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# Diet-based defensive secretions in Harvestmen

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by

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**Abstract**

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Diet-Based Defensive Secretions in Harvestmen

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Harvestmen are known to secrete a wide range of defensive chemicals in order to protect themselves from predators. An earlier study examined the phylogenetic pattern of defensive secretions produced by 22 species of harvestmen. This research, however, assumed that there is a genetic link between the defensive secretions. I wished to determine whether harvestmen defensive secretions may be diet-based by introducing several irritants into their food and then testing their secretions in the same manner. I performed a GC-MS analysis on 13 samples from the *Vonones sp*. But I found no initial GC-MS readings that showed this species of harvestmen to contain any irritants in their secretions. The absence of irritants does not allow any evaluation of whether harvestmen secretions are genetically or dietarily based.

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## Introduction

Many organisms produce defensive secretions in order to ward off predators, but the source of these secretions may vary considerably. For example, 28 species of parasitic alloxystine wasps were shown to produce genetically based defensive secretion with the same chemical patterns in their mandibular secretions regardless of their host (Hubner et al. 2001). An alternate method of utilizing defensive secretions can be seen in the larvae of leaf-feeding beetles (*Neolema sexpunctata*). These beetles use primary and secondary host-derived compounds attained from their diets as a chemically deterrent shield (Morton 1998). In my thesis I chose to examine the origin of defensive secretions produced by harvetmen.

Harvestmen (Class Arachnida, Order Opiliones, Subphylum Chelicerata, Phylum Arthropoda) are eight legged creatures related to spiders. Harvestmen generally forage on dead insects at night and reside under logs during the day. Many harvestmen produce chemicals to defend themselves against predators. Harvestmen emit a gut liquid along with a stored irritant in the form of two globs on either side of their body (Hara and Gnaspini, 2003; Schultz, 2000). These defensive emissions are emitted from a pair of scent glands positioned at laterofrontal angles to the cephalothorax (Clawson 2005). Harvestmen quickly dab this secretion onto their legs in order to ward off certain predators. This secretion is capable of deterring ants, but not larger predators such as wolf spiders (Machado 2005).

**Defensive Secretions**

In a recent study of the defensive secretions of harvestmen, Hara et al. (2005) attempted to group harvestmen phylogenetically according to the chemical compositions of their secretions. From 22 species of Gonyleptidae harvestmen, Hara et al (2005) characterized 37 defensive compounds. Many compounds were present in the spectrum of harvestmen families, but the three most common are methylbenzoquinones: 2,3-dimethyl-1,4-benzoquinone, 1,4-benzoquinone, and 2,3,5-trimethyl-1,4-benzoquinone (Eisner 1977). It is of note that two of these compounds are solid at room temperature and must be kept together in order to remain a liquid within the harvestmen. This storage combination is seen in eight different species of harvestmen. The compounds were then mapped onto phylogenetic trees of the species proposed by Roach et al (1980) and Duffield et al (1981). Hara et al (2005) found that several of the compounds were produced in multiple, distantly related species. Hara et al (2005) proposed that the production of several compounds had evolved independently in different lineages. There is, however, an alternative possibility that seems more likely: that defensive secretions are diet based instead of genetically determined. My thesis research aimed to test this hypothesis.

Thus, although Hara et al. (2005) admittedly had scarce data and a scattered phylogenic tree, they raised the possibility of a larger study being more conclusive on connecting harvestmen species based upon defensive secretions. Our study conversely aims to prove that defensive secretions are diet based instead of genetically pre-determined.

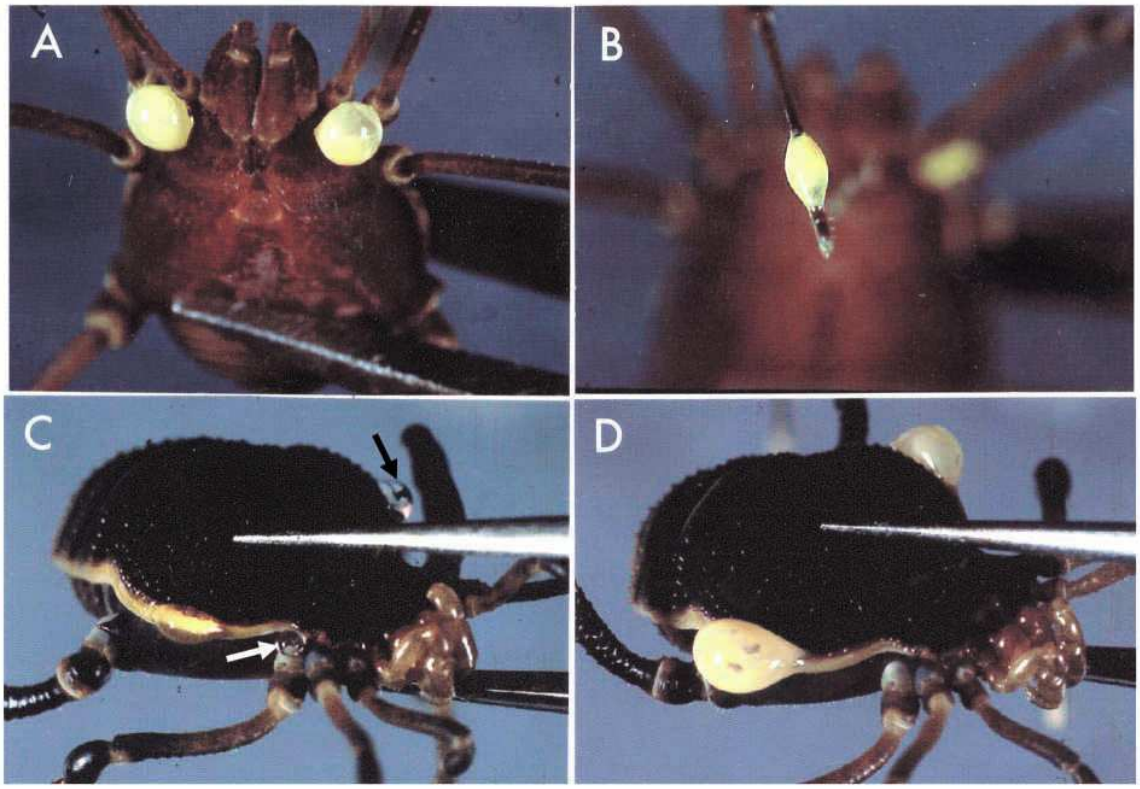

Figure 1 – *Vonones sayi* secreting a milky white defensive secretion (Eisner 2004)

## Methods

In order to test this hypothesis, a single species will be examined first for a baseline reading of the compounds, and then after it has ingested another common defensive secretion. This should show whether harvestmen can sequester different irritant compounds after ingestion. Several complications could arise with this experiment because the secretion of multiple methylbenzoquinones is thought to be necessary for the storage of them in their liquid states, but some species have bypassed this requirement (Hara et al., 2005).

### Harvestmen Care

James Wetterer collected the harvestmen under logs in Stuart, Florida and identified them as *Vonones ornata*. I maintained approximately 30 *Vonones ornata* in a terrarium for two months, feeding them a diet of non-quinone containing ants and mealworms. The terrarium was kept moist to simulate the typical harvestmen environment. After the secretions are analyzed, I planned to feed these harvestmen a diet of irritant containing mealworms. This would be accomplished by first finding a similar irritant to the one(s) excreted. The irritant would be fed by setting up several small dishes containing mealworms and a dilute solution of irritant and water. The concentrations would most likely have to be varied depending on the irritant but will range between 1mM and 1µM solutions. The harvestmen would be given two weeks to adequately ingest enough irritant so that it too can be excreted. Other irritants might also need to be attempted if the first proves to be incompatible with the harvestmen.

Figure 2 –*Vonones ornate* < *http://bugguide.net/node/view/12435*>

**Secretion Extraction**

I extracted defensive secretions were extracted by gently applying pressure dorso-ventrally and then micro-pipetting the secretion, typically between one and four microliters. I then transferred the secretions to individual vials and added 100 µL of $CHCl_2$. The secretions were emitted clear and remained clear even if the subject was repeatedly aggravated or if pressure was applied for up to two minutes. This is disconcerting because harvestmen have been known to emit clear gut liquid and then inject their irritant from a separate gland, making the secretion dark, cloudy, or milky (Eisner 2004).

Two other secretion extraction methods reported in the literature were freezing and dissecting the harvestmen (Hara et al. 2005). I froze three individuals but no secretion was released as in the reported text. Micro-dissection proved too hard due to the harvestmen's size and lack of micro-dissection tools. However, one harvestman was dissected into quadrants and the anterior quadrants were run after immersion in dichloromethane.

**GC-MS**

Gas chromatography-mass spectroscopy is a powerful tool that analyzes a liquid sample for a variety of compounds based upon retention time and quantity of the molecules. The gas chromatograph portion separates molecules based upon retention times. Separated molecules are then passed on to the mass spectrometer in a series of bands for analysis. The mass spectrometer analyzes molecules by ionizing the molecules and detecting fragmentation patterns based on their mass to charge ratio. The GC-MS outputs a series of retention time peaks and computer based approximations of what compounds are present.

Two sets of five samples were run in the NAME* at TEMP RANGE*. Each sample contained only a single individual's secretion. Before each set a blank of dichloromethane was run for reference. Major peaks were analyzed using the computer's built-in compound recognition system which operates by making educated guesses as to a compound's identity based on mass fragmentation patterns. A third set of samples was run containing another blank,

a concentrated collection of ten harvestmen secretions, and two solutions containing soluble components of the anterior quadrants.

## Results

Unfortunately, the initial GC-MS attempts proved fruitless. Of the fourteen samples run, none contained any of the reported irritants found in other harvestmen, or any related quinones/phenols. The only common substance in multiple samples was iron monocarbonyl but this proved to have originated from impurities in the solvent. Even the two samples with the dissected quadrants lacked any clear peaks.

## Discussion

I detected no defensive chemicals in the secretions of all the harvestmen tested. The lack of secretion detection could be due to multiple factors. First and foremost the GC-MS had recently undergone major repair the week before the samples were run. Calibration runs did show that the machine was working properly, but after major repairs all of the issues may not be fully worked out. Two other possibilities are that the harvestmen either did not ingest any irritant with which to secrete or the species *Vonones ornata* does not release irritant and the gut liquid is an artifact. Although Eisner et al. (2004) claimed that harvestmen can hold dozens of doses of irritant, it is possible that the sample harvestmen did not retain their irritant over the two months they were kept and fed non-irritant containing ants and mealworms. If this species typically does secrete quinones then their lack of quinones after isolation for two months may serve as proof that the irritants are diet-based rather than genetically synthesized.

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