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ORIGINAL PAPER

Earwigs (*Labidura riparia*) mimic rotting-flesh odor to deceive vertebrate predators

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Abstract Many insects repel predators with caustic chemicals, while insects mimicking odors of wastes/dead insects to fool predators have not been documented. We found that the shore earwig, *Labidura riparia* (Dermaptera: Labiduridae) when bitten by anole lizards, *Anolis carolinenesus*, spits a rotting-flesh odor that deceives these insectivores into rejecting prey. Once a lizard attacked and rejected an earwig, the lizard did not attack another earwig during several weeks despite consuming other prey, indicating associative learning after one trial. The fetid odor was found in the head-prothorax containing salivary glands of both male and female earwigs and was comprised of ~100 ng dimethyl disulfide and ~600 ng dimethyl trisulfide. Nymphs had <5 ng of either compound. Adults also spit odorous sulfides after prolonged attacks by harvester ants, *Pogonomyrmex rugosus*, who were only deterred by the earwig's forceps. Sulfides released by the earwig are similar to odors of carrion/feces, which may be innately repulsive to some vertebrate predators. The mean initial discharge percentage (IDP) of sulfides from a cohort of earwigs was 62 %; however, IDPs of individuals were highly variable (3–99 %; mean 57 %). The discharge refill time (DRT) to refill 50 % of the earwig's allomone reservoir was estimated at 13 h. A positive relationship in sulfide amounts with body weight was found only in females in 2009, suggesting metabolic cost tradeoffs were revealed when sulfide content was half that in 2010. This is the first report of insects releasing sulfur-containing compounds that may mimic carrion-fecal odors as a deceptive defense against vertebrate predators.

Keywords Associative learning · Defensive allomones · Innate aversion · Mimicry · Predators · Vertebrate learning

Introduction

The shore earwig, *Labidura riparia* (Pallas) (Dermaptera: Labiduridae) is a large insect 20 to 30 mm long found throughout the world in many agricultural crops (Schlinger et al. 1959; Langston and Powell 1975; Waddill 1978; Radi and Linsenmair 1991; Johri and Johri 2012). The earwig is mainly nocturnal and a voracious predator of both soil-dwelling and aboveground insects including grubs, caterpillars, and aphids (Schlinger et al. 1959; Shepard et al. 1973; Ammar and Farrag 1974; Ruberson et al. 1994). During capture of a female *L. riparia*, we noticed a strong odor similar to carrion-feces. The volatiles in the odor were collected by solid-phase microextraction, analyzed by gas-chromatography mass spectrometry, and shown to consist mainly of dimethyl disulfide and dimethyl trisulfide. These two volatiles are known from carrion and dung (Urru et al. 2011; Jürgens et al. 2013) as well as from carrion-mimicking flowers and stinkhorn fungus (Moré et al. 2013; Pudil et al. 2014) that may deter herbivores (Lev-Yadun et al. 2009). While some carnivores such as vultures that feed on carrion have evolved specialized adaptations to counter high levels of potentially dangerous microbes in their diet (Roggenbuck et al. 2014), other animals may have evolved behavioral aversions to odors of rotting tissue and feces to avoid bacterial

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infection (Brand et al. 1988; Reed and Rocke 1992; Hussain et al. 2013). Therefore, we hypothesized that the earwig evolved a defensive strategy that uses a carrion/fecal-like odor to deceive insectivorous vertebrates into rejecting an otherwise suitable live prey. Such a “dishonest” mimicry of dead insect/feces odor would be unusual and in contrast to the common and more “honest” defense strategy of employing toxic irritants (Eisner 1960; Pasteels et al. 1983; Aldrich 1988; Eisner et al. 2000; Byers 2006).

A more effective defense against predators may be mounted by individuals with large amounts of defensive volatiles compared with those with more limited amounts. Some studies have suggested there are relatively larger metabolic costs for producing semiochemicals in smaller insects compared with larger individuals (Byers 2005, 2006; Jaffe et al. 2007; Byers and Levi-Zada 2011). Therefore, the first objective was to determine whether a positive relationship exists between body weight of *L. riparia* and amounts of the major volatile dimethyl trisulfide for males and females.

The initial discharge percentage (IDP) was hypothesized as an inherent characteristic of species that employ alarm and defensive volatiles, giving a relatively consistent mean release (Byers 2006). For example, insect species employing defensive compounds during a predator attack might have a large IDP and release a large proportion of their glandular contents in an “all-out” defense strategy (e.g., hemipteran bugs), while other species might have smaller IDP due to smaller but multiple releases in a short time (e.g., ants defending their colony). The mean IDP due to molestation can be determined by solvent extraction of two cohorts of individuals, one group unmolested (full glands) and another group molested (expelled glands). IDP of individuals have not been estimated previously but could be determined by quantifying amounts in air collected from a molested individual followed by solvent extraction of that individual (the two amounts equal the initial unmolested amount). Therefore, the second objective was to investigate individual variation of earwig IDPs and compare the corresponding mean to the mean IDP calculated from the cohort extraction method. A third objective was to explore another hypothesized species characteristic, here termed the discharge refill time (DRT) that is the time required for the insect to replenish its defensive compounds after release in preparation for the next predatory challenge. Additional objectives were as follows: (a) quantify the amounts of dimethyl disulfide and dimethyl trisulfide and other similar compounds in both sexes of *L. riparia*, (b) investigate whether insect predators (harvester ants) or insectivorous vertebrate predators (anole lizards) would elicit sulfide release from the earwig and whether the sulfides would deter further attack or feeding, (c) determine whether earwig nymphs use the same defensive chemicals as adults, and (d) identify the body region where the allomones are stored.

Materials and methods

Insects

Adult *L. riparia* were collected in 20 pitfall traps (5-cm diameter×10-cm deep) in alfalfa fields (33° 4′ N, 111° 59′ W) in October 2008 and from August to October in 2009 and 2010. Both sexes were held in plastic boxes (29×14×11 cm) from 1 to 14 days after collection until used in experiments. Earwigs were fed ad libitum with water (10-ml tubes with cotton) and dried dog food. In addition, second instar caterpillars of *Trichoplusia ni* (Hübner) were introduced daily (about one per adult earwig). Nymphs (first to fifth instar, sex undetermined) were also collected from the pitfall traps and used immediately in experiments in 2010. Sex of adults was determined by the presence in males of a single tooth near the middle of each large cercus on the inner side and in females a series of smaller teeth along the inner sides of the less stout cerci, also called forceps or pincers (Langston and Powell 1975).

Lizard predation tests with adult earwigs

The anole lizard, *Anolis carolinensis* Voigt, feeds exclusively on living insects in southeastern USA (Lovern et al. 2004). Six female and four male anole lizards were purchased from pet stores in Phoenix, AZ, and each individual was placed alone in a plastic box with screened top (45×30×30 cm). The enclosures with lizards were maintained at 28 °C under natural light conditions. Care and maintenance of the lizards complied with guidelines of the National Academy of Sciences, USA (Garber et al. 2011). The lizards had access to water in a 60-mm Petri dish and on the first test day, earwigs were introduced to half the lizards and the other half was given fourth or fifth instar mealworms (*Tenebrio molitor* L.). Every 2 days for a total of 12 test days in November 2008, the prey types were reversed with respect to the lizards ($N=60$ observations for each prey type). The presented earwig or mealworm was removed after 5 minutes if it had not been eaten by a lizard. The numbers of successful and unsuccessful predation events by the lizards were recorded for earwigs and mealworms and compared by a Fisher’s exact test (Statistica 5.1, OK, USA).

Solvent extraction, identification, and quantification of earwig volatiles

To determine amounts of the major volatiles responsible for the odor of the earwigs, individual earwigs were extracted and analyzed by gas chromatography-mass spectrometry (GC-MS). Individuals (entire nymphs or body sections of adult males or females) were extracted in 100 μl hexane with an internal standard of (+)-carvone (1 ng/μl, Sigma-Aldrich, MO, USA). Earwig sections were macerated in hexane in a

Wheaton 0.1-ml thick-walled glass V-vial (0.3 ml capacity; NJ, USA) with a blunt, nickel-plated tapestry needle (Prym-Dritz Corp., SC, USA). To avoid enzymatic degradations, the supernatant hexane from the macerated earwig was transferred by pipette into a glass insert (W.R. Grace, Columbia, MD) in a 2-ml vial (Byers 2006). Chemical analysis of volatiles was carried out by autosampler injection (Varian 8400, CA, USA) of 1 μl of each extract into a Varian 3800 GC coupled to a Varian Saturn 2000 MS (ion trap at 70 eV). The GC column was a chiral fused-silica capillary (Cyclodex-B, J&W Scientific, CA, USA) 60 m long×0.25 mm ID coated with 0.25 μm permethylated β-cyclodextrin. Helium at a constant flow of 1.2 ml/min was used as carrier gas. The injection port was held at 250 °C, and the analysis was performed in the splitless mode for 0.75 min then 60:1 split to 5 min. The GC-oven temperature program was 40 °C for 2 min, then 15 °C/min to 100 °C and held 10 min, then 3 °C/min to 150 °C, then 20 °C/min to 230 °C and held 10 min. Dimethyl disulfide (7.70 min, RI=853), dimethyl trisulfide (13.95 min, RI=1065), and 3-(methylthio)-propanal (13.60 min, RI=1056) in earwigs were identified by comparison of retention times and mass spectra to those of commercial standards (all >98 % purity, Sigma-Aldrich) using the NIST08 (National Institute of Standards, USA) spectral reference library. These compounds in earwigs were also analyzed by a nonpolar column and matched RI of standards. Other sulfur-containing compounds in trace amounts were tentatively identified by spectral references and reported RI. To quantify chemicals in solvent extracts, the total ion chromatogram areas at the respective retention times were adjusted for quantities and MS response factor sensitivities (Byers 2006) and compared with areas of the internal standard. Selected monitoring of ions 61, 79, 94, 126, and 158 were used to estimate smaller amounts of the sulfides sometimes co-eluting with other volatiles (Byers 2005).

Individual earwigs collected from alfalfa fields in 2009 were brought to the laboratory and separated by sex, weighed on a microbalance to nearest 0.01 mg, and then cooled and frozen (−20 °C) to prepare them for extractions. Frozen earwigs were cut by a razor blade into head, thorax, and anterior and posterior abdomen sections that were each extracted in hexane and analyzed by GC-MS as described above. Because significant amounts of the sulfides were found in the thorax, it was portioned differently in 2010. The adults (*N*=33 males, *N*=51 females) collected at that time were weighed, frozen, and sectioned into head+prothorax (including the first pair of legs) and meso+metathorax (with second and third pairs of legs). The abdomen was discarded as no sulfides were found in earlier extractions above. Various earwigs molested by ants and pinches of legs by forceps were similarly extracted after airborne collection or solid-phase microextraction (SPME) fiber absorption of headspace volatiles (described subsequently). Differences between extracted amounts of the two sulfides between the sexes or within a sex between unmolested and molested individuals for each experiment were analyzed by Wilcoxon rank-sum tests (SAS Institute JMP 11.1.1, NC, USA). Scatter plots of fresh weight versus dimethyl trisulfide content were analyzed separately for each combination of insect sex and year of collection by linear regression with TableCurve 2D version 5.01 (Systat Software Inc., IL, USA).

Collection of airborne volatiles from molested earwigs

To determine whether adult earwigs will release their sulfide volatiles upon molestation by ants alone, or by a combination of ants and pinching of earwig legs by forceps, SPME and GC-MS analysis was performed. SPME collections were done to investigate relative emission amounts among individuals of adult males (*N*=39), adult females (*N*=51), and nymphs in 2010. Adults of both sexes of *L. riparia* were individually weighed and placed in a 25-ml vial with Teflon lid that had a 0.5-mm hole. Three harvester ants, *Pogonomyrmex rugosus* Emery, then were added which proceeded to attack the adult earwig. Earwigs that managed to prevent the ants from inflicting injury for several minutes had their legs pinched with a forceps as ants crawled over them which invariably elicited release of the sulfides detected initially by the foul smell. Immediately thereafter, the vial was capped with Teflon liner and a SPME 23-gauge needle was inserted into the vial containing the earwig. After 30 s of odor equilibrium, the fiber (1 cm×60 μm polyethylene glycol; Supelco, Bellfonte, PA, USA) was exposed in the vial for 1 min before volatile desorption in the GC injector (250 °C). The earwigs were held for 20 min after molestation and SPME collection to allow the expelled sulfides to evaporate from their cuticles before extraction of internal amounts. This time was sufficient as shown by sequential 10-min air collections on Porapak Q presented subsequently. Control SPME collections from individual unmolested earwigs (*N*=5 each sex) were also performed.

To estimate sulfide contents and releases of nymphs, first to fifth instars were weighed, placed in 2-ml vials, molested by attacks of two *Pogonomyrmex californicus* (Buckley) ants, and then SPME was performed as above. The amounts of the sulfides in molested (*N*=131) and unmolested nymphs (*N*=52) grouped by weight class (<10, 10–20, 20–40, and 40–90 mg) were found by solvent extraction as above. The GC oven temperature program for SPME analysis was 40 °C for 2 min, then 15 °C/min to 80 °C, then 3 °C/min to 140 °C, then 20 °C/min to 230 °C and held 5 min. Other GC-MS conditions were as described above.

Adult earwigs (*N*=30) each had a leg pinched by forceps in the presence of two *P. rugosus* ants to determine absolute quantities of sulfides released individually in response to molestation. Laboratory air was passed from an activated charcoal trap (Alltech Associates, IL, USA) through Teflon tubing and a flow meter to an adapter fitting with Teflon ferrules (Swagelok, Solon, OH) for a 5-cm long × 1.27-cm diameter glass tube. The earwig and harvester ants were placed inside this glass tube, and after successful molestation (smell of sulfides), the other end of the glass tube was immediately fitted to another adapter connected to a 2.7-cm long Teflon tube filter filled with Porapak Q adsorbent (80/100 mesh, Alltech Associates), which was connected to a vacuum line. Volatiles were collected at 25 °C for 10 min at an air flow of 0.2 l/min. No breakthrough of the sulfides was observed into a second Porapak plug. Two successive 10-min collections on different filters were done to determine the rate of evaporation of the sulfides before the earwig was removed and extracted. After each airborne collection, the Porapak Q filter was removed and 200 μl of hexane with internal standards was passed through the adsorbent into a glass insert for subsequent GC-MS analysis.

IDP of defensive contents

IDP experiments were conducted in September and October 2010. The cohort IDP of defensive compounds from an insect species was based on the mean amounts in extracts of an unmolested cohort (33 males or 51 females) compared to mean amounts extracted from a molested cohort (39 males or 51 females) according to Byers (2006):

$$\mathrm{IDP} = \frac{U - M}{U} \times 100 \tag{1}$$

where U is the mean extracted amount of the unmolested group and M is the mean extracted amount of the molested group. Mean amounts in extracts were determined by GC-MS.

Individual IDP ($N=30$ mixed sexes) was measured differently by molesting an earwig inside a glass tube so that the emitted odors could be collected by Porapak Q as described above. After odor collection for 20 min (two successive 10-min Porapak Q collections), the individual was extracted and the IDP calculated according to:

$$\mathrm{IDP} = \frac{P_M}{M + P_M} \times 100 \tag{2}$$

where P_M is the amount collected by the Porapak after molestation and M is the amount extracted from the adult after the Porapak collection.

DRT of defensive contents

The time to replenish the defensive compounds after molestation was estimated by comparing the mean amounts of dimethyl trisulfide in molested insects based on three cohorts ($N=6$ each) that were extracted at either 6, 11, or 48 h after molestation. The amount of this sulfide in molested insects at 0 h (mean of both sexes: 232.6±28.8 ng/insect, $N=114$) was subtracted from the amounts of the above cohort means. The data were then subjected to nonlinear regression using TableCurve 2D and fit to a kinetic first-order function (Byers 2013): $Y=a(1-\exp(-bX))$, where Y is the estimated content of dimethyl trisulfide at the time of extraction, X is time after molestation, and a is the estimated maximum content of dimethyl trisulfide. This function was solved for X using $Y=50$ or 95 % of a. Thus, the time to replenish 50 % (DRT_{50}) or 95 % (DRT_{95}) of the defensive compounds is given by

$$DRT_P = \frac{-\ln\left(-\left(a \cdot P / 100 - a\right) / a\right)}{b} \tag{3}$$

where P is percentage refill and a and b are from the fitted regression function.

Results

Lizard predation tests with adult earwigs

The ten anole lizards ate 52 of the 60 mealworms presented but none of the 60 earwigs ($P<0.001$, Fisher's exact test). Seven lizards attacked, bit, and rejected an earwig on the first introduction; two lizards rejected an earwig on the second introduction; and one lizard rejected an earwig on the third introduction. Thus, all ten lizards bit and immediately spat out an earwig. In all attacks, a distinct odor of rotting flesh could be smelled immediately thereafter and the earwigs appeared uninjured. No lizard attacked more than one earwig during the tests, but mealworms were eaten readily throughout the testing regardless of earlier exposure to an earwig.

Solvent extraction, identification, and quantification of earwig volatiles

Heads of female earwigs ($N=31$) collected in August 2009 contained a mean (±SE) of 24±4 ng of dimethyl disulfide and 122±20 ng of dimethyl trisulfide, and thoraxes averaged 37±13 and 121±38 ng, respectively. The anterior abdomen had a mean of 15±9 and 34±20 ng of the respective sulfides while the posterior abdomen had none. However, 28 of the 31 females had no sulfides in their anterior abdomen, suggesting the three with large amounts may have been cut imprecisely. Heads of males ($N=10$) averaged 42±14 and 142±37 ng of the two sulfides, and thoraxes averaged 18±7 and 46±11 ng. Only a trace of dimethyl trisulfide was found in the anterior abdomen of males, and none was found in the posterior abdomen. Thus, most of the sulfides reside in the head and thorax. The mean amounts of the two sulfides in females were 76±21 and 277±55 compared to 60±18 and 188±47 ng in males. There were no significant differences between the sexes in mean disulfide (S=215, df=1, P=0.89) or trisulfide content (S=200, df=1, P=0.77). Linear regression of body weight and dimethyl trisulfide amount in females had a positive slope (Fig. 1) that was significant ($F_{1,29}$=7.13, P=0.012) with 19.7 % (R^2=0.197) of the variation explained. The regression for males was not significant ($F_{1,8}$=0.29, P=0.6) with only 3.5 % of the variation explained. In 2010, however, linear regressions of weight versus dimethyl trisulfide amounts in unmolested males and females did not show significant linear relationships (males $F_{1,31}$=2.33, P=0.14; females $F_{1,49}$=0.018, P=0.89).

Unmolested nymphs sampled in 2010 had only small quantities of dimethyl disulfide and dimethyl trisulfide (Fig. 2). The amounts of sulfides appeared greater in heavier nymphs, while none of the first instar (2 to 7 mg weight) unmolested nymphs (N=24) contained detectable amounts of the two sulfides. Corresponding nymphs that had been molested by ants in 2010 had similarly low amounts of the two sulfides (Fig. 2). In the confines of the 2-ml vials nymphs repeatedly escaped attacks by *P. californicus* ants but after several minutes 57 of 131 nymphs were eventually captured and killed by the ants.

In 2010, unmolested adult earwig males had a mean weight of 168.1±9.1 mg and corresponding females a mean weight of 144.9±4.2 mg. The unmolested adult males had significantly greater mean amounts of the two sulfides than the molested adult males (Fig. 3). The loss of sulfides due to molestation gave mean IDPs for males of 50 % for dimethyl disulfide and 52 % for dimethyl trisulfide (Fig. 3). The unmolested adult females also had significantly greater mean amounts of the sulfides than the molested females (Fig. 3). The female cohort IDP for the two sulfides was 75 % for dimethyl disulfide and 71 % for dimethyl trisulfide (Fig. 3). The amounts of the two sulfides were not significantly different between the unmolested sexes (S=1301, df=1, P=0.36, and S=1374, df=1, P=0.80) or between molested sexes (S=1903, df=1, P=0.30, and S=1978, df=1, P=0.10). The unmolested male meso+metathorax contained only 4.9 and 5.4 % as much dimethyl disulfide and dimethyl trisulfide, respectively, as their head+prothorax. The unmolested female meso+metathorax contained even less of the two sulfides (0.1 and 0.2 %,

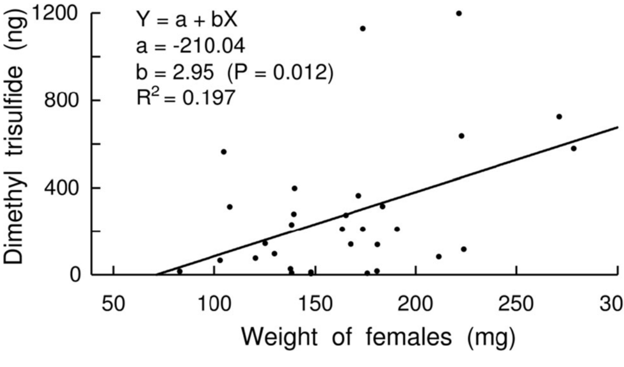

Fig. 1 Relationship of body weight of female *Labidura riparia* and amount of dimethyl trisulfide extracted from these individuals after collection from alfalfa fields (August 2009, Maricopa, AZ)

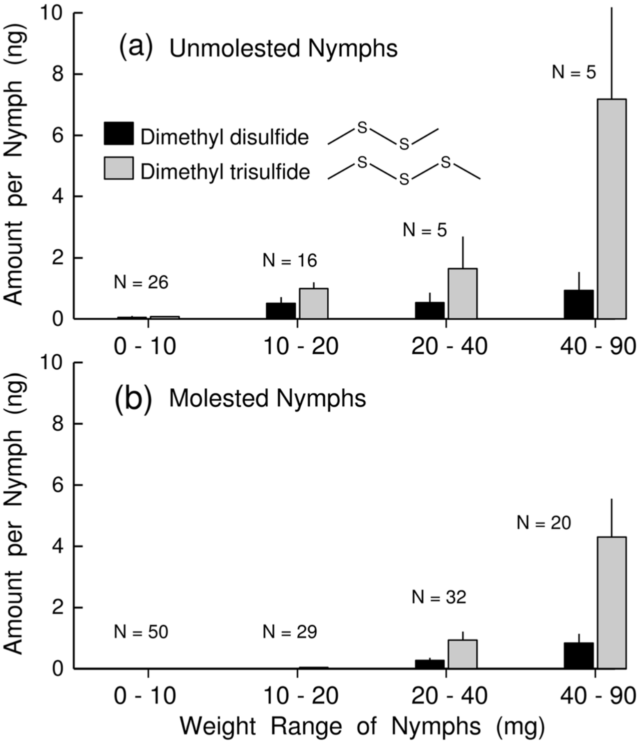

Fig. 2 Mean amounts (histogram bars+SE lines above) of dimethyl disulfide and dimethyl trisulfide extracted from earwig nymphs *Labidura riparia* of various weight ranges (collected from alfalfa fields 25 Aug–19 Oct 2010). **a** Nymphs not molested. **b** Nymphs molested by exposure to attacking harvester ants (*Pogonomyrmex californicus*)

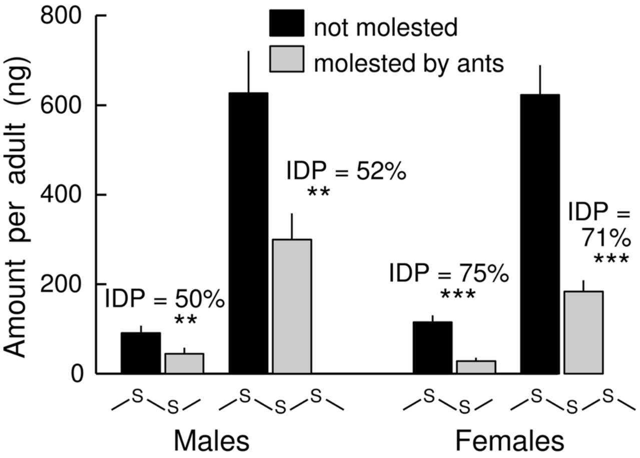

Fig. 3 Mean amounts (+SE) of dimethyl disulfide ($C_2H_6S_2$) and dimethyl trisulfide ($C_2H_6S_3$) extracted from *Labidura riparia* adult males unmolested and molested (N=33 and N=39, respectively) and similarly from adult females (N=51 and N=51, respectively) collected from alfalfa fields Sep–Oct 2010 (Maricopa, AZ). *Asterisks* indicate differences in amounts within a chemical and sex between unmolested and molested groups (**P<0.01 and ***P<0.001, Wilcoxon); cohort initial discharge percentage (IDP) from Eq. 1

respectively) compared with their head+prothorax. These males and females contained almost identical mean amounts of dimethyl trisulfide (about 620 ng), which was greater than amounts observed in 2009. In unmolested adults in 2010, there was considerable variation between individuals in allomone amounts. For example, the major component (dimethyl trisulfide) had a coefficient of variation (CV=SD/mean) of 76 % in females and 86 % in males. In unmolested adults (N=84), dimethyl trisulfide was the major sulfide component (625 ng=85.4 %) compared to dimethyl disulfide (14.4 %), and very small amounts of methyl methylthio methyl disulfide (0.14 %) and dimethyl tetrasulfide (0.004 %). Also, 3-(methylthio)-propanal was rarely detected in extracts and then only in trace amounts.

Collection of airborne volatiles from molested earwigs

The adult earwigs in the 25-ml vials repeatedly used their pincers to grasp ants and prevent attacks. *P. rugosus* ants in the vial usually needed several minutes to catch and bite an earwig usually on its leg, which then induced volatile sulfide release. At that time, a small droplet was often seen emitted from the earwig's mouth onto the wall of the glass vial. The emission of volatiles by the earwig did not appear to prevent ants from continuing their attacks, which failed to kill the earwig. Of the 51 molested females, 33 (65 %) were induced to release volatiles by the attacking ants, while 18 (35 %) had to be pinched by forceps with ants present to induce release. The observations of 39 molested males were similar, except 14 (35 %) were induced naturally by ants and 25 (64 %) had to be pinched by forceps with ants present, suggesting that males were more reluctant to release allomone than females when only attacked by ants (P=0.01, Fisher's exact test).

The mean amounts of dimethyl disulfide and dimethyl trisulfide collected on the SPME fiber sampling volatiles from molested males (N=39) was 0.24±0.04 and 3.69±0.89 ng and for females (N=51) 0.26±0.03 and 3.12±0.40 ng, respectively, with no significant differences between sexes for either compound (S=1727, df=1, P=0.70, and S=1303, df=1, P=0.64). Molested nymphs released a tiny fraction of that by molested adults as indicated by SPME collections. For example in 2010 a mean of 0.12±0.03 ng dimethyl trisulfide per molested nymph (40–90 mg, N=20) was collected on SPME in 2-ml vials. SPME collected even less (0.03±0.01 ng) from smaller nymphs (20–40 mg, N=32), and only a trace (0.003±0.001 ng) was detected for nymphs under 20 mg (N=79). It is well known that quantities collected by SPME are a small proportion of the total released and rather indicate relative amounts released by individuals. There was considerable variation in amounts of dimethyl trisulfide collected by SPME (CV=151 % for males and 91 % for females). Numerically, there was more variation in amounts of dimethyl trisulfide extracted from molested adults than from unmolested adults (for males CV=107 and 86 %, respectively; for females CV=112 and 76 %). Unmolested adult males and females (N=5 each sex) sampled by SPME and GC-MS did not release detectable amounts of the sulfides. Linear regressions did not indicate relationships between weights of molested earwigs and amount of dimethyl trisulfide on SPME for males ($F_{1,37}$=0.25, P=0.62) or females ($F_{1,49}$=0.13, P=0.72). One sulfur-containing compound, 3-(methylthio)-propanal that was rarely detected in extracts was absorbed in small amounts by the SPME fiber (means of 0.07 ng from females and 0.05 ng from males).

IDP of defensive contents

The variation in amounts of sulfides released, determined by Porapak adsorption, and amounts remaining in the earwig varied considerably and gave IDPs of individuals that ranged from 3 to 99 % (Fig. 4). Some individuals with low initial amounts were able to release high proportions for a large IDP, while other individuals with initially large amounts released small proportions for a small IDP (Fig. 4). The mean IDP of males was 59±10 % (N=12) and 56±9 % for females (N=18). The mean IDP of individuals of pooled sexes was 57 ±6 % (95 % CI=44–70 %), which was similar to the IDP estimated from the means of molested and unmolested cohorts (62 % average of males and females).

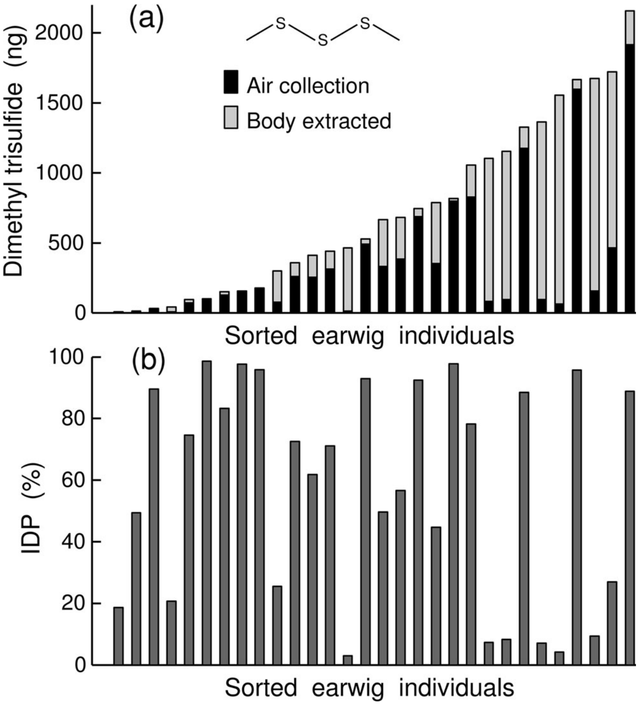

Fig. 4 **a** Amounts of dimethyl trisulfide released from adult *Labidura riparia* individuals that were molested (as determined by Porapak Q collection) compared to the same individuals extracted in solvent and sorted by total amounts (Sep–Nov 2010). **b** Initial discharge percentage (IDP) of corresponding individuals based on ratios of amounts released and retained in body (Eq. 2)

DRT of defensive contents

The first-order kinetic function (Eq. 3) relating mean amounts of dimethyl trisulfide in extracted cohorts at various times after molestation was significant ($F_{1,2}$=18.07, P=0.05; Fig. 5). Solving for X at 50 or 95 % of a, the estimated maximum, gave estimated times in hours for the earwigs to refill their defensive secretion to 50 or 95 % (DRT_{50}=13 h, DRT_{95}=56 h). However, the estimated time to refill the glands to 95 % of capacity should be interpreted with caution because it was outside the range of observations.

Discussion

The anole lizard, *A. carolinensis*, is a potential predator of the shore earwig, *L. riparia*, since both species inhabit areas of the southeastern USA (Schlinger et al. 1959; Waddill 1978; Lovern et al. 2004). The shore earwig's defense appears unique by mimicking the distinct odor of rotting flesh that because of microbial growth could be dangerous for insectivorous vertebrates to eat (Brand et al. 1988; Reed and Rocke 1992; Hussain et al. 2013). Whether the aversive response of the anole lizard to the immediate release of the earwig's defensive allomone would occur with other vertebrate predators is unknown but worthy of study. Dozens of insectivorous lizard species, including several geckos, co-occur with the earwig in desert areas in the southwestern USA where cotton and alfalfa are grown (Brennan and Holycross 2006). Predation by diurnal lizards would likely be limited because the earwig is nocturnal, but two common species of gecko are nocturnal, the native western banded gecko, *Coleonyx variegates*, and the introduced Mediterranean gecko, *Hemidactylus turcicus* (Brennan and Holycross 2006). These exclusively insectivorous geckos would be candidates for further study to see if the responses observed here are more general among these types of predators. In addition several small mammals such as the insectivorous desert shrew, *Notiosorex crawfordi* (Armstrong and Jones 1972), mice, *Peromyscus* spp. (Hoffmeister 1986), and grasshopper mice, *Onychomys* spp. (Rowe and Rowe 2006) overlap in distribution with the earwig and deserve further examination.

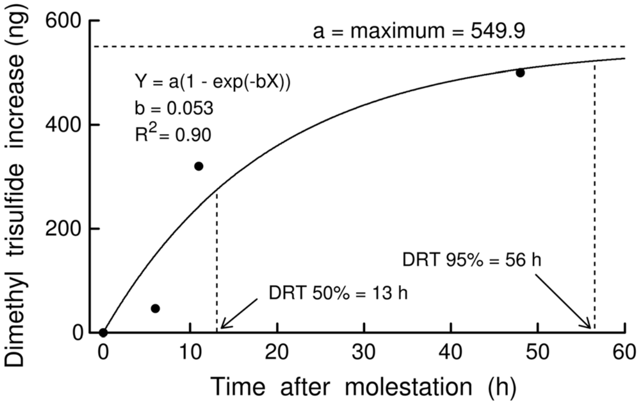

Fig. 5 Increase in dimethyl trisulfide in *Labidura riparia* adults after initial molestation at 0 hours. The discharge refill time (DRT) for 50 and 95 % replenishment of dimethyl trisulfide in adults after molestation was estimated by a kinetic formation equation. Adults were collected from alfalfa fields (20–27 September 2010) and maintained in the laboratory until molested (N=114 extracted at 0 h), or extracted at 6, 11, or 48 h after molestation (N=6 each time). Observed means of cohorts were reduced by 232.6 ng (mean of 0 h cohort) to estimate content change over time

The anole lizards exhibited an innate aversion to bitten earwigs that released dimethyl disulfide and dimethyl trisulfide. Innate aversion responses to specific odors/tastes have been shown in vertebrates such as fish, reptiles, birds, and mammals (Cooper and Hardegen 2000; Hile et al. 2004; Kobayakawa et al. 2007; Aihara et al. 2008; Shanbhag et al. 2010; Hussain et al. 2013). None of the anole lizards after biting an earwig attempted to attack another earwig over several weeks suggesting the lizards learned to associate putrid odors of unpalatable food with the earwig after only one trial. Rapid learning associating innately aversive odors/tastes with objects and colors (or species) has been demonstrated after only one trial in some other vertebrates (Roper and Marples 1997; Laska and Metzker 1998; Shanbhag et al. 2010).

In 2009, most of the dimethyl disulfide and dimethyl trisulfide amounts were found in the head and thorax of both sexes. About 97 % of the sulfides were found in the head+prothorax extracts compared with 3 % in the meso+metathorax. The digestive system of *L. riparia* was described by Johri and Johri (2012) as a straight tube from the esophagus in the head to the crop (foregut) that then extends through the thorax to the first abdominal segment. They show a medial reservoir at the back of the head connected to a pair of salivary glands extending into the prothorax. Because the earwig inside the vial was seen spitting droplets of liquid on the glass wall when attacked by ants, and the sulfides were primarily found in the head+prothorax, this suggests that the defensive secretion is found in the salivary glands. The crop was not found to contain the sulfides (Byers unpublished). Additional study will be necessary to confirm the source of the defensive compounds.

Moth species with greater body size are associated with higher pheromone titers in females, suggesting costs that smaller-bodied species are less able to maintain (Symonds et al. 2011). A positive relationship between body weight and semiochemical amounts was found in females of cotton aphid *Aphis gossypii*, plant bug *Lygus hesperus*, moth *Neoleucinodes elegantalis*, and wasp parasitoid *Leiophron uniformis*, suggesting metabolic costs that were difficult to achieve by smaller individuals (Byers 2005, 2006; Jaffe et al. 2007; Byers and Levi-Zada 2011). Condition-dependent costs of pheromone signaling also were suggested because larger individuals have more pheromone than smaller individuals, or release more pheromone when reared on high-quality diet (Ming and Lewis 2010; Harari et al. 2011; Blaul and Ruther 2012). However, the ecological implications and physiological relationships are suggestive and still unresolved. In *L. riparia*, a significant positive relationship between body weight and dimethyl trisulfide was not found in males, while females exhibited a positive relationship in 2009 but not in 2010. While evidence for metabolic cost tradeoffs for defensive chemicals in the shore earwig was inconclusive, the positive relationship in females may have been revealed in 2009 when sulfide content was 48 % of that in 2010. The high interindividual variation in amounts of sulfides in the earwig (Fig. 4) appears similar to variation in venom amounts in heads of the braconid wasp *L. uniformis* (Byers and Levi-Zada 2011). Because the earwigs were field-collected, some of the observed variation in sulfide amounts could have resulted from inclusion of earwigs that recently experienced a predation attempt that elicited defensive emission. A better understanding of the variation may come from studies on the earwig's salivary glands and the biosynthesis of the sulfur-containing volatiles.

Nymphs of weights from 2 to 10 mg (first–second instars) had practically no sulfides that could be used for defense. The fourth and fifth instar nymphs (40–90 mg) had only a few ng of dimethyl trisulfide available for defense (Fig. 2) compared to adults weighing from 100 to 300 mg that contained a mean of 625 ng, with some adult individuals containing >1000 ng. Perhaps the younger nymphs do not expend resources for a sulfide defense due to little benefit because they are sheltered in the maternal burrow (Schlinger et al. 1959; Shepard et al. 1973; Ammar and Farrag 1974; Radi and Linsenmair 1991) and have limited exposure to vertebrate predators.

Two species of harvester ants were used as models of an insect predator and were able to attack and kill earwig nymphs but not adults. The ants had difficulty catching the earwigs even over several minutes in the small vials, while the pincers of adult earwigs appeared effective in defense against ants, also observed for the European earwig, *Forficula auricularia* (Eisner 1960). However, harvester ants that bit an adult earwig and elicited sulfide emissions did not appear to be injured or deterred from continuing to attack. Dimethyl disulfide, the sulfide found at about 17 % of the major component dimethyl trisulfide, has been reported to repel a mirid bug *Apolygus lucorum* (Pan et al. 2013). Dimethyl disulfide is reported to cause insecticidal neurotoxicity through mitochondrial dysfunction and activation of potassium-ATP channels in some insects (Dugravot et al. 2004). Although not investigated, it could be that the earwig uses its neurotoxic dimethyl disulfide in saliva to paralyze prey, as well as a defense against vertebrate predators.

Solvent extractions of cohorts of unmolested and molested adult earwigs estimated a mean IDP for dimethyl trisulfide of 71 % for females and 52 % for males. These sexual IDPs estimated by the cohort method can be averaged as 62 % since there was no significant difference in sulfide contents between the sexes whether molested or unmolested. However, the IDPs of individuals based on Porapak Q odor collections and extractions were surprisingly variable ranging from 3 to 99 % (mean 57 %). Total amounts (odor collection and extracted) of dimethyl trisulfide also varied substantially among these individuals (9–2157 μg). The results suggest individual IDP of this earwig are largely independent of the individual's initial content of sulfides, raising questions as to the consistency of the cohort IDP of 62 % for this species. However, the variation in individual IDPs that resulted from molestation by a combination of ant attacks and pinching with forceps could be different from that in response to vertebrate predators. The estimated mean IDP of the earwig is similar to the cohort mean IDP for metathoracic secretions by the plant bug *L. hesperus* (60 %) in response to attack by ant predators (Byers 2006).

Once the defensive secretion is discharged, it would be beneficial to rapidly biosynthesize new secretion to regain protection. The question is how long to replenish glandular capacity. Like many biosynthetic reactions (Berg et al. 2002), the DRT may be under feedback control and thus may recharge according to a first-order kinetic formation function. This type of function gives a coefficient that represents the theoretical maximum amount of secretion and can be used to solve for X (time) at which the glands are refilled to 50 or 95 % of maximum capacity. These results are preliminary and should be expanded for *L. riparia* and other insects to determine whether the kinetic formation function has broad application for predicting glandular refill.

Some other earwigs are known to produce defensive compounds that are caustic. For example, the European earwig emits benzoquinone compounds from glands on two mid-abdominal tergites to repel harvester ants (Eisner 1960). However, these defenses were not effective against several vertebrate predators. Another earwig, *Doru taeniatum*, has a pair of defensive glands on the fourth abdominal tergite that discharge benzoquinones (Eisner et al. 2000). However, no earwigs previously have been shown to use compounds with sulfur. In fact, insect use of sulfur-containing semiochemicals has been found in only a few species. Robbins et al. (2003) reported methyl 2-(methylthio)benzoate as a unique sulfur-containing sex pheromone for a scarab beetle, *Phyllophaga crinita*. Sulfur-containing components of the sex pheromone of the cockroach *Nauphoeta cinerea* are 2-methylthiazolidine and 2-methyl-2-thiazoline (Sreng 1990; Sirugue et al. 1992). The ant *Paltothyreus tarsatus* produces dimethyl disulfide and dimethyl trisulfide biosynthesized from methionine; however, the biological function of these compounds is unknown (Crewe and Ross 1975). In contrast, results herein demonstrate the previously unreported use of sulfur-containing compounds for insect defense from predation. Thus, it seems likely that *L. riparia* has evolved a novel chemical defense that mimics decaying flesh and fecal odors, and that this defense is innately repulsive to many of its likely insectivorous vertebrate predators. The repellent chemicals involved in this deceptive strategy and other similar systems open new avenues for research into developing non-lethal management of wildlife in conflict with humans (Baker et al. 2005).

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Data accessibility Data deposited in figshare repository: http://figshare.com/preview/_preview/1308556

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