CHEMICAL DEFENCE OF THE SPECIALIST ASCOGLOSSAN *COSTASIELLA* SP. FROM THE INDIAN COST

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ABSTRACT

Extracts of the green alga *Avrainvillea erecta* and the gastropod *Costasiella* sp. deterred both herbivorous and carnivorous fishes and also the sea star *Protoreaster lineti*. This investigation demonstrates the ability of the ascoglossan, *Costasiella* sp., to sequester the repugnant metabolite from the chemically defended dietary alga *A. erecta* and employ the same to deter potential predators. The host plant specialisation protects the prey by sequestering the defensive metabolites as in the case of the *Avrainvillea-Costasiella* relationship and also through behavioural sequestering by the association of *Avrainvillea-Ampithoe* which ultimately lead into predator deterrence or avoidance.

INTRODUCTION

A widely accepted hypothesis suggests that avoidance or deterrence of natural enemies is a major factor selecting for host-plant specialisation among terrestrial insects (Price *et al.* 1980; Bernays & Graham 1988; Bernays 1989). Recent studies on marine herbivores also support this hypothesis (Hay *et al.* 1990; Hay 1992). In the marine system, ascoglossan (=sacoglossan) gastropods are the only group of herbivores with a high proportion of specialised feeders. They feed almost exclusively on a few genera of chemically defended green seaweeds (Jensen 1980; Faulkner & Ghiselin, 1983; Hay *et al.* 1990b). They sequester functional algal chloroplasts (Trench 1980; Stirts & Clark 1980), and also effectively use chemical deterrents sequestered from their hosts as defences against their own predators (Paul & Van Alstyne 1988a, b; Hay *et al.* 1990b; Gavagnin *et al.* 1994). Several authors have reported the association of specialist ascoglossans with *Halimeda macroloba*, *Caulerpa sertularioides*, *C. prolifera,* and *Avrainvillea longicaulis* (Lewin 1970; Paul & Van Alstyne 1988a; Hay *et al.* 1990b; Gavagnin *et al.* 1994). The present investigation is designed to unravel the functional relationship between the chemically defended green alga *Avrainvillea erecta* and the herbivores *Costasiella* sp. and *Ampithoe* sp. from the Indian Coast. This investigation forms the first and only report on the chemical ecology of opisthobranchs from the Indian coast.

MATERIALS AND METHODS

Study sites and organisms

The green algae *Avrainvillea erecta* (Berk.) Gepp is distributed at certain islands in the Gulf of Mannar region and found growing among seagrass meadows from the intertidal region to a depth of about 2 m.

The algae *A. erecta*, *Caulerpa scalpelliformis* (R.Br.) Web.V. Bosse, *Halimeda macroloba* Decaisne, *Udotea flabellum* Howe, *Ulva lactuca* Linn., and the seagrass *Enhalus acoroides* (L.f.) Royle were collected from the Krusadai Island (9°15' N; 79°12' E) in the Gulf of Mannar. The cryptic species *Costasiella* sp. (Opisthobranchia: Ascoglossa) was found in association with *A. erecta*. The amphipod, *Ampithoe* sp. is a tube-dwelling species also associated with the *A. erecta*. The macrophytes and ascoglossans were collected through SCUBA diving and snorkelling. The ascoglossans were gently separated from the algae and kept in a 2.5 l container with sea water for further experiments.

Preparation of extracts

Immediately after collection, the surface of *Avrainvillea* was blotted dry, cut into small pieces and preserved in diethyl ether. The *Costasiella* sp. was also preserved separately in diethyl ether and transported to the laboratory. Both species were separately extracted, thrice with diethyl ether. The extracts were pooled together and evaporated to dryness under reduced pressure at 40 ± 2 °C and stored at 4 °C.

Algal preference assay

Laboratory experiments were conducted to ascertain the most preferred diet of *Costasiella* sp. Six different species of marine macrophytes, available in the vicinity, *i.e.*, *Avrainvillea erecta*, *Caulerpa scalpelliformis*, *Halimeda macroloba*, *Udotea flabellum*, *Ulva lactuca*, and *Enhalus acroides* were subjected to preference assay. The thallus was cut into discs of 1 cm in diameter and placed randomly in a glass container with 1 litre of sea water. Groups of 15 ascoglossans were released into the experimental container. After 4 h, the presence or absence of number of ascoglossans on each piece of the plants were scored. This assay was carried out in 6 replicates. Statistical comparisons were made through one-way ANOVA with Duncan Multiple Range test.

Feeding deterrent assay

This assay was conducted by a method similar to Hay *et al.* (1988). *Ulva lactuca*, a highly palatable alga, was used in this manipulative experiment. This alga was cut into small pieces (290-300 mg) of uniform size (2 mm width) and were coated with diethyl ether extracts of *Avrainvillea erecta* and *Costasiella* sp. The control was coated with diethyl ether only. Three pieces each of treated and control thalli of *Ulva lactuca* were woven into 3 strands of 0.5 m length and 4 mm thickness white polypropylene lines. Three separate lines were prepared for the control, *Avrainvillea* and *Costasiella* extracts, and were placed 0.2 m apart. Fifteen lines of these triplets were placed in a protected area of the harbour about 0.5 m apart and at 1 m depth. Periodic monitoring of the experimental lines through snorkelling was carried out to ensure the grazing only through fishes. After 5 h, the ropes were retrieved and the amount of algae consumed was calculated. The results of the assay were analysed by the Wilcoxon Signed Ranks Test for paired comparisons.

In order to assess the palatability of alive *Costasiella* and its egg mass to the fish *Therapan jarbua*, 5 separate 2 litre containers with sea water were set up with one fish in each. Each fish was offered the ascoglossan and the egg mass individually for 6 times with a gap of 10 minutes each. The acceptance or rejection of the material was recorded.

Chemotactic response assay

The diethyl ether extracts of *Avrainvillea* and *Costasiella* were evaluated for chemotactic tube-foot response of sea star, *Protoreaster lineti* de Blainville. For evaluating chemotactic response the method reported by McClintock *et al.* (1994) was used with slight modification. The individual animal was placed onto the aboral side in a 30 cm diameter container with 3 litres of sea water. The diethyl ether extracts were mixed with an inert silicone grease in a 1:1 ratio (w/w). A round and smooth tipped glass rod was thinly coated with silicone grease-extract mixture and used as the tool to evaluate the tube-foot response. Initially a plain glass rod was used to elicit prodding motion of the tube foot and also acclimatise the animal to experimental conditions. Subsequently a plain silicone grease coated glass rod (control) was used to record the tube-foot retraction time. For each treatment the experiment was terminated after 1 min from the extruded tube-foot sensing the glass rod. The response to diethyl ether extracts of the algae and ascoglossan was tested, in 10 randomised trials. In most of the occasions the tube-foot located towards the tip of the arm was subjected to this assay. The observations were subjected to a Kruskal-Wallis one-way ANOVA for ranks followed by pairwise comparison with a Mann-Whitney U test.

RESULTS

Distribution of Avrainvillea and Costasiella

A. erecta is sparsely distributed around several islands of the Gulf of Mannar region. It grows within seagrass beds dominated by *Enhalus acroides* and *Cymadocia* sp. In addition several species of algae were also growing in this environment. An extensive survey on the occurrence of *Avrainvillea* revealed that an average of 2 plants m^{-2} was present in the Krusadai Island, where this investigation was carried out. It was also observed that one in seven individual algae harboured ascoglossans. The number of these molluscs varied from 1 to 5.

Algal preference of Costasiella

The ascoglossan appeared to live and feed exclusively on *Avrainvillea* and is cryptic in nature. On extensive search none of these molluscs were associated with any other algae growing in the vicinity. One-way ANOVA showed that there is a significant difference in the algal preference of the molluscs (F ratio = 64.6387, $P<0.01$), among the 6 species of macrophytes offered. Subsequently Duncan multiple range test revealed that the ascoglossans showed significant preference for *A. erecta* (Tab. 1).

Though the animals tend to settle on other algae, it was not permanent. If the molluscs were manually placed on *A. erecta* they did not shift the position in all occasions. But mostly they crawled out after being placed on other species of macrophytes.

Feeding deterrence:

Fig. 1 shows the results of the *in situ* experiments on the herbivore deterrent activity of the diethyl ether extracts of *A. erecta* and *Costasiella* sp. By the end of the experimental period, 84.15 % of the control alga (*U. lactuca*) was consumed. Only 44.88 % of the same palatable alga coated with diethyl ether extracts of *Costasiella* sp., and 19.11 % of *A. erecta* extract coated algae were consumed. This shows that the extracts of both *Costasiella* sp. and *A. erecta* had deterrent chemicals. Wilcoxon Matched-Pairs Signed-

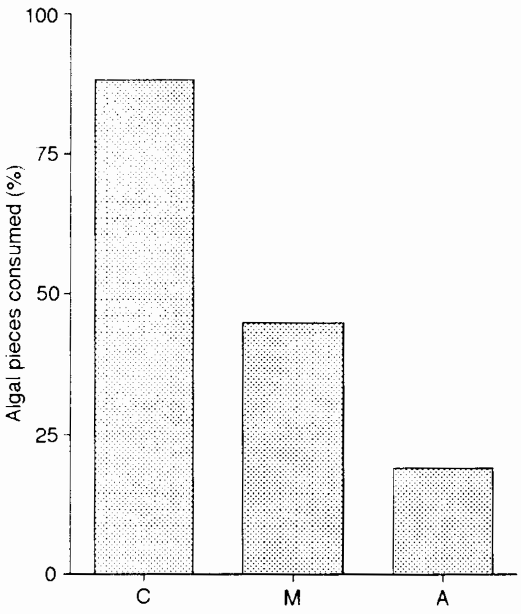

Figure 1. Feeding deterrent effects of the extracts of *Avrainvillea erecta* (A) and *Costasiella* (M) towards herbivorous fishes. C = control alga, *U. lactuca*.

Table 1. Patterns of algal selection by *Costasiella* sp. when given choice of six species of macrophytes. Means with the same subscript do not differ significantly.

Sl.No	Groups of macrophytes	Mean preference ± SE	Subscripts
1	*Caulerpa scalpelliformis*	0.50 ± 0.22	a, b
2	*Halimeda macroloba*	1.67 ± 0.49	b, c
3	*Udotea flabellum*	2.33 ± 0.42	c
4	*Ulva lactuca*	0.00 ± 0.00	a
5	*Avrainvillea erecta*	10.00 ± 0.86	d
6	*Enhalus acroides*	0.50 ± 0.34	a, b

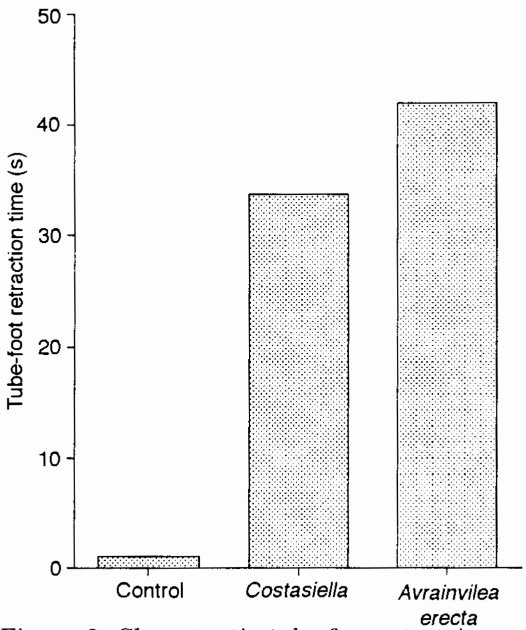

Figure 2. Chemotactic tube-foot retraction response (time) of the sea star, *Protoreaster lineti* to the extracts of *Costasiella* sp. (M) and *A. erecta* (A) compared to the control, silicone (C).

Ranks test assessing that the deterrent activity was significant for algal extract (Z = 5.8413, P<0.01) and the extract of ascoglossan (Z = -5.7171, P<0.01), when compared with the control. Likewise, the comparison of algae and ascoglossan also showed significant (Z = -5.0515, P<0.01) deterrence. Comparatively, the extract of *A. erecta* showed higher deterrence than that of *Costasiella* sp. The attempt made to assess the palatability of alive *Costasiella* and its egg mass for carnivorous fishes, resulted in the fish *Therapon jarbua* rejecting both the mollusc and egg mass after taking them into the mouth. On repeated offering on all occasions the fish did not try it again.

Chemotactic response

The tube-feet of the sea star showed significant retractile response to the extracts of *A. erecta* and *Costasiella* sp. Comparatively, *A. erecta* elicited stronger retraction (41.9 sec) than *Costasiella* sp. (33.6 sec) (Fig. 2).

Pairwise comparison made through Mann Whitney U test showed significant tube-foot repellence for algal (Z = -3.8649; P<0.01) and ascoglossan (Z = -3.8633; P< 0.01) extracts. A Kruskal-Wallis analysis of variance of ranks test also showed significant ability to retract (P<0.01) for both the extracts.

DISCUSSION

This investigation demonstrates the ability of the ascoglossan *Costasiella* sp. to sequester the repugnant metabolite from the chemically defended dietary alga, *A. erecta* and employ the same to deter potential predators.

The extracts of algae and ascoglossans deterred both the herbivorous and carnivorous fishes and also the sea star. The tube building amphipod, *Ampithoe* sp. received protection from the larger predators by associating with *A. erecta*.

Seaweeds are complex in structure and morphology (Taylor 1960) and synthesise diverse secondary metabolites (Faulkner 1988). The green alga *A. erecta* produces a brominated diphenylmethane derivative, avrainvilleol showing antibacterial, feeding deterrent, and ichthyocidal activities (Sun *et al.* 1983). Small herbivores can reduce predation if they are rarely encountered by predators, or if they are not attacked when encountered. By specialising on chemically defended plants that are seldom visited by herbivores, sedentary mesograzers may reduce encounters with herbivores. This strategy could become even more effective if the mesograzers were cryptic on their host plants.

Opisthobranch gastropods of the order Ascoglossa are among the widely investigated specialised marine herbivore gastropods. They are the only group of herbivores showing a high degree of feeding specialisation in the marine environment. They sequester both the defensive compounds and functional chloroplasts from marine algae. The present investigation revealed that *Costasiella* sp. tend to show significant feeding preference for *A. erecta*, though it had free access to other species of green algae. The unique radular structure and buccal anatomy vary among the ascoglossans and may relate to feeding success on different green algae (Jensen 1981; Clark & De Freeze 1987). Ascoglossans feed by sucking algal sap rather than by chewing. Most species were reported to feed on *Caulerpa* spp. or other chemically rich green algae (Jensen 1980, 1983), in the families Halimedaceae and Udoteaceae (*e.g.*, *Halimeda*, *Udotea*, *Penicillus*, *Chlorodesmis*, *Avrainvillea*). Natural history observations, laboratory feeding assays and chemical analyses suggest that most of the ascoglossans feed on one or a few related algae (Jensen 1980, 1984; Paul & Van Alstyne 1988a; Hay *et al.* 1989, 1990b). So the feeding specialisation of *Costasiella* sp. conforms to earlier reports on ascoglossans in general.

In spite of wide assumptions, that ascoglossans are defended from predation through the release of sequestered algal metabolites contained in mucus, while molested and also in the cerata, rigorous investigations on this behaviour are rare. In Guam, the ascoglossan *Elysia halimedae* appears to feed exclusively on the chemically defended algal genus *Halimeda*, and is most often associated with *H. macroloba*. Once consumed, the major metabolite of *Halimeda*, halimedatetraacetate, present in 1-2 % algal dry mass, is converted to a related diterpene alcohol that the ascoglossan sequesters as up to 7 % of its dry mass. This compound significantly deterred feeding both by herbivorous and carnivorous fishes (Paul & Van Alstyne 1988a).

In the Caribbean, the ascoglossan *Costasiella ocellifera* and the crab *Thersandrus compressus* were found only on the green alga *Avrainvillea longicaulis*, which produces a feeding deterrent, the diphenylmethane derivative avrainvilleol. The gastropod sequesters avrainvilleol from its algal host and uses it against the predators. But the crab doesn't sequester the defensive metabolite; however it is camouflaged when on *Avrainvillea* and thus also experiences less predation when associated with this alga (Sun *et al.* 1983; Hay *et al.* 1990b). Recent studies of two Australian ascoglossans specialising on the chemically defended green alga *Chlorodesmis fastigiata* showed that alga-sacoglossan-predator interactions are not always so straight forward (Hay *et al.* 1989; Roussis *et al.* 1990). One of the investigations showed that the sacoglossan was chemically defended; but its defence did not seem to be algae derived. The other study revealed that the sacoglossan sequestered algal metabolites, but it could not be established that an alga-derived compound was the cause of feeding deterrence to fishes.

As observed by Hay *et al.* (1990b), from the Caribbean coast, the present investigation from the Gulf of Mannar also shows that the specialist herbivore *Costasiella* sp. feeds exclusively on the chemically defended alga *A. erecta* and might have sequestered avrainvilleol, which deters both herbivorous and carnivorous fishes. But unlike Caribbean observation, *A. erecta* from the Indian coast do not harbour the crab *T. compressus*. Instead it has a tube-dwelling amphipod *Ampithoe* sp. living on it. The amphipod may not be sequestering the chemical defence, as it was readily consumed by the carnivorous fish when offered in the aquaria. So the association of *Ampithoe* sp. with *A. erecta* may favour it to avoid predation.

Similar to the occurrence of *Ampithoe* sp. on the Indian coast, the crab *Caphyra rotundifrons* on Australia's Great Barrier reef lives cryptic only on the chemically defended green alga *Chlorodesmis fastigiata*. But it does not sequester the feeding deterrent chlorodesmin (Hay *et al.* 1989). In addition, the amphipod *Pseudamphithoides incurvaria* lives in a mobile, bivalved domicile that it constructs from the chemically defended brown alga *Dictyota bartayresii*, which produces dictyol-class diterpenes, that deterred feeding by reef fishes (Lewis & Kensley 1982; Hay *et al.* 1990a). On line with the previous reports, it can be assumed that the herbivore *Ampithoe* sp., associated with *A. erecta*, also developed "behavioural sequestering" of algal chemical defences as the amphipod can not sequester it metabolically. Few species of opisthobranchs appear to allocate defensive metabolites to egg masses as a protective measure (Roesener & Scheuer 1986; Paul & Van Alstyne 1988a). So, the pale coloured egg mass laid by *Costasiella* sp. sequestered with the dietary metabolite would provide protection from predators.

Contact chemoreception has been reported in all asteroids (Sloan & Campbell 1982) and the echinoderm tube-feet are one of the primary sites for perception of chemical stimuli (Lawrence 1975; Sloan & Campbell 1982, McClintock *et al.* 1994). The dermal chemosensitivity in the tube-feet of asteroids is fundamentally a defensive response and probably evolved as a mechanism to protect the organism as it encounters significant alteration in its sensory environment (Sloan 1980). The significant tube-foot retractile response of sea star shows the presence of alarm substances or deterrent metabolites in the extracts of *Costasiella* sp. and *A. erecta*. It can be inferred that the defensive metabolite synthesised by the algae and sequestered by the mollusc could deter carnivorous predators too.

The *Costasiella-Avrainvillea* relationship is comparable with terrestrial plant-insect systems. *Costasiella* appears to be highly specialised for living with *Avrainvillea*, an alga avoided by most generalist predators and herbivores, and sequester both chloroplasts and toxins from its host and uses it for nutrition and defence. Similarly, many monophagous terrestrial insects living on chemically defended plants use defensive metabolites for locating their hosts and as feeding stimulants (Harborne 1978). In the present study, both laboratory trials and natural history observations showed that *Costasiella* sp. preferentially feed on *Avrainvillea*. This is probably due to the chemical cues originating from *Avrainvillea* which may also act as a feeding stimulant. The marine environment has few specialised feeders compared to terrestrial systems (Bernays 1989). Given the tremendous effects the herbivorous gastropods, sea urchins and fishes have on seaweeds (Lubchenco & Gaines 1981; Hay 1985; Carpenter 1986) most seaweed chemical defences probably evolved in response to diffuse herbivory (Fox 1981) from diverse types of herbivores and not in response to the few more specialised herbivores encountered.

The host plant specialisation protects the prey through sequestering of the defensive metabolites as in the case of the *Avrainvillea-Costasiella* relationship and also through behavioural sequestering by the association of *Avrainvillea-Ampithoe* which ultimately lead into predator deterrence or avoidance. These relationships clearly suggest that predation may be a prime selecting factor for feeding specialisation in marine communities.

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