Masculinization and Breeding of the WW Xenopus[1]

In earlier reports[2,3] it was shown how, by the implantation of a small testis into Xenopus larvae, genetic females (ZW) may be converted into functional males, without changing their ZW constitution. Breeding normal ZW females with ZW convert-males, offspring are obtained of which about one-fourth are WW females. The latter can only be distinguished from the normal ZW females – which, as expected, make up about half of each culture – by further breeding tests. So far, seventeen WW females have been singled out[4]. Now arises the question, whether or not also WW larvae – lacking the Z chromosome which is so characteristic for normal males – may be induced to take a male course of development, i.e. change from ovarian to testicular development.

delivers offspring consisting of males only[3]. *Combination 3*, both parents sex-converted, produces equal numbers of males and females. From *combination 4*, consisting of two heterozygous partners, are obtained females and males in the ratio of 3:1[2,3]. The WW females can be recognized and separated from their ZW sisters only by breeding with normal ZZ males (*combination 5*). Their offspring must consist of 100% females (ZW), while the sister combination (ZW female and ZZ male) gives equal numbers of males and females (this latter combination is not shown in the Figure). The process of separating out the WW females evidently is quite time consuming. To raise the first offspring of *combination 4* to sexual maturity takes over one year. The offspring of any following combination have to be tended another 3 months to bring them to a stage at which gonadal sex easily may be recognized. However, once a stock of certified WW females

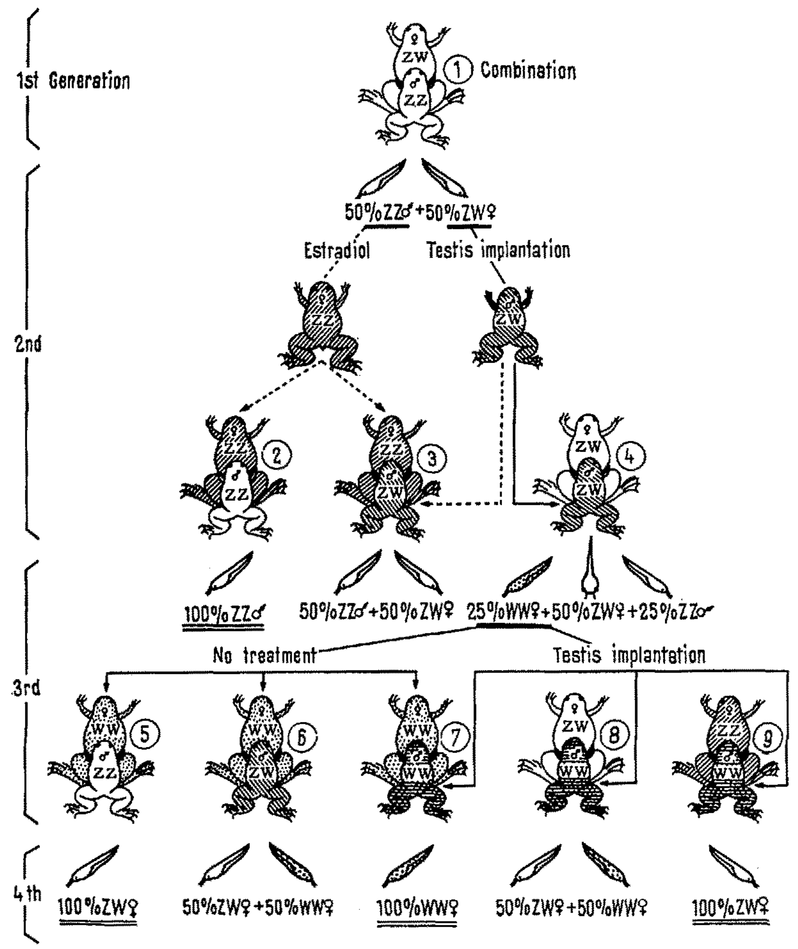

Diagram of experiments with Xenopus. Sex reversal and breeding tests. *White* – normal animal; *hatched, down left* – with estradiol feminized male; *hatched, down right* – by testis graft masculinized female; *hatched horizontally* – by testis graft masculinized WW animals. *Stippling* indicates WW constitution.

Materials and methods. As materials for the here presented experiments served various normal and sex-converted animals of our laboratory stocks. The methods of induction of sex-conversion are the same as previously described[2]. The recognition of WW females and males in genetically mixed groups is possible only by time-consuming progeny tests.

Results. The history of the experiments, which were designed to provide, eventually, offspring consisting entirely of either males or of females, is outlined in the Figure. Starting from a normal culture (*combination 1*) estradiol feminization leads to *combination 2*, which

has become available, it is possible to raise all-female ZW cultures in unlimited numbers by repeated breedings.

It became now the particular aim of the present study to attempt masculinization of WW larvae by the implantation of testes from recently metamorphosed males. The results which then might be obtained by breeding

1 With the support of a grant by the Ford Foundation.
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4 K. MIKAMO, Am. Zool. *3*, 490 (1963).

WW convert-males with the three possible types of females are represented in the Figure, *combinations 7–9*. Actually, the following is the way of procedure.

First, the assumption that the offspring of *combination* 4 contain about 25% larvae of WW constitution was ascertained by the above mentioned breeding test with ZZ males. It resulted in the identification of 17 WW females and 37 ZW females. Implanting testes into 120 undifferentiated larvae we could therefore expect that among them about 30 were of the WW constitution, 60 of ZW, and 30 of ZZ. The mortality was 15%. In a considerable number (46) it was early recognized that the testes failed to arrest severely ovarian development, obviously because of poor takes of the grafts. All these were discarded, as were also another 23 which showed no trace of testicular development at the age of maturity (22 months). Of the remaining 32 males the progeny tests prove that 19 are ZZ males, 12 are ZW convert-males and only 1 is a WW convert-male. Another animal with male external characters proves to be a hermaphrodite. Though it copulates vigorously with stimulated females, it cannot fertilize eggs, probably because no patent efferent ductules have become established.

At the present it is still impossible to explain the relatively much too low number of WW convert-males. But this first case of its type furnishes already full proof that by the method of testicular implants also WW larvae can be induced to develop testes. This evolves from the following breeding record of this animal.

Combination 9
(male WW × female ZZ)
first offspring: 24 females (100%)
second offspring: 89 females (100%)

Combination 7
(male WW × female WW)
third offspring: 146 females (100%)
fourth offspring: 84 females (100%)

Discussion. The general importance of the fact that all three possible chromosome-gene combinations (ZZ, ZW, WW) can develop and become fertile as females as well as males will be discussed in a forthcoming paper that deals with the chromosomes of Xenopus[5]. The ease with which one can now breed one-sexed cultures and be sure of normal sex, male or female, at any stage from egg to metamorphosed frog, calls for a reinvestigation of the embryology of gonad differentiation. There will no longer be any guesswork about the earliest testicular and ovarian stages. More important, experimental work on sex changes will no longer have to rely on statistics. It is a fortunate circumstance that Xenopus can so easily be induced to breed under laboratory conditions and may be raised to and maintained at maturity with a minimum of caretaking.

Zusammenfassung. Xenopuslarven mit genetischer WW-Konstitution können durch Implantation von Hoden junger Fröschchen vermännlicht werden. Ein erstes WW-Männchen gab zweimal völlig weibliche Nachkommenschaften mit ZZ-Weibchen und desgleichen mit WW-Weibchen. Alle drei möglichen diploiden Kombinationen der Geschlechtsfaktoren sind jetzt als Männchen sowohl als Weibchen bis zur Geschlechtsreife gezüchtet und als fortpflanzungsfähig befunden worden.

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Anatomisches Institut, Universität Basel (Switzerland), August 11, 1964.

[5] K. MIKAMO and E. WITSCHI, in preparation.

The Action of Iodine upon Thyroidectomized Frog Larvae

In 1919 SWINGLE[1] found that feeding a mixture of wheat flour and iodine could induce metamorphosis of thyroidectomized frog larvae which otherwise would not have metamorphosed. He suggested that iodine itself functioned as the hormone. DVOSKIN[2] has also found that elemental iodine can act like thyroxine in the rat and chick. The growth of thyroidectomized rats can be stimulated by high concentrations of iodide, and EVANS et al.[3] suggested that iodide has thyroxine-like activity.

Another explanation for the activity of iodine and iodide is that they could be converted to thyroxine *in vitro* or in the tissues of a thyroidectomized organism. Thyroxine could not be detected in thyroidectomized frog larvae incubated in radioactive sodium iodide[4], but the failure to detect thyroxine formation does not mean that it is absent. LIPNER and HAZEN[5] found that iodide or iodine-treated flour induces metamorphosis of thyroidectomized larvae of *Rana grylio*, but chromatography of aqueous extracts of the treated flour revealed the presence of thyroxine. It is known from the work of LUDWIG and VON MUTZENBECHER[6] that incubation of iodine, casein and sodium bicarbonate at 37°C, pH 7–9, followed by alkaline hydrolysis, allows the detection of thyroxine, diiodotyrosine and monoiodotyrosine. These results imply that thyroxine may be formed *in vitro*, and that it is the compound that promotes metamorphosis of thyroidectomized larvae.

One purpose of this investigation was to feed iodine to thyroidectomized larvae of *Rana pipiens*, then incubate

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