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Induction of sexual behavior in male fish (*Rhodeus ocellatus ocellatus*) by amino acids

Review Article

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Summary. A new function of amino acid in fish behavior was found. Amino acids induced sexual behavior in male rose bitterling (*Rhodeus ocellatus ocellatus*). Two types of sexual behavior which were pecking and sperm release were observed. Amino acids are known as feeding stimulants in some fish. The pecking behavior of male fish induced by amino acids is similar to the feeding behavior but it was sexual. Only male bitterling showed pecking and sperm release but the female showed no response to the amino acids. 10 out of 20 amino acids induced sexual behavior and both pecking and sperm release were induced by the same amino acids. These two kinds of behavior changed alternately depending on the light conditions. It is of interest that non-specific material such as some amino acids function like sex pheromone.

Keywords: Amino acids – Sexual behavior – Fish – Sex pheromone – Feeding stimulants

Introduction

A large number of studies on feeding stimulants in fish have been done and it has been reported that some amino acids function as feeding stimulants (Konosu et al., 1968; Car et al., 1977; Adron et al., 1978).

We tested amino acid preference in rose bitterling and found that induced pecking behavior was different from feeding behavior. Induced behavior of the fish by amino acids was examined and role of amino acids in reproductive behavior of rose bitterling was discussed.

Induction of pecking behavior by amino acids

At first, we constructed a bioassay system by using dialysis tubes (Kawabata et al., 1992a). The dialysis tube filled with amino acid solution and a control tube containing distilled water were suspended in the test tank under dark illumination of 2 to 3 luxes. It was observed that the fish approached and showed pecking behavior towards the amino acid containing tube. The position of the pair of tubes were changed at random to avoid the effect of learning of fish on the number of pecking counts. The pecking number were counted three times with each concentration of amino acid.

The number of pecking times increased linearly with the concentration up to 0.1 M, and became constant thereafter. The pecking times for 20 amino acids were counted at the concentration of 0.1 M except the low solubility ones, Trp, Asp and Glu which were used at saturated concentration. Cys, Ser, Ala, Lys and Gly had strong pecking behavior-inducing activity, and Thr, Ile, Gln, Met and Arg had slightly less. Another 10 amino acids had little activity.

To grade these amino acids by inducing activity of the pecking behavior, we introduced a competitive assay. A pair of dialysis tubes containing different amino acid solutions were suspended in the tank together. The activities of the amino acids were graded based on the comparison of pecking counts between two amino acids. The count was performed three times for each combination. Then the grade of five amino acids with high activity was determined as follows, Cys > Ser > Ala > Gly > Lys, and they were placed in the first group. The second group which consisted of Thr, Ile, Gln, Met and Arg could not be graded because of their low activity but all of the amino acids in the second group had lower activity compared with the last grade of the first group Lys.

Through these experiments, the difference of pecking from feeding behavior in some points was noticed by careful observation. The fish did not open their mouths when pecking at the dialysis tube and the movements of the fish during pecking were very slow and gentle compared with their feeding behavior. Then there arose a possibility that the pecking is not a feeding behavior.

Sperm release induced by amino acids

When illuminance was gradually increased, the males began to release sperm against the dialysis tube containing amino acid solution (Kawabata et al., 1992b). The same amino acids which induced pecking also induced sperm release. Especially male bitterling actively displayed sperm release to the five amino acids, which had high pecking-inducing activity.

Pecking behavior was not feeding but sexual

Induction of sperm release in the male rose bitterling by amino acids strongly suggests that the pecking behavior induced by amino acids is not a feeding but sexual. The possibility of pecking behavior resulting from the sexual behavior of male fish was examined (Kawabata et al., 1992b).

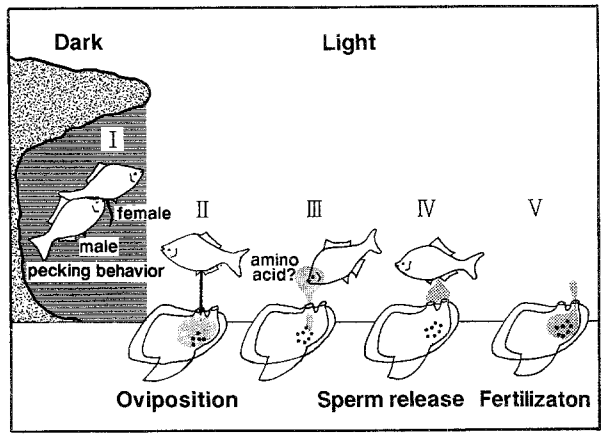

Fig. 1. Model for role of amino acids in reproductive behavior of rose bitterling. Pecking behavior of male fish was induced under dark condition and sperm release was induced under light condition by amino acids. It is suggested that female fish releases amino acids in a series of sexual behavior as signals to tell egg maturation and timing of sperm release to male fish (Reprint from Nippon Suisan Gakkaishi 58: 839–844, Kawabata et al., 1992b)

Induction of the pecking behavior in female fish was tested (Kawabata et al., 1992a). Female fish showed no response to any amino acid-containing tubes. The pecking behavior was not induced in female by amino acids.

The bitterlings at various growth stages were tested (Kawabata et al., 1992b). If the pecking is a feeding behavior, pecking will be observed at all stages of their growth because fish need to feed at all stages. On the other hand, pecking will be observed only in matured individuals if pecking is a sexual behavior. The result was that pecking was not induced in juveniles but in grown individuals.

In the observation through a series of reproductive behavior in rose bitterling, the pecking behavior of male fish to female fish was observed. The male chased the female and pecked lightly at the abdomen from which a short ovipositor was elongating before spawning.

The results of these experiments and observations can be summarized as follows. 1.) The same amino acids induced both pecking behavior and sperm release. 2.) Pecking was not induced in the female. 3.) Amino acids did not induce pecking in immature individuals. 4.) Pecking behavior was observed to be different from feeding behavior 5.) Males pecked females at the abdomen near the ovipositor during reproductive behavior.
females at the abdomen near the ovipositor during reproductive behavior.

Thus it can be concluded that the pecking behavior of male rose bitterlings induced by amino acids is not a feeding behavior but a sexual behavior.

As pecking behavior is similar to feeding behavior, the pecking should not be mistaken for feeding behavior. Even if pecking were induced by amino acids, it should not be taken for simply a feeding behavior. Careful observation is necessary. It is not difficult to confirm whether the behavior which is feeding or sexual. It can be checked by performing bioassay on males and females independently.

Induction of sexual behavior by non-specific materials

Many different amino acids induced sexual behavior in male bitterling (Kawabata et al., 1992a). Structual characteristics and properties of these amino acids differ. In general, pheromone has its own species-specificity which comes from its specific structure. For example, steroid hormone has been identified and known to function as sex pheromone in fish (Stacy and Sorensen, 1986; Kobayashi et al., 1986; Stacy, 1991). Therefore it is not reasonable to think that many amino acids act as analogues of a certain sex pheromone of rose bitterling. The most noticeable finding is that such substances seem not to have a significant specificity but function like sex pheromone. Why do many free amino acids induce sexual behavior? This question can be answered by considering as follows. Many different amino acids may possibly function as signals to induce sexual behavior.

Induced sexual behavior consists of pecking and sperm release. In the former case, amino acids may function to form breeding pairs in reproductive behavior by letting males know the egg maturation in the female. In the latter, they let males know the timing for sperm release. In the stage of immature egg cells, follicular cells are connected to each other through intercellular matrix protein in the ovary, but the egg cells become separated at the time of spawning. Proteins in the intercellular matrix should be hydrolysed by protease(s) during the maturation stage, especially in ovulation (Oshiro and Hibiya, 1975). Thus amino acids or short peptides will be produced as hydrolysate of intercellular matrix proteins and play roles as signals of egg maturation and timing of sperm release.

Effects of illuminance on pecking behavior and sperm release

The pecking behavior of the male bitterling was only induced by amino acids under dark conditions at 2 or 3 luxes. It was also found that sperm release was only induced under light conditions of 50 luxes. According to increase of illuminance, the pecking counts decreased and the number of sperm release increased. These two types of sexual behavior interchanged definitely by changing the light and dark conditions (Kawabata et al., 1992b). The light dependent change of pecking behavior and sperm release possibly comes from the habit of rose bitterling.

Rose bitterling usually prefer dark places to bright places except during spawning, however ovulation occurs at night. Light pecking behavior at the abdominal part of females before spawning was observed under dark conditions. The rose bitterling has the unique nature of spawning into shellfish with ovipositor in the daytime. When spawning is performed into the shellfish in shallow and light places which shellfish inhabit, amino acids or short peptides are possibly released along with the eggs. The amino acids or short peptides are discharged from the exhalent siphon of the shellfish along with water. The male stimulated by them releases sperm toward the exhalent and inhalent siphons of the shellfish. The sperm released enters the shellfish through the inhalent siphon for fertilization.

To conclude, the role of amino acids in the reproductive behavior of rose bitterling was discussed above, based on the character of induced sexual behavior and a series of reproductive behavior. But this is just a start of studies into examine the function of amino acids that induce sexual behavior in fish. There are still a lot of unknown things and many problems still to be investigated. We have just noticed and opened a door for the subject of study on a new role of amino acids in the reproductive behavior of fish.

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