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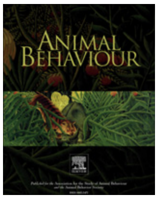

(*E*)-Methylgeranate, a chemical signal of juvenile hormone titre and its role in the partner recognition system of burying beetles

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In recent years, studies have shown that animals can communicate their physiological state or condition by means of chemical signals. However, as the chemicals involved in the condition-dependent signals have rarely been identified, evolutionary mechanisms that ensure their reliability are not well understood. We identified a volatile chemical signal that may serve as a reliable indicator of hormonal state in burying beetles, and is involved in their partner recognition system. Burying beetles reproducing on carcasses are able to distinguish between their breeding partners and infanticidal conspecifics. This discrimination depends on breeding status, which is positively linked to juvenile hormone III (JH III) titre. Breeding *Nicrophorus vespilloides* beetles, in contrast to nonbreeding ones, emitted considerable amounts of (*E*)-methylgeranate. The amount of emitted (*E*)-methylgeranate was positively correlated with juvenile hormone titres known from other burying beetle studies. Moreover, our behavioural experiments showed that dummies treated with methylgeranate induced tolerant behaviour, whereas control dummies were treated aggressively. The fact that (*E*)-methylgeranate and JH III share a conspicuous structural similarity and the same biosynthetic pathway may explain how the reliability of the signal for JH titre is ensured. We discuss the implications of our results in the light of theoretical work on the evolution of chemical communication, particularly on the origin of chemical signals.

Signal evolution has been studied intensely in the areas of sexual selection, aposematism and dominance signalling over the past quarter century (e.g. Espmark et al. 2000; Ruxton et al. 2004; Tibbetts & Dale 2004; Macias Garcia & Ramirez 2005; Laidre & Vehrencamp 2008). Most theoretical and empirical investigations of the evolution of animal signals, however, have focused on visual and auditory communication. Studies of chemical signals are less common (Johansson & Jones 2007; Symonds & Elgar 2008), although chemical signalling is regarded as the most ancient and widespread form of communication in the animal kingdom (Hölldobler 1984; Bradbury & Vehrencamp 1998; Wyatt 2003). Most work on chemical signals has focused attention on long-range mate attraction and sex recognition (Johansson & Jones 2007; Symonds & Elgar 2008). Chemical signals, however, are also important in several other contexts. Animals are known to release chemical substances that provide information about a specific physiological state, such as stage of oestrous cycle, copulatory readiness, dominance status, fertility, nutritional condition or health (e.g. Moore et al. 1997; Dietemann et al. 2003; Stacey et al. 2003; Kortet & Hedrick 2005; Fisher & Rosenthal 2006; Johansson & Jones 2007; Meyer et al. 2008). However, most of the chemical substances serving as condition-dependent signals have not been identified, and thus little is known about their evolution, design and reliability. Because individuals sending and receiving signals frequently have conflicting interests, one of the central questions in the evolution of communication is how honesty of information transfer is maintained given that senders may benefit by sending false information (Lachmann et al. 2001; Maynard-Smith & Harper 2003). From an evolutionary perspective, stable communication systems require phenotypic traits that convey, on average, honest information; otherwise, receivers will be unable to gain fitness benefits and stop attending to signals. Regarding species and sex recognition, both senders and receivers frequently share the same interest and there is no need for specific mechanisms to ensure the reliability of a signal (Johansson & Jones 2007). When advertising a certain physiological state or quality, however, there is ample inducement for deception, as, for example, when two individuals are competing for the same limited resource. The factors that have been proposed

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to ensure honesty of a signal are: (1) costs associated with producing or maintaining the signal (Zahavi 1975, 1977; Grafen 1990) and (2) physiological constraints, in which the signal cannot mislead because it is linked to the specific condition it advertises (Maynard-Smith & Harper 2003). However, empirical evidence for such mechanisms in chemically mediated communication is rare. Few studies have tried to assess costs of chemical signalling, because the costs of producing chemical signals are generally regarded to be low (Cardé & Baker 1984; Alberts 1992; but see Rantala et al. 2003). With respect to the 'constraints hypothesis', there is often too little knowledge about the metabolic pathways of pheromone production and the metabolism of the substances that induce the physiological state (e.g. hormones). Studies that integrate behaviour, physiology and chemical ecology in an attempt to understand signal design are lacking.

Insects are an attractive system with which to study chemical communication because they display a rich repertoire of behaviours mediated by olfaction. In addition, it has been demonstrated or at least suggested that physiological conditions that are advertised are linked to juvenile hormone (Sledge et al. 2004; Lengyel et al. 2007; Kou et al. 2008; Mas & Kölliker 2008), a pivotal endocrine regulator in insects (Flatt et al. 2005). Such knowledge provides the basis for testing the 'constraints hypothesis' as a mechanism to ensure signal reliability, by elucidating the metabolic pathways of both the hormone and the putative pheromone. In burying beetles, for example, juvenile hormone III (JH III) titre is positively correlated with reproductive condition and parental care (Trumbo et al. 1995; Trumbo 1997, 2002; Scott et al. 2001; Scott & Panaitof 2004). In addition, there is evidence that information about breeding state is transmitted to conspecifics by an external chemical signal (Müller et al. 2003; Scott et al. 2008; Steiger et al. 2008, 2009).

Burying beetles exhibit elaborate parental care (Pukowski 1933; Eggert & Müller 1997). Normally, a pair of beetles cooperate in burying a vertebrate carcass, a small bird or mammal, and rear a brood on it (Pukowski 1933). Both the male and the female feed and defend the larvae, which undergo their development on the buried carcass (Pukowski 1933; Fetherston et al. 1994; Scott 1998; Smiseth & Moore 2004; Trumbo 2006). When a conspecific intruder discovers and successfully takes over a carcass, it commits infanticide and rears its own young on the resource. However, resident males and females attempt to drive off intruders. The beetles are able to recognize their breeding partners and to distinguish them from intruding infanticidal conspecifics (Müller et al. 2003). The discrimination depends on the breeding status of the encountered beetle: breeding beetles of the opposite sex are generally accepted as partners, whereas nonbreeding ones of both sexes are attacked (Müller et al. 2003). Beetles undergo physiological changes during a breeding attempt on a carcass, which include a dramatic increase in JH III level until a peak is reached on the first days on which larvae are present on the carcass, and the intensity of parental care is highest (Scott et al. 2001; Trumbo 2002; Scott & Panaitof 2004). Hence, advertising hormonal state would be a reliable means of informing a partner about breeding state. In recent studies, bioassays have provided direct evidence that chemical cues are involved in the 'nestmate recognition' system of the burying beetles *Nicrophorus vespilloides* (Steiger et al. 2009) and *Nicrophorus orbicollis* (Scott et al. 2008). Moreover, previous studies suggest that cuticular hydrocarbons, specifically polyunsaturated hydrocarbons, signal breeding state (Steiger et al. 2007, 2008; Scott et al. 2008). However, the question of how these hydrocarbons could be a reliable indicator of breeding or hormonal state remains unsolved. It is possible that the production of specific hydrocarbons is necessarily linked to a high JH III titre, but, to date, the underlying metabolism producing such a constraint is unknown. The emission of a terpenoid, on the other hand, could reliably signal JH III titre because it is known from other insect species that this group of chemicals is linked to the biosynthesis of JH III as it shares the same biosynthetic pathway as the hormone (Seybold & Tittiger 2003; Bellés et al. 2005). In some mammals, fish and crab species, it has been demonstrated that a hormone itself or a derivative of it functions as a sex pheromone (see references in Haynes & Potter 1995).

Whereas the previous studies on the partner recognition system of burying beetles have concentrated on the investigation of nonvolatiles (Steiger et al. 2007, 2008; Scott et al. 2008), the current study tested the hypothesis that the beetles also produce volatiles during breeding, specifically terpenoid volatiles. In a follow-up behavioural experiment, we examined the role of these volatiles in partner recognition.

METHODS

Study Organisms

Experimental *N. vespilloides* beetles were the first-generation offspring of beetles collected from carrion-baited pitfall traps in a deciduous forest near Freiburg, Germany (48°00′ N, 07°51′ E). Beetles were maintained in temperature-controlled chambers at 20 °C with a 16:8 h light:dark regime. Prior to experiments, groups of up to five adults of the same sex and family were kept in small plastic containers (10 × 10 cm and 6 cm high) filled with moist peat and fed freshly killed mealworms twice a week. All experimental subjects were between 20 and 50 days of age. The size of the beetles was relatively uniform as they had been reared under standardized laboratory conditions (see Eggert et al. 1998).

Generation of Parental Beetles

We defined nonparental beetles as individuals having no access to a carcass suitable for reproduction, whereas parental beetles were individuals breeding on a carcass (dead mouse) and caring biparentally for young. To generate parental beetles, pairs of beetles (one male and one female) were placed into plastic containers filled with moist peat and provided with a 10 g mouse carcass. Mice were reared in our own laboratory and killed with a high concentration of CO_2. Once the carcass was buried, the containers were kept in darkness and the following manipulations performed under dim red light. After 48 h, each pair was transferred to a new box along with its carcass. To facilitate observations of behaviour on and around the carcass, the new containers were filled with a compact layer of 1–2 cm of moist peat. The previous containers, which contained the eggs, were checked for the presence of newly hatched larvae four times a day. Once we observed larvae, we placed 10 first-instar larvae on the carcass with the respective pair of beetles. Some of the larvae added were not the pair's own offspring. The use of unrelated larvae is of no consequence to the present study because parental beetles do not discriminate between their own and unrelated larvae, provided they appear at the right time (Müller & Eggert 1990). To standardize the parental beetles' breeding condition, pairs were left to care for the larvae for a certain period of time (12–20 h). For all experiments, we used only beetles that were present on the carcass after the standardized period of parental care. Pairs of beetles in which the male, the female or both were hiding in the peat were excluded from the experiment as they might not have been involved in biparental care.