

Developmental-Stage Series of Axolotl Embryos

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In 1969 Harrison's staging series for *Ambystoma maculatum* (*Amblystoma punctatum*), with drawings by Lisbeth Krause, was published. Harrison divided embryonic development into 46 stages based on external morphology and on features visible in dissected embryos. That paper became the model for comparable series for the axolotl published later by Schreckenberg and Jacobson (1975) and by Bordzilovskaya and Dettlaff (1975, 1979). The present chapter represents a revision of the 1979 paper.

Schreckenberg and Jacobson divided the development of the axolotl into 40 stages, based on external morphological features. Timing was measured at 18°C. Their series is illustrated with photographs that are accompanied by brief descriptions.

The staging series originally published by Bordzilovskaya and Dettlaff is also based on external morphology. It furthermore provides a ratio (t_n/t_0) that allows the time and duration of the various stages to be predicted for various temperatures. Such prediction is possible because the duration of different developmental periods in many species changes in a proportional manner in the optimal temperature range (about 16°–25°C) (Dettlaff and Dettlaff, 1960, 1961; Dettlaff, 1964, 1976; Dettlaff and Rudneva, 1973). Such a proportionality has been established for the axolotl as well (Cate, 1956; Skoblina, 1963; Valouch et al., 1971). If the duration of some one period or stage is known at various temperatures, and the durations of the remaining stages are known at one constant temperature (20°C), the duration of each stage at various temperatures can be estimated. The duration of one mitotic cycle during synchronous cleavage division is t_0 (Dettlaff and Dettlaff, 1961; Dettlaff, 1976), corresponding in the axolotl to the interval between the first appearances of the first and second cleavage furrows on the egg surface (Rott, 1973). Skoblina (1963, 1965) determined t_0 at various temperatures, as depicted in Fig. 19-1. At 20°C, t_0 varies from 87 to 93 minutes (M. N. Skoblina, unpublished data). In this chapter, $t_0 = 92$ minutes is used in all calculations. The time from the appearance of the initial cleavage furrow on the egg surface to the onset of a given devel-

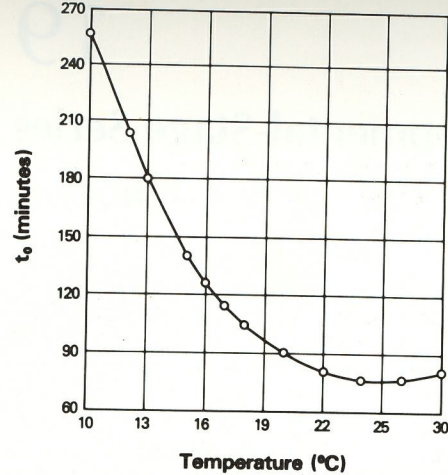


Fig. 19-1. Variation in t_0 , the duration (in minutes) of one mitotic cycle in the period of synchronous cleavage divisions, for the axolotl (vertical axis) with the temperature in degrees Celsius (horizontal axis). (After Skoblina, 1965.)

opmental stage is t_n . By multiplying the ratio (t_n/t_0) by the value of t_0 at the temperature of interest, obtained from the graph in Fig. 19-1, the time in minutes to any developmental stage at any temperature in the optimal range can be determined.

PROCEDURES EMPLOYED FOR STAGING

Eggs from natural spawnings were collected within 20-40 minutes of being laid. Timing began with the first appearance of the initial cleavage furrow on the animal pole. The stages have been designated in accordance with the numbering system proposed by Harrison (1969) for *Ambystoma maculatum*. Features common to both species and those that best distinguished successive stages and allowed a precise determination of time of transition were chosen to characterize the stages. Fixed material was used to define the features and timing of the later stages more accurately. Embryos were fixed up to stage 35, at an interval of $0.5-1t_0$ at first, then at a $5t_0$ interval, and in the latest stages at intervals from 10 to $20t_0$ with reference to precisely definable features. Three to five eggs that began to cleave at different times were fixed for every stage. A greater number of eggs and a greater number of observations were used to define transitional stages such as the beginning of gastrulation, the stage with a slitlike blastopore, and the closure of the neural folds. Size indications are the average of several measurements (Fig. 19-2). The times used in the table were determined on the more advanced embryos in each sample because they have been shown to provide more precise and reproducible results (Dettlaff and Dettlaff, 1961; Zubova, 1962; Ignatieva, 1974).

In some cases, the variation in duration of a certain stage among individual embryos in a sample exceeded the time interval between successive stages. These stages are marked with an asterisk in Table 19-1. In most cases, this variation was less than 10% of the total duration of a given stage.

The descriptions of diagnostic features and the drawings (Plates I-VI) of the

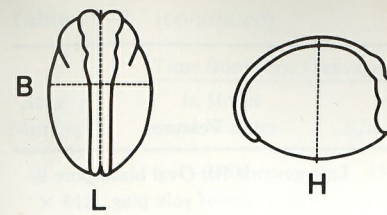


Fig. 19-2. Scheme of measurements. L, length; B, breadth; H, height.

Table 19-1 Stages of Normal Development of *Ambystoma mexicanum* (incubation at ^{20°C} ~~29°C~~)^a

Stage Number	Time from First Cleavage		Size (mm)	Features
	In Hours and Minutes	t_n/t_0		
1			1.85-2.0	Freshly laid fertilized egg in jellycoat.
1½			2.0	Activated egg: a broad perivitelline space has formed.
2*	0.0	0	2.0	First appearance of the first cleavage furrow on the animal pole; timing begins.
2	0.65	0.72	2.0	2 cells.
3	2.40	1.7	2.0	4 cells.
4	4.12	2.7	2.0	8 cells.
5	5.22	3.5	2.0	16 cells.
6	6.45	4.5	2.0	32 cells.
7	8.26	5.5	2.0	64 cells.
8	16.06	10.5	2.0	Early blastula (fall of mitotic index in animal blastomeres: Rott and Beritashvili, 1975).
9	21.28	14	2.0	Late (epithelial) blastula; surface is smooth.
9½	24.32	16	2.0	Morphogenetic function of nuclei begins (Ignatieva, 1972).
10	26.00	17	2.0	Early gastrula I: First signs of dorsal blastopore lip are visible.
10½	32.10	21	2.0	Early gastrula II: Invagination continues; blastopore is a nearly horizontal slit in one quadrant.
10%	37.00	24	2.0	Middle gastrula I: Dorsal lip forms a semicircle.
11	38.30	25-26	2.0	Middle gastrula II: Blastopore covers three quadrants. Lateral lips are formed; ventral lip is marked only by pigment accumulation. Yolk plug reaches its maximum diameter (1.2 mm).
11½	40-42	27-28	2.0	Late gastrula I: Blastopore is circular; invagination continues; yolk plug diameter, 0.6 mm.
12	47.30	31	2.0	Late gastrula II: Blastopore has an oval or circular shape; size of yolk plug, 0.40×0.45 mm.

