

# THE BIOLOGICAL BASIS OF SEXUAL BEHAVIOUR IN AMPHIBIA

## II. THE INDEPENDENCE OF OVULATION AND OF COUPLING (THE MATING REFLEX) IN *XENOPUS LAEVIS* (THE SOUTH AFRICAN CLAWED TOAD)

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IN the previous paper in this series (Shapiro, this *Journal*) a technique was described for inducing the mating reflex in *Xenopus laevis* (the South African clawed toad) by means of extracts of pregnancy urine or of anterior pituitary tissue. In this way the mating reflexes of the animals, and ovulation on the part of the females, can be controlled adequately and observed conveniently.

Literature which deals with the exact relationship between ovulation and copulation is scanty, but observations available at the time have been reviewed by Marshall and Jolly (1905). In the rabbit no ovulation is said to occur without copulation (Heape, quoted by Marshall, 1904), but unpublished experiments by the present author have failed to confirm this. Marshall (1904), who is confirmed by Robinson (1918), reports that no ovulation occurs without copulation in the ferret. This is also stated to be the case, under certain circumstances, in the sheep (Marshall, 1903); and Longley (1911) states that pairing is necessary in the cat for all the eggs destined to undergo normal extra-ovarian development. Harper (1904) observed that, in the pigeon, ovulation is delayed until mating.

Little, if anything, however, is known of the relationship between coupling and ovulation or oviposition in amphibians.

Rostand (1934) states (p. 78) that ovulation can be retarded if the female is prematurely uncoupled. He quotes Nussbaum, however, to the effect that ovulation in the common frog is sometimes spontaneous.

The technique for evoking the mating reflex in the laboratory with ease at all times of the year has made it possible to investigate this point in *Xenopus*, an animal which never under any circumstances ovulates spontaneously under captivity conditions in the laboratory even at the height of the breeding season.

The type of animal employed for the experiments, and the extracts of pregnancy urine and of anterior pituitary used, have already been described in the previous paper in this series (Shapiro, this *Journal*).

Pairs of clawed toads were injected with pregnancy urine extract and anterior pituitary extract. As soon as the male had firmly clasped the female the coupling

pair was removed, pithed and immediately examined. In all, five pairs of toads which responded to injection with pregnancy urine extract and four pairs which responded to anterior pituitary extract were examined. Control uninjected pairs were also pithed and examined.

In no case were ova found in the oviduct of a female which had recently been embraced by a male. The ovaries were hyperaemic, and also the oviducts, especially the pars uteri of the oviduct. In the males the testes showed a marked hyperaemia.

The uninjected controls showed no such vascular activation of the gonads or secondary sex organs.

It is well established that ovulation or oviposition can occur without the presence of the male and without the mating reflex. This response is the basis of the test for pregnancy on *Xenopus laevis* which has been described by Shapiro and Zwarenstein (1933, 1935).

The experiments described above equally definitely indicate that the mating reflex can take place before ovulation begins. This suggests either that the same chemical substance in the extracts initiates coupling before ovulation and oviposition, or else that two different substances are present in the extract, the one acting before the other.

In the experiments described in the first paper in this series, females and males were found which although injected repeatedly for several days failed to couple. Of fifteen such animals which were killed thirteen were found to have severely atrophied ovaries. Of these, the ovaries of a few animals contained large unhealthy-looking ova, some of which had made their way into the oviducts.

It therefore seems that the ovaries may degenerate sufficiently to give no oviposition response at all or only a mild ovulation intraoviducally. But such animals do not couple. These facts suggest that of the two reactions, ovulation and oviposition depend on a gonad stimulating activity of the extracts and that the mating reflex depends on the presence of normal ovaries, *i.e.* the principle in the extracts responsible for coupling acts by stimulating normal gonads.

The testes of all the males in the above experiments showed the usual marked hyperaemia characteristic of the coupling male.

Further experiments on castration are being carried out.

#### SUMMARY.

1. Ovulation and oviposition can occur in *Xenopus laevis* without the mating reflex.
2. Conversely, the mating reflex is shown to occur before intraoviducal ovulation has taken place.
3. Females with atrophied ovaries which may yet respond to the ovulating principle in the extracts fail to evoke the mating reflex in the males.
4. This is to be regarded as additional evidence of the fact that the extracts probably exert their effect on the mating reflex through the intermediation of the normal gonads.

5. The failure of females with atrophied ovaries to couple although intra-oviducal ovulation may have occurred suggests that a second ovarian mechanism, which is present in the normal but not in the atrophied ovary, is necessary for the establishment of the mating reflex.

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