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Experiments on the Nuptial Coloration of the Common Japanese Newt, *Triturus pyrrhogaster* (Boie)¹⁾

By

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(With 2 textfigures)

Several papers have recently been published concerning the breeding habit and the secondary sexual characters of the Japanese newt, *Triturus pyrrhogaster* (Boie). Ueki (1930), having measured the lengths and the weights of various organs of this form, found several differences in the two sexes. Tsutsui (1931) described in detail several differences in the dermal glands, skin and tail of the two sexes at ordinary times and during the breeding season, and also made interesting observations on their breeding habit. Recently Nagasaka (1935) published microscopical observations on the dermal glands distributed on the cheek and on the hair-like appendages found in the cloaca of the male during its breeding season. But so far as the present writer is aware, no experimental work has been done on the secondary sexual characters of *T. pyrrhogaster* and therefore the following investigations were undertaken as experimental works on the secondary sexual characters of this species. Before proceeding further, the writer wishes to record his thankfulness to Prof. Tohru Uchida under whose constant and helpful guidance the research was carried out.

I. The Principle External Sexual Differences Due to Gonadic Hormones

(1) *The external sexual differences.*

The principle external sexual differences of *T. pyrrhogaster* have

1) Contribution No. 109 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.

already been described by the above mentioned investigators. The male is marked by the remarkable development of such dermal glands in its head region as the parotoid, the globular and the scapular glands. In the abdominal region of the male the dorso-vertebral ridge, the dorso-lateral and the ventro-lateral ridges are distinct but in the female only the dorso-vertebral ridge exists. On account of these dermal ridges, the cross section of the body of the male through its trunk shows a pentagonal form; but in the female it takes the shape of a section of chestnut. The skin of the female is beady and coarse, while that of the male is soft and smooth with many transverse wrinkles especially on its lateral ridges. The tail of the male is broader than that of the female in lateral view, the dorsal and ventral free margins forming fins. In the female these fins are absent. The most remarkable change occurring in the male during the breeding season, is the appearance of the nuptial coloration, a whitish blue or violet hue conspicuous from the head region to the tail. The coloration is observed in captivity early in the winter. It is maintained through the winter, becomes brighter and spreads in the following spring to almost all parts of the body surface except the dark dorsal side and the red belly. After the breeding season, the coloration disappears gradually during the summer months.

In the female no particular change of external sexual character is observable during the breeding season except the swelling of the trunk which is due to the development of the ovary.

(2) *The effects of castration on the secondary sexual characters.* In about 50 males the gonads were removed on both sides. The operations were performed late in the autumn, or in early winter, when the violet shade was absent in the normal male. In about 60-70% of the castrated males no nuptial violet was detected in the following spring, but in the rest of them faint violet hue was observed on the trunk and the tail. The dermal ridges on the sides of the trunk gradually disappeared after the castration and the male trunk became like that of the female, but in the tail no change of shape occurred.

It is remarkable to note that similar changes of the secondary sexual characters in the male took place when the animals were hypophysectomized. The gonads of the hypophysectomized males became reduced to one-tenth, or even less, of the volume of the gonads in the normal males (Fig. 1, A). When sections of these gonads



Fig. 1 A. Testes of the normal (left) and hypophysectomized (right) males. $\times 3/5$.



Fig. 1 B. Section of the testis of the hypophysectomized male.

were examined, the majority of the germ cells were found to be in an inactive condition (Fig.1, B). Moreover, in none of the hypophysectomized males did the nuptial coloration appear. Kopeč (1927) reported in the case of *Phoxinus laevis* Agassiz that the appearance of the nuptial colour notwithstanding the removal of the gonads was due to the hormones which had already been liberated from the testicules, or the ovaries before the castration was performed. Guyénot and his collaborators (1932) pointed out that the hypophysis influences the appearance of the pads on the thumbs of *Bombinator pachyypus*. In the present experiments on *T. pyrrhogaster*, however, it could not be determined whether the appearance of the violet coloration in the castrated males depends upon the residual hormone substances or upon the direct influence of the hypophysis. At any rate the fact that the nuptial coloration sometimes appears in castrated males, but not in hypophysectomized ones and, further, that it appears after the injection of Prolan (*vide infra*), suggests some intimate relationship between the hypophysis and the nuptial coloration.

In the female no remarkable change in its secondary sexual characters was observed after spaying.

II. Effects of Injecting Various Hormone Preparations into Castrated Males

Various hormone preparations were injected into castrated males devoid of violet hue or dermal glands. These injections were

performed in most cases in spring when the nuptial coloration of the normal male attains its maximum extent.

(1) *Brayed testes*. Testes of the normal males, with prominent nuptial colorations, were brayed to milky juice and $\frac{1}{2}$ cc of this was injected every alternate day into castrated males. In two animals nuptial violet colour appeared prominently after the second, or the third injection; but in most cases the animals died without showing any change of coloration which was perhaps due to the imperfect sterilization of the substance injected into them.

(2) *Spermatin* (prepared by "Y. Teikokusha" Japan). One-fourth or $\frac{1}{2}$ cc of Spermatin was injected on alternate days into castrated or hypophysectomized males which did not bear the violet colour. In a few days all the injected animals showed distinct violet coloration on their tails and trunks. Sometimes the violet coloration induced by the hormonal injection was more beautiful than that of the normal male during the breeding season and lasted for three to four weeks after injection. Though Spermatin was so effective in producing the nuptial coloration, it had no influence upon the dermal glands that had disappeared in the castrated males, because they were just in the same condition before and after the injection of the hormonal substance.

(3) *Oophorin-extract* (prepared by "Dr. Freund & Dr. Redlich. Organpräparate, Berlin"). The injection of Oophorin was not so effective in inducing the appearance of nuptial coloration as Spermatin was. But in all animals injected with Oophorin the violet colour appeared to more or less extent excepting one case which died a few days after the injection.

(4) *Aphrodine hydrochloride = Yohimbine Spiegel*. (prepared by "Chemische Fabrik Güstrow"). Yohimbine was injurious to the newt; all the injected animals having died the following day after the injection without showing any definite colour reaction in the skin.

In the female newts, normal or spayed, no change of any external sexual character was observable after a similar procedure. In regard to the nuptial coloration the skin of the female newt suggests itself to be in a different "*forma*" from that of the males.

The artificially induced nuptial coloration above referred to was generally observed within six months after the castration. But the response of the skin to the hormone stimulus was quite different in

animals that had lived for one or two years after the removal of their gonads, although the conditions of experimentation were alike in the two cases. The writer also experimented with a mixture of Prolan (prepared by "Bayer-Meister Lucius") and Spermatin, but no change in the coloration of the body surface was produced. The injection had no influence upon any character of the castrate. Violet hue to a faint degree was, however, exhibited by one specimen that had been injected with Prolan without admixture with Spermatin.

The seeming inconsistency of the two facts that the injection of the same hormone was effective during a certain period after castration and ineffective thereafter is supposed to be due to the different physiological phase of the skins at varying periods. As already mentioned, the injection of hormones into the female has no influence upon its colour reaction whether the animal is spayed or normal, but in the castrated male the skin which was at first soft and velvety becomes coarse and beady, and the wrinkles on the lateral ridges of the trunk disappear—the whole appearance of the male becoming female-like. It seems probable that the male skin when kept in such a condition for a long time gradually loses its physiological potency of response to the hormone stimulus. The condition of the skin in such animals must, therefore, be assumed to resemble that of the females in respect to the colour reaction. The writer, however, regrets that microscopically he could not find any morphological differences between the skins of the male and the female, or between that of the castrated and the normal individual.

III. Reciprocal Transplantations of the Tail Skins between the Male and Female

The experiments on reciprocal transplantations of the tail skin made between the males and females under various conditions yielded results in agreement with those obtained from the injection experiments. They are enumerated below.

(1) Pieces of skin from the tails of the females (obtained from near the junction of tail with the trunk) were transplanted on corresponding positions on the bodies of the males (Fig. 2, A). The operations were performed in early winter when the nuptial colour of the male is absent. In the following spring, when the skin of

the host male showed brilliant nuptial coloration, the transplanted female skin did not show any violet hue.

(2) In the same manner, pieces of skin from males were transplanted on that of females (Fig. 2, B). The transplants showed no change of coloration in the following breeding season.

(3) In another experiment the skins of males were transplanted on the bodies of females, whose ovaries had been replaced with a testis some months before (Fig. 2, C). In some of these animals clear indications of the violet coloration appeared on the grafted male skins only, but not on the skins of the host females.

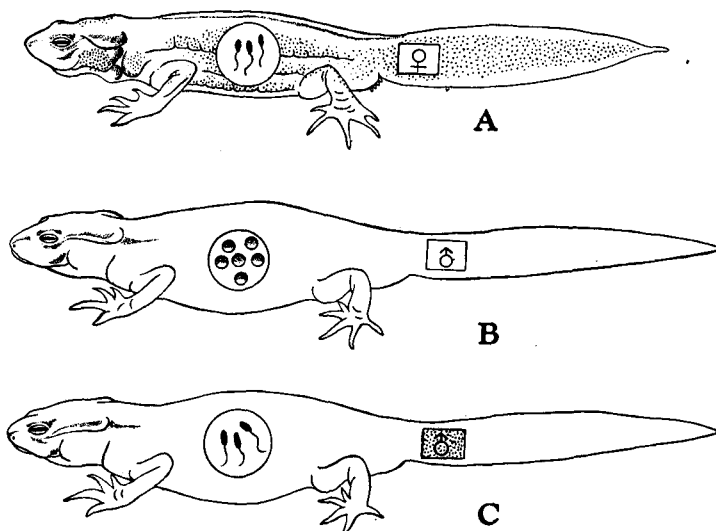


Fig. 2. A schema showing the relationships between the coloration of the grafted skins and the gonads. A, A piece of female skin grafted on a normal male; no violet coloration on the graft. B, A piece of male skin grafted on a normal female; no violet coloration on the graft. C, A piece of male skin grafted on a female, whose ovaries were replaced by a testis; violet coloration appeared only on the graft.

It is noticeable, however, that the bits of female skin, when grafted upon normal males, showed no coloration during the first breeding season, but acquired the responding property in the second and third years of the reproductive cycle, and consequently becomes indistinguishable from the skin of the hosts. Judging from these results, it seems that the male skin has some physiological property

making an easier response to the male hormone stimulus than the female skin. These relations between the grafted skins and the gonads of the host call to mind the experiment made by Danforth and Foster (1929) on different races of domestic fowls. They found that the colours of the feathers on the grafted skins are determined according to the genetical constitution of the donors, but as regards the sexual differences in shape they are under the control of the gonads of the host birds.

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