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RAPID COMMUNICATION

Chemical Composition of Inks of Diverse Marine Molluscs Suggests Convergent Chemical Defenses

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Abstract Some marine molluscs, notably sea hares, cuttlefish, squid, and octopus, release ink when attacked by predators. The sea hare *Aplysia californica* releases secretions from the ink gland and opaline gland that protect individuals from injury or death from predatory spiny lobsters through a combination of mechanisms that include chemical deterrence, sensory disruption, and phagomimicry. The latter two mechanisms are facilitated by millimolar concentrations of free amino acids (FAA) in sea hare ink and opaline, which stimulate the chemosensory systems of predators, ultimately leading to escape by sea hares. We hypothesize that other inking molluscs use sensory disruption and/or phagomimicry as a chemical defense. To investigate this, we examined concentrations of 21 FAA and ammonium in the defensive secretions of nine species of inking molluscs: three sea hares (*Aplysia californica*, *Aplysia dactylomela*, *Aplysia juliana*) and six cephalopods (cuttlefish: *Sepia officinalis*; squid: *Loligo pealei*, *Lolliguncula brevis*, *Dosidicus gigas*; octopus: *Octopus vulgaris*, *Octopus bimaculoides*). We found millimolar levels of total FAA and ammonium in these secretions, and the FAA in highest concentration were taurine, aspartic acid, glutamic acid, alanine, and lysine. Crustaceans and fish, which are major predators of these molluscs, have specific receptor systems for these FAA. Our chemical analysis supports the hypothesis that inking molluscs have the potential to use sensory disruption and/or phagomimicry as a chemical defense.

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Introduction

Animals use a variety of defenses against predators, among them chemical defenses. Marine molluscs, including sea hares, squid, octopus, and cuttlefish, have a striking defensive behavior—releasing ink when attacked. Unlike cephalopods, sea hares often simultaneously release secretions from two glands, the ink and opaline glands (Johnson and Willows 1999). As the cephalopod ink sac is not homologous with the sea hare secretory glands, any functional similarities in the secretions would be the result of convergent evolution.

Sea hare secretions function against predators in at least three ways: chemical deterrence, sensory disruption, and phagomimicry. Chemical deterrents, which are typically unpalatable or toxic, inhibit feeding of predators on sea hares (Johnson and Willows 1999; Kicklighter and Derby 2006). Sensory disruption results from secretions massively stimulating the predator's chemosensory systems, preventing normal function, which leads to confusion and cessation of attack by the predator (Kicklighter et al. 2005). Phagomimicry results from secretions stimulating the predator's sensory pathways involved in feeding, thus causing the predator to attend to the secretions as if they were food and thus affording the sea hare an opportunity to escape (Johnson 2002; Kicklighter et al. 2005). The active compounds involved in sensory disruption and phagomimicry include free amino acids (FAA) and ammonium, which are extraordinarily concentrated in ink and opaline of the sea hare *Aplysia californica* and are powerful stimulants of the chemosensory neurons and feeding behavior of the spiny lobster *Panulirus interruptus*, a sympatric predator (Kicklighter et al. 2005).

Cephalopod inks function as anti-predatory visual stimuli, either as "smoke screens" or distracting decoys (Caldwell 2005). Some have proposed that cephalopod inks may contain compounds that disrupt a predator's chemical senses, but evidence is fragmentary or anecdotal (Caldwell 2005).

Our hypothesis is that inks from diverse cephalopods serve not only as visual defenses but also as chemical defenses against a diversity of predators, through sensory disruption, phagomimicry, or other mechanisms similar to those elucidated for *A. californica* against *P. interruptus*. As a first test, we analyzed FAA composition of the inks of three species of sea hares and six species of cephalopods, and compared these with FAA in typical food of predators of molluscs.

Methods and Materials

Aplysia californica individuals were field-collected in California (Marinus Scientific Inc., Garden Grove, CA, USA). Secretions from the ink and opaline glands were collected and pooled from one set of 15 individuals, and from a second set of 10 animals. Hemolymph from *A. californica* was collected from five animals. Mucus was collected from the skin of five animals. Ink and opaline glands were also collected from five *A. californica* raised in the NIH/University of Miami National Resource for *Aplysia* Facility and fed the red alga

Gracilaria ferox. The ink and opaline glands were taken from 10 *Aplysia dactylomela* collected in the Florida Keys. The opaline glands were taken from eight *Aplysia juliana* collected in waters off New Zealand.

Ink collected from the ink sacs of the following squid species were analyzed: three *Lolliguncula brevis* (collected near Galveston, TX, USA); three *Loligo pealei* (collected near Woods Hole, MA, USA); and one *Dosidicus gigas* (collected in California). In addition, ink was collected from the ink sacs of two *Octopus bimaculoides* (National Resource Center for Cephalopods) and one *Octopus vulgaris* (collected in waters near St. Augustine, FL, USA). Ink from three cuttlefish *Sepia officinalis* (collected near Galveston, TX) was also analyzed.

All species were collected in the field, except for the one group of *A. californica* raised in the NIH *Aplysia* facility, and *S. officinalis* cultured at the National Resource Center for Cephalopods. All animals, except for *L. brevis*, were adults and were alive until immediately before collection of the tissue or secretions. Sea hares were cooled until immobilized and then injected with isotonic magnesium chloride. Cephalopods were anesthetized by cooling on ice. Animals were dissected to remove the tissues of interest, which were frozen until used. Frozen samples were thawed, and secretions were collected. Ink and opaline were collected as previously described (Kicklighter et al. 2005; Kicklighter and Derby 2006). Hemolymph was collected from sea hares by first de-inking the animals to avoid contamination of hemolymph with ink and opaline, cold anesthetizing them, washing an area of skin between the rhinophores with 70% ethanol, making a small incision in that region, and collecting the released hemolymph into a sterile tube. Mucus was collected from sea hares by gently scraping the surface of the skin with a blunt probe. Secretions or tissue collected from multiple animals were pooled. Frozen samples were lyophilized and stored until used, at which time they were diluted in deionized water. Proteins were removed from the samples by dialysis. Concentrations of FAA and ammonium in the samples were analyzed by using a Beckman Model 6300/7300 amino acid analyzer by Scientific Research Consortium, Inc. (http://www.aminoacids.com). This system uses an ion exchange column, post-column ninhydrin reaction at 131°C, followed by absorbance measurements at 440 and 570 nm. (*S*)-2-Aminoethyl-1-cysteine and glucosaminic acid were used as internal standards. Data were acquired and managed with Beckman System Gold 8.10 chromatography software.

Multidimensional scaling (MDS) was used to examine the degree of similarity in the FAA composition of molluscan secretions and fluids, and food tissues. Our analysis used the proportional (%) compositions of the 20 secretions in Table 1 plus an equidose mixture (E). E, which contains the same 21 FAA as the mixtures in Table 1 but has all FAA at the same relative concentration (4.76), was included in this analysis for comparison. MDS was performed by using SPSS-X software and Euclidean values as a distance measure. The dimensionality of an acceptable MDS solution is the minimum that produces a stress value <0.10 (Kruskal and Wish 1978). In our analysis, a 3-dimensional solution produced a stress value of 0.072 and was thus accepted.

Results and Discussion

Sensory disruption requires that a secretion has the type and amount of FAA sufficient to strongly stimulate the sensory pathways of predators, although not necessarily mimicking the composition of food. Phagomimicry requires that a secretion's absolute and relative

Table 1 Chemical composition of molluscan secretions and other tissues[a]

Chemical in μM[a]	*A. californica* Wild opaline #1 So1	*A. californica* Wild opaline #2 So2	*A. californica* Wild ink #1 Si1	*A. californica* Wild ink #2 Si2	*A. californica* Mariculture ink Si3	*A. californica* Hemolymph Sf1	*A. californica* Mucus Sf2
Taurine	231,200 (74.5)	221,000 (54.7)	7,830 (52.9)	5,994 (49.3)	1,743 (86.0)	194 (27.1)	90 (15.9)
L-Aspartic acid	2,512 (0.8)	11,222 (2.8)	2,231 (15.1)	1,636 (13.5)	62 (3.1)	8 (1.1)	84 (14.9)
L-Threonine	236 (0.1)	182 (0)	193 (1.3)	173 (1.4)	3 (0.2)	10 (1.4)	6 (1.0)
L-Serine	68 (0)	270 (0.1)	214 (1.4)	123 (1.0)	7 (0.4)	13 (1.8)	26 (4.6)
L-Asparagine	41 (0)	0 (0)	51 (0.3)	0 (0)	1 (0.1)	6 (0.8)	4 (0.7)
L-Glutamic acid	1,616 (0.5)	3,970 (1.0)	1,166 (7.9)	1,394 (11.5)	27 (1.3)	4 (0.6)	14 (2.5)
L-Glutamine	9 (0)	0 (0)	121 (0.8)	72 (0.6)	6 (0.3)	17 (2.4)	8 (1.4)
L-Proline	7 (0)	122 (0)	131 (0.9)	124 (1.0)	8 (0.4)	0 (0)	2 (0.4)
L-Glycine	791 (0.3)	3,390 (0.8)	181 (1.2)	170 (1.4)	62 (3.1)	31 (4.3)	164 (29.1)
L-Alanine	339 (0.1)	416 (0.1)	1,024 (6.9)	879 (7.2)	21 (1.0)	64 (9.0)	29 (5.1)
L-Valine	56 (0)	328 (0.1)	301 (2.0)	189 (1.6)	15 (0.7)	10 (1.4)	12 (2.1)
L-Cystine	16 (0)	62 (0)	84 (0.6)	54 (0.4)	2 (0.1)	3 (0.4)	9 (1.6)
L-Methionine	35 (0)	102 (0)	122 (0.8)	29 (0.2)	6 (0.3)	4 (0.6)	1 (0.1)
L-Isoleucine	96 (0)	0 (0)	135 (0.9)	151 (1.2)	1 (0)	3 (0.4)	1 (0.1)
L-Leucine	10 (0)	160 (0)	327 (2.2)	144 (1.2)	4 (0.2)	6 (0.8)	2 (0.4)
L-Tyrosine	14 (0)	74 (0)	297 (2.0)	142 (1.2)	13 (0.6)	3 (0.4)	84 (15.0)
L-Phenylalanine	648 (0.2)	0 (0)	130 (0.9)	39 (0.3)	1 (0.1)	8 (1.1)	1 (0.1)
L-Tryptophan	0 (0)	0 (0)	5 (0)	0 (0)	0	1 (0.1)	0 (0)
L-Lysine	65,190 (21.0)	145,320 (35.9)	0 (0)	2 (0)	1 (0.1)	20 (2.8)	9 (1.6)
L-Histidine	7,185 (2.3)	17,237 (4.3)	255 (1.7)	844 (6.9)	20 (1.0)	14 (2.0)	15 (2.7)
L-Arginine	340 (0.1)	512 (0.1)	0 (0)	0 (0)	23 (1.1)	296 (41.4)	2 (0.4)
Total (TFAAs) μM	310409	404366	14798	12159	2026	715	563
Total (TFAAs) mM	310.4	404.4	14.8	12.2	2.0	0.7	0.6
Ammonium (μM)	6,810	5,336	24,360	32,305	15,180	272	2,852

concentrations of FAA adequately mimic that of the predators' food. Both mechanisms are supported by our chemical data.

The concentrations of FAA in ink and opaline of wild-caught sea hares are in the high millimolar range (Table 1). Opalines of wild-caught *A. californica* and *A. dactylomela* have the highest concentrations, 310–448 mM total FAA (TFAA), and opaline of *A. juliana* has 3.2 mM TFAA. Inks of wild-caught *A. californica* and *A. dactylomela* have 12–15 mM TFAA. Ink of maricultured *A. californica* contains about 15% of the TFAA in wild-caught animals, but the relative amounts of FAA acids are similar. Ink or opaline from two groups

Table 1 (continued)

A. dactylomela Opaline	*A. dactylomela* Ink	*A. juliana* Opaline	*Sepia officinalis* Ink	*Loligo pealei* Ink	*Lolliguncula brevis* Ink	*Dosidicus gigas* Ink
So3	Si4	So4	C1	C2	C3	C4
215,350 (48.1)	4,900 (41.3)	1,384 (43.2)	2,088 (82.1)	1,264 (75.5)	274 (60.2)	1,773 (83.9)
21,300 (4.8)	1,388 (11.7)	52 (1.6)	32 (1.3)	30 (1.8)	15 (3.3)	14 (0.7)
186 (0)	177 (1.5)	31 (1.0)	17 (0.7)	15 (0.9)	10 (2.2)	8 (0.4)
189 (0)	138 (1.2)	38 (1.2)	20 (0.8)	15 (0.9)	9 (2.0)	11 (0.5)
0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
83,800 (18.7)	1,780 (15.0)	469 (14.6)	135 (5.3)	66 (3.9)	26 (5.6)	71 (3.4)
0 (0)	104 (0.9)	0 (0)	2 (0.1)	4 (0.3)	1 (0.2)	6 (0.3)
170 (0)	159 (1.3)	28 (0.9)	49 (1.9)	107 (6.4)	8 (1.8)	37 (1.8)
2,554 (0.6)	735 (6.2)	47 (1.5)	6 (0.2)	8 (0.5)	4 (0.8)	26 (1.2)
2,063 (0.5)	859 (7.2)	139 (4.3)	134 (5.3)	88 (5.3)	59 (12.9)	43 (2.0)
312 (0.1)	273 (2.3)	33 (1.0)	30 (1.2)	21 (1.2)	14 (3.0)	11 (0.5)
14 (0)	250 (2.1)	2 (0.1)	0 (0)	5 (0.3)	0 (0)	0 (0)
17 (0)	323 (2.7)	15 (0.5)	0 (0)	1 (0)	0.4 (0.1)	14 (0.7)
118 (0)	108 (0.9)	20 (0.6)	10 (0.4)	15 (0.9)	9 (1.9)	7 (0.3)
188 (0)	195 (1.6)	29 (0.9)	17 (0.7)	29 (1.7)	20 (4.4)	12 (0.6)
71 (0)	0 (0)	14 (0.4)	0 (0)	0 (0)	0 (0)	0.7 (0)
75 (0)	343 (2.9)	13 (0.4)	0 (0)	3 (0.2)	4 (1.0)	2 (0.1)
496 (0.1)	18 (0.2)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
118,850 (26.6)	110 (0.9)	832 (26.0)	2 (0.1)	1 (0.1)	0.8 (0.2)	6 (0.3)
1672 (0.4)	0 (0)	10 (0.3)	0 (0)	0.0	0.6 (0.1)	11 (0.5)
212 (0)	0 (0)	48 (1.5)	0 (0)	2 (0.1)	1 (0.3)	61 (2.9)
447637	11,860	3203	2542	1675	455	2114
447.6	11.9	3.2	2.5	1.7	0.46	2.1
36,400	12,000	185	4,595	310	216	423

of 10–15 wild/caught *A. californica* collected at different times and places had similar TFAA, demonstrating a relative constancy at the population level.

Taurine, a sulfonic amino acid, is the major component in most secretions, constituting 41–86% of the TFAA. A few other FAA are in relatively high concentration, notably aspartic acid, glutamic acid, alanine, and lysine. Ammonium levels in *A. californica* and *A. dactylomela* ink or opaline are also high, 5–36 mM.

Cephalopod ink has lower millimolar levels of TFAA: 132 mM in *Octopus vulgaris*, 14.4 mM in *O. bimaculoides*, 2.5 mM in *Sepia officinalis*, 2.1 mM in *Dosidicus gigas*, 1.7 mM in *Loligo pealei*, and 0.5 mM in *Lolliguncula brevis* (Table 1). Taurine is typically

Table 1 (continued)

Octopus bimaculoides Ink C5	*Octopus vulgaris* Ink C6	*Callinectes sapidus* Muscle[b] T1	*Penaeus duorarum* Muscle[b] T2	*Crassostrea virginica* Body[b] T3	*Mugil cephalus* Muscle[b] T4
1,171 (8.1)	62,448 (47.1)	11,300 (3.9)	33,000 (17.5)	33,100 (62.7)	11,400 (39.1)
802 (5.6)	2,512 (1.9)	220 (0.1)	830 (0.4)	980 (1.9)	340 (1.2)
768 (5.3)	3,384 (2.6)	2,420 (0.8)	640 (0.3)	110 (0.2)	480 (1.6)
862 (6.0)	3,840 (2.9)	1,590 (0.5)	1,340 (0.7)	670 (1.3)	490 (1.7)
555 (3.9)	1,872 (1.4)	1,170 (0.4)	1,000 (0.5)	230 (0.4)	340 (1.2)
941 (6.5)	5,696 (4.3)	1,700 (0.6)	1,650 (0.9)	1,010 (1.9)	420 (1.4)
318 (2.2)	2,112 (1.6)	17,700 (6.1)	6,300 (3.3)	770 (1.5)	510 (1.7)
686 (4.8)	4,232 (3.2)	33,400 (11.5)	11,400 (6.0)	2,450 (4.6)	470 (1.6)
608 (4.2)	2,728 (2.1)	68,000 (23.5)	93,700 (49.7)	4,790 (9.1)	3,050 (10.4)
1,092 (7.6)	7,400 (5.6)	128,000 (44.2)	21,200 (11.2)	7,320 (13.9)	2,640 (9.0)
937 (6.5)	3,904 (2.9)	1,910 (0.7)	2,300 (1.2)	80 (0.2)	280 (1.0)
42 (0.3)	440 (0.3)	[c]	[c]	[c]	[c]
361 (2.5)	2,656 (2.0)	2,350 (0.8)	1,040 (0.6)	20 (0)	70 (0.2)
732 (5.1)	3,624 (2.7)	640 (0.2)	1,060 (0.6)	40 (0.1)	140 (0.5)
1,467 (10.2)	8,376 (6.3)	1470 (0.5)	1,900 (1.0)	80 (0.2)	260 (0.9)
173 (1.2)	2,936 (2.2)	610 (0.2)	920 (0.5)	50 (0.1)	40 (0.1)
948 (6.6)	3,408 (2.6)	560 (0.2)	540 (0.3)	10 (0)	80 (0.3)
265 (1.8)	304 (0.2)	790 (0.3)	0 (0)	0 (0)	0 (0)
662 (4.6)	4,944 (3.7)	1,360 (0.5)	490 (0.3)	330 (0.6)	1,180 (4.0)
510 (3.5)	912 (0.7)	830 (0.3)	320 (0.2)	100 (0.2)	6,720 (23.0)
481 (3.3)	4,728 (3.6)	13,700 (4.7)	8,820 (4.7)	620 (1.2)	280 (1.0)
14381	13,2456	289,720	188,450	52,760	29,190
14.4	132	290	188	53	29
1,307	7,208	[c]	[c]	[c]	[c]

[a]Value for the concentration of each chemical is in micrometer (μm), followed (in parentheses) by the concentration expressed as a percentage of the total free amino acids, to one decimal place.

[b]Values from Carr (1988)

[c]Not measured

the most abundant FAA in cephalopod inks. *O. bimaculoides* is unusual in that its taurine content is low and no single FAA dominates. Ammonium levels in cephalopod inks are also in the low millimolar range.

The major FAA in molluscan inks are those to which their major predators—crustaceans and fish—are sensitive, and their concentrations are well above response threshold, in many cases higher than response saturation. This is true of both the antennular (olfactory) and mouthpart (gustatory) chemoreceptor neurons of crustaceans (Voigt and Atema 1992; Derby 2000). Taurine and ammonium are noteworthy, as they are in such high concentrations in all of the molluscan secretions, and crustaceans have specialized receptor systems for them and are sensitive to submicromolar concentrations. Fish olfactory and gustatory systems are likewise highly sensitive to the FAA in these secretions, and have specialized receptors for several of them (Caprio 1988). These observations are consistent with the potential function of these secretions as sensory disruptors of molluscan predators. Furthermore, *A. californica* ink and opaline have much higher levels of TFAA (310–404 and 12–15 mM, respectively) compared to *A. californica* hemolymph and mucus (0.7 and

0.6 mM FAA, respectively). This finding is consistent with the idea that the FAA of sea hare ink and opaline are not reflective of the composition of other fluids or secretions of sea hares, but are more concentrated by up to several orders of magnitude, thus further supporting the idea that ink and opaline have evolved as a chemical defense.

Phagomimicry requires that the secretions contain chemicals in absolute and relative concentrations sufficient to mimic the food of predators. The molluscan secretions contain the same FAA as food, and they are generally present at similar absolute concentrations. TFAA in ink and opaline of *A. californica* and *A. dactylomela* (12–448 mM) and the inks of cephalopods (0.5–132 mM TFAA) are within or close to the range of TFAA in tissues of typical food of fish and crustacean predators (crab, shrimp, oyster, and mullet: 29–290 mM). This comparison of the FAA compositions of molluscan secretions and food tissues, together with behavioral results showing that an artificial mixture of *A. californica* ink or opaline with the FAA composition shown in Table 1 evokes feeding behaviors of the predatory spiny lobster *Panulirus interruptus* (Kicklighter et al. 2005), shows that molluscan secretions can be food-like in their TFAA.

Multidimensional scaling (MDS) was used to compare the relative FAA compositions of molluscan secretions with each other and with the prey species (Fig. 1). This analysis reveals several features of these secretions and tissues. First, different batches of the same secretion have similar proportions of FAA. *A. californica* ink samples Si1 and Si2, each representing samples pooled from 10–15 wild-caught animals, are similar to each other. A similar trend is seen for *A. californica* opaline samples So1 and So2. This shows relative constancy in the proportion of FAA for groups of animals collected at a different time and place. Second, secretions of a particular type (e.g., sea hare opaline, sea hare ink, or cephalopod ink) tend to be more closely grouped to each other, and thus have FAA composition more similar to each other than to those in a different type of secretion or tissue. The sea hare opalines (especially So2/So4) are clustered close to each other, the ink of three species of wild-caught sea hares (Si1, Si2, Si4) are clustered, and five of the six cephalopod inks (C1–C4, C6) are clustered. This supports the idea that although molluscan defensive secretions have quantitative and qualitative compositional similarities to food, the different molluscan secretions (molluscan opaline vs. molluscan ink, sea hare ink vs. cephalopod ink) have some differences in their FAA compositions. Third, whereas all of these molluscan secretions are similar to food in having high absolute concentrations of TFAA, the relative compositions (i.e., FAA proportions) of some molluscan secretions are more similar than others to food of their predators. All of the sea hare inks (Si1–Si4) and five of the cephalopod inks (*S. officinalis* C1, *L. pealei* C2, *L. brevis* C3, *D. gigas* C4, *O. vulgaris* C6) group close to each other and to oyster *Crassostrea virginica* (T3) and mullet *Mugil cephalus* (T4), indicating their FAA proportions are most similar. Sea hare inks and tissues from oyster and mullet also have some compositional similarity to the sea hare opalines, although less so than to each other. Relatively different in composition from all of these are sea hare hemolymph (Sf1), *Callinectes sapidus* muscle (T1), *Penaeus duorarum* muscle (T2), and *O. bimaculoides* ink (C5), and the equidose mixture (E). Thus, this MDS analysis, together with the absolute concentrations of FAA, shows that all molluscan secretions have FAA compositions highly similar to that of food, with some molluscan inks being more similar to food than are others.

Taurine, lysine, alanine, glycine, and glutamine contribute most to the compositional differences in the secretions and tissues in Fig. 1, as indicated by a linear multiple regression model of the MDS data ($P<0.001$) (Kruskal and Wish 1978). The concentration of taurine is relatively high in sea hare ink and opaline and in cephalopod inks, and relatively low in sea hare mucus and hemolymph and the crustacean muscle tissues. (The

exception is *O. bimaculoides*, which is not dominated by any component and thus is compositionally similar to the equidose mixture, E.) Regarding the other FAA in relatively high concentrations, lysine is high in sea hare opaline compared to all the other secretions and tissues. Alanine is relatively high in sea hare ink and cephalopod ink but not in sea hare opaline. Glutamine is absent in sea hare opaline but present in other secretions and food tissues. Glycine is relatively high in sea hare mucus and in the four food tissues.

The FAA composition of the molluscan defensive secretions and the sensory abilities of predators of these molluscs suggest that these secretions have the potential to function as chemical defenses through mechanisms that may include sensory disruption and/or phagomimicry. The focus of our paper is FAA and ammonium, but other chemicals in these secretions may also have defensive properties. A combination of mechanisms may enhance the effectiveness of the secretions (Kicklighter et al. 2005). For example, ammonium can be an inhibitor of feeding behavior of crustaceans (Zimmer-Faust 1987) and thus might also function as a deterrent in addition to its role as a sensory disruptor. Our demonstration of the presence in cephalopod ink of millimolar concentrations of feeding

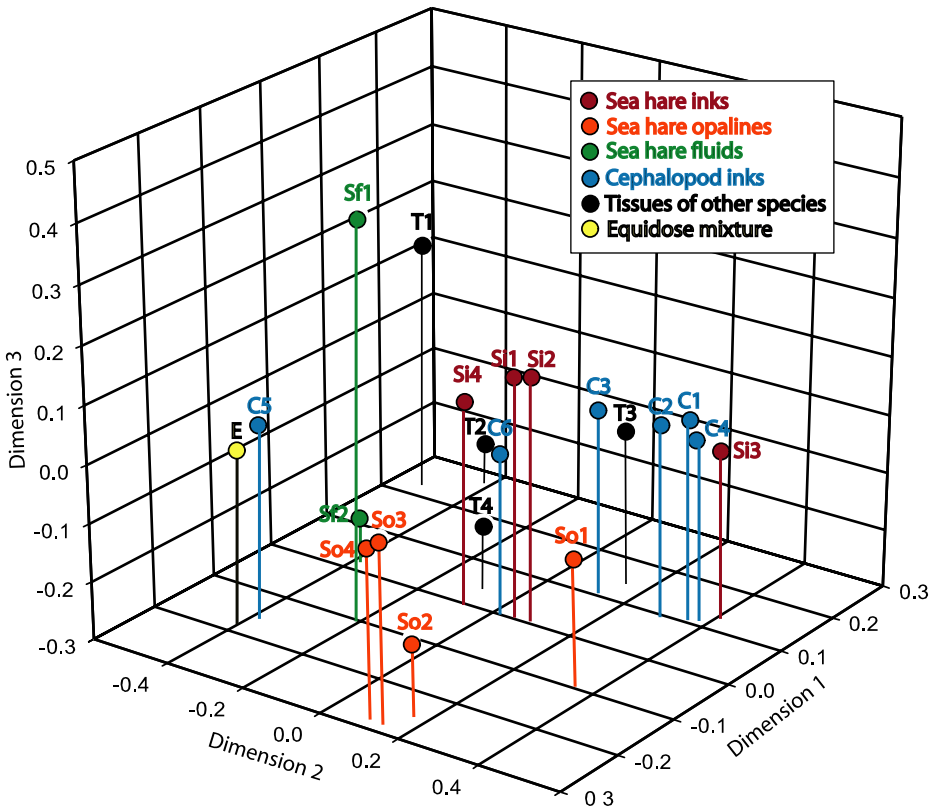

Fig. 1 Degree of similarity in the chemical composition of molluscan secretions and fluids, and food of predators of molluscs, based on multidimensional scaling. The identities of the secretions and tissues associated with the labels in this figure are coded according to type (e.g., Si=sea hare inks, So=sea hare opalines, C=cephalopod inks, T=food items), and the identity of each of the individual secretions and tissues (e.g., Si1, C4) are given in Table 1 and Methods and Materials. The values on the dimensions are derived from the analysis and are not strictly FAA concentration. See text for an explanation of the MDS analysis and results

chemostimulants of fish raises the possibility that cephalopod ink might have convergently evolved antipredatory effects through a combination of chemical mechanisms that may include sensory disruption or phagomimicry. Confirmation awaits behavioral testing.

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