COMMENTARY

Chemical or nematocyst-based defence in the nudibranch Cratena peregrina? – a reply to B.K. Penney

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Aguado & Marin (2007) analysed the interaction between Cratena peregrina (Gmelin, 1791) and predatory fish in laboratory and field assays, using both live aeolids and artificial models. Penney’s comment (2009) on the article reopens the old question of the role of nematocyst-based defence in nudibranchs. Penney suggests that the defensive mechanism of C. peregrina is chemical because the nematocysts used in assays could be unarmed. Penney argues that (1) Aguado & Marin (2007) obtained nematocysts by macerating Eudendrium hydroids with a mortar and pestle, and this cannot be considered equivalent to kleptocnidia isolated from C. peregrina; (2) the method by which the authors attempted to incorporate nematocysts into the test food is unclear; and (3) regardless of how nematocysts were added to the artificial food models, it is possible that no functional nematocysts would remain by the time fish encountered them.

The main purpose of our article was to demonstrate that fish learn to avoid the warning coloration pattern of C. peregrina, due to associating bad taste or unpleasant experience with the colour pattern. Nematocysts are contained in the defensive exudates and in the external part of the body of C. peregrina, which can only be regarded as circumstantial evidence that these defensive cells or the molecules that they contain represent a deterrent for predators. Of course, the article does not provide experimental evidences that the source of deterrence is the nematocysts, but there is no proof, either, that chemical defences play an active role. If we support the general idea that a positive result for deterrence alone cannot be taken as evidence for a nematocyst-based defence, the same argument must apply to chemical-based defence. We must classify nematocyst-based defences as a special form of chemical defences because nematocysts contain harmful molecules. From this point of view, C. peregrina transfers chemicals from food to the cnidosacs at the tip of the ceras.

There is no evidence that C. peregrina contains secondary metabolites other than the nematocyst toxins. Chemical analysis supports the general hypothesis that the nematocysts are the main weapons. Cimino et al. (1980) studied three species of the hydroid Eudendrium (E. rameum, E. racemosum, E. ramosum) in the Bay of Naples (Italy), which are prey of C. peregrina. These authors found the same pathway of polyhydroxylated steroids (Cholest-4-en-4, 16β, 18, 22R-tetrol-3-one 16,18-diacetate) in the hydroids and in the nudibranchs. Unfortunately these steroids do not play a defensive role in hydroids or nudibranchs. Some aeolids contain pigments and secondary metabolites from their prey, which probably have no chemical defence role either (e.g. Goodwin & Fox, 1955; McBeth, 1972: the compounds are ubiquitous and conserved amongst numerous non-deterrent species; Cimino et al., 1980: the compounds are located within internalized structures and thus not quickly encountered by predators). Sequestered compounds can occur at concentrations lower than threshold feeding deterrent levels (Karuso, 1987; Rogers & Paul, 1991). Thus, metabolite accumulations within opisthobranchs may represent a mechanism to store toxic chemicals with impunity for subsequent elimination, and not necessarily to defend the organism (Pennings & Paul, 1993).

Penney suggests that because E. carrnenum from North American possesses chemical extracts that deter feeding by pinfish, extracts from Eudendrium employed with models of C. peregrina could be unpalatable because they contain chemicals and not active nematocysts. Stachowicz & Lindquist (2000) indicated that crude extracts from Eudendrium deterred feeding, ‘demonstrating’ that defensive chemistry plays a significant role in the unpalatability of these species. To test their hypothesis that unpalatable secondary metabolites represent alternative antipredator defences for hydroids, they compared the palatability of intact polyps with those in which the nematocysts had been chemically stimulated to discharge. Hydroid polyps defended solely by nematocysts should become palatable when their nematocysts are discharged before offering them to predators, whereas the palatability of chemically defended hydroids should not change after treatment. However, neither the lipophilic (DCM + butanol soluble compounds) nor the water-soluble fractions of Eudendrium deterred feeding. These authors suggested that there is an additive or synergistic effect among compounds in the two fractions, or that the deterrent compounds decomposed during the partitioning of the crude extract. In my opinion these results do not demonstrate that defensive chemistry plays a significant role in the unpalatability because toxins contained in nematocysts could also act as a chemical deterrence. If there truly are active chemicals in Eudendrium, the lipophilic and the water-soluble fractions must be clearly deterrent to fish, but this is not the case. Secondary metabolites contained in the discharged nematocysts could be responsible for fish rejection.

The biosynthesis of defensive metabolites is costly for organisms. It is not surprising that species showing de novo biosynthesis of defensive metabolites use these metabolites for other purposes. This is the case in the nudibranch Tethys fimbria, which synthesizes prostaglandin derivates (Marin, Di Marzo & Cimino, 1991). The structural variety of the lactones and the data on their distribution in the body of T. fimbria suggest a range of different biological functions: to contract smooth muscle fibres, defence allomones of the ceratal secretion, and in basic physiological functions (e.g. ion regulation, renal function and reproductive biology). These arguments do not mean that other Aeolidoidean nudibranchs should also contain defensive metabolites. The aeolid nudibranch Phyllodesmium guamensis feeds on the soft corals Sinularia maxima and S. polydactyla. Phyllodesmium guamensis sequesters a diet-derived diterpene 11β-acetoxyypukalide selectively within various body parts. Levels were highest in the cerata, with moderate and low to non-existent levels in the mantle and viscera, respectively. Laboratory tests showed that 11β-acetoxyypukalide deters feeding by the puffer fish Canthigaster solandri at a given concentration (0.5% dry mass), while feeding field experiments with...
nudibranch tissues exhibited species-specific reactions to the nudibranch tissues and their isolated metabolite, which ranged from non-deterrence to emesis (Slattery et al., 1998). Even if active deterrent metabolites are present in C. peregrina, it is very unlikely that chemical defence is the sole defensive mechanism, because retaining functional nematocysts must be energetically expensive. Several studies in opisthobranchs have indicated that chemical and physical defences commonly co-occur and can function either additively, or synergistically, to reduce susceptibility to predators. In the nudibranch Doris verrucosa, several defensive strategies occur at the same time. This nudibranch is perfectly mimetic in its habitat and its mantle is protected by spicules and by the presence of two toxic molecules, verrucosin-a and -b (Cimino et al., 1988). Many algae and invertebrates display redundant defence mechanisms against predation (Hay et al., 1994; Schupp & Paul, 1994). Nudibranchs utilize both physical (spicules, tunic toughness) and chemical (secondary metabolites, acidity) defences and suffer relatively little predation by generalist predators.

The method by which we incorporated nematocysts into the test food was clearly described: ‘the artificial models were bathed in hydroid sauce’ (Aguado & Marín, 2007). We used the word impregnation incorrectly because it suggests that the hydroid sauce entered and spread completely through the artificial food. The Eudendrium sauce was applied as a coat to the outside of models, because if nematocysts were mixed with the artificial food before solidification the high temperature could have degraded them. This method was chosen because nematocysts are not released into the cnidosac of the aeolid, but into large epithelial cells called cnidophages. The release of nematocysts from aeolids is caused by a contraction of the muscle complex surrounding the cnidosac, and entire cnidophages are released (Greenwood & Mariscal, 1984). These cnidophages are forced towards the ceras tip where they are pushed into a cellular matrix and through the epithelium, before being released from the ceras. The application of isolated nematocysts to artificial food might be considered inappropriate, because it does not reflect the defensive mechanism of aeolid nudibranchs. The coating of artificial models with hydroid sauce is more realistic, because it simulates the expulsion of the entire cnidophage in the aeolids, rather than a bioassay with isolated nematocysts. It is also suggested that any nematocysts that might have adhered to the models could be washed off when the models came into contact with the water of the aquarium or field site and that it is possible that no functional nematocysts would remain by the time fish encountered the models. Penney’s criticism is in error, because our personal observation indicates that a high percentage of nematocysts remains undischarged in the models. However, just a small percentage of active nematocysts might be sufficient to deter fish in the bioassays with artificial models. In addition, Martin (2003) in a study of the management of nematocysts in C. peregrina found that large proportions of these nematocysts were undischarged in the alimentary tracts of the snails. This author even describes that the faeces from snails contained large numbers of undischarged nematocysts that appeared structurally intact. Nematocysts were isolated from the sea anemone Calliactis tricolor maintained under different feeding schedules or in different degrees of salinity, in an attempt to determine how these culture conditions influence the discharge of isolated nematocysts. The nematocysts isolated from individuals from two separate populations of C. tricolor responded significantly differently to 5 mM EGTA and to de-ionized water, and these responses also depended upon the isolation solution used. This study provides evidence that the environment or feeding state of an anemone affects the discharge of isolated nematocysts. Inherent differences in ionic and osmotic characteristics among nematocysts could explain some of the ambiguities when comparing past studies of isolated nematocyst discharge (Greenwood et al., 2002). Penney also suggests that nematocysts obtained by macerating Eudendrium cannot be considered equivalent to kleptocnidae isolated from C. peregrina. However, Martin (2003) in an exhaustive study of C. peregrina found, when feeding on polyps of the hydrozoan Eudendrium, that phagocytosis of nematocysts and food particles by digestive cells is rather non-selective. In the course of over two years of this study there was no evidence of selective exclusion of specific nematocyst types by cnidophagous cells.

REFERENCES


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