

Intracolony variation in chemical defenses of the sponge *Cacospongia* sp. and its consequences on generalist fish predators and the specialist nudibranch predator *Glossodoris pallida*

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ABSTRACT: On Guam, the nudibranch *Glossodoris pallida* specializes in feeding on the branching sponge *Cacospongia* sp., which contains scalaradial and desacetylscalaradial as major secondary metabolites. The nudibranch, which accumulates both compounds for its own defense, is typically found on the base of the sponge. In this study, we quantified intracolony variation in the production of secondary metabolites (percent yield of crude extract, filtered extract, scalaradial, and desacetylscalaradial), structural material (fiber and ash content), and protein content in *Cacospongia* sp. in order to assess the consequences that such variation has on feeding responses of generalist fish predators, the specialist nudibranch predator *G. pallida*, and on the distribution of the nudibranch on the sponge. Levels of secondary metabolites varied among the parts of the sponge analyzed (tip, base, surface, and matrix). The mean percent yield of crude extract was higher in the surface of the sponge, while filtered extract and desacetylscalaradial were higher in tips than in bases. Structural materials were highest in the base and in the surface of the sponge. In contrast, the inner part of the sponge contained higher levels of protein than the surface, but no differences were found between tips and bases. Compared to a control, the crude extract of *Cacospongia* sp. significantly deterred fish feeding at the lowest concentration found in the sponge (base concentration) in field assays. When offered a choice between base and tip concentrations, both the pufferfish *Canthigaster solandri* in laboratory assays and the natural reef fish assemblage in the field showed no preference for extracts of bases over tips (i.e. lower levels over higher levels of chemical defenses). However, when offered a choice between pieces of sponge from the base and from the tip, the nudibranch *G. pallida* preferred bases over tips, which agrees with the nudibranch distribution on the sponge. Alternative factors may shape the observed distribution of nudibranchs on *Cacospongia* sp. Nudibranchs at the bases may suffer lower levels of predation because they are less accessible to predators than nudibranchs at the tips of the sponge. Therefore, we also tested whether small disks of artificial squid food located on tips and bases of the sponge are equally accessible to generalist fish predators. The reef fish *C. solandri* in laboratory assays and the natural fish assemblage at the *Cacospongia* sp. community consumed equal amounts of food from tips and bases. Our data demonstrate that *Cacospongia* sp. extracts deter feeding by fishes at the lowest concentration found in the sponge, which suggests that the higher concentration in the tips is not a mechanism to avoid consumption by generalist fish predators. In contrast, avoidance of higher levels of chemistry appears to be the factor behind the selection of bases over tips by the specialist nudibranch predator *G. pallida*. Intracolony variation in chemical defenses seems to determine the nudibranch distribution on the sponge.

KEY WORDS: Chemical defenses · Within-colony variation · Antipredatory role · Generalist versus specialist predators · *Glossodoris pallida* · *Cacospongia* sp.

INTRODUCTION

Studies on terrestrial chemical ecology have widely documented variation in the production of chemical compounds associated with numerous physical and

biological factors (see reviews by Rhoades & Cates 1976, Rice 1984, Cates & Redak 1988, Bryant et al. 1991 among others). Chemical variation in marine environments has received less attention. General patterns of production of chemical defenses in organisms

from different communities or geographical regions have been documented (Coll et al. 1982, Pawlik et al. 1987, Uriz et al. 1991, Steinberg 1992, Harvell et al. 1993). However, evidence for intraspecific variation in the production of chemical defenses is still scarce. The importance and need for such studies have been pointed out (Hay 1996). Studies on intraspecific chemical variation are valuable for understanding the factors affecting the production of chemical defenses, as well as providing insights into the ecological consequences and evolutionary implications of such variation. Intraspecific patterns of variation have been reported over temporal (Turon et al. 1996), between-colony (Maida et al. 1993, Becerro et al. 1995), or within-colony (Paul & Van Alstyne 1988, Harvell & Fenical 1989, Van Alstyne et al. 1994, Turon et al. 1996) scales. Although other functions cannot be ruled out, most data available support an antipredatory role of secondary metabolites (Paul 1992) and point to predation as one of the factors affecting intraspecific variation (Hay 1996).

The study of within-colony variation in chemical defenses is particularly interesting from a predator-prey point of view since it determines the actual levels of defenses that predators encounter at the 'bite' level. Chemical defenses are often concentrated in the outer layers of the producers (Tugwell & Branch 1989, Uriz et al. 1995, Turon et al. 1996), the least structurally defended regions (Harvell & Fenical 1989, Meyer & Paul 1995), and biologically valuable regions (such as growing and reproductive regions; Hay et al. 1988, Paul & Van Alstyne 1988, Tugwell & Branch 1989, Meyer & Paul 1992, 1995). These data are consistent with an antipredatory role of secondary metabolites in nature (Paul 1992, Hay 1996). However, when variation has been reported and specifically tested for an effect against consumers, data do not always support the antipredatory role of secondary metabolites. For example, quantification of the secondary metabolites in 3 *Caulerpa* spp. showed that uprights have higher levels of compounds than runners (Meyer & Paul 1992). This distribution was consistent with the optimal defense hypothesis (Feeny 1976, Rhoades 1979): the most valuable (photosynthesis, sexual reproduction, highest energetic value) part of the alga, which also is the most exposed to macroherbivores, is the most defended. However, feeding experiments failed to support this hypothesis, since the levels of secondary metabolites had no consequences on herbivorous reef fishes (Meyer & Paul 1992).

The tropical sponge *Cacospongia* sp. provides a particularly good system for the study of intracolony variation in defenses and its consequences on predators. First, *Cacospongia* sp. inhabits coral reefs, an ecosystem known for its high levels of predators including

generalist and spongivorous fish predators (Jones et al. 1991). Furthermore, *Cacospongia* sp. is preyed upon by the nudibranch *Glossodoris pallida*. On Guam, this nudibranch specializes in feeding on *Cacospongia* sp., on which it is exclusively found (Rogers & Paul 1991). Second, *Cacospongia* sp. has a variety of sesquiterpenes that may be responsible for the deterrent properties of the sponge (Rogers & Paul 1991). In fact, *G. pallida* sequesters the major metabolites scalaradial and desacetylscalaradial from the sponge and, at the average concentration in nudibranchs, scalaradial deters predation by crabs and fishes (Rogers & Paul 1991, Avila & Paul 1997). Third, *Cacospongia* sp. displays an erect, branching morphology. Variation in defenses at an individual level occurs across broad phylogenetic groups with such morphology, the general trend being a decrease in the production of chemical defenses from distal to basal portions (algae: Hay et al. 1988, Meyer & Paul 1995; gorgonians: Harvell & Fenical 1989, Van Alstyne et al. 1992; soft corals: Wylie & Paul 1989; trees: Coley 1983). Whether this distribution occurs in sponges, and specifically in *Cacospongia* sp., is unknown. To this effect, it is worth noting that the specialist nudibranch predator *G. pallida* is confined to the base of the sponge, a distribution that could be caused by chemical variation within *Cacospongia* sp. In this study we quantified the levels of secondary metabolites, specifically the 2 major metabolites scalaradial and desacetylscalaradial, within colonies of *Cacospongia* sp. We also quantified structural material and protein content since chemical, structural and nutritional characteristics of organisms affect predation (Duffy & Paul 1992, Pennings et al. 1994, Schupp & Paul 1994). Our main goal is to address the consequences that intracolony variation in *Cacospongia* sp. has on (1) feeding responses of generalist fish predators and the specialist nudibranch *G. pallida* and (2) the distribution of the nudibranch on the sponge.

MATERIAL AND METHODS

Study site and organisms. *Cacospongia* sp. (Family Thorectidae, Order Dictyoceratida) (hereafter *Cacospongia*) was collected at Sponge Mound, a pinnacle in Apra Harbor, Guam, USA (13° 25' N, 144° 55' E). On this pinnacle, *Cacospongia* occurs at depths between 20 and 25 m, where it is among the most dominant sponges in the community (Thacker et al. in press). This sponge is an undescribed *Cacospongia* species (M. Kelly-Borges pers. comm.) with branching morphology due to its erect, thick, and compressible lobes. Maximum size is under 40 cm high. The surface of *Cacospongia* is gray-black while the sponge matrix and fibers are golden.

Glossodoris pallida is a dorid nudibranch that on Guam is exclusively found on *Cacospongia* [Rogers & Paul 1991 (sponge identified as *Hyrtios erecta*), Avila & Paul 1997]. Maximum size is under 10 mm long. *G. pallida* is typically found at the base of the sponge feeding on the surface and leaving behind fibers, which are exposed to the exterior of the sponge. Other possible predators in Sponge Mound include generalist fish species such as *Canthigaster solandri*, *Cheilinus fasciatus*, *Cheilinus trilobatus* and *Zanclus cornatus*.

Intracolony variation: analysis of chemical and structural components. *Cacospongia* has a variety of sesterterpenes that seem to play an antipredatory role in nature (Rogers & Paul 1991, Avila & Paul 1997). In laboratory and field tests on Guam, at Fingers Reef the extract of *Cacospongia* was deterrent while it had no effect in field tests at Haps Reef (Rogers & Paul 1991). Several compounds isolated from the crude extract were also tested with surprising results: scalaradial was deterrent, scalarin stimulated feeding, and a 1:1 mixture of scalaradial:scalarin was highly deterrent (Rogers & Paul 1991). Scalaradial and desacetyl-scalaradial are sequestered by *Glossodoris pallida*, which incorporates them in the mantle for its own defense (Avila & Paul 1997). Scalaradial also deterred feeding by crabs (Avila & Paul 1997). We analyzed intracolony variation in the production of secondary metabolites in *Cacospongia* by quantifying the amount of scalaradial and desacetyl-scalaradial as the major metabolites in the sponge sequestered by the nudibranch, but we also quantified filtered extract to measure all relatively nonpolar materials in the sponge and crude organic extract to account for the more polar lipids as well. We quantified these 4 variables in tissues from bases and tips of the branches ($n = 9$), and choanosome and ectosome of the sponge ($n = 5$). We call tip tissues those in the 3 most distal cm of the branches and base tissues those in the 3 most basal cm of the bottom of the sponge. Ectosome refers to the grey-black outer layer of the sponge while choanosome refers to the golden inner matrix. We obtained ectosome and choanosome tissues from the middle portion of the sponge, which refers to the zone between tips and bases.

Sponge tissues were individually soaked in a 1:1 mixture of dichloromethane:methanol for about 2 to 3 h. This was repeated 3 times to assure the sponge was exhaustively extracted (extracts of sequential extractions compared by TLC, thin layer chromatography). The extracts were pooled, filtered, dried in a rotary evaporator, and weighed. Extracted *Cacospongia* tissues were dried at 50°C for 48 h and weighed. Percent yield of crude extract was calculated as the mass of extract divided by the total mass of dried sponge tissue plus crude extract. To prepare filtered extracts,

crude extracts were dissolved in 25:75 ethyl acetate:hexanes and filtered through short (1 to 2 cm) silica gel columns, dried, and weighed. Percent yield of filtered extract was calculated as the mass of filtered extract divided by the total mass of dried tissues plus crude extract. All samples followed the same procedure and comparative TLC showed that the column retained the most polar compounds in the crude extracts, while scalaradial and desacetyl-scalaradial were totally eluted by the ethyl acetate:hexanes mixture.

To quantify scalaradial and desacetyl-scalaradial, filtered extracts were dissolved in 25:75 ethyl acetate:hexanes at a concentration of 1 mg per 20 μ l. Ten μ l of this solution was injected into a quantitative high performance liquid chromatography system (HPLC, column: Econosphere[®] silica 5 μ , 250 \times 4.6 mm, eluant: 25:75 ethyl acetate:hexanes, refractive index detection). Peak areas were calculated by a Beckman 427 integrator. Amounts of the 2 compounds were determined by comparison to standard curves calculated from known concentrations of the pure compounds. Percent yield of scalaradial and desacetyl-scalaradial was calculated as the amount of compound divided by the total mass of dried tissues plus crude extract.

We also quantified protein, fiber, and ash content in bases, tips of the branches, choanosome, and ectosome of the sponge as an estimate of the nutritional value of these parts and the allocation to structural materials. Five sponges were frozen immediately after collection, freeze-dried, and weighed (dry mass). Freeze-dried tissues were then introduced for 1 wk into a 25:75 mixture of ammonium hydroxide (30% solution):hydrogen peroxide (3% solution) to remove non-structural materials (Uriz pers. comm.), dried, weighed (structural mass), and placed into a muffle furnace at 450°C for 48 h to obtain ash mass. Fiber content was calculated as percentage of fiber mass (obtained by subtracting ash mass from total structural mass) per dry mass. Ash content was calculated as percentage of ash mass per dry mass.

We used a standard procedure (NaOH-soluble protein content; Bradford 1976) to quantify the protein content in the sponge tissues. Briefly, tissues from 5 sponges (10 to 15 mg of freeze-dried material) were individually digested in 5 ml of a 1 N NaOH solution. After 12 h, 100 μ l of the resulting protein-NaOH mixture was added to 5 ml of Bio-Rad Protein Solution and absorbance analyzed in a DU[®]-65 Beckman spectrophotometer at $\lambda = 595$ nm. Protein percentage was calculated by comparison to a calibration curve run with standards. Standards were prepared by adding 100 μ l of NaOH with known quantities of bovine serum albumin to 5 ml of Bio-Rad Protein Solution.

For the statistical analyses, base versus tip of the branches and choanosome versus ectosome were com-

pared within each sponge and tested for significant differences by paired *t*-tests or Wilcoxon's signed-ranks test when data did not meet parametric assumptions. Differences in structural components and protein content among tip, middle portion, and base per sponge were analyzed by a 2-way analysis of variance, factors being sponge (block) and zone within sponge. Systat 5.2 for Macintosh was the software used for the analyses.

Intracolony variation: effects on potential predators. Field feeding experiments: To determine whether *Cacospongia* extracts deter fish feeding at the lowest concentration found in the sponge, we conducted a field feeding deterrence experiment using methods similar to those used by Meyer & Paul (1995). We added 0.75 g of extract (in 2 ml of ethyl acetate) from the bases of 5 sponges to an artificial diet consisting of 5 g of ground catfish pellets (Kruse's Perfection Brand), 2.5 g of carrageenan and 80 ml of water (10% extract concentration per dry mass, which mimics the mean concentration in the base of the sponge). Control foods were prepared by adding 2 ml of solvent to the carrageenan-food diet. The diet, which is 19% protein by dry mass and has a dry mass/volume ratio of 0.11 g ml⁻¹, approximates the protein content and dry mass/volume of the sponge (14% and 0.13 g ml⁻¹ respectively). The mixture was poured into 1 cm³ molds containing a rubber O-ring, which allowed the use of safety pins to attach cubes to ropes. Each rope contained either 4 control or 4 treated food cubes. We placed 20 pairs of control and treated ropes on the reef of Western Shoals, Apra Harbor, Guam. The pairs were removed when approximately half of the cubes were eaten in any of the treatments. We used Wilcoxon's signed-ranks test for paired comparisons to test for significant differences in the number of cubes eaten in the 2 treatments.

We also tested for the feeding preference of the natural assemblage of reef fishes at Western Shoals for extracts in the tips and bases of *Cacospongia*. To run this experiment, we used the methods described above. The only difference was that instead of a control treatment we used a tip treatment by adding 1.20 g of extract from the tips to the artificial diet described above (16% extract concentration, which mimics the mean concentration of the tips of the sponge). We quantified the number of cubes eaten by fishes in the tip and base treatments and used Wilcoxon's signed-ranks test for paired comparisons to test for significant differences between treatments.

Laboratory feeding experiments: The pufferfish *Canthigaster solandri* was offered a choice between an artificial diet containing *Cacospongia* extract from tips and an artificial diet containing *Cacospongia* extract from bases at their natural concentrations. *C.*

solandri is a generalist predator that feeds on benthic algae, ascidians, and other invertebrates (Amesbury & Myers 1982). It also adapts well to laboratory conditions and has previously been used to test deterrent properties of *Cacospongia* and *Glossodoris pallida* (Rogers & Paul 1991). A total of 14 sponges were collected and their tissues from tips and bases individually extracted. We added 60 mg of tip extract from one sponge to 250 mg of ground catfish pellets, 125 mg of carrageenan and 4 ml of water (16% dry mass concentration). After mixing all components, the mixture was poured into a mold backed with fiberglass window screening to form a small strip that covered 120 squares of the window screening. The identical protocol was followed with 37.5 mg of base extract from the same sponge (10% concentration), which was poured next to the tip treatment. Both tip and base food treatments from the same sponge were placed in 9.5 l flow-through tanks and offered as a choice to an individual pufferfish. Strips were removed when approximately half of the squares were eaten in any of the treatments or after 24 h. We used paired *t*-tests to test for significant differences in the percentage of squares eaten in the 2 treatments.

Nudibranch distribution: To test whether the nudibranch *Glossodoris pallida* actively selects bases over tips, we used seventeen 500 ml flow-through tanks containing a small (4 to 6 cm³) piece of the tip and of the base of the same *Cacospongia* specimen. Each piece was located in opposite corners of the tanks. Seventeen nudibranchs were individually introduced into the tanks and placed in the middle of each tank. Many nudibranchs fed on the first piece of sponge they found, but many others passed by the sponge pieces several times. We recorded a nudibranch choice as the first piece of sponge which a nudibranch fed on, independent of whether it was the first piece of sponge a nudibranch contacted or not. Data were compared to a binomial distribution ($n = 17$, $p = q = 0.5$) to test for a significant preference.

Our goal was to test whether this distribution was due to significant differences in the chemical composition between bases and tips. However, a direct test for this hypothesis was unfeasible because of the specificity in the feeding response of nudibranchs. Artificial diets, agar plates with *Cacospongia* extracts, or even extracted *Cacospongia* in which *Cacospongia* extracts were re-incorporated proved unsuccessful methods of feeding the nudibranchs. We wondered whether factors extrinsic to the sponge could be relevant for the distribution of the nudibranch, specifically whether predators have equal accessibility to nudibranchs on tips and bases of *Cacospongia*. To test this hypothesis, we measured levels of consumption by fish of an artificial squid diet designed to mimic nudibranch shape,

size, and color placed on tips and bases of *Cacospongia*. We used nudibranch mimics because *Glossodoris pallida* is partially defended against predators and is mobile, factors that may hinder the feasibility of our experimental design. Nudibranch mimics were made by adding 35 g of fresh, ground squid to 50 ml of a 5% carrageenan solution. After heating and mixing, the squid solution was spread on a 3 mm thick mold to form a squid sheet from which small disks (6 mm in diameter) were made. We placed 6 disks on tips and bases of *Cacospongia* specimens and quantified their consumption by fish in both laboratory and field experiments.

In a laboratory experiment, each of five 115 l aerated aquaria with running sea water was haphazardly assigned a sponge and a specimen of the pufferfish *Canthigaster solandri*. The experiment was run over 24 h, after which we evaluated the amount of material eaten in the disks by counting the number of visible squares within a disk when placed over a grid of fiberglass window screening. Data (percentage of squares eaten) met the assumptions for parametric analyses and were analyzed by 2-way mixed model analysis of variance (ANOVA), factors being aquarium (random factor) and zone (fixed factor; levels: tip and base).

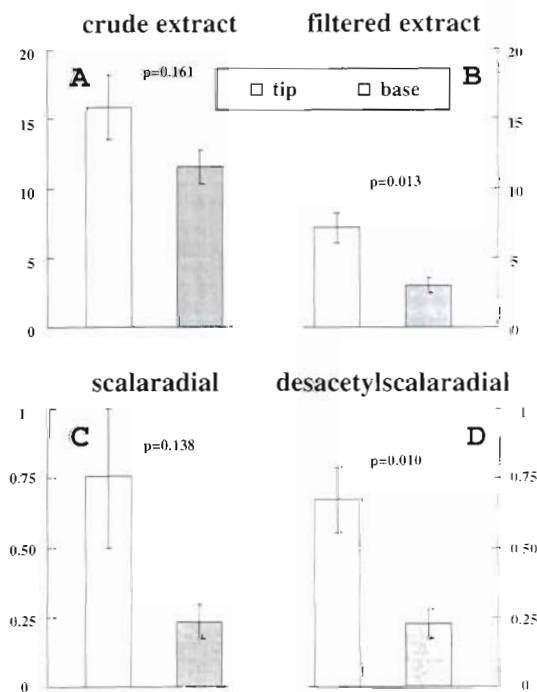


Fig. 1 Mean percent yield per dry mass (± 1 SE) of (A) crude extract, (B) filtered extract, (C) scalaradial, and (D) desacetylscalaradial in tissues from tips and bases of *Cacospongia* specimens ($n = 9$). Probabilities calculated by paired t -test (crude and filtered extract) or Wilcoxon's signed-ranks test (scalaradial and desacetylscalaradial). Two-tailed p -values reported. Note changes in scale among graphics

In a field experiment, 5 sponges were placed at Sponge Mound, where *Cacospongia* and *Glossodoris pallida* naturally occur. The experiment was run over 30 min, after which we evaluated the amount of material eaten in the disks as explained previously. Data (percentages) met the assumptions for parametric analyses and were analyzed by 2-way mixed model ANOVA, factors being specimen and zone.

RESULTS

Distribution of chemical and structural components

Tips of *Cacospongia* have significantly higher mean percent yields of filtered extract and desacetylscalaradial than bases ($t = -3.154$, $p = 0.013$ and $Z = 2.547$, $p = 0.010$ respectively; Fig. 1B, D), while differences for mean percent yields of crude extract and scalaradial were not statistically significant ($t = -1.542$, $p = 0.161$ and $Z = 1.481$, $p = 0.138$ respectively; Fig. 1A, C). Compared to the choanosome of the sponge, the ectosome also has higher mean percent yield of crude extract ($t = -5.104$, $p = 0.006$; Fig. 2A) while filtered extract ($t = -1.339$, $p = 0.251$), scalaradial ($t = 0.829$, $p = 0.453$), and desacetylscalaradial ($t = -0.596$, $p = 0.583$) have similar mean percent yields.

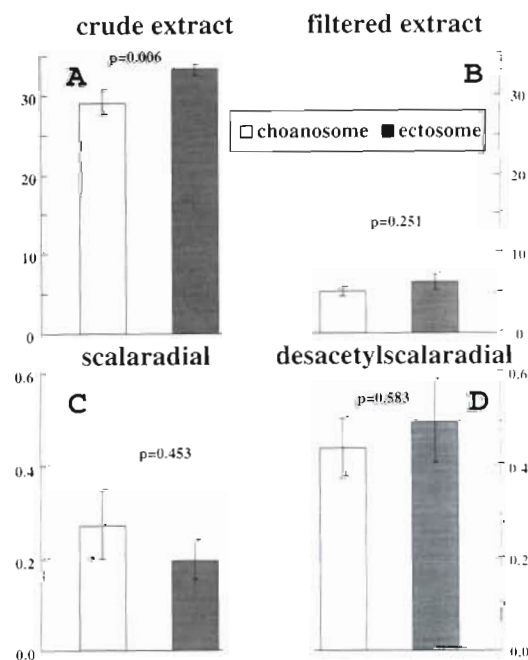


Fig. 2 Mean percent yield per dry mass (± 1 SE) of (A) crude extract, (B) filtered extract, (C) scalaradial, and (D) desacetylscalaradial in tissues from choanosome (matrix) and ectosome (surface) of *Cacospongia* specimens ($n = 6$). Probabilities calculated by paired t -test. Two-tailed p -values reported. Note changes in scale among graphics

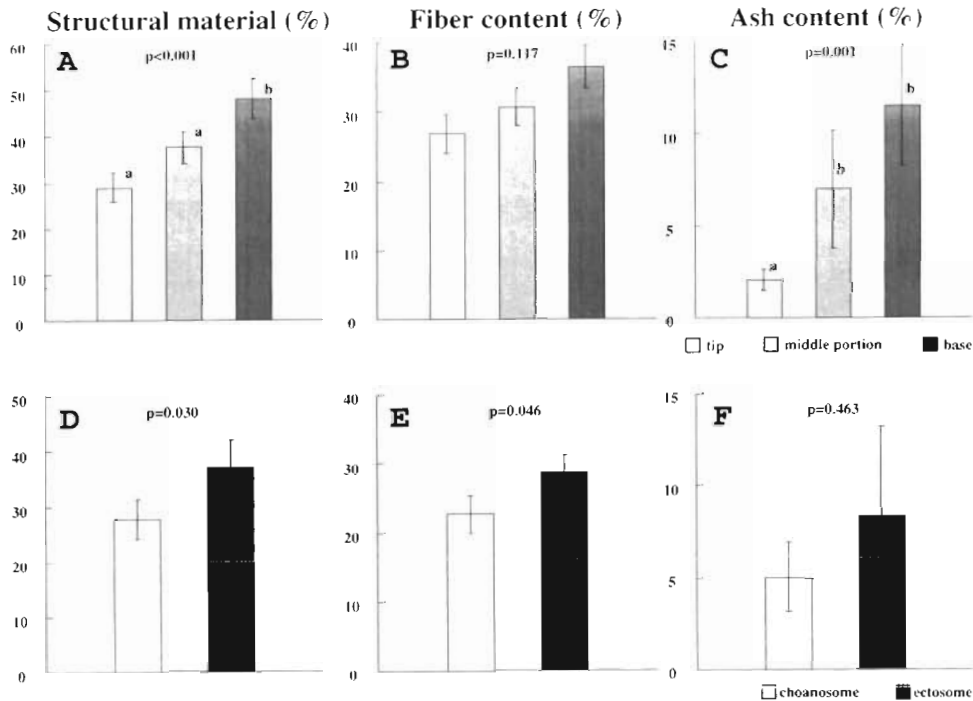


Fig. 3. Mean percentage per dry mass (± 1 SE) of (A) structural mass, (B) fiber content, and (C) ash content in tip, middle, and base sponge parts. Analyzed by 2-way ANOVA (blocked by sponge) followed by Tukey's. Bars sharing the same letters above them (a, b) do not differ at $\alpha = 0.05$. (D) Structural mass, (E) fiber content, and (F) ash content in choanosome (matrix) and ectosome (surface) of the sponge. Probabilities calculated by paired *t*-tests (structural mass) or Wilcoxon signed-ranks test (fiber and ash content). Two-tailed *p*-values reported. Note changes in scale among graphics

Compared to the chemical variables, structural materials followed a contrasting pattern (Fig. 3). In a gradient from tips to bases, significant differences are found in structural mass and ash content ($F = 15.669$, $p < 0.001$ and $F = 14.008$, $p = 0.001$ respectively; Fig. 3A, C) while fiber content remains constant throughout the sponge ($F = 1.214$, $p = 0.117$; Fig. 3B). In the ectosome versus choanosome comparison, higher levels of structural mass ($t = -2.990$, $p = 0.030$; Fig. 3D) and fiber content ($Z = -1.992$, $p = 0.046$; Fig. 3E) are found in the ectosome of the sponge while ash content remains constant ($Z = 0.734$, $p = 0.463$; Fig. 3F).

Similar levels of protein are present in tips, middle portions and bases ($F = 1.049$, $p = 0.386$; Fig. 4A), while the matrix of the sponge shows significantly higher protein content than the surface ($t = -2.630$, $p = 0.046$; Fig. 4B).

To correct for the possible effect that differences in structural material among parts of the sponge may have in measuring the concentration of compounds (i.e. higher concentrations of compounds may reflect the higher organic content of different parts of the sponge), we calculated the mean percent yield of the 4 chemical variables per ash free dry mass by dividing their percent yield by their corresponding ash free dry mass percentage. Although mean percent yields

changed, results are basically the same as before with significant differences for filtered extract and desacetylscalaradial in the tip-base comparison (crude extract: tip = 16.19 ± 2.35 , base = 13.03 ± 1.38 , $t = -1.071$, $p = 0.315$; filtered extract: tip = 7.29 ± 1.09 , base = 3.33 ± 0.62 , $t = -2.835$, $p = 0.022$; scalaradial: tip = 0.77 ± 0.26 , base = 0.26 ± 0.07 , $t = -1.821$, $p = 0.106$; desacetylscalaradial: tip = 0.69 ± 0.12 , base = 0.25 ± 0.06 , $t = -3.328$, $p = 0.010$). In the choanosome-ecto-

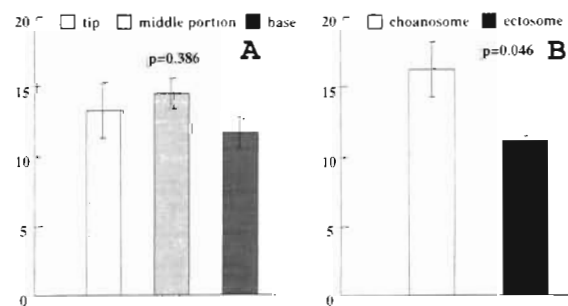


Fig. 4. (A) Mean percentage per dry mass (± 1 SE) of protein content in tip, middle, and base sponge parts. Analyzed by 2-way ANOVA. (B) Mean percentage per dry mass (± 1 SE) of protein content in the choanosome (matrix) and ectosome (surface) of the sponge. Probability calculated by paired *t*-tests. Two-tailed *p*-values reported

some comparison, significant differences are again limited to the crude extract (crude extract: choanosome = 30.55 ± 1.55 , ectosome = 36.31 ± 0.74 , $t = -6.762$, $p = 0.002$; filtered extract: choanosome = 5.42 ± 0.53 , ectosome = 6.94 ± 1.05 , $t = -1.547$, $p = 0.197$; scalaradial: choanosome = 0.29 ± 0.08 , ectosome = 0.21 ± 0.05 , $t = 0.744$, $p = 0.498$; desacetylscalaradial: choanosome = 0.46 ± 0.07 , ectosome = 0.54 ± 0.10 , $t = -0.766$, $p = 0.486$).

Effects on potential predators

Feeding experiments

Compared to controls, *Cacospongia* extracts significantly deterred feeding by the reef fish assemblage (control = 3.05 ± 0.26 , treatment = 1.95 ± 0.35 , $Z = -2.139$, $p = 0.032$). The reef fish population showed no preference between food pellets containing natural concentrations of *Cacospongia* extracts from the base and food pellets containing natural concentrations of *Cacospongia* extract from the tips of the branches (tip = 2.31 ± 0.31 , base = 1.62 ± 0.27 , $Z = -1.396$, $p = 0.162$). Laboratory feeding experiments gave similar results; the pufferfish *Canthigaster solandri* also showed no preference between tip and base treatments (tip = 38.10 ± 6.99 , base = 48.45 ± 4.56 , $t = -1.527$, $p = 0.150$).

Nudibranch distribution

In laboratory experiments, the nudibranch *Glossodoris pallida* significantly preferred bases over tips (tip: 4, base: 13, $p = 0.024$, binomial distribution). The pufferfish *Canthigaster solandri* ate similar percentages of squid disks located on tips and bases of *Cacospongia* specimens (tip = 41.66 ± 7.42 , base = 41.66 ± 7.89 ; ANOVA: zone, $F < 0.001$, $p = 1.000$; aquarium, $F = 2.647$, $p = 0.184$; zone \times aquarium, $F = 1.161$, $p = 0.339$). Field experiments showed similar results; the natural fish assemblage of Sponge Mound consumed the same amounts of food from tips and bases of *Cacospongia* specimens (tip = 56.67 ± 9.20 , base = 43.45 ± 9.18 ; ANOVA: zone, $F = 0.611$, $p = 0.478$; sponge, $F = 1.160$, $p = 0.445$; zone \times sponge, $F = 1.948$, $p = 0.117$).

DISCUSSION

The production of chemical defenses is one of a number of mechanisms by which benthic species can reduce the effect of predators (Bakus et al. 1986, Hay &

Fenical 1988, Paul 1992). However, it is not just the presence and concentration of chemical defenses but the variability in the producer which constitutes the basis for the complexity of the predator-prey interaction (Laca & Demment 1991). Although quantification of the levels of secondary metabolites in marine benthic species has received increasing attention, the lack of information on intraspecific variation in chemical defenses and its ecological consequences is notable and the need for such studies has been already pointed out (Hay 1996). In our study, we provide evidence for intracolony variation in chemical defenses in the branching sponge *Cacospongia* sp. and assess the consequences of such variation on generalist fish predators and the specialist nudibranch predator *Glossodoris pallida*.

Few chemical studies have quantified the levels of compounds within or among individuals. Most studies have focused on algae, gorgonians, and soft corals (Hay et al. 1988, Paul & Van Alstyne 1988, Harvell & Fenical 1989, Meyer & Paul 1992, 1995, Van Alstyne & Paul 1992, Harvell et al. 1993, Maida et al. 1993, Yates & Peckol 1993, Cronin & Hay 1996). Sponges produce a large number of unique natural products (Faulkner 1997 and previous reviews). Some studies have examined intraspecific variation in secondary metabolites (Thompson et al. 1987, Teeyapant & Proksch 1993, Chanas et al. 1996), but within-sponge variation remains almost uninvestigated (Unson et al. 1994, Bewley et al. 1996).

Although intracolony variation in secondary metabolites in sponges could be expected, our study is the first to quantify levels of compounds at a within-individual scale, providing a first estimate of the chemical variance at this level. We measured about 2 times more desacetylscalaradial in the tips of the branches than in the base of the sponge. While a similar trend was found for scalaradial, the variance for this compound was quite large. Whether this level of variation is present in other sponges is unknown. Medium-size, sciaphilous specimens of the Mediterranean sponge *Crambe crambe* are 2 times more toxic than small, photophilic specimens (Becerro et al. 1995), while the outer layer of this encrusting sponge is 5 times more toxic than its choanosome (Uriz et al. 1996). Unfortunately, the relationship between levels of toxicity and concentration of secondary metabolites is not clear, which hinders the comparison of variances between both sponges. Variation at the individual scale has been reported for other groups (Phillips & Towers 1982, Cronin & Hay 1996, de Nys et al. 1996).

Our results support previous observations on intracolony variation of defenses of branching organisms. Concentrations of secondary metabolites are generally higher at distal portions in algae, soft corals, gorgoni-

ans, and trees (Coley 1983, Harvell & Fenical 1989, Wylie & Paul 1989, Meyer & Paul 1995, de Nys et al. 1996, but see Van Alstyne & Paul 1992). Structural materials usually follow a contrasting pattern, decreasing in concentration from the distal end to the base. Since structural components can also deter predators (Harvell et al. 1988, Van Alstyne et al. 1992, Pennings et al. 1996), the negative relationship between structural and chemical defenses is often interpreted as a trade-off in allocation to both defenses (Sammarco et al. 1987, Harvell & Fenical 1989, Meyer & Paul 1995), although different predation pressures may also explain such a pattern (Schupp & Paul 1994, Pennings et al. 1996). In *Cacospongia* sp., there is significantly more structural material in the base than in the distal part. Accordingly, secondary metabolites seem to be concentrated in the distal part of the sponge, where structural material is less abundant. However, levels of both chemical and structural components (although not the secondary metabolites we analyzed) are higher in the surface than in the inner part of the sponge. Similarly, sciaphilous specimens of the sponge *Crambe crambe* invest more in both chemical defenses and structural material than their photophilic counterparts (Becerro et al. 1995, Uriz et al. 1995).

Few studies have evaluated the ecological consequences of intracolony variation in chemical defenses. The amphipod *Amphitoe longimana* and the sea urchin *Arbacia punctulata* preferred young apices over older middles of their diet alga *Dictyota ciliolata* (Cronin & Hay 1996). Secondary metabolites were significantly higher in the middles, which seems to explain the feeding preference of the herbivores since plant morphology and toughness proved irrelevant (Cronin & Hay 1996). The green alga *Neomorisa annulata* has significantly higher concentrations of secondary metabolites in tips than in bases (Meyer & Paul 1995). Food containing *N. annulata* extracts at tip concentration deterred feeding by herbivore fishes while it failed to deter feeding at base concentration (Meyer & Paul 1995). However, intra-plant variation may have no consequences on some herbivores (Meyer & Paul 1992). In our study, field experiments show that *Cacospongia* extracts deterred feeding by the natural reef fish community at the lowest concentration found in the sponge (base concentration). However, when offered a choice between food containing *Cacospongia* extract at base and tip concentrations (i.e. low and high concentration of secondary metabolites), the pufferfish *Canthigaster solandri* in laboratory experiments and the natural fish community in field experiments showed no preference for bases over tips.

On Guam, the nudibranch *Glossodoris pallida* exclusively feeds on *Cacospongia*, where it can be found in small groups (Rogers & Paul 1991). Nudibranchs are

generally found near the sponge base, a distribution that can be formed by both intrinsic (nutritional value, defenses) and extrinsic (differential predation rates on base and tip) sponge characteristics. In laboratory experiments, *G. pallida* significantly preferred bases over tips. Structural defenses and nutritional factors may shape this distribution, but they seem unlikely in this study. Higher levels of structural material are found in the base of the sponge. When feeding, nudibranchs leave behind the structural material, which is exposed to the exterior and can be easily observed in the field. Sponge structural material has been shown to play a role against predation (Uriz et al. 1996, but see Chanas & Pawlik 1995, 1996), besides reducing the nutritional value of the sponge (McClintock 1987). Protein content does not seem to be responsible for the nudibranch distribution on the sponge, since similar protein levels were found throughout *Cacospongia*. The higher levels of compounds in the tips provide the strongest explanation for the distribution of *G. pallida* on the sponge. Scalaradial and desacetylsclerodiol are 2 major metabolites of the sponge and both of them are incorporated by the nudibranch for its own defense (Rogers & Paul 1991, Avila & Paul 1997). Levels of desacetylsclerodiol are higher in tips than in bases, although whether or not this compound alone is responsible for the feeding behavior of the nudibranch is unknown.

Factors extrinsic to the sponge may also shape the distribution of the nudibranch on the sponge. Nudibranchs at the tips of the sponge may be more accessible to predators than nudibranchs at the bases of the sponge. Although *G. pallida* is chemically defended (Rogers & Paul 1991, Avila & Paul 1997) and there is no need for an associational defense (Hay 1996), the sponge may prevent predators from reaching nudibranchs at the bases and contribute to the distribution of the nudibranch on the sponge. The actual levels of consumption of nudibranchs by fish predators are unknown but our data show that fish predators reach the base of the sponge and consume the same amount of food in tips and bases, which fails to support differential consumption rates as a factor affecting the distribution of the nudibranch on the sponge.

Many marine organisms produce secondary metabolites that may play an antipredatory role. However, it is the variation in their production, the concentration of chemical defenses in the parts of the animal exposed to predators, which determines whether or not an organism is eaten. We have quantified different levels of secondary metabolites and structural material within the branching sponge *Cacospongia* sp. The ecological consequences of such variation are difficult to ascertain. Generalist predators seem to be unaffected by the different levels of compounds, while variation in

defenses within *Cacospongia* sp. seems to be responsible for the distribution of *Glossodoris pallida* on the sponge. Whether chemically defended sessile organisms can deter not only generalist predators but also specialist predators from feeding is unknown. In our opinion, *G. pallida* could be a first example of a true specialist predator deterred from feeding by the chemistry of the sponge it lives on exclusively. Our data also show that prey can present predators a variable environment of nutrients and defenses. Investigating this pattern of variation and its consequences on predators will provide insights into predatory-prey interactions.

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