Chemical communication and ecology in Eupnoi harvestmen

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Abstract

Communication via chemical signals and cues is a widespread modality in animals. Producing, transmitting, receiving and processing chemical compounds impose important challenges. Nonetheless, certain arthropods rely almost exclusively on this channel for intra- and inter-specific communication. Through a preliminary literature review, I summarize here the morphological, behavioral and evolutionary implications of chemical communication in harvestmen (the arachnid order Opiliones), with particular emphasis in one group: the Eupnoi or “Daddy Long-legs”. This group has a unique secretory gland that opens in an ozopore in their dorsum. While relying mostly on short-range olfaction and contact chemoreception using different setae, some harvestmen are known to use chemicals in defense, alarm, spatial marking, recruitment, or reproduction. I then propose future research direction on the mechanisms of production and the evolutionary history of these traits. Specific questions can include (1) are chemical signals used as alarm pheromones in Eupnoi aggregations? (2) Do harvestmen rely on chemicals to mark their traditional roosting sites? If so, what are the chemicals involved and how do those differ from the ones used as alarm signals? Also, (3) what are the specific functions of the chemical communication during in female-male communication and multi-specific roosting aggregations? And (4) to what extent the use of chemical defenses imposes a trade-off with other defense mechanisms such as ‘playing dead’ or voluntarily releasing legs. Overall, these harvestmen provide a unique opportunity to test comprehensive and interdisciplinary hypotheses to understand the evolution of chemical communication, as well as the importance of chemical ecology on species diversification in arthropods.

Key Words: Arachnida, Daddy Long-legs, defenses, Opiliones, perception.
I. Chemical communication in terrestrial environments

Communicating via chemical channels is challenging. However, this modality is thought to be the most ancestral form of transmitting signals and cues (Bradbury & Vehrencamp 2011).

Molecules have a long path in these channels; organisms need to produce them, either metabolically or aided by other organisms, or sequester them from the environment. After synthesis, chemical compounds have to be stored, secreted, sent, and received (Agosta 1992). Transmission can occur using current flows of air or water, by simple diffusion, or by deposition on the substrate. Finally, received chemicals are to elicit neural responses and cognitive process in the receiver.
Animals in terrestrial environments use either the airflow intended for olfactory reception, and diffusion or direct deposition for contact reception to transmit their pheromones (those chemicals intended for conspecifics) and allelochemicals (interspecific communication). Substrate-born signals (or cues, depending on the context) have environmental constraints, including the lack of temporal patterns and modulation (when compared with auditory and visual channels), as well as the necessity for the receiver to physically contact the pheromone source. Such pheromones are physiologically costly, and important trade-offs between their composition, shape, molecular weight, and other chemical properties modulate how the compounds persist, are transmitted and received in different topographic, climatic and stratigraphic environments (Bradbury & Vehrencamp 2011). Lastly, the use of localized and sessile signals is risky, because the signaler can be followed by an eavesdropping predator.

II. Chemical communication in arthropods

Chemical communication in invertebrates is associated with social interaction, including reproduction. For instance, ants have numerous and highly context-specific glands and pheromones (Hölldobler & Wilson 1990). Pseudo-social or no-social invertebrates have fewer glands, which are used mostly in species recognition, and reproductive tasks. In arachnids, chemical communication plays an important role in intra- and inter-specific interaction. In spiders, this topics has been recently reviewed (Uhl & Elias 2011, Fischer 2019). Hence, this review aims to focus on a particular group within the arachnid order of Opiliones, the Eupnoi. Nonetheless, I will extract available information on all Opiliones to summarize our current understanding of this topic.

In general, spider use chemicals to locate and evaluate potential mates, avoid predators and attract prey by mimicking prey such as ants (revision in Uhl & Elias 2011). During male-female interaction, both can recognize age and geographic precedence, suggesting the specificity of the chemical signals. Surprisingly, the mechanisms and structures used to produce pheromones are mostly unknown in spiders (Uhl & Elias 2011). Glands and spigots associated with silk production, and the silk itself, are thought to convey information. Research has focused on the sensory biology in spiders, especially in the physiological and neuro-ethological aspects of
receiving and processing mechanical stimuli involved in foraging (Barth 2002). As for reception, tip pore sensilla in distal regions of legs and pedipalps are thought to perceive stimuli.

Certain clades of spiders are specialized in social life and rely on chemical communication to distinguish between prey and conspecific, and even differentiate the latter in familiar and unfamiliar individuals, which mediates agonistic behavior within the colony (Trabalon 2013). However, solitary spider species also use chemical channels to mediate aggressive behavior. For instance, theraphosids change the cuticular component ratios throughout maturation, which increases their level of aggression (revision in Trabalon 2013).

Other arachnid orders have similar chemical communication mechanisms. Although they do not produce silk, harvestmen, scorpions, pseudoscorpions and other arachnids possess morphological and physiological adaptations that assist in producing, emitting and receiving chemical signals.

III. Harvestmen systematics and biology

With over 6500 currently described species, the order Opiliones have four suborders (from the basal to the more derived clade, and in increasing order of number of species): Cyphoptalmi, Dypsnoi, Eupnoi and Laniatores (Giribet & Kury 2007, Giribet & Sharma 2015). The presence of secretory gland and their respective lateral opening, the ozopore, are synapomorphies of harvestmen. With more than two-thirds of those species, and notorious reproductive and ecological processes, Laniatores have been the most studied group. Interestingly, Eupnoi harvestmen are a widely distributed group with distinctive life history traits, such as grouping behavior and autotomy, the voluntary release of legs (Machado & Macías-Ordoñez 2007, Escalante et al. 2013, Domínguez et al. 2016).

Harvestmen are considered to be well defended despite lacking venom or silk. Morphological, behavioral and chemical channels allow them anti-predator defense on different contexts (day/night, active/roosting, solitary/aggregated) (Machado & Macías-Ordoñez 2007). Traditionally, hierarchical “lines of defense” are thought to mediate their defense depending if the interaction with predator escalates. Their defenses include aposematic coloration and grouping, the hard spines and exoeskeleton, giving a pinch with the coxae and phemora or
chelicerae, performing thanatosis, stridulations within cheliceral segments, and lastly the secretions of chemicals irritants (Gnaspini & Cavalheiro 1998, González et al. 2004, Pomini et al. 2010). Additionally, a trade-off between those strategies could allow species to rely more on certain means of defense (Machado & Pomini 2008).

Chemical signaling is perhaps the best channel for communication for some harvestmen (Machado & Macías-Ordoñez 2007). This because most harvestmen are nocturnal, have long slender antenniform legs, and lack associated structures to perform visual or acoustic communication. Also, since grouping behavior occur in certain clades, harvestmen can rely on a combination of chemical cues from feces, pheromones, substrate impregnated with conspecifics’ odor, and cuticular compounds, as reported for other arthropods (cockroaches and spiders) (Machado & Macías-Ordoñez 2007). Although these stimuli may act as chemical cues in aggregations, their roles in mutual attraction remain to be experimentally tested (see Harvey et al. 2017), as well as if scent-gland secretions functions as aggregation pheromones.

IV. Production of chemical signals in harvestmen

Here I will focus on Eupnoi harvestmen, the long-legged harvestmen, commonly known as “Daddy Long-legs”, to outline future research on chemical communication and ecology in this group. As most research dealing with these topics has been done on another suborder (the Laniatores), I also aimed to highlight the many unexplored avenues of research and unknown topics in Eupnoi.

Pheromones and allelochemicals are produced by harvestmen in their unique secretory, repugnatory or defensive glands. Glands are connected to the hemolymph, in which chemicals travel, as well as the sequestrated compounds from food sources and symbiotic organisms. Wouters et al. (2013) found that either microorganisms or enzymes are involved in the production of pyranyl kenotes in one gonyleptid (Laniatores). This suggest that harvestmen contain biosynthetic routes of secondary metabolites production. Additionally, harvestmen have special cuticular cells, the oenocytes in the epidermis, which may be related to carbohydrate production for use in the epicuticle and as pheromones (Shultz & Pinto-da-Roca 2007). The chemical composition of the cuticle bears individual information. The production of compounds
deposited in the cuticle has proven to be sex-, age-, and nutrition dependent in harvestmen (revision in Trabalon 2013); although no gland has been identified as the source yet.

Producing chemical defenses is physiologically costly. In Acutiosoma longipes Nazareth & Machado (2015) found that females undergo crucial reproductive and defensive trade-offs when producing the secretions. Additionally, producing secretions is condition-dependent, as poorly fed individuals produced secretions with lower mass and concentration of benzoquinones (Nazareth et al. 2016).

The secretory glands are sacs derive from the ectoderm, and have three layers (revision in Gnaspini & Hara 2007). Although the gland lack accessory muscles, Clawson (1988) suggested that secretion expulsion is facilitated by hydrostatic pressure from muscles compressing dorsal and ventral exoskeleton. Secretion seems to occur by exocytosis from secretory granules, and transport of fluids through glandular cells from hemocoel to the canaliculi (Clawson 1988). The openings of each secretory gland, the ozopore, is located on the lateral portion of the prosoma, either in the carapace or in the supracoxal pleural region (Shultz & Pinto-da-Rocha 2007). The ozopores in Eupnoi are located between the coxa I and II, in the dorsal side.

Harvestmen release pheromones or allelochemicals directly through the ozopore. Most of the chemicals released are intended for contact reception, although short-range olfaction is also used (Willemart & Chelini 2007, Gainett et al. 2017). Harvestmen use many behaviors to release chemicals, aided by morphological traits such as accessory appendages (Gnaspini & Cavalheiro 1998, Schaider et al. 2011). For example, several ridges and aphophyses in the latero-ventral side assist and regulate the process of releasing fluids (Gnaspini & Hara 2007).

V. Chemicals compounds

Pheromones are organic compounds with basic carbon skeleton. Some contain functional groups with oxygen atoms, including alcohols, aldehydes or ketones (revision in Gnaspini & Hara 2007). Other pheromones are acidic or esters with two oxygen atoms (Bradbury & Vehrencamp 2011). Chemical properties of each compound determine their function and limitations. For instance, airborne molecules evaporate easily (volatile). Also, airborne molecules are typically small and light (maximum molecular weight: 300 dalton, and 20 carbon atoms) (Bradbury &
Additionally, airborne pheromones are hydrophobic and lipophilic. Hence, substrate-transmitted molecules have the opposite properties. Research technics to study chemicals involved in harvestmen are noted in Box 1.

Heavy and complex non-volatile molecules can be highly specific. For example, closely related species differ only in one functional group in the main component in sexual pheromones or the ratios of components. If those blends contain the same compounds, accessory components are important (Bradbury and Vehrencamp 2011). The size and polarity of the

**BOX 1. How to study chemical ecology and communication in harvestmen?**

The first way to study chemical communication in harvestmen is by isolating and identifying the chemicals involved. There are three basic ways to do this (Raspotnig et al. 2010, 2015):

1. Directly dabbing secretions on small pieces of filter paper immediately after the emission from the ozopores after gentle squeezes to the harvestmen. Then, filter papers are extracted in hexane for 30 min.
2. Whole body extraction of individuals in 150 µl of hexane or methylene chloride for 30 min.
3. Extraction of excised single glands in methylene chloride for 30 h.

Several classic and modern research technics in chemistry are now available to study the chemical compositions of animal secretions, and to be able to infer their functions. As for harvestmen, extensive research has enabled several technics, even in species that release small volumes of chemicals, such as Eupnoi. Certain technics have proven successful in giving profiles and ratios of the components present in the chemical blends extracted from secretions, including:

- Gas chromatography – mass spectrometry (GC-MS) (Raspotnig et al. 2015)
- Proton nuclear magnetic resonance spectroscopy (H NMR)
- NMR (Rocha et al. 2011)
- High resolution mass spectroscopy (HRMS) (Machado et al. 2005, Wouters et al. 2013)

Additionally, combining these with scanning electron microscopy (SEM) and magnetic resonance imaging (MRI) provide morphological confirmation of the associated structures, and their potential functions. See Fischer (2019) for detailed descriptions of available methods to study chemical compounds in spiders.

Finally, extensive field observations and behavioral ecology approaches using choice tests in experimental arenas and bioassays to look for the chemicals that elicit observed behaviors will complement the research on chemical communication in harvestmen.
molecules affect their persistence time in the environment. When vapor pressure decreases with increasing size or polarity, the evaporation rate declines, and consequently, the signal can be emitted for a longer time. Also, larger molecules diffuse slower, allowing the signal to stays for longer (Trabalon 2013).

Although variable among clades, the secretions of harvestmen are usually a mixture of compounds (see Table 10.1 in Gnaspini & Hara 2007). Raspotnig (2012) comprehensively reviewed the diversity of scent gland-derived chemical components in harvestmen. The author found a great variation on the number of components present in secretions between clades. Furthermore, Raspotnig (2017) provided support for a hypothesis about the evolutionary trajectory of chemical compound groups in harvestmen. For instance, naphthoquinones are thought to be scent gland compounds of common ancestry, which have been lost in certain groups. On the other hand, benzoquinones have appeared independently at least twice in harvestmen. Ultimately, reconstructing the evolutionary trajectory of the chemical compounds used secretions in harvestmen (‘phylogenetic chemosystematic’, Schaider et al. 2018) provides a unique opportunity for very interesting interdisciplinary research.

Secretions in Cyphophthalmi harvestmen include naphthoquinones and methyl ketones, in Laniatores alkylated benzoquinones, phenolics, vinyl ketones, and small tobacco alkaloids (Raspotnig et al. 2015). Additionally, other compounds known contain alkaline benzoquinones, quinones, phenols, naphthoquinone, anthraquinones, heptenones, hexenones, pentanones, vinyl ketones, phenylethylamine and bornyl esters (Ekpa et al. 1985, costa et al. 1993, Hara et al. 2005, Raspotnig 2012, Raspotnig et al. 2010, 2014a,b, 2015, Rocha et al. 2011, Wouters et al. 2013). Alkaloids, nicotinoid alkaloid anabaseine and nitrogen-containing components were found in basal groups of cladonychiid harvestmen (Laniatores), and thought to be the ancestral allomones in Laniatores. Then, those compounds were evolutionary “replaced” and reduced by a phenol- and benzoquinone-rich chemistry in derived clades (Raspotnig et al. 2011).

Notorious exceptions to the patterns listed above exist. For instance, volatile secretions composed mostly of octanes in a naphthoquinone matrix in some Dyspnoi species (Raspotnig et al. 2014). Along with Dyspnoi, the Eupnoi harvestmen scent gland have been poorly investigated,
compared with Laniatores (Raspotnig et al. 2014). Sclerosomatidae Eupnoi species secrete mostly short-chain acyclic ketones and alcohols. Leiobuninae species have highly volatile, short acyclic ethyl ketone-rich secretions. Phalangiidae produce bezoquinones, caprylic acid, and naphthoquinones, considered to be rare as natural products (revision in Hara et al 2005, Gnaspini & Hara 2007, Raspotnig et al 2010, 2015). For example, the phalangiid *Rialena triangularis* have benzoquinone-based chemical defenses, along with naphthoquinone and caprylic (=octanoic) acid (Raspotnig et al. 2015).

VI. Transmission of chemical signals in harvestmen

Chemical communication is challenging for animals in terrestrial environments since the medium (either air or solid substrate) impose stronger barriers for long distance perception than water. Additionally, the prevalence time is variable between microclimates. For instance, alarm or aggregation signals transmit between 1-10 cm in small colonial insects (Bradbury & Vehrencamp 2011). Environmental factors affect the chemical transmission, for instance (1) ambient wind and currents, (2) temperature, which increases the pressure of volatiles and higher temperatures cause faster spread and sooner fade (3) humidity and rain, (4) vegetation structure, since complex tridimensional environment, and (5) topography and surface structure.

The transmission of substrate-born semiochemicals varies with the morphological arrangement of an animal’s secretory structures, its behaviors, and function. Harvestmen have two main strategies of transmitting defensive compounds: (1) create a chemical shield around the body, and (2) direct the liquid toward the offending agent, i.e. ‘jet emission’ (Segovia et al. 2015). These strategies are variable among harvestmen (Gnaspini & Hara 2007). Eupnoi harvestmen have shown to emit a secretion globule that then evaporates, as well as the emission of a fine spray that moistens its dorsum, displacement of liquid through ventral integumentary grooves, and delivering the secretion by retracting a leg toward the harvestmen’s body (revision in Gnaspini & Hara 2007). Additionally, inter-sexual variation in jet emission was recently reported for *Phareicranaus calcariferus* (Moore et al. 2018), specifically in the propensity to jet, and the composition (enteric v. creamy secretions).
Harvestmen also use colorless enteric fluids secreted through the mouth opening to assist the transmission of chemical signals (Acosta et al. 1993). These fluids are often mixed with the white-milky, yellowish secretions from the secretory gland, and displaced towards the end of the body by lateral channels or sallow slits (Clawson 1988, Segalerba & Toscano-Gadea 2016). This process dilutes the secretion and increases the evaporation surface. Mixing the compounds is useful because (1) it dilutes the costly (for production and storage) compounds produced in the glands, and (2) some compounds, like benzoquinones, are unstable in water (Gnaspini & Hara 2007). During agonistic interactions, Eupnoi harvestmen can use the coxal aphophyses direct the secretions towards the ventral side of the body (Shultz & Pinto-da-Rocha 2007).

VII. Chemical reception in harvestmen

Receiving chemical stimuli is complex. First, adequate sensory structures are required. Then animals then have to be able to discriminate between innumerable chemical compounds in the environment and couple the relevant ones that will elicit appropriate responses. Finally, associated neuron and neural pathways for those relevant stimuli should quickly transmit precise information to central nervous systems.

Chemical channels used by arthropods, however, seldom transmit reliable information about the source or location of the emitting source, especially because chemical compounds do not follow any intensity pattern or gradients. Therefore, relying on olfactory reception (smell) is not as distributed as contact reception (taste) in arthropods. The olfaction in Lepidopterans and hymenopterans is assisted by an extensive development of antennae or other hair-like projections throughout their body that provide a maximized exposure volume for sensilla and other structures and cells to airborne chemicals. Certain receptors in forelegs and near the mouth assist in foraging, whereas other receptors (such as bristles with pores) in legs and dorsal and ventral areas serve for communication.

The sensory capacities among harvestmen are varied, and each group seems to rely differently on certain modalities. Some gonyleptids (Laniatores) used olfaction when food was placed in their container (Santos & Gnaspini 2002, Willemart 2002). While hunting, live prey is searched using direct physical contact with mechanoreceptors. Willemart and Chelini (2007)
experimentally found on a gonyleptid that both close-range olfaction and contact chemoreception are important while foraging. Depending on the intensity of the stimuli, these harvestmen used leg II to smell and leg I to taste. As for olfaction on Eupnoi, Chelini et al. (2009) found that the response of *Eumesosoma roeweri* to predatory cues (spider scent) was not different than to conspecific or control treatments. This suggests that chemical reception (at least long-range olfaction) is not extensively developed in Eupnoi (but see Gainett et al. 2017). However, in long-legged Eupnoi distant mechanoreception might also be important. Detection of vibration through the substrate was also experimentally demonstrated in phalangiid Eupnoi harvestmen (Immel 1955).

**VI. a. Sensory structures**

Harvestmen have a unique, elaborated, and diverse array of hair-like structures and slits for chemosensory reception, mostly located in the distal end of legs, pedipalps, and chelicerae (Shultz & Pinto-da-Rocha 2007). Willemart et al. (2009) provide a very extensive and detailed revision and analysis of those structures, and the demonstrated and assumed sensory capacities. The authors conclude that harvestmen seem to be strongly dependent on contact chemoreception for reproductive interactions, hunting, and predator avoidance. However, close-range olfaction assists certain groups (especially Laniatores) while scavenging or foraging for food with a strong odor (Willemart et al. 2009). Additionally, Gainett *et al.* (2017) recently proposed the importance of several pored-sensilla on olfaction reception for this group of arachnids.

While harvestmen pedipalps act primarily as tactile organs, harvestmen have sensory structures in their legs (especially the forelegs, I and II), prosoma and opisthosoma, in both ventral and dorsal surfaces (Shultz & Pinto-da-Rocha 2007). The cuticular surface have setae and seta-covered aphophyses associated with prey capture. Harvestmen setae are diverse in form, shape and sensitivity, but in general are hair-like projections of the cuticle with basal articulation (Willemart et al 2009). In Eupnoi setae are thought to be the most important and widespread sensory structure (Box 2).
Once the chemical compound has been coupled from the medium to the sensors, binding proteins attach it to the sensory cell’s membrane (for example, the G protein coupled receptor GPCR; similar to rhodopsin in photoreceptor cells). This coupling then depolarizes the membrane of those neurons and fire action potentials (Barth 2002, Shultz & Pinto-da-Rocha 2007).

Reception proteins are sensitive to specific functional groups, molecule sizes, or shapes. Therefore, the receptor is more intricated than a simple lock-key system. Additionally, the receptor responds to a variety of ligands, and the match with the cell’s requirements affect the

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**BOX 2. Main sensory structures of Eupnoi harvestmen**

- **Slit (lyrifissures) sensilla**: measure cuticular strain, self-generated muscular or haemolymphal movement (propioreceptors) or from external sources (exteroception). Located mostly in the basal segments of all appendages, and near the articulations. Not arranged in true lyriform organs, but occur either in solitary or groups.

- **Campaniform sensilla**: circular or oval modifications of slit sensilla. Located in all legs, with concentration near the plane of autotomy. Leg II in *Phalangium* had similar numbers than other legs.

- **Sensilla chaetica**: located in the whole body. Contain a socket membrane, and these setae are probably gustatory contact mechanoreceptor because they have an internal lumen associated with a pore tip.

- **Sensilla basiconica**: located on the dorsal side of the metatarsus of all legs, and also in the dorsal side of the pedipalps of *Phalangium opilio*. A short shaft inserts in a large sockets. Its function is unknown.

- **Spicer’s tarsal organ**: A longitudinal series of microdenticles on the ventral surface of the pedipalps. Their pores lack innervation, and this organ is thought as an olfactory receptor. However, without further examination, this structure can also be a glandular opening.

- Other structures in harvestmen include scattered macrosetae or spines, clumps of microsetae at the tip of pedipalps and legs, microsetae with final projections in pedipalps (plumose or pinnate setae) present exclusively in Eupnoi, and setae associated with gland in Dyspnoi that assist the secretion of adhesive fluids to capture prey (Shultz & Pinto-da-Rocha 2007).

See Gainett et al. (2017) for more description of sensilla associated with olfaction.
excitatory output (amplitude, rate, and duration of neural spike trains) (Bradbury & Vehrencamp 2011).

The electric impulse then heads to a glomerulus (which centralizes the impulses coming from many receptor cells). The glomeruli save information of where the receptor was, although no evidence has been found about a chemotopic ordering in the CNS, as for the case of tactile or visual receptors (Bradbury & Vehrencamp 2011). The way how arthropods can discern between chemical compounds is currently not resolved. However, the interaction between many receptor cells and glomeruli, and the action of inhibitory interneurons suggest that multi-source pathways allow for the specificity of the responses to different compound blends. Harvestmen sensillae can have one or more basal neurons (Shultz & Pinto-da-Rocha 2007). However, in spiders, tarsal chemoreceptor sensory hairs are usually innervated by up to 21 sensory cells (Trabalon 2013).

This may activate the olfactory bulb with only a specific signature pattern of glomeruli activity, analogous to a tri-dimensional bar code (Bradbury & Vehrencamp 2011).

VIII. Phylogenetic implications and approaches

Chemical compounds and the ratios of each in a mix, known as the “pheromone blend” or “odor signature” (Bradbury & Vehrencamp 2011), can be species-specific, especially in those clades with complex molecules. Therefore, secretions can be informative traits to elucidate phylogenetic relationships, as well as the evolutionary trajectory of production mechanisms and functions. Different states of the trait can inform of speciation, radiation, and even convergence in pheromone composition. Additionally, like many other phenotypic traits, chemical compounds are expected to differ greatly between species if it’s driven by sexual selection than if they are involved in alarm signaling. But how to make species-specific chemical compounds? Animals can (1) modify the molecule properties. Large molecules with the unique structure or chemical composition, for example, adding amino acids; and (2) change the blend of compounds. Infinite unique mixtures can ten arise when the main and minor components are present in different ratios.

Traits related to the chemical communication and ecology of harvestmen have been used in systematic studies. For example, the chemical compounds, the morphology of the gland
opening in the tegument, the behaviors employed during predation attempts to evaporate or jettison the secretions and their combinations apparently have phylogenetic signals (Acosta et al. 1993, Hara & Gnaspini 2003; Gnaspini & Hara 2007, Pomini et al. 2010). For example, the chemical composition of the secretion has been used, along with morphometrical, genetic, ecological and/or behavioral data to elucidate the phylogenetic patterns in the families Gonyleptidae (Hara et al. 2005, Caetano & Machado 2013) and Phalangiidae (Wachter et al. 2015). However, chemical compounds also bring intriguing results, mostly because of the lack of comprehensive phylogenetic and pheromone identity information. In 22 species of Gonyleptidae 37 compounds were found, but only half of them were identified (Hara et al. 2005). Some of them revealed symplesiomorphies, while others autopomorphies. Interestingly, one acyclic compound that was also found in a sclerosomatid Eupnoi (Hara et al. 2005). In Dyspnoi harvestmen Shear et al. (2014) found different chemicals in species of Travunioidea, suggesting phylogenetic signal of chemical compounds, and separation of these during clade diversification. In another clade of eastern US species the major component was anabaseine, an alkaloid related to nicotine. In the western species, the major component was a phenylethylamine. In Eupnoi, no phylogenetic effort including chemical data has been made, although systematics of Sclerosomatidae has been recently studied (see Hedin et al. 2012b).

IX. Functions of chemical communication in harvestmen

Harvestmen engage in both intra- and inter-specific chemical communication using volatile and non-volatile pheromones. For several life history activities, the chemical channel is their only mean of transmitting signals. Fischer (2019) extensively reviewed the importance and functions of chemical communication for spiders. In Opiliones, predator and parasite deterrence and alarm signaling are the most frequently studied functions. Secretions can have more than one function. For instance, in Eupnoi the secretions are thought to serve to mark trails as a mean of intraspecific communication with the clear fluid, and as defense with the milky one, as suggested for Leiobunum aldrichi (Bishop 1950).

For harvestmen intra-specific communication occurs during the scent-marking of grouping sites, prey attraction, and various reproductive tasks (marking of oviposition sites, parental care, and male-female courtship interactions) (Juberthie et al. 1991, Gnaspini & Hara
However, the functions of those chemicals vary among suborders. For instance, whereas all harvestmen use chemicals as defense, only Laniatores are known so far to use them extensively in alarm (Machado et al. 2005, Da Silva Fernandes & Willemart 2014), and only Eupnoi species used them to mark roosting sites (Donaldson & Grether 2007). Below I will expand on most of those functions.

IX. a. Defense

The chemical secretions can be useful to deter certain types of potential predators (revision in Gnaspini & Hara 2007). For example, benzoquinones of the harvestmen Goniosoma longipes (Gonyleptidae) may serve as a chemical shield. When tested in lab trials, such shield deterred ants, wandering spiders and frogs but not opossums (Machado et al. 2005). Secretions have also experimentally demonstrated to be successful to deter flatworm attacks in Myschonyx cuspidatus (Gonyleptidae) (Silva et al. 2018). Besides the chemical shielding and jettison, some behaviors may enhance the secretion as defense. The “bobbing” behavior of intense and frequent vibrations (Gnaspini & Hara 2007) may have two mechanisms of anti-predator defense: (1) confuse predators, and (2) release and diffused unnoticed secretions (as suggested by Chelini et al. 2009). However, chemical secretions did not increase survival in M. cuspidatus harvestmen exposed to wandering spiders that had their gland opening clogged (Dias & Willemart 2013).

IX. b. Alarm signaling

The function of quinones as alarm pheromones was recorded in the gonyleptid Goniosoma proximum. By exposing aggregations with the exudate 73.3% of the aggregations responded (Machado et al. 2002). Also, the time of reaction was inverse to group size (Machado et al. 2002). Gregariousness is prerequisite for the evolution of alarm signals, as noted in many other arthropods. Since the alarmed harvestmen bump into each other while fleeing, the alarm reaction is also mechanically spread, resulting in a general erratic scattering of the group, as occur in fish and insects (revision in Machado & Macias-Ordoñez 2007). Alarm pheromone can simultaneously act as a defense mechanism, and in fact could be a by-product of the defensive function. Evolutionary, once grouped, harvestmen might have taken advantage of the chemical channel and transformed the defensive response and use it as intra-specific communication.
However, Segovia et al. (2015) found that the chemical secretions of *D. pectinifemur* do not function as an alarm signal.

**IX. c. Site marking and recruitment**

Harvestmen secretions may function as site marking to attract conspecifics. For instance, Schaider & Raspotnig (2009) found the presence of an atrium outside the ozopore and the possibility of solid secretions of chemicals in the Dypsnoi *Trogulus tricarinatus* (Trogulidae), suggesting site marking in this soil-dwelling species. Species of *Leiobunum* have been seen rubbing the defense tubercle over the area where lay eggs, potentially to mark sites and repel others (Gnaspini & Hara 2007). Also, Donaldson & Grether (2007) suggested that the Eupnoi *Prionostemma* can mark the roosting site continuously. However, the site marking does not necessarily have to involve communication.

**IX. d. Reproductive tasks**

The occurrence of sexually dimorphic glands was reported in Cosmetidae and Gonyleptidae (Da Silva Fernandes & Willemart 2014, Dias & Willemart 2016). Males rub parts of the legs that matched with the gland openings, which was interpreted as the release of a pheromone into the substrate, which may amplify the signal towards females. Additionally, the authors found the males were waving the leg, which may be important for short-range communication. Also, males of *Iporangaia pustulosa* can control the release of secretions of the metatarsal gland IV (Murayama & Willemart 2015). However, the phenotypic traits or individual identification of those chemical cues remain to be studied. Additionally, Dias & Willemart (2016) did not find evidence of contact chemical release during mating in two species of Opiliones, despite having sexually dimorphic glands.

Ultimately, chemical signals can simultaneously serve for different purposes. For instance, *Formica* ants spray formic acid used in defense and recruiting colony members to the source of danger (Wilson 1971). However, these differences could be achieved with small modifications in the chemical compounds, their blends or ratios. Therefore, behavioral essays and proper identification of the chemicals used in different biologically relevant context are necessary to test the multifunctionality of chemical communication in harvestmen.
Marking the substrate with individual information have been found for reproductive tasks in Eupnoi harvestmen. Willemart & Hebets (2012) found that the Eupnoi *Leiobunum vittatum* rubs their body in the substrate. The authors found sex-specific responses to conspecific cues, suggesting the possibility of sexual communication mediated by chemical cues. Males exposed to conspecific cues performed two behaviors never recorded in females. Males spent more time, engage in more scrapping with leg I, and did more pedipalps tapping when faced with conspecific vs control cues. Females spent more time tapping their palps in the presence of male cues rather than female cues, suggesting their ability to distinguish them. But there was no difference between winning or losing males. Certain Eupnoi (*Leiobunum vittatum* and *Phalangium opilio*) apparently can differentiate males and females after contact (Macías-Ordoñez 1997; Willemart et al. 2006).

Expanding of the importance of intraspecific communication, Fowler-Finn et al. (2014) found a behavioral interplay with repeated leg touching and grabbing between male and female *Leiobunum vittatum*. Also, the production and offer of a nuptial gift—probably derived from enteric fluids secreted by the mouth—by the male was key in continuing the mating. This nuptial gift can be high in enzyme content (Kahn et al. 2018). Additionally, Kahn et al. (2018) found that aminoacid content of gifts is variable among taxa and better predicted by reproductive morphology and behavior, instead of the phylogeny. Overall, this recent information also suggests the importance of chemical communication for reproductive purposes.

Additionally, pheromones of *Prionostemma* harvestmen seem to be sex-specific and females appear to be attracted to female scent, while males are attracted equally to male and female scent (G. Grether, *pers. comm.*). The sex ratio in aggregations of *Prionostema* is commonly 1:1, although some groups are either male or female skewed (Grether et al. 2014b). Harvey et al. (2017) experimentally demonstrated that this skew is caused by sex-specific differential recruitment through chemical signals. Together, these data suggest that sclerosomatids can form aggregations for several reasons, and females can return to the more aggregations more frequently, can be more sensitive to disturbance, or have different microhabitat preferences than males (as suggested by Grether et al. 2014b).
Since many species of harvestmen form aggregations, the use of chemical as communication seems to follow an evolutionary pathway favoring initial steps to social life. Also, harvestmen often form multispecific aggregations (Grether et al. 2014b, Harvey et al. 2017, Domínguez et al. 2016, I. Escalante pers. obs.). Some species apparently do not release pheromones in the groups, while other do. The differential release of alarm pheromones may explain multispecific aggregations; species that do not release pheromones can take advantage of the ones that do, and their presence favors other species by diluting the effect of risk in the aggregations (Machado & Macías-Ordoñez 2007). However, this idea remain untested.

In aggregations, the use of pheromones may be key in three contexts: (1) communication during recruitment, (2) defense and (3) alarm signals. However, evidence on the multifunctional of pheromones in this context is still preliminary. The attraction of conspecific to daytime roosting sites in spiny palms in an undescribed species of the Neotropical Prionostemma (Sclerosomatidae) genus, which inhabits the rainforest of Central America, have been investigated. These sclerosomatids leave the groups at dusk and forage on the ground (Wade et al. 2011), and at day re-group, although not necessarily in the same site (Donaldson & Grether 2007). Harvestmen apparently mark the substrate (palm fronds) with pheromones (Donaldson & Grether 2007, Grether and Donaldson 2007). Such sites persist as a grouping location for even several years (Teng et al. 2012). Prionostemma harvestmen have also high site tenancy to particular “traditional” sites, even though sever predations and detrimental changes in preferred ecological factors were simulated and experimentally tested (Grether et al. 2014a); suggesting that roosting site can become “ecological traps”. Additionally, new unmarked individuals frequently join the aggregation (Grether et al. 2014a). Nonetheless, Grether et al. (2014b) found a decrease in recruitment over time after experimentally removing individuals. They suggest that marking pheromones can also include alarm informational. Nonetheless, what is noticeable in these findings is the function of pheromones in communication. It is worth noting that the untested proposed mechanism involved contact chemoreception of potentially heavy and non-volatile pheromones. Donaldson & Grether (2007) report frequent observations of Prionostemma harvestmen rubbing their sides and posterior end of the carapace against palm
fronds, which could be interpreted as the scent-marking behavior. However, other species have been found to not rely on conspecific markings or plant volatile compounds to select roosting sites (i.e. the Eupnoi *Jussara*, Pagoti et al. 2017). Regardless of this, in arthropods, constant marking of a site creates a long-term and spatial overlap of single puffs that allows conspecific to find and follow the intended sites or trials (reviewed for ants and copepods in Bradbury & Vehrencamp 2011).

**XI. Conclusions and future directions**

Besides anecdotal observations of reduced recruitment (Grether et al. 2014b), and research on distant Laniatores harvestmen (Machado et al. 2002), the alarm function of pheromones in Eupnoi harvestmen remains untested. The presence of alarm pheromones will be beneficial for group living and will provide information on the evolutionary maintenance of this trait. By exposing groups to concentrated pheromones, the alarm function of their secretions could be correlated with (1) their fleeing behavior, (2) site tenancy, measured as the return throughout the days, (3) the transmission of the alarm signal by either olfactory or contact receptions, (4) the potential importance of bobbing behavior (Gnaspini & Hara 2007, Escalante et al. 2019) as a way to disseminate the alarm pheromone (an untested strategy so far), and finally (5) the importance of the position in the group. Therefore, future research will provide important insights into the interaction between behavior, chemical communication, morphology (in the form of the extremely elongated legs in Eupnoi) in harvestmen. Consequently, this research will benefit the knowledge of the factors mediating the grouping behavior as a widespread trait in animals.

As for the defense function of pheromones in harvestmen, both the translucent and the milky secretion have been seen in the Neotropical *Prionostemma* and the North American *Nelima paessleri* (Escalante, pers. obs.). Both systems provide the opportunity to test the importance of chemical communication during mating, as well as during recruitment and defense. These Eupnoi harvestmen frequently autotomy their legs (Domínguez et al. 2016, Escalante et al. *In prep.*), which brings questions regarding the potential trade-offs of defensive strategies in this group. Finally, these clades are also known to form multispecies aggregations,
which will allow testing for the importance of chemical ecology and chemo-systematics on species diversification and the overall evolutionary trajectory of these arachnids.

Proper extraction and identifications of the compounds are necessary in order to understand the nature of these chemical signals. The context-dependent differences in the major and minor chemical compounds (and/or their proportions in the blends) in the secretions will inform the chemical variation of these signals. Specifically, to identify the chemicals released as alarm pheromones during a disturbance to the groups (perhaps by emulating a predatory attempt), and the one deposited in the roosting sites at day, that can serve as intra-specific communication to mark the sites. This will test for the multifunctionality of secretions as well as the diversity of compound used by *Prionostemma* and other Eupnoi harvestmen. Ultimately, this will suggest interesting coupling mechanisms in harvestmen, as well as their ability to fine-scale discrimination using the elaborate, diverse, and chemically different secretions.

The use of intersexual chemical communication has been suggested in harvestmen in the form of pheromones and nuptial gifts. Although Eupnoi species have shown to discriminate between male and female chemical signals, the properties of their signals, their use and importance have not been addressed. These topics can be studied by conducting reproductive trails (as in Fowler-Finn et al. 2014) and extract the secretions and nuptial gifts as those are presented during the interactions. Then, chemical analyses will provide the identity of the compounds in the secretion. Finally, to test the function of the secretions in communication can be investigated with ablation experiments and removal of nuptial gifts. Consequently, information of this kind will inform of the importance of courtship and fitness of the chemical traits on Eupnoi harvestmen as well as in invertebrates that use chemical channels for reproduction.

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XIII. References


