誘引および忌避物質混合物のドジョウの行動への影響

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Effects of Attractant and Repellent Mixtures on Behavior of the Oriental Weatherfish *Misgurnus anguillicaudatus*[*1]

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The effects of mixtures of attractants (L-lysine and L-alanine) and repellents (L-glutamic acid, L-cysteic acid, *n*-butyric acid and DL-malic acid) were examined on the behavior of the oriental weatherfish *Misgurnus anguillicaudatus*.

The chemotactic activity of either 100 mM of L-lysine or L-alanine was significantly affected by the addition of more than 10 mM of each repellent. There was no difference in the activity of repellents. Similarly, the activity of 10 mM of L-glutamic acid was affected by the addition of 1, 10 and 100 mM of either L-lysine or L-alanine similar to each other. The same was true for repellents such as L-glutamic acid and DL-malic acid. It seems that the effects of repellents were more significant than those of attractants in the same concentrations.

A large number of feeding attractants and/or stimulants have been identified for various marine organisms from their prey animals.[*3] However, feeding repellents and/or deterrents have attracted little attention from researchers, though prey organisms contain both attractants and/or stimulants, and repellents and/or deterrents in many cases.[*3] Therefore, we attempted to look into the contribution of repellents to overall activity of attractants for the oriental weatherfish *Misgurnus anguillicaudatus*.

This paper deals with the effects of repellents (L-glutamic acid, L-cysteic acid, *n*-butyric acid and DL-malic acid) on the chemotactic activity of attractants (L-lysine and L-alanine) in the oriental weatherfish.

Materials and Methods

Test Animals

Oriental weatherfish (average body length 8.9 cm) were obtained from an aquaculture farm in Shiga prefecture. Forthy (two groups, 20 individuals in each) and sixty individuals were kept in two experimental aquaria. They were fed with formulated eel feed (Nihon Haigoshiryo Co. Ltd.).

Test Samples

Two representative attractants,[1] L-lysine and L-alanine, and four representative repellents,[2] L-glutamic acid, L-cysteic acid, *n*-butyric acid and DL-malic acid were used. Original solutions were prepared at concentrations of 200 mM each. The solutions were, if necessary, adjusted to pH 6.5 by dilute sodium hydroxide or hydrochloric acid. The final volume of test solutions was made up to 7.5 m*l*.

Estimation of Attraction and Repellence Activities

Chemotactic activities were estimated according to the previous papers.[3,4] First of all, the method for estimating the chemotaxis index, attraction and repellence, was as follows. To conduct duplicate experiments at the same time, the aquarium was divided into two chambers, each chamber consisting of one resident and two test compartments. Twenty fishes were put in each resident chamber, and a test sample and dummy were introduced to the test compartment of each chamber. The experiment was repeated by changing the compartment where the test sample and dummy were placed, according to the Latin square. 7.5 m*l* of the test sample was absorbed into a crumpled gauze ball (a sheet, 25×25 cm), and an untreated ball was used as a dummy for control. The gauze ball was placed in the test

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compartment on the opposite side of the resident compartment. Water was continuously introuduced at flow rate of 500 ml/min into each test compartment through the water inlet pipe during experiments. The number of fish entering the test compartment 1 min after application of a test sample was counted. The entering time-course to estimate the chemotaxis index was obtained by integrating each number for ten minutes.

To estimate only the attraction index, the above-mentioned method was slightly modified. Briefly speaking of some different points, the experimental aquarium was composed of one resident and four test compartments. Four test samples containing dummies were applied according to the Latin square. The remaining time-course was obtained by integrating each number with the passage of time.

A logistic curve $y=g/\{1+\exp[-r(x-a)]\}$ was applied to both the entering and the remaining time-courses mentioned above. The fitness of each time-course was checked by a chi-square test, at the 0.100 level. In the former entering time-course, a chemotaxis index Ra (C. I. *Ra*), *i. e.* the ratio of dummy-*a* to sample-*a* was calculated. The C. I. *Ra* shows an attraction index of below 1 and a repellence index of over 1. On the other hand, in the latter remaining time-course, an attraction index *a* (A. I. *a*), *i. e.* coefficient *a*, was estimated.

Results

When 100 mM of L-lysine or L-alanine containing various concentrations of repellents including 1, 10 and 100 mM were tested, chemotactic activity of attractants was found to be significantly affected by the addition of repellents, as shown in Fig. 1. There was no difference in the activity of repellents. In particular, when concentrations of repellents exceeded 10 mM, the activity of attractants diminished to a greater extent. Among the repellents, the effects of *n*-butyric acid was most significant. Next, 10 mM of L-glutamic acid containing various concentrations of either L-lysine or L-alanine were tested. The two results were quite similar to those obtained in the first experiments (Fig. 2). It is noted that the effects of repellents on fish behavior were greater than those of attractants. As shown in Fig. 3, there seemed to be no synergistic or additive effect for either attractant.

Finally, attraction activity was estimated for

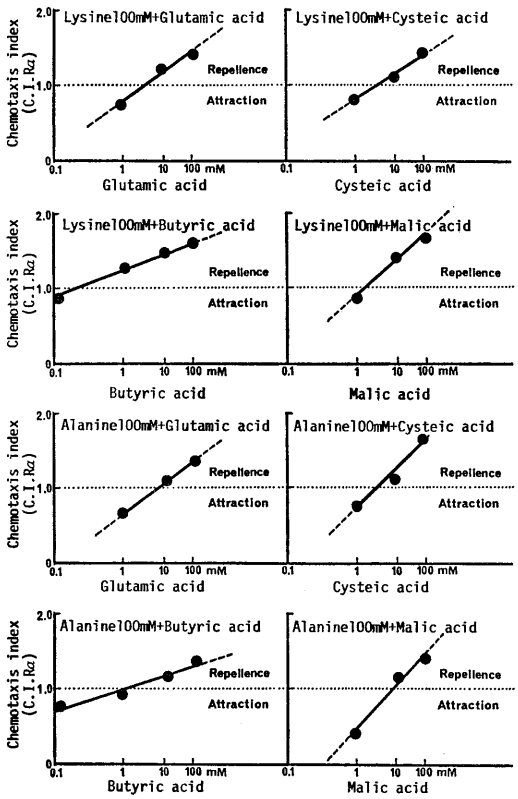

Fig. 1. Chemotactic activity in the mixture of one attractant (constant concentration) and one repellent (variable concentration).

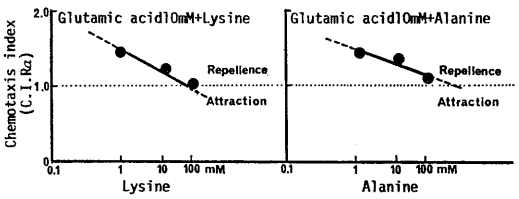

Fig. 2. Chemotactic activity in the mixture of one attractant (variable concentration) and one repellent (constant concentration).

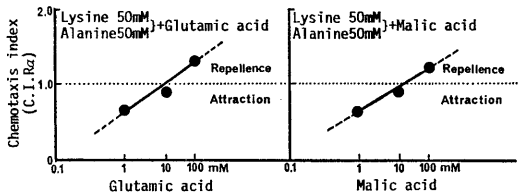

Fig. 3. Chemotactic activity in the mixture of two attractants (constant concentration) and one repellent (variable concentration).

Table 1. Attraction activity in the mixture of attractant (constant concentration) and repellent (variable concentration)

Attractant		+Repellent		Attraction index
(mM)		(mM)		(A.I.*a*)
Dummy				2.0
Lysine	(100)			2.8
Lysine	(100)	+Cysteic acid	(1)	2.0
Lysine	(100)	+Cysteic acid	(10)	1.5
Dummy				2.1
Lysine	(100)			2.9
Lysine	(100)	+Butyric acid	(1)	2.1
Lysine	(100)	+Butyric acid	(10)	1.6
Dummy				1.7
Alanine	(100)			2.1
Alanine	(100)	+Glutamic acid	(1)	1.9
Alanine	(100)	+Glutamic acid	(10)	1.5
Dummy				2.1
Alanine	(100)			3.2
Alanine	(100)	+Malic acid	(1)	2.3
Alanine	(100)	+Malic acid	(10)	2.0

mixtures containing 100 mM of attractant and various concentrations of each repellent. As shown in Table 1, attraction indices agreed well with Fig. 1.

Discussion

From the present experiments, it is obvious that repellents significantly affected the activity of attractants. In natural prey organisms, the attractants L-lysine and L-alanine for oriental weatherfish are contained in relatively large amounts. A naturally repellent amino acid, L-glutamic acid is also present in large concentrations, as shown in Table 2. In some species, L-glutamic acid is contained in higher amounts than are L-lysine or L-alanine.

In fact, the marine worm *Perinereis brevicirrus* and the short-necked clam *Tapes japonica* were not attractants for oriental weatherfish.[14] Furthermore this also supports the validity of the repellence effect of the mixtures of L-lysine and L-glutamic acid, and L-alanine and L-glutamic acid in the two model systems.

According to a review* of feeding activators (attractants, stimulants and so on) and inhibitors (repellents, suppressant and so on) so far, the attractant and/or stimulant activity of L-lysine have been reported for yellowtail *Seriola quinqueradiata*, cichlid *Telapia zillii* and several species of aquatic invertebrate animals, in addition to oriental weatherfish. Similarly the attractant and/or sitimulant activity of L-alanine have also been reported for more than ten species of fresh water fish such as the cichlid in addition to oriental weatherfish, brackish water fishes such as the Japanese eel *Anguilla japonica*, seawater fish such as the yellowtail, and many species of other

Table 2. The contents of representative attraction and repellence amino acids for oriental weatherfish in the feed materials recorded in literatures

Feed materials*	Attraction amino acid		Repellence amino acid
	Lysine	Alanine	Glutamic acid
Aquatic invertebrates		(mM)	
Marine worm-"Isogokai"-*Perinereis brevicirrus*[5]	4.4	32.0	13.3
Squid-"Surumeika"-*Ommastrephes sloani pacificus*[6]	0.6	8.4	1.8
Short-necked clam-"Asari"-*Tapes japonica*[7]	1.7	13.5	6.9
Snow crab-"Zuwaigani"-*Chionoecetes opilio*[8]	1.7	21.0	1.2
Euphausid-"Tsunonashiokiami"-*Euphausia pacifica*[9]	14.0	19.2	0.5
Green sea urchin-"Bafun'uni"-*Strongylocentrotus pulcherrimus*[10]	14.7	29.3	7.0
Aquatic vertebrates			
Sardine-"Maiwashi"-*Sardinops melanosticta*[11]	0.8	2.2	0.8
Common mackerel-"Masaba"-*Scomber japonicus*[12]	1.2	2.5	1.8
Stone flounder-"Ishigarei"-*Kareius bicoloratus*[13]	0.3	2.1	0.5

* Common name-"Japanese name"-Scientific name.

* K. Harada: Feeding activators and inhibitors for aquatic animals. Sugiyama Chemical & Industrial Lab. Ann. Rep., 69–117 (1990).

aquatic invertebrates. On the contrary, the repellent activity of L-alanine has been reported for the Arctic char *Salvelinus alpinus* and the snail *Biomphalaria glabrata*.

On the other hand, L-glutamic acid is effective as a repellent and/or suppressant for the starfish *Asterias rubens*, the lobster *Homarus americanus*, the marine worm *Nereis virens*, and Hydra *Hydra littoralis*, in addition to oriental weatherfish. Acidic compounds containing carboxylic group such as L-glutamic acid serve as a repellent and/or suppressant for many aquatic animals. On the contrary, L-glutamic acid is effective as an attractant and/or stimulant to the cichlid, naked goby *Gobiosoma bosci*, the lobster, octopus *Octopus vulgaris*, and the snail. Accrdingly L-glutamic acid is thought to be a specific compound which serves as an attractant or repellent depending on the species of aquatic animals.

In this connection, it is conceivable that materials which are attractive for all aquatic animals should contain a large amount of L-lysine and L-alanine, and a smaller amount of L-glutamic acid. This finding affords one proof that crustaceans such as the snow crab and the euphausid are the most suitable feed for fish and shellfish.

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