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THE HATCHING PROCESS IN *ALYTES*, *ELEUTHERO-* *DACTYLUS* AND OTHER AMPHIBIANS

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The amphibian embryo enclosed within its egg capsules would not be able to free itself at the proper moment for hatching were it not equipped with some mechanism for breaking through the capsules. This mechanism is not the same throughout the Amphibia and the hatching process has been adequately described in only a few forms. The present paper embraces a description of the hatching in two well-known exotic Salientia in which the process had been previously either incorrectly or incompletely reported.

Alytes obstetricans, the common Mid-Wife Toad of Europe, has been assumed to escape from its egg capsules by biting its way out (Boulenger, 1897). I have watched the hatching of a single batch of *Alytes* eggs and have definitely determined that these larvæ did not utilize their larval teeth in the hatching process. The eggs were secured some days before the hatching began. They varied in size from 4×5 mm. to 5×6 mm. The larvæ fitted tightly in the capsules and could rotate only slightly. Their heads in all cases lay near one of the poles (gelatinous strings). The most striking feature of these larvæ was their lack of vascularity. No external gills or vasculated tail fin lay flattened against the capsules as in the encapsuled larvæ of many higher Salientia.

The hatching of each egg was comparatively slow requiring from twenty-five minutes to over a half hour. These eggs were kept on wet filter paper and the process of hatching studied under the binocular. As shown in Fig. 1b, the first sign of hatching was an elongation of the egg. Then the cephalad end became pointed in the region of the nostrils. The egg capsules at this pointed end softened slightly as I determined by pressing with a blunt needle. The bulging was not due to a breaking down of the inner capsule. At this stage I removed the outer capsule and found the inner intact. After some fifteen or twenty minutes the surface of the capsule directly between the nostrils, or more frequently to one side, nearer one external naris or the other, suddenly liquified. The integument at this spot bulged out, frequently carrying the adjacent nostril with it (Fig. 1b). In another moment the larva burst forth from the capsule.

During this hatching process the mouth was kept closed or slightly opened. Buccal movements occurred but the teeth did not grip the capsule. In all cases the mouth came through the rent after the nostril region. There was no escaping the conclusion that something in the internostril region brought about a softening of the capsules.

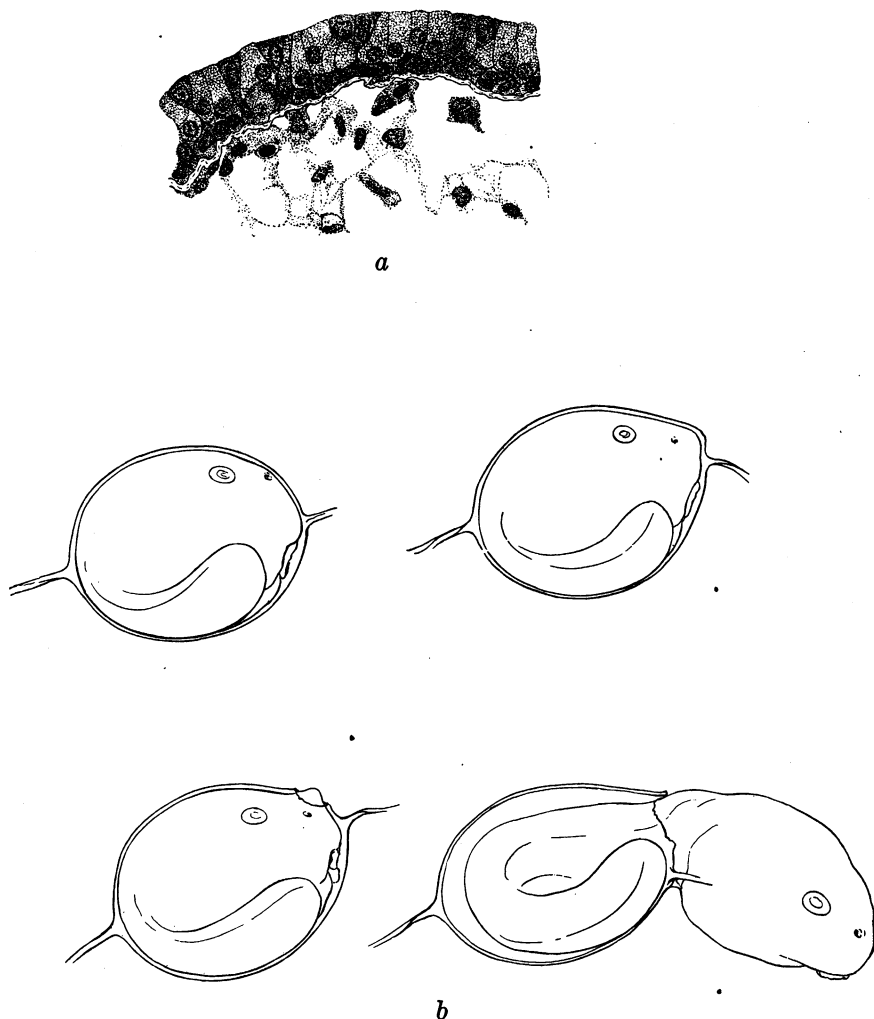


Fig. 1. Hatching of *Alytes obstetricans* (Laurenti).

a. The frontal organ of *Alytes obstetricans*. The eosinophils are less heavily stippled than the other epidermal cells.

b. The digestion of the egg capsules by the frontal organ in *Alytes obstetricans*.

At first I thought it possible that the hatching could have been initiated by buccal fluids forced out through the nares. No such fluids could be detected even though the capsules were very transparent. Eggs were turned in all directions. Three with their heads directly upward and their bodies in contact with the wet filter paper hatched exactly as the others. This disintegration of the capsule is very local and not due merely to water in contact with the capsule.

Sections of eggs before and after hatching showed that the internal nares are well guarded by valves. It is possible but not probable that liquids could be forced by these to initiate the hatching process. However, these sections show other structures which give a definite clue as to the mechanics of hatching. Scattered over the head between the eyes and the tip of the snout are a number of eosinophil cells of large size. These are especially numerous between the external nares (Fig. 1a). These cells are obviously secretory in nature. They cannot be confused with Leydig cells because of their superficial position and detailed structure. These cells are remarkably similar to the cells of the frontal organ of *Rana* and *Bufo*. Such cells have been shown by Bles (1906) in *Xenopus*, and by Jaensch (1921) in two species of *Rana*, to initiate the hatching process. They differ from the frontal organ cells of these genera by their wide distribution over the snout and by the absence of pigment associated with them.

Fahrenheit (1925) has recently described these eosinophil cells in *Alytes* and compared them with the frontal organ cells of other Salientia. In none of my larvæ do these cells form a distinct organ as well differentiated as Fahrenheit indicates (1925, Fig. 4). Further, my sections show that the eosinophil cells extend considerably beyond the limits shown by his figures. They lie much nearer the nostrils and pineal organ than he indicates. They are most numerous in a band between the eyes and in a Δ -shaped cluster on the snout such as he shows. It would seem that Fahrenheit's specimens showed externally some evidence of their frontal organs. It is, nevertheless, also clear that the limits of this organ cannot be determined in any specimens of *Alytes* without a microscopical examination.

My material fixed in Zenker's solution does not exhibit the distinct cuticle which Fahrenheit (1925, Fig. 5) figures. Further, the supporting cells are not more elevated than the eosinophil cells. The outer edge of both supporting and eosinophil cells are on the same level (Fig. 1a). The distal cytoplasm of the supporting cells is denser than that of the eosinophils but it does not form a typical cuticle.

It has recently been denied that the frontal gland initiates the hatching process in certain species which hatch in an immature condition (Fernandez-Marcinowski, 1921). It is by no means easy to determine this possibility by the macroscopic examination of hatching tadpoles. The fact should be stressed that the function of the frontal gland has been proved by Bles (1906) in the case of *Xenopus* and the gland will probably be found in all early hatching aquatic tadpoles if sectioned. The gland is known to occur in *Bombina bombina* (Goette, 1875, Jaensch, 1921), *Xenopus laevis* (Bles, 1906), *Bufo vulgaris* (Adler, 1901), *Rana fusca* (Hinsburg, 1901, Jaensch, 1921), *Rana esculenta* (Corning, 1899), and in *R. arvalis* (Jaensch, 1921). I have found it in recently hatched larvæ of *Rana pipiens*, *R. sylvatica* and *Scaphiopus holbrookii*, although the frontal stripe is frequently not visible in specimens of the latter species fixed in formol or in Zenker's fluid. In *Scaphiopus holbrookii* swollen and apparently secreting cells are found, not only in the frontal gland region but also between this band and the adhesive organs. We may conclude that the frontal gland is probably present in the tadpoles of all Salientia whether these hatch at a very early stage before the external gills have developed (*Rana pipiens*, *Scaphiopus holbrookii*), or later when the external gills are well formed (*Rana sylvatica*). Frontal gland tissue seems to be present even in tadpoles which hatch at a very advanced stage (*Alytes*), but this tissue lacks the pigment which is found in the frontal gland of early stages. Nevertheless, to judge from my observations, the latter apparently functions in the hatching process.

Salientia which hatch fully formed from the egg capsules lack the frontal gland as I have determined in sections of recently hatched young of *Eleutherodactylus abbotti*. Unlike aquatic larvæ they have a well-developed horny layer to the epidermis which becomes modified to produce a new type of hatching mechanism, the egg tooth. It would thus seem possible that the presence of a horny layer prevented the elaboration of frontal gland tissue.

The eggs of the various species of *Eleutherodactylus* may be laid between leaves of epiphytes, palms, bananas, etc., or on the ground under rocks, logs, vegetation, or merely in a hole in the bank. The breeding site may be near water or far away from it. As a rule, each species has a definite habitat preference for its eggs, but this is not constant. I have found in Santo Domingo that two species, *E. abbotti*, and *E. flavescens*, may lay their eggs among leaves above the ground or on the ground at the base of reeds. Development in these species direct. All species of *Eleutherodactylus* fully investigated have been found to have an egg

tooth lying directly over the symphysis of the premaxillæ (Fig. 2). This may vary in size and shape in the different species. I have found that *E. abbotti* on hatching rips first the inner capsule and then the outer with its egg tooth. *E. inoptatus*, on the other hand, has a very small egg tooth and one bunch of eggs on which I experimented could not be induced to hatch without the application of water. Then the eggs elongated, the outer capsule broke first and the inner only some time later. It would therefore seem that forms such as *E. abbotti* may hatch any time the encapsuled larva has the strength to rip the capsules, while in nature, *E. inoptatus* must wait for the rains. The first species sometimes lays its eggs among reeds in such a position as to be well protected from the rains. The



Fig. 2. Egg teeth of *Eleutherodactylus*.

- a. *E. abbotti* Cochran. The egg tooth has the form of a pair of bulls' horns in miniature.
b. *E. inoptatus* (Barbour). The egg tooth is very small, and hatching in this species is initiated by the rains.

latter always (three observations) lays its eggs in shallow holes in the bank. Such situations become soaking wet with every rain. The eggs of *E. inoptatus* would therefore become wet long before they are ready to hatch. Thus there is probably a time factor which prevents the recently laid eggs from splitting open at the first soaking. In other Salientia the egg capsules after a long soaking are much less resistant than recently laid ones.

Direct development has been independently attained in various families of Salientia (Noble, 1925). It is probable for this reason that egg teeth of the various forms are not homologous. In *Eleutherodactylus* the egg teeth have the form shown in Fig. 2 and lie on the premaxillary symphysis. In *Rana opisthodon* the egg tooth is pointed and is found at the end of the snout. In a Madagascan species referred by Boettger (1913) to *Hyperolius* the egg tooth occurs on the upper arm. Thus the method of mechanical rupture seems to be constant for all Salientia which practice direct development, but the tools are fashioned out of epidermis overlying different parts of the body in the various groups.

Urodeles are not known to possess egg teeth or digestive cells to initiate the hatching process. I have examined sections of recently hatched *Desmognathus fuscus* and could find no cells comparable to the

eosinophils on the snout of *Alytes*. Further, Wintrebert (1912) has studied the process of hatching in *Ambystoma* and believes that the disintegration of the capsules is correlated with an absorption of water. Archey (1922) has described the hatching of *Liopelma*. It is a slow process correlated with a disintegration of the capsules. The froglet lacks egg teeth. It makes little effort to free itself from the capsules. The actual hatching cannot be caused by any sudden movements of the frog. In the majority of cases the tail comes out first. While we do not know what causes the capsule disintegration in *Liopelma*, the whole process seems more urodele than frog-like.

In the Discoglossidæ, and especially in their derivatives, the Pipidæ, a series of unicellular digestive glands have already appeared and assumed the rôle of initiating the process. In all higher frogs which have tadpoles hatching into the water the frontal organ probably functions as the releasing mechanism. This frontal organ is frequently indistinct and without a histological examination its limits cannot be determined.

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