COMMENTARY

A comment on F. Aguado & A. Marin: 'Warning coloration associated with nematocyst-based defences in aeolidioidean nudibranchs'

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In their paper concerning defences of the nudibranch *Cratena peregrina* (Gmelin, 1791), Aguado & Marin (2007) assert in the title and text that the deterrence observed is due to the kleptocnidae of the nudibranchs. However, the source and treatment of the nematocysts used in assays make this unlikely, and a positive result for deterrence alone cannot be taken as evidence for a nematocyst-based defence. Kleptocnidae have often been presumed to be defensive (Edmunds, 1966; Harris, 1973; Thompson, 1976; Todd, 1981), but direct observational and experimental evidence to support this is lacking (Todd, 1981; Miller & Byrne, 2000). Many nudibranchs possess chemical defences (Avila, 1995; Cimino & Ghiselin, 2001) and the relative importance of chemical *vs* kleptocnidal defence has been debated since the discovery that some nudibranchs possessed active nematocysts (Harris, 1973). Assays using whole nudibranchs cannot distinguish between these possibilities (Edmunds, 1966; Hand, 1994–1996) and for several reasons Aguado and Marin's experiments with models also fail in this regard.

First, the authors obtained nematocysts by macerating *Eudendrium* hydroids with a mortar and pestle (Aguado & Marin, 2007) and this cannot be considered equivalent to kleptocnidae isolated from *C. peregrina*. Many nudibranchs sequester only particular nematocyst types from their prey and may keep nematocysts from several prey species simultaneously (Harris, 1973; Todd, 1981; Frick, 2003). *Eudendrium* is only one of many prey species reported for *C. peregrina* (McDonald & Nybakken, 1997) and at least one species of these hydroids is known to deter fish via chemical defence rather than nematocysts (Stachowicz & Lindquist, 2000), so *C. peregrina* may not obtain nematocysts useful against fish from this prey species. Also, some nematocysts discharge in response to mechanical stimulation (Hessinger, 1988) such as grinding with mortar and pestle. While a greater percentage discharge when appropriate chemical factors are present (Kass-Simon & Scappaticci, 2002), we do not know whether different nematocyst types are more likely to discharge with mechanical stimulation alone. Beyond this, studies of antipredator defence should ensure that the treatment used in the bioassay matches the concentration normally found in the organism of concern (Hay *et al*., 1998). The authors do not describe how they attempted to match concentrations of *Eudendrium* extract with concentrations found in the average slug. Therefore, the method used by Aguado and Marin leads to isolation of a subset of nematocysts that may not represent the cnidome seen in *C. peregrina* in the field.

Second, the method by which the authors attempted to incorporate nematocysts into the test food is unclear; they describe two very different methods, one which might denature nematocysts and the other where nematocysts may not stay on the artificial food for the assays. On page 24 they state that the artificial food models were 'made distasteful by impregnation with nematocysts,' suggesting that nematocysts were mixed throughout the entire volume of artificial food, presumably before it solidified. The artificial food recipe used includes boiling water (Aguado & Marin, 2007). The potential alteration of nematocyst discharge with temperature change is not well investigated (McKay & Anderson, 1988), but nematocysts are composed of a number of proteins crucial for their function (Tardent, 1995; Kass-Simon & Scappaticci, 2002) and boiling water denatures proteins, so it seems unlikely that any nematocysts so incorporated would be functional. Even if they were, many nematocysts would be buried within the food too deeply to be effective. Conversely, on page 25 the authors state that the artificial food models were 'bathed in hydroid sauce' in order to add nematocysts. If this was the case, it is quite possible that any nematocysts that might have adhered to the models could be washed off when the models contacted the water of the aquarium or field site. There is no indication that the authors checked the models after preparation to ensure functional nematocysts were included.

Third, 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. Nematocysts isolated from their cnidocytes discharge differently from those still *in situ* (Thorington & Hessinger, 1988), and in many cases they discharge upon contact with seawater (Todd, 1981; Martin, 2003). Therefore, all nematocysts added may have already discharged once the food was introduced to the aquaria or water at the field site.

Taken together, these concerns suggest that the antipredator deterrence observed by Aguado and Marin may stem from a factor other than nematocysts, and a chemical defence seems possible. Application of extract to an artificial food before solidification or as a coat on the outside of such food are both proven methods for testing chemical defence against predators (Hay *et al*., 1998). Given the aforementioned chemical defence of a North American *Eudendrium* species (Stachowicz & Lindquist, 2000), this seems a quite likely explanation for Aguado and Marin's results. However, we cannot be certain that the chemical defence found in the *Eudendrium* extract used is the same as any chemical defence that *C. peregrina* might have. Other nudibranchs are known to modify dietary metabolites or to synthesize their own chemical defences *de novo* (Avila, 1995; Cimino & Ghiselin, 2001; Cimino & Gavagnin, 2006); although this ability has not yet been documented in aeolids, chemical defence in general has not been as well-investigated in this group as in other nudibranchs (Cimino & Ghiselin, 2001) and biosynthetic origin of defensive compounds may not have been explicitly tested.

Aguado and Marin's paper nicely shows that *C. peregrina* is deterrent to fish predators and that fish can associate shape and colour with deterrent factors, and their conclusion that defences could arise via individual selection has been noted previously (Penney, 2004). However, a true test of the defensive efficacy of kleptocnidae awaits the ability to separate this factor from other potential defences in manipulative experiments.

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