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Volatiles Emitted by Calling Males of Burying Beetles and *Ptomascopus morio* (Coleoptera: Silphidae: Nicrophorinae) Are Biogenetically Related

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Abstract In burying beetles, *Nicrophorus* spp. (Coleoptera: Silphidae: Nicrophorinae) mate finding is mediated by male produced volatile compounds. To date, pheromone components of only two species have been identified. In an attempt to better understand the evolution of male pheromone signaling in burying beetles, we investigated the male released volatiles of ten *Nicrophorus* species and one closely related nicrophorine species, *Ptomascopus mori.* Volatiles emitted by calling males were collected in the laboratory by means of solid phase micro extraction and analyzed using gas chromatography coupled with mass spectrometry. Identified volatiles included short chain esters of 4-methylcarboxylic acids, terpenoids, and some other aliphatic compounds. The long-range volatile signals of the burying beetle species included in this study are blends of two to seven components. We found that methyl or ethyl esters of 4-methylheptanoic acid and 4-methyloctanoic acid are produced by eight of the ten investigated *Nicrophorus* species. These esters may play a key role in chemical communication. Their widespread occurrence suggests that these compounds did not evolve recently, but appeared relatively early in the phylogeny of the genus. Although *Ptomascopus* is considered the sister genus of *Nicrophorus, P. morio* males do not produce any of the *Nicrophorus* compounds, but release 3-methylalkan-2-ones, which are absent in *Nicrophorus*. A better understanding of the evolution of burying beetle pheromones, however, will only be possible once more species have been studied.

Keywords Silphidae · Nicrophorinae · 4-methylcarboxylates · 3-methylalkan-2-ones · Pheromone · Volatile organic compounds

Introduction

Burying beetles (*Nicrophorus* spp., Silphidae: Coleoptera) are known for their complex brood care behavior, which involves the search and burying of small vertebrate carcasses, the preparation of the buried carrion for their offspring, and elaborate care during the time when the larvae are feeding at the carcass. Mate finding is mediated by volatiles emitted by males (Pukowski 1933; Bartlett 1987; Eggert and Müller 1989). Various ecological implications of male pheromonal advertising in burying beetles have been studied (Eggert 1992; Trumbo and Egggert 1994; Beeler et al. 1999, 2002; Walling et al. 2009), however, research on the chemical nature of the male pheromones in *Nicrophorus* is still scarce. To date, male pheromone constituents of only two species are known. The first components identified were ethyl 4-methylheptanoate and geranylacetone, emitted by *N. vespilloides* (Haberer et al. 2008). A behaviorally active pheromone component, emitted by *N. humator* males, was identified to be methyl 4-methyloctanoate, whereas the role of the second component, isovaleric acid, found in several but not all samples of this

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species, remains to be clarified (Haberer et al. 2011). As calling *Nicrophorus* males also attract conspecific males (Müller and Eggert 1987), the corresponding chemical signals are considered aggregation pheromones rather than sex pheromones. Cross-attraction of congeneric beetles via long-range pheromones is a phenomenon also observed in *Nicrophorus* males (Müller and Eggert 1987; Haberer et al. 2011). This kairomonal response is thought to be adaptive in the context of interspecific competition (Trumbo and Bloch 2002) for rare breeding resources.

Nicrophorus males can employ different behavioral tactics to find a mate (Eggert 1992): Since adults of both sexes are attracted to carcasses (Kalinová et al. 2009), one option is to search for a carcass suitable for reproduction, on which a conspecific female may already be present. If that is not the case, males will start to release pheromones to attract females, since males cannot utilize the carcass on their own (Pukowski 1933). An alternative mate-finding tactic for males involves calling for females without having located a carcass (Müller and Eggert 1987). On a carcass, the attractiveness of male pheromones may be synergistically enhanced by odors emanating from the substrate itself; however, calling without a breeding resource is likely to contribute significantly to reproductive success (Eggert and Müller 1989; Eggert 1992; Beeler et al. 1999; Müller et al. 2007; Walling et al. 2009).

The aim of our study was to establish an initial inventory of volatiles released by calling males of several *Nicrophorus* species. This was accomplished by collecting headspace volatiles from beetles showing calling behavior by using solid phase microextraction (SPME) and analyzing the samples by gas chromatography coupled with mass spectrometry (GC/MS). The study includes five North American and five Central European species (Table 1) as well as one species of the sister genus of *Nicrophorus*: the Asian *Ptomascopus morio*. Unlike burying beetles, *P. morio* exhibits only rudimentary brood care behavior and is considered a facultative brood parasite of *Nicrophorus concolor* (Trumbo et al. 2001; Suzuki and Nagano 2005). Mate-calling behavior, however, is similar in *Nicrophorus* spp. and *P. morio* (unpublished observations). At a species-specific time of the day, males typically perch at elevated locations, extend their hind legs, raise their abdomen, and release volatiles for about one to 3hrs. Diurnal and crepuscular species like *N. defodiens, N. interruptus, N. pustulatus, N. tomentosus, N. vespilloides*, and *N. vespillo* exhibit this calling behavior in the late afternoon and in the evening, at the end of their daily activity period, in contrast to strictly nocturnal species such as *N. humator* and *N. orbicollis*, which typically signal later, during the night (Haberer, unpublished observations; Müller and Eggert 1987; Beeler et al. 1999; Mulrey et al. 2015).

Methods and Materials

Beetles, Origin and Rearing Beetles used in this study were either caught in the wild or were laboratory-reared (Table 1). Individuals were maintained separately at 20 °C under a 16:8 h light:dark regime in transparent polystyrene containers (10x10x6cm) filled with moist peat and fed decapitated mealworms twice weekly.

Volatile Collection Headspace sampling was accomplished using solid phase micro extraction (SPME) fibers (Supelco, coating: polydimethylsiloxane/divinylbenzene, Sigma-Aldrich). Volatiles were collected in the plastic containers in which beetles were housed. An SPME fiber was inserted into the container through a perforated lid and mounted close to the beetles. Under laboratory conditions, three main phases of male behavior can be observed during their active time of the day (Eggert 1992). First, the beetles appear at the surface

Table 1 Species included in the headspace analyses. N = number of individuals of which volatiles have been collected

Species	N	Wild-caught or Laboratory-bred	Origin
Ptomascopus morio	16	lab	Iwakura, Kyoto, Japan
Nicrophorus defodiens	7	lab	Powassan, northern Ontario, CAN
N. defodiens	16	lab	Moran, Wyoming, USA
N. humator	19	wild, lab	Freiburg, Germany
N. interruptus	4	wild	Gravières, Ardèche, France
N. interruptus	3	wild	Gennetines, Allier, France
N. interruptus	3	wild	Illmitz, Austria
N. interruptus	2	wild	Freiburg, Germany
N. investigator	2	wild	Bielefeld, Germany
N. marginatus	2	wild	Lexington, Illinois, USA
N. orbicollis	17	wild, lab	Lexington, Illinois, USA
N. pustulatus	14	wild, lab	Lexington, Illinois, USA
N. tomentosus	10	wild, lab	Lexington, Illinois, USA
N. vespillo	12	wild	Freiburg, Germany
N. vespilloides [a]	11	wild, lab	Freiburg, Germany

[a] data from Haberer et al. 2008

of the substrate, run around and repeatedly attempt to fly. Subsequently, the males choose an elevated site, remain there more or less stationary and show the typical calling behavior: head down, hind legs extended, and abdomen extended and raised. The last abdominal segment, otherwise mostly concealed within the penultimate segment, is extruded and may appear wet on the surface. After this calling phase, which typically lasts 1–3 h, the beetles may move around again on the surface before disappearing into the peat, where they remain inactive until the next calling period on the following day. To identify volatiles emitted only during calling, headspace samples obtained from calling beetles were compared to samples taken before or after calling.

Chemical Analysis The SPME-fiber, loaded with volatiles, was inserted at the injection port of a gas chromatograph (HP series 6890, Hewlett Packard, Palo Alto, California) equipped with a DB-5 column (30 m × 0.25 mm × 0.25 μm, J & W, Folsom, California), coupled to a quadrupole mass spectrometer (HP series 5973, Hewlett Packard, Palo Alto, California), which was run at 70 eV. The injection temperature was 250 °C, and the column temperature was ramped from 50° to 250 °C by 10 °C/min with a final hold of 5 min at 250 °C. Chemical structures of target compounds were inferred from diagnostic fragments and compared to those reported in the literature. Retention times, retention indices, and fragmentation patterns of the substances detected were compared to those of authentic reference compounds: ethyl (9*Z*)-hexadecenoate (Tokyo Chemical Industry Co., Japan), (2*E,*6*Z*)-dodecadienal, ethyl tetradecanoate, geranylacetone, *n*-heptadecane, isovaleric acid (Fluka GmbH, Switzerland), and methyl geranate (Chemos GmbH, Germany). Racemates of 4-methylheptanoic acid and 4-methyloctanoic acid were synthesized according to Joung et al. (1998) and esterified according to laboratory standards - the plotted 70 eV mass spectrum of the new isoamyl 4-methylheptanoate is shown in the Supplement, along with its interpretation and a comment. 3-Methylundecan-2-one was synthesized according to Midgley and Thomas (1987); fuscumyl acetate was synthesized from geranylacetone according to Li et al. (2003); 3-methyl-2-oxopentanoic acid was obtained by acidification of sodium 3-methyl-2-oxopentanoate (Aldrich, Gillingham, Dorset, UK) with acetic acid; (9*Z*)-octadecenal and hexadecanal were synthesized by oxidation of the corresponding alcohols (Roth, Germany) according to Corey and Suggs (1975). (2*E*)-Dodecenal was provided by Prof. Wittko Francke, Univ. Hamburg. Methyl (2*E,*6*E*)-farnesoate and (*E,E*)-farnesylacetone were tentatively identified by comparing their mass spectra with reference data reported in the Wiley data base of mass spectra (Wiley & Sons Inc., 6th edition). Structures of 3-methyldodecan-2-one and 3-methyltridecan-2-one were identifed according to their mass spectra, which were very similar to that of 3-methylundecan-2-one, which we had identified by means of a synthetic reference compound.

Results

Volatiles Detected in the Headspaces of Calling Beetles and Proportions in Released Blends Volatiles emitted by males of *Nicrophorus* spp. comprise 5 alkyl 4-methylalkanoates, 5 terpenoids, 7 saturated and unsaturated acetogenins, and 2 compounds that may be related to amino acids (Table 2). *P. morio* males do not produce any of the compounds detected in *Nicrophorus* spp. but emit three 3-methylalkane-2-ones (Table 2), among which 3-methylundecan-2-one is quantitatively dominant (Fig. 1i).

The number of volatiles released by males range from two in *N. defodiens, N. humator*, *N. marginatus, N. vespillo,* and *N. vespilloides* to a maximum of seven components in *N. pustulatus* (Table 2, Fig. 1).

Esters of 4-methyl-branched carboxylic acids were detected in eight of the ten *Nicrophorus* species included in this study (Table 2). Ethyl 4-methylheptanoate is released by six species, and predominant among the volatiles of *N. pustulatus*, *N. tomentosus,* and *N. vespilloides* (Fig. 1c, d, h). *N. investigator* also produces ethyl 4-methylheptanoate, along with (2*E*,6*Z*)-dodecadienal (Table 2), but information on the ratio of these compounds in the blend cannot be provided here, because the ratios strongly differed between the two specimens available. In the three-component blends obtained from two male *N. marginatus* (Table 2), we found primarily ethyl 4-methyloctanoate but also small amounts of ethyl 4-methylheptanoate and ethyl (9*Z*)-hexadecenoate. As in *N. investigator*, more individuals of this species must be analyzed to reliably estimate volatile proportions. In all studied *Nicrophorus* species, the quantitative composition of blends was highly variable among individuals (Fig. 1), especially in *N. defodiens*, *N. tomentosus*, *N. vespillo,* and *N. vespilloides* (Fig. 1a, d, g, h). However, *P. morio* showed little interindividual variation (Fig. 1i).

With the exception of *N. humator* and *N. vespillo,* all *Nicrophorus* species released acetogenins or terpenoids (Table 2). *N. vespillo* released isoamyl 4-methylheptanoate as a unique ester.

Discussion

Our study yielded six principal results: (1) None of the species in the survey releases a single-component signal, but blends, containing two to seven compounds. (2) The volatiles involved are chemically diverse; they represent different classes of compounds and, according to their carbon skeletons are produced via distinct biosynthetic pathways. (3) *P. morio*

Table 2 Volatiles emitted by calling males of ten *Nicrophorus* species and *Ptomascopus morio*

No	RI [a]	RI references	Substances	Species
			Alkyl 4-methylacylates	
[1]	1071		Methyl 4-methylheptanoate [b]	pus
[2]	1156		Methyl 4-methyloctanoate [b]	hum
[3]	1157		Ethyl 4-methylheptanoate [b]	int, inv, vsp, mar, pus, tom
[4]	1241		Ethyl 4-methyloctanoate [b]	mar, pus
[5]	1403		Isoamyl 4-methylheptanoate [b]	vso
			Terpenoids	
[6]	1324	1322 (Adams 2007)	Methyl geranate	pus
[7]	1451	1452 (Babushok et al. 2011)	Geranylacetone	int, vsp, orb, pus
[8]	1573	1573 (Fonseca et al. 2010)	Fuscumyl acetate [b]	def
[9]	1783		Methyl (2*E*,6*E*)-farnesoate [b, c]	pus
[10]	1913	1919 (Lazari et al. 2000)	(*E*,*E*)-Farnesylacetone [c]	def, orb, pus
			3-Methylalkan-2-ones	
[11]	1346		3-Methylundecan-2-one [b]	P.mor
[12]	1436		3-Methyldodecan-2-one [b, c]	P.mor
[13]	1536		3-Methyltridecan-2-one [b, c]	P.mor
			Acetogenins	
[14]	1451	1465 (Dharmawan et al. 2009)	(2*E*,6*Z*)-Dodecadienal	int, inv
[15]	1469	1468 (Babushok et al. 2011)	(2*E*)-Dodecenal	int
[16]	1700		*n*-Heptadecane	orb
[17]	1792	1795 (Adams 2007)	Ethyl tetradecanoate	inv
[18]	1970		Ethyl (9*Z*)-hexadecenoate	inv, mar
[19]	1819	1817 (Babushok et al. 2011)	Hexadecanal	orb, tom
[20]	1999		(9*Z*)-Octadecenal	int, inv, orb, tom
			Other	
[21]	843	827 (Adams 2007)	Isovaleric acid	int, inv, hum
[22]	928		3-Methyl-2-oxopentanoic acid [b]	vso, orb

def *N.defodiens*, hum *N.humator*, int *N.interruptus*, inv *N.investigator*, mar *N.marginatus*, orb *N.orbicollis*, pus *N.pustulatus*, tom *N.tomentosus*, vsp *N.vespilloides*, vso *N.vespillo*, P.mor *Ptomascopus morio*

[a] *RI* retention index calculated according to Van Den Dool and Kratz (1963) on a DB-5MS column (Agilent Technologies Inc., Santa Clara, California)

[b] stereochemistry to be clarified

[c] tentatively identified

males release a blend of three 3-methylalkan-2-ones, but none of the compounds found in *Nicrophorus*. (4) Several of the compounds present in *Nicrophorus* are not unique to a single species, but occur in several species. (5) The blends of some but not all *Nicrophorus* species include terpenoids. (6) In most (8 of 10) of the *Nicrophorus* species studied, male volatiles include esters of 4-methyloctanoic acid and 4-methylheptanoic acid, which may play a pivotal role in pheromone signaling in the genus. *N. defodiens* and *N. orbicollis* do not produce such esters but terpenoids, whereas the two-component blend of *N. vespilloides* contains both.

The occurrence of esters of 4-methyloctanoic acid and 4-methylheptanoic acid is not restricted to *Nicrophorus*. These esters have also been identified as male pheromone components in several rhinoceros beetles species of the genus *Oryctes* (Gries et al. 1994; Hallett et al. 1995; Rochat et al. 2004; Saïd et al. 2015). The terpenoids identified here are also known as components of male pheromones of a number of other organisms. Methyl geranate (*N. pustulatus*) occurs in the pheromone of male stink bugs in the genus *Chlorochroa* (Ho and Millar 2001a, b), as does methyl (2*E*,6*E*)-farnesoate (*N. pustulatus*). This latter ester is also produced by male African milkweed butterflies (Schulz et al. 1993) and male African fruit fly species of the *Ceratitis* complex, where its biological activity has been confirmed in electrophysiological studies (Břízová et al. 2015). The nor-terpenoid geranylacetone (*N. interruptus, orbicollis, pustulatus,* and *vespilloides*), is found in many different organisms, ranging from microorganisms and plants to insects and even mammals. Fuscumyl acetate, the main volatile in *N. defodiens* is, along with the corresponding alcohol, a male produced pheromone component of longhorned beetles (Silk et al. 2007; Fonseca et al. 2010). Farnesylacetone (*N. defodiens*, *orbicollis*, and *pustulatus*) is less common, but has been identified among plant seed volatiles attractive to beetles (Adhikary et al. 2015), the dorsal gland of an antelope (Burger et al. 1981), and the androgenic glands of male crabs *Carcinus maenas* (Ferezou et al. 1977).

Deciphering the biological significance of these terpenes in *Nicrophorus* will require more in-depth studies. Laboratory and field tests are required to assess which of the volatiles detected in this study are actually perceived by potential

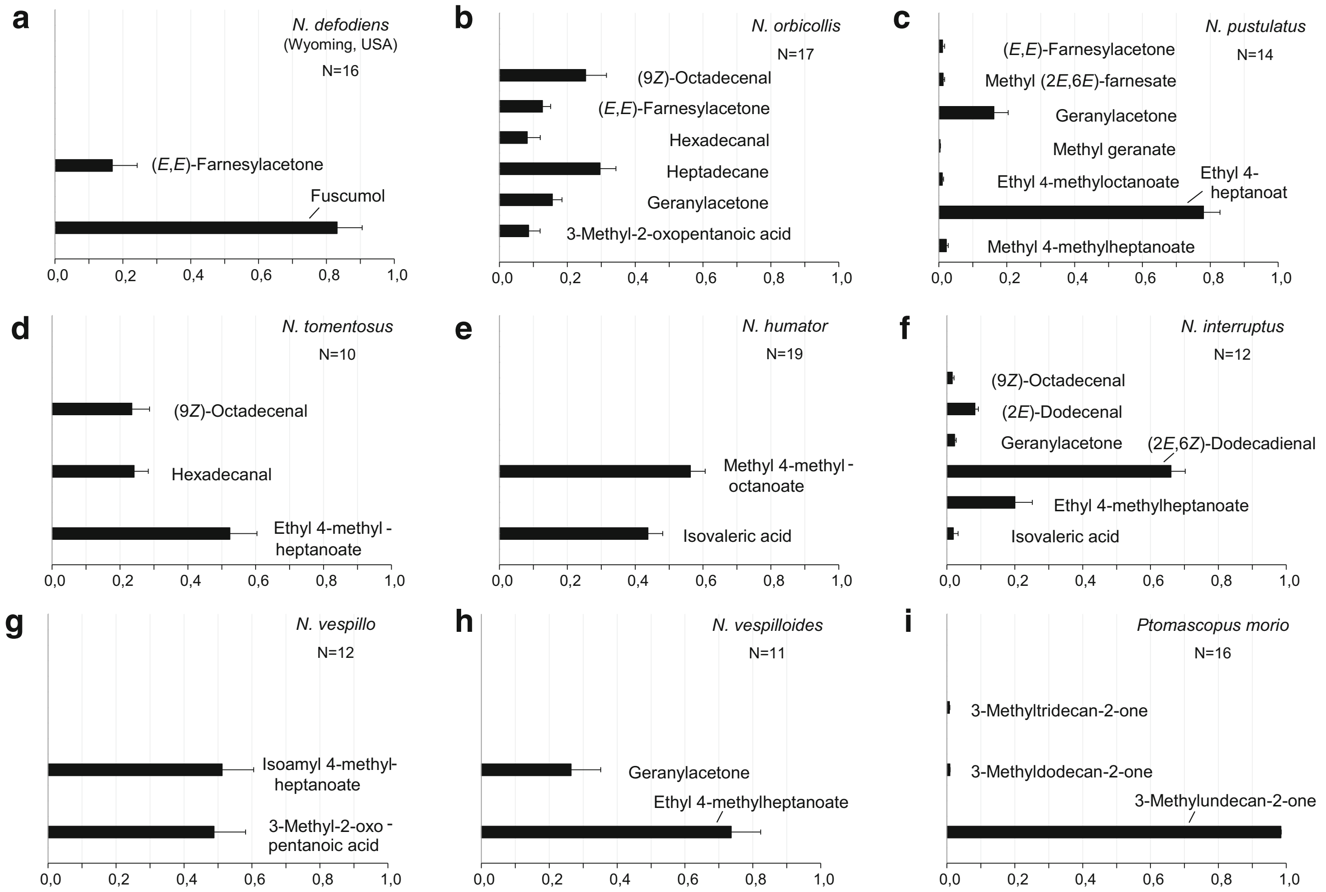

Fig. 1 Proportions of headspace components collected from calling *Nicrophorus* spp. and *Ptomascopus morio.* Mean and standard error of the integrated peak areas of the total ion chromatograms. The X-axis shows relative proportions of volatiles generated by SPME-GC/MS-analysis (peak areas in ion-chromatograms, data not corrected)

recipients and modify their behavior. To date, only pheromones identified from *N. vespilloides* and *N. humator* have been tested in the field (Haberer et al. 2008; Haberer et al. 2011). However, these results must be interpreted with caution, since the stereochemical configuration of the respective main components, ethyl 4-methylheptanoate and methyl 4-methyloctanoate, has not yet been determined. This limitation applies also to the two other alkyl 4-methylcarboxylates identified in this study.

The composition of pheromone blends produced by male *Nicrophorus* appears to vary greatly between individuals. This raises an important issue concerning the significance of such variation: does it convey information about characteristics of the signaling individual such as size, health, reproductive history, or nutritional status to the signal recipient? A recent study on *N. vespilloides* revealed that such factors substantially affect amounts and proportions in the two-component blend, which significantly affect attractiveness to females (Chemnitz et al. 2015). Moreover, it would be particularly beneficial for females to distinguish between males that have located a carcass and males that have not. A carcass, buried by the monopolizing male, is olfactorily "invisible" or at least difficult to detect (Trumbo and Bloch 2002), and thus, a semiochemical advertising ownership could be a way for the male to communicate the presence of the hidden resource to the intended receiver only (however, conspecific males and congeners might be able to intercept this information, see Eggert and Müller 1989). Candidate compounds for such advertising are substances derived from carcass material that the beetles ingest during the first hours on the carcass. Isovaleric acid, emitted by males of *N. humator, N. investigator*, and *N. interruptus* may be such a candidate compound as it most probably originates from the amino acid L-leucine. Its production may, thus, depend on the access to a protein-rich diet. Similar considerations apply to 3-methyl-2-oxopentanoic acid in *N. orbicollis* and *N. vespillo* as this compound is the immediate transamination product of isoleucine (Francke and Schulz 1999). Further studies are necessary to test this hypothesis.

Several *Nicrophorus* species show remarkable overlap in blend constituents. Ethyl 4-methylheptanoate was detected in six of ten species from Europe and America. Presumably, this ester and esters of its homologue, 4-methyloctanoic acid, appeared early in the evolution of burying beetles. The distribution of species producing these esters across several different major branches in the phylogenetic tree of *Nicrophorus* (Sikes and Venables 2013) supports this hypothesis. Several species appear to have evolved additional pheromone constituents that facilitate species-specific recognition. The recent finding that breeding *N. vespilloides* females produce similar esters with as of yet unknown biological function (Haberer et al. 2014) also suggests a long evolutionary history of signaling with these esters in *Nicrophorus*. Apparently, these substances are used to convey information in very different contexts. More chemical details such as the stereochemical configurations of the compounds involved, but also fundamental ecological information about niche differentiation and mating systems are needed to understand the evolution of long-range recognition in burying beetles, especially among sympatric species that share pheromone constituents.

Although *P. morio* produce 3-methylalkan-2-ones that are chemically distinct from the compounds found in *Nicrophorus* so far, a comparison of the likely biosynthetic pathways of these ketones and that of the alkyl 4-methylcarboxylates in *Nicrophorus* reveals a close relationship. The biosynthesis of the 4-methylcarboxylates of *Nicrophorus* and of the 3-methylalkan-2-ones of *P. morio* involve the incorporation of propanoate, similar to the formation of the methyl group in the 4 position of 4-methylnonan-1-ol, the sex pheromone of the yellow mealworm, *Tenebrio molitor* (Islam et al. 1999). The 4-methylacyl-motif, formed in a propanoate - acetate - sequence as the final steps during chain formation, would directly yield 3-methylalkan-2-ones upon β-oxidation and decarboxylation of the corresponding 4-methyl-3-oxocarboxlic acids. *Ptomascopus* is the sister genus to *Nicrophorus* (Sikes and Venables 2013) and has retained some ancestral characteristics in morphology (Peck 1982) and behavior (Suzuki and Nagano 2006). Several studies on the breeding behavior of *P. morio* have been carried out (Trumbo et al. 2001; Suzuki et al. 2005; Nagano and Suzuki 2008), but we are just beginning to understand its mating system (Suzuki et al. 2005).

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References

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream

Adhikary P, Mukherjee A, Barik A (2015) Attraction of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) to four varieties of *Lathyrus sativus* L. seed volatiles. Bull Entomol Res 105:187–201

Babushok VI, Linstrom PJ, Zenkevich IG (2011) Retention indices for frequently reported compounds of plant essential oils. J Phys Chem Ref Data 40:43101-1–43101-47. https://doi.org/10.1063/1.3653552

Bartlett J (1987) Evidence for a sex attractant in burying beetles. Ecol Entomol 12:471–472

Beeler AE, Rauter CM, Moore AJ (1999) Pheromonally mediated mate attraction by males of the burying beetle *Nicrophorus orbicollis*: alternative calling tactics conditional on both intrinsic and extrinsic factors. Behav Ecol 10:578–584

Beeler AE, Rauter CM, Moore AJ (2002) Mate discrimination by females in the burying beetle *Nicrophorus orbicollis*: the influence of male size on attractiveness to females. Ecol Entomol 27:1–6

Břízová R, Vaníčková L, Faťarová M, Ekesi S, Hoskovec M, Kalinová B (2015) Analyses of volatiles produced by the African fruit fly species complex (Diptera, Tephritidae). ZooKeys 540:385–404

Burger BV, Le Roux M, Spies HSC, Truter V, Bigalke RC (1981) Mammalian pheromone studies. IV. Terpenoid compounds and hydroxy esters from the dorsal gland of the springbok, *Antidorcas marsupialis*. Z Naturforsch C 36:340–343

Chemnitz J, Jentschke PC, Ayasse M, Steiger S (2015) Beyond species recognition: somatic state affects long-distance sex pheromone communication. Proc Roy Soc B Biol Sci 282:20150832. https://doi.org/10.1098/rspb.2015.0832

Choi H-S (2003) Character impact odorants of *Citrus* Hallabong [(*C. unshiu Marcov*×*C. sinensis* Osbeck)x*C. reticulata* Blanco] cold-pressed peel oil. J Agric Food Chem 51:2687–2692. https://doi.org/10.1021/jf021069o

Corey EJ, Suggs JW (1975) Pyridinium chlorochromate, an efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. Tetrahedron Lett 31:2647–2650

Dharmawan J, Kasapis S, Sriramula P et al (2009) Evaluation of aroma-active compounds in Pontianak orange peel oil (*Citrus nobilis* Lour. var. microcarpa Hassk.) by gas chromatography-olfactometry, aroma reconstitution, and omission test. J Agric Food Chem 57:239–244. https://doi.org/10.1021/jf801070r

Eggert A-K (1992) Alternative male mate-finding tactics in burying beetles. Behav Ecol 3:243–254

Eggert A-K, Müller JK (1989) Mating success of pheromone-emitting *Necrophorus* males: do attracted females discriminate against resource owners? Behaviour 110:248–257

Ferezou JP, Berreur-Bonnenfant J, Meusy JJ, Barbier M, Suchý M, Wipf HK (1977) 6,10,14-Trimethylpentadecan-2-one and 6,10,14-trimethyl-5-trans,9-trans-13-pentadecatrien-2-one from the androgenic glands of the male crab *Carcinus maenas*. Experientia 33:290

Fonseca MG, Vidal DM, Zarbin PHG (2010) Male-produced sex pheromone of the cerambycid beetle *Hedypathes betulinus*: chemical identification and biological activity. J Chem Ecol 36:1132–1139

Francke W, Schulz S (1999) Pheromones. Mori K (ed) Comprehensive Natural Products Chemistry 8:197–261. https://doi.org/10.1016/B978-0-08-091283-7.00052-7

Gries G, Gries R, Perez AL et al (1994) Aggregation pheromone of the African rhinoceros beetle, *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae). Z Naturforsch C. Biosci 49:363–366. https://doi.org/10.1515/znc-1994-5-614

Haberer W, Schmitt T, Peschke K et al (2008) Ethyl 4-methyl heptanoate: a male-produced pheromone of *Nicrophorus vespilloides*. J Chem Ecol 34:94–98. https://doi.org/10.1007/s10886-007-9406-y

Haberer W, Schmitt T, Schreier P, Müller JK (2011) Intended and unintended receivers of the male pheromones of the burying beetles *Nicrophorus humator* and *Nicrophorus vespilloides*. Entomol Exp Appl 140:122–126. https://doi.org/10.1111/j.1570-7458.2011.01143.x

Haberer W, Steiger S, Müller JK (2014) Dynamic changes in volatile emissions of breeding burying beetles. Physiol Entomol 39:153–164. https://doi.org/10.1111/phen.12059

Hallett RH, Perez AL, Gries G et al (1995) Aggregation pheromone of coconut rhinoceros beetle, *Oryctes rhinoceros* (L) (Coleoptera, Scarabaeidae). J Chem Ecol 21:1549–1570. https://doi.org/10.1007/BF02035152

Ho H-Y, Millar JG (2001a) Identification and synthesis of a male-produced sex pheromone from the stink bug *Chlorochroa sayi*. J Chem Ecol 27:1177–1201

Ho H-Y, Millar JG (2001b) Identification and synthesis of male-produced sex pheromone components of the stink bugs *Chlorochroa ligata* and *Chlorochroa uhleri*. J Chem Ecol 27:2067–2095

Islam N, Bacala R, Moore A, Vanderwel D (1999) Biosynthesis of 4-methyl-1-nonanol: female- produced sexpheromone of the yellow mealworm beetle, *Tenebrio molitor* (Coleoptera: Tenebrionidae). Insect Biochem Mol Biol 29:201–208

Joung MJ, Ahn JH, Lee DW, Yoon NM (1998) Coupling reaction of alkenes with alpha-bromo carboxylic acid derivatives using nickel boride and borohydride exchange. J Org Chem 63:2755–2757

Kalinová B, Podskalská H, Růzicka J, Hoskovec M (2009) Irresistible bouquet of death–how are burying beetles (Coleoptera: Silphidae: *Nicrophorus*) attracted by carcasses. Naturwissenschaften 96:889–899. https://doi.org/10.1007/s00114-009-0545-6

Lazari DM, Skaltsa HD, Constantinidis T (2000) Volatile constituents of *Centaurea pelia* DC ., C . *thessala* Hausskn. subsp. *drakiensis* (Freyn & Sint.) Georg. and *C. zuccariniana* from Greece. Flavour Fragr J 15:7–11

Li Y, Lu B, Li C, Li Y (2003) First total synthesis of (±)hedaol B. Synth Commun 33:1417–1423. https://doi.org/10.1081/SCC-120018704

Midgley G, Thomas CB (1987) Selectivity of radical formation in the reaction of carbonyl compounds with manganese(III) acetate. J Chem Soc Perkin Trans II:1003–1108

Müller JK, Eggert A-K (1987) Effects of carrion-independent pheromone emission by burying beetles (Silphidae: *Necrophorus*). Ethology 76: 297–304

Müller JK, Braunisch V, Hwang W, Eggert A-K (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, Nicrophorus vespilloides. Behav Ecol 18:196–203. https://doi.org/10.1093/beheco/arl073

Mulrey TEP, Eggert A-K, Sakaluk S (2015) Switching tactics: phenotypic plasticity in the alternative mate-finding tactics of burying beetles. Anim Behav 108:175–182. https://doi.org/10.1016/j.anbehav.2015.07.025

Nagano M, Suzuki S (2008) Number and size of clutch in *Ptomascopus morio* (Coleoptera: Silphidae: Nicrophorinae), with comparisons to *Nicrophorus*. Entomol Sci 11:153–157. https://doi.org/10.1111/j.1479-8298.2008.00267.x

Peck B (1982) The life history of the Japanese carrion beetle *Ptomascopus morio* and the origins of parental care in *Nicrophorus* (Coleoptera, Silphidae, Nicrophorini). Psyche 89: 107–112

Pukowski E (1933) Ökologische Untersuchungen an Necrophorus F. Z Morphol Ökol Tiere 27:595–618

Rochat D, Mohammadpoor K, Malosse C et al (2004) Male aggregation pheromone of the date palm fruit stalk borer *Oryctes elegans*. J Chem Ecol 30:387–407

Saïd I, Hasni N, Abdallah Z et al (2015) Identification of the aggregation pheromone of the date palm root borer *Oryctes agamemnon*. J Chem Ecol 41:446–457

Schulz S, Boppré M, Vane-Wright RI (1993) Specific mixtures of secretions from male scent organs of African milkweed butterflies (Danainae). Phil Trans Royal Soc London B 342:161–181

Sikes DS, Venables C (2013) Molecular phylogeny of the burying beetles (Coleoptera: Silphidae: Nicrophorinae). Mol Phylogenet Evol 69: 552–565. https://doi.org/10.1016/j.ympev.2013.07.022

Silk PJ, Sweeny JD, Wu J, Price J, Gutowski JM, Kettela EG (2007) Evidence for a male-produced pheromone in *Tetropium fuscum* (F.) and *Tetropium cinnamopterum* (Kirby) (Coleoptera: Cerambycidae). Naturwissenschaften 94:697–701

Suzuki S, Nagano M (2005) Host defense in *Nicrophorus quadripunctatus* against brood parasitism by *Ptomascopus morio* (Coleoptera: Silphidae: Nicrophorinae). Popul Ecol 48:167–171. https://doi.org/10.1007/s10144-005-0245-9

Suzuki S, Nagano M (2006) Resource guarding by *Ptomascopus morio*: Simple parental care in the Nicrophorinae (Coleoptera : Silphidae). Eur J Entomol 103:245–248

Suzuki S, Nagano M, Trumbo ST (2005) Intrasexual competition and mating behavior in *Ptomascopus morio* (Coleoptera: Silphidae Nicrophorinae). J Insect Behav 18:233–242. https://doi.org/10.1007/s10905-005-0477-8

Trumbo ST, Bloch PL (2002) Competition between *Nicrophorus orbicollis* and *N. defodiens*: resource locating efficiency and temporal partioning. Northeast Nat 9:13–26

Trumbo ST, Eggert A-K (1994) Beyond monogamy: territory quality influences sexual advertisement in male burying beetles. Anim Behav 48:1043–1047

Trumbo ST, Kon M, Sikes D (2001) The reproductive biology of *Ptomascopus morio*, a brood parasite of *Nicrophorus*. J Zool 255: 543–560. https://doi.org/10.1017/S0952836901001637

Van den Dool H, Kratz PD (1963) A generalizitaion of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11:663–471

Walling CA, Stamper CE, Salisbury CL, Moore AJ (2009) Experience does not alter alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. Behav Ecol 20:153–159. https://doi.org/10.1093/beheco/arn127