Basis of the sexual and territorial behavior in males of Oreochromis niloticus and Oreochromis mossambicus

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Basis of the Sexual and Territorial Behavior in Males of *Oreochromis niloticus* and *Oreochromis mossambicus*

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Abstract

This study compared the social hierarchy of three groups of five males of two tilapia species: *Oreochromis niloticus* and *Oreochromis mossambicus*. Measurements of circulating steroid (testosterone, 11-KT and 17.20P) showed high testosterone levels in dominant males of *O. niloticus*, whereas no significant difference was observed in the levels of testosterone between dominant and dominated males of *O. mossambicus*. In *O. niloticus*, highly aggressive and territorial behaviors are correlated whereas *O. mossambicus* has low endocrine levels and does not have a highly hierarchical social behavior. In *O. niloticus*, the color of the dominant male is testosterone-dependent and has an appeasing function: pre-spawning females adopt this color after a sharp increase in testosterone and are thus able to enter the nest guarded by the male.

The use of different steroid implants confirms the role of testosterone in the aggressive behavior of the tilapia, allowing some fish to rise in the social hierarchy. The effects of 11-KT are more obvious for nest-digging activities than for chasing behavior.

Introduction

Previous studies have shown a correlation between gonadal cycles, circulating steroid levels and sexual behavior in many fish, especially in salmonids (Liley and Stacey 1983).

In cichlids, Aronson (1945, 1951) described the role of external stimuli (visual stimuli, sound emission, contact between the lateral lines and chemical communication) produced by other mates on spawning frequency. In *Oreochromis mossambicus*, Silverman (1978a, 1978b) suggested an effect comparable to pheromones as shown by Solomon (1977) in other species. In *O. mossambicus*, males as well as females can increase spawning frequency.

This study compares the social behavior and the endocrine levels of two species of tilapia: *Oreochromis niloticus* and *O. mossambicus*.

Materials and Methods

Fish

The fish used in the experiments were *O. niloticus* from a laboratory strain originally coming from Bouake (Côte d'Ivoire). All animals came from the same culture batch and were one year old. They measured 14.5±1.29 cm and weighed 96.3±3.5 g (mean body weight). Experiments were performed every year between April and September from 1989 to 1992.

For *O. mossambicus*, the animals came from a strain developed in Louvain-la-Neuve (Belgium) by Ursula Falter. In this experiment, 10 females of *O. mossambicus* and eight females of *O. niloticus* were used as well as 15 males of each species.

Culture Conditions

Outside experimental periods, males and females were separated and placed in tanks containing 0.5 m^3 recirculated water kept at 27°C. These tanks contained only tilapias. During experimental periods, the animals were placed in 300-l glass tanks containing adsorbing sand at the bottom and were transferred during tests to an 800-l tank also containing adsorbing sand at the bottom.

Experimental Protocols and Ethograms

Males were divided into groups of five animals. An ethogram was used to establish the social hierarchy for each of these groups and to visualize the social relationships within the group. For easy identification, the animals were tagged by fixing a nylon string with different colored beads forming a determined pattern on the posterior part of the head. The fish were anesthetized and a blood test was done before and after a series of behavioral tests conducted over a five-day period to evaluate their social hierarchy. These tests were triplicated for each groups.

On the ethogram where the five fish are represented in a circle (see Fig. 1), the dominant male, i.e., that which directs the greatest number of activities towards its conspecifics, is placed at the top. The dominated animal, i.e., that which sustains the greatest number of aggressive actions without reciprocation, is placed at the bottom. Interactions between animals are represented by arrows oriented in the direction of the action. The thicker the line, the higher the number of aggressive actions between fish. The number of fish is indicated in circles.

Social and Sexual Behavior

The different elements of the social and sexual behavior were recorded during a series of 10-minute tests every hour, eight times a day. This behavior was characterized by pursuits, mouth-to-mouth contacts, mouth-to-flank contacts, parallel swimming, erection of the dorsal fin and nest-digging with removal of sand.

Each group studied was composed of five males of similar size and body weight.

Blood Tests

The animals were anesthetized individually with a solution of phenoxyethanol at 2 $ml \cdot l^{-1}$ of water poured in a 10-l water tank. Blood was taken (1-1.5 ml) between 0900 and 1100 hours using a 2-ml heparinized syringe, and preserved in an ice box before centrifugation of all samples in a refrigerated centrifuge (4°C) for 10 minutes at 2,500 revolutions per minute.

Hormone Assay

Hormone concentrations were determined by radio-immunology. Testosterone and 11-KT were measured using the method developed by Fostier et al. (1982), giving a coefficient of variation of 8.12% (18.4 $ng \cdot ml^{-1}$, n=15) for testosterone and 6.37% (17.5 $ng \cdot ml^{-1}$, n=15) for 11-KT. The method described by Fostier et al. (1981) was used to measure 17.2P with a coefficient of variation of 5.74% (9.25 ng, n=15).

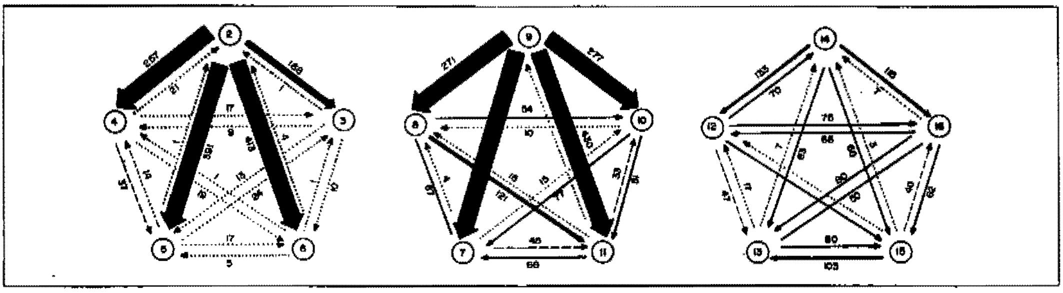

Fig. 1. Ethograms for three groups of five males of *Oreochromis niloticus*. The numbers in circles indicate individual fish. Numbers along arrows indicate the numbers of aggressive actions (see text).

Statistical Analysis

Paired comparison was done after an analysis of variance and Barlett's test. Data were compared using a *t*-test. Unless otherwise indicated, the level of significance was P=0.05.

Results

Ethograms of the Male Groups

The study of the three groups of five males clearly showed the presence of a dominant male in two groups, whereas social relationships in the third group were much more homogeneous although a dominant male could be recognized. Interactions between the fish also showed a second dominant animal. This was confirmed by the withdrawal of the dominant animal. Fig. 1 shows three diagrams summarizing all activities directed to the other fish within the groups: pursuits, mouth-to-flank and mouth-to-mouth contacts, and erection of the dorsal fin when approaching another fish.

Hormone Levels and Social Hierarchy in O. niloticus *and* O. mossambicus

The comparison of social hierarchies observed during pursuits showed clear differences between the two species of *Oreochromis*.

In *O. niloticus*, the social hierarchy is clearly established with a strictly dominant fish, with the greatest number of activities. Only the dominant male has access to the substrate and digs the nest. Its body color pattern is pearl white, with tinges of salmon pink, and the extremity of the fins is black. The other fish, males and females, are consigned to the upper corner of the aquarium. They are stressed and their color pattern ranges from greenish dark gray to black.

In contrast, *O. mossambicus* shows little signs of pursuit and there is no apparent social hierarchy in this species. Interactions between individuals are few and similar in all animals.

The comparison of hormone levels for the three steroids in these two species (Fig. 2) showed a very high level of testosterone in *O. niloticus* (52 $ng \cdot ml^{-1}$) in comparison with *O. mossambicus* (8 $ng \cdot ml^{-1}$). Levels of 11-KT and 17.20P were also higher in *O. niloticus* than in *O. mossambicus*.

Moreover, in *O. niloticus*, plasma levels of testosterone were very different, depending on whether the animals are dominant or dominated, whereas in *O. mossambicus*, no significant difference was observed. The same differences were observed, although somewhat reduced,

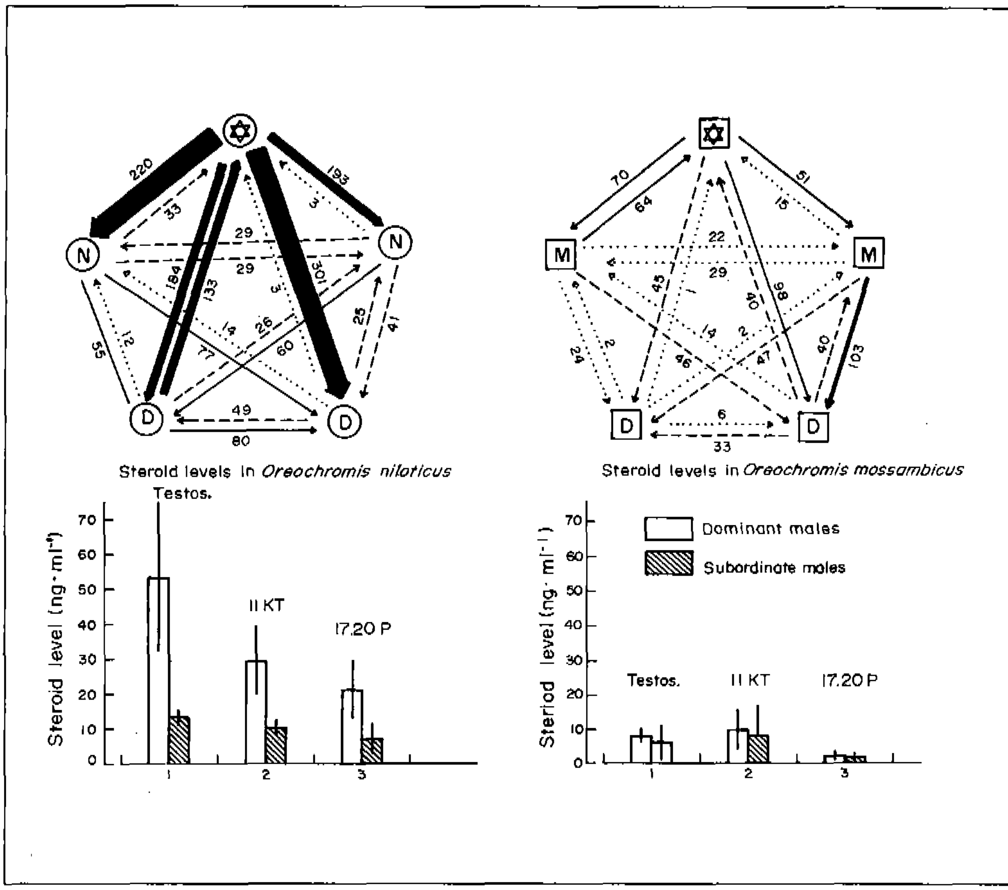

Fig. 2. Sterold hormone levels and social hierarchy in *Oreochromis nlloticus* and *O. mossamblcus*. On the ethograms, ✡ = dominant, N and M refer to the species used, and D = dominated (see text for discussion of comparisons and significance of differences).

in the levels of the two other steroids (11-KT and 17.20P).

Hormone Levels and Sound Emission In Males

In a collaborative study with U. Falter and Olivier Dufayt from the University of Louvain-la-Neuve (Belgium), we have for the first time established a correlation between sound emissions in males of these two species and other behavior patterns.

The male defending its territory chases the other fish (other males or females that are not ready to spawn) and emits a brief sound for less than half a second. The frequency of this sound emission is high: 400-700 Hz in *O. niloticus* and approximately 250 Hz in *O. mossamblcus*.

These emissions which are characteristics in dominant males are, like in the defense of the territory, dependent on plasma testosterone. Only the dominant males can emit these sounds.

Hormone Levels and Acceptance of the Female by Territorial Males

In *O. nilotlcus*, the color taken by the pre-spawning females (which is identical to that of the dominant male) is related to the high level of aggressive behavior in this species. This color pattern inhibits the aggressive behavior of the male that accepts the female of the same color. We have observed that some males with strong color patterns are sometimes accepted in the nest of the territorial male. The analysis of the female hormone levels (Fig. 3) showed that the change in color is correlated to an important testosterone level, particularly when the female is ready to spawn.

In *O. mossambicus*, although the same high testosterone level has been observed in the pre-spawning females, changes in the color pattern cannot be perceived by the human eye. The other steroids, circulating at relatively low levels, involve very few changes regardless of the species.

Effect of Steroid Implants on the Social Behavior of **O. niloticus**

In order to complete our study and to confirm the action of steroids on the social behavior of fish, we used implants with various quantities of several steroids in the fish and studied their effect by observing the corresponding ethogram. Three animals out of a group of five received steroid implants while the two others were used as control.

First, we studied the dynamics of release of pure testosterone from the implants at 0.5 $mg \cdot 100\ mg^{-1}$ implant 50 ml^{-1} saline solution. Between 6 and 36 hours, the quantity of hormone released was relatively stable for 1.75± 0.25 $ng \cdot ml^{-1}$. The animals receiving testosterone implants showed increased aggressiveness, thereby rising in the social hierarchy (Fig. 4), the number of pursuits increasing four hours after the implantation of steroids. The nest-digging behavior did not vary significantly in the three days following the implantation (Fig. 5).

The controls' aggressive behavior decreased significantly in terms of pursuits and nest-digging activities. This reduced activity can be explained by increased activity and aggressiveness of the animals with implants living with them (Fig. 6).

There was also a significant increase in nest-digging activities in the animals with implants of 11-KT after implantation (Fig. 7). In contrast, the implantation of 17.20P did not cause any significant change in behavior aside from a general reduced level of activity (Fig. 8).

Conclusion

These results show the significant role of testosterone in the territorial behavior of males and in the species' level of aggressiveness. The sounds emitted by dominant males during pursuits against conspecifics, or to accompany and guide the female ready to spawn to the nest, are testosterone-dependent. This hormone is also responsible for the change in color pattern in the dominant male. This phenomenon is also observed in pre-spawning females which can thus enter the nest. The adoption of this male-specific color by the female has an inhibitory effect on the aggressive behavior of the territorial male.

In *O. mossambicus*, the aggressive behavior is practically nonexistent and endocrine levels are also much lower. The female does not need to take on the male-specific color pattern to be accepted by the male.

Ketotestosterone has a more obvious effect on nest-digging activity. Hence, the various hormones have each a specific action on social and reproductive behaviors. However, the steroid 17.20P does not seem to have any direct action on male behavior when implanted.

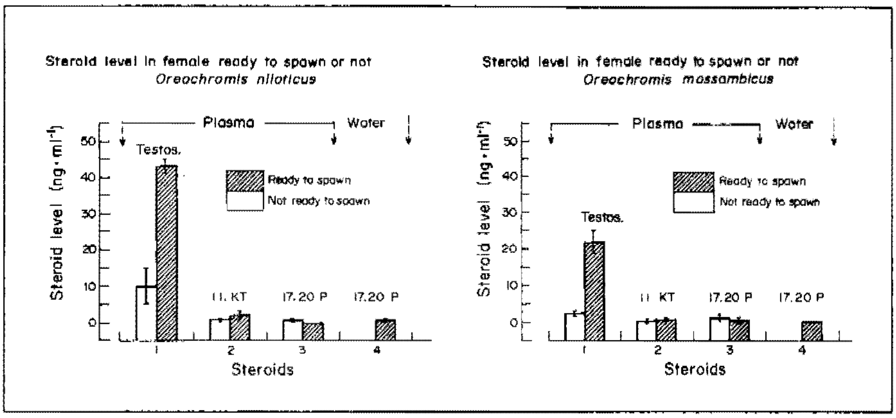

Fig. 3. Comparative study of steroid levels in female of *Oreochromis niloticus* and *O. mossambicus*.

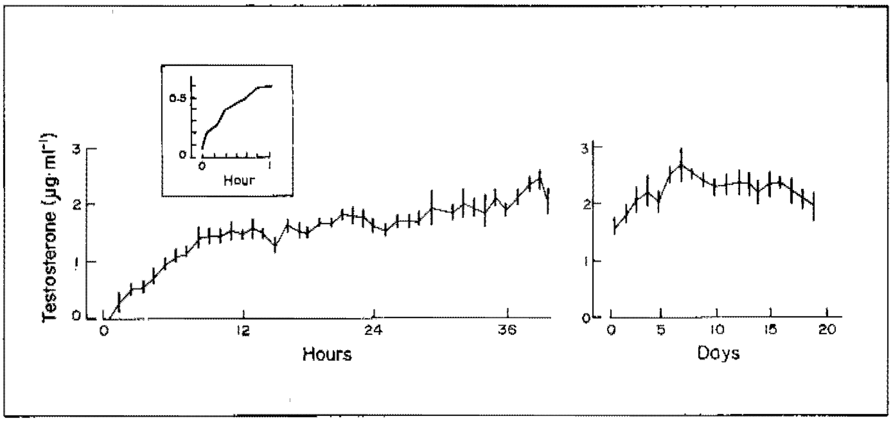

Fig. 4. Dynamics of in vitro release of testosterone implants (0.5 mg T·100 mg^{-1} implant·50 ml^{-1} saline solution).

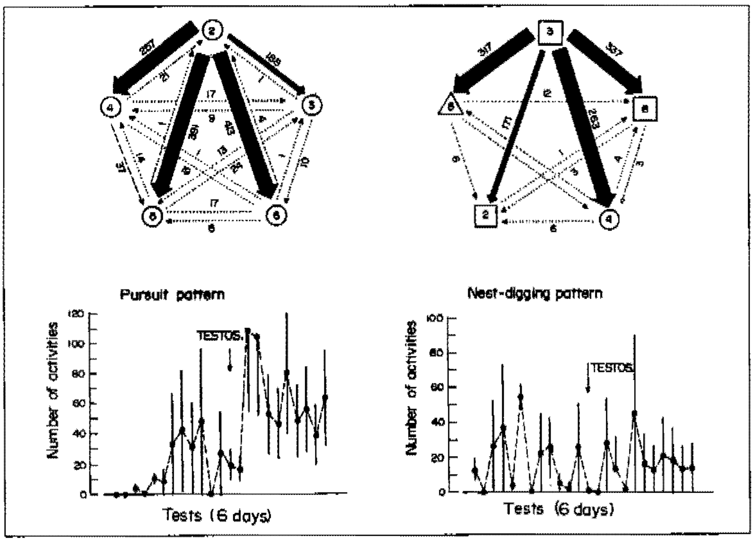

Fig. 5. Effect of implants (testosterone 0.25 mg) on the social behavior of *Oreochromis niloticus*.

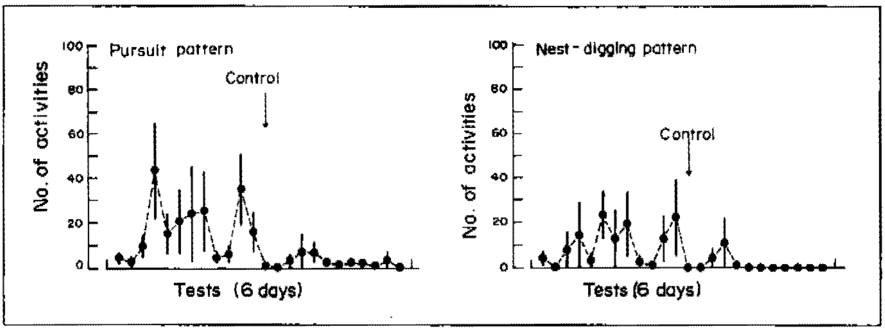

Fig. 6. Effect of implants (control) on the social behavior of *Oreochromis niloticus.*

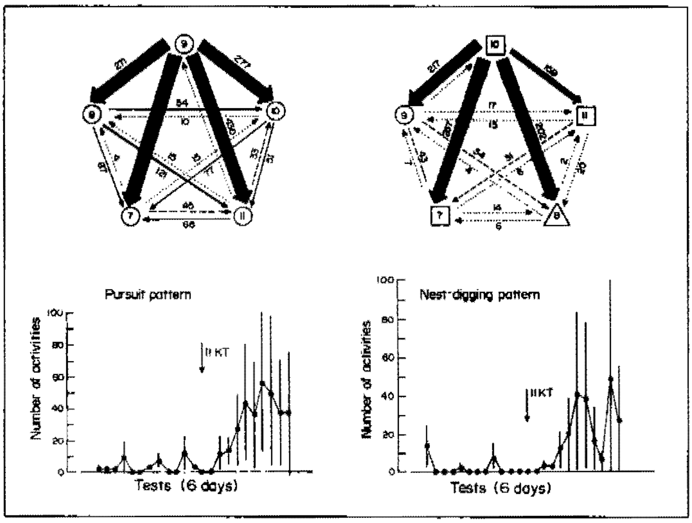

Fig. 7. Effect of implants (11-KT 0.25 mg) on the social behavior of *Oreochromis niloticus.*

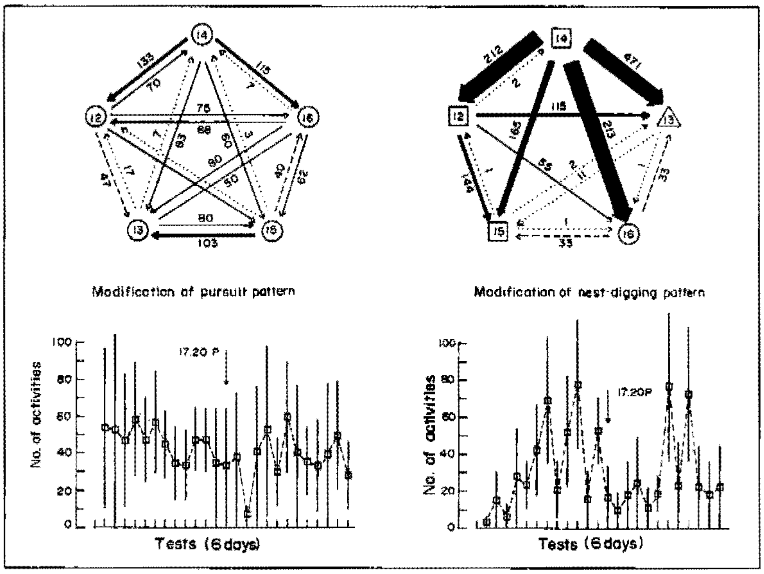

Fig. 8. Effect of implants (17.20P 0.25 mg) on the social behavior of *Oreochromis niloticus.*

Yet, studies by Stacey and Sorensen (1991) have shown that this steroid may act as a pheromone in goldfish (*Carassius auratus*). The different steroids thus appear to have a specific role in the different sequences of behavior involved in the defense of the territory and in the development of the reproductive behavior.

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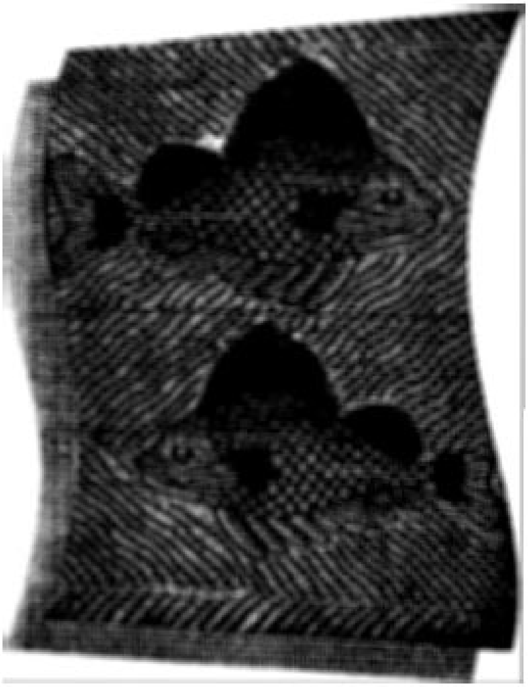

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