Social environment determines degree of chemical signalling

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Few studies have attempted to distinguish between cues and signals in the context of chemical communication. A number of chemical substances have been shown to vary with physiological state, such as stage of oestrus cycle, fertility, dominance status or nutritional condition, but little is known about whether this variation is incidental or adaptive. Here, we provide evidence of a substance whose emission varies with breeding status but is not merely an incidental by-product of physiological state, but rather, an evolved signal. Breeding females of the facultative biparental burying beetle, Nicrophorus vespilloides, release methyl geranate, a substance that helps males to identify breeding status and to distinguish between their female partners and non-breeding intruders. We demonstrate that females respond flexibly to their social environment and emit high amounts of methyl geranate only in the presence of a male partner, i.e. a receiver. In contrast, cuticular hydrocarbons, which also have been shown to change with breeding status, are not modulated and do not differ between single and paired breeding females. Receiver-dependent chemical signalling is expected to evolve when costs are involved in the production or transmission of the signal; such signal modulation might be more common than previously thought.

Keywords: chemical communication; chemical signal; signal modulation; parental care; burying beetle; juvenile hormone

1. INTRODUCTION

Like other communication traits, chemical signals are often costly to produce, maintain or transmit [1]. Costs may be metabolic, removing energy or chemical precursors necessary for other important biosynthetic pathways, or ecological, making the organism more conspicuous to predators [2]. One strategy for efficiently minimizing these costs is to produce or transmit the signal in a specific context only, e.g. in the presence of a desirable receiver. Modulation of pheromone emission has been shown predominantly for sex pheromones [3], but often it is not the presence or absence of conspecifics that directly affects the pheromone emission, but rather a time-specific pattern of pheromone release occurs that corresponds to the activity time of the receivers [4–6]. Here, we present a novel example of receiver-dependent signal transmission involving a social pheromone that is crucial for cooperative brood defence.

Burying beetles exhibit elaborate biparental care [7] and usually, a pair of beetles cooperates in burying a vertebrate carcass and rearing a brood on it. Both the male and the female feed and defend the larvae, which undergo their development on the buried carcass. The beetles are able to recognize their breeding partners and to distinguish them from intruding infantilid conspecifics [8]. This discrimination depends on the breeding status of the encountered beetle, which is positively linked to the titre of juvenile hormone III (JH III): breeding beetles of the opposite sex are accepted as partners, whereas non-breeding beetles are attacked [8–10]. In a recent study, we found evidence that the monoterpenoid methyl geranate, which is structurally related to JH III and therefore appears to provide information about an individual’s hormone titre [11], is involved in the recognition mechanism. We showed that breeding females emit high amounts of methyl geranate in contrast to non-breeding females, and demonstrated that breeding males accept non-breeding females when the latter are treated with synthetic methyl geranate [11].

Apart from biparental care, female uniparental care occurs regularly in the field [12]. In those cases in which the female rears offspring on her own, there is no need for signal breeding status, as a male partner (i.e. a receiver) is not present. Moreover, if single females can switch off methyl geranate production irrespective of their physiological condition, this would rule out the possibility that the substance is simply an unavoidable by-product of breeding state and would indicate that methyl geranate production is under selection for communication. To test whether female economize on signal production and/or transmission, we compared the amount of methyl geranate produced by female Nicrophorus vespilloides breeding on a carcass with and without a male partner (i.e. with and without a receiver). In a companion chemical analysis, we investigated possible quantitative differences in cuticular hydrocarbons (CHCs) between single and paired females. CHCs have shown to change with breeding status and may play an additional role in the partner recognition system of burying beetles [13,14].

2. MATERIAL AND METHODS

(a) Generation of parental beetles

Experimental N. vespilloides 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). Females were each held with a male for 48 h in a small plastic container to ensure that they received sufficient sperm to fertilize their eggs. During their daily afternoon activity period on the third day, females were either placed alone ('single females') or with a male partner ('paired females') on a freshly killed 10 g mouse placed on top of moist peat. Once the carcass was buried, the containers were kept in darkness and all following manipulations made under dim red light. After 48 h, each female or pair was transferred to a new box along with its carcass. The previous containers, which contained the eggs, were checked for the presence of newly hatched larvae three times a day. Once larvae were observed, we placed 10...
first-instar larvae on the carcass with the respective single or paired female. To standardize the parental beetles’ breeding condition, females and pairs were left to care for the larvae for a defined period of time (18–26 h). There was no difference in time to care between single and paired females (t-test, $t_{21} = 0.49$, $p = 0.65$).

(b) Chemical analyses
We performed headspace analyses on two different groups of beetles: parental single females ($n = 13$) and parental paired females ($n = 17$). For sampling, analysing and quantifying chemicals, we followed the protocol described in Haberer et al. [11] (see also electronic supplementary material).

For analysis of CHCs, parental single ($n = 12$) and paired ($n = 11$) females were freeze-killed and soaked individually in 3 ml n-pentane (>99%, Fluka, Switzerland) for 15 min on an oscillator. The extracts were analysed by GC–MS (full description of chemical analysis are provided in the electronic supplementary material). Peaks of 89 components were manually integrated. For the statistical analysis of chemical data, the relative peak area of each of the 89 peaks with respect to an internal standard (n-eicosane) was calculated. A discriminant analysis (DA) was performed to determine whether single or paired females could be discriminated on the basis of their cuticular profile. To reduce the number of variables prior to the DA, we performed a principal components analysis (PCA).

(c) Behavioural experiment
We established 65 pairs of parental $N$. vespilloides beetles. Once they had cared for larvae for approximately 24 h, the female was carefully removed from the carcass and replaced with either a freshly killed parental female that had been breeding alone on a carcass (‘single female’, $n = 34$) or a freshly killed parental female that had been breeding with a male partner (‘paired female’, $n = 31$). In each case, the body of the female was placed in close proximity to the carcass. We observed the male’s behaviour towards the female body in the first five encounters and classified their reactions as aggressive or tolerant. The behaviour was considered aggressive if the male grasped and bit the female’s body in at least four of the five encounters, and tolerant if there was no aggressive behaviour in at least four of the five encounters. The bioassay was performed blind to treatment.

3. RESULTS
On average, females who were breeding in the company of a male partner emitted 200 times more methyl geranate than single females (t-test, $t_{28} = 4.43$, $p < 0.001$; figure 1). DA was used to determine whether single females could be distinguished from paired females based on their CHC profiles, after first reducing the number of variables (=89 CHCs) using PCA. The PCA produced 14 principal components with eigenvalues of more than one, explaining 95.6 per cent of the total variance. The DA performed on the 14 principal components could not significantly differentiate between the two groups ($\text{Wilks' } \lambda = 0.24$, $\chi^2_{14} = 20.11$, $p = 0.13$). Even when each substance was tested separately, single and paired females did not differ significantly in any of the 89 CHCs (ANOVA; $\forall p > 0.05$; electronic supplementary material, table S1). Also the total amount of CHCs did not differ between single and paired females (t-test, $t_{31} = -0.15, p = 0.88$).

In our behavioural experiment, we found that parental males behaved more aggressively towards dead females that had been breeding alone on a carcass than towards dead females that had been reproducing with a male partner ($\chi^2 = 8.8, p < 0.01$; figure 2). Nevertheless, over 50 per cent of the single females were tolerated by the males.

4. DISCUSSION
Our results demonstrate that methyl geranate is not only a cue, but a signal evolved to convey information about a female’s physiological state to a male breeding partner. Although single females readily bred on carcasses and reared young, they emitted only trace amounts of methyl geranate, a result that shows that methyl geranate is not merely an unspecified metabolic by-product of breeding state. Instead, female methyl geranate emission is specifically activated when a receiver is present, as only paired females released high amounts of methyl geranate. Because breeding males only emit trace amounts of methyl geranate [11], the pheromones emitted by paired breeding females cannot be attributed to inadvertent transfer of odours from their male partners. This result has three implications: (i) breeding females are able to assess their social environment, i.e. whether they breed alone or with a male partner, (ii) they possess enzymes whose syntheses or activities depend on females’ social environment, and (iii) they minimize the costs of signal production and/or transmission and emit the chemical signal only when it is necessary, i.e. in the presence of a breeding partner.

**Figure 1.** Peak area of methyl geranate emitted by paired ($n = 17$) and single females ($n = 13$) breeding on a carcass (mean ± s.e.). y-Axis is logarithmically scaled for better visualization.

**Figure 2.** Proportion of parental paired ($n = 31$) and single females ($n = 34$) that were treated aggressively by breeding males. Dead females were used in the behavioural assay.
The costs of methyl geranate production or emission are currently unknown. It is unlikely that the chemical signal makes female burying beetles more prone to predation, as they are underground in a crypt during signal emission. Rather, we believe that the costs are primarily metabolic. JH III and methyl geranate have common precursors, and pheromone production may negatively affect JH titre in the current or in a future breeding attempt.

In a former study, we found that not only the quantity of methyl geranate is affected by the breeding status, but also the relative and absolute amount of CHCs in the beetles’ cuticle [13]. However, these latter changes occur irrespective of the presence or absence of a male partner as the results of the current study show that the cuticular pattern of single breeding females does not differ from that of paired females. Thus, we cannot determine whether the change in CHCs constitutes a cue or a signal on the basis of existing data. However, the finding that there was no difference in CHCs between both groups of females may explain why 50 per cent of the single females were rejected as a breeding partner.

In conclusion, burying beetle females advertise their breeding status via the emission of methyl geranate, but do so only when a receiver, a male partner, is present. Receiver-dependent signal transmission has presumably evolved to reduce the costs of maintaining or transmitting a signal and may play an important role in other signalling systems.

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