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Mucous Trail Following in 2 Intertidal Nudibranchs

Yasuhiro NAKASHIMA
Department of Zoology, Faculty of Science, Kyoto University, Kyoto 606-01, Japan

Gastropods are generally known to be able to follow mucous trails. They follow the trails of their own for homing (McFarlane 1981; Chelazzi et al. 1987), of the same species for mating (Townsent 1974) or of other species for predation (Paine 1963; Gonor 1967). But according to Cook (1977), "trail following may be widespread [but] it need not necessarily be a general feature of a gastropod's normal behavioural repertoire." Trail-following behavior in opisthobranchs, especially for mating, has been referred to in only a few studies (Hadfield & Switzer-Dunlap 1984) and not certified experimentally. For example, *Stylocheilus longicauda* (Switzer-Dunlap & Hadfield 1979) and *Bursatella leachii pleii* (Lowe & Turner 1976) were observed to follow conspecific trails in the field. Todd (1979) considered that *Onchidoris bilamellata* might find mates by following trails, but the results of his laboratory tests were inconclusive.

Dendrodoris nigromaculata and *D. nigra* are common sponge feeders in the intertidal zone of southern Japan. They are annual species, as is common among nudibranchs, and are sometimes observed in aggregations during the spring mating season. As nudibranchs are simultaneous hermaphrodites, they need not distinguish the sex of other individuals for mating. In the present study, I examined whether they utilize water-borne substances of the same species or mucous trails to find their mate.

Materials and Methods

I conducted the laboratory experiments at the Seto Marine Biological Laboratory, Shirahama, Japan, from April to June 1978. During this period *Dendrodoris nigromaculata* and *D. nigra* were seen copulating and laying eggs at the intertidal rocky shore near the laboratory. I captured the nudibranchs that were found singly and not laying eggs. The nudibranchs were kept singly in 1000-ml plastic cups. They were not fed, and were utilized for the experiments between the 2nd and 7th day from the collection. I replaced the sea water in the cup every day. No individual was used repeatedly in each experiment.

The test apparatus (hereafter referred to as the Y-maze) was a Y-shaped open passage made of acryl resin, a modification of the Y-maze described by Davenport (1950). It consisted of a 12-cm stem and 2 perpendicular 12-cm arms. The stem and the arms were 3 cm in height. Each arm was connected to a 3000-ml plastic cup filled with filtered sea water or test solution. The water or solution in the cup was siphoned into the arms 1 min after an experimental animal was introduced to the stem. Then the test started and lasted for 10 min. About half of the water had flowed out by the end of the test. Animals that did not enter either arm of the Y completely were recorded as no choice.

In experiment 1, a control experiment, filtered sea water was siphoned to both arms. This was to determine whether the nudibranchs exhibited a preference to either arm of the Y-maze. In experiment 2, the test solution was siphoned to either the left or right arm and sea water to the opposite arm. Three small cups of the sea water, in which the nudibranchs of the same species as the test animal had been kept for a day, were filtered and gathered into a large cup to use as test solution. A conspecific (in experiments 3 and 5) or heterospecific (in experiment 4) mucous trail was prepared by the following procedure. After blocking an arm of the Y-maze with a glass microscope slide, filtered sea water was siphoned to the open arm and a nudibranch was positioned so as to crawl the whole length of the stem and the unblocked arm. When a test animal in experiment 1 crawled to the end of an arm without entering the other arm, the Y-maze was also utilized in experiments 3 or 4. Each experiment started within 10 min after removing the 1st nudibranch. Filtered sea water was siphoned to both arms in experiments 3 and 4, sea water to the arm with the mucous trail and the test solution to the other arm in experiment 5. The Y-mazes used for experiments were thoroughly washed and rinsed in clean running sea water for a day.

Results and Discussion

The results of the experiments are shown in Table 1. Both species did not show any significant preference for either arm in experiment 1 or any other experiments (Fisher's exact probability test; $P=.803$ in experiment 1 of *D. nigromaculata*; $P=.352$ in experiment 4 of *D. nigra*; $P>.999$ in all other experiments). In experiment 2, the test solution, which was assumed to contain water soluble chemical substances of the conspecific individuals, had no effect on the behavior of the test animals (comparison of experiments 1 and 2 by χ^2 test; $P=.806$ in *D. nigromaculata*, $P=.888$ in *D. nigra*). In experiment 3, the test animals of both species significantly preferred to enter the arm with a conspecific trail (comparison of with and without trail by

Table 1 Results of Y-maze experiments. Statistical analyses are shown in the text. (con trail=conspecific trail, hetero trail=heterospecific trail)

a) *Dendrodoris nigromaculata*

Exp. number	Left arm	Right arm	N	choice		
				L	R	No choice
1	sea water	sea water	53	17	14	22
2	test solution	sea water	30	8	10	12
	sea water	test solution	30	8	8	14
3	con trail	no trail	20	16	1	3
	no trail	con trail	20	1	15	4
4	hetero trail	no trail	20	8	6	6
	no trail	hetero trail	20	7	9	4
5	con trail	hetero trail	15	12	0	3
	hetero trail	con trail	15	0	15	0

b) *Dendrodoris nigra*

Exp. number	Left arm	Right arm	N	choice		
				L	R	No choice
1	sea water	sea water	40	6	5	29
2	test solution	sea water	20	3	4	13
	sea water	test solution	20	3	3	14
3	con trail	no trail	28	16	5	7
	no trail	con trail	28	3	13	12
4	hetero trail	no trail	20	2	6	12
	no trail	hetero trail	22	5	6	11

Fisher's exact probability test; P<.0001 in *D. nigromaculata*, P=.015 in *D. nigra*). In experiment 4, the test animals of both species did not show significant preference for the heterospecific trail (comparison of with and without trail by Fisher's exact probability test; P=.617 in *D. nigromaculata*, P=.751 in *D. nigra*). In experiment 5, the test solution had no effect on the behavior of the test animals (comparison of experiments 3 and 5 by Fisher's exact probability test; P=.497 in *D. nigromaculata*).

D. nigromaculata entered either arm significantly more often than *D. nigra*, when there was no conspecific trail in the Y-maze (comparison of entering either arm and no choice by Fisher's exact probability test; P<.01 in experiments 1 and 4, P=.024 in experiment 2). When there was a conspecific trail, significant difference was not detected between the behavior of both species because the majority of test animals entered the arm with a trail (Fisher's exact probability test; P=.103 in experiment 3). Heterospecific trails did not affect the mobility of the test animals in either species (comparison of experiments 1 and 4 by Fisher's exact probability test; P=.124 in *D. nigromaculata*, P=.113 in *D. nigra*).

The test animals of both species distinguished conspecific and heterospecific mucous trails and followed only the former. Several animals turned back in the Y-maze without entering either arm and finally returned to the starting point in experiments 1 and 2. In contrast, no animal was observed to turn back in experiment 3. This suggests that both nudibranchs can detect directional information of the trail as does *Ilyanassa obsoleta* (Trott & Dimock 1978) or *Onchidium verruculatum* (McFarlane 1981), although it was not certified experimentally. *Aplysia californica* is reported to release water-borne pheromones during and just after oviposition and becomes attractive to other individuals (Audesirk 1977; Pennings 1991). *Dendrodoris* spp. did not react to the water soluble substances from nonlaying individuals in experiment 2. It is still probable that egg laying *Dendrodoris* spp., especially *D. nigromaculata* (which was less mobile when there was no chemical stimulus), releases chemical substances that facilitate trail-searching behavior in conspecific animals.

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