KOREAN JOURNAL OF ZOOLOGY
Vol. 20, No. 1 49–56 (1977)

The Mexican Axolotl (*Ambystoma mexicanum*) as Experimental Material for Studies in Embryology I. General Introduction

Hae-Moon Chung and George M. Malacinski*
(Dept. of Biology, Busan National University,
*Dept. of Zoology, Indiana University, U.S.A)

發生學 硏究用實驗動物로서의 Mexican axolotl에 대한 一般資料

鄭 海 文 · G.M. Malacinski*
(부산대 생물학과, *美 인디아나大 動物學科)
(Received February 28, 1977)

摘 要

Mexican axolotl (*Ambystoma mexicanum*)의 생활사, 기원, 실험실내에서의 유지 및 교배방법 등을 소개한다. 이 종은 난자형성과 발생의 연구면에서 몇가지 대단히 유리한 점을 가지고 있다. 즉 큰 염색체를 가진 대형의 난자를 보유하고 있는 점, 난자가 수란관을 통과하는 동안 총배설강내에서 수정하는 점, 초기 배아의 실험발생학, 세포학 및 생화학적 연구에 쉽사리 이용할 수 있다는 점과, 무엇보다도 난자형성과 배발생동안의 여러가지 중요한 기능에 영향을 미치는 30여개 이상의 유전자를 이용하여 유전자조절현상을 밝혀낼 수 있다는 점이다.

A neotonous salamander, the Mexican axolotl is a favorable laboratory animal for research in several areas of both classical and contemporary biology. The Mexican axolotl does not ordinarily undergo metamorphosis and become adapted to a terrestrial existence. It reaches sexual maturity while retaining the larval or juvenile body form, and spends its entire life in water (Smith, 1969). Other members of the genus *Ambystoma*, for example the tiger salamander (*Ambystoma tigrinum*) which is found in the western United States and in Mexico, do indeed undergo metamorphosis and achieve a terrestrial existence.

The neotonous character of the Mexican axolotl makes it a practical and useful laboratory animal. Adult animals can be kept for their entire lifespan individually

or in large numbers in small aquaria or large tanks. Fig. 2 displays animals which are at the young larval, intermediate, and adult stages of development. Animals which are reared in the laboratory in the temperature range of 18~22°C usually reach sexual maturity within 1~1$^1/_2$ years. Exceptional cases have, however, been observed in which animals have been successfully mated at only 9~10 months of age.

Ambystoma mexicanum is indigenous to Lake Xochimilco, a large clearwater lake near Mexico City, Mexico. It has been distributed to several laboratories in both North America and Europe. The Mexican government, fearing the extinction of its native population, has enacted legislation which limits the export of the axolotl. It is more practical, therefore, to obtain breeding stock from one of the amphibian facilities in the United States (*e.g.*, the Indiana University Axolotl Colony, Bloomington, Indiana), or Europe (*e.g.*, the Hubrecht Laboratory, Utrecht, The Netherlands).

Laboratory Maintenance:

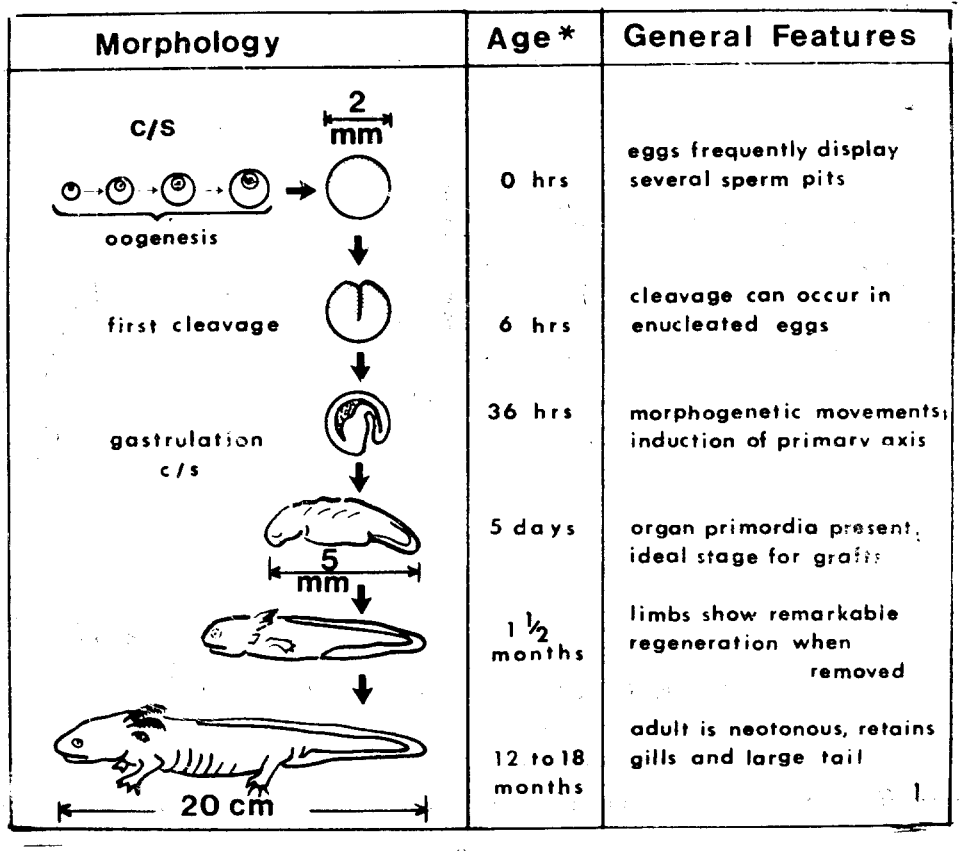


Fig. 1. Life cycle of the axolotl. *20°C

The aquatic mode of life facilitates the laboratory cultivation of the axolotl. The life cycle is diagrammed in Fig. 1. After hatching, young larvae are reared on a diet of either washed, freshly hatched brine shrimp, or small Tubifex worms. They eat liberally, and should be fed every day if a maximal growth rate is desired. As soon as they are capable of ingesting larger pieces of food, usually by $1^1/_2$ months, they should be switched to a diet or chopped beef liver, beef heart, or commercially available canine pet foods. Other high protein diets, such as fish parts or other mammalian tissues which would be easily ingested and digested should provide adequate substitutes for beef tissues. Also, earthworms are an excellent food. Adult animals should be fed every 2~3 days. Larvae and adults can be grown and maintained singly in individual aquaria, or as mass cultures in large tanks. An adequate supply of fresh (non-chlorinated) water should be provided to animals at all stages of their life cycle.

Adult animals are mated by placing them in a small aquarium. Adult animals can be sexed by examination of the cloacal opening (Fig.3). The aquarium should contain a layer of coarse sand or gravel at the bottom, so that the spermatophores emitted by the male can easily become attached to a solid substrate. A male will usually deposit several spermatophores.

The female brings her cloacal opening over a spermatophore, and makes temporary contact with the mass of sperm. The spermatozoa make their way into the spermathecal tubules which open into the dorsal portion of the cloacal chamber, and are stored there. As the eggs are shed into the cloaca from the oviducts they come in contact with sperm. As the eggs are shed into the water they are, therefore, already fertilized. The first cleavage division usually occurs within 6 hours after the eggs are shed.

From a typical mating several hundred eggs are produced. All the eggs of a single spawning are ordinarily shed over an 8~24 hour period. The quality of the eggs is usually quite high—eggs displaying sperm pits (small craters on the surface of the egg where the sperm penetrated the cortex, Fig. 4.) develop normally with very high frequencies (ca. 80-95%). The size of the egg and early embryo is relatively large (Fig. 1), so it is amenable to various types of surgical manipulations.

As well as natural spawnings, fertile eggs, albeit in more limited quantities, can be obtained by artificial insemination. Mature eggs can be bathed in a macerate of sperm, in much the same manner that is routinely used for other amphibia, particularly anurans (Humphrey, 1962; Newrock and Brothers, 1973).

A developmental stage series for early *Ambystoma mexicanum* embryogenesis is available (Schreckenberg and Jacobson, 1975). A more complete series for a related species, *Ambystoma maculatumhais*, has also been published (Hamburger, 1966).

Karyotype and Sex Determination:

Frankhauser and Humphrey (1942) provided the chromosome count of 28 for the diploid organism. Subsequently, Signoret (1965) and Callan (1966) provided descriptions of the mitotic chromosomes. As well, Callan (1966) has compiled a map of the lampbrush chromosomes of the axolotl oocyte.

In the axolotl, unlike anurans or even mammals for that matter, the female rather than the male is heterogametic. Evidence on this point was provided by Humphrey (1945). He converted a piece of ovary to a functional testis by grafting, and scored the progeny of a test mating. Both male and female progeny were observed, indicating that the female is heterogametic. Further evidence was also obtained by grafting primordial germ cells among embryos. Subsequent test matings substantiated the hypothesis on sex determination (Humphrey, 1957). Female heterogamety has also been discovered for another urodele, *Pleurodeles*, by Gallien (1951).

Research Applications:

Amphibian embryos have long been a favorite experimental system of developmental biologists. The ease with which a developmental pattern can be observed, the simple culture techniques required for development through the entire life cycle, and the availability of large numbers of eggs from a single spawning contribute to the general popularity of this experimental material. In addition, over three dozen mutant genes are available in highly inbred stocks of the axolotl (Humphrey, 1975; Chung and Malacinski, 1977). A variety of genetic manipulations can be employed to produce haploid or polyploid individuals (Humphrey *et al.*, 1950; Fankhauser, 1945), and nuclear transplantation can be employed for a variety of experiments (Signoret *et al.*, 1962).

There are three major research areas for which the axolotl has been particularly useful. One area—the problem of how the pattern of early morphogenesis is built into the cytoplasm of the unfertilized egg—is among the oldest in experimental embryology. The so-called "morphogenetic determinants" which are incorporated into the egg during oogenesis direct the major morphological changes which occur during the stages of development up to and including neurulation. The discovery in the axolotl of genes which exhibit maternal effects and give rise during oogenesis to deficiencies in the egg cytoplasm has led to the development of bioassays which are useful for the isolation of some determinative substances (Malacinski and Brothers, 1974).

Another research area in which the axolotl is contributing is the study of the regulation of macromolecular synthesis during embryogenesis. Eggs and embryos

can be exposed, by straightforward microinjection techniques, to isotopes and metabolic inhibitors. Previously, concepts dealing with gene expression in early development were most profitably examined with marine invertebrate eggs. Now, however, methods are available for examining protein and nucleic acid synthesis directly in the eggs and early embryos of amphibians such as the axolotl.

A third important research area in embryology to which the axolotl is contributing concerns the definition of the biochemical, cytological, and anatomical events that occur during the development of the tissues and organs of the adult organism. Tissue interactions and organ development are especially convenient to study in amphibians. In addition, genetic mutations that affect the development of such organs as the eye, heart, kidney, and limbs are available in the axolotl (Chung and Malacinski, 1977). The availability of these mutant genes is providing a set of model systems for studies on embryological events which are common to allhigher vertebrates, including mammalian species such as humans.

In all, over 3 dozen mutant genes are available in the axolotl. All of these genes are recessive, and many of them display, in the homozygous condition, effects on specific stages of development. The maternal effect genes are potentially important as tools for analyzing the morphogenetic organization of the egg. There are a total of five known maternal effect genes in the Mexican axolotl. One of these genes (*o*) is particularly interesting, because it is thought to be responsible for the accumulation in the oocyte of a protein which functions to control gene expression in the late blastula stage of development. Another group of genes affect the devlelopment of specific organs or tissues. In a few cases these genes are known to affect a specific phase in the development of a single organ. For example, genes have been discovered which affect the induction of either the heart, eyes, or hypothalamus.

In addition to those types of genes, there is available in the axolotl an assortment of genes which affect pigment cells, and other genes which apparently exert lethal effects on all parts of the embryo or larva.

The combination of the favorable features of the amphibian as experimental material for embryology, and the availability of a wide variety of mutant genes, promise to insure that the axolotl will maintain an important position in the animals of developmental biology.

ACKNOWLEDGEMENTS

The authors express their appreciation to Mr. Larry Lawrence for preparing the photographs. Financial assistance of NSF and Fulbright-Hays Commission grants are greatly appreciated.

REFERENCES

Callan. H.G., 1966. Chromosomes and nucleoli of the axolotl, *Ambystoma mexicanum*. *J. Cell Sci.* **1** : 85—108.

Chung, H-M., and G.M. Malacinski, 1977. The Mexican axolotl (*Ambystoma mexicanum*) as experimental material for studies in embryology. II. Mutant genes. Manuscript in preparation.

Fankhauser, G., 1945. The Effects of Changes in Chromosome Number on Amphibian Development.

Gallien, L., 1951. Sur la descendance unisexuee d'une femelle de *Pleurodeles waltlii* Michah. avant subi pendant sa phase larvaire l'action gynogene du venzoate d'estradiol. *C. R. Hebd. Seances Acad. Sci. Ser. D.Sci. Nat.* **233** : 828—830.

Hamburger, V., 1966. A Manual of Experimental Embryology. University of Chicago Press Chicago, Illinois.

Humphrey, R.R., 1945. Sex determination in ambystomid salamanders: A study of the progeny of females experimentally converted into males. *Am. J. Anat.* **76** : 33—66.

Humphrey, R.R., R. Briggs and G. Fankhauser, 1950. Sex differentiation in triploid *Rana pipiens* larvae and the subsequent reversal of females to males. *J. Exptl. Zool.* **115** : 399—423.

Humphrey, R.R., 1957. Male homogamety in the Mexican axolotl: A study of the progeny obtained when germ cells of a genetic male are incorporated into a developing ovary. *J. Exptl. Zool.* **134** : 91—102.

Humphrey, R.R., 1962. Mexican axolotls, dark and white strains: Care of experimental animals. *Bull. Phila. Herpetol. Soc.* **10** : 21—25.

Humphrey, R.R., 1975. The axolotl, *Ambystoma mexicanum*, *In*: Handbook of Genetics, (R. C. King, editor). Plenum Press, New York.

Malacinski, G.M. and A.J. Brothers, 1974. Mutant genes in the Mexican axolotl. *Science* **184** : 1142—1147.

Newrock, K.M. and A.J. Brothers, 1973. The artificial induction of out of season breeding in the Mexican axolotl, *Ambystoma mexicanum*. *Amer. Soc. Zool. Newsletter*, October.

Schreckenberg, G.M. and A.G. Jacobson, 1975. Normal stages of development of the axolotl, *Ambystoma mexicanum*. *Develop. Biol.* **42** : 391—400.

Signoret, J., 1965. Etude des chromosomes de la blastula chez Paxolotl. *Chromosoma* **17** : 328—335.

Signoret, J., R. Briggs and R.R. Humphrey, 1962. Nuclear transplantation in the axolotl. *Develop. Biol.* **4** : 134—164.

Smith, H.M., 1969. The Mexican axolotl; some misconceptions and problems. *Bio. Science* **19** : 593—598.

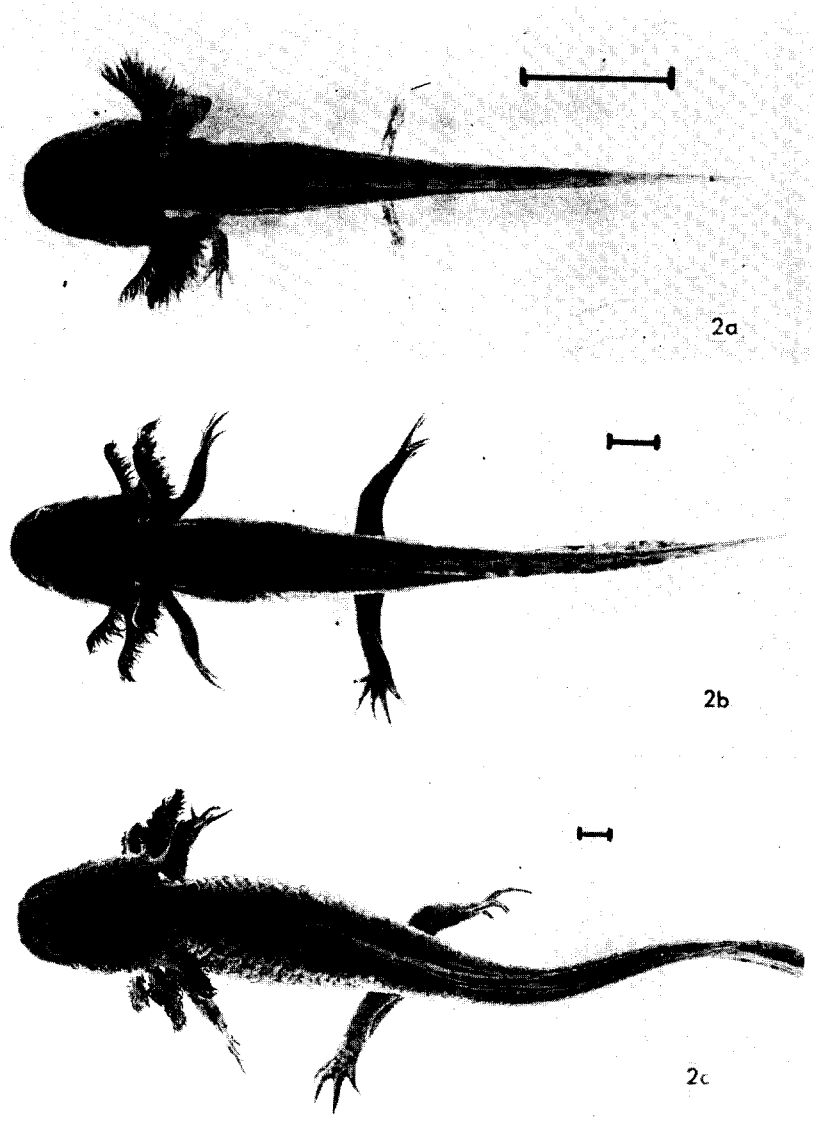
EXPLANATION OF FIGURES

Fig. 2. The Mexican axolotl at various stages of development. Top, 9 weeks; middle,11 months; bottom, 22 months. The gills are visible and prominant at all stages. The adult displays a wide tail. The scale represents 1 cm.

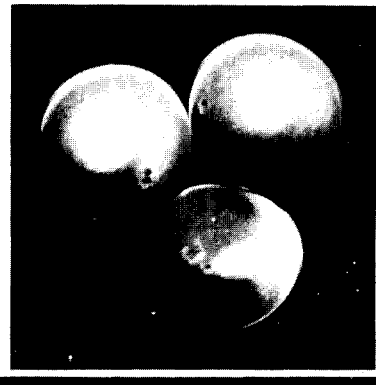
Fig. 3. Examination of the cloacal region reveals the difference between the male and female sexes. Male (top) displays pronounced cloacal glands surrounding the opening. Female (bottom) cloaca is small compared to male.

Fig. 4. Eggs display pronounced sperm pits. These craters mark the entrance of the sperm, and provide an early indication as to whether the eggs are fertile.

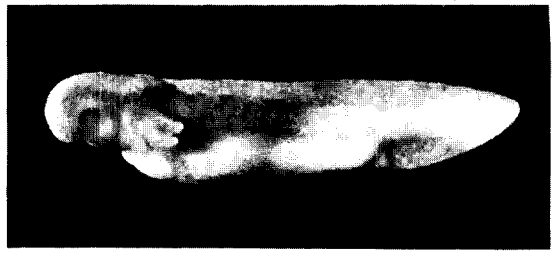
Fig. 5. Embryo at 7 days of development. Note the formation of gill primordia.


2a
2b
2c

3a 3b



4



5