

FURTHER OBSERVATIONS ON ARTIFICIAL PARTHENOGENESIS IN FROGS

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1. We have repeated our efforts to raise parthenogenetic frogs during the past season in order to be able to determine their sex, but with less success than we had last year. None of the parthenogenetic tadpoles which we produced this year lived as long as those of the previous year.

During the past two seasons we have punctured the eggs of *Rana sphenoccephala*, *R. pipiens*, *R. silvatica*, *Chorophilus feriarum* and of *Bufo americanus*. Only the eggs of the first two species could be utilized for our purpose, inasmuch as it is possible in them alone to obtain swimming parthenogenetic tadpoles from the punctured eggs. If the eggs of the other species mentioned are punctured they may begin to segment but they do not reach the tadpole stage. The frogs were caught in the open while copulating, the sexes separated and sent immediately to the laboratory. Some of the frogs (*R. pipiens*) were obtained in Long Island and some were sent from Chicago. The results were in both cases the same, in spite of the fact that the eggs of the frogs sent from Chicago must have remained in the uterus several days. From 600 to 1000 eggs of each female were punctured. The results were similar to those described in our last paper,¹ except that this spring all the swimming parthenogenetic tadpoles died when they were from twenty to thirty days old. Some of the eggs were smeared with blood before they were punctured. While

¹ Jour. Exp. Zool., vol. 14, p. 275.

the percentage of the eggs which underwent early segmentation was possibly augmented by this procedure, the percentage of the eggs which developed into tadpoles was not increased in this way. Practically all the eggs of the same females that had been fertilized with sperm developed into tadpoles, many of which are healthy and alive today.

2. We were anxious to obtain parthenogenetic frogs in order to test the statement of Kuschakewitsch,² to which Professor Morgan called our attention, that the young frogs are often hermaphrodites, or intermediate forms. If Kuschakewitsch's statement is correct, the presence of ova in the genital glands is not sufficient proof that the young frog is going to develop into a female. He gives some criteria for the discrimination between real females and the intermediate form which may develop into males. On the basis of his statements we examined once more the sections of the sexual glands of last year's parthenogenetic frog. We also utilized the slides we had made from the sexual glands of a young frog produced by fertilization with sperm from an egg of the same female from which the parthenogenetic tadpole and frog originated.

The gonads of the parthenogenetic frog which died at the close of metamorphosis were fairly well preserved. An examination showed that there were eight or nine genital spaces. According to Kuschakewitsch eight genital spaces are characteristic for the intermediate form while six are characteristic for the pure female. In addition there were many degenerating ova, which Kuschakewitsch mentions as a characteristic for the intermediate form. Comparatively few of the oöcytes had entered the growth period. The right gonad, which was smaller and in an earlier developmental stage than the left one, had nineteen larger oöcytes while the left gonad had only fifteen. Most of the oöcyte nests (or oögemmae as Kuschakewitsch calls them) did not contain any large oöcytes. If Kuschakewitsch's criteria are correct, the parthenogenetic frog which we described is a hermaphrodite or intermediate.

²Festschr. f. Richard Hertwig, Bd. 2, p. 61.

According to Kuschakewitsch these intermediates may develop into either males or females, and there are indications in the sections of the parthenogenetic frog that this transformation may have been under way. In the anterior part of the left gonad of the parthenogenetic frog the sections show all the appearances which Kuschakewitsch (p. 145) considers characteristic for the early stages of the transformation of the intermediate form into males. The endothelial lining of the secondary genital spaces is thicker than usual, more convoluted, and mitotic figures may be seen in places. In some regions considerable masses of undifferentiated embryonic tissue have been formed which are continuous with the endothelium of the secondary genital spaces and occupy almost the whole cross section of the gonad except for a few patches of germinative endothelium. If we may rely upon the statements of Kuschakewitsch, our frog would have developed into a male if it had survived.

The gonads of the parthenogenetic tadpole were so poorly fixed that nothing could be made out about them except that they contained many more large oöcytes than those of the frog. It could not be determined whether this individual was a female or belonged to the intermediate form.

The frog which had originated from a fertilized egg of the same female from which the parthenogenetic tadpole and frog were obtained, was killed about four months after metamorphosis. It had grown rapidly. The gonads were evidently those of a male. The spermatoc follicles contained cells in all stages of spermatogenesis from spermatogonia to spermatids with greatly elongated nuclei. In addition almost every section showed follicles which were nearly filled with a large oöcyte in the early growth period. Thus it appears that this male may have developed from a hermaphrodite or intermediate, if Kuschakewitsch's statements are correct.

In the European species, *R. esculenta*, the formation of spermatozoa does not take place for several years after metamorphosis while in the control frog this process was well under way about four

months after metamorphosis. It would seem, then, that in *Rana sphenocephala*, which is a southern species, sexual differentiation and sexual maturity set in a little more rapidly than in *Rana esculenta*.

It is obvious that further observations are needed to determine definitely the sex of parthenogenetic frogs.