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AN ANTI-PREDATOR CHEMICAL DEFENSE OF THE MARINE PULMONATE GASTROPOD *TRIMUSCULUS RETICULATUS* (Sowerby)

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Abstract: Anti-predator defenses of the marine pulmonate gastropod *Trimusculus reticulatus* (Sowerby) were studied in the laboratory. By repeatedly presenting the seastars *Pisaster ochraceus* (Brandt) and *P. giganteus* (Stimpson) with one *Trimusculus reticulatus* and an individual of some species of prosobranch limpet, it was shown that seastars tended to avoid eating *T. reticulatus* ($P < 0.001$). When attacked, *T. reticulatus* secretes a milky-white mucus. After this mucus was spread on the shells of prosobranch limpets, those limpets were eaten by *Pisaster* significantly less often than clean limpets of the same species ($P < 0.001$). This defensive mucus contains a compound which temporarily stuns the tube feet of an attacking seastar. Seastars suffered no lasting effects from repeated exposure to this defensive mucus, and those which ate *Trimusculus reticulatus* showed no ill effects. Feeding preference experiments with the crab *Pachygrapsus crassipes* (Randall) indicated that the mucous defense of *Trimusculus reticulatus* is not effective against this predator.

Key words: chemical defenses; predation; pulmonate; *Trimusculus reticulatus*

INTRODUCTION

Intertidal gastropods have been demonstrated to employ a wide variety of tactics in dealing with predators. In addition to morphological adaptations, these include both behavioral (Feder, 1963; Margolin, 1964a; Phillips, 1976; Harrold, 1982; Watanabe, 1983) and chemical defenses (Thompson, 1960; Ireland & Faulkner, 1978; Faulkner & Ghiselin, 1983). The particular type of defense used may have great effect on the morphology and ecology of the prey species (Phillips, 1976; Vermeij, 1978), as well as on the behavior and ecology of the predator (Harrold, 1982). For these reasons, an understanding of the defensive strategies available to prey species is important to understand their ecology. In this paper, I report on a previously undescribed defensive strategy used by a little studied gastropod.

Trimusculus reticulatus (Sowerby) is a marine pulmonate limpet found along the west coast of North America. It lives in dense populations on the roofs of caves and on the undersurfaces of overhangs. Populations near Santa Cruz California occur from + 4.0 to − 1.0 ft in the intertidal zone. The first extensive observations of this limpet and its habitat were made by Yonge (1958, 1960). He reported that these animals tend to move

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up and away from water and that, although they are relatively sessile in captivity, they move around in the wild to feed. More recently, Walsby (1975) demonstrated that *T. reticulatus* feeds by using a mucous net to capture suspended material in the water. This reduces the need to be mobile and Walsby emphasised the essentially sessile nature of the adult limpets.

The range of *T. reticulatus* overlaps substantially with that of the predatory seastar *Pisaster ochraceus* (Brandt), and the two frequently share the same rock crevices. Many of the prosobranch limpets in this range exhibit predator-specific escape behaviors when near seastars (Feder, 1963; Margolin, 1964a; Phillips, 1975, 1976). These escape behaviors generally consist of chemically detecting and moving away from an approaching seastar. *Trimusculus reticulatus* is able to form large, densely packed populations without resorting to this type of escape behavior.

In this report, I present evidence that *T. reticulatus* is able to secrete a defensive compound in a mucus (hereafter referred to as defensive mucus) which interferes with the ability of *Pisaster* to detect and capture it.

MATERIALS AND METHODS

All animals were collected on intertidal rocky platforms near Santa Cruz (California, U.S.A.) between June 1983 and June 1984. These included 45 *Trimusculus reticulatus*, 100 prosobranch limpets and 15 seastars. Animals were kept in tanks with running sea water. The individuals of *T. reticulatus* used were kept completely submerged.

In the laboratory, seastars were presented with two limpets placed 5 to 10 cm apart on a rock. A seastar was allowed to move across each limpet and the outcome was observed. Carrying out this procedure once constituted one trial. A trial was not counted if the seastar did not eat either limpet. Nor was a trial counted unless the seastar passed over both limpets.

In the first set of trials, a seastar (*Pisaster giganteus*, Stimpson) was presented with an individual of *Trimusculus* and one prosobranch limpet (prosobranchs used included *Collisella scabra*, Gould; *C. digitalis*, Rathke; *C. pelta*, Rathke). The seastars used had been starved for ≈ 2 months.

To test the specific effectiveness of the defensive mucus secreted by *Trimusculus* at deterring seastars, a second set of trials was carried out. In these, seastars (some *Pisaster ochraceus* and some *P. giganteus*) were presented with two specimens of *Collisella scabra*. One of these had been coated with the mucus obtained by pulling a specimen of *Trimusculus reticulatus* off the rock and teasing its mantle fold, the other was clean. If the mucus covered limpet survived one trial, it was washed off and used as the clean limpet in the next trial. Individuals of *Collisella scabra* were used because, like *Trimusculus*, they do not evade approaching seastars (Feder, 1963).

Further observations of the ways in which the defensive mucus hinders a seastar's attack were made by placing a specimen of *Trimusculus reticulatus* on a glass plate, allowing it to become attached, then lowering the plate into a tank of seastars. With the plate sitting vertically in the tank, close observations of attack and defense could be made.

Those seastars which succeeded in eating *Trimusculus* were closely watched to detect any ill effects which this might cause them. In addition, one specimen of *Pisaster giganteus* was kept in a separate tank and supplied with nothing but individuals of *Trimusculus*. Each limpet was left in the tank until the seastar succeeded in eating it and the seastar's health was observed over a 4-month period.

Feeding preference experiments were also carried out with the crab *Pachygrapsus crassipes* (Randall). In these trials, the crab was presented with an individual of *Trimusculus* and one of *Collisella pelta*. The outcomes were observed as in the *Pisaster* experiments.

Results

The results of the first set of experiments, in which seastars were presented with a specimen of *Trimusculus reticulatus* and some prosobranch limpet, are shown in Table I. In most cases (7) the seastar passed over *Trimusculus* without appearing to notice it. Seastars sometimes sat covering *Trimusculus* while eating the other limpet. In each case that it encountered a seastar, a specimen of *T. reticulatus* would produce large amounts of milky-white mucus. In the two cases in which *T. reticulatus* was eaten, it took the

Table I

Results after 10 repetitions of experiments in which an individual of *Pisaster* was presented with one specimen of *Trimusculus reticulatus* and a prosobranch limpet: *T. reticulatus* was eaten significantly less often than the other limpets ($P < 0.001$).

	Eaten	Not eaten	Total
T. reticulatus	2	8	10
Prosobranch	10	0	10
Total	12	8	20

Table II

Results of experiments in which a specimen of *Pisaster* was presented with two *Collisella scabra*: one coated with *Trimusculus* mucus, the other clean; mucous limpets were significantly ($P < 0.001$) less likely to be eaten than clean limpets.

	Eaten	Not eaten	Total
Mucous	8	30	38
Clean	37	1	38
Total	45	31	76

seastar much more time to pull it off the rock than to remove the prosobranch limpet (30 to 60 min for *T. reticulatus*, as compared to 1 to 5 min for *Collisella*).

The presence of *Trimusculus* defensive mucus on the shell significantly reduced the probability of a prosobranch limpet being eaten (Table II). Again, seastars often passed over the mucus-covered limpet without appearing to notice it.

Close observation showed that a seastar's tube feet will pull back and often remain inactive for up to 10 min when they encounter the defensive mucus of *T. reticulatus*. Only those tube feet which contact the mucus are affected but, because much mucus is secreted during an attack, the seastar's entire arm may be stunned (Fig. 1). By artificially applying the defensive mucus to various parts of a seastar's arm, I found that the tip of the tube foot must encounter the mucus if there is to be an effect.

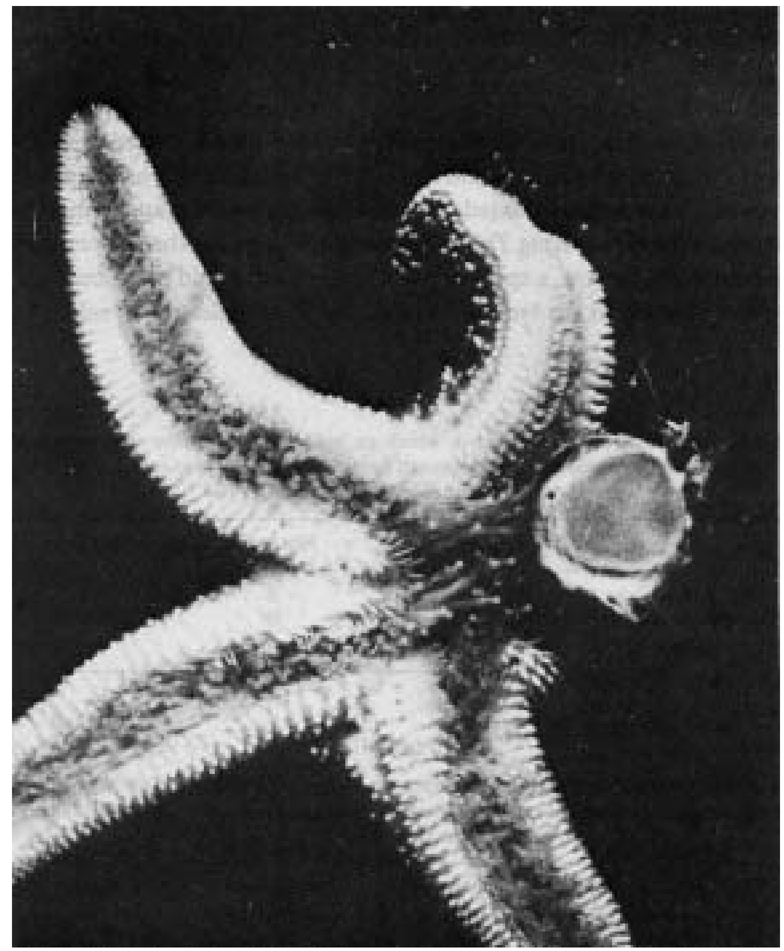

Fig. 1. *Pisaster ochraceus* attacking *Trimusculus reticulatus* (seen from below): note the white defensive mucus around the limpet's foot and the seastar's reaction.

The defensive mucus is milky-white and is secreted from around the mantle fold. As noted by Walsby (1975), secretion is initiated by mechanical pulling on the shell as when the animal is removed from a rock.

Mucus secretion is not elicited by seastars which are nearby but not touching the limpet. Furthermore, no defensive response occurred when severed tube feet were introduced under the limpet's shell. Apparently *Trimusculus* cannot chemically detect seastars and can only begin to defend itself after an attack is initiated.

Those seastars which ate *Trimusculus* did not suffer any ill effects. The individual of *Pisaster giganteus* which was fed nothing but *Trimusculus* did not suffer from 4 months of this treatment. Over this time, the seastar ate many limpets but generally took from 2 to 5 h to manipulate the limpet so that it could be digested. This entails pulling the prey off the rock, moving it to the oral region and turning it over so that the limpet's foot faces the seastar's mouth. This last act often took an hour or more. By contrast, seastars were able to begin digesting *Collisella* within 5 to 20 min of initiating an attack. Thus, it appears that *Trimusculus reticulatus*' defense, even when ultimately unsuccessful, greatly increases the handling time which a seastar must go through to eat it.

Experiments with the crab *Pachygrapsus crassipes* indicated that the limpet's mucus defense is not effective against this predator. In 10 trials, *Trimusculus reticulatus* was eaten in all cases and *Collisella pelta* was eaten in 9. The crab ate *Trimusculus reticulatus* as quickly as it did *Collisella* and showed no ill effects.

Discussion

Both Yonge (1958) and Walsby (1975) noticed the secretion of large amounts of mucus when *Trimusculus reticulatus* is removed from a rock. Walsby attributed this to glands in the foot being distorted when the animal is dislodged, leading to uncontrolled secretion. My work shows that this mucus is secreted from the mantle fold and contains some compound which acts as a defense against predatory seastars. The defensive mucus is a different consistency and color (milky-white) than the feeding mucus described by Walsby. Furthermore, it does not appear that the limpets ever secrete this mucus except when being attacked.

Most chemical defenses employed by intertidal gastropods are general in terms of the predators which they deter. Toxic or distasteful secretions (Ireland & Faulkner, 1978; Faulkner & Ghiselin, 1983) and acids (Thompson, 1960) are effective against a variety of predators. *T. reticulatus* diverges from this trend by producing a predator-specific defense which takes advantage of the specific mode of attack used by *Pisaster*.

Some other gastropods use mucous surfaces as a defense against seastars; notably the limpet, *Diodora aspersa* (Margolin, 1964b), and the snails *Natica catena* (Edmunds, 1974) and *Calliostoma ligatum* (Harrold, 1982). These all pull some part of the mantle or foot over the shell, thus presenting an attacker with a slick surface which is difficult to grasp. These animals combine this with a running (Edmunds, 1974) or twisting

(Harrold, 1982) response. Thus, the slimy surface serves only to enhance the effectiveness of some behavioral defense.

Some pulmonates of the genus *Onchidella* have been shown to produce a defensive compound which is distasteful to predators and acts as an antibacterial agent (Ireland & Faulkner, 1978). This sort of defense has the advantage that it does not require any behavioral complement.

Trimusculus reticulatus is different in that it produces a defensive compound which interferes only with the ability of its predators to capture it. The effect is like that of a local anesthetic which renders an attacker's tube feet temporarily functionless. It is important to note that the presence of mucus in the water does not hinder stomach extrusion by *Pisaster* or digestion of the limpet. Its only effect is to make *Trimusculus* hard to detect (if the shell is already coated) and hard to pull off the rock once detected.

In those cases in which a seastar succeeded in eating *Trimusculus*, the limpet's mucous defense forced it to take significantly more time than it would to eat *Collisella* or some other unprotected limpet. In fact, the entire defensive strategy might best be thought of as a mechanism to increase the seastar's handling time, usually to a point where it gives up. This is relevant in light of the fact that *Trimusculus* lives in very dense populations (≈ 30 individuals per square decimeter). The effect of handling time on predator feeding efficiency is greatest for high prey densities. Furthermore, if predator population size is not determined by the density of a particular prey species, then with a long handling time, predation rates will not increase as a function of prey densities for any prey population larger than a few animals. It seems reasonable that *Pisaster* population size is constrained by parameters other than the density of *Trimusculus*. Thus, simply by increasing handling time, the mucous defense may help to make clumping an advantageous strategy for *Trimusculus*.

Another important consequence of this sort of defense is that it allows the limpet to deter predators without moving. This, along with the feeding mechanism described by Walsby (1975), allows *Trimusculus* to maintain its sessile life-style. Because of their lack of ctenidia, these limpets are probably not very efficient at respiring under water. Despite this, populations may be found as far down as -1.0 ft in the intertidal zone. Perhaps by maintaining a sessile life-style and making extensive use of mucus, which is energetically cheap to produce, *Trimusculus* is able to maintain a low enough energy budget that availability of oxygen does not significantly restrict its range.

Perhaps because its habitat (living upside down on the roofs of caves and undersurfaces of overhangs) makes it relatively inaccessible to many predators (such as birds), *T. reticulatus* has developed a predator-specific defense against seastars. Given that seastars are such important predators in intertidal ecosystems (Paine, 1966), it seems plausible that other gastropods might have evolved similar defenses. The closely related Australian pulmonate *T. connica* has many morphological and ecological similarities to *T. reticulatus* (Haven, 1973). In particular, *T. connica* appears to be sessile and to live in dense populations. Thus, it might well use a similar defensive strategy.

Another candidate for this sort of defense is the prosobranch *Acmaea mitra* (Rathke).

Margolin (1964a) reports that this limpet, though more mobile than *Trimusculus*, does not retreat from approaching seastars. In his experiments, seastars would initially not eat *Acmaea mitra* and would only do so after being exposed to the limpets for several days. Thus, non-poisonous chemical defenses among gastropods may be more varied than has been reported.

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