A Possibility of Species Discrimination by Olfaction in the Cyprinid Fish Genus *Tribolodon*

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There is no doubt that chemical emissions may be involved in a wide variety of social interactions in fish (Umezu, 1966; Solomon, 1977; Liley, 1982; Pfeiffer, 1982). Sexual pheromones are especially interesting. Those emitted by ovulating females attract males and stimulate their spawning activity (Tavolga, 1956; Newcombe and Hartman, 1973; Okada et al., 1978; Honda, 1979, 1980, 1982 a, b; Teeter, 1980). Those emitted by mature males in some species also induce the dramatic onset of spawning activity (Okada et al., 1978; Stacey and Hourston, 1982). Not surprisingly those pheromones may play an important role in the discrimination of conspecifics from other species to avoid hybridization (Bloom and Perlmutter, 1978; Honda, 1982a, b).

Such chemical information is received by olfactory receptors in many cases (Pfeiffer, 1982). The olfactory responses can be measured electrophysiologically by recording stimulant-induced waves from the olfactory bulb (Hara, 1973). However, it is not always easy to know the degree of olfactory discrimination by electrophysiological methods. The quantitative method of cross adaptation may be an exceptional method which enables us to measure the degree of discrimination electrophysiologically.

The Far Eastern cyprinid genus *Tribolodon* includes four species. Three of them, *T. hakonensis* (Günther), *T. ezoe* Okada et Ikeda and *T. brandti* (Dybowsky), often coexist with one another and spawn en masse in the same spawning grounds in Hokkaido (Ito, 1975). Although *T. brandti* partially hybridizes with the two other species, most individuals of the three species spawn with conspecifics without hybridizing (Sakai and Hamada, 1985; Sakai, 1987). Mutual recognition of conspecifics may be achieved not only visually but also through olfaction. To ascertain the possibility of olfactory recognition of species, cross adaptation experiments were carried out to measure the degree of olfactory discrimination in *Tribolodon*.

Materials and methods

Material. Olfactory responses were recorded from two male specimens of *T. ezoe* (164.0 and 172.0 mm SL) caught in the Mukawa River, Hokkaido, one week before the experiments were undertaken.

Preparation of stimulating solution. Eggs and male urine were collected from mature spawning runners (listed in Table 1) caught at the same time and locality as the male specimens. Eggs were gently squeezed out of each ovulating female, and care was taken to avoid contamination. The eggs were then washed with a 0.1 mM $CaCl_2$ solution (perfusing solution, see below) of the same weight as the eggs. This egg wash was used as the stock solution for providing the stimulus. Male spawning runners were also squeezed discharging copious amounts of urine before releasing sperm. Each urine sample was stocked individually as an undiluted stimulating solution.

Records of olfactory responses. The two male specimens were weakly anesthetized with urethan and immobilized with an intramuscular injection of D-tubocurarine (about 3 mg/kg body weight). The

Table 1. Standard length (SL) of *Tribolodon* specimens from which the stock stimulating solutions were collected, and the dilution of each stimulating solution.

	Egg wash		Male urine	
	SL (cm)	Dilution of stimulating solution ($\times 10^{-4}$)	SL (cm)	Dilution of stimulating solution ($\times 10^{-4}$)
T. hakonensis	22.1	1.8	25.9	5.0
T. brandti	35.0	1.2	30.6	1.0
T. ezoe 1	23.2	0.8	18.8	1.0
T. ezoe 2	17.1	1.6	17.4	1.0
T. ezoe 3	15.9	2.0		

gills were irrigated with aerated water during the experiment. The stimulant-induced waves (bulber responses) were recorded by sticking a pair of tungsten bipolar electrodes into the olfactory bulb of the fish (Fig. 1). The olfactory tract was cut to eliminate centrifugal influences on the bulb. The responses were amplified through a conventional AC amplifier, passed through an integrator (time constant 0.3 s), and displayed on a pen recorder. The olfactory epithelium was irrigated with a perfusing solution (0.1 mM $CaCl_2$ of pH 6.5) at the flow rate of 20 ml/min. The general operation method followed Hara (1973).

Cross adaptation experiments. In the cross adaptation experiment, one stimulus was first applied to the olfactory epithelium. The bulber response to the stimulus used in the present study was so tonic that it could not easily be lowered to spontaneous levels. The first stimulus was repeatedly given until the response declined to the horizontal level, and then another stimulus was applied secondarily (Fig. 2). The reverse cross adaptation experiment of the two stimuli was then carried out. The peak height of the integrated response was the magnitude of the response. The magnitude of the response to the second stimulus expressed in % of that of the first stimulus was used as the degree of discrimination (100 B/A in

Fig. 2). When the concentrations of the two stimulating solutions were not well balanced, the peak height of the response for the first stimulus was not equalized between the original and the reverse cross
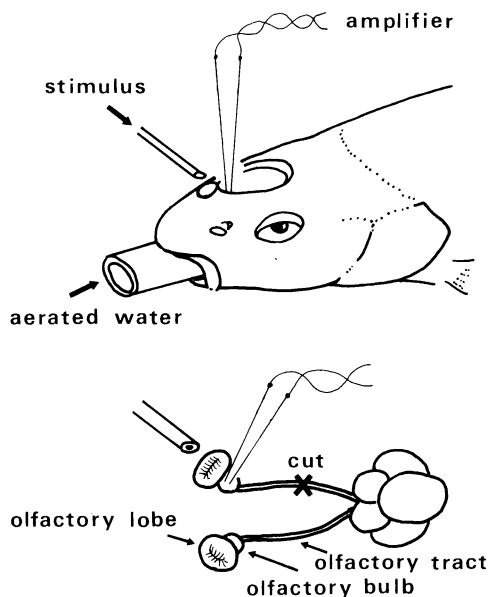

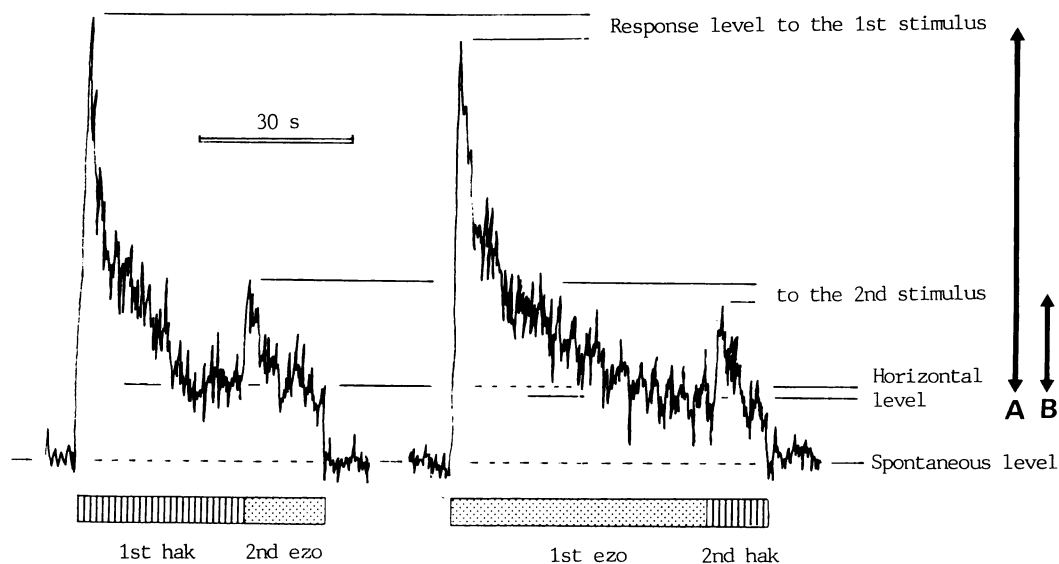Fig. 1. The operation method for recording the olfactory responses. As for practical procedure, see text.

Fig. 2. Typical records of the cross adaptation experiments between egg wash stimuli of two species, *Tribolodon hakonensis* and *T. ezoe*. Left, original, and right, reverse experiments. Bars at the bottom of the records represent the duration of the applications of the stimuli. A, the magnitude of the response to the 1st stimulus; B, the magnitude of the response to the 2nd stimulus; hak, *T. hakonensis*; ezo, *T. ezoe*.

adaptation experiments, and the response to the second stimulus was not obviously recorded in the original or in the reverse cross adaptation experiment. Therefore, it was critical to regulate the concentrations appropriately by diluting the stock solutions with the perfusing solution and checking the response to the second stimulus repeatedly through trial experiments. The regulated concentrations of the stimulating solutions are shown in Table 1.

All the experiments were carried out at 20 ± 1℃.

Results and discussion

Using the egg wash stimuli, the cross adaptation experiments of three interspecific and three intraspecific combinations were carried out on each male specimen, *T. ezoe* No. 1 and No. 2. *T. ezoe* No. 1 was also subjected to the experiments using the urine of four interspecific and one intraspecific combinations. *T. ezoe* No. 2 died of overanesthetization in the midst

Table 2. Degree of discrimination revealed by the cross adaptation experiments in *Tribolodon ezoe* (ezo No. 1 and No. 2). hak, *T. hakonensis*; bra, *T. brandti*; ezo, *T. ezoe*.

Stimuli	Degree of discrimination	
1st–2nd	ezo No. 1	ezo No. 2
Egg wash		
hak–bra	53.2	17.1
bra–hak	27.3	16.7
hak–ezo1	30.9	23.3
ezo1–hak	27.9	33.3
bra–ezo1	21.0	24.3
ezo1–bra	39.7	29.4
ezo1–ezo2	22.2	14.9
ezo2–ezo1	19.5	13.2
ezo1–ezo3	26.2	18.8
ezo3–ezo1	17.0	15.8
ezo2–ezo3	21.7	14.9
ezo3–ezo2	12.7	17.7
Male urine		
hat–bra	42.9	
bra–hak	35.0	
hak–ezo1	45.7	
ezo1–hak	31.0	
hak–ezo2	46.8	
ezo2–hak	69.4	
bra–ezo1	26.2	
ezo1–bra	40.8	
ezo1–ezo2	20.5	
ezo2–ezo1	17.6	

of the experiments before it could be stimulated by the urine. Results are shown in Table 2 in units of the degree of discrimination.

In both specimens, the degree of discrimination observed among the interspecific combinations was much higher than that observed among the intraspecific combinations. The difference in the mean degree of discrimination between inter- and intraspecific combinations in *T. ezoe* No. 1 was statistically significant in the egg wash stimulating experiments (mean 33.3 vs. 19.9, $p < 0.05$) as well as in the urine stimulating experiments (42.2 vs. 19.1, $p < 0.05$). The difference was more significantly supported when the results of the experiments for both stimuli were calculated together (38.4 vs. 19.7, $p < 0.001$). In *T. ezoe* No. 2, the difference of the mean was also significant between inter- and intraspecific combinations (24.0 vs. 15.9, $p < 0.02$). The results clearly indicate that the male fish were able to differentiate between the stimulating solutions of the different species. It is highly probable that chemicals dissolved in the egg wash (ovarian fluids) or in the urine of mature fish are cues (pheromones) which stimulate their reproductive behavior (pointedly reviewed by Liley, 1982, and Pfeiffer, 1982). Moreover, the mature fish must make use of those cues to discriminate between conspecifics and other species to avoid hybridization (Bloom and Perlmutter, 1978; Honda, 1982a, b).

Individual recognition has also been demonstrated to be an aspect of fish olfaction (e.g. Richards, 1974; Liley, 1982; Pfeiffer, 1982). The degree of intraspecific discrimination indicated in the present study may reflect such an ability in the subject fish.

At any rate, the cross adaptation method employed in the present study is considered effective in evaluating the relative degree of species discrimination at the olfactory receptor level. To determine the behavior after discrimination, another experiment such as a Y-maze test may be desirable.

Acknowledgments

Literature cited

Bloom, H. D. and A. Perlmutter. 1978. Possible phero-mone mediated reproductive isolation in two species of cyprinid fishes of the genus *Brachydanio*. J. Fish Biol., 13(1): 47–50.

Hara, T. J. 1973. Olfactory responses to amino acids in rainbow trout, *salmo gairdneri*. Comp. Biochem. Phys-iol., 44A: 407–416.

Honda, H. 1979. Female sex pheromone of the ayu, *Plecoglossus altivelis*, involved in courtship behaviour. Bull. Japan. Soc. Sci. Fish., 45(11): 1375–1380.

Honda, H. 1980. Female sex pheromone of rainbow trout, *Salmo gairdneri*, involved in courtship behaviour. Bull. Japan. Soc. Sci. Fish., 46(9): 1109–1112.

Honda, H. 1982a. On the female sex pheromones and courtship behaviour in the bitterling *Rhodeus ocellatus ocellatus* and *Acheilognathus lanceolatus*. Bull. Japan. Soc. Sci. Fish., 48(1): 43–45.

Honda, H. 1982b. On the female sex pheromones and courtship behaviour in the salmonids, *Oncorhynchus masou* and *O. rhodurus*. Bull. Japan. Soc. Sci. Fish., 48(1): 47–49.

Ito, Y. 1975. Notes on the spawning habits of three·species of genus *Tribolodon*, in Hokkaido. Sci. Rep. Hokkaido Fish Hatch., (30): 39–42. (In Japanese.)

Liley, N. R. 1982. Chemical communication in fish. Can. J. Fish. Aqu. Sci., 39: 22–35.

Newcombe, C and G. Hartman. 1973. Some chemical signals in the spawning behavior of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Bd. Canada, 30(7): 995–997.

Okada, H., D. K. Sakai and K. Sugiwaka. 1978. Chemical stimulus on the reproductive behavior of the pond smelt. Sci. Rep. Hokkaido Fish Hatch., (33): 89–99. (In Japa-nese with English summary.)

Pfeiffer, W. 1982. Chemical signals in communication. Pages 307–326 *in* T. J. Hara, ed. Chemoreception in fishes: development in aquaculture and fisheries science, 8. Elsevier Scientific Publ., Amsterdam.

Richards, I. S. 1974. Caudal neurosecretory system: pos-sible role in pheromone production. J. Exp. Zool., 187: 405–408.

Sakai, H. 1987. Population genetics of hybridization in the genus *Tribolodon*, Cyprinidae. Pages 18–30 *in* N. Mizuno and A. Goto, eds. The freshwater fishes of Japan. Tokai Univ. Press, Tokyo. (In Japanese.)

Sakai, H. and K. Hamada. 1985. Electrophoretic dis-crimination of *Tribolodon* species (Cyprinidae) and the occurrence of their hybrids. Japan. J. Ichthyol., 32(2): 216–224.

Solomon, D. J. 1977. A review of chemical communication in freshwater fish. J. Fish Biol., 11: 363–376.

Stacey, N. E. and A. S. Hourston. 1982. Spawning and feeding behavior of captive Pacific herring, *Clupea har-engus pallasi*. Can. J. Fish. Aqu. Sci., 39: 489–498.

Tavolga, W. N. 1956. Visual, chemical and sound stimuli as cues in the sex discriminatory behavior of the gobiid fish *Bathygobius soporator*. Zoologica, 41: 49–64.

Teeter, J. 1980. Pheromone communication in sea lam-preys (*Petromyzon marinus*): implications for population management. Can. J. Fish. Aqu. Sci., 37: 2123–2132.

Umezu, T. 1966. Behavioral responses of fishes to chemical stimuli. Bull. Japan. Soc. Sci. Fish., 32(3): 252–376. (In Japanese.)

ウグイ属魚類における嗅覚による種識別の可能性

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ウグイ属魚類における嗅覚による種識別の可能性を，交差順応法 (cross adapatation method) により，電気生理学的に調べた．被験魚はエゾウグイで，嗅覚刺激としては，ウグイ，マルタおよびエゾウグイの，排卵雌の卵洗浄液と成魚雄の尿を用いた．その結果，いずれの嗅覚刺激においても，種内に比較して種間の差異を大きく識別し，嗅覚によって種を区別できることが示唆された．今回用いたような嗅覚刺激が，成魚の産卵行動を誘発することはよく知られている．成熟魚が，同時にその情報を，同種の認知に適用していることは，大いにありうることと思われる．

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