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GENERAL BIOLOGY

Comparison of Taste Perception and Behavior in Two Forms of the Three-Spined Stickleback *Gasterosteus aculeatus, trachurus* and *leiurus*

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A high species specificity of taste preferences and taste behavior of fish has been demonstrated in numerous examples. However, studies on the intraspecific variation of taste perception has not shown considerable differences in the taste spectra of fish of consecutive generations, from different populations, of different sexes, or with different feeding experiences [1, 2]. On the other hand, distinct individual deviations of fish taste responses and their dependence on some external factors have been observed [1, 3]. We compared taste preferences and taste behavior in two morphological/biological forms of the three-spined stickleback *Gasterosteus aculeatus*, *trachurus* and *leiurus*. Our study was the first to demonstrate a pronounced similarity of these forms in taste preferences and taste behavior, despite their difference in the way of life: the *leiurus* form is predominantly freshwater, and the *trachurus* is predominantly marine.

Experiments were performed on mature three-spined stickleback, including seven *trachurus* (mean length, 6.5 cm) caught at the mouth of a small creek during the spawning migration from the sea and nine *leiurus* (mean length, 6.0 cm) caught in an old quarry filled with fresh water and isolated from the sea. The *trachurus* three-spined sticklebacks had, on average, 22–23 lateral plates and a distinct keel on the tail; *leiurus* had no keel and only three to five lateral plates in the anterior part of the body. Both sites where the fish were caught were located near the White Sea Biological Station of Moscow State University (the Bay of Kandalaksha of the White Sea). At the station, the fish were placed together in large tanks filled with fresh water; seven days later, each individual fish was placed into a separate small tank (4 l). Water temperature during the experiments was 11–13°C. The water was partly replaced every two days. The fish were fed with mosquito larva once a day (after the experiments) ad libitum.

During the first one or two days, the fish were taught to catch granules put into the water one at a time. The granules were made out of 2% agar gel containing a water extract of mosquito larva (175 g/l) and Ponceau 4R dye (5 μM). In the experiments, we used granules containing one of the classic taste substances (5% citric acid, 10% sodium chloride, 10% calcium chloride, or 10% sucrose) or one 21 amino acids in a free form (*L* isomers, 0.1–0.001 M). We also used control granules (containing the dye but not taste substances) in each experiment. The data recorded in each experiment were the following: whether a granule was eaten or rejected, how many times in a row the fish caught the granule, and how long the fish held the granule in the mouth after catching it for the first time and in total after all successive catches. Granules with different taste irritators were given in random order. Granules that the fish rejected or did not catch were removed from the tank immediately after the experiment. The experimental procedure, preparation of the gel, the conditions of its storage, and the calculation of the gustatory attractiveness index are described elsewhere [2, 4].

We performed a total of 4320 experiments, including 1890 experiments with the form *trachurus* and 2430 experiments with the form *leiurus*. The significance of difference from the control was estimated using the χ^2 test (granule consumption) and Student's *t* test (duration of holding the granule and the number of catches of the same granule). Correlations were estimated by the nonparametric Spearman's rank-order correlation coefficient (r_S).

Citric acid was the most effective gustatory irritator for *G. aculeatus* among the classic taste substances studied. The consumption of granules containing citric acid was as high as 62.9% in *trachurus* and 40.0% in *leiurus*, which was considerably higher than that for control granules (10.0 and 1.1%, respectively). Sucrose

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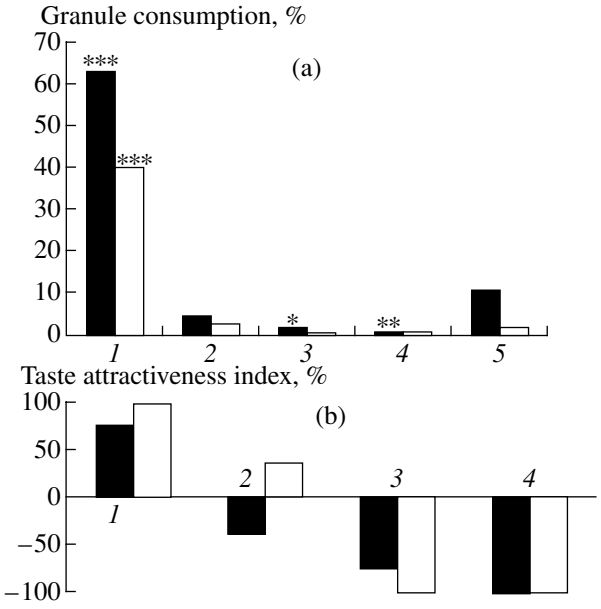

Fig. 1. (a) Consumption of granules containing classic taste substances and (b) the indices of gustatory attractiveness of these substances for two morphological/biological forms of *G. aculeatus.* Solid and open bars show data on *trachurus* and *leiurus*, respectively. Taste substances: *1*, citric acid (5%); *2,* sucrose (10%); *3*, sodium chloride (10%); *4*, calcium chloride (10%); *5*, control. One, two, or three asterisks indicate a significant difference from the control value ($p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively).

was an indifferent gustatory irritator for both forms of *G. aculeatus*: its inclusion into granules had no significant effect on their consumption. Calcium chloride and sodium chloride significantly decreased the consumption of granules by the form *trachurus* but did not cause noticeable change in the consumption of granules by *leiurus* (Fig. 1).

None of the 21 amino acids tested had a deterrent effect. Most of them (18 and 17 in experiments with *trachurus* and *leiurus* forms of *G. aculeatus*, respectively) were indifferent. Cysteine, glutamic acid, and aspartic acid caused a significant increase in granule consumption by both forms of *G. aculeatus*, and glutamine had this effect only on *leiurus* (table).

The taste behavioral responses proper to granules containing classic taste substances and free amino acids were also similar in the two forms of *G. aculeatus.* All the fish had rejected and caught every granule, including a control one, many times before they finally swallowed or rejected it. The number of repetitive catches was the largest in the experiments with the substances that were attractive for the fish. On average, the fish from the forms *trachurus* and *leiurus* successively caught each granule containing citric acid five and six times, respectively, during an experiment. In experiments with amino acids, this parameter varied from 1.9 to 1.6 for *trachurus* and from 5.0 to 4.8 for *leiurus* and was correlated with the consumption of granules ($r_S = 0.90$, $p < 0.001$ and $r_S = 0.80$, $p < 0.001$, respectively).

The duration of holding the granule, another parameter of the fish taste response, varied widely but within similar ranges. In experiments with amino acids, *trachurus* held the granule after they caught it for the first time for 1.3–3.9 s, and *leiurus*, for 1.5–5.9 s. The summary duration of holding the granule in the mouth during an experiment varied, respectively, from 2.4 to 14.3 and from 2.2 to 10.7 s. Both parameters were closely related to the gustatory attractiveness of the granules: in the *G. aculeatus* form *trachurus*, the coefficients of correlation of the duration of holding the granule after the first catch and the summary duration of holding it with the granule consumption were 0.90 ($p < 0.001$) and 0.95 ($p < 0.001$), respectively; in the *G. aculeatus* form *leiurus*, these correlation coefficients were 0.91 ($p < 0.001$) and 0.90 ($p < 0.001$), respectively.

These results show that the taste preferences of the two *G. aculeatus* forms, *trachurus* and *leiurus*, are similar to each other. Citric acid is the most attractive for both of them; their attitude to the tastes of various amino acids was also almost the same: *G. aculeatus* perceived most of these substances as gustatorily inert and displayed taste preference for only some of them. The positive correlation between the amino acid preference spectra of the *trachurus* and *leiurus* forms was weak ($r_S = 0.60$, $p < 0.01$), probably, because the fish altogether rejected granules containing many of amino acids (14 and 12 amino acids in experiments with *trachurus* and *leiurus*, respectively). The absence of deterrent amino acids, a large number of indifferent amino acids, and a narrow spectrum of gustatorily preferred amino acids are also common characteristics of taste reception of the compared forms of *G. aculeatus.*

The attitude to the tastes of some substances studied was the same in *G. aculeatus* and in other species of fish studied earlier. For example, citric acid is also gustatorily attractive for *Cyprinus carpio, Salvelinus alpinus erythrinus, Tinca tinca, Salmo trutta, Poecilia reticulata*, and *Pungitius pungitius.* Many of these fish, like *G. aculeatus*, display gustatory preference for cysteine, aspartic acid, and glutamic acid [1–10]. However, *G. aculeatus* differs from other species studied in the attitude to the tastes of most substances, including both free amino acids and classic taste substances. The widths and compositions of gustatory spectra, as well as the ratios between positive, negative, and indifferent substances are different for these species. For example, for many fishes, unlike *G. aculeatus*, deterrent amino acids, sometimes numerous, exist. Amino acids attractive for *Oncorhynchus keta, P. pungitius*, and some other species are considerably more numerous than those attractive for *G. aculeatus* [2, 5]. The taste behavior per se of *G. aculeatus* also substantially differs from that of other fishes.

Thus, we may conclude that different morphological/biological forms of *G. aculeatus, leiurus* and *tra-*

Consumption of agar granules containing free amino acids by the *G. aculeatus* forms *trachurus* and *leiurus*

Amino acid	Concentration, M	Consumption of granules, %	
		trachurus	*leiurus*
Cysteine	0.1	82.9 ± 4.5***	54.4 ± 5.3***
Glutamine	0.1	2.9 ± 2.0	17.8 ± 4.1***
Valine	0.1	2.9 ± 2.0	0
Threonine	0.1	2.9 ± 2.0	0
Arginine	0.1	0	0
Proline	0.1	0	0
Alanine	0.1	0	1.1 ± 1.1
Serine	0.1	0	0
Asparagine	0.1	0	1.1 ± 1.1
Phenylalanine	0.1	0	0
Methionine	0.1	0	2.2 ± 1.6
Histidine	0.1	0	0
Norvaline	0.1	0	0
Lysine	0.1	0	1.1 ± 1.1
Glycine	0.1	0	0
Glutamic acid	0.01	41.4 ± 5.9***	34.4 ± 5.0***
Aspartic acid	0.01	40.0 ± 5.9***	38.9 ± 5.2***
Isoleucine	0.01	1.4 ± 1.4	4.4 ± 2.2
Leucine	0.01	0	0
Tryptophan	0.01	0	0
Tyrosine	0.001	0	0
Control	–	0	2.2 ± 1.6

*** Significant difference from the control ($p < 0.001$).

churus, are similar to each other in taste perception and taste behavior. The results obtained confirm the assumption that taste preferences of fish are a species-specific trait, which is common for fish of different sexes and from different generations and populations [1, 10].

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