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ALARM SUBSTANCE OF THE MARINE MUD SNAIL, *Nassarius obsoletus*: BIOLOGICAL CHARACTERIZATION AND POSSIBLE EVOLUTION[1]

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Abstract—The gastropod snail *Nassarius obsoletus* shows a dramatic self-burial response to the presence of crushed conspecifics. After it was shown that this burial alarm response could be reliably replicated in laboratory tests, a further characterization of the alarm substance was undertaken. Dilution experiments showed a very high response threshold resulting in a short effective radius of the substance in agreement with earlier field reports. Longevity experiments showed that the substance had lost some activity after 16 hr standing over marsh mud in sea water at room temperature; it became inactive after 24 hr. Superthreshold concentration in sea water was not necessary to keep the snails buried: Mud apparently provides an adsorption surface which can remain a stimulus source for previously unalarmed snails, and snails tend to remain buried after a short exposure to alarm substance, even when given a fresh environment. The substance is present in the snail's blood and tissues and is passively released. A potential natural predator capable of such release is *Carcinus maenas*, the green crab. Predator odor alone did not cause burial alarm responses. Preliminary chemical analysis indicates that the substance is water soluble, heat stable, and of high apparent molecular weight (over 100,000). A comparison with fish alarm substance and response is made in a discussion of the possible evolution of chemically triggered alarm responses. It is argued that *N. obsoletus* may have developed an alarm response to an existing nonspecific substance rather than a true alarm pheromone.

Key Words—*Nassarius obsoletus*, snails, alarm substance.

[1] This paper is based in part upon a thesis submitted by D.S. to Boston University for the degree of Master of Arts.

INTRODUCTION

Alarm responses elicited by chemical substances passively released from wounded conspecifics have been described for aquatic animals of widely different taxa, such as fish (von Frisch, 1938, 1941a, b; Pfeiffer, 1963, review), amphibian tadpoles (Eibl-Eibesfeldt, 1949; Hrbácek, 1950; Kulzer, 1954), gastropod snails (Kempendorff, 1942; Snyder, 1967; Snyder and Snyder, 1971; Atema and Burd, 1975; Stenzler and Atema, 1977), sea urchins (Snyder and Snyder, 1970), sea anemones (Howe and Sheikh, 1975), and turbellarians (Heinz, 1954). Their independent evolution in so many different groups is sufficient evidence for the biological significance of these substances. It is generally assumed that alarm substances serve as a defense system against predators (Snyder, 1967), although direct evidence for their effectiveness to reduce predator efficiency has not been demonstrated experimentally. Supporting evidence for the predator defense hypothesis can be found in the specificity with which prey species respond to alarm substances. For example, the fresh water minnow *Phoxinus laevis* quickly flees from areas where a wounded conspecific has left alarm substance; the intensity and pattern of the alarm response depends on the quantity of substance released as well as on a number of other factors (von Frisch, 1941a,b). The same minnow "freezes" and sinks slowly to the bottom when faced with the odor of its natural predator, the pike (Göz, 1941). There are two important aspects to this: (1) Responses to alarm substance are different from responses to predator odor, each having its own adaptive significance, and (2) the responses appear modifiable under varying environmental and physiological conditions.

Another example of predator-specific responses can be observed in the gastropod snail, *Nassarius vibex*. This snail buries itself in response to conspecific body juices (Snyder, 1967), but emerges from the sand and moves away from the odor of the predatory snails, *Fasciolaria tulipa* and *F. hunteria*, and the sea star, *Luidia alternata* (Gore, 1966). When actually touched by these predators or excised parts of them, *N. vibex* shows a violent flipping response. Other sea stars, *Astropecten duplicatus* and *Echinaster sentus*, do not elicit these responses in *N. vibex* (Gore, 1966). The responses are adaptive because all three predators could probably uncover a buried *N. vibex* and establish a good hold on it. Moving away is thus the safest avoidance. However, if contact does take place, violent flipping can prevent the predator from attaching to the snail. The burial response to conspecific body juice was apparently enforced by other predators, whose identities are thus far unknown. The other two sea stars are probably not predatory on *N. vibex*: *E. sentus* is not sympatric with *N. vibex*, and *A. duplicatus* may not pose such a serious danger to the snail population as to warrant the evolution of a

behavioral defense stimulated by odor. This leads to two related questions, which will be discussed at the end of this paper: (1) Why would predator odor detection alone not be sufficient since it could trigger a "selfish" escape response, and (2) how could potentially altruistic behavior of maintaining alarm substance have evolved?

The typical response of the mud snail, *Nassarius obsoletus*, to the presence of conspecific body juices is burial (Snyder, 1967; Atema and Burd 1975; Stenzler and Atema, 1977). The juices of other molluscan species from its salt marsh habitat elicit attraction (Atema and Burd, 1975). Snyder (1967) found that *N. obsoletus* had a long-lasting alarm response and a high threshold compared to those of the many gastropod snails he tested. Atema and Burd (1975) also noted that the alarm response in the field lasted perhaps as long as 24 hr, thus surviving two tidal flushings. This parallels the observation that minnows sometimes avoided their feeding area in the lake for days after alarm substance was released there (von Frisch, 1938, 1941a,b).

Attempts to characterize and identify an aquatic alarm substance have been made for fish. It was concluded that club cells in the skin of ostariophysine fishes are the source of their alarm substance, which is passively released by predator damage (Reutter and Pfeiffer, 1973). The substance was identified as a pterin with a molecular weight below 500, perhaps isoxanthopterin (Pfeiffer and Lemke, 1973). An alarm substance of the sea anemone, *Anthopleura elegantissima*, was described and identified with a molecular weight of 213.5 (Howe and Sheikh, 1975). The present paper describes biological and some chemical properties of the alarm substance of *N. obsoletus*, followed by a discussion of their evolution and biological significance.

METHODS AND MATERIALS

Characterization of Alarm Substance: Threshold, Longevity under Natural Conditions, Mud Adsorption, Lasting Response to Short Exposure

For laboratory testing, *Nassarius obsoletus* (Say) and mud were collected periodically from the flats in Little Sippewissett Marsh, West Falmouth, Massachusetts. The snails, whose average wet weight without the shell was 0.9 g, were kept on mud in a shallow tank with running ambient seawater. The water temperatures in the holding tank ranged from 4 and 23°C with the seasons. Tests were conducted with freshly collected snails and marsh mud in flat trays (40 × 40 cm) with new plastic liners for each test. A layer of 1 cm of mud was placed in the trays and covered with 4.5 liters of seawater, to a depth of 3 cm. Clean glass tubes introduced air into the water and

caused thorough mixing as indicated by dye tests. Each experiment was replicated four times unless otherwise indicated.

For data analysis and representation, the number of snails visible at any recording time in the tests was expressed in percentage. At time 0 min, the number of snails visible was always recorded as 100% visible. Visible snails are defined as those which were neither totally nor partly buried. Only the burial alarm response was used in these tests, disregarding the less common response of crawling away from the source. This results in a conservative measure of the total response, especially at 2 min. The Kramer Duncan Multiple Range Test has been used for all the tests of significance (unless otherwise indicated) at the $\alpha = 0.05$ level of significance.

To calculate the threshold concentration of alarm substance required to elicit the alarm response in *Nassarius obsoletus*, an extract was prepared by homogenizing 1.1 g wet weight of snail meat (without shell) in 5 ml distilled water. After filtration, 4 ml were introduced into prepared test trays without snails. After 10 min, when the extract was distributed throughout the tray, a half mussel was placed in the center as a focus for feeding attraction and 10 *N. obsoletus* were placed directly around it (time 0 min). The snails' activities were recorded at 2, 5, and 10 min after their introduction. The final concentration in the tray was calculated as 200 mg of snail meat/liter. Further dilutions of the original extract permitted the testing of concentrations of 75 mg/liter and 20 mg/liter. As a control identical tests were run, but with 4 ml distilled water added to the test trays instead of crushed snail extract.

The results show that only concentrations of 200 mg of snail tissue/liter elicited responses which were similar to the field alarm response (Atema and Burd, 1975) and the standard crushed snail bioassay (Stenzler and Atema, 1977) at 2, 5 and 10 min. Lower concentrations did not elicit the burial response, never differing significantly from controls at any time interval (Figure 1).

To test the longevity of alarm substance in seawater standing over marsh mud, its natural environment, the threshold test procedure was used with a snail extract concentration of 200 mg/liter. But here, the time interval between introduction of the snail extract and half mussel plus 10 mud snails was varied from 10 min to 1 hr, 11 hr, 16 hr, and 24 hr. The control was the same as in the threshold test. In longer tests the temperature in the test trays varied from 11 to 18°C due to daily temperature changes in the laboratory.

The test results showed a significant difference between control and test responses at all recording times for 10-min-, 1-hr-, 11-hr-, and 16-hr-old snail extract. There was no significant difference between the 10-min, 1-hr, and 11-hr responses at the 5-min and 10-min recording. Thus, the alarm substance lost some activity between 11 and 16 hr, and became inactive between 16 and 24 hr (Figure 2).

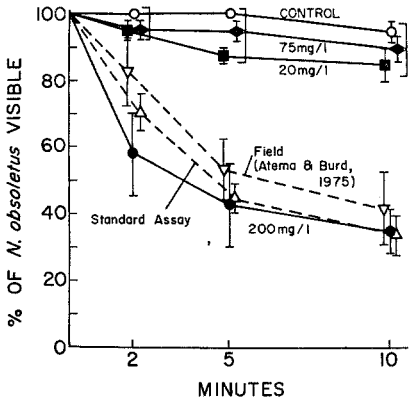

FIG. 1. The mean percent ±SE of *Nassarius obsoletus* visible after their introduction into water with a conspecific extract concentration of 200 mg snail meat/liter (●), 75 mg/liter (◆), and 20 mg/liter (■). The control snails were placed in trays free of snail extract (○). Values which are not significantly different are bracketed. For reference, the percent of *N. obsoletus* ±SE visible after introduction of a crushed conspecific in the laboratory standard bioassay (Stenzler and Atema, 1977) (△), and in the field (Atema and Burd, 1975) (▽).

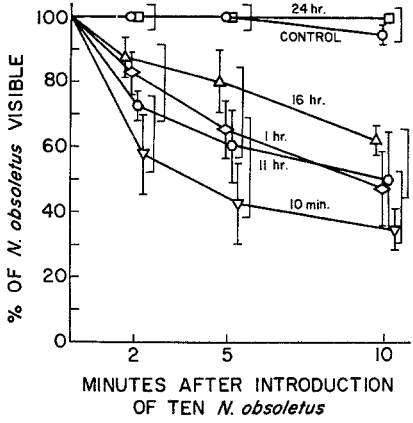

FIG. 2. The mean percent ±SE of *Nassarius obsoletus* visible after their introduction into water with a conspecific extract concentration of 200 mg snail meat/liter. The time intervals between the extract and *N. obsoletus* introductions were 10 min (▽), 1 hr (◇), 11 hr (○), 16 hr (△), and 24 hr (□). The control snails were placed in trays free of snail extract (○). Values which are not significantly different are bracketed.

To determine whether the long-lasting burial alarm reaction is in response to a short exposure to alarm substance, or is maintained by the constant presence of alarm substance in the water column, or by adsorption onto the mud, the following tests were done.

1. Superthreshold Concentration of Alarm Substance in the Water Column. Ten *Nassarius obsoletus* were placed in a freshly prepared test tray and given 20 hr acclimation. After a 15-min mussel-attraction period, a crushed conspecific was introduced and the snails' behavior was recorded 2, 5, and 10 min after that introduction. The water was then siphoned from the tray and the half mussel attractant and crushed *N. obsoletus* stimulus were removed. Then 4.5 liters of clean seawater were siphoned into the tray (water change took about 10 min) and a new half mussel was placed in the center of the tray. The number of mud snails visible was again recorded at 0, 2, 5, and 10 min after the introduction of the second mussel. In the control tests an identical procedure was followed, except that the same water was siphoned directly back into the tray from which it came, thus maintaining the same concentration of alarm substance. A second control for the possible effect of water changing itself was done by adding oyster chips to a test tray instead of crushed *N. obsoletus*. A clean water change was performed for this second control group.

These experiments showed the not surprising result that before water changes the responses of the two groups of *N. obsoletus* exposed to crushed conspecifics did not differ significantly (30% and 26% visible after 10 min) and that both were significantly different from controls (90% visible after 10 min). However, after the two different water change procedures, there was still no significant difference between the numbers of snails visible in the clean water tests and in control (same water) tests at all recording times (about 30%), while both groups remained significantly different from the oyster chip controls (about 90%).

2. Mud-Adsorbed Alarm Substance. To test this hypothesis, the same experiment was performed as above but without adding the 10 test snails for the first part. After the mussel and crushed snail were removed, the water was replaced with clean water. Ten mud snails which happened to be buried in the holding tank were manually unburied, rinsed 5 times in clean seawater, and placed in the tray without mussel present (time 0 min). Recordings were made at 2, 5, and 10 min after their introduction. As a control, 10 buried snails were manually unburied from the holding tank, rinsed, and placed in a fresh tray without mussel or crushed snail.

The tests for mud adsorption showed that when fresh snails were placed on possibly contaminated mud from the clean-water-change experiment, the number of *N. obsoletus* visible at 5 and 10 min differed significantly from controls (Figure 3).

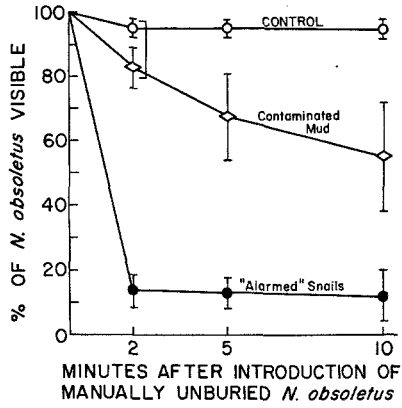

FIG. 3. The mean percent ±SE of *Nassarius obsoletus* visible in fresh trays after they were manually unburied from the holding tank (CONTROL) (○), and from test trays which were treated with crushed *N. obsoletus* and a clean water change ("Alarmed" Snails) (●). *N. obsoletus* were also manually unburied from the holding tank and placed in trays which were treated with crushed *N. obsoletus* and a clean water change (Contaminated Mud) (◇). Values which are not significantly different are bracketed.

3. Lasting Response to Short Exposure. Snails which had remained buried at the end of the first experiment after the clean water change were manually unburied, rinsed 5 times in clean seawater, and placed in a freshly prepared tray without mussel present. Recordings were made as before. Control as in the second experiment.

The results of the single dose response tests showed that most of the previously "alarmed" snails that were manually unburied and rinsed, buried again immediately in the fresh trays. The number of these snails at 2, 5, and 10 min differed significantly from control, which had not been exposed to alarm substance (Figure 3).

These last three tests show that a superthreshold concentration of alarm substance in the water column is not necessary to maintain the buried state. Apparently, the marsh mud provides an adsorption surface for the alarm substance, which remains a stimulus source for unalarmed snails, and previously alarmed snails stay in an alarmed state in the absence of the alarm stimulus.

Source and Mode of Release

To determine the source of the alarm substance in *Nassarius obsoletus*

we used the standard bioassay (Stenzler and Atema, 1977) with the following modifications: at time 0 min, 4 ml of filtered extract were pipetted over the feeding snails while 4 ml of filtered seawater served as the control. Two major portions of the snail were examined: the foot, which is protrusible through the aperture of the shell, and the visceral mass, which is permanently contained within the shell. Extracts were made by homogenizing these tissues in a tissue grinder with 5 ml of seawater. In tests with blood as a stimulus, a total of 0.5 ml of snail blood was taken from the feet of about 10 mud snails by making a few fine pin holes in their extended feet. This method was adopted after it was shown to yield similar results to blood drawn directly from the heart. The 0.5-ml sample was introduced at time 0 min in the test trays.

To test whether the alarm substance is actively released, a mud snail was hit between two large rocks so as to put a visible crack in its hard shell. This unusual treatment was considered alarming, while no damage to the inner tissues was observed. The crack-shell snail was then used as a stimulus source and placed in the bioassay test tray at time 0 min. In the control, chips of oyster shell were introduced to simulate the mechanical part of placing the mud snail into the test tray.

A more natural way to test for active release was to place a natural predator, the moon snail, *Lunatia heros*, with a mud snail in a bowl with mud substrate and aerated seawater. They contacted each other within 5 min. Afterwards, the mud snail was placed in a test tray to serve as a stimulus for 10 conspecifics to test if the alarm substance was being released actively as a result of this encounter. The oyster chip control was the same as in the standard bioassay.

Since active release of alarm substance may cease immediately after breaking contact with the predator, a longer test was done by again placing the two in the bowl. Within 18 hr, the *L. heros* was in a boring–feeding position and the mud snail concealed by the moon snail's massive foot (Stenzler and Atema, 1977). A 4-ml water sample was taken from the bowl at that time and tested for alarm substance release using the liquid extract bioassay. Water samples were tested 3 times per day for the following 3 days while the moon snail was still in the feeding position. Introductions of 4 ml of seawater served as controls.

To test if moon snail odor alone causes mud snails to actively release the alarm substance or to exhibit the alarm reaction, a *L. heros* was kept for 20 hr in a bowl with 1 liter of seawater. Four ml of this *L. heros* odor water were pipetted into the test tray, the normal test procedure was used, and 4 ml of seawater served as controls.

When another marsh predator was discovered, the green crab, *Carcinus maenas* (Stenzler and Atema, 1977), similar tests were conducted with crab

odor and mud snail predation water using a slightly different procedure, in which response snails were placed in stimulus water. This modification did not introduce significant changes in the snails' responses. One *C. maenas* and 5 *N. obsoletus* were placed in a 20-cm-diameter finger bowl with mud substrate and 1 liter of seawater. During the first 30 min, the crab had eaten one snail. The crab and the remaining snails were then removed, and 10 naive mud snails were placed in the bowl. Their behavior was recorded 2, 5, and 10 min after their introduction. The snails were then removed, mud stirred, and slime trails cleaned from the sides of the bowl. Five trials with new snails were recorded in that bowl. As a control, a crab and 5 snails were kept mechanically isolated from each other by a perforated plastic screen. Water from this bowl was tested as above after 30 min. Water with 30-min snail odor alone and crab odor alone were also tested. Since unusual responses were observed after 20 hr of exposure to crab plus snail odor, the responses of snails to 20-hr snail odor alone and crab odor alone were also examined.

RESULTS

The *Nassarius obsoletus* response to both conspecific foot and visceral mass tissue extracts and to blood differed significantly from the control at all time intervals. There was no significant difference between the responses to foot extract, visceral mass extract, and blood (Figure 4). The alarm substance is apparently present throughout the snail's tissues.

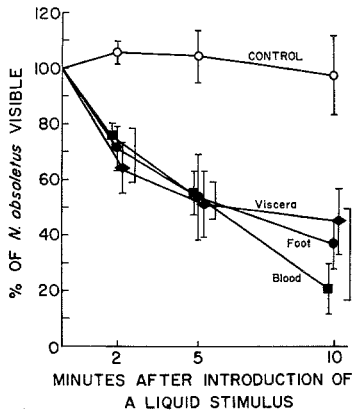

FIG. 4. The mean percent ±SE of *Nassarius obsoletus* visible after the introduction of 4 ml of seawater control (○) (6 replicates), *N. obsoletus* viscera extract (◆), foot extract (●), and 0. 5ml of blood (■). Values which are not significantly different are bracketed.

Active release of alarm substance does not appear to take place, since burial behavior was not significantly different from controls, whether the stimulus consisted of cracked-shell snails, or snails which had been in contact with *Lunatia heros*, or water from a bowl with a feeding *L. heros*. Similarly, 30-min crab plus snail odor (screen-separated animals) and 30-min crab or snail odor, or 20-hr snail odor alone had no effect. Only when *Carcinus maenas* crushed and began feeding on *N. obsoletus* did the surrounding water induce the alarm response in other snails. This indicates that physical damage to the snail's body is necessary to release the alarm substance. In fact, even when the shell of a mud snail was carefully peeled away completely except for a small attachment to the columellar muscle, the naked snail joined its feeding conspecifics without causing them to alarm. However, as soon as the last piece of shell was cut from the muscle, the alarm response was induced. This example dramatically illustrates the passive nature of alarm substance release.

When moon snail odor alone was introduced into the test trays, an unusual number of burials *and* unburials were observed (unlike the response to the alarm substance). Since only the number of snails visible at any time interval was used in the analysis, the overall response to this odor is not borne out in this representation. When 20-hr green crab odor was presented to the snails, they began to crawl out of the test bowls, but showed no signs of burying. Thus, distinctly different responses to predator odor and to alarm substance were observed in *Nassarius obsoletus*.

Preliminary Chemical Analysis

To determine some general chemical characteristics of the alarm substance, filtered snail homogenate was boiled for 5 min over a flame. The resulting precipitate was filtered out. The filtrate was tested and found as active as regular alarm substance.

In a second series of tests the filtered homogenate was extracted with hexane. The activity was recovered from the water contained in the resultant emulsion which formed above the water fraction. Neither the water fraction nor the hexane fraction was active. When the active fraction was boiled for 5 min in a water bath, the activity remained.

An attempt was made to determine molecular weight by running the hexane-purified extract through various grades of Sephadex columns. The activity remained at the eluant front even when G-100 was used, indicating an apparent molecular weight of about 100,000 or greater. Minor activity was recovered in a fraction of lower molecular weight. Further analysis is in progress.

DISCUSSION

This study has shown that the alarm response of *Nassarius obsoletus* occurs equally well and with a similar time course in the laboratory and in the field. Based on such tests further characterization of the alarm response and substance could be undertaken. Our threshold results agree with Snyder's (1967) in that a stimulus dilution of 75 mg snail meat/liter or less did not elicit the alarm response. In other words, the substance should cause a short-range effect. This is confirmed by the field observation that an area of only 50-cm radius is affected (Atema and Burd, 1975). Snyder found that the *N. obsoletus* response had the highest threshold of all those tested in his snail alarm survey study.

When Atema and Burd (1975) noted that few snails were visible the following day in previously tested areas on the marsh, they suggested that the alarm effect survived two tidal flushings. In the laboratory the alarm substance did not lose its activity until left standing 16–24 hr in seawater over marsh mud at room temperature. Further tests showed that the substance also adsorbed onto the mud and still retained its activity. Finally, "memory" may also be involved, since the snails remained buried after a brief exposure to the alarm substance, which was subsequently removed. Together, these results could easily explain the long-lasting response in the field. Similar observations on fish were made by von Frisch (1938), who noted that minnows (*Phoxinus laevis*) avoided a feeding area in the lake for several days after being exposed to alarm substance there. These fish appear to have a space memory connected with the alarm response.

The alarm responses of the mud snail and of the ostariophysine fishes are similar in that the alarm substances are likely only passively released. However, while in fish the substance is released from specialized cells in the skin of the animal (Reutter and Pfeiffer, 1973), in *Nassarius obsoletus* the substance appears to be present in the blood and all its tissues. Perhaps it should be mentioned that von Frisch (1941b) found low levels of alarm substance present in *Phoxinus* ovaries (100 times less than in skin), muscle tissue (20 times less) and gills (5–10 times less), which could lead us to believe that in fish the substance is not present *only* in the specialized club cells of the skin.

The release of the alarm substance was caused by *Carcinus maenas* (feeding on mud snails) but not by *Lunatia heros*. An explanation may be that the massive foot of *L. heros* (or mucus coating) sealed off the whole feeding area, or that its proboscis sealed off the bored hole through which it feeds (Stenzler and Atema, 1977), thus causing insufficient release of alarm substance in the surrounding water. *C. maenas*, on the contrary, is a sloppy feeder which scatters food particles while ripping and "chewing" its food

(Stenzler and Atema, 1977). It can be assumed that a predator like *C. maenas*, rather than like *L. heros*, selected for the evolution of the *N. obsoletus* alarm substance. In the field, the responses to predator odor alone are probably not meaningful, since mud snails are not likely to come into contact with anything like 1 liter of water that has surrounded a predator for 20 hr.

Another difference between *Phoxinus* and *Nassarius* alarm substances is their chemistry. As far as determined, the fish substance is a relatively small molecule with a molecular weight below 500 (Pfeiffer and Lemke, 1973), while the mud snail substance appears to be of high molecular weight, perhaps over 100,000. Snyder (1966) found the alarm substance of another gastropod *Helisoma duryi* to be of relatively high (5000–10,000) molecular weight. It has been shown that other high-molecular-weight compounds play an important role in eliciting proboscis extension in *Nassarius* (Carr, 1974). Therefore, both feeding behavior and alarm responses can be elicited by high-molecular-weight molecules, something not frequently considered in the past. The nutrition-dependent balance between feeding attraction and alarm repulsion (Stenzler and Atema, 1977) could further tempt us to speculate that perhaps the two responses are released by similar or even related molecules. This is not so strange as it may at first appear. *Nassarius obsoletus*, being a facultative scavenger, shows good feeding attraction to various snail body juices including in exceptional cases its own conspecific juices (see also Stenzler and Atema, 1977). It may be reasonable to assume that all this snail has done is evolve the behavioral alarm response to an already existing substance and its sensory processing. The following discussion of the possible evolution of alarm responses may provide further context for this speculation.

Evolution of Alarm Response and Substance

The simplest assumption about the evolution of alarm responses is that detection of conspecific body juices decreases the probability of conspecific individuals becoming prey. The response thus increases the fitness of the species, presumably because the presence of these juices would indicate that a predator was actually "at work." This would make a difference from detecting a predator by its odor, since the latter would cause snails to stop feeding and bury whenever the predator is near, even when he does not "hunt." Snyder (1967) and Snyder and Snyder (1971) have speculated along these same lines, arguing that the balance between time and energy spent on feeding versus alarming must be subjected to predator pressure. The snail's choice at the individual level may be starvation versus being eaten. When this pressure is not too great and when feeding is time consuming, the snails benefit from not responding to predator presence per se. Perhaps in terms of the whole population the resulting loss of few individuals is outweighed by the avail-

ability of a greater number of well-fed individuals. In other words, whenever we find alarm responses but not predator odor detection, as in *Nassarius obsoletus*, we can assume relatively light predator pressure on a population that must graze extensively to feed itself.

For a species to arrive at this state of conspecific body juice detection, the individuals must develop (1) a chemoreceptor site capable of recognizing a conspecific metabolic product which is liberated in the environment during predation, and (2) the appropriate escape behavior. It was speculated above that *N. obsoletus* may already have had the proper receptor sites, and only needed to modify the behavioral response to its input.

This may be the approximate state in which we find contemporary *Nassarius* species. It was shown that three species of the *Nassarius* genus respond to each other's alarm substance, that they respond strongest to the conspecific substance and not to juices from other gastropod genera (Stenzler and Atema, 1977). The one exception to this rule was the strong response of *N. trivittatus* to the juices of the sympatric, but taxonomically not closely related, *Urosalpinx cinerea* (Stenzler and Atema, 1977). This exception supports the assumption of the evolutionary state of *Nassarius*; we do not have to invoke complex molecular relationships between *Nassarius* and *Urosalpinx* juices if we assume that it became advantageous for *N. trivittatus* to respond to *U. cinerea* juices. Sharing a common predator would be sufficient "reason" for this development. *Nassarius* species seem to be predisposed to develop this type of a response.

If indeed the receptor sites and the appropriate response are the only evolutionary adaptations, then the alarm substance does not serve a specific signal function. In fact, detection of alarm substance appears very similar to prey or predator detection. In the latter case it seems very clear that no "intentional" (chemical) signal has evolved to establish "communication" between prey and predator (Burghardt, 1970). Thus, the alarm substance should not be considered part of a communication system and should not be classified as a pheromone.

The situation changes when we consider fish alarm substances. Although these substances may be present in small amounts in various tissues and organs (von Frisch, 1941), there are good arguments that the main source of it is in the club cells of the skin. These cells are thus far not known to have any function (Reutter and Pfeiffer, 1973). If such a function cannot be demonstrated, we can argue that in the course of evolution the substance has taken on a special signal function, carried largely if not entirely for the purpose of warning conspecifics. The fish alarm substance would thus be a true pheromone. Since a metabolic price must be paid for the production and maintenance of club cells and pheromone, we now deal with a case of altruism: It is likely that the victim does not itself benefit from the metabolic

expense incurred. The altruistic aspects of this evolution are even more striking when we see that a varying threshold has evolved in the responding fish population: Some low-threshold individuals, usually the perhaps more expendable males, start darting away long before their schoolmates detect the alarm substance. The behavior of these "alarmists" acts as an amplifier and visually causes alarm in the rest of the school. It also draws the attention of the predator, which will inevitably attack the "odd one" (Gandolfi, Mainardi and Rossi, 1968a,b).

Finally it should be mentioned that alarm responses seem to be rather common among gastropod snails (Snyder, 1967), and it is reasonable to assume that this group is predisposed to develop the appropriate receptor sites, neurobiology, and behavior. The same could be said for the ostariophysine group of fishes (Pfeiffer, 1963). Perhaps the most intriguing questions we are left with are the mechanisms by which these and other chemoreceptor sites evolve and the neurobiological processes that make such dramatic shifts in behavior possible.

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