tumour cells by inducing large amount of DNA damages. Therefore, the relative concentration of GSH and SOD in normal and tumour cells will be an important factor in determining therapeutic index while BLM is used as an anticancer drug.


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Direct development in the rhacophorid frog, Philautus variabilis (Gunther)

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Direct development was observed in the tree frog, Philautus variabilis under laboratory conditions at 25°C temperature and relative humidity 76–85%. Eggs were unpigmented creamy white in colour and large in size. Development has been divided into 12 stages. Cleavage started after 7 hours of oviposition. Further, the morphological changes during the development of eyes, limbs, gills, appearance of pigmentation and embryonic behaviour were observed. There was no free swimming tadpole stage and the froglets hatched after 12 days of development. The present study provides evidence of direct development for the first time in the tropical Indian anuran.

ANURAN amphibians exhibit a wide variety of reproductive patterns ranging from the species that lay large clutches of small-sized pigmented eggs, which require aquatic media for development, to species that lay small clutches of large-sized unpigmented eggs which develop under terrestrial conditions. It is well known that the majority of frogs lay their eggs in water which subsequently develop into tadpoles that metamorphose later into adults. But in some anurans the larval stage has been modified or is totally absent and these forms exhibit direct development. This pattern has evolved in major families of frogs. The species belonging to the genus Eleutherodactylus exhibit most advanced type of direct development and these have been studied extensively. From the literature it is apparent that the developmental biology studies in India to date are concentrated on the anurans that develop into adult via a free-swimming tadpole stage. Philautus variabilis is a small sized (30 ± 4.5 mm SVL) tree frog. The female exhibits parental care by collecting the eggs beneath the
abdomen using fore and hind limbs. It also aggressively attacks the intruder male to protect the eggs\(^{11}\). The present work was undertaken to record the direct development in \textit{P. variabilis}. To the best of our knowledge this is the first report on direct development in Indian anurans.

During our field studies from 1994 to 96 (June–August) in the vicinity of Dharwad (15°17'N; 75°3'E), five amplexed pairs of \textit{P. variabilis} were collected and brought to the laboratory. Frogs were released into the large terraria which simulated the natural habitat, i.e. with damp soil, dead leaves and small plants. Ovulation occurred after 8–10 hours and the clutch contained 54–62 large eggs. After oviposition, frogs (parents) were taken out from the terraria and the eggs were allowed to develop. Various developmental stages, i.e. from fertilized egg to froglet were observed daily with the help of dissecting microscope (0.8X/3.2X). Developing stages were observed at regular intervals of 24 hours. Stages were marked on the basis of 24 hour-intervals. From the day of oviposition to the day of complete development, each day the developing stages were collected and preserved in Bouin’s fluid from a single clutch (62) of eggs. Jelly layers were removed for the camera lucida drawings and photography. Eggs developed at the laboratory temperature which varied between 22 and 24°C and a relative humidity of about 76–85%. Observations on the live and preserved stages were made.

Eggs were unpigmented, creamy white in colour, large in size and measured 4.1 ± 0.2 mm (\(N = 30\)) in diameter. After 12 days of development, the tiny froglets with remnants of tail came out of the eggs.

After 7 hours of development, reticulate cleavage furrows appeared. Neural plate (Figure 1\(a\)) and neural folds (Figures 1\(b\), 2\(a\)) were observed after 25 and 35 h of development respectively. Morphological changes in the various developing organs such as eyes, limbs, gills, tail, appearance of pigmentation and the behaviour of the developing embryo were as follows:

**Eyes.** Eyes appeared as large anterior bulges in the cephalic region in the Stage 3 (Figures 1\(c\), 2\(b\), c) and became prominent by Stage 4 (Figures 1\(d\), 2\(d\), e). These bulged areas developed into eyes in the Stage 5 (Figure 2\(g\)). Pigmentation of the iris began to appear in the Stage 6 (Figure 2\(k\)). With the increase in the pigmentation, iris darkened progressively by Stages 10 and 11 (Figure 2\(n\), o). By Stage 12 the iris became totally dark and pupil gained light pigmentation (Figure 2\(r\)).

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Figure 1. Photographs of \textit{P. variabilis} embryos. a, Stage 2 (arrow shows neural plate); b, Stage 2 (note the neural groove with neural folds); c, Stage 3; d, Stage 4, lateral view; e, Stage 6, dorso lateral view; f, Stage 7, dorsal view; g, Stage 9, dorsal view; h, Stage 10, dorsal view; i, Stage 12, dorsal view (note the reduced tail); j, Stage 12, ventral view.
Figure 2. Camera lucida drawings of *P. variabilis* embryos. a, Stage 2 (24 h), dorsal view; b, Stage 3 (48 h), dorsal view; c, Stage 3, lateral view; d, Stage 4 (72 h), dorsal view; e, Stage 4, lateral view; f, Stage 5 (96 h), dorsal view; g, Stage 5, lateral view; h, Stage 6 (120 h), dorsal view; i, Stage 6, lateral view; j, Stage 7 (144 h), dorsal view; k, Stage 7, lateral view; l, Stage 8 (168 h), dorsal view; m, Stage 8, lateral view; n, Stage 9 (192 h), dorsal view (note elongated tail); o, Stage 10 (216 h), ventral view; p, Stage 11 (240 h), lateral view; q, Stage 12 (264 h), dorsal view (note the reduction of tail); r, Stage 12, ventral view. Scale line (below a) = 1.00 mm; fb, fore limb bud; hb, hind limb bud; e, eye; mt, membranous tail.
Gills. A pair of gills appeared first time as tiny buds in Stage 5 and became distinct by Stage 7. Gills remained unbranched and regressed rapidly by Stage 8. Overall gills were existing for less than one-fourth of the developmental period.

Pigmentation. Melanophores marked their way in the developmental process first time in Stage 5 (Figure 2 f) on the head and trunk region. Later pigmentation was restricted to the same areas and also extended between the front and hind limbs in the Stage 6 (Figure 2 h, i). It began to scatter on both the sides of the mid-dorsal area (Figures 1 f-h, 2 j, k). Further, the pigmentation progressed on the lateral sides and by 10th stage onwards it extended to ventral side (Figure 2 n, o). At the time of hatching the whole froglet gained the pigmentation. However, it was light on limbs and ventral surface as compared to the dorsal surface (Figures 1 i, 2 q, r).

Limbs. Limbs made their appearance on the 3rd day as small buds on the lateral side (Figures 2 b, c). The fore and hind limb buds developed simultaneously. At the beginning the limb buds appeared as circular swellings and were slightly separated from the neural tube (Figure 2 b). These swellings further increased in size and became more oblong and later joined the trunk by the Stages 4 and 5 (Figure 1 d, 2 d, f). Further, the limbs began to elongate (Figure 1 e) and by Stage 7 the foot paddles, constrictions of elbow and knee joints made their appearance (Figures 1 f, 2 j, k). By 8th Stage the nubs/points on three digits on front and hind feet appeared (Figure 2 m). With the progress of the development, limbs and toes further elongated and at the time of hatching these reached full length (Figure 1 j). The forelimb buds remained smaller than hindlimb buds throughout the development.

Tail. On day 3, development of tail was noticed and it adhered to the main body of the embryo (Figure 2 b). It was slightly curved on one side. Later the tail curving became prominent on one side and it was adhered to the body of the embryo until the completion of Stage 4 (Figures 2 d, e). By Stage 5 (Figure 2 f) it became totally free from the body. The membranous tail fin developed in the Stage 5 itself (Figure 2 g). The tail attained its maximum size in Stage 8 (Figures 2 l, m) and remained unchanged till Stage 10. From the Stage 11 (Figure 2 p) the tail began to regress and at the time of hatching it was reduced to almost half of its previous size. The tail was highly vascularized and the tail fin was translucent throughout the development. It was unpigmented except the main stem of the tail of later stages.

Behaviour. In the course of development the behaviour of the live embryos was observed. The movement of the embryo began after Stage 2. By the Stage 3, the embryos began to rotate within the egg capsules and movement continued till the end of the Stage 4 and finally ceased by Stage 5. The first evidence of flexing of mid-body and tail movement was apparent in the Stage 7. Although the tail thrashing was marked in the Stage 7, it became rapid by the Stage 8 and continued through the Stage 9. Flexing of the mid-body with limbs was observed by the Stage 10 and continued up to hatching.

Terrestrial breeding of frogs provides opportunities for analysis by both developmental and evolutionary biologists. Larval stage as a functionally distinct stage has been eliminated by some species in almost every family of amphibians in all three orders. In caecilians this has been accomplished by viviparity, in frogs by direct development, and in salamanders by viviparity and direct development. Some species belonging to the genera Cryptobatrachus, Hemiphractus, Stefania, Eleutherodactylus and Gastrotheca are egg-brooding frogs and exhibit direct development. The most extreme case of loss of larval features is found in the genus Eleutherodactylus. In some species of this genus, external gills make fleeting appearance but these never develop at all and the gills fail to break through. Gills are highly reduced in E. antilenis and E. martincipens. Eleutherodactylus coqui possess a pair of prominent gills with 2–4 convolutions13, whereas in P. variabilis gills are highly reduced and do not possess any terminal convolutions. In E. coqui as the rapid regression of gills coincides with the appearance of large membranous tail, it is reported that the respiratory function is carried out primarily by the tail15. This may also be the case with P. variabilis since the gills not only rapidly regress with appearance of membranous tail but also do not possess the terminal convolutions. The pigmentation in the present species begins by Stage 5. Some species of Eleutherodactylus hatch in about 12 days which is considered to be the most rapid complete development known in vertebrates14. In a similar way, P. variabilis froglets hatch in 12 days. The present study suggests that there is direct development in the tree frog P. variabilis.

Evidence of ductile shearing from the extensional crenulation cleavage: An example from Zawar area, the Aravalli mountain

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Extensional crenulation cleavage marked by asymmetric kinks and ductile shears occurs at low angle to steep S1 planes in the mica schist of Zawar area in the Aravalli mountain. It is developed due to extension along the S1 planes consequent to large-scale ductile shearing. The compressive stress of such shearing is deduced to be subhorizontal in NNE-SSW direction. Early fold axes assume variable trend due to shearing.

Small-scale microfolds, termed as crenulation, develop on pre-existing slaty cleavage or schistosity due to superposed folding. The rocks, with crenulations, have a striped appearance due to the preferential localization of mica flakes in the limbs–cleavage domain of such folds. The cleavage domains are discrete and often marked by shear fractures. These constitute the crenulation cleavage (CC) and bear similar significance as axial plane cleavage. The coarse fold pleats, enriched with quartz, between the cleavage domains define microlithons. The CC is genetically related to buckling where a multi-layered sequence undergoes layer parallel shortening. Extensional crenulation cleavage (ECC) morphologically resembles a normal CC. However, they occur at a low angle (<45°) to s-planes compared to high angle of disposition (45° to 90°) of the other. ECC owes its origin to shortening normal to s-planes (i.e. extension along layering) and the associated microfolds (single or conjugate kinks) develop because of shearing. A type of internal buckling may also be caused by extension along foliation to develop such microfolds. The ECC is synonymous to shear band cleavage or 'C' fabric in a ductile shear zone and is analogous to the structure proposed by Means and William with a layer normal shortening in salt mica multilayer. We report here ECC from Zawar area which evidences ductile shearing in this part of the Aravalli mountain.

Zawar is 35 km south of Udaipur in Rajasthan and is well known for Proterozoic Pb–Zn mineral deposit. The area is represented by Aravalli Supergroup of rocks, including mica schist, dolomite and quartzite, which are impressed upon by polyphase deformation (Figure 1). Summarily, the area shows the presence of three generations of folding. The first generation folds, F1, are isoclinal and predominantly oriented in WNW-ESE direction (Figure 1A). The dominant schistosity, S1, is axial planar to this fold. The F2 and F3 folds, representing the second and third generation structures, are in NNE-SSW and WNW-ESE directions respectively. Crenulation and fracture cleavages mark the axial direction of F2 and F3 folds. This property is used to differentiate the F3 axial planar cleavage from that of F1, though they trend in the same direction. Apart from this, wide-scale ductile shearing parallel to the axial plane of F3 fold is observed constituting a prominent ductile shear zone in the study area (Figure 1, near Har Mandir). Offsetting of the bedding planes and quartz veins, presence of S-C fabric and, rotation of fold axis and intersection lineation of first generation from NNE-SSW direction in the east and to northwesterly direction in the west characterize

Figure 1. Geological map of Zawar area (Insets. Map of India for location of Zawar; A, 115 F1 axial plane and S1 schistosity, maximum strikes in WNW-ESE direction, contours 1-3-5-7% of 1% area; B, F3 conjugate axial planes with the spot S1 plane; ε1, ε2 and ε3 indicate the direction of maximum, intermediate and minimum compressive stress respectively.)