but unerupted tooth crowns. Newborns examined also had not yet erupted their teeth. The chondrocranium was well formed. Since all froglets examined were late in development, attention was paid to ossification centers in serially sectioned heads. Ossification of angular, squamosal, dentary, and exoccipital had begun in all specimens, and was most pronounced in the dentary and angular. The 6.5 mm froglet showed slight ossification of the premaxillary, maxillary, and pterygoid. I do not find in any froglet skull the frontal, parietal, or quadratojugal splints seen by Lynn (1942) in *nubicola* four days before hatching. Therefore the ossification sequence of *jasperi* differs from that of *nubicola* in that premaxillary ossification begins after that of the dentary and exoccipital, and frontal, parietal, and quadratojugal ossification in *jasperi* occurs after maxillary and pterygoid ossification, and perhaps that of other bones, has begun. Not only is the sequence slightly different, but ossification appears to be a bit delayed relative to that of *nubicola.* The problem of correlating stages of development in aquatic developers, direct developers, and live-bearers is considered below.

The egg tooth: All of the froglets examined, both oviducal and newly born, had a well keratinized, unpigmented or slightly pigmented, bifurcated egg tooth (Fig. 4). Some resorbtion or sloughing of the structure was indicated in specimens of greater than 7.0 mm snout-vent length. Emergence from the egg membrane occurs when the froglet is 4.5 to 5.0 mm snout-vent length. Lynn (1948, 1961) and Lynn and Peadon (1955) report that loss of the egg tooth is among the short-lived metamorphic events under thyroxin control. It is of interest that *E. jasperi,* which has an egg tooth presumably to facilitate emergence from rather tough egg membranes (determined by comparative dissections), retains the tooth during the rest of its time in the thin-walled oviduct. Further, it is of interest that loss of the tooth, presumably thyroxin-mediated, occurs after birth. On the other hand, the egg tooth is small and not extremely heavily keratinized, and lacks the outer layer illustrated by Adamson et al. (1960) so it may not be effective in puncturing the egg membranes. All direct-developing *Eleutherodactylus* have an egg

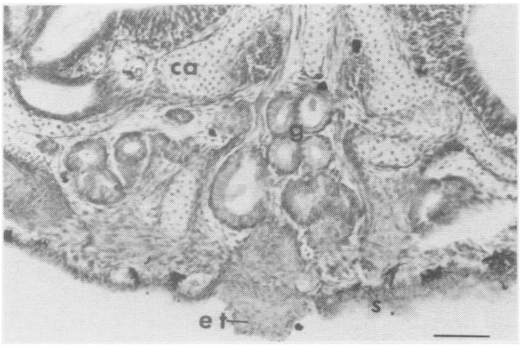

FIGURE 4. Section through the anterior tip of the head, showing the egg tooth. Line = 40 μ. Note that the skull is comprised of cartilage in this nearly metamorphosed froglet. The egg tooth is small, but a distinctly keratinized bifurcate structure. Abbreviations: ca = cartilage, et = egg tooth, g = glands, s = skin.

tooth, whether it is used to rasp membranes or not. Noble (1925) comments that the egg tooth is small in species whose hatching is triggered by rain and whose egg membranes are thin, and that the tooth is larger in species whose membranes are heavier and whose hatching seems less directly environmentally induced.

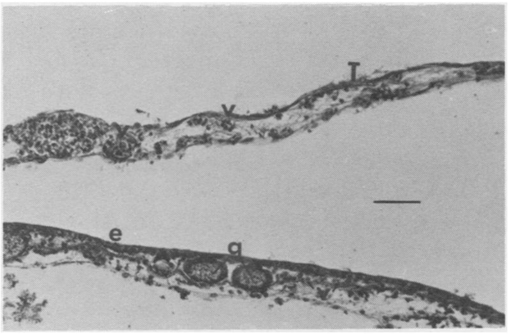

FIGURE 5. The relationship of the fetal tail to the body surface of the froglet. Line = 45 μ. Note that the tail is highly vascular. Contact with the thin epidermis of the froglet and especially with the distended oviduct wall may provide considerable surface for gaseous exchange between fetus and maternal female. Abbreviations: e = epidermis, g = skin gland, t = tail, v = blood vessel.

The skin-tail-oviduct relationship: The tail is thin and highly vascularized (Fig. 5). Capillaries are well distributed and often just below the epidermis, which is one cell layer deep. The skin on the back and on the abdomen of the froglet is also highly vascularized. Capillaries lie just below the thin epidermis (Fig. 5). Skin glands are well developed. The dilated oviduct wall is also adequately though not extensively vascularized. The retention of the broad, flattened tail throughout virtually all of the oviducal period of life in the froglet and its association with well vascularized skin and oviducal surfaces suggests that the tail may be a significant organ for gaseous exchange between froglet and adult female. The tail has also been suggested as a respiratory organ during development in the direct-developing species.

Yolk retention and visceral organ development: The developmentally advanced froglets retain considerable yolk. The abdominal cavity of the newborn froglets was dominated by the yolk mass, and that mass was even larger in smaller oviducal froglets. It has been noted (Bogert, 1969) that *E. decoratus* froglets retain yolk after hatching. In the smallest *jasperi* froglet sectioned, liver buds and a pancreas rudiment were present. In the largest froglet, liver development had progressed substantially, but was not of adult size and proportions (Fig. 6). The gall bladder had also formed. Only at birth did liver development appear to accelerate. At that time the rate of yolk resorbtion also increased, and yolk masses were crowded posteriorly and laterally in the abdominal cavity.

The lungs of unborn froglets are very small sacculated structures. In newborns they are more elongate, but still very small and undilated, relative to adult proportions, though I presume that they are functional. These points are significant for it seems that development of these organs in *E. jasperi* occurs at a different rate than in *E. nubicola* (Lynn, 1942).

FIGURE 6. The developing liver in the abdomen filled with yolk. Line = 80 μ. Yolk is retained through metamorphosis and until after birth. Liver development appears to be slow and restricted. Abbreviations: l = liver, y = yolk.

DISCUSSION

The only other species of frogs previously known to retain developing young in the oviducts are members of the African montane genus *Nectophrynoides,* family Bufonidae. The developing young of *E. jasperi* and of three species of *Nectophrynoides* show some of the general characteristics of direct developing, non-aquatic forms. Mouthparts are poorly developed and denticles and adhesive organs absent; the external gills and spiracle are transitory; rates of development are modified (Lutz, 1947; Orton, 1949). There are, however, marked differences in the mode of development and maternal maintenance between *jasperi* and the *Nectophrynoides.* These can be summarized as follows:

1. *E. jasperi* produces few young per clutch (3-5); *Nectophrynoides* produces substantially more—*occidentalis* 4-35 (Angel and Lamotte, 1944, 1948), *tornieri* 9-35, and *vivipara* 114-135 (Barbour and Loveridge, 1928).
2. The period of development in *jasperi* is short, less than one month (Drewry and Jones, 1976) but is longer in *Nectophrynoides.* Barbour and Loveridge (1928) reported two to three months for *tornieri* and *vivipara*; Angel and Lamotte (1944, 1948) and Lamotte and Xavier (1972) report nine months for *occidentalis,* encompassing the time the maternal female is underground during the dry season, and a period following her emergence. Lamotte and Xavier report that development proceeds slowly for the first six months, and accelerates greatly during the last three.
3. Eggs of *jasperi,* though small for an *Eleutherodactylus* (2.7-5.0 mm dia), are larger than those of *Nectophrynoides*—0.5-0.6 mm in *occidentalis* (Angel and Lamotte, 1948), 0.9 mm in *vivipara* and 2.0 mm in *tornieri* (Gallien, 1959).
4. Intra-oviducal nutrition in *jasperi* is dependent on yolk throughout development; metamorphosed young following birth retain considerable yolk. *Nectophrynoides* mobilize the yolk early during development and are subsequently nourished by ingesting a mucoprotein "uterine milk" secreted by the oviducal epithelial glands (Vilter and Lugano, 1959). Corpora lutea are present in the ovaries of *Nectophrynoides* females carrying young, and apparently regulate the oviducal nutrient secretion (Lamotte et al., 1964). No corpora lutea were seen in *E. jasperi.* Since maternal nutrition is provided in *Nectophrynoides,* they would be considered truly viviparous (according to definitions that require maternal nutrition but no placentation), and *jasperi* is ovoviviparous, since no post-yolk nutrition is provided.
5. Development in *jasperi* differs considerably from that in *Nectophrynoides. Jasperi* has a large, fan-like tail, probably used for intra-oviducal gaseous exchange. It has an egg tooth, possibly of use in hatching from the egg membranes. Both of these features are legacies of the *Eleutherodactylus* lineage. *Nectophrynoides* have long thin tails (Noble, 1927, and Orton, 1949, for *tornieri*; Vilter and Lugano, 1959, for *occidentalis*), and mouths surrounded by papillae (Lamotte and Xavier, 1972). Vilter and Lugano (1959) state that the mouth and gastro-intestinal tract develop usually quickly in *occidentalis,* probably to facilitate ingestion and assimilation of the uterine milk. There are numerous other smaller differences in developmental pattern and mechanism of maternal maintenance between *E. jasperi* and the species of *Nectophrynoides.* These largely indicate convergence in two separate lineages on the reproductive mode of egg retention through metamorphosis, so differences in features of the mechanism are not surprising.

The discovery of a live-bearing species in the genus *Eleutherodactylus,* previously known to be comprised only of direct-developing species, poses a number of questions. The most fundamental is that of the selective pressures that caused the shift in reproductive mode resulting in the evolution of ovoviviparity in one species in a lineage. Questions of seasonality of reproduction, clutch size, and habitat of species of *Eleutherodactylus* must be considered to determine if correlations with the shift in reproductive mode exist. Questions relating to the physiology and morphology of live-bearing systems also arise regarding the mechanism of egg retention through metamorphosis and the events that might trigger birth. Several species of *Eleutherodactylus* practice forms of parental care of their eggs. The shift to ovoviviparity as another mode of parental care must be examined.

Salthe and Duellman (1973) discussed reproductive modes in frogs and reached several conclusions. These include:

1) small body size in frogs is a preadaptation for reproductive experimentation;
2) small to middle-sized frogs are better able to enter terrestrial adaptive zones;

3) selection favors increased clutch size in large frogs and increased ovum size in small frogs;
4) greatest diversity of reproductive modes occurs in the tropics;
5) increased ovum size is associated with terrestrial environments;
6) there are positive correlations of a) ovum size and female snout-vent length, b) clutch size and female snout-vent length, and c) ovum size and size of hatchlings, the latter regardless of reproductive mode, the former two within a given mode as defined by Salthe (1969);
7) there are negative correlations between a) clutch size and ovum size and b) ovum size and rate of development.

These generalizations may be used to analyze the situation in *Eleutherodactylus* and to examine the conditions involved in the change of reproductive mode in the lineage. Data used in this analysis are gathered from the literature (cited throughout this paper and also Crump, 1974; Dunn, 1926, 1931; Goin, 1947, 1954; Lynn and Grant, 1940; Myers, 1969; Noble, 1925, 1931) and from dissection of some twenty species in the collections of the Museum of Vertebrate Zoology at the University of California, Berkeley. Partial information is therefore available for some fifty-five species of *Eleutherodactylus* on female snout-vent length, clutch size, ovum size, hatching size, time to hatching, and characters of developing embryos. The following analysis is not meant to be exhaustive, but is appropriate in that it gives considerable information about the reproductive biology of the genus of use in interpreting the shift to ovoviviparity in *E. jasperi.*

Several aspects of *Eleutherodactylus* reproductive biology correspond to Salthe and Duellman's ideas. All of the species of *Eleutherodactylus* except *jasperi* are direct developers with eggs laid in terrestrial sites; *jasperi* is a small species of *Eleutherodactylus* that has performed a 'reproductive experiment'; the genus is tropical in distribution so the diversity of derived reproductive modes is not unexpected. Increased ovum size characterizes direct developers that lay eggs on land relative to aquatic breeders (Lutz, 1947; Matthews, 1958; Orton, 1949, 1957); e.g., species of *Eleutherodactylus* have smaller clutches of larger eggs (see figures 7, 8, 9) than do *Rana* with hundreds of smaller eggs. The generalization of selection favoring increased clutch size in large frogs and increased ovum size in small frogs may be apparent when all groups of frogs are considered, but it does not completely hold within the genus *Eleutherodactylus.* Figure 7 lumps clutch size and snout-vent length data for several species and for several individuals of different sizes within species. There is a positive correlation, significant at .95. Larger species and larger individuals within species do have larger clutches; that generalization holds for the genus. Note that *E. jasperi,* with small clutches carried by small females, fits the trend for the genus, though it has modified its mode of reproduction. Its small clutch is correlated with small body size and, in addition, with the trend toward reduced clutch size in live-bearing forms. Figure 8 shows that smaller frogs, as species or individuals, do not have increased ovum size. Ovum size is significantly correlated with body size at the .95 level. Eggs ranged from 2.7 to 5.0 mm in diameter. This may be associated with direct development as well as ovoviviparity, in which large amounts of yolk are 'packaged' and are available through metamorphosis in the absence of a free-living, feeding tadpole stage in all species in the genus. There is no significant correlation between clutch size and ovum size (Fig. 9) in the *Eleutherodactylus* sample ex-

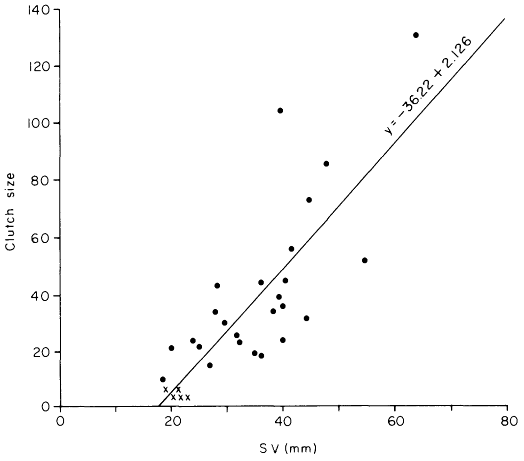

FIGURE 7. Regression of clutch size on female snout-vent length for individuals of several species of *Eleutherodactylus. E. jasperi* are indicated by the symbol X, other species by ● in figures 7 and 9. Correlation (r) = .802; significant at .95.

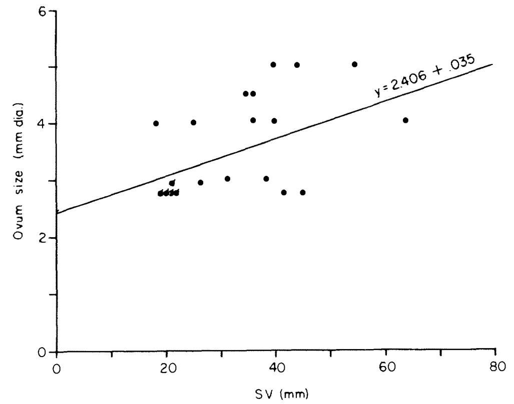

FIGURE 8. Figure 8. Regression of ovum size on female snout-vent length for individuals of several species of *Eleutherodactylus*. Correlation (r) = .498; significant at .95. *E. jasperi* is indicated by flagged symbols.

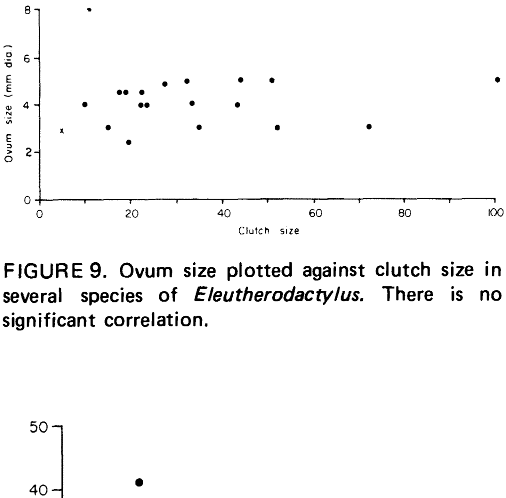

FIGURE 9. Ovum size plotted against clutch size in several species of *Eleutherodactylus*. There is no significant correlation.

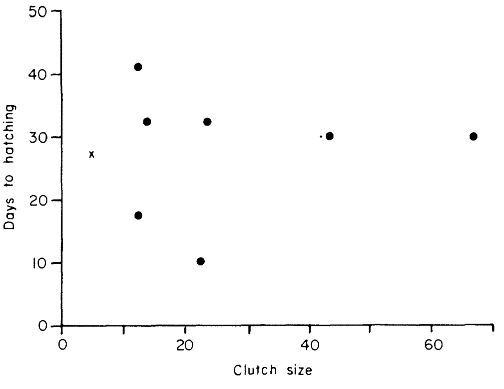

FIGURE 10. Days to hatching (rate of development) plotted against clutch size in several species of *Eleutherodactylus*. Though clutch size in *jasperi* (symbol X) is low, developmental time is comparable to that of other species, and in fact falls at the mean for this sample ($\overline{X}$ = 27.5 da).

amined. This may be associated with the relatively great yolk requirement of direct developers and in the live-bearing species.

Insufficient data were available to test the positive correlation of ovum size and size of hatchling and the negative correlation of ovum size and rate of development. However, the relationship of *jasperi* to other *Eleutherodactylus* for which there are data on clutch size, ovum size, and development shows several phenomena. Size at hatching for *jasperi* was considered to be the size at which the froglet emerged from the egg membranes into the lumen of the oviducal chamber. Rate of development was construed as the time to hatching following fertilization. *E. jasperi* hatches at a smaller size relative to both ovum size and clutch size than other *Eleutherodactylus*. Thus it may be increasing its overall rate of metamorphosis, for hatchlings are virtually fully metamorphosed, though not ossified. Smaller size at hatching appears to be correlated with the time spent in the oviduct after hatching, for size at birth is comparable to hatching size in non-live-bearing *Eleutherodactylus*. The rate of development of *jasperi* is comparable to that of other species of *Eleutherodactylus* (Fig. 10).

The comparison of development of live-bearing frogs with direct developers, and especially with aquatic developers, poses several problems. Data presented above indicate that developmental rate and sequence differ in the live-bearing species relative to direct developers in the same genus, probably because developmental environments are significantly different, especially with regard to oxygenation and other demands on the developing young. Comparisons with aquatic developers are even more difficult because of the elimination of the larval period and, concomitantly, a number of structures such as bones, mouthparts, and lateral line organs. Care has been taken to compare data that appear significant to the question of the acquisition of ovoviviparity in *jasperi*.

The data imply that the ovoviviparous *jasperi* is exploiting its small body size, reducing its clutch size and ovum size relative to clutch size, and shortening its time to hatching but providing for post-hatching oviducal growth until an optimum size for emergence into the environment is reached. Drewry and

Jones (1976) found *jasperi* only in bromelaids. This sort of habitat-specificity is frequent in the tropics, but I see little direct correlation to the shift in reproductive mode, for bromeliads are the frequent habitat of egg-laying-with-tadpole frogs and direct developers, as well as the live-bearing species. I do not have any information on the impact of environment on the reproductive biology of the species. Drewry and Jones (1976) suggest that the presence of the bromeliads is correlated with climatic and edaphic effects that produce heavy dew. The bromeliads would likely contain some water throughout the year under those conditions, providing adequate habitat for the frogs, and an exploitable 'new' habitat.

Information on seasonality of reproduction is lacking as previously noted, for year-round collections have not yet been made. Throughout the period spanned by collection, mid-April to early August, gravid females carrying oviducal young have been collected. All of the *Eleutherodactylus* that I examined that had fully yolked eggs also had a complement of smaller vitellogenic eggs. It is significant that active spermatogenesis continues throughout the period during which females are carrying young. Several alternative strategies are possible: 1) females in the population are receptive over a long period of time, and males have a long period during which mature sperm are present; 2) females produce two clutches per year, as suggested by Drewry and Jones (1976), so males maintain spermatogenesis for a long period; 3) reproduction is acyclic, so males will have active spermatogenesis throughout the year. Since females can be carrying oviducal froglets while they have a fully yolked ovarian clutch and a second set of eggs in vitellogenesis, multiple clutches and/or reproduction throughout the year is possible. These hypotheses would be substantiated or negated by examination of animals collected from October thorugh February; such specimens are not yet available.

The mechanism of maintenance of the developing young in the oviduct is not known. Hormonal mediation must be involved, but the data on vitellogenesis do not suggest a feed-back system that would suppress activity in the ovary. Events triggering birth are also unknown, though a volume of young relative to female volume ratio may be reached that initiates a stress response. Also, birth of oviducal young is apparently not necessary to trigger vitellogenic events in the ovary. The pelvic girdle must distend during birth, for measurements of pelvic diameters in a female carrying froglets indicates that the pelvis is smaller than the diameter of the froglets in 'fetal' position. This, too, suggests hormonal mediation.

Internal fertilization is presumed in *jasperi,* since development is oviducal and the number of young is almost exactly that of the fully-yolked ovarian eggs. The mechanism of fertilization has not been observed in the species, however. Amplexus is in a standard axillary position in other species of *Eleutherodactylus* (Goin, 1947; Lynch, 1971; P. Starrett, pers. comm.). It is possible that internal fertilization may occur in other species, for several workers (Adamson *et al.,* 1960; P. Licht, pers. comm., and P. Starrett, pers. comm.) report that attempts to induce ovulation by injection of hormones have resulted in dilation of oviducts, etc., but not in ovulation or oviposition. It is possible that the stimulus of courtship and possible internal fertilization might be necessary for normal reproduction, though observed aspects of amplexus do not suggest internal fertilization.

It is, indeed, possible that other species of *Eleutherodactylus* are live bearers. Lynn and Grant (1940) reported collecting several adult *E. orcutti* near Chester Vale, Jamaica. On returning from the night's collecting, six newly hatched young were in the bottle with the adults. Lynn suspected ovoviviparity, but this was not supported by his dissections of all of the females collected, so he considered the appearance of the hatchlings 'a mystery.'

A number of species of *Eleutherodactylus* practice parental care (summarized by Salthe and Mecham, 1974). Several demonstrate female guarding of the clutch, a few male tending. Salthe and Mecham suggest that bromeliad nesters may abandon their clutches (though noting Drewry's observation of possible male tending in one species), while species in terrestrial dwelling sites may or may not care for the clutch. They consider the bromeliad site as utilization of a mechanism for extending development sites. They suggest that parental care is a means of adapting to new developmental sites, and that subsequently clutch size decreased with an increase of ovum size. In that case, bromeliad dwellers would have abandoned parental care subsequent to exploitation of the new site. Other *Eleutherodactylus* species are found in bromeliads in the area where *jasperi* occurs. It is possible that ovoviviparity evolved in that species partly to compete by means of an alternative kind of parental care. Obviously, Salthe and Mecham's suggestion that parental care facilitates egg oxygenation and reduces yolk stratification is also operable in live-bearing species—these consequences are met for internally carried developing eggs. Fecundity and clutch size are very low in *jasperi.* In the absence of information on juvenile mortality, the question of live-bearing as a mechanism to insure reproductive success in a low-fecundity species cannot be answered. In any case, the several advantages of parental care suggested by Salthe and Mecham with regard to clutch-tending are also provided by the live-bearing mode.

The acquisition of that mode by a small, direct developing, low fecundity, internally fertilizing species in a site-competitive situation can reasonably be effected by retention of the eggs by the adult female during the developmental period, and is therefore postulated as the condition by which *E. jasperi* became ovoviviparous.

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