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The Role of Steroid Hormones in Sex Recognition during Spawning Behaviour of the Goldfish, *Carassius auratus* L.

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1. Sex recognition mechanisms in goldfish were examined using hypophysectomized male fish.

2. After hypophysectomy, Methyltestosterone (m.t.) and estradiol-17β at the concentration of 10 ppm of the diet were orally administered for two weeks.

3. The m.t.-treated fish discriminated estradiol-treated fish and chased them within 6 min after pairing.

4. The olfactory epithelium of the m.t.-treated fish became thick and olfactory cells were regularly arranged having clearly visible microvilli or cillia.

5. The estradiol-treated fish had thread-like testes with no functional activity but the kidney of these fish showed conspicuous changes suggesting secretory function.

6. It is surmised that m.t.-treated fish having enhanced olfaction recognize estradiol-treated fish which secrete odour or a sex pheromone from the kidney.

Introduction

One of the most remarkable phenomena in the spawning of fishes is that they easily identify the opposite sex as they start their specific spawning behaviour.

In stickleback for example, visual cue is reported to be involved in the sex recognition (Noble 1938). Difference in body colour and movement pattern in the spawning season have important significance in sex recognition in jewel fish (Noble & Curtis 1939).

In general, females show less changes of body colour or shape as compared with male fish. Therefore, males might recognize their mates through some other cues rather than visual one. Tavolga (1956) reported that the males of *Bathygobius soporator* identify females by olfactory cues by unknown chemicals secreted from the ovaries. Chemoreception has also been proposed as a method of sex recognition in some fishes (Breder 1935, Losey 1969, Bloom & Perlmutter 1977). The information on the sex recognition, however, is meagre and fragmentary in fishes. The present study was carried out to elucidate the role of steroid hormones in the mechanism of the sex recognition during spawning behaviour of the goldfish, *Carassius auratus*.

Material and Methods

Male goldfish, *Carassius auratus* of 8–9 cm in standard length were used in the present study. The experiments were carried out during March to June 1978. Nine mature

males were hypophysectomized and reared for about 3 weeks at water temperatures of 18–25°C. After becoming whitish in body colour due to hypophysectomy, they were divided into 3 groups of 3 fish each. One group served as the control. The second group was fed with diet supplemented with estradiol-17β at a concentration of 10 ppm. The third group was fed with diet containing methyltestosterone (m.t.) at the same concentration as in the second group. Each group was fed to satiation daily for 2 weeks.

After two weeks of administration of the hormones, each fish was paired with another taken at random from the other groups, i.e. in total $3 \times 3 \times 3 = 27$ pairs, and their behaviour was observed for almost a continuous 15 min period starting just after pairing. The observations were done in an aquarium 30 cm wide, 30 cm deep and 45 cm long.

To examine the role of the olfactory sense organs in the spawning behaviour, a cotton ball was plugged into the nostril of the fish treated with methyltestosterone and the fish with the cotton plugs were paired with estradiol-treated fish to observe the behaviour. Also observation on the same pairs was carried out after removing the cotton plugs.

After observation of behaviour, all the fish used were checked for completeness of hypophysectomy. Olfactory regions, kidneys and testes were fixed with Bouin's solutions and cut at 7 μm. The sections were stained with Delafield's hematoxylin and eosin for histological observation.

Intact immature goldfish ranging from 8 to 9 cm in standard length were used for another experiment to study the effect of methyltestosterone at the concentration of 10 and 30 ppm and estradiol-17β at the concentration of 10 ppm of diet on olfactory regions of the fish. Hormonal diets were administered for 2 weeks, then the olfactory regions were fixed with Bouin's solution, sectioned and stained with Delafield's hematoxylin and eosin to observe histological changes of olfactory epithelium after treatment with sex steroids.

Results

Behaviour

Four types of behaviour were recognized in paired fish from different groups.

1. *Indifference :* The paired fish behave independently and show no interest in each other.
2. *Orientation :* Fish orientates itself toward the other but does not show any sign of chasing behaviour.
3. *Pecking :* Fish pecks and noses the other around the urinogenital opening or posterior part of the body.
4. *Chasing :* Fish chases the other, touching and sometimes rubbing its head on the posterior part of the partner.

The behaviour of the 27 pairs was recorded according to the categories described above. Control and estradiol-treated pairs showed no change in their behaviour and this was recorded as indifferent in all combinations between these two groups. Chasing behaviour was observed only in pairs of methyltestosterone (m.t.)-and estradiol- treated fish. The results are summarized in figure 1. When m.t.-treated fish were paired with control fish, they showed either orientation or pecking behaviour except one fish (Mt. 1) which showed chasing toward No. 3 control fish (Co. 3). On the other hand, when paired with estradiol-treated fish, they showed violent chasing behaviour within 6 min in all pairings (figure 1). Furthermore, m.t.-treated fish chased only estradiol-treated fish even when they were put together with control fish in an experimental aquarium. These results clearly indicate that the m.t.-treated fish could identify estradiol-treated fish, and estradiol-treated fish being chased, behaved as a female partner.

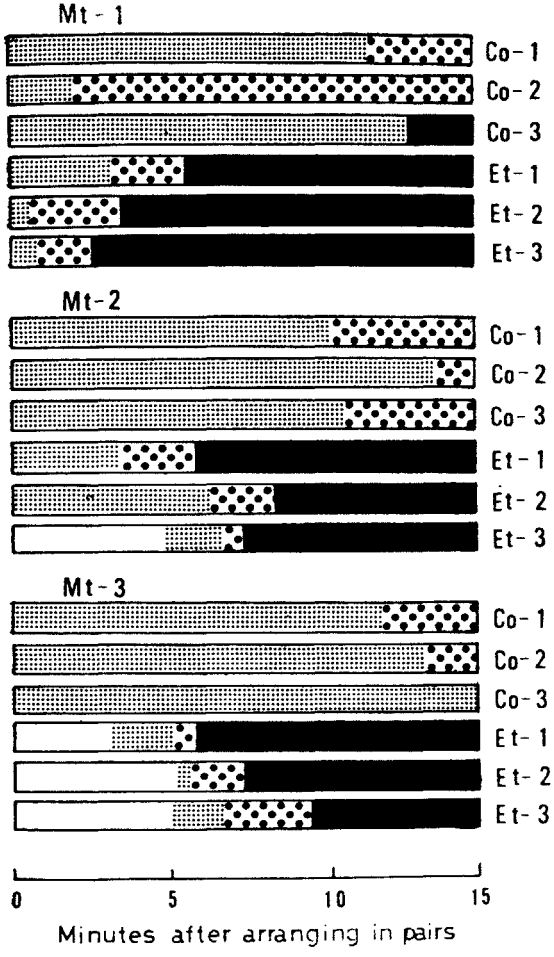


Figure 1 Behavior response of hypophysectomized male goldfish treated with 10 ppm methyltestosterone (Mt 1-3) toward hypophysectomized male goldfish treated with 10 ppm estradiol-17β (Et 1-3) or hypophysectomized control (Co 1-3)

□ : Indifference; ■ : Chasing

▒ : Orientation ⁘ : Pecking

Effect of plugging of nostrils on the behaviour

The effect of cotton balls plugged in the nostril of m.t.-treated fish on the chasing behaviour toward estradiol-treated fish is illustrated in figure 2. No chasing behaviour was observed when the cotton ball was plugged in the nostril of the m.t.-treated fish. The m.t.-treated fish could not identify estradiol-treated fish and behaved indifferently toward both control and the estradiol-treated fish. When the cotton balls were removed, the sex recognition was restored and the fish showed interest in the estradiol-treated fish (figure 3). This suggests that there is an olfactory stimulus involved in sex recognition by males.

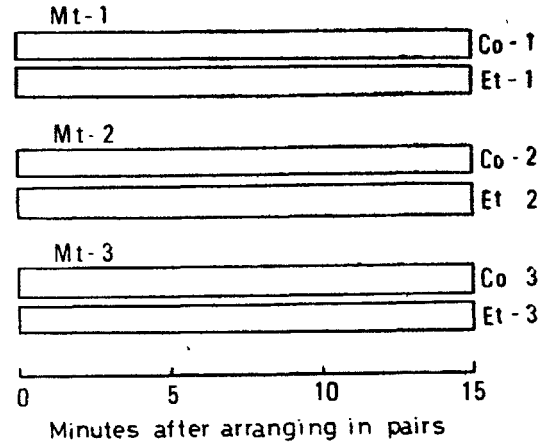


figure 2 Behaviour response of hypophysectomized male goldfish treated with 10 ppm methyltestosterone and the nostrils plugged with cotton balls (Mt 1-3) toward estradiol-treated fish or controls when put together in an aquarium

□ : Indifference

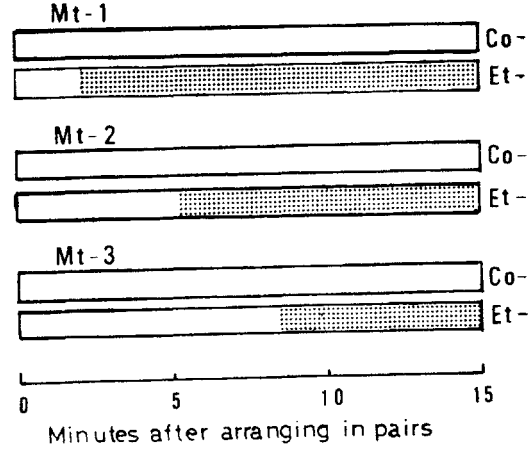


Figure 3 Behavior response of hypophysectomized male goldfish treated with 10 ppm methyltestosterone when plugs removed from the nostrils

□ : Indifference; ▒ : Orientation

Histological observation

Olfactory epithelium: The olfactory epithelium of the hypophysectomized control fish showed regressive features such as disordered arrangement of olfactory cells. The free surface of the epithelium of the hypophysectomized estradiol-treated fish was also regressive—which was similar to the controls.

On the other hand, the epithelium of hypophysectomized m.t.-treated fish revealed that the free surface of the epithelium became clear and microvilli of the olfactory cells were clearly visible. The nucleus of the olfactory cells was round or ellipsoid in contour and regularly arranged suggesting that the m.t. treatment affects the olfactory epithelium and activates olfaction.

This was verified by the experiment using intact immature fish which were treated with 30 ppm and 10 ppm m.t. or 10 ppm estradiol. The results (figure 4) show that the olfactory epithelium became thick in m.t.-treated fish while no change was observed in either estradiol-treated or control fish. This leads to the conclusion that the methyltestosterone activates the function of olfactory epithelium.

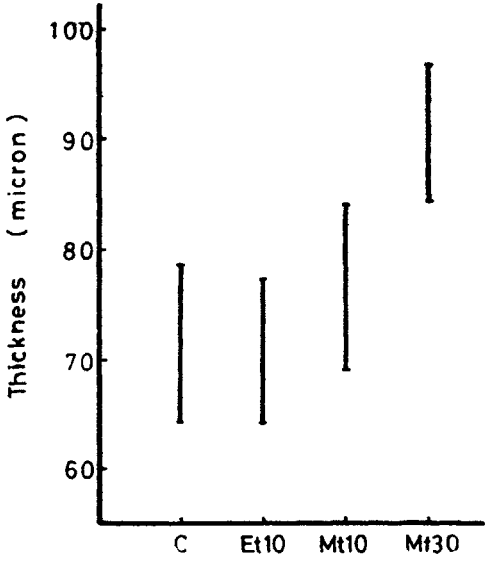


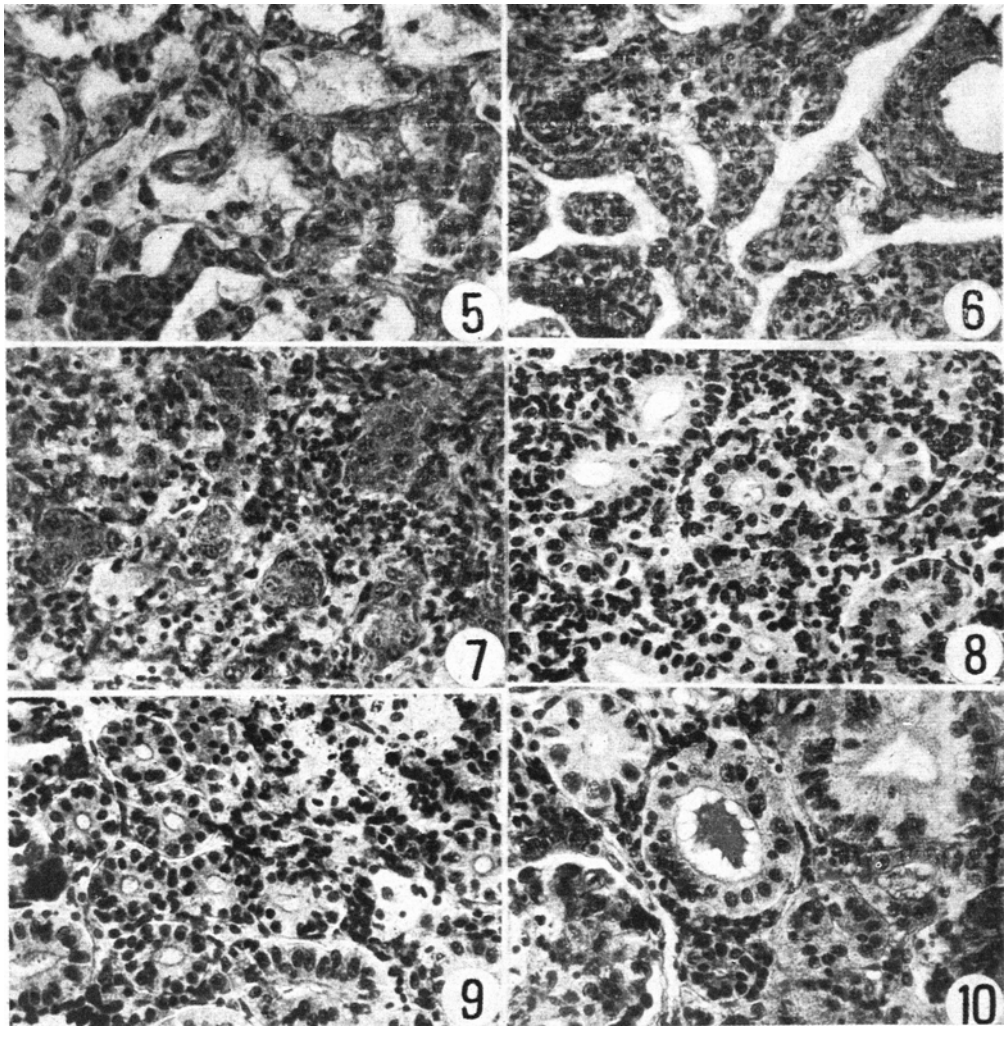
Figure 4 Ranges of thickness of olfactory epithelium : *C*, intact controls, *Et 10*, intact fish treated with 10 ppm estradiol-17β; Mt 10, intact fish treated with 10 ppm methyltestosterone (m.t.); Mt 30, intact fish treated with 30 ppm m.t.

Testes: Histological observation of nine hypophysectomized fish used for the behaviour experiments revealed that the testes were thread-like (figures 5–7). Even the testes of m.t.-treated were almost the same as those of estradiol-treated and the control fish in having fibrous connective tissue, with a few inactive spermatogonia.

Kidney: No change was observed in the kidneys of the fish treated with m.t. and the control fish (figures 8, 9). While in the kidneys of estradiol-treated fish, conspicuous changes were observed in the glomerulus, renal tubules of the proximal portions and hematopoietic tissues. Capillaries of the glomerulus of lymphoid tissue were greatly extended. Congestion was recognized in both glomerulus and lymphoid tissues. Eosinophilic substance was found in some parts of the renal tubules (figure 10). Degenaration and regeneration of the renal tubules were extensive.

Discussion

Sex-recognition is a fundamental factor in the initiation of the spawning behaviour in fishes. This recognition is divided into two categories, viz. (i) female's and (ii) male's recognition of the opposite sex. It is assumed that the bright colours of the males are attractive or stimulating to the females. From this viewpoint, the visual impression must be important in sex recognition of males in species in which conspicuous colour changes occur during the spawning season, e.g. jewel fish (Noble 1938). The female sticklebacks respond to a certain movement of the males (Leiner 1930). Noble and Curtis (1939) concluded that female jewel fish recognize their mates by visual cues.



Figures 5-10 (× 180) **5-7**, Testis of hypophysectomized male : **5**, control; **6**, treated with 10 ppm methyltestosterone; **7**, treated with 10 ppm estradiol (e.t.); **8-10**, Kidney of hypophysectomized male : **8**, control; **9**, treated with 10 ppm m.t.; **10**, treated with 10 ppm e.t.

In goldfish, however, there might be no sex recognition through visual cue at the spawning season because except for pearl organs which appear in the operculum and pectoral fins of males, no sexual dimorphism in colour or appearance is detectable. In the present study, all the experimental fish used were hypophysectomized and changed in colour from red to white. Further, there appeared to be no changes in body colour or form even after treatment with either estradiol or methyltestosterone. These facts indicate that in goldfish sex recognition is performed through means other than visual cues.

The present study clearly indicates that hypophysectomized males treated with estradiol were chased by androgen-treated males.

The testes of these fish were thread-like with no functional activity due to surgical removal of the pituitary gland. Therefore, the gonads themselves are not directly involved in sex recognition, and may only act as a source of sex steroids. Fish whose nostrils were plugged with cotton balls showed no chasing and sex identification behaviour, but after removing the cotton plugs, the sex recognition recovered. Therefore, olfaction is clearly involved. The chasing behaviour was demonstrated to be under the control of androgen. But the androgen is not enough by itself to induce chasing behaviour because androgen-treated fish or mature males never show any sign of chasing behaviour if they were kept in an aquarium without estrogen-treated fish or gravid females but become excited and actively engage in chasing behaviour when estradiol-treated fish or gravid females are introduced in the aquarium. These facts further suggest that the olfactory cue of the male is involved in sex-recognition and in the initiation of chasing behaviour of females. This supposition is also supported by the fact that the olfactory epithelium became thick and the microvilli or cillia of the free surface of the olfactory cells became clear when fish was treated with methyltestosterone, suggesting that sensitive olfaction is enhanced by the action of androgen.

The foregoing discussion further raises the question as to what is the substance that the estrogen-treated fish or mature females produce as a sign of maturation which affects sensitive olfaction in androgen treated or mature males. In the present study the estrogen-treated fish which were chased by the m.t.-treated fish and behaved like females were hypophysectomized males. This means that the substance or odour is secreted from tissues other than ovaries, which have a connection to urinogenital opening, because the chasing fish nose or peck quite often around the urinogenital opening during the chasing behaviour. The histological observation revealed conspicuous changes in the urinary duct or lymphatic tissues of the kidney of estrogen-treated fish suggesting that the kidney might be the possible source of the substance or the odour. This substance is a sex-attractant and can be classified as a sex pheromone.

In conclusion, in sex recognition of goldfish, only mature males can recognize mature females secreting sex pheromones but they do not recognize genetic females which are immature. The sex pheromone might be species-specific and should be extracted and chemically analysed in a future study. This substance not only has various applied possibilities in fish culture but also is useful for basic studies on the spawning behaviour of fish.

Acknowledgement

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