HANDBOOK OF GENETICS

EDITED BY ROBERT C. KING

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Volume 4
Vertebrates of Genetic Interest

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Preface

The purpose of the first four volumes of the *Handbook of Genetics* is to bring together collections of relatively short, authoritative essays or annotated compilations of data on topics of significance to geneticists. Many of the essays will deal with various aspects of the biology of certain species or species groups selected because they are favorite subjects for genetic investigation in nature or the laboratory. Often there will be an encyclopedic amount of information available on such species, with new papers appearing daily. Most of these will be written for specialists in a jargon that is bewildering to a novice, and sometimes even to a veteran geneticist working with evolutionarily distant organisms. For such readers what is needed is a written introduction to the morphology, life cycle, reproductive behavior, and culture methods for the species in question. What are its particular advantages (and disadvantages) for genetic study, and what have we learned from it? Where are the classic papers, the key bibliographies, and how does one get stocks of wild type or mutant strains? Lists giving the symbolism and descriptions for selected mutants that have been retained and are thus available for future studies are provided whenever possible. Genetic and cytological maps, mitotic karyotypes, and haploid DNA values are also included when available.

Volume 4 deals with certain vertebrate species that have been studied in considerable detail from the standpoint of genetics or molecular cytogenetics. Such data are available for only a relatively few vertebrates. So little is known concerning the genetics of species belonging to the Agnatha, the Chondrichthyes, and the Reptilia that these classes are not represented. Among the Osteichythes genetic information is only available for species representing two of the forty-one orders. The amphibia are well represented. Various species of *Xenopus* are employed frequently in

current research utilizing the sophisticated techniques of molecular biology, and studies on the lampbrush chromosomes of certain salamanders have generated results of tremendous importance in elucidating the functional anatomy of the eukaryotic chromosome. An abundance of genetic data is available for domesticated species of birds. Among the Mammalia, species from six of the seventeen orders have been studied. Attempts to obtain chapters dealing with domesticated sheep, swine, and cattle proved unsuccessful, but the genetics of their blood group systems is covered in Chapter 21. Six chapters are devoted to rodents and four to carnivores.

Of course, the vertebrate for which the most genetic information is available is *Homo sapiens.* Some idea of this massive scientific literature can be gained from perusing in Chapter 23 the titles of the 381 books that have been published in the English language during the past dozen years concerning the genetics of our own species. While this literature is overwhelming in amount, it is readily accessible, and for this reason the chapters dealing with *Homo sapiens* are, for the most part, annotated tables and bibliographies.

Volume 4 concludes with chapters describing animal viruses of genetic interest and cataloguing important animal cell lines which are kept in continuous culture in various laboratories throughout the world. The techniques derived from microbial genetics are being applied to such systems, and the results are currently revolutionizing our understanding of mammalian genetics and virology.

In volume 5 the early chapters will provide explanations of the advantages and shortcoming of some of the techniques of molecular biology as applied to genetics. The later chapters will be authoritative essays dealing with the structure and functioning of the genetic molecules that reside in the nucleus and in the mitochondria and chloroplasts of eukaryotes.

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AMPHIBIA

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The Axolotl, *Ambystoma mexicanum*

RUFUS R. HUMPHREY

Introduction

The Mexican axolotl (*Ambystoma mexicanum*), a widely used laboratory animal, is a native of Lake Xochimilco, a large clear-water lake at Mexico City. The various scientific names which have been applied to it are discussed at length by Smith (1969) in a recent article. The common name, axolotl, of Aztec origin, has been variously interpreted as water dog, water twin, water sprite, or water slave. The last interpretation ("slave of the water") is in one sense particularly appropriate: Since the Mexican axolotl does not ordinarily metamorphose and become adapted to a terrestrial existence, it must spend its life in water, in contrast with its many relatives of the genus *Ambystoma*. The term axolotl, however, is loosely applied to unmetamorphosed or larval stages of the various subspecies of *Ambystoma tigrinum* found in the western part of the United States and in Mexico; hence, in applying the term to *Ambystoma mexicanum,* it is well to qualify it by saying "Mexican axolotl."

Animals which attain sexual maturity while retaining the larval or juvenile body form are termed neotenous. Neoteny in the Mexican axolotl is the outstanding characteristic which has led to its extensive use as a

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laboratory animal. Its aquatic mode of life is highly advantageous. Adults can be kept singly in containers (fish bowls or aquaria) of one-gallon size, although somewhat larger ones might be preferable. They require no provision for a period of hibernation as do their terrestrial relatives. They reach sexual maturity in 10–15 months, as compared to two or more years for terrestrial species. An adult male and female placed together in a small aquarium or an ordinary dish pan will mate and produce fertile eggs in numbers up to several hundreds. It is necessary to provide a thin layer of coarse gravel or crushed stone in the container, to which the spermatophores emitted by the male may be attached. The spermatophores must be kept in the upright position for the female to make contact with the packet of spermatozoa that caps the conical or pyramidal gelatinous base which is secreted by the cloacal glands of the male. The female does not remove the packet of spermatozoa *in toto* from the spermatophore; her cloacal opening is brought down over the sperm mass, whose surface coating is probably quickly dissolved by action of cloacal secretions (enzymes?), permitting the spermatozoa to make their way into the spermathecal tubules which open into the dorsal portion of the cloacal chamber. Here the spermatozoa are stored until spawning begins, at which time a few are expelled against the surface of the eggs as they come into the cloacal chamber from the oviducts. Spawning may begin in from 10 to 24 hours after the animals are placed together, and will usually be completed in 24 hours.

When it is found impossible to obtain progeny from apparently mature axolotls by the usual mating procedures, resort may be had to artificial insemination. This ordinarily involves the sacrifice of both animals, since eggs cannot be obtained from axolotl females by stripping, as is possible with frogs, and sperm suspensions must be obtained by removing and mincing the ductus deferens. The usual procedures for artificial insemination, together with instructions for feeding and care of the young, have been published elsewhere (Humphrey, 1962*a*). The developmental stages of axolotl embryos and larvae are comparable to those of Harrison's series for *Ambystoma maculatum*; for figures of these see Hamburger (1966).

Among the advantages for genetic studies to be credited to the axolotl, as contrasted with the majority of other urodeles, is its relatively easy maintenance and breeding under laboratory conditions. A decided advantage, as compared with most avian or mammalian species, is the large number of fertile eggs (up to several hundreds) which may be obtained from a single mating. A genetic ratio may thus be determined without the use of numerous animals or the lapse of months or years of time. Further, the entire development of the young, from the first cleavage

division of the zygote to the attainment of the adult state, is open to observation. Mutant genes determining abnormalities in cleavage or during early embryonic or larval stages may, therefore, be detected in a fashion impossible for the embryos of amniotes, which are concealed *in utero* or in eggs with nontransparent coverings.

The Mexican axolotl is not without disadvantages, however, as a subject for genetic studies. A serious one, of course, is the time required for the individual to attain sexual maturity, as compared with the fruit fly, the mouse, or other common laboratory organisms. One must expect about 1 year from generation to generation, although in exceptional cases a female may be capable of spawning at 10 or 11 months of age and a male may be mature enough at that age to deposit normal spermatophores. A second disadvantage is that it is sometimes difficult to provide the essential or optimal conditions for normal matings: The chemical nature of the water supply, the temperature, the character of the food supply, the handling of the animals, etc. all may influence the mating responses.

Chromosome Number, Karyotype, and Crossing Over

The diploid number of chromosomes in the Mexican axolotl (28) was determined by Fankhauser and Humphrey (1942) from counts made in stained tail tips of triploid, diploid, and haploid larvae. The axolotl karyotype was described by Signoret (1965), and shortly thereafter by Callan (1966). Signoret's description and figures of the mitotic chromosomes were based on preparations of blastula cells treated for 100 hours at 0°C, a procedure which results in characteristic secondary constrictions which make individual chromosomes more easily recognizable. Callan pictured mitotic chromosomes from liver cells with and without cold treatment, as well as those from epithelial cells of larval tail tips; he also described and pictured the lampbrush chromosomes of oocytes. In these he found chiasmata occurring at a high frequency, with an average of 113 per nucleus for the five chromosome complements fully analyzed. A working map of lampbrush chromosomes of the axolotl appears on p. 74.

The occurrence of numerous chiasmata in the oocytes of the axolotl would indicate that crossing over might be expected with considerable frequency. Proof that crossing over actually occurs in the female axolotl as well as in the male was obtained for the closely linked genes *f* and *g* (see pages 10 and 11). The rate of crossing over in females in which these linked genes were "in repulsion" (*F g/f G*) was determined as 2.97 percent, while the rate for males of the same genotype was 1.63 percent (Humphrey, 1959).

Sex Determination in the Mexican Axolotl

The mode of sex determination in the axolotl is unlike that in frogs or mammals in that the female rather than the male is heterogametic. This was first demonstrated by mating a normal female with a female in which one ovary had been converted into a functional testis through the action of a testis graft (Humphrey, 1945). If the axolotl female were homogametic (X/X), as in frogs and mammals, all the progeny obtained from such a mating (XX × XX) should have been females. Instead, the sex ratio was 1 male : 3 females, a ratio which would be expected if the female axolotl were heterogametic (Z/W) in genotype; the offspring then expected would be 1 Z/Z : 2 Z/W : 1 W/W. Females of genotype W/W were found to be indistinguishable from the ordinary Z/W type, but were identified by the fact that they produced only female offspring (Z/W) when mated with normal males (Z/Z).

Further proof of male homogamety was later obtained by incorporating primordial germ cells of dark embryos into one gonad of white recipients. Germ cells from male donors incorporated in an ovary gave rise to ova; these carried the dominant gene for dark color and only Z chromosomes if the axolotl male donor was homogametic. From such grafted white females mated with ordinary white males (Z/Z), all the dark offspring obtained were males (Humphrey, 1957).

Since the female axolotl is heterogametic, any difference between the Z and W chromosomes must be sought in the cells of that sex. Brunst and Hauschka (1963) and Hauschka and Brunst (1965), studying mitotic chromosomes, stated that in the thirteenth pair, the longer arm of one member is longer than that arm in the other. Callan, from his studies on the lampbrush chromosomes of the axolotl, concluded that no such difference existed between chromosomes of the thirteenth or any other chromosome pair. It is quite probable that the Z and W chromosomes differ in nothing except the members of a gene pair involved in stimulating gonadal development in either the male or female direction; this difference need result in no measurable difference in chromosome length.

Proof of male homogamety in *Pleurodeles* was obtained by Gallien (1951) through reversal of larval males to females by treatment with estradiol; such females, genetically males (Z/Z), when mated with normal males (Z/Z), produced only male offspring. Whether the male is homogametic in all Urodele species as in the axolotl and *Pleurodeles* remains undetermined.

Mutant Genes

Mutant genes have been found in the Mexican axolotl in strains maintained for numerous generations under laboratory conditions. Some