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Research

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The Millipede-Predation Behavior of *Promecognathus* and Exceptional Cyanide Tolerance in *Promecognathus* and *Metrius* (Coleoptera: Carabidae)

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Abstract

Promecognathus (Carabidae) includes beetles that are specialist predators whose prey are polydesmidan millipedes that produce highly toxic hydrogen cyanide and benzaldehyde as a defense, and it is unknown how *Promecognathus* overcomes these chemicals. We observed *Promecognathus laevissimus* (Dejean, 1829) and *P. crassus* (LeConte, 1868) in the laboratory and found that they did not use behaviors to avoid the chemical defenses of their prey, *Xystocheir dissecta* (Wood, 1867) (Polydesmida: Xystodesmidae). We tested benzaldehyde as a feeding deterrent and found noticeable deterrence in all carabid beetles tested except *Promecognathus* species and *Metrius contractus* (Eschscholtz, 1829). A total of 18 carabid species were exposed to cyanide vapors in an enclosed chamber for 10 min to determine their relative tolerances. *Promecognathus* and *M. contractus* were unaffected by HCN exposures 7–15 times greater than quantities that knocked down all other species. *Promecognathus laevissimus* and *M. contractus* were then exposed to high levels of HCN for 2 h, and while individuals of *M. contractus* succumbed, all *P. laevissimus* were still moving after 2 h. It is possible that *Promecognathus* evolved a high tolerance to cyanide as part of a suite of adaptations related to millipede predation. However, we have no plausible explanation for the high tolerance in *Metrius*, for which there is no evidence of millipede feeding. This is the first documented case of predatory insects that exhibit high tolerance and potential resistance to cyanide. Possibly, these beetles have a detoxification mechanism that is not cyanide specific, as their tolerance level far exceeds any dose they would encounter in their natural habitat.

Key words: Carabidae, chemical ecology, predator–prey relation, toxicology

Millipedes (Diplopoda) are a diverse and ancient group of arthropods that are known to produce a wide range of defensive allomones, making them distasteful to predators (Rodriguez et al. 2018). Millipedes generally have few predators, but some animals have evolved to be facultative or specialized predators of millipedes and can avoid or tolerate their chemical defenses using a variety of mechanisms. For example, glowworm beetles (Coleoptera: Phengodidae) prey on spirobolid millipedes without risking exposure to noxious chemicals by first injecting the millipede with a paralytic fluid, preventing it from discharging its chemical defense (Eisner 1998). Millipede assassin bugs (Hemiptera: Reduviidae: Ectrichodiinae) also hunt millipedes by injecting toxic saliva into their prey (Forthman and Weirauch 2012). The giant whipscorpion *Mastigoproctus giganteus* (Lucas, 1835) (Thelyphonida: Thelyphonidae) preys on millipedes and may be protected by its own waxy cuticle (Carrel and Brit 2009). Among vertebrates, some rodents are known to prey on bioluminescent millipedes (Marek and Moore 2015), and there remains the mysterious 'Robespierre', an unknown vertebrate that apparently selectively feeds on the glandless and chemically undefended heads of millipedes, leaving behind decapitated bodies (Eisner 1978, B. Weary, unpublished data). Some predators may exploit millipede chemical defenses as kairomones. Millipede-eating dung beetles locate prey by the scent of the millipede's chemical defense and prefer to attack injured individuals (Bedoussac et al. 2007, Larsen et al. 2009). In the vast majority of cases, however, it is unknown how millipede specialists respond to their prey's chemical defense.

The ground beetle genus *Promecognathus* (Coleoptera: Carabidae) is composed of two species native to western North America that are known millipede specialists. The genus is included in the tribe Promecognathini, a disjunct group, that

has a presumably relict distribution with genera related to *Promecognathus* found in Spain and South Africa. The lineage is thought to date back at least 150 mya (Ribera et al. 2005). *Promecognathus* are winter and spring active, nocturnal hunters as adults. Larval habits are unknown, with only a single 1st instar specimen described (Bousquet and Smetana 1986). The adults feed on millipedes in the order Polydesmida (MacSwain and Gardner 1956, LaBonte 1983), and based on our observations, in the San Francisco Bay area, the most abundant prey species is *Xystocheir dissecta* (Wood, 1867) (Polydesmida: Xystodesmidae) (Fig. 1). This species can produce cyanide (Pavlov et al. 2020), and it is unknown how *Promecognathus* tolerates this chemical defense.

Polydesmidans are the most diverse group of millipedes with approximately 3,500 species (Shear 2011) and are capable of producing a potent cyanide chemical defense (Guldenstedeen-Egeling 1882). Polydesmidans synthesize cyanogenic compounds such as mandelonitrile or benzoyl cyanide, which are secreted into a reaction chamber gland (Eisner et al. 1963). In the reaction chamber, the cyanogenics are mixed with enzymes that catalyze the dissociation of the compounds into HCN and benzaldehyde, and then the toxic mixture is ejected from the ozopores on the millipede's flanks. There is no doubt that cyanide is an effective poison; it inhibits cytochrome oxidase, an essential component of the respiratory electron transport chain that is ubiquitous in organisms that use aerobic respiration (Beasley and Glass 1998). A 1 g millipede could produce 18 times the lethal dose of cyanide for a 300 g pigeon and six times the dose for a 25 g mouse, and insects kept in the same container as a polydesmidan will often succumb within minutes (Eisner et al. 1963). Polydesmidans are tolerant to their own HCN and probably have a unique cytochrome oxidase system (Hall et al. 1971). Benzaldehyde, which is released as a by-product, is a component of almond extract and smells strongly of amaretto flavor. It may be used as an aposematic signal to warn predators of the cyanide defense (Brown 1992).

Cyanide is typically toxic to beetles (Parkin et al. 1937), so *Promecognathus* must somehow overcome the chemical defense of its millipede prey. *Promecognathus* beetles have been observed attacking millipedes by plunging their long thin mandibles down behind the head to presumably sever or crush the ventral nerve cord and then feeding from the head posteriorly (LaBonte 1983). It is possible that this stereotyped prey handling behavior could paralyze the millipede before it discharges its chemical defense, similar to the behavior in glowworm beetles (Eisner 1998). *Promecognathus* might also be able to tolerate cyanide by detoxifying it. Rhodanese is a ubiquitous enzyme that detoxifies cyanide by converting it to thiocyanate (Westley 1973), and *Promecognathus* might have elevated rhodanese activity. Many plants produce cyanogenic compounds and the metabolic pathways for detoxifying cyanide have been found in plant-feeding insects (Zagrobelny et al. 2004). Bamboo lemurs (*Hapalemur* spp. (Primates: Lemuridae)) are also known to feed on cyanide-rich plant material, but the mechanism for their tolerance is unknown (Yamashita et al. 2010). However, there are no documented cases of a predator that has an enzyme-based resistance to cyanide.

Fig. 1. *Promecognathus laevissimus* feeding on a subdued *Xystocheir dissecta*.

If the *Promecognathus* beetles overcome the millipede chemical defense in part or entirely by avoidance, then we expect that there would be observable behaviors during prey handling and prey consumption that are consistent with preventing the release of the secretions or minimizing and bypassing contact with defensive chemicals. If avoidance is not employed or is only a minor part of how the beetles cope with the millipede's chemical defense then we expect that there would be a difference in cyanide tolerance between *Promecognathus* and other similar carabid beetles. In order to investigate how *Promecognathus* overcomes the chemical defense of the polydesmidan *X. dissecta*, we 1) made repeated, detailed observations of *Promecognathus* in the laboratory setting to learn how they handle millipedes during capture and how they feed on the millipedes, 2) used predation trials to establish the efficacy of *X. dissecta*'s defense against predation when faced with a variety of carabids other than *Promecognathus* that co-occur with the millipedes in the field, 3) conducted feeding trials using benzaldehyde-laced food to determine whether this chemical alone was a feeding deterrent, and 4) exposed *Promecognathus* and other carabid beetles to various quantities of HCN to establish their relative tolerances.

Materials and Methods

Selection of Beetle Species

In addition to *Promecognathus crassus* and *P. laevissimus*, which are known millipede feeders, seven other carabid species were included in the predation and feeding deterrence tests. These were selected because 1) they have been repeatedly observed to prey and scavenge, at night, on small arthropods comparable to millipedes in size, and 2) they are known to have abundant and highly active adults in the same area and at the same time of year as the millipedes are active (except *Laemostenus complanatus* (Dejean, 1828) (Coleoptera: Carabidae), which is typically from areas lacking millipedes). Nine more species were included in the cyanide tolerance test to expand the sample to cover a range of body size that slightly exceeds (both larger and smaller) the size range of *Promecognathus* species, and species known to produce different classes of defensive chemicals from their pygidial glands (Supp Material [online only]). All are species endemic to the San Francisco Bay Area and Diablo Range of California except for *L. complanatus*, which is a long-established, accidentally introduced European species (Bousquet 2012).

Specimen Acquisition and Field Methods

Millipedes and beetles were collected at sites in California listed in the Supp Material (online only) and transferred to laboratory facilities at UC, Berkeley. Vouchers of each species are deposited in the Essig Museum of Entomology (EMEC), UC, Berkeley.

Animal Husbandry

Live beetles were segregated by species and brought to lab facilities at UC, Berkeley where they were kept, two beetles each, in

2 oz plastic, lidded condiment containers, with moist soil. When beetles were not being used in a feeding trial, they were fed small pieces of dry, commercial dog food once every 3 d. Prior to use in a feeding or predation trial, individual beetles were isolated in a container and not fed for a minimum of 3 d. All millipedes were kept in a large aquarium with moist leaf litter, decaying hardwood, and soil substrate taken from the collection site. The substrate was moistened every 3 d or when drying was apparent. All arthropods were kept at room temperature (approximately 18–21°C) and with a 12:12 (L:D) h light cycle. Individual beetles were used for only one replicate of a given test and then released or killed and preserved as a specimen.

Weight

As a rough approximation for relative size across the species in the study, an average weight was calculated from the weight taken on an electronic balance of one to five beetle individuals of a given species that had been knocked out with HCN or chilled for 3 min in a –20 freezer (Fig. 4).

Behavioral Observations

To observe the hunting behavior in the lab, we placed an individual *Promecognathus* beetle in a 4-inch diameter container with 10 mm of moist substrate, and left it in complete darkness for at least 30 min. In order to match the apparent circadian rhythm activity of the beetles and millipede as seen in the field, trials were conducted after 6:00 p.m. PST in the typical evening activity period of predation. We then placed an adult *X. dissecta* millipede in the container arena and observed both using red light that neither the millipede nor the beetle appears to be sensitive to as indicated by a lack of photophobic behaviors. We observed 12 trials and recorded detailed behavioral notes in each, particularly the beetle's responses to the millipede, the length of time of the ensuing struggle, and the final outcome of the trial. If a beetle stopped responding to the millipede for several minutes or if the millipede stopped moving, the trial was concluded. Four beetles that successfully subdued their prey were left undisturbed for 14 h so that the prey remains could be examined the next day.

Millipede-Predation Trials

Promecognathus crassus (n = 5), *P. laevissimus* (n = 5), *Metrius contractus* (n = 5), *Laemostenus complanatus* (n = 5), *Pterostichus vicinus* Mannerheim, 1843 (n = 5), and *Scaphinotus interruptus* (Ménétriés, 1843) (n = 10). An additional 10 individuals of *Pterostichus* that were not identified to species, but were a mix of *Pt. vicinus* and *Pt. californicus* (Dejean, 1828) were also tested. To determine if beetles of each species would feed on undefended millipedes, pre-killed *X. dissecta* were placed in the container with the beetle. To test for predation on a chemically defended millipede, individual beetles were placed in a condiment container with a single live *X. dissecta* millipede. In both cases, after 4 d the status of the millipede was recorded.

Feeding Deterrence Trials

Beetle species listed in Fig. 3 were included in the feeding deterrence trials. All are known predators/scavengers that readily eat commercial dog food in the laboratory with or without the application of water (K.Will, unpublished data). Dry dog food was partially crushed and particles of 1–2 mm diameter were selected for feeding trials. Lids from standard 0.2 ml PCR reaction tube strips were cut apart and used as a 'dish' to hold the dog food particles for application of water or benzaldehyde and to present the food to the beetles. Two or three particles of dog food were placed in the lid dish and treatments of 1, 2, and 4 µl of ≥98% benzaldehyde or 4 µl distilled water were applied to the food. The dish of chemically treated or water control-treated food was placed in the container with the beetle. The status of the food was checked after 24 h and 48 h.

Cyanide Tolerance Trials

To determine the concentration of cyanide in the headspace of the test chambers would be very challenging (Ma and Dasgupta 2010, Pavlov et al. 2020) and beyond the scope of this study. Our tests are intended to show relative cyanide tolerance between carabid beetles species, not measure absolute cyanide tolerance. To test the relative effects of cyanide on *Promecognathus* and other carabids, we constructed a chamber that would allow us to expose various species of beetles to similar quantities of cyanide gas while we observed them.

Short-Exposure Trial

Species and number of replicates used in the 10 min exposure trials are listed in Fig. 4. A chamber for the trials was made from a 360 ml glass bowl with a small weighing tray set in the center. The pre-weigh NaCN salt (2.3–100 mg) was placed in the center of the weighing tray. The beetles were placed in the chamber in the area outside the weighing tray so that there was no direct contact with the NaCN and then the bowl was sealed tightly with parafilm. A syringe was used to pierce the parafilm above the NaCN and a few drops, approximately 1.5 µl, of a 2% H_2SO_4 solution was placed on the NaCN salt. The acid solution reacts with NaCN to rapidly produce HCN gas, exposing all beetles in the sealed chamber to the cyanide. The beetles were observed for 10 min, and any erratic behaviors were recorded. After 10 min, if a beetle was immobilized it was recorded as a knockdown. All beetles were then carefully removed from the chamber and placed in separate dishes for observation to assess any apparent impact from the exposure.

In each replicate, trial beetles that had not been previously exposed were used. If any individual of a given species was not immobilized after the trial, then an additional replicate of that species would be tested in subsequent trials with a higher quantity of cyanide introduced into the chamber. When a trial resulted in complete knockdown for all individuals for a given species, that species was not tested further, and the quantity of cyanide was recorded as its maximum exposure. At least two individuals per species were tested per quantity of NaCN, and each species was tested in at least two separate trials, even if the first trial resulted in a complete knockdown. In such a case, the smallest quantity of cyanide that resulted in a complete knockdown was recorded as the maximum exposure tolerance. Up to 10 individual beetles of mixed species were used in the same chamber for each trial.

Long-Exposure Trial

A lidded, plexiglass box arena with a volume of 1,275 ml was used. In 2 oz condiment cups without lids, five individuals each of *P. laevissimus*, *M. contractus*, *Pt. vicinus*, and *Pt. californicus* were placed around a central tray with KCN salt (Fig. 2). Initially, 50 mg of KCN was placed in the chamber and approximately 1.5 µl of 2% H_2SO_4 was placed on the KCN salt and the lid closed. After 10 min an additional 50 mg of KCN was added. After 40 min, an additional 200 mg of KCN salt was added and an additional 1.5 µl of 2% H_2SO_4 was placed on the KCN salt. At the 2 h point, two *Brachinus*

Fig. 2. Long-exposure chamber arrangement. Centrally is a tray with KCN salt. Clockwise from upper left are containers with *Pt. vicinus*, *Pt. californicus*, *P. laevissimus*, and *M. contractus*.

mexicanus Dejean, 1831 and one *B. gebhardis* Erwin, 1965 (Coleoptera: Carabidae) placed in a condiment cup in the arena.

Results

Behavioral Observations

Of the 12 *Promecognathus* beetles observed, two did not respond to the presence of *X. dissecta* and did not display any actions that could be interpreted as predatory behaviors. The other 10 beetles engaged in the stereotyped predatory behavior (LaBonte 1983). Six of these beetles did not successfully subdue their prey, and eventually stopped their pursuit of the millipede. Four of the beetles subdued and killed their prey. Predator-prey aggression and defense episodes were observed to last between 5 and 45 min. After 14 h, millipede carcasses had been consumed almost entirely.

Millipede Predation

All beetles presented with a pre-killed millipede largely or entirely consumed the prey by day 4. At the end of 4 d, the live *X. dissecta* millipedes were only killed and consumed entirely by *Promecognathus crassus* (5 of 5 eaten) and *P. laevissimus* (4 of 5 eaten). The only other cases of predation were in *Pterostichus vicinus* (1 of 5) and *Pt. californicus* (1 of 5). In both of these instances, only the millipede body segments anterior to the ozopore-bearing segments were partially eaten. In the additional, mixed sample of 10 *Pterostichus* individuals of *Pt. vicinus* and *Pt. californicus* there was no feeding on live millipedes (0 of 10). Neither *M. contractus* (0 of 5) nor *L. complanatus* (0 of 5) fed on live millipedes. In a single case, *Scaphinotus interruptus* (1 of 10) fed on the millipede placed with it. However, it was noted that the millipede was inactive when placed with the beetle and, therefore, probably died during the trial and was eaten.

Feeding Deterrence

All beetles presented with water-treated dog food as controls readily fed, consuming the food in 24 h. While each species shows a slightly

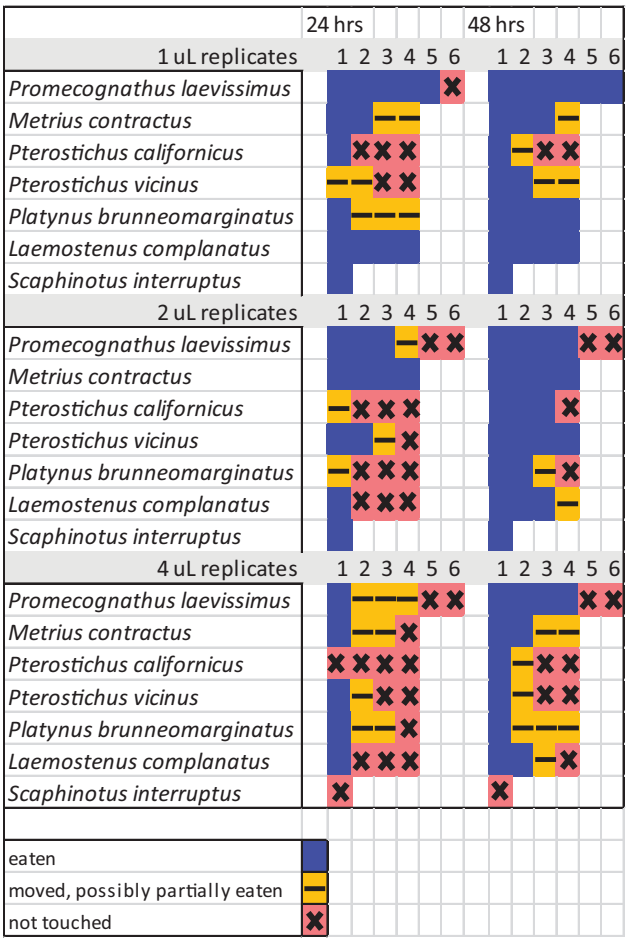

Fig. 3. Cells show status of food for benzaldehyde feeding deterrence test results. For each species listed one to six replicates at three treatment levels at 24 and 48 h. Filled cells, food eaten; cells with a dash, food moved, often fragmented into particles, but were not obviously eaten; and cells with an X, food showed no sign of being touched.

different pattern of feeding (Fig. 3), across all species after 24 h there was much greater occurrence of feeding in the 1 µl application (14 of 27) than in the 4 µl treatments (5 of 27). After 48 h the majority of 1 and 2 µl treated food was consumed (1 µl, 21 of 27; 2 µl 23 of 27) while feeding rate remained low in the 4 µl treatments (9 of 27). During the first 24 h at the 1 and 2 µl levels *P. laevissimus*, *M. contractus*, and *L. complanatus* ate or manipulated the food in most cases, while other species only rarely fed. At the highest application level (4 µl) only *P. laevissimus* and to a lesser degree *M. contractus* fed at a high rate.

Cyanide Tolerance

In both the short and long exposures, beetles of all species were observed to recover and return to typical behaviors within minutes or hours after exposure, with the only exception being the three individuals of *Brachinus* in the long-exposure trial. All three *Brachinus* died.

Short-Exposure Trial

Fifteen of the 18 species tested in the 10-min exposure were very susceptible to HCN and only *P. crassus*, *P. laevissimus*, and *M. contractus* were not knocked down in those tests (Fig. 4). Body size, as estimated by weight, was correlated with exposure tolerance

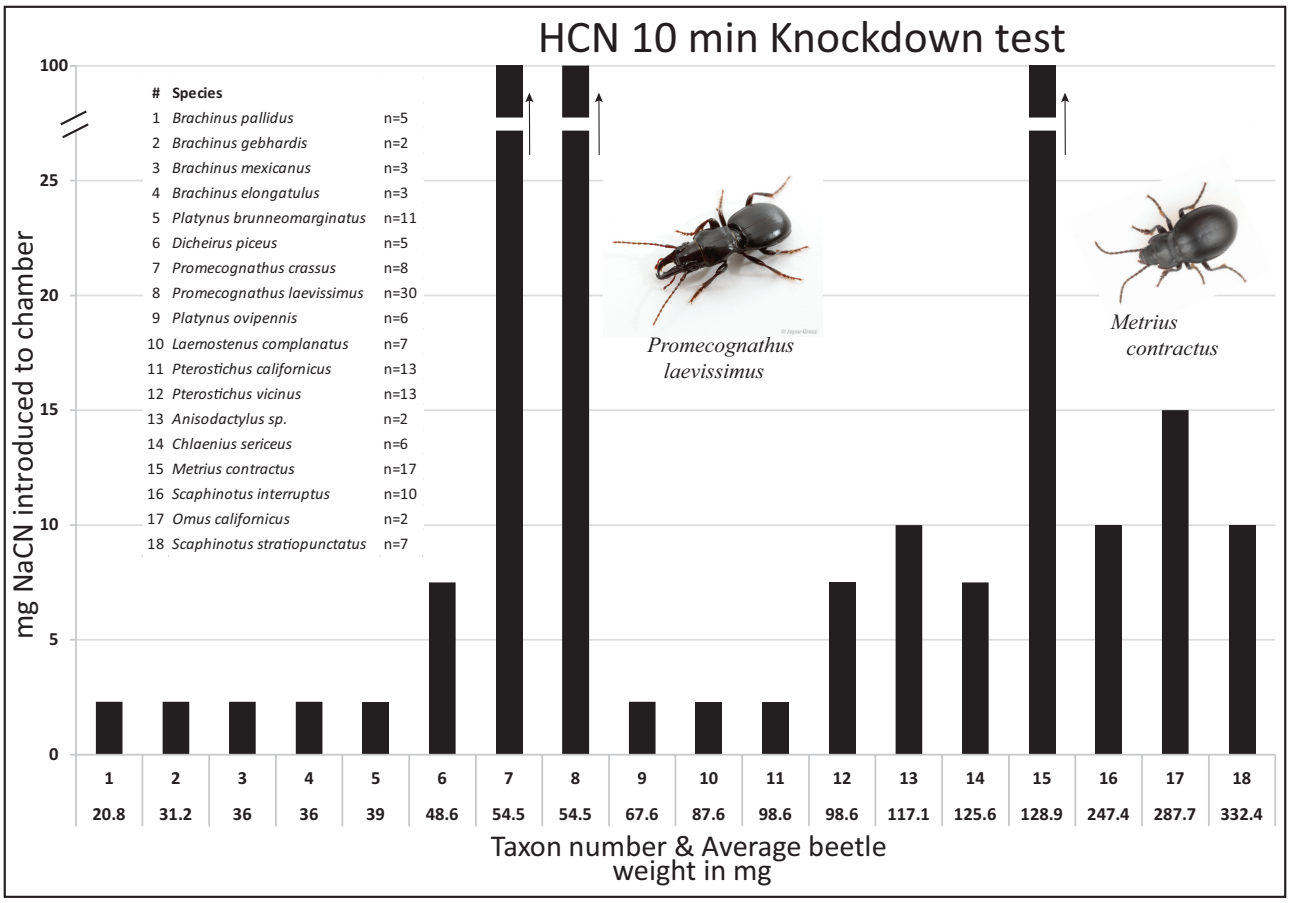

Fig. 4. HCN tolerance in short-exposure trials. Bars represent the quantity of NaCN placed in the chamber that was able to knockdown a given species. Taxa are listed above by number and the number of replicates for each is given. The *Y*-axis has the taxon number and is ordered by increasing weight. For *Promecognathus crassus, P. laevissimus,* and *Metrius contractus,* a broken bar is used to represent the maximum exposure even though none of these were knocked down in the 10 min trial at any level.

in the susceptible species. The three highly tolerant species were exposed in the chamber to approximately seven times the amount of HCN of the much larger species tested without showing any sign of reduced activity. Small-sized beetles, such as the *Brachinus* and *Platynus* (Coleoptera: Carabidae) species, were highly susceptible and typically would be knocked down in 4 min or less even at the low-exposure dose. There was no apparent correlation between the seven different pygidial gland chemical product classes (Supp Material [online only]) and exposure tolerance.

Long-Exposure Trial

Initially, five each of *P. laevissimus*, *M. contractus*, *Pt. vicinus*, and *Pt. californicus* were placed in separate dishes in the arena, the 50 mg of KCN, the H_2SO_4 added and the lid closed. After 10 min, there was no significant effect on any beetles and an additional 50 mg of KCN was added. At the 20 min point, the *Pterostichus* were apparently agitated and had noticeably increased the frequency of mouthpart cleaning. At the 25 min mark, the first *Pt. californicus* was knocked down and by 38 min all 10 *Pterostichus* were knocked down. At that point, neither the *P. laevissimus* nor the *M. contractus* showed any sign of being affected. At the 40 min mark, the *Pterostichus* were removed and set aside to recover and an additional 200 mg of KCN crystals and H2SO4 solution were added to the arena. There was no sign of effect in either the *P. laevissimus* nor the *M. contractus* until the 77 min mark. At that time, several *M. contractus* began to show difficulty moving their legs and began walking convulsively. By 82 min all but one *M. contractus* were fully knocked out. One *M. contractus* continued to occasionally move and attempt to walk until the 110 min point. At the 120 min point, all the *P. laevissimus* were still moving, the three largest individuals without any apparent impairment. The two smallest *P. laevissimus* appeared at this time to be having some difficulty walking. To check the potency of the cyanide vapors at the 2 h point, two *B. mexicanus* and one *B. gebhardis* were placed in the arena. All three *Brachinus* were knocked down in 7 min. The experiment was then terminated.

Discussion

Behavioral Observations

When collecting beetles and millipedes at various field sites, we have quite frequently observed *Promecognathus* feeding on *X. dissecta*, but rarely witness predatory behaviors. LaBonte (1983) describes the prey handling behavior of *Promecognathus* in which the beetle climbs onto the dorsal surface of the millipede and severs the ventral nerve cord by biting the millipede with its elongate jaws. We set out to reproduce these results. In the laboratory, we observed the early steps in the stereotyped prey handling behavior described in LaBonte (1983) in nearly all cases. If attacking from the posterior end of the millipede, the beetle would rapidly clamber along the dorsal surface

while biting the millipede repeatedly. The beetle would stop several body segments short of the head and clamp down tightly with its long mandibles. If attacking from the anterior end, the beetle would perform the same behavior, stopping several segments after the head and then turning around to face the same direction as the millipede. The beetle then inserts its mandibles between the segmental sclerites and then cuts the millipede in a scissor-like fashion. The millipedes appear to die from bleeding and exhaustion. There was no evidence of paralysis in any of the hunts observed. The millipedes invariably continued to struggle for at least 5 min after being initially attacked, and in one exceptional case, the predation bout lasted 45 min.

The millipedes have two main methods of defense against *Promecognathus*: chemicals and physical size and strength. An attacked millipede releases its chemical defense profusely as gas that the attacker is exposed to during the course of the predation bout. A human observer can easily smell the cyanide and benzaldehyde from a meter away, and the smell was quite apparent, and an irritating effect was felt during observations in the closed laboratory room. As anticipated, the chemical defense appeared to have no effect on *Promecognathus* beetles during their hunts, although sometimes the beetle could be observed cleaning its antennae for several minutes after subduing its prey. The millipede's most effective defensive maneuver was to dislodge an attacking *Promecognathus* by twisting back on itself. The millipede rapidly loops back and under its posterior body region such that the beetle is pushed forcefully against the millipede's posterior body segments and is knocked off if not gripping tightly enough. Not all *Promecognathus* are knocked off this way, and most were able to re-mount the millipede. In one observation period, the beetle successfully subdued the millipede after being dismounted eight times. These are somewhat artificial conditions because the arena is small and closed, such that the millipede cannot escape after dislodging the beetle. In an open setting, the millipede will have an opportunity to flee, and the beetle may not get the chance to re-mount after being knocked off.

Promecognathus beetles consume the millipede starting at the head and working posteriorly, leaving only exoskeleton fragments. The beetle is presumed to eat the millipede's chemical defense reservoir glands and secretory cells since we did not observe any residual tissue in the area of the ozopores. The gland reservoirs do not contain cyanide; they contain the precursor molecules that react to form benzaldehyde and HCN. As the glandular parts of the millipede are eaten, some remaining benzaldehyde and HCN may be produced if enzymes are mixed when tissues are disturbed. The beetles were observed, leaving and returning to the carcass several times. It is possible that residual chemicals volatilize during these breaks, and it is also possible that the prey carcass is relocated by the beetle sensing these chemicals.

In the observation of predation, we confirmed that *Promecognathus* uses much of the stereotyped hunting behavior described in LaBonte (1983) to subdue prey. We did not, however, find any evidence that *Promecognathus* uses this behavior to minimize its exposure to cyanide. The predation events were protracted, and the millipedes produced their chemical defense in profuse amounts, enough to adversely affect a human observer. The beetle is not likely to be 'holding its breath' because it is performing a high-intensity activity for 5 min or longer, which would demand respiration. Instead, we propose a non-chemical related explanation for the hunting behavior. There is a large size disparity between predator and prey, and one would expect the predator to evolve a specialized method of incapacitating its prey. The millipede is stronger than the beetle, and in some observed hunts, it was able to successfully push the beetle off and away. It is harder for the millipede to push the beetle away if the beetle is on its dorsal surface. If the beetle does sever the ventral nerve cord, thereby inducing paralysis, it is to stop the millipede from struggling, not to stop it from producing a chemical defense, since typically those have already been released at the first moment of the encounter.

Millipede Predation

In order to show that *Promecognathus* uniquely overcomes the chemical defense of polydesmidan millipedes, we first needed to verify that the chemical defense of *X. dissecta* is effective against other predatory carabid beetles. In the millipede-predation trials, *Promecognathus* readily and reliably ate *X. dissecta*, both alive and pre-killed. Other carabids tested did not eat or only very rarely ate the live millipedes, but they did eat pre-killed millipedes. Pre-killed millipedes are chemically undefended because they cannot release HCN gas as that would have been expended prior to death during handling. In the two exceptional cases where *Pterostichus* preyed on live millipedes, the beetles only fed on the chemically undefended pre-ozopore segments (ozopores on xystodesmids are not present on segments 1–4). This is evidence that *X. dissecta* is generally well-defended against most carabid beetles except for *Promecognathus*. This experiment does not rule out defensive coiling as the millipede's primary defense against beetles since we did not conduct direct observation of all species and their interactions with millipedes. It is possible that *Promecognathus* is the only carabid able to hold on and consistently penetrate the exoskeleton of *X. dissecta*.

Feeding Deterrence

In order to control for defensive coiling behavior of millipedes and isolate part of the chemical defense as the possible deterrent, we tested if the carabids would eat benzaldehyde-treated dog food. Generally, we found that carabids were less likely to consume dog food if it was treated with more benzaldehyde and during the time period when the benzaldehyde concentration was highest. The tendency to consume the food after 48 h is most likely due to the volatilization of the benzaldehyde to the point it was tolerated by the beetles. We considered that the benzaldehyde treatment made the dog food unpalatable due to moisture, but we ruled this out with the water-treatment control, as all beetles readily consumed water-treated dog food. *Promecognathus* and *Metrius* were more tolerant of benzaldehyde, feeding on treated food at high rates even at the highest doses. We tested benzaldehyde anticipating that it might be an allomone used by polydesmidans to warn predators (Brown 1992). Although it is less toxic than cyanide, it is well established that insects are sensitive to the compound as either a repellent or attractant (e.g., Townsend 1963, Yang et al. 2018). It is not surprising that *Promecognathus* is not deterred by the chemical signal produced by its favorite prey. *Promecognathus* may recognize its prey by the scent, and one avenue for further research would be to test if it uses benzaldehyde as a kairomone. It is more surprising that *Metrius* is little deterred by benzaldehyde, as we have no evidence that *Metrius* will prey on live millipedes. *Metrius* may be unable to successfully prey on millipedes due to their defensive coiling or other factors not tested or observed in our study.

Cyanide Tolerance

Metrius contractus and *Promecognathus* species tolerated the maximum quantity of cyanide they were exposed to in the short-exposure trial with seemingly no side effects, and they tolerated cyanide in the long-exposure trial exceptionally well. Based on the long-exposure trial, *Promecognathus* does have a higher cyanide tolerance compared to *Metrius*. No individuals of *Promecognathus* were knocked down, but *Promecognathus* is not completely immune to cyanide

because some behavioral abnormalities were observed towards the end of the long-exposure trial. Although cyanide tolerance is found among herbivorous insects (Zagrobelny et al. 2004), this is the first recorded instance of cyanide tolerance in predatory insects, or any predatory animal as far as we know. To what degree other predatory animals reported to eat xystodesmid millipedes, such as rodents (Marek and Moore 2015) or birds (Kobayashi et al. 2018), avoid or tolerate cyanide has not been established. As more polydesmidan specialist predators are discovered and studied, new cases of exceptional cyanide tolerance will likely be found in nature. Possibly, there are cases of complete resistance.

The doses of cyanide these beetles were exposed to far exceed what they would encounter naturally in the wild. *Promecognathus* and *Metrius* are functionally immune to the cyanide-component of a polydesmidan millipede's chemical defense. *Promecognathus* probably evolved exceptional cyanide tolerance, long mandibles, a unique prey handling behavior, and similar seasonal life cycle to overcome its prey's arsenal of defenses and take advantage of a food source that few other animals competed for. *Metrius* is functionally immune to a millipede's cyanide and is also little deterred by benzaldehyde, so one would expect *Metrius* to be undeterred by a polydesmidan's chemical defense. It may not be affected by the millipede's chemicals, but *Metrius* lacks the morphological and behavioral adaptations *Promecognathus* has to specialize on polydesmidan millipede prey. *Metrius* does not specialize on hunting millipedes (Moore and Di Giulio 2008), but its tolerance to cyanide may allow it to facultatively prey on them, especially sick or injured individuals. This is plausible since some phorid flies (Hash et al. 2017) and scarab beetles (Bedoussac et al. 2007, Larsen et al. 2009) also prey on injured millipedes.

Carabids are well-known for producing a wide array of defensive compounds (Dazzini-Valcurone and Pavan 1980, Dettner 1985, Will et al. 2000). Given that they synthesize and store rather toxic compounds like quinones and phenols, it is conceivable that species producing a particular allomone class might also have an innate ability to tolerate a variety of compounds. An example would be the giant whipscorpion *Mastigoproctus giganteus*, which evolved a waxy cuticle that protects it against its own acetic acid spray as well as the quinones of millipedes (Carrel and Brit 2009). However, we found no evidence of a correlation between pygidial gland chemicals and cyanide tolerance. Specifically, *M. contractus* is a bombardier beetle capable of spraying a hot quinonoid secretion as a defense (Eisner et al. 1977), and it is highly tolerant of cyanide. However, we also included three species of *Brachinus*, which are also bombardiers, though not closely related to *M. contractus*. These proved highly susceptible to cyanide, consistent with their body size (Fig. 4). The *Chlaenius sericeus* (Forster, 1771) (Coleoptera: Carabidae) we tested are a similar size as *M. contractus* and produce phenols, but they were found to be very susceptible to cyanide (Fig. 4). *Promecognathus*, *Scaphinotus*, and *Pterostichus* species all produce similar pygidial gland secretions, largely higher saturated and unsaturated carboxylic acids (Supp Material [online only]), but again, there was no correlated greater resistance to cyanide.

The biochemistry of *Promecognathus* is not well understood, and there are many avenues for further research. Studying how *Promecognathus* detoxifies cyanide could lead to new medical treatments for cyanide poisoning. It would also be important for understanding the evolution of cyanide resistance in animals. *Promecognathus* and *Metrius* are not closely related, and cyanide tolerance or resistance may have evolved independently multiple times in carabids and other beetles.

Conclusion

We showed that *Promecognathus* can prey on the cyanide-defended *Xystocheir dissecta* millipedes, and other carabid beetles present in the same habitat do not or do so only very rarely and then feed only on selected, undefended tissues. Predation by these generalist predator/scavenger species likely happens only when the millipede's defenses are weak. While the tested carabids will scavenge dead millipedes, only *Promecognathus* kills live, robust millipedes, and we observed that the beetles' behavior appears to do little to minimize exposure to the millipede's chemical defense. Benzaldehyde, which is produced by the millipedes, was shown to vary as a feeding deterrent, but its deterrence was least apparent in *Promecognathus* and *Metrius*. Of the 18 species of carabids tested, that range in body size and their pygidial gland chemical defense, 15 were highly susceptible to cyanide, with a tolerance level coincident with their body size. Two *Promecognathus* species and *Metrius contractus* all tolerated extremely high quantities and long exposure to HCN. Cyanide did not seem to affect the behavior of *Promecognathus* in any way, and therefore we propose that cyanide tolerance in this millipede specialist is likely part of its suite of adaptations to millipede hunting. *Metrius contractus* has nearly as high of tolerance of HCN as *Promecognathus*, but we found no evidence of millipede predation in this species. The significance and possible explanation for HCN tolerance in *Metrius* remain to be explored.

Supplementary Data

Supplementary data are available at *Annals of the Entomological Society of America* online.

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References Cited

Beasley, D. M., and W. I. Glass. 1998. Cyanide poisoning: pathophysiology and treatment recommendations. Occup. Med. (Lond). 48: 427–431.

Bedoussac, L., M. E. Favila, and R. M. López. 2007. Defensive volatile secretions of two diplopod species attract the carrion ball roller scarab *Canthon morsei* (Coleoptera: Scarabaeidae). Chemoecology. 17: 163–167.

Bousquet, Y. 2012. Catalogue of Geadephaga (Coleoptera, Adephaga) of America, north of Mexico. Zookeys. 245: 1–1722.

Bousquet, Y., and A. Smetana. 1986. A description of the first instar larva of *Promecognathus* Chaudoir (Coleoptera: Carabidae). Syst. Entomol. 11: 25–31.

Brown, J. W. 1992. Two new species of *Gnamptogenys* and an account of millipede predation by One of Them. Psyche. 99: 275–289.

Carrel, J., and E. Britt. 2009. The Whip Scorpion, *Mastigoproctus giganteus* (Uropygi: Thelyphonidae), preys on the chemically defended Florida scrub millipede, *Floridobolus penneri* (Spirobolida: Floridobolidae). Fla. Entomol. 92: 500–502.

Dazzini-Valcurone, M., and M. Pavan. 1980. Glandole pigidiali e secrezioni difensive dei Carabidae (Insecta Coleoptera). Publicazioni dell'Ist Entomol Univ di Pavia. 12: 1–36.

Dettner, K. 1985. Ecological and phylogenetic significance of defensive compounds from pygidial glands of Hydradephaga (Coleoptera). Proc. Acad. Nat. Sci. Philad. 137: 156–171. Retrieved from http://www.jstor.org/stable/4064888. August 30 2020.

Eisner, T., H. E. Eisner, J. J. Hurst, F. C. Kafatos, and J. Meinwald. 1963. Cyanogenic glandular apparatus of a millipede. Science. 139: 1218–1220.

Eisner, T., T. H. Jones, D. J. Aneshansley, W. R. Tschinkel, R. E. Silberglied, and J. Meinwald. 1977. Chemistry of defensive secretions of bombardier beetles (Brachinini, Metriini, Ozaenini, Paussini). J. Insect Physiol. 23: 1383–1386.

Eisner, T., D. Alsop, K. Hicks, and J. Meinwald. 1978. Defensive secretions of millipedes. pp. 41–72. *In* S. Bettini (ed.), Arthropod Venoms. Handbook of Experimental Pharmacology / Handbuch der experimentellen Pharmakologie, vol 48. Springer, Berlin, Heidelberg, Germany.

Eisner, T., M. Eisner, A. B. Attygalle, M. Deyrup, and J. Meinwald. 1998. Rendering the inedible edible: circumvention of a millipede's chemical defense by a predaceous beetle larva. Proc. Natl. Acad. Sci. U. S. A. 95: 1108–1113.

Forthman, M., and C. Weirauch. 2012. Toxic associations: a review of the predatory behaviors of millipede assassin bugs (Hemiptera: Reduviidae: Ectrichodiinae). Eur. J. Entomol. 109: 147–153.

Guldenstedeen-Egeling, C. 1882. Uber Bildung von Cyanwasserstoffsaure bei einem Myriapoden. Pflugers Arch. Physiol. 28: 576–579.

Hall, F., R. Hollingworth, and D. Shankland. 1971. Cyanide tolerance in millipedes: the biochemical basis. Comp. Biochem. Physiol. B 38: 723–737.

Hash, J. M., J. G. Millar, J. M. Heraty, J. F. Harwood, and B. V. Brown. 2017. Millipede defensive compounds are a double-edged sword: natural history of the millipede-parasitic genus *Myriophora* Brown (Diptera: Phoridae). J. Chem. Ecol. 43: 198–206.

Kobayashi, S., Y. Morita, Y. Nakaya, T. Nagamine, M. Onuma, T. Okano, A. Haga, I. Yamamoto, M. Higa, T. Naruse, M. Izawa. 2018. Dietary habits of the endangered Okinawa rail. Ornithol Sci. 17: 19–35.

LaBonte, J. R. 1983. Feeding behavior in the carabid beetle, *Promecognathus*. Bull. Oregon Entomol. Soc. 86: 681–682.

Larsen, T. H., A. Lopera, A. Forsyth, and F. Génier. 2009. From coprophagy to predation: a dung beetle that kills millipedes. Biol. Lett. 5: 152–155.

Ma, J., and P. K. Dasgupta. 2010. Recent developments in cyanide detection: a review. Anal. Chim. Acta 673: 117–125.

MacSwain, J. W., and W. V. Garner. 1956. Notes on two millipede-feeding carabids. Pan-Pac Entomol. 32: 54.

Marek, P. E., and W. Moore. 2015. Discovery of a glowing millipede in California and the gradual evolution of bioluminescence in Diplopoda. Proc. Natl. Acad. Sci. U. S. A. 112: 6419–6424.

Moore, W., and A. Di Giulio. 2008. *Metrius* Eschscholtz (Carabidae: Paussinae) is not a millipede specialist. Pan-Pac Entomol. 84: 33–34.

Parkin, E. A., and J. R. Busvine. 1937. The toxicity of hydrogen cyanide to certain wood-boring insects. Ann. Appl. Biol. 24: 131–143.

Pavlov, J., S. Xu, K. Will, B. Weary, and A. B. Attygalle. 2020. HCN emission by a polydesmid millipede detected remotely by reactive adsorption on gold nanoparticles followed by laser desorption/ionization mass spectrometry (LDI-MS). J. Chem. Ecol. 46: 455–460.

Ribera, I., J. Mateu, and X. Belles. 2005. Phylogenetic relationships of *Dalyat mirabilis* Mateu, 2002, with a revised molecular phylogeny of ground beetles (Coleoptera, Carabidae). J. Zool. Syst. Evol. Res. 43: 284–296.

Rodriguez, J., T. H. Jones, P. Sierwald, P. E. Marek, W. A. Shear, M. S. Brewer, K. M. Kocot, and J. E. Bond. 2018. Step-wise evolution of complex chemical defenses in millipedes: a phylogenomic approach. Sci. Rep. 8: 3209.

Shear, W. 2011. Class Diplopoda de Blainville in Gervais, 1844. In: Zhang, Z.-Q. (Ed.) Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Zootaxa. 3148:159–164.

Townsend, G. F. 1963. Benzaldehyde: a new repellent for driving bees. Bee World. 44:146–149.

Westley, J. 1973. Rhodanese. Adv. Enzymol. Relat. Areas Mol. Biol. 39:327–368.

Will, K. W., A. B. Attygalle, and K. Herath. 2000. New defensive chemical data for ground beetles (Coleoptera: Carabidae): interpretations in a phylogenetic framework. Biol. J. Linn. Soc. 71: 459–481.

Yamashita, N., C. L. Tan, C. J. Vinyard, and C. Williams. 2010. Semi-quantitative tests of cyanide in foods and excreta of three *Hapalemur* species in Madagascar. Am. J. Primatol. 72: 56–61.

Yang, C. Y., J. Kim, and H. H. Kim. 2018. Benzaldehyde synergizes the response of female *Xyleborinus saxesenii* (Coleoptera: Curculionidae, Scolytinae) to ethanol. J. Econ. Entomol. 111: 1691–1695.

Zagrobelny, M., S. Bak, A. V. Rasmussen, B. Jørgensen, C. M. Naumann, and B. Lindberg Møller. 2004. Cyanogenic glucosides and plant-insect interactions. Phytochem. 65: 293–306.