Burying Beetle Parents Adaptively Manipulate Information Broadcast from a Microbial Community

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Abstract: Microbial volatiles provide essential information for animals, which compete to detect, respond to, and perhaps control this information. Burying beetle parents have the opportunity to influence microbiably derived semiochemicals, because they monopolize a small carcass for their family, repairing feeding holes and applying exudates that alter the microbial community. To study adaptive manipulation of microbial cues, we integrated mechanistic and functional approaches. We contrasted gas chromatography–mass spectrometry (GC–MS) volatile profiles from carcasses that were or were not prepared by a resident pair of Nicrophorus orbicollis. Methyl thioctanate (MeSCN), the primary attractant for burying beetles seeking a fresh carcass, was reduced 20-fold by carcass preparation, while dimethyl trisulfide (DMTS), which deters breeding beetles, was increased 20-fold. These results suggest that parental care serves to make previously public information more private (crypsis, MeSCN) and to disinform rivals with a deterrent (DMTS). Functional tests in the field demonstrated that carcass preparation reduced discovery and use by congeners (threefold) as well as by dipteran rivals. Because microbes and their chemicals influence nearly every aspect of animal ecology, animal manipulation of microbial cues may be as widespread as manipulation of their own signals.

Keywords: semiochemical, deception, resident advantage, parental care, carrion ecology, microbi ally derived volatile organic compounds.

Introduction

Organisms compete to detect, use, and control information. Success can be achieved by being highly sensitive to important cues or by being fast to respond (Bytheway et al. 2016; Potier et al. 2018). Success is also possible by controlling information—making information more private or disinforming rivals. Deception occurs when a sender produces a signal that increases its own fitness but lowers the fitness of the receiver (Wiley 1994). If the organism itself is the source of a cue, it is possible to manipulate the cue directly. For example, larval butterflies in the genus Maculina are social parasites of Myrmica ants, using acoustic mimicry to be fed by or to feed on colony members (Barbero et al. 2009). Numerous species of corpse-mimicking plants produce fetid odors to bring falsely informed carrion insects to the flower as pollinators (Jürgens et al. 2013; Kite and Hetterscheid 2017).

Microbes can inform eukaryotes by producing volatile organic compounds (Tomberlin et al. 2012; Davis et al. 2013). Microbes alter pheromone release in diverse organisms (Theis et al. 2013; Engl and Kaltenpoth 2018; Whittaker et al. 2019), potentially providing information to social partners and rivals. Odor cues from microbes in the external environment guide organisms to oviposition sites (Liu et al. 2016) or food (Grunseich et al. 2020), in some cases by providing cues that can be learned (Russell and Ashman 2019). We assume that these semiochemicals affect animal fitness and that animals might adaptively control this information, but the latter assumption is not well supported. Nest hygiene (a form of social immunity) can effectively reduce pathogens (Cotter and Kilner 2010a; Fialho et al. 2018; Nuotclà et al. 2019), demonstrating that adaptive control of the microbiota is possible. Beyond causing disease, microbes in the nest may have additional negative consequences by releasing odors that alert predators, parasitoids, or rivals (Eichholz and Koenig 1992; Adams and Six 2008). The specific chemicals, however, are rarely identified (but see Cortez et al. 2017; Shukla et al. 2018) and we know of no field demonstration of the efficacy of control of such microbi ally derived semiochemicals.

Odors in the external environment, such as from carrion, are generally considered public information (Rhinesmith-Carranza et al. 2018). Burying beetles (Nicrophorus spp.), however, might be expected to exhibit resident advantage...
because they monopolize a small vertebrate carcass. Carcass preparation alters the microbial community (N. orbicollis, Hoback et al. 2004; Hall et al. 2011; Nicrophorus vespilloides, Wang and Rozan 2017; Vogel et al. 2017; Shukla et al. 2018) by a number of mechanisms including upregulating antimicrobial peptides and lysozymes in secretions applied to the carcass (Arce et al. 2012; Jacobs et al. 2016; Shin et al. 2017; Heise et al. 2019), repairing feeding holes in the carcass (Trumbo 2017), and inoculating the carcass with selective gut symbionts (Duarte et al. 2018; Miller et al. 2019). The present study was undertaken to examine whether the burying beetle, N. orbicollis, uses its opportunity to adaptively alter microbial semiochemical release to keep public information private and to disinform rivals, with concomitant fieldwork to assess the effectiveness of volatile manipulation by parents.

The value of carrion to organisms is apparent by its rapid disappearance and recycling in an ecosystem (Barton and Bump 2019) as well as by the diverse competitive adaptations of carrion feeders (Merritt and De Jong 2015). Burying beetles breed by using a small vertebrate carcass—rare, ephemeral, and protein rich (Wilson 1971). As with carrion flies, burying beetles are highly sensitive to sulfur compounds (Kalinova et al. 2009; Podskalska et al. 2009) produced by microbial metabolism of the amino acids methionine and cysteine during decomposition (Stutz et al. 1991; Paczkowski et al. 2012; Cernosek et al. 2020). These compounds include methyl thiocyanate (MeSCN), S-methyl thioacetate, and the dimethyl sulfides (mono-, di-, tri-, and tetra-). Burying beetles have the ability to search for resources for long periods (Nicrophorus vespilloides; Attisano et al. 2015), covering up to 6 km in a night (Nicrophorus americanus; Bedick et al. 1999). Adaptations for detecting and searching do not ensure success because there are far more congeners than resources suitable for breeding. A larger rival will attempt to take over the carcass, expel the resident(s), and kill any larvae in the nest (Trumbo 1990; Suzuki 1999). Breeding burying beetles also have to contend with attracted flies that oviposit, in addition to nuisance carrion insects that do not contest for the resource but visit the nest area.

On discovering a suitable carcass, a single female or male-female pair delay feeding while working to take the resource underground, hence, their common name (Fabre 1899). Hair or feathers are removed from the carcass, which is typically rounded into a ball. Underground, the male and especially the female feed on the carcass, using their mandibles to open holes through the skin. This is necessary for rapid ovarian development (females may produce a clutch equivalent to 20% of body mass, while still increasing their body mass prior to postnatal care; Trumbo and Rauter 2014). These incisions could greatly accelerate microbial decay of the resource and the production of microbial volatiles (Vass 2001; Brodie et al. 2016), which would lead to poor larval growth if not repaired. The parents, however, are quick to close up their feeding holes, in addition to holes made by other carrion feeders (Trumbo 2017), and to apply anal and oral secretions to these locations as well as to the entire carcass surface (Pukowski 1933).

Two chemicals have recently been identified that have a major effect on whether N. orbicollis locates a fresh carcass for breeding. MeSCN, rarely examined in carrion studies, increases the discovery of a fresh carcass nearly threefold when used as a chemical supplement. DMTS, a commonly studied carrion volatile, is a deterrent for breeders, decreasing discovery three- to sixfold (Trumbo and Steiger 2020), likely because it is released in higher quantities during active decay, when maggot activity precludes use of the resource by a breeding burying beetle (Paczkowski et al. 2012; Trumbo and Dicapua 2020). Little is known about how carcass preparation alters the production of MeSCN and DMTS, or whether carcass preparation can reduce discovery in the field by congeners and other carrion rivals.

To study adaptive manipulation of microbial cues, we integrated mechanistic and functional approaches. Gas chromatography–mass spectrometry (GC–MS) was used to compare the release of microbiologically derived volatiles from carcasses that were or were not prepared by a nesting male-female pair of N. orbicollis. Three field experiments tested the hypothesis that parental modification of the carcass reduces the discovery and use of a found resource by congeners and dipterans. We narrowed the scope of possible adaptations to parental modification of the carcass by placing the laboratory-prepared carcasses in the field without parents (excluding direct defense) and by including an experiment that examined discovery of carcasses previously used for feeding by nonbreeding N. orbicollis.

**Material and Methods**

**Experiment 1: Does Carcass Preparation Alter the Volatile Profile?**

In experiment 1, we collected and analyzed the volatile organic compounds deriving from mouse carcasses that had been either prepared by pairs of Nicrophorus orbicollis beetles (prepared treatment; n = 13) or not (control treatment; n = 14). We used hairless mouse carcasses (24.1 ± 3.6 g; Zoobedarf Hitzegrad, Krefeld, Germany) that were pierced with a scalpel on the dorsal side near the hip and shoulder for both the control and prepared treatments. Carcasses were pierced to mimic feeding holes that parents would make prior to preparation. This allows us to model the volatile profile that would occur if a burying beetle
found a carcass, buried it in soil, and opened feeding holes, but did not repair the damage. This can be compared with the prepared treatment where the parents repair the experimental holes as well as any additional feeding holes they might make. Because it is common for burying beetles to find a carcass in the field that has been opened by other carrion insects, the opening of holes prior to the trial also represents a natural test of a microbial challenge. Hairless carcasses were used because carcass preparation removes hair, leaving it in a ring around the carcass. When a typical carcass is taken from the breeding box to the test arena (or to the field), the hair would be left behind, resulting in an improper contrast with nonprepared carcasses that retain their hair. By using hairless carcasses to begin both treatments, this confounding factor is eliminated. The N. orbicollis beetles used in the experiments were laboratory-reared organisms descended from wild beetles caught in a forested area near Lexington, Illinois (40°39′57″N, 88°53′49″W). At the beginning of the experiment, adult beetles were 32–42 days postemergence.

The experiment was conducted in a climate chamber at a constant temperature of 20°C. Carcasses were transferred to plastic containers (10 × 10 × 6 cm) half filled with soil originating from a deciduous forest close to Gießen, Germany. In the prepared treatment, a pair of beetles was introduced and allowed to manipulate the carcass for 3 days. In the control treatment, the carcass was buried under the soil for the same amount of time. Cadaveric volatiles were collected using a standard dynamic headspace method. Directly after the carcasses were retrieved from the soil, they were packed into commercial oven bags, through which air was pumped at a flow rate of 200 mL/min using a membrane pump (DC 12/16FK, Fürgt, Aichstetten, Germany) for 60 min. Incoming air was cleaned by an activated charcoal filter (Orbo 32, Supelco, Bellefonte, PA); the effluent airstream also passed an activated charcoal filter (Orbo 32, Supelco, Bellefonte, PA), in which the volatiles emitted from the carrion resource were collected. The charcoal filters were rinsed with dichloromethane, and 10 µg of methyl undecanoate (Sigma-Aldrich) was added as an internal standard. Chemical analyses were performed on a coupled gas chromatograph–mass spectrometer system (Agilent 7890/5977A), which was equipped with a nonpolar capillary column (DB-Wax, 30 m, 0.25 mm i.d., 0.25 µm film thickness; Agilent Technologies, Germany). Samples were injected with a split ratio of 1 : 5 at an injector temperature of 250°C. Helium was used as carrier gas at a constant linear velocity of 50 cm s⁻¹. The temperature program of the GC oven started at 40°C, which was held for 5 min and then increased by 5°C min⁻¹ up to 230°C. The MS was operated in electron impact mode at 70 eV and scanned a mass-range between 30 and 500 m/z⁻¹. Substances were identified using authentic reference compounds and by comparison of mass spectra with the NIST Research Library.

**Experiment 2: Does Carcass Preparation Reduce Discovery by Congeners?**

Experiments 2 and 3 were field tests that examined how preparation of a carcass by N. orbicollis affects discovery by free-flying competitors. Experiments were conducted in a mixed deciduous woodland in Bethany, Connecticut (41°27′36″N, 72°57′37″W), that was conducive for flying insects to locate a target using volatile cues (Conover 2007; flat area without significant topographical obstacles, small- to medium-diameter trees with a semi-open canopy). Mouse carcasses were supplied by Rodent Pro (Inglefield, IN). Nicrophorus orbicollis were laboratory-reared organisms descended from wild-caught beetles from the field site.

In experiment 2, we simulated what would happen if burying beetle parents made feeding holes in a discovered resource and did not repair their feeding holes during carcass preparation. Two holes were made on the dorsal side (shoulder and hip) of hairless mouse carcasses (19–21 g) to represent feeding holes. Hairless carcasses were used for the same rationale as in experiment 1, and holes were created in the same manner. In the laboratory, carcasses were either prepared by a male-female pair of N. orbicollis over 3 days (rounding, application of oral and anal secretions, repair of holes) in a breeding chamber (35 × 11 × 18 cm) half filled with soil from the field or buried in soil from the field without beetles (no repair or general preparation). Soil from the field, rather than commercially produced soil, was used to present a natural microbial challenge (Lauber et al. 2014). After 3 days, the carcasses were transported to the field (without parents) and placed along two transects. Each transect had three points, with 20 m between points. A single transect consisted of multiple carcasses (three) of the same treatment; transects were >200 m apart to reduce cross-attraction between transect-treatments. Cups (10 cm diameter, 12 cm height) were initially half filled with 6 cm of soil from the field site and buried so that the rim was flush with the ground surface. The carcass was placed on top of this soil, and 4 cm of additional loose soil was then placed over the carcass and patted down such that the top of the soil was 2 cm below the rim of the cup. Treatments were rotated randomly through four transects, such that each transect was used twice for each treatment during the course of the experiment. Carcasses were placed in the field at 17:00. To terminate the trial, the soil contents of each container were examined for the presence and number of burying beetles at 09:00 the day following placement. Each carcass was recorded as undiscovered, discovered by free-flying
burrowing beetles (carcass buried and beetle[s] present), or discovered by a scavenger (carcass removed). All containers were removed from the field after each trial and cleaned before the following trial to remove residual cues; unscavenged carcasses were also removed. The trials were run on 8 days (June 20–July 4, 2016), with each day an experimental replicate (24 carcasses per treatment in total).

Experiment 3: Can a Nonparental Activity Explain the Reduction in Carcass Discovery?

To investigate whether a nonparental activity, such as a generalized secretion, might be sufficient to explain the reduced discovery of prepared carcasses in experiment 2, newly emerged N. orbicollis adults were used. Hairless mouse carcasses (17–18 g) were initially left in the laboratory in soil from the field site for 3 days. In one treatment, a male-female pair of N. orbicollis prepared a carcass over the 3-day period, as in experiment 2. In the second treatment, a newly emerged male and female N. orbicollis were placed on a carcass. Newly emerged beetles are not reproductively competent; they will feed on the carcass, opening holes and producing anal secretions, but will not prepare a carcass for nesting. Carcasses were placed, without beetles, on two three-point transects (one treatment per transect) as before. The experiment was conducted over 8 days (July 23–August 20, 2015), with each day an experimental replicate (24 carcasses per treatment in total).

Experiment 4: Does Carcass Preparation Deter Dipteran Competitors?

The effect of burying beetle preparation of a carcass on the broader carrion community was investigated in two experiments (4 and A2) conducted in a fallow garden in Cheshire, Connecticut (41°31'42"N, 72°53'19"W). Carcasses were not hairless, to provide typical stimuli to carrion flies. For prepared carcasses, the hair removed by the parents was transported, as best as possible, to the field site by including a small amount of soil from the nest made in the laboratory.

A standard forensic measure of decomposition is biomass loss of a carcass (De Jong and Hoback 2006). In experiment 4, the effect of carcass preparation on loss of biomass to dipteran consumption was assessed. Carcasses (21–24 g) were initially weighed after thawing. On a 2 × 6 m grid, six carcasses prepared by a N. orbicollis pair for 2 days and six 2-day-old nonprepared carcasses were exposed on the soil surface at 10:00 on each of three dates (August 11, September 2, and September 9, 2010; n = 18 per treatment). After 48 h (carcasses were placed in an enclosed container each evening to protect them from scavengers and insects and returned to the surface each morning), carcasses were removed and transported to the laboratory and then placed on a wire mesh on top of soil from the field site in enclosed containers. After all fly larvae had left the carcass, the wire mesh was lifted and the remains were scraped into a glass dish and weighed.

Statistical Analysis

We analyzed the cadaveric volatile profiles of carcasses using R version 3.5.2 (R Core Team 2018). To compare the profiles of prepared and nonprepared carcasses, we performed a permutational multivariate analysis of variance (PERMANOVA; adonis function in the vegan package) as a multivariate nonparametric test. To test which substances differed, we used Wilcoxon rank sum tests.

Field experiments involving three-point transects (experiments 2 and 3) were analyzed in two ways. The frequency of carcass discovery by burying beetles was contrasted between treatments using Fisher’s exact test (scavenged carcasses excluded). In addition, a discovery score (total number of beetles discovering carcasses on a transect-treatment on a given day, divided by the number of unscavenged carcasses on that transect on that day) was computed for each experimental replicate. These scores were not normally distributed, contained many zero values, and were highly skewed; standard transformations did not result in near-normal distributions. A nonparametric test (Wilcoxon matched-pairs signed rank test) was therefore employed to examine treatment differences in discovery scores (SAS Institute 2007).

The proportion of carcass biomass consumed by dipteran larvae (experiment 4) was analyzed by a two-way ANOVA (treatment and date effects) after a logit transformation of proportions (SAS Institute 2007). Data underlying all analyses and figures have been deposited in the Dryad Data Repository (https://doi.org/10.5061/dryad.vq83bk3qx; Trumbo et al. 2020).

Results

Experiment 1: Does Carcass Preparation Alter the Volatile Profile?

The volatile profiles of prepared and nonprepared carcasses differed significantly (PERMANOVA, F1,25 = 37.00, P < .001). Out of 20 cadaveric compounds detected in the headspace samples, 10 volatiles differed quantitatively between treatments (table A1, available online). Focusing on the sulfur-containing substances, we found that prepared carcasses emitted less S-methyl thioacetate (Wilcoxon rank sum test, W = 16, P < .001) and MeSCN (W = 10.5, P < .001), but emitted more DMTS (W = 182, P < .001)
and dimethyl tetrasulfide (DMQS; $W = 182, P < .001$; fig. 1).

**Experiment 2: Does Carcass Preparation Reduce Discovery by Congeners?**

In experiment 2, hairless carcasses with two simulated feeding holes that were not repaired by *N. orbicollis* parents were discovered by free-flying conspecifics at a higher rate (88.9% of 18 unscavenged carcasses) than similar carcasses that had been prepared by a male-female pair (27.3% of 22 unscavenged carcasses, $P < .001$, Fisher’s exact test). Nonprepared carcasses attracted three times the number of beetles per night as prepared carcasses. There was not a single night (eight replicates) in which the discovery score (number of beetles per available [unscavenged] carcass) was greater for prepared than for nonprepared carcasses (fig. 2A).

![Figure 1: Emission of sulfur volatiles by nonprepared (solid) and prepared (hatched) carcasses. A, S-methyl thioacetate (MeSAc), methyl thiocyanate (MeSCN), and dimethyl tetrasulfide (DMQS). B, Dimethyl disulfide (DMDS) and dimethyl trisulfide (DMTS). Shown are medians (horizontal lines), the middle quartiles (boxes), and outliers (open circles). The upper stem and cap bars represent the upper quartile + 1.5 × interquartile distance. * $P < .001$, Wilcoxon rank sum test.](image_url)
None of the 48 carcasses were scavenged in experiment 3. Carcasses were discovered much more readily when exposed to nonparental newly emerged beetles (91.7%) than when prepared by parental beetles that repair their feeding holes (12.5%, $P < .001$, Fisher’s exact test). The numbers of beetles per trap-night (discovery scores) also differed between treatments (fig. 2B).

**Experiment 4: Does Carcass Preparation Deter Dipteran Competitors?**

Fly larvae consumed significantly more biomass on carcasses that had not been prepared by burying beetles.
compared with carcasses prepared for 2 days by a beetle pair (fig. 3; two-way ANOVA: treatment: $F_{1,30} = 24.80, P < .001$; date: $F_{2,30} = 12.11, P < .001$; treatment × date: $F_{2,30} = 0.11, P = .89, n = 18$ per treatment).

**Discussion**

Complex adaptations of animals to the microbiota are most often associated with endo-, nutritional, or cultured symbionts (Currie et al. 1999; Baumann 2005; Engel and Moran 2013). Resource specialists like burying beetles, however, consistently encounter an evolutionarily predictable external microbiota, so we might expect similar complexity. Adaptive associations with noncultured external microbes and adaptive host manipulation of semiochemicals from microbes have been little explored. Breeding burying beetles radically alter the microbial community on a prepared carcass (Hall et al. 2011; Vogel et al. 2017; Wang and Rozen 2017; Shukla et al. 2018), producing a community that is less species diverse and rich than on a nonprepared carcass (Duarte et al. 2018; Miller et al. 2019). Such simplified microbial communities are hypothesized to be easier to manipulate for the benefit of the host (Figueiredo and Kramer 2020). Our study demonstrates that parental care decreases emission of a microbially derived attractant (MeSCN) and increases a deterrent (DMTS and possibly DMQS). Furthermore, field experiments demonstrate that this behavior reduces discovery and use of the resource by congeners and carrion flies. Experiment 3 points to care-specific adaptations to control volatile emissions and not, for example, to general antimicrobial secretions present in nonbreeding burying beetles (Hoback et al. 2004; Hall et al. 2011; Steiger et al. 2011). We first discuss the general implications and follow with details from the study.

The ubiquity of microbes and their chemicals that serve as interkingdom cues suggests that many organisms may have evolved mechanisms of manipulation, although information on specific microbes, chemicals, and mechanisms of manipulation are incomplete for any one system. The importance of microbially derived volatiles for plants has long been understood. For example, bean plants (*Phaseolus vulgaris* L.) that form a symbiotic association with the mycorrhizal fungus *Funneliformis mosseae* C. Walker & A. Schüßler are better able to attract predators of herbivores through microbial compounds (Schausberger et al. 2012). Rhizobacteria are well-known growth promoters of plants, and do so, in part, by release of volatiles (Wintermans et al. 2016). How plants are able to favor specific microbes, whether they can adaptively bias volatiles emitted by microbes, and whether adaptation requires a long history of association are important questions to

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**Figure 3**: Percentage of carrion biomass consumed by dipteran larvae for prepared versus nonprepared carcasses aggregated across three dates. Shown are medians (horizontal lines), the middle quartiles (boxes), and outliers (open circles). The upper stem and cap bars represent the upper quartile + 1.5 × interquartile distance. ‘$P < .001$ for effect of carcass preparation, ANOVA.”
understanding plant competitive ability, their invasive potential, and the development of reduced-pesticide crops (Hammerbacher et al. 2019).

The dynamic interaction of host and microbe, each of which produces signals that cue third parties, creates similar difficulties in studies of adaptation in animals (Carthey et al. 2018; Engl and Kaltenpoth 2018). The failure to understand how individuals modify the olfactory environment by altering the microbiota leaves large gaps in our knowledge of animal decisions about diet, resource use, social partners, nesting, and habitat selection. Voigt (2005) hypothesized that male sac-winged bats (Saccopteryx bilineata Temminck) control their own sexual scent by a daily cleaning and redeposition of microbes into their wing sacs. Glands harboring a rich microbiota important in intraspecific communication are found in bats (Gaona et al. 2019), birds (Whittaker and Theis 2016; Maraci et al. 2018), and diverse organisms (Theis et al. 2013; Li et al. 2018). Control of volatiles by personal hygiene is likely to have a role in enhancing benefits.

Nest hygiene might also adaptively alter volatile emissions. In birds, the uropygial glands harbor a rich microbiota and produce secretions that change noticeably at the onset of incubation (Martin-Vivaldi et al. 2010) in a similar way that burying beetle secretions change during nesting (Cotter and Kilner 2010; Steiger et al. 2011). While one function in both systems is likely to control pathogens, the changing volatile profile should reduce discovery of the nest (Reneerkens et al. 2005). Microbially derived cues from a nest can attract parasitoids (Boone et al. 2014; Cha et al. 2016) or be used in selection of a mate (Lehtonen and Kvarnemo 2015) or to identify nest mates (Richard et al. 2007). Identification of specific microbially derived cues and mechanisms to control broadcasted information will broaden our understanding of parental adaptations.

Adaptive signaling can be honest or deceptive, and we expect comparable outcomes when microbially derived cues are modified. Recipients can be deceived about manipulated microbially derived cues for the same reasons they are deceived by animal signals. If deceptive cues are rare compared with accurate cues, then recipients will still have a net benefit by responding (Johnstone 1998). In the case of burying beetles, the vast majority of carrion biomass at one time is not being handled by another burying beetle; breeding beetles remain well-equipped to avoid unsuitable carcasses and to respond to carcasses appropriate for reproduction, even if they are occasionally misdirected.

Even when signalers (or, in this case, individuals manipulating microbial cues) have a conflict of interest with receivers, deceitful signals can evolve into honest signals when signals have a cost and there is selection on receivers for enhanced discrimination (Johnstone 1998). The original description of carcass preparation by burying beetles speculated that residents’ secretions mark a carcass as occupied to deter other carrion insects, that is, that the broadcasted information is honest (Pukowski 1933). We do not feel that either the beetles’ secretions or the distinctive manipulated volatile blend is honest, at least in regard to congeners. Rather, we view the decrease in MeSCN and the increases in DMTS and DMQS as disinformation for several reasons. Foremost, burying beetles show no hesitation in using a prepared carcass marked by another beetle, a common experimental procedure in the laboratory and a common occurrence following an infanticidal takeover (Trumbo 1990). In addition, subordinate females that are driven from a carcass attempt to parasitize the brood, reducing the fitness of residents (Müller et al. 2007), and an unpaired male that releases a sex pheromone can be exploited by conspecific males and congeners that eavesdrop and then attempt to displace the resident male from his carcass (Haberer et al. 2011). When a large number of potential breeders have not found any reproductive opportunity, the release of honest location cues from a discovered resource would be risky. The combination of low MeSCN, the most reliable cue for a fresh carcass, and high levels of DMTS and DMQS, indicators of an older (or larger) unusable carcass, serves to deceive rival breeders, in our opinion.

Carcass preparation has wider effects on the carrion community. DMTS and DMQS might be expected to attract nuisance carrion–frequenting flies and beetles (Kalinova et al. 2009; Podskalska et al. 2009; von Hoermann et al. 2016; Yan et al. 2018), which would seem to be a detriment to a breeding burying beetle. We note that despite the increase in DMTS and DMQS from prepared carcasses, these carcasses resulted in fewer visits by carrion flies (fig. A1, available online) and less consumption of carrion by maggots (experiment 4). Carcass preparation by Nicrophorus vespilloides appears to have a similar effect on fly visits (Suzuki 2000). Because burying beetles and their phoretic mites will clear carcasses of fly eggs when the number of eggs is small, it is possible that fly deterrence benefits the beetle and reduces unprofitable oviposition by flies. We therefore withhold speculation on whether the volatile profile honestly conveys a poor choice for carrion insects other than breeding burying beetles.

The most striking laboratory finding in the present study was the 20-fold reduction in MeSCN when a parental pair of Nicrophorus orbicollis prepared a carcass. Recently, MeSCN was shown to be an important cue that free-flying beetles use to locate a carcass on which to breed (Trumbo and Steiger 2020). The value for resident beetles in limiting the broadcast of this semiochemical seems straightforward.

Parental preparation of a carcass also resulted in a greater than 20-fold increase in DMTS and up to a 50-fold increase in DMQS.
increase in DMQS (because DMQS was detected from only two nonprepared carcasses, the increase may be an overestimate as the actual values from nonprepared carcasses were unlikely to have been zero). We hypothesize that enhanced DMTS production is adaptive, because high levels of DMTS deter free-flying burying beetles searching for a fresh carcass on which to breed (Trumbo and Steiger 2020). Despite the value of the hidden resource, the manipulated volatile blend is uncharacteristic of a suitable carcass (disinformation). DMQS needs to be tested in the field for its effect on breeding burying beetles, as do putrescine and cadaverine, two compounds decreased by carcass preparation in N. vespilloides (Shukla et al. 2018). DMTS and DMQS are cues that peak during the late bloat or later stages of decomposition, often associated with maggot activity (Kalinova et al. 2009; von Hoermann et al. 2016). Maggot activity, in fact, can lead to a burst of DMTS release (Recinos-Aguilar et al. 2019), presumably by opening up holes, which facilitates microbial proliferation (Brodie et al. 2016). Breeding burying beetles tend to avoid such carcasses. Breeders placed on maggot-infested carcasses are not aggressive (Chen et al. 2020), their ovaries do not fully develop (Wilson and Knollenberg 1984), and, if they and their phoretic mites cannot clear the carcass of maggots, the resource is used for feeding (Ito 2020). Additional evidence that DMTS and DMQS represent post-fresh stages of decomposition is provided by other deceptive uses. DMTS is part of a foul-smelling defense mechanism by the earwig, Labidura riparia (Byers 2015), and both compounds are used by stinkhorn fungi to attract flies that disperse spores (Kakumyan et al. 2020) and, famously, by plants that mimic a well-rotted corpse to attract pollinators (Jürgens et al. 2013; Kite and Hetterscheid 2017).

Carcass preparation also reduced S-methyl thioacetate (methyl thiolacate) release. This compound acts synergistically with DMTS to attract carrion beetles associated with active decay of a carcass (Trumbo and Dicapua 2020) and is part of the bouquet of carrion-mimicking plants (Kite and Hetterscheid 2017). Unlike DMTS, S-methyl thioacetate does not deter breeding burying beetles from a carcass (Trumbo and Steiger 2020), so there would be no benefit to manipulating its increase. The combination of high levels of DMTS and low levels of S-methyl thioacetate from a prepared carcass could minimize attraction of the biggest threat—infanticidal congeners—without attracting more nuisance carrion insects associated with later stages of decomposition. The chemical analysis indicated additional compounds that were altered by carcass preparation (table A1: 2-butanol, 1-propanol, 1-decanol, dimethyl pyrazine). These are not known to be important for carrion beetles but deserve further study. Additional work on volatile emission from buried versus unburied carcasses would also be of interest. Deeply buried carcasses are extremely difficult to discover because of the scarcity of cues; shallowly buried carcasses are discoverable by burying beetles (present study) and flies (Szpila et al. 2010).

The specific microbial origins of the altered volatile profile are uncertain, as the community of a beetle-prepared carcass is highly diverse. One intriguing potential contributor to the elevated levels of DMTS is an ascomycetous yeast (Yarrowia sp.), identified as a mutualist that is part of the core microbiota of burying beetles (Shukla et al. 2018). The silphids are the only family of beetles known to harbor this symbiont (Kaltenpoth and Steiger 2014), and this symbiont is found only in beetle guts and on beetle-prepared carcasses (Vogel et al. 2017; Heise et al. 2019). The related free-living Yarrowia lipolytica (Wick., Kurtzman & Herman) produces appreciable levels of DMTS and is used in the cheese-making industry for this purpose (Cholet et al. 2008; Hebert et al. 2013).

Microbes are well-known manipulators of animal communication (Cooley et al. 2018) and odor (De Moraes et al. 2014; Engl and Kaltenpoth 2018). Animals, however, are not recognized as having the same effect on microbes. The present study demonstrates control of microbial semiochemicals and uncovers a new form of resident advantage whereby first colonizers avoid contests with rivals by altering microbially derived cues. Experimental manipulation of microbes or their volatiles may provide amenable experimental systems for testing hypotheses of signaling and deception. Unlike direct manipulation of a signaler (e.g., modification of bird plumage), the manipulation of the microbially derived cue may be carried out without altering the signaler directly. The identification of two manipulated volatiles that are rarely considered in carrion studies (MeSCN and MeSAc) may provide insight into mechanisms of ecological succession on a carcass, which require new theories (Michaud and Moreau 2017), information for developing time-of-death models using volatiles (Fredericks et al. 2012), and tools for monitoring the American burying beetle and other carrion insects of special concern (Lomolino et al. 1995). Although examples of manipulation of microbial semiochemicals are rare, we expect it to be common because microbes and their chemicals influence nearly every aspect of host ecology (Ezenwa and Williams 2014; Jordan et al. 2015).

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Statement of Authorship
S.T.T., S.S., P.K.B.P., and J.S. designed the experiments. S.T.T. and S.S. carried out the experiments and analyzed the data. S.T.T. wrote the manuscript, which S.S. and P.K.B.P. critically revised.

Data and Code Availability
Data and coding are available through the Dryad Data Repository (https://doi.org/10.5061/dryad.vq83bk3qx; Trumbo et al. 2020) the OpenCommons@uconn (data set: trumbo-pository (https://doi.org/10.5061/dryad.vq83bk3qx; Trumbo Data and coding are available through the Dryad Data Re-

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A dead mouse before (left) and after (right) preparation by a male-female pair of *Nicrophorus orbicollis*. The tail of the mouse can be seen in the lower half of the carrion ball. Photo by Stephen T. Trumbo.