Responses of Four Arthropod Prey Species to Mechanosensory, Chemosensory and Visual Cues from an Arachnid Predator: A Comparative Approach[1]

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Abstract: Comparisons of multiple invertebrate prey species to direct predator sensory cues are relatively uncommon. We compared prey responses to arachnid predators (Araneae: Lycosidae) of four species: *Blattella germanica* (Blattodea: Blattellidae), *Acheta domesticus* (Orthoptera: Gryllinae), *Armadillidium vulgare* (Oniscidea: Armadillidae), and *Porcellio laevis* (Oniscidea: Porcellionidae). Prey experienced combinations of direct mechanosensory, chemosensory or visual cues. All species responded to all cues, but response structure differed among species. Mechanosensory and chemosensory predator cues elicited frequent shifts between behaviors, whereas visual stimuli tended to diminish responses. Mechanosensory stimuli produced the most extreme responses, particularly in crickets and cockroaches, but responses to mechanosensory stimuli diminished when accompanied by visual stimuli. Crickets were particularly sensitive to chemical cues and reduced activity in response to them. Isopods were sensitive to visual stimuli, reducing activity in response. These behavioral similarities and differences provide insight into the sensory ecology of different species of arthropod.

Key Words: Predator, Prey, Behavior, Lycosidae, Blattodea, Gryllinae, Oniscidea

Introduction

Predators and prey create mutual selection pressures, leading to the evolution of sensory adaptations for detection and capture/avoidance (Edmunds 1974, Vermeij 1994, Downes and Shine 1998). These adaptations may be tuned to specific predators or prey when closely interacting populations coevolve, such as the crab, *Platytelphusa armata* A. Milne-Edwards, 1887 and its gastropod prey (*Spekia*, *Neothaum*, *Lavigeria*, and *Paramelania* spp.) in Lake Tanganyika (West et al. 1991). However, adaptations that increase efficiency of predation or predator avoidance can also be generalized, as in background matching that is effective against multiple antagonistic species (Venesky and Anthony 2007).

Sensory cues generated by predators are diverse, as are the mechanisms for their detection. Some sensory information is immediate and direct (e.g., visual

and auditory), whereas other cues may be indirect and asynchronous (e.g., chemical). This is particularly apparent in arthropods, as the phylum is characterized by tremendous diversity in mechanisms for detection of visual, chemical, and mechanical stimuli (Bate et al. 1978).

Vision. Arthropod simple and compound eyes vary in form and function with respect to size and shape, retinal sampling, the extent of the visual field, and spectral or polarization sensitivity (Warrant and McIntyre 1993). Variation in eye morphology can be associated with predatory behavior, as seen in more pronounced odonate frontal acute zones of quick-flying dragonflies (Anisoptera) relative to slower-flying damselflies (Zygoptera, Sherk 1978).

Chemoreception. Diverse arthropod species use chemical cues to avoid or escape predators. For example, two-spotted spider mites, *Tetranychus urticae* C. L. Koch, 1836, do not oviposit in substrates previously inhabited by the predatory mite *Phytoseiulus persimilis* Athias-Henriot, 1957 (Dicke and Grostal 1999). Direct chemical cues from predators arise from exuviae, secretions, excrement or other deposits (Kortet and Hedrick 2004), whereas they may indirectly originate from dead or injured conspecifics (Kats and Dill 1998). Efficacy of chemical information varies with environmental conditions (Wilder et al. 2005) and may be less effective with time and distance (Barnes et al. 2002).

Mechanoreception. Arthropod predators and prey typically use antennae or sensory bristles to directly detect objects (Pelletier and McLeod 1994, Camhi and Johnson 1999) or indirect substrate vibrations (Chidrawi and Mercer 2003). The subgenual organs in the tibiae of diverse species are particularly sensitive to substrate borne vibrations (Autrum 1941, Autrum and Schneider 1948).

Although a number of studies have compared sensory morphology (e.g., Pinter 1972, Crouau 1997, Olliver et al. 2004), there are fewer comparative studies of arthropod antipredator behavior (but see Hardy and Shaw 1983). In one comparative study, Bucher et al. (2014) demonstrated that antipredator behavior can be species and modality specific by exposing more than twenty arthropod prey species to indirect chemical cues from different predatory spiders. In the present study, we examine four arthropod species: house crickets, *Acheta domesticus* Linnaeus, 1758 (Orthoptera: Gryllinae), German cockroaches, *Blattella germanica* Linnaeus, 1767 (Blattodea: Blattellidae), and terrestrial isopods *Armadillidium vulgare* Latreille, 1804 (Oniscidea: Armadillidiidae) and *Porcellio laevis* Latreille, 1804 (Oniscidea: Porcellionidae) that directly interacted with predatory wolf spiders (Araneae: Lycosidae).

House crickets, *Acheta domesticus,* are crepuscular/nocturnal insects with widespread distribution in urban environments, fields, and forests (Pinter 1972). Crickets often escape predators by kicking the rear legs in response to mechanical stimulation to the cerci (Dangles et al. 2008). Cricket subgenual organs also respond to substrate vibrations, including low-frequency airborne sounds transduced into the substrate (Autrum 1941). Antennal chemoreception

is used for mating and interaction with conspecifics (Hardy and Shaw 1983). We therefore expected that crickets would be particularly sensitive to mechanical and chemical predator cues.

German cockroaches, *Blattella germanica,* are gregarious nocturnal cockroaches. Vision in *B. germanica* is used to detect light change that causes scattering (Koehler et al. 1987). Cockroach antennae are complex sensory appendages that contain mechanoreceptors, chemoreceptors, thermoreceptors, hygroreceptors, and several types of proprioceptors (Schaller 1978, Toh 1981, Toh and Yokohari 1985). Cockroach subgenual organs may be among the most sensitive insect vibration detectors (Autrum and Schneider 1948) and potentially detect airborne sound (Shaw 1994). Cockroaches have ventral abdominal mechanoreceptors responsive to substrate vibration (Florentine 1967, Kehler et al. 1970), and like crickets, cockroaches possess highly mechanosensitive cerci that elicit a running response when stimulated (Camhi et al. 1978, Watson and Ritzmann 1994). This suggests that cockroaches should be particularly responsive to predator mechanical cues.

The terrestrial isopods used in this study, *Armadillidium vulgare* and *Porcellio laevis,* are primarily nocturnal and inhabit leaf litter and other detritus. Mixed olfactory-gustatory organs on the second antennae have social and foraging functions (Seelinger 1983, Zimmer et al. 1996, Linsenmair 2007), and it seems possible these chemosensory organs might also detect predators. Terrestrial isopods respond to substrate vibration (Houghtaling and Kight 2006), with mechanoreceptors that more resemble insect mechanosensitive cells than those of aquatic crustaceans (Crouau 1997). Oniscid compound eyes, however, are relatively simple and may not be used for vision (Abbott 1918). The two isopod species have distinctive antipredator morphological and behavioral adaptations: the body of *A. vulgare* can be flexed into a defensive ball, whereas this is not possible for the more dorsoventrally compressed *P. laevis*, which must flee predators or exhibit a "death feint" (Sutton 1972, Hal and Beal 1982). In the latter case (tonic immobility), there can be variation within and between species, as discussed by Quadro et al. (2012) for the isopods *Balloniscus glaber* Araujo and Zardo, 1995, *B. selowii* Brandt, 1833, and *P. dilatatus* Brandt, 1833*,* and among conspecifics, as discussed by Tuf et al. (2015) in *P. scaber* Latreille, 1804. The morphological and behavioral differences between the Armadillidiidae and Porcellionidae suggest that *A. vulgare* should exhibit less locomotion than *P. laevis* following predator detection, but we anticipated that neither species would be sensitive to visual cues.

The wolf spiders (Araneae: Lycosidae) used in this study, *Hogna antelucana* (Montgomery, 1904), *H. carolinensis* (Walckenaer, 1805) (Figure 6, p. 134), *H. lenta* (Hentz, 1844), *Rabidosa rabida* Walckenaer, 1837, *Tigrosa annexa* (Chamberlin and Ivie, 1944), are wandering spiders that capture and overpower prey (Edgar 1969). Wolf spiders capture prey by pouncing and using the walking legs and chelicerae for restraint, thereafter immobilizing prey with

the venom apparatus. Wolf spiders are generalists and prey upon a wide variety of small arthropods (Uetz et al. 1992), with specialized forelimbs for capture and handling of diverse prey species (Rovner 1980, Persons and Uetz 1997, Storm and Lima 2008). In our preliminary observations and experimental trials, all wolf spiders used in this study (9-23 mm) consistently attacked all four prey species. The spiders also consumed all species except the isopod *A. vulgare*, presumably due to its conglobation and thick cuticle (Csonka et al. 2013).

There is a need for studies in which prey species interact directly with living predators, as studies of antipredator behavior tend to use simulated (e.g., Wilson et al. 2010, Dupuy et al. 2011, Hedrick 2013; but see Camhi et al. 1978, Carbines et al. 1992) or indirect predator cues (e.g., Hoefler et al. 2012, Hegarty and Kight 2014). For example, Beleznai et al. (2015) found that leafhoppers, *Psammotettix alienus* Dahlbom, 1850, changed foraging movement patterns in the direct presence the spider, *Tibellus oblongus* Walckenaer, 1802. In the present study, we manipulated combinations of visual, chemical and mechanical cues to examine how the sensory environment influences arthropods with diverse antipredator mechanisms and sensory systems. By using a common stimulus predator, we were able to directly compare behavioral responses of different prey species. The selected prey species are nocturnal, and we predicted a general responsiveness to mechanosensory and/or chemical cues, but less response to visual cues. Deviations from this expected pattern, and differences among species, should therefore provide insight into the unique sensory ecology of each prey species.

Methods

All animals were purchased from commercial suppliers between September 2013 and February 2014. Isopods and wolf spiders were collected by the suppliers (Carolina.com and TarantulaSpiders.com, respectively) from wild populations in North Carolina and Florida, USA. House crickets (7-20 mm) and German cockroaches (5-15 mm) came from captive bred populations. Isopods (*A. vulgare*, 5-12 mm; *P. laevis*, 7-15 mm) were maintained in small cardboard containers, fed carrots, and misted with water *ad libitum* to maintain adequate humidity. Crickets were kept in a 38 l glass terrarium and fed commercial cricket food *ad libitum*. Cockroaches were kept in a 37 x 22 x 25 cm plastic container (Pet Keeper™) with a cardboard refuge tube and fed commercial dry cat food *ad libitum*. Wolf spiders were housed in individual 15 x 15 x 12 cm plastic containers, along with damp paper towels for humidity. Spiders were provided with water and fed one to two crickets on the same day every week at least three days before being used in a trial. Housing and all experiments took place at 21ºC under laboratory lighting conditions.

Behavioral Recording. All trials took place within an arena (Figure 1) with an outer circular wall constructed of aluminum flashing fashioned into a cylinder (22 cm diameter). This outer cylinder was covered with paper and the

upper rim coated with petroleum jelly to prevent escape. A 20 cm tall, 7 cm diameter inner cylinder was constructed from transparent plastic and placed inside the outer wall with 15 cm of space between the outer and inner cylinders. Cylinders were open at the top to permit observation.

Before each trial began, a spider was placed between the two cylinders beneath a small aluminum blind attached to a string. A focal prey animal was then introduced into the inner cylinder and permitted to acclimate for approximately 20 seconds. The string was then retracted to remove the aluminum blind, allowing the predator access to the area between the inner and outer cylinders. Prey behavioral patterns were recorded for 120 seconds, after which both animals were removed from the arena.
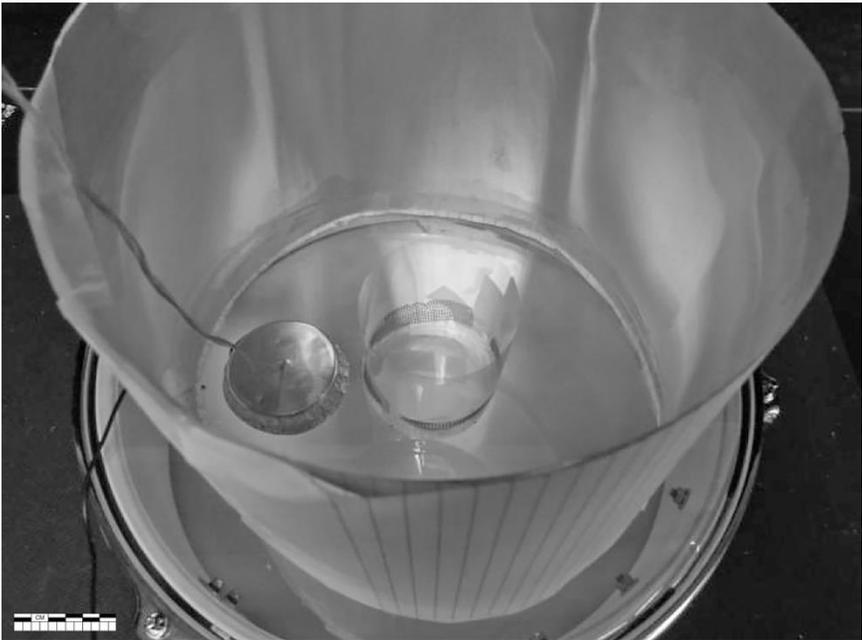

Figure 1. Experimental arena for modulation of seismic, visual, and chemical predator cues. Focal prey animals were introduced into the inner cylinder. Stimulus spiders were initially held beneath an aluminum blind (7 cm, pictured left of the inner cylinder) and then released into the space between the inner and outer cylinders as each trial began. Scale bar represents 6 cm, each small subdivision 0.5 cm.

The arena could be adjusted to modulate the transmission of vibrational (seismic), visual and volatile chemical information between predator and prey:

Seismic Cues. Responses to seismic cues (S+) were assessed by placing the arena upon a 33-cm diameter snare drum (Pulse™ Piccolo) with the snare removed. Trials not involving the assessment of seismic cues (S-) were

conducted with the arena upon the drumhead removed from the drum resonating chamber and positioned atop a vibration dampening mat.

Visual Cues. Responses to visual cues (V+) were assessed by using clear transparency sheets to construct the inner cylinder of the arena. For trials in which visual cues were excluded (V-), the transparent inner cylinder was occluded with a black Sharpie™ marker (allowing 24 hours to elapse for dissipation of solvent odors). Although the model species were nocturnal, it was necessary to conduct all trials under room lighting and during the photophase in order to modulate spider visual cues without occluding the vision of focal animals.

Chemical Cues. Responses to chemical cues (C+) were assessed by modifying the inner cylinder with small (<5 mm) holes and 5 mm horizontal slits above the line of sight. Trials excluding chemical cues (C-) took place in an arena without holes or slits. Chemotactile cues could not permeate the cylinder wall, so any chemical cues in the inner cylinder were volatile.

These modifications were used to create eight experimental conditions (Table 1). Treatments ranged from control trials, in which all three sensory cues were damped, to treatments in which all three sensory cues were available.

Prey animals were randomly selected for each trial. Spiders were selected the morning of each experiment (at least three days after the last feeding) and used as stimulus animals until exhibiting signs of stress (e.g., low crouching without movement). In these cases, the spider was removed from further trials that day. Each experiment included 20 trials for each of the four prey species. Each trial lasted for 120 seconds. Between each trial, all surfaces were cleaned with 70% isopropyl alcohol to eliminate potential chemical cues left from the preceding trial. Frequency and duration of relevant behavioral patterns were recorded using Noldus Observer™ 2.0 event recording software.

Behavioral Patterns. The frequency and duration of antennal movement and walking (behaviors common to all four prey species) were recorded during each trial. Walking was usually regular and relatively slow, but was sometimes punctuated with short bursts of faster locomotion. Antennal movements included displacement of antennae in any direction.

Behavioral frequency and duration can be used to characterize the general activity patterns of focal animals. Highly active animals that often switch between behavioral states are characterized by high frequency of behaviors. Animals that exhibit long bouts of a specific behavioral pattern are characterized by low frequencies (few switches between behaviors) and long durations (total time per trial) of the favored behavior.

Statistical Analysis. All statistical analyses were performed in JMP Pro (v 11.0) statistical software (SAS Institute, Cary, North Carolina, USA). Behavioral frequencies and durations were analyzed for pairwise correlations. Because all dependent variables were significantly correlated (see Results) we transformed the data with Principal Component Analysis (PCA) and

subsequently analyzed the resulting principal component scores with factorial ANOVA using the JMP least squares fit model platform. Post-hoc comparisons of least squares means were conducted in JMP with the Tukey HSD procedure with α = 0.05.

The sample size for each experimental treatment was 20 randomly selected individuals of each prey species, for a total of 80 prey individuals per experimental treatment (Table 1) and a total of 640 individuals for the entire study. Each individual was used in only one trial.

Table 1. Experimental treatments: combinations of visual (V+/V-), seismic (S+/S-), and/or chemical (C+/C-) cues.

Experiment	Sensory Conditions
V+/S-/C-	Visual cues available, but seismic and chemical cues damped.
V-/S-/C+	Chemical cues available, but seismic and visual cues damped.
V-/S+/C-	Seismic cues available, but chemical and visual cues damped.
V+/S-/C+	Visual and chemical cues available, but seismic cues damped.
V+/S+/C-	Visual and seismic cues available, but chemical cues damped.
V-/S+/C+	Chemical and seismic cues available, but visual cues damped.
V+/C+/S+	All cues available. No cues damped.
V-/C-/S-	No cues available. All cues damped. (Control)

Results

Principal Components. Pairwise comparisons of frequency and duration of antennal movement and walking across all species and treatments indicated significant correlations among all dependent variables (Table 2). We therefore conducted Principal Component Analysis to transform these correlated variables into a set of linearly uncorrelated component variables. PCA revealed four principal components. The first (PC1, Eigenvalue 2.3877) and second (PC2, Eigenvalue 1.0593) principal components explained 59.7% and 26.5% of total variance, respectively. The third (PC3, Eigenvalue 0.4744) and fourth (PC4, Eigenvalue 0.0785) principal components explained 11.9% and 1.9% of total variance, respectively, and were not used in further analysis.

Table 2. Correlations among dependent variables.

Variable	By Variable	Correlation	P value
Antenna Duration	Antenna Frequency	0.5522	< 0.0001
Walking Frequency	Antenna Frequency	0.8702	< 0.0001
Walking Frequency	Antenna Duration	0.3220	< 0.0001
Walking Duration	Antenna Frequency	-0.3509	< 0.0001
Walking Duration	Antenna Duration	-0.4789	< 0.0001
Walking Duration	Walking Frequency	-0.0942	0.0171

Table 3. Principal component analysis eigenvectors and loading values. For the purposes of interpretation, loading values of less than 0.5 were considered to not contribute meaningfully to the respective principal component.

	Principal Component			
	One	Two	Three	Four
Eigenvectors				
Antenna Frequency	0.60790	0.25391	-0.11115	0.74407
Antenna Duration	0.48505	-0.38004	0.77295	-0.15113
Walking Frequency	0.51796	0.54261	-0.18252	-0.63559
Walking Duration	0.35624	0.70475	0.59739	0.13980
Loading Values				
Antenna Frequency	0.93934	0.26133	-0.07656	0.20854
Antenna Duration	0.74951	-0.39115	0.53240	-0.04236
Walking Frequency	0.80036	0.55857	-0.12572	-0.17814
Walking Duration	-0.55047	0.72535	0.41147	0.03918

The frequency and duration of antennal movement and the frequency of walking contributed positively, but walking duration negatively, to PC1 (Table 3). Hence as PC1 scores increase, focal animals tended to switch between behavioral patterns more often, and spend more time moving antennae but less

time walking. In contrast, walking frequency and duration contributed positively, but antennal duration negatively, to PC2 (Table 3). As PC2 scores increase, focal animals tended to start and stop walking more often, and spend more time walking but less time moving antennae.
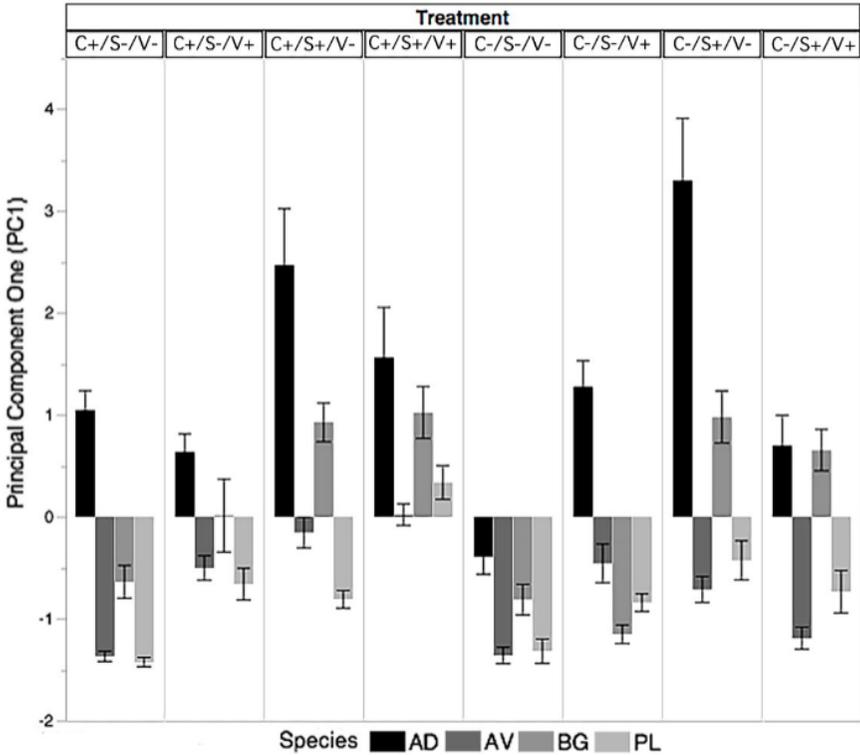

Figure 2. Mean principal component one (PC1) scores for experimental treatments. Treatments are combinations of visual (V+/V-), seismic (S+/S-), and/or chemical (C+/C-) cues. AD = *Acheta domesticus*, AV = *Armadillidium vulgare*, BG = *Blattella germanica*, PL = *Porcellio laevis*. Error bars represent one standard error on the mean.

Experimental Treatments. To compare results of the eight experimental treatments (Table 1), separate analyses of variance were conducted for the influence of experimental treatment on PC1 or PC2 component scores. There were significant effects of experimental treatment on both principal components (Figure 2, PC1, $F_{(7, 632)} = 16.5224$, $p < 0.0001$; Figure 3, PC2, $F_{(7, 632)} = 9.4894$, $p < 0.0001$). Post-hoc comparisons of PC1 scores indicated that treatments with seismic cues generally had significantly greater scores than

treatments without seismic cues (Table 4): animals in trials with seismic cues tended to switch to antennal movement more often and spend more time moving antennae. A notable exception were the lower PC1 scores in the (V+/S+/C-) treatment with paired seismic and visual cues, but damped chemical cues. In this trial, antennal movement occurred with unusually low frequency relative to other trials with seismic cues.
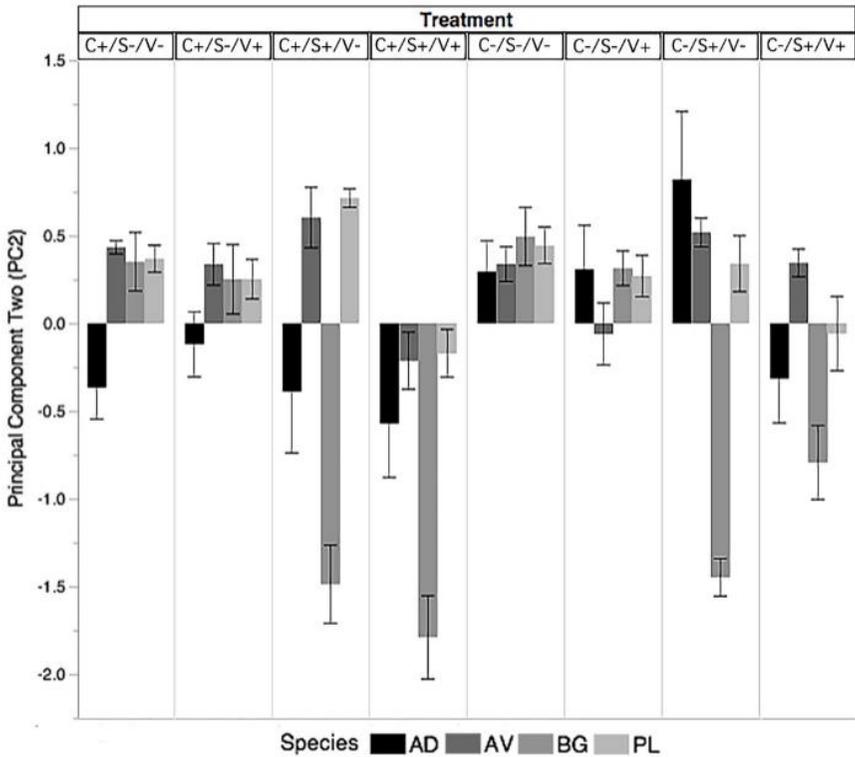

Figure 3. Mean principal component two (PC2) scores for experimental treatments. Treatments are combinations of visual (V+/V-), seismic (S+/S-), and/or chemical (C+/C-) cues. AD = *Acheta domesticus*, AV = *Armadillidium vulgare*, BG = *Blattella germanica*, PL = *Porcellio laevis*. Error bars represent one standard error on the mean.

Table 4. Post-hoc comparison of experimental treatments. Treatments are combinations of visual (V+/V-), seismic (S+/S-), and/or chemical (C+/C-) cues. Levels not connected by the same letter are significantly different.

Level				Least Squares Mean
Principal Component One (PC1)				
C-/S+/V-	A			0.7852516
C+/S+/V+	A			0.7361835
C+/S+/V-	A			0.6091388
C+/S-/V+		B		-0.1279586
C-/S+/V+		B		-0.1425736
C-/S-/V+		B	C	-0.2931413
C+/S-/V-		B	C	-0.5958244
C-/S-/V-			C	-0.9710759
Principal Component Two (PC2)				
C-/S-/V-	A			0.3924181
C-/S-/V+	A	B		0.2072215
C+/S-/V-	A	B		0.1963533
C+/S-/V+	A	B		0.1791911
C-/S+/V-	A	B		0.0578666
C+/S+/V-		B		-0.1400465
C-/S+/V+		B		-0.2062436
C+/S+/V+			C	-0.6867607

Trials with only visual (V+/S-/C-) or only chemical cues (V-/S-/C+) had low PC1 scores that did not differ significantly from control trials (V-/S-/C-), which had the lowest PC1 scores (Table 4). Antennal behavior was infrequent and of short duration in these trials. In contrast, control trials had the highest PC2 scores, associated with the longest durations of walking behavior.

We next examined prey responses in more detail by conducting separate factorial analyses of variance on the influence of four independent variables (seismic cues, chemical cues, visual cues, prey species) on PC1 and PC2 component scores. Definitions and levels of variables are found in Table 5. ANOVA F-values and significance levels are found in Appendix I.

Table 5. Dependent variables and independent variables

Dependent (Response)	Definition
PC1	Principal component one
PC2	Principal component two
Independent Variable	**Levels**
Seismic Cues	Available (S+) or damped (S-)
Chemical Cues	Available (C+) or damped (C-)
Visual Cues	Available (V+) or damped (V-)
Prey Species	*Acheta domesticus, Blattella germanica, Porcellio laevis,* or *Armadillum vulgare*

Sensory Cues. There were significant main effects of seismic cues and chemical cues on PC1 and significant main effects of all three sensory cues on PC2 (Appendix I). In general, PC1 scores were significantly greater, but PC2 scores lower, in trials with seismic cues or trials with chemical cues. Trials with seismic or chemical cues were characterized by more frequent and longer antennal movement and shorter walking duration. PC2 scores were also significantly lower in trials with visual cues, which were characterized by longer antennal movement but shorter walking duration.

There were significant interactive effects between seismic and visual cues on both principal components (Appendix I). Trials with both seismic and visual cues had significantly lower PC1 and PC2 scores than trials with only seismic cues. This was associated with lower frequencies of antennal movement and walking in seismic trials that also included visual cues. There was also a significant interaction between chemical and visual cues on PC1, indicating that PC1 scores were significantly greater in trials with both chemical and visual

cues. This was associated with more frequent and longer antennal movement, but shorter walking duration, in visual trials that also included chemosensory cues. These patterns were further reflected in a significant interactive effect between all three sensory cues on PC1.

   Species. There were main effects of species on both PC1 and PC2. There also were several significant interactive effects between species and sensory cues on both PC1 (Figure 4) and PC2 (Figure 5), including an interaction between all four factors on PC1 (Appendix I).
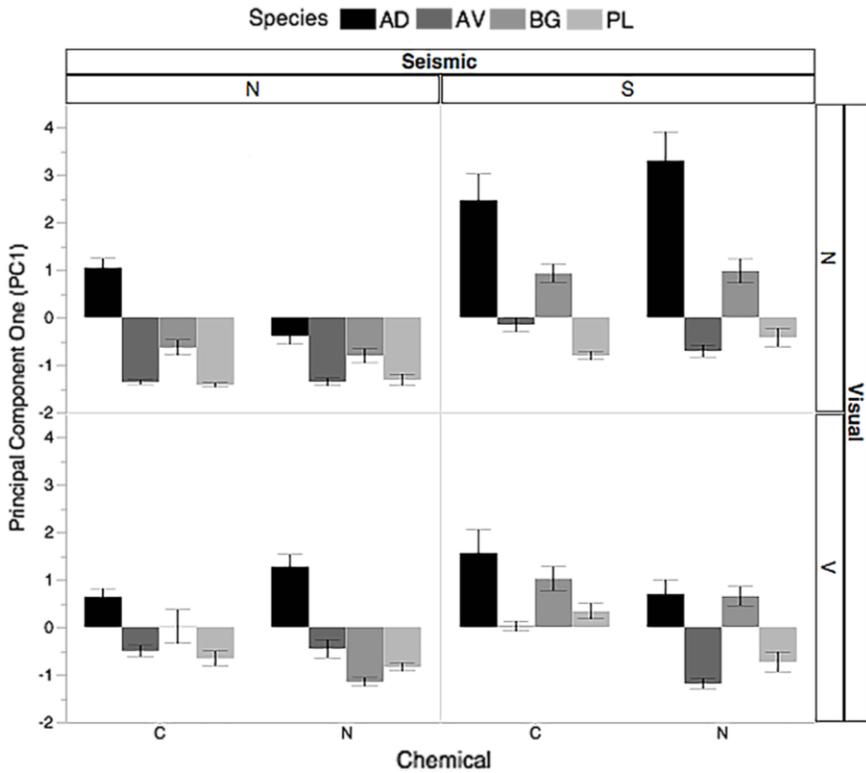

Figure 4. Mean principal component one (PC1) scores for interactions among sensory cues. Cue states are visual cues present (V) or not (N), seismic cues present (S) or not (N), and chemical cues present (C) or not (N). AD = *Acheta domesticus*, AV = *Armadillidium vulgare*, BG = *Blattella germanica*, PL = *Porcellio laevis*. Error bars represent one standard error on the mean.

   Crickets exhibited significantly greater PC1 scores than other species, with generally more frequent antenna movement and walking, but shorter walking duration. Unlike other species, crickets had significantly lower PC2 scores when

exposed to chemical cues, which was associated with shorter and less frequent walking. Crickets also appeared to be more sensitive to interactions between seismic and visual cues, exhibiting significantly lower PC1 and PC2 scores in trials with both cues than in seismic-only trials. This was reflected in lower frequencies of antennal movement and walking in trials with seismic and visual cues. Crickets were the only species to have consistently positive PC1 scores in all trials except controls. In control trials, all species had negative PC1 scores (Figure 2). Crickets in control trials had markedly less frequent and shorter antennal movement than crickets in any other experimental treatment.
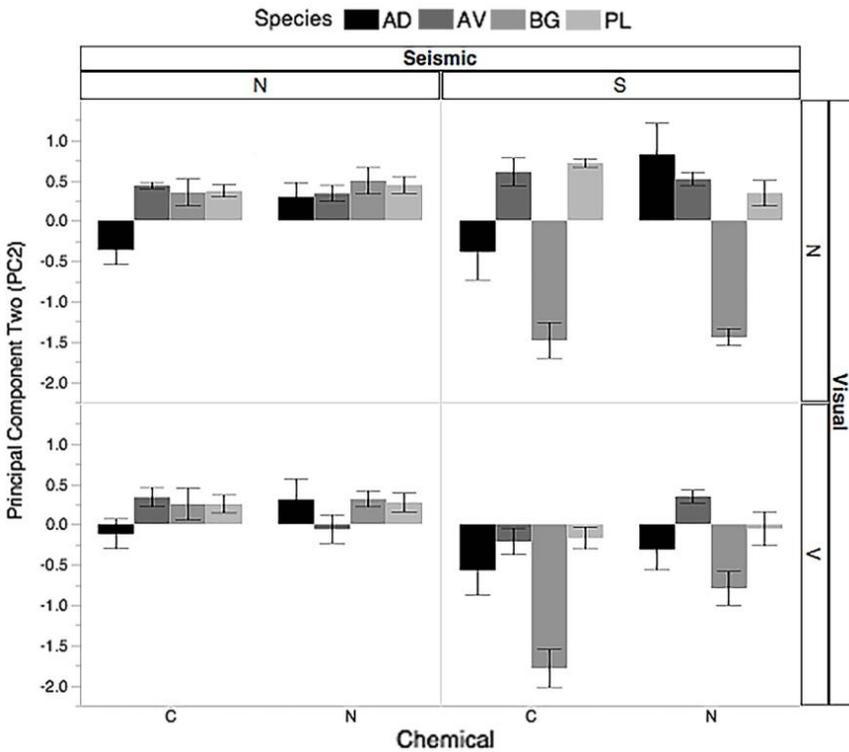

Figure 5. Mean principal component two (PC2) scores for interactions among sensory cues. Cue states are visual cues present (V) or not (N), seismic cues present (S) or not (N), and chemical cues present (C) or not (N). AD = *Acheta domesticus*, AV = *Armadillidium vulgare*, BG = *Blattella germanica*, PL = *Porcellio laevis*. Error bars represent one standard error on the mean.

Cockroaches exhibited significantly lower PC2 scores in general than other species, due in part to very long durations of antennal movement. Although all species exhibited significantly higher PC1 scores when exposed to seismic cues,

the effect was most pronounced in cockroaches. Unlike other species, cockroaches also had significantly lower PC2 scores when exposed to seismic cues. These effects are associated with very long duration of cockroach antennal movement in trials with seismic cues.

Isopods exhibited significantly greater PC2 scores in general, but lower PC1 scores, than other species. The two isopod species generally did not differ from one another: both species had less frequent and shorter antenna movement, and longer walking duration than other species. In trials with visual cues, both isopod species had significantly higher PC1 scores, as indicated by longer antennal durations but shorter walking durations in visual trials. Isopods were also the only species to have consistently negative PC1 scores in all trials except (V+/S+/C+), where all three sensory cues were simultaneously presented (Figure 2). In these trials, isopod antennal duration was longer and walking duration shorter than all other experimental treatments. The only treatment in which the two isopod species differed from one another was the (V-/S+/C+) experiment, in which *A. vulgare* had significantly greater PC1 scores than *P. laevis*. In this treatment, *A. vulgare* exhibited greater frequencies of both antennal movement and walking, with long durations of antenna movement but short walking duration.

Discussion

All four prey species behaviorally responded to all three sensory cues, but the structure of responses differed among species. In general, vibrational stimuli produced the strongest behavioral responses. However, responses differed in some cases when vibrational stimuli were presented in concert with other stimuli, particularly visual cues. There were also a number of species-specific response patterns to combinations of cues.

The testing arena appears to have effectively damped the strength of seismic, chemical, and visual spider cues in experiments designed to exclude particular stimuli. The arena did not modulate other kinds of sensory information (e.g., airborne auditory cues) that may have also have been detected by focal animals. For all three modulated sensory channels, prey species exhibited main and interactive behavioral effects in trials with and without cues. We therefore assume that prey animals detected visual, chemosensory and/or mechanosensory cues differently depending on the experimental treatment. We note, however, that the inclusion of a negative control treatment, in which no spider was present, would have provided a useful baseline for discriminating between prey responses to predator cues and cues that might have been generated by the focal animal.

Seismic Cues. All prey species seemed highly sensitive to substrate vibrations produced by spiders. Crickets and cockroaches, in particular, altered behavioral patterns in response to substrate vibrations. Presumably, this was an antipredator reaction, although it is possible that responses were more general.

The strong response to seismic cues (relative to chemical and visual cues) may have been influenced in part by the construction of the arena. Trials with seismic cues took place on a drumhead over a resonating chamber. Vibrations may therefore have propagated more efficiently than in natural habitats. However, even small arthropods walking across dry leaf litter can produce vibrations and sounds that are detectable to humans, suggesting that relatively intense mechanosensory cues are part of the sensory ecology of the arthropods in this study. Indeed, some wolf spiders, such as *Hygrolycosa rubrofasciata* Ohlert, 1865, are known to use leaf litter to propagate sexual "drum" signals to attract females from a distance (Parri et al. 2002).

Chemical Cues. Cockroaches and isopods did not respond noticeably to chemosensory predator cues, exhibiting similar behavioral patterns in trials with and without them. In contrast, crickets exhibited less walking activity when presented with predator chemical cues. Crickets are known to avoid chemical cues of funnel spiders (*Gryllus integer* Scudder, 1902, Kortet and Hedrick 2004) and centipedes (*Acheta domesticus*, Hoefler et al. 2012), particularly when the centipedes previously fed upon conspecific crickets. Experience with chemical cues left by predators can have ultimate consequences: Bucher et al. (2014) found that in wood crickets, *Nemobius sylvestris* (Bosc d'Antic, 1792), experience with spider cues led to increased foraging activity and body mass, and lower rates of predation. In the present study, it is possible that crickets exhibited more chemosensory responses than other species because stimulus spiders were maintained on a cricket diet. However, a minimum of three days elapsed between the time a spider was fed and when it was used in a trial. This presumably minimized chemical cues from previously ingested crickets.

Visual Cues. The only species that responded to visual cues (as a main effect) were the terrestrial isopods. This was surprising, because crickets, for example, are known to use visual cues to detect predators. For example, wood crickets, *N. sylvestris*, were less likely to detect and escape a simulated predator strike when their eyes were covered (Dupuy et al. 2011). However, the crickets in the present study did not have their vision occluded, they merely could not see a predator on the other side of an opaque barrier. Because the prey species in this study are primarily nocturnal, their responses to visual stimuli might have been influenced by the illuminated environment.

Both terrestrial isopod species spent significantly less time walking in trials with visual predator cues than trials without them. Terrestrial isopods have relatively simple compound eyes, with only 20 ommatidia in *Porcellio scaber* (Nemanic 1975). While the compound eyes respond to light for phototaxis (Warburg 1968), less is known about terrestrial isopod vision. Abbott (1918) concluded that terrestrial isopods do not use the compound eyes for vision because animals with removed antennae did not avoid obstacles, but blinded individuals with intact antennae successfully avoided obstacles. While reduced walking behavior in the present study suggests that isopods detected visual

predator cues, we cannot rule out phototaxis. Even though the arena was illuminated from above in all treatments, perhaps isopods merely responded to the presence or absence of dark pigmentation that was used to occlude the walls of the inner arena, or the dark pigmentation elicited nocturnal-associated behavior.

Species-specific behavioral patterns. Crickets shifted between behavioral patterns more often than other prey species in almost every experiment. We observed a consistent pattern in which walking crickets frequently stopped, moved their antennae, and then started walking again. Breaks in walking might improve discrimination between substrate vibrations from predators and those produced by the cricket itself, or improve binaural comparisons of auditory signals for sound localization by eliminating sounds produced during locomotion (Givois and Pollack 2000).

An interesting outcome of this study was the interaction of seismic and visual cues on cricket behavior. Crickets in trials with both visual and seismic cues exhibited lower frequencies of antennal movement and walking relative to trials in which seismic cues were presented without visual cues. Although the functional significance of this effect is unclear, one speculation is that a distant predator should only be detectable by substrate vibrations, which might elicit an active antipredator response. When predators are close enough to be detected visually, but not chemically, a reduction in activity might avoid detection by the predator. However, when all three sensory cues are detected simultaneously, the predator should be in close proximity, which might elicit an escape response.

Cockroaches seemed largely unresponsive to visual and chemical cues from spiders, and responded almost exclusively to seismic cues. It is possible that any response to visual or chemical cues was subtle and not reflected in the particular behaviors we recorded. An incomplete array of sensory cues might also preclude responsiveness. American cockroaches, *Periplaneta americana*, Linnaeus, 1758, exposed to visual, seismic and chemical cues of the toad *Bufo marinus* [currently known as *Rhinella marina* (Linnaeus, 1758)] did not exhibit escape behavior unless these cues were accompanied by wind puffs generated by the striking predator (Camhi et al. 1978).

One of the most peculiar outcomes we observed was that behavior of the two isopod species almost never significantly differed from one another in any trial. During most trials, both isopod species exhibited long bouts of walking, and if frequency or duration of walking changed in response to predator cues, the effect was similar for both species. Indeed, in our observations, both isopods spent relatively little time during the two minute trials in stasis. This is surprising for *A. vulgare*, which can flex the body into a "ball" position when attacked by predators, whereas *P. laevis* cannot conglobate and must flee, seek cover or exhibit a "death feint" (Sutton 1972; Hal and Beal 1982). The only exception was the V-/S+/C+ treatment, in which *A. vulgare* differed from *P. laevis* in all behaviors. There may be species-specific responses to coincident

chemical and seismic information associated with differences in antipredator behavior and morphology.

Conclusions

Crickets, cockroaches, and terrestrial isopods evolved diverse sensory and antipredator modifications, which presumably underlie differences in responsiveness to mechanosensory, chemosensory, and visual aspects of the sensory environment. Nonetheless, there are similarities among species, particularly in response to predator induced substrate vibrations. This suggests either convergence or conservation of substrate mechanoreception as an antipredator adaptation among diverse arthropod species.

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Figure 6. Female Carolina wolf spider, *Hogna carolinensis* (Walckenaer, 1805) Lycosidae.

Appendix I. Effect test F-values generated from factorial ANOVA for Principal Component One (PC1) and Principal Component Two (PC2) in response to chemical cues (C), seismic cues (S), visual cues (V), and prey species (Sp). DF Error = 608. (*) = $P<0.05$, (**) = $P<0.01$, (***) = $P<0.001$.

Response			
Factor	**DF**	**PC1**	**PC2**
C	1	13.4876***	11.6505***
S	1	137.9845***	54.4074***
V	1	1.0390	14.6825***
C*S	1	0.2297	2.9524
C*V	1	6.2294*	0.1877
S*V	1	33.0737***	5.2954*
C*S*V	1	13.9661***	2.9046
Sp	3	131.5549***	32.0833***
C*Sp	3	0.5735	5.7724***
S*Sp	3	10.4038***	39.8267***
C*S*Sp	3	3.7944**	1.2649
V*Sp	3	8.0306***	2.0436
C*V*Sp	3	1.9563	2.8868*
S*V*Sp	3	8.9779***	3.1456*
C*S*V*Sp	3	7.2861***	2.3399