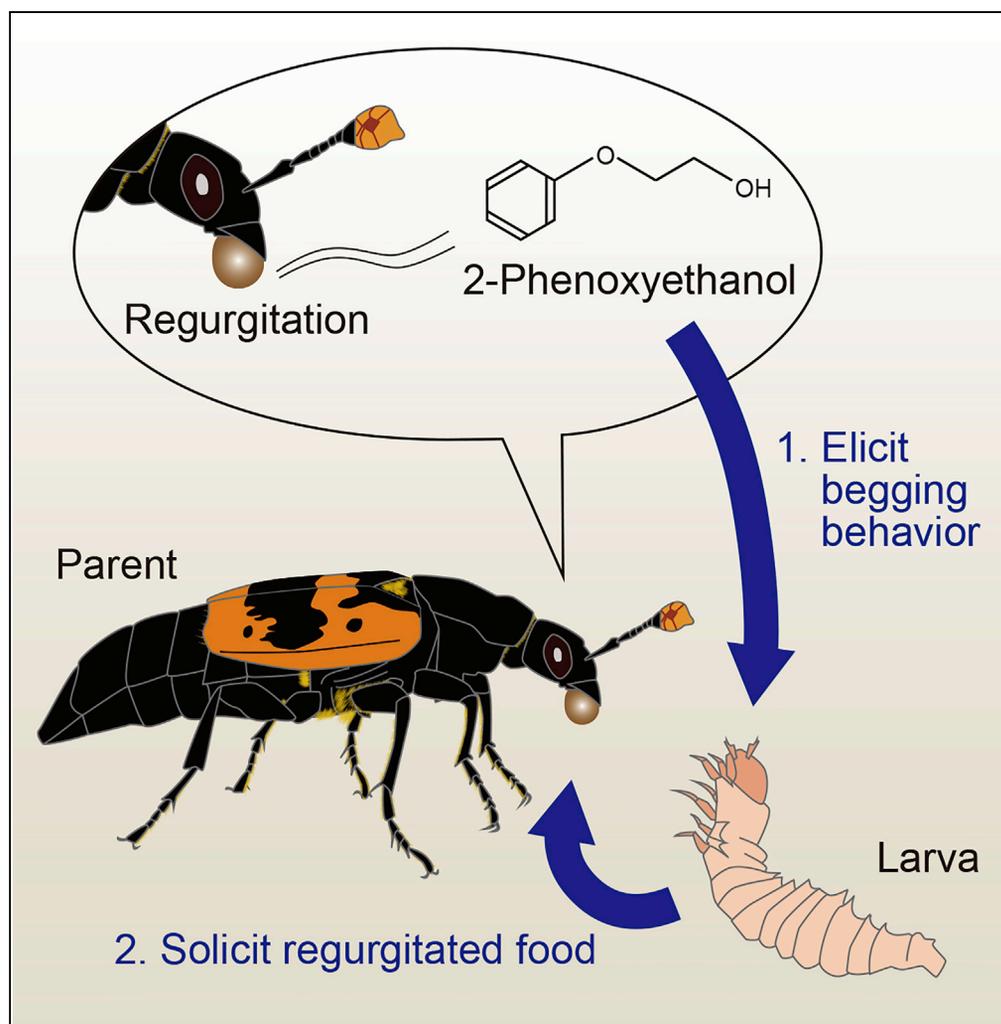


## Article

## A Parental Volatile Pheromone Triggers Offspring Begging in a Burying Beetle



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**HIGHLIGHTS**

Burying beetle parents emit a volatile pheromone that elicits larval begging

Parents secrete the pheromone into their regurgitation, which is consumed by offspring

This pheromone benefits the parents by reducing the cost of larval begging

Article

# A Parental Volatile Pheromone Triggers Offspring Begging in a Burying Beetle

Mamoru Takata,<sup>1,5,6,\*</sup> Yuki Mitaka,<sup>2,5</sup> Sandra Steiger,<sup>3</sup> and Naoki Mori<sup>4</sup>

## SUMMARY

Parental care is a notable aspect of reproductive effort in many animals. The interaction between offspring begging and the parental feeding response is an important communication mechanism that regulates offspring food supply, and reducing the cost of superfluous begging is beneficial to both parents and offspring. Here we concluded that parents of the burying beetle *Nicrophorus quadripunctatus* inform their offspring of their preparation for provisioning by emitting “provisioning pheromone.” Female parents emitted an antimicrobial aromatic compound, 2-phenoxyethanol, in their regurgitation before provisioning, and this compound elicits begging behavior from their offspring. Furthermore, begging incurs growth and survival costs, and parents spent more than 85% of their time in close proximity to their offspring without provisioning. Therefore, it is suggested that limiting offspring begging during provisioning is beneficial to both parents and offspring. We report here a novel aspect of parent-offspring communication in family life.

## INTRODUCTION

Parent-offspring communication is essential to family processes. As the interests of parents and offspring conflict with regard to the allocation of parental resources (Godfray, 1995a; Trivers, 1974), offspring solicitation for provisioning and the parental feeding response are particularly important communication mechanisms that regulate the amount of food provided to each offspring. Theoretically, costly begging behavior exhibited by offspring is a key mechanism for resolving the evolutionary conflict and maintaining the signal's reliability (Godfray, 1995b, 1991; Kilner and Johnstone, 1997; Parker et al., 2002). Offspring can access more food by sending stronger begging signals; however, they simultaneously suffer as a result of the increased costs it incurs. Indeed, these costs have been observed among birds (Chappell and Bachman, 2002; Kilner, 2001; Noguera et al., 2010; Rodríguez-Gironés et al., 2001). Several models have predicted that these costs prevent the escalation of begging signals, and that the begging signal's strength eventually reaches an equilibrium whereby the offspring obtains the greatest benefit for the least cost (reviews in Chappell and Bachman, 2002; Godfray, 1995a; Mock and Parker, 1997). As the benefits vary depending on the offspring's needs or conditions, these costs ensure the inherent honesty of the begging behavior. However, those costs may in turn have implications for the evolution of parental signals: if offspring engage in begging behavior when their parents are unable to provide food immediately, the offspring incur redundant begging costs. Therefore, offspring should only engage in begging behavior when their parents are ready to feed, and it is also beneficial for parents to reduce energy expenditure among their offspring. Consequently, we may predict the evolution of a mechanism that prevents superfluous begging behavior on the part of offspring. For example, there may be a parental signal indicating that parents are ready to provision, and this signal may elicit begging behavior in the offspring. Three studies in birds (Leonard et al., 1997; Madden et al., 2005; Maurer et al., 2003) have reported that offspring exhibit begging in response to parental acoustic stimuli. However, no study has examined the benefit of emitting the stimulus to parents or discussed whether the stimulus is a parental cue or a signal, which is essential for assessing its importance (Laidre and Johnstone, 2013).

Burying beetles exhibit elaborate parental care, including offspring provisioning (Eggert and Müller, 1997; Scott, 1998). Burying beetles breed on the carcasses of small vertebrates, and both male and female parents provide care (Figure 1). Because the carcasses are colonized by detrimental microbes (Rozen et al., 2008), adult beetles secrete antimicrobial substances that suppress microbial growth during breeding (Arce et al., 2012; Cotter et al., 2013; Cotter and Kilner, 2010; Jacobs et al., 2016; Palmer et al., 2016), thereby enhancing the survival rate of their larvae (Arce et al., 2012). Parents feed predigested carrion to each larva by means of regurgitation. Larvae beg for parental provisioning by waving their legs toward the mouthparts of the parents while raising their heads (Rauter and Moore, 1999; Smiseth

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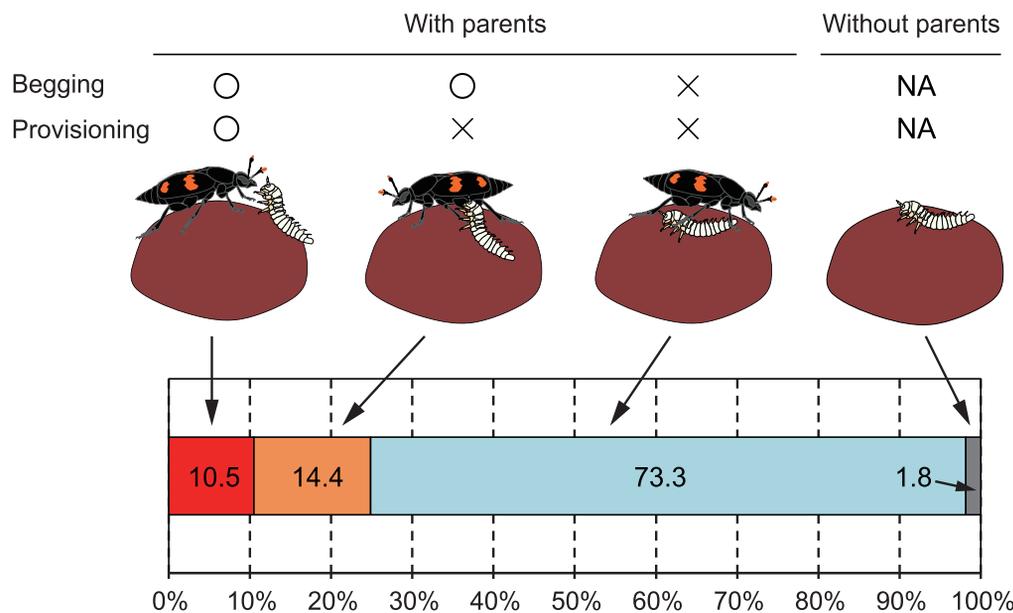
**Figure 1. Female Burying Beetle Provisioning Larvae**

A female beetle feeds its larvae via regurgitation.

et al., 2003). The intensity of the larval begging reflects the larva's hunger level (Smiseth and Moore, 2008, 2004), and parents allocate more food to hungrier larvae (Smiseth and Moore, 2008). Although the energetic and opportunity costs of begging have not hitherto been observed in burying beetles (Smiseth and Parker, 2008), larval begging increases the risk of mortality owing to the larva's increased likelihood of becoming a target for filial cannibalism (Andrews and Smiseth, 2013). Because care behavior is theoretically coordinated among family members to maximize the benefits of each discrete care event and to minimize any costs associated with redundant actions, larvae should avoid begging when the parents are unprepared for provisioning. Consequently, we may predict the existence of a provisioning signal that prevents superfluous begging among larvae and regulates their investment in begging signals.

Pheromones play a crucial role in communication among members of burying beetle families. At the beginning of their breeding cycle, adult males emit sex pheromones (in *Nicrophorus vespilloides*, a mixture of ethyl 4-methyl heptanoate and (*E*)-geranylacetone) to attract adult females (Haberer et al., 2008). The quantities and the ratio of these components reflect the quality of the male and determine his attractiveness to females (Chemnitz et al., 2015). During the larval provisioning period, female parents emit an antiaphrodisiac pheromone (methyl geranate), which deters males' mating attempts (Engel et al., 2016); the quantity of pheromone emitted reflects the females' temporal infertility (Engel et al., 2016). Larvae distinguish breeding from non-breeding female parents via chemical cues and engage in begging in response to chemical stimuli from breeding parents (Smiseth et al., 2010). Furthermore, larvae discriminate between female and male parents and beg more from females than from males (Paquet et al., 2018; Suzuki, 2015). However, whether or not larvae only beg from parents carrying food remains unclear. Hence, parents' provisioning status has hitherto been overlooked and the existence of a provisioning signal has been unattested. If parents use provisioning signals to regulate larval begging, they are most likely to take the form of pheromone signaling.

Herein, we reveal that female parents among the burying beetle *Nicrophorus quadripunctatus* produce a pheromone that signals their readiness for provisioning, and that their larvae respond to this pheromone by exhibiting begging behavior. Furthermore, we identify an active compound of this pheromone, determine its emission source, and demonstrate that the pheromone is instrumental in reducing the costs associated with larval begging.



**Figure 2. Time Spent in Female-Parental Care and Offspring Begging Behavior in *Nicrophorus quadripunctatus* Families**

Instantaneous scan sampling of 42 broods showed that female parents spent 98.2% of their time in close proximity to larvae (red + orange + blue bars). Larval begging was observed 24.9% of the time (red + orange bars) and accessing parental provisioning 10.5% of the time (red bar). Parents spent only 1.8% of their time away from the larvae (gray bar).

## RESULTS

### Parental Care and Offspring Begging

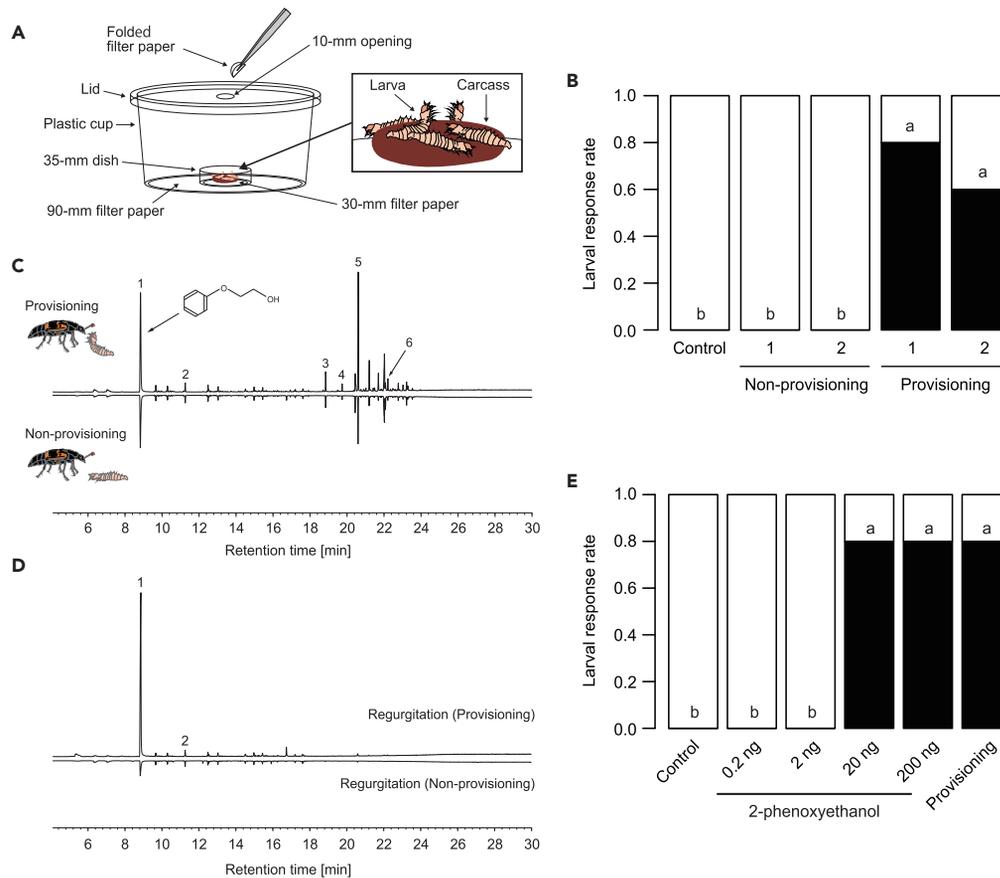
Before testing whether females of *N. quadripunctatus* emit a chemical provisioning signal, we observed the behavioral sequence of parental provisioning and investigated the frequency of parent-offspring feeding. The provisioning sequence was as follows (Video S1): first, parents fed directly on a carcass and digested it (step 1). During this step, they remained continuously in close proximity to the larvae. Subsequently, they left the larvae for a few seconds (step 2), before returning to close proximity to the larvae (step 3). Larval begging typically began at this stage, and most larvae commenced begging synchronously. Finally, parental provisioning occurred (step 4). Observation of the processes of parental care and offspring begging in burying beetle families revealed that larvae in the begging group spent 24.9% of their time begging, despite their parents being present most of the time, and that parents spent only 10.5% of their time provisioning (Figure 2). These results imply that parents regulate larval begging by emitting a chemical provisioning signal.

### Larval Responses to Extracts from Provisioning and Non-provisioning Parents

To verify the existence of a chemical signal aimed at eliciting larval begging behavior, we compared larval reactions to the hexane crude extracts from provisioning parents (i.e., the individuals that approached larvae to feed and from which the larvae begged) and non-provisioning parents (i.e., the individuals from which the larvae did not beg) (see Transparent Methods for details), using our bioassay device (Figure 3A). Larvae were observed to engage in more begging behavior in response to extracts from provisioning females than to those from non-provisioning females, without touching the filter paper impregnated with the extracts (Fisher's exact test with Benjamini-Hochberg correction,  $p < 0.05$ ; Figure 3B). Therefore, we inferred that the highly volatile components in the parent extracts elicited larval begging behavior.

### Chemical Profiles of Extractions of Provisioning and Non-provisioning Females

To identify the volatile compounds that elicited begging behavior, we analyzed the chemical profiles of the whole-body extracts from provisioning and non-provisioning parents using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis revealed that several of the compounds detected were common



**Figure 3. Bioassays and Chemical Analysis of the Pheromone Emitted from Parental Extracts**

(A) Schematic of the bioassay device.

(B) Larval responses to female extracts. Extracts from provisioning females (two samples) elicited larval begging, but those from non-provisioning females (two samples) did not (Fisher's exact test with Benjamini-Hochberg correction,  $p < 0.05$ ). Each treatment was replicated 10 times. Different letters indicate significant differences.

(C) Representative gas chromatograms of the whole-body extracts of provisioning (upper) and non-provisioning (lower) females. Compounds 1–6 were identified as 2-phenoxyethanol (1: retention time [ $t_R$ ] = 8.83 min),  $\beta$ -elemene (2:  $t_R$  = 11.26 min), heneicosane (3:  $t_R$  = 18.85 min), docosane (4:  $t_R$  = 19.74 min), tricosane (5:  $t_R$  = 20.61 min), and pentacosane (6:  $t_R$  = 22.21 min), respectively (refer to Figure S1 for the mass spectrum of 2-phenoxyethanol). No other compounds were identified.

(D) Representative gas chromatograms of the regurgitation extracts derived from provisioning (upper) and non-provisioning (lower) females. For peak numbers, refer to (C).

(E) Larval begging behavior was elicited by the authentic standard of 2-phenoxyethanol at 20 and 200 ng per paper, as well as the extract from a provisioning female (sample 1 in Figure 2) (Fisher's exact test with Benjamini-Hochberg correction,  $p < 0.01$ ). Each treatment was replicated 10 times. Different letters indicate significant differences.

to all beetle extracts, and six compounds (compounds 1–6) were identified altogether: 2-phenoxyethanol (1),  $\beta$ -elemene (2), heneicosane (3), docosane (4), tricosane (5), and pentacosane (6) by comparing the retention times and mass spectra of their compounds to those of authentic standards (Figure 3C). Also, because larval begging behavior is directed toward the parental mouthparts shortly before parents regurgitate pre-digested carrion, we predicted that the chemical that elicits begging is contained in the parental regurgitation (i.e., the larvae's food). To test this prediction, the chemical profiles of regurgitated food from provisioning and non-provisioning females were compared. We identified only 2-phenoxyethanol and  $\beta$ -elemene in these regurgitation extracts and found that 2-phenoxyethanol was contained specifically in the regurgitations from provisioning females (Figure 3D). Therefore, we surmised that 2-phenoxyethanol functions as a provisioning signal.

### Identification of the Active Component of the Provisioning Pheromone

To determine whether or not 2-phenoxyethanol is an active compound of the provisioning pheromone, larval reactions to filter papers impregnated with authentic 2-phenoxyethanol in serial doses of 0.2, 2, 20, and 200 ng per paper were investigated. The authentic sample elicited larval begging at 20 and 200 ng per paper (approximately equivalent to 0.002 and 0.02 individuals, respectively) as did the extracts from provisioning parents (Fisher's exact test with Benjamini-Hochberg correction,  $p < 0.01$ ; [Figure 3E](#) and [Video S3](#)), whereas the larvae showed no response to 0.2 and 2 ng of 2-phenoxyethanol, as did the hexane control ([Video S4](#)).

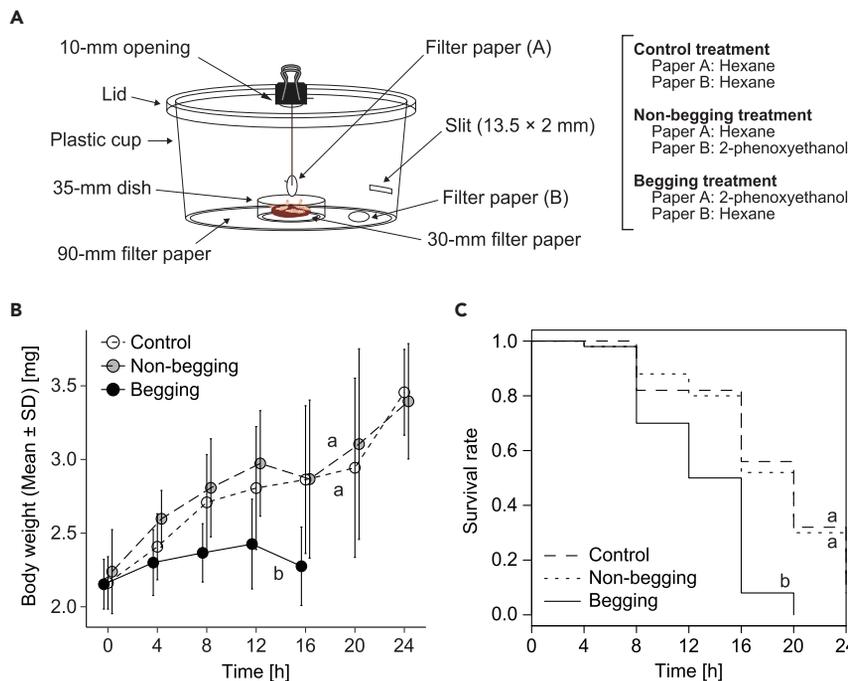
### Costs of Larval Begging

Begging behavior costs have not hitherto been detected in the burying beetle *N. vespilloides* ([Smiseth and Parker, 2008](#)) and have not been tested in *N. quadripunctatus*. However, as the provisioning pheromone that we identified impelled the larvae to engage in begging behavior, we had the unique opportunity to identify any begging costs that may affect *N. quadripunctatus*. We compared larval growth and survival rates among the begging treatment (larvae were forced to beg by directly presenting the authentic pheromone), non-begging treatment (the pheromone was introduced into a bioassay device but larvae were not directly subjected to the pheromone), and control treatment (the pheromone was not introduced). As a result, larval begging was observed only in begging treatment, and larval growth was suppressed to a greater degree by the begging treatment than by the non-begging and control treatments (generalized linear mixed model [GLMM] followed by multiple pairwise-comparisons of interaction between treatment and time with Bonferroni correction,  $p < 0.01$ ). Moreover, the survival of the larvae was reduced by the begging treatment compared with the non-begging and control treatments (Kaplan-Meier survival analysis followed by multiple pairwise comparisons with Bonferroni correction,  $p < 0.001$ ) ([Figure 4](#)), and the number of begging larvae decreased over time ([Table S1](#)). These results suggest that the begging treatment reduced the larval survival duration due to enforced begging, not due to the toxicity of highly concentrated 2-phenoxyethanol.

### DISCUSSION

Burying beetles provide post-hatching parental care and family members communicate via pheromones ([Chemnitz et al., 2015](#); [Engel et al., 2016](#); [Haberer et al., 2008](#); [Steiger et al., 2007](#)). In this study, we verified that parents of the burying beetle species *N. quadripunctatus* inform their offspring of their preparation for provisioning by emitting a volatile signal, which we term the "provisioning pheromone," inducing offspring begging. Our behavioral observations revealed that no larva exhibited begging more than 70% of the time, even though the female parent was in close proximity ([Figure 2](#) and [Video S2](#)); however, most larvae commenced begging synchronously shortly before the provisioning ([Video S1](#)). Our GC-MS analyses and bioassays demonstrated that the regurgitation of provisioning females contains an aromatic compound, 2-phenoxyethanol, and that this compound itself elicits begging behavior ([Figure 3](#)). Furthermore, using synthesized 2-phenoxyethanol, we demonstrated that superfluous begging incurs growth and survival costs ([Figure 4](#)). Because parents spent more than 98% of their time in close proximity to their offspring but spent only 10.5% of their time in provisioning ([Figure 2](#)), these results suggest that limiting offspring begging during provisioning is beneficial to both parents and offspring because it reduces superfluous begging. Our results demonstrated that 2-phenoxyethanol is a parental signal but not a cue, and that it is secreted into the parental regurgitation before the provisioning event.

The temporary increase of the provisioning pheromone before parental feeding ([Figures 3C](#) and [3D](#)) and the limited exhibition of begging behavior ([Figure 2](#)) attest that the pheromone serves as a mechanism for avoiding superfluous begging in burying beetle offspring. Interestingly, a previous study showed that burying beetle parents emit acoustic signals that attract young larvae ([Niemitz and Krampe, 1972](#)). The acoustic signal would reinforce the effectiveness of the provisioning pheromone by gathering their larvae to a place where parents visit to feed. Although our hypothesis postulated that begging incurs costs, a previous study using *N. vespilloides* did not detect such a cost ([Smiseth and Parker, 2008](#)). To elicit larval begging, the authors presented the dead bodies of parents to larvae for 105 min. Because the dead parent-derived chemicals that elicit offspring begging degrade over time, it would be difficult to prolong the treatment sufficiently to detect the energetic costs of larval begging. Here, on the other hand, using an artificial pheromone enabled us to prolong the exposure period compared with that in the above-mentioned previous study, and we verified that superfluous begging is costly to larvae of this species



**Figure 4. Costs of Begging Behavior**

(A) Schematic of the bioassay device used to evaluate the costs of begging.

(B) Effect of begging on larval growth. Black, gray, and white circles represent the begging, non-begging, and control groups, respectively. Data are means ± SD. Growth in larval body weight was suppressed by begging behavior. Different letters indicate significant differences (GLMM; pairwise comparisons of interaction between the experimental group and time by log rank test followed by Bonferroni correction:  $p < 0.01$ ).

(C) Kaplan-Meier survival analysis of larvae in the begging, non-begging, and control groups. Solid, dotted, and dashed lines represent the begging, non-begging, and control groups, respectively. Larvae in the begging group had a shorter survival time. Different letters indicate significant differences (pairwise comparisons by log rank test followed by Bonferroni correction,  $p < 0.001$ ).

(Figure 4). In our experimental device, we supplied larvae a piece of carcass to mimic their breeding chamber and allowed them to self-feed. However, even in non-begging and control treatment, survival rate at 24 h from hatching was only ca. 30% (Figure 4C). The results are consistent with previous studies in burying beetles. Unlike the *N. vespilloides* larvae (Capodeanu-Nägler et al., 2016; Eggert et al., 1998), the *N. quadripunctatus* larvae have poor ability to self-feed and most larvae are not able to survive to dispersal stage (ca. 120 h from hatching) in the absence of parents (Satou et al., 2001). The poor self-feeding ability would contribute to detect the cost of begging, because the *N. quadripunctatus* larvae waste their energy reserves as they move to beg but could not obtain foods. As a result, all larvae were dead by 20 h in begging treatment. Our results suggest that the provisioning pheromone benefits parents and offspring by reducing the offspring's energy expenditure by conveying the information about when and where to beg, as the pheromone is contained in the parental regurgitation. These mechanisms aimed at preventing superfluous begging in offspring may be common among species wherein the parents provide food directly and gain fitness benefits by limiting offspring begging at provisioning and/or indicating location of the target to beg. For example, previous studies in bird species reported that parental stimuli before provisioning trigger offspring begging (Leonard et al., 1997; Madden et al., 2005; Maurer et al., 2003) and the associated costs (Chappell and Bachman, 2002; Kilner, 2001; Noguera et al., 2010; Rodríguez-Gironés et al., 2001). Furthermore, the red bill spot on gull parents is a well-known trait that offspring beg toward it (reviewed by ten Cate, 2009), although whether those stimuli are a signal or a cue is unclear. Thus, parental stimuli can be considered signals if the parents benefit from a reduced cost of offspring begging (Laidre and Johnstone, 2013). Determination of the parental benefit obtained by regulating offspring begging is needed to enhance our understanding of parent-offspring communication.

Our findings also offer a suggestion with regard to the provisioning pheromone's origin. As burying beetles breed on microbe-rich carcasses in an underground breeding chamber, antimicrobial strategies are crucial

for larval survival and growth (Arce et al., 2012; Rozen et al., 2008). Indeed, adult beetles secrete antimicrobial substances during breeding and suppress microbial growth (Arce et al., 2012; Cotter et al., 2013; Cotter and Kilner, 2010; Hall et al., 2011; Jacobs et al., 2016; Palmer et al., 2016). The reduction of microbes in parental regurgitations is particularly important, because these are a major food source for larvae in the first and second instar phases, which are vulnerable stages with regard to microbial challenge (Arce et al., 2012). The key component of the provisioning pheromone, 2-phenoxyethanol, is well known as a germicidal agent (Lowe and Southern, 1994; McNamara et al., 2009; Siebert and Harke, 2009), and we observed its secretion into parental regurgitations. A previous study reported that parental oral secretions, including regurgitation, contain a variety of antimicrobial components, although 2-phenoxyethanol has never hitherto been detected (Degenkolb et al., 2011). Consequently, it is likely that it serves primarily as a form of social immunity, but has secondarily evolved to also fulfill a communicative function (Steiger et al., 2011; Stökl and Steiger, 2017). Many group-living insects have been observed to feed their broods with anti-parasitic components (Meunier, 2015), but examples that use 2-phenoxyethanol as a semiochemical are rare. For example, the 2-phenoxyethanol contained in ball-point pen ink acts as the analog of the trail pheromone in termites (Chen et al., 1998). It also functions as a fixative in rabbit chin gland secretions, slowing the release rate of the more volatile constituents, and causing a dominant animal's scent to persist in the environment (Hayes et al., 2003). Because the biosynthesis pathway of 2-phenoxyethanol is still unknown in any organism, it is important to ascertain how burying beetles acquire 2-phenoxyethanol, which gland secretes the compound, and when they began to use the compound as a pheromone. Within the subfamily Nicrophorinae, which includes the burying beetle (genus *Nicrophorus*) (Sikes and Venables, 2013), there is some interspecies variation with regard to the extent of parental care, ranging from care without provisioning (e.g., in *Ptomascopus*; Suzuki and Nagano, 2006) to care including provisioning that is facultative or obligate for offspring survival (e.g., in *Nicrophorus*; Capodeanu-Nägler et al., 2016). Thus, interspecies comparison of the provisioning pheromone's presence promises to be beneficial in investigating the evolutionary relationship between the extent of parental care and pheromone activity.

In this study, we identified a particular mode of parent-offspring interaction and shed light on the communication system's origins. Parents in burying beetle families do not occupy a passive position (i.e., passively receiving and responding to begging signals by following their behavioral reaction norms; Smiseth et al., 2008), as has been suggested in previous theoretical reports, but rather actively affect offspring behavior via a provisioning signal. This identification of the provisioning pheromone uncovers a new aspect of parent-offspring communication in family life.

### Limitation of the Study

The larvae typically exhibit begging just before the provisioning event (Video S1), suggesting that the emission period of the provisioning pheromone is limited. To facilitate the collection of the pheromone, the identification of secretory organ will be needed. In addition, to uncover the communication among family members, further studies will be needed to investigate whether male parents also use the pheromone.

### METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

### SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.isci.2019.06.041>.

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### AUTHOR CONTRIBUTIONS

Conceptualization, M.T., Y.M., and S.S.; Methodology, M.T., Y.M, N.M., and S.S.; Validation, M.T. and Y.M.; Formal analysis, M.T. and Y.M.; Investigation, M.T. and Y.M.; Resources, M.T.; Writing – Original Draft, M.T.

and Y.M.; Writing – Review and Editing, M.T., Y.M., S.S., and N.M.; Visualization, M.T. and Y.M.; Supervision, S.S. and N.M.; Project administration, M.T.; Funding Acquisition, M.T.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

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## **Supplemental Information**

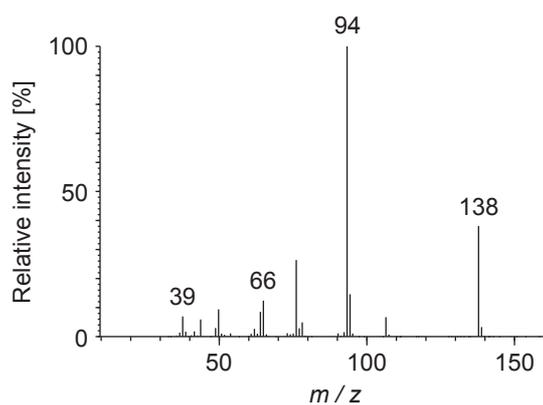
**A Parental Volatile Pheromone Triggers**

**Offspring Begging in a Burying Beetle**

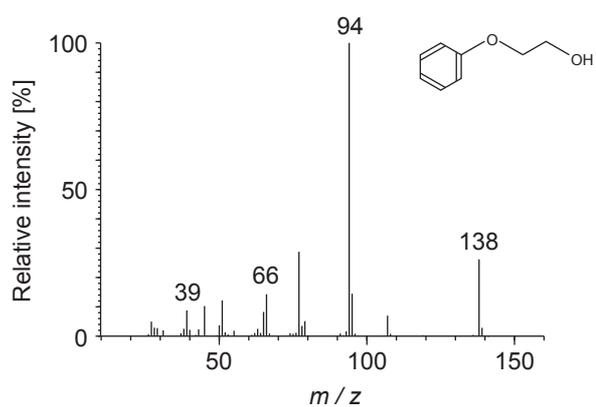
**Mamoru Takata, Yuki Mitaka, Sandra Steiger, and Naoki Mori**

## Supplemental Information

Provisioning female



Authentic standard



**Figure S1. Related to Figure 3.** Mass spectra of 2-phenoxyethanol from a provisioning female (derived from Fig. 3A) and from the authentic standard.

**Movie S1. Related to Figure 3.** Behavioral sequence of provisioning in *N. quadripunctatus*. A female burying beetle preparing food and provisioning her larvae. Upon her approach, her larvae begin to exhibit begging behavior. Subsequently, the female beetle feeds her larvae by mouth-to-mouth regurgitation.

**Movie S2. Related to Figure 3.** Non-provisioning *N. quadripunctatus* female. The female was in close proximity to her larvae but they did not beg from her.

**Movie S3. Related to Figure 3.** Larval response to the filter paper impregnated with 200 ng of 2-phenoxyethanol.

**Movie S4. Related to Figure 3.** Larval response to the filter paper impregnated with hexane.

**Table S1. Related to Figure 4.** Mean numbers of surviving and begging larvae and proportion of begging larvae during the experiment in begging treatment to determine the costs associated with begging.

Time [h]	0	4	8	12	16	20	24
Mean number of surviving larvae	5.0	4.9	3.5	2.5	0.4	0.0	0.0
Mean number of begging larvae	3.3	3.3	1.6	0.2	0.0	0.0	0.0
Proportion of begging larvae	0.7	0.7	0.5	0.1	0.0	NA	NA

## **Transparent Methods**

### **Beetle preparation**

The burying beetles used in the experiments were the third- and fourth-generation offspring of over 200 wild-caught adult *Nicrophorus quadripunctatus* Kraatz that were collected using baited pitfall traps in Tokyo, Japan, in June 2017. The beetles were maintained individually in small transparent plastic cups (height 4 cm, diameter 6 cm) at  $20 \pm 1^\circ\text{C}$  under a 14:10 h light/dark cycle. Following their emergence as adults, they were fed 200 mg of freshly killed mealworms (*Zophobas atratus*) three times a week.

Pairs of non-sibling, sexually mature virgin adult males and females were randomly selected two weeks after the date of eclosion. Each pair was placed in a plastic cup (height 80 mm, diameter 115 mm) containing 2 cm of moist peat and  $4.0 \pm 0.5$  g of a whole-body mouse carcass (Cyber Cricket, Shiga, Japan). The beetles normally breed  $3.2 \pm 0.8$  larvae (mean  $\pm$  SD) on carcasses of this size (Takata et al., 2016). The beetles in the plastic cups were kept in a dark incubator at  $20 \pm 1^\circ\text{C}$  for 72 h. During this period, female beetles laid eggs in the soil near the carcass. Subsequently, the female and the carcass were transferred to a second, new plastic cup with moist peat while the eggs were left to hatch in the original plastic cup. The male was removed from the original plastic cup at this stage. The larvae's hatching was monitored, with checks at 2 hourly intervals, and five newly hatched larvae were transferred onto the carcass with their female parent.

### **Behavioral observation of parental care**

Observation of female-parental and larval behavior was conducted under a red light 24 h ( $\pm$  15 min) after the larvae had been placed on the carcass with a female parent in a plastic cup (height 80 mm, diameter 115 mm) containing 2 cm of moist peat ( $n = 42$  broods). First, we recorded the behavioral sequence of parental provisioning using a video camera. Subsequently, to investigate the frequencies of offspring begging and parental provisioning, we used instantaneous scan sampling every 1 min for 30 min (Martin and Bateson, 1986). Each scan recorded four types of parental and offspring behavior: (1) the offspring begged and the female parent provisioned, (2) the offspring begged but the parent did not provision, (3) the offspring did not beg and the parent did not provision, and (4) the parent was away from the larvae. Parental provisioning was defined as mouth-to-mouth contact between the parent and at least one larva (Rauter and Moore, 1999). When the distance between the parent and the nearest larva exceeded 5 mm (i.e., almost

equal to the parent's pronotum width), we assumed that "the parent was away," since begging occurs only when parents are in close proximity to the larvae (Rauter and Moore, 1999; Smiseth and Moore, 2002). The percentage of time spent engaging in each type of behavior was calculated by dividing the number of scans in which that focal behavior was observed by the total number of scans and multiplying by 100.

### **Chemical extraction**

We prepared whole-body crude extracts from provisioning and non-provisioning female parents 12 h after the introduction of five newly hatched larvae. One living beetle was dropped and shaken in a glass centrifuge tube containing 500  $\mu$ L of n-hexane (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) for 5 min. The provisioning parent was defined as a breeding parent that was approaching (but had not yet made contact with) the larvae exhibiting begging behavior (step 3 in the behavioral sequence of provisioning; Movie S1). The non-provisioning parent was defined as a breeding parent that was in close proximity to their larvae but was not targeted with begging behavior (Movie S2). The extracts from both provisioning and non-provisioning females were replicated twice. More than 2  $\mu$ L of regurgitation was collected from each provisioning and non-provisioning female by applying pressure to their abdomens. Approximately 2  $\mu$ L of regurgitation was immediately dropped and shaken in a glass centrifuge tube containing 500  $\mu$ L of n-hexane for 5 min. The regurgitation extracts from both provisioning and non-provisioning females were also replicated twice.

Each crude extract of hexane was transferred into a new glass centrifuge tube for further processing. For the chemical analyses, the 100  $\mu$ L aliquots of hexane extracts were concentrated to 10  $\mu$ L aliquots using a gentle stream of nitrogen, and the samples were then subjected to GC-MS analysis for chemical identification.

### **Chemical analysis**

GC-MS was performed using an Agilent Technologies 5975 Inert XL Mass Selective Detector combined with an Agilent Technologies 6890N Network GC system equipped with an HP-5MS (30 m  $\times$  250  $\mu$ m, 0.25- $\mu$ m film thickness; Agilent Technologies, Santa Clara, CA, USA). The column temperature was increased from 60°C (2 min) at 10°C/min to 290°C (5 min). One portion (1  $\mu$ L) of hexane extract was injected for each sample. The injector used was in splitless mode, with helium as the carrier gas

(flow rate: 1 mL/min). The injection port temperature was 240°C. MS data were obtained under the following conditions: ionization energy, 70 eV; scan range, 33–550 *m/z*. The GC-MS system was controlled using GC/MSD ChemStation software (G1701DA version D.02.00.275; Agilent Technologies). Compounds were identified through comparison of their retention times and fragmentation patterns with those of the authentic standards.

### **Bioassay**

A bioassay device was designed to evaluate the pheromonal activity of the sample solution (Fig. 3A). Five 12-h-old larvae were placed in this device on a piece of carcass surface (~100 mg), on which a 30-mm filter paper moistened with distilled water had been placed in a 35-mm plastic dish. The plastic dish was located on the center of a 90-mm filter paper moistened by distilled water, which was placed on the bottom of a plastic cup (115 × 80 mm). The cup had an opening (diameter, 10 mm) in the center of the lid, and the opening was covered with the lid of the plastic dish, except during experiments, to avoid disturbing the larvae with air inflow. We introduced a folded filter paper (diameter, 10 mm) impregnated with a sample solution into the cup through this opening, using a pair of tweezers, and the filter paper was held suspended over the larvae to gauge the larval responses. In all treatments, the filter paper was treated with a total amount of 10 µL of solution, and subsequently held suspended over the larvae after the solvent had been volatilized for around 3 min.

First, to test the pheromonal activities of the crude extracts from provisioning and non-provisioning parents, the folded filter paper was treated with 10 µL of sample solution (0.02 individuals equivalent of provisioning female or non-provisioning female). As a control, the filter paper was treated with the same amount of the solvent (hexane). Ten replications were performed for each treatment. Subsequently, to test the dose-dependency of 2-phenoxyethanol (FUJIFILM Wako Pure Chemical Corporation), a standard solution containing 20 µg of authentic 2-phenoxyethanol diluted in 1 mL of hexane was prepared, and this standard solution and 10- to 1000-fold diluted hexane solutions containing it were used for sample treatments. Hexane was used for a negative control treatment, and the crude extract from provisioning females was used as a positive control treatment. Each treatment was replicated ten times.

Comparison of the response rates (i.e., the proportion of the number of replications in which at least one larva exhibited begging behavior to the total number of replications)

was performed using Fisher's exact tests and the p-values were adjusted for multiple testing according to the Benjamini–Hochberg method (Benjamini and Hochberg, 1995). All statistical analyses were performed and graphs were generated using R software v.3.3.3 (R Core Team, 2016).

### **Analyzing the cost of begging behavior**

To test whether larval begging incurs energetic costs, we manipulated the begging behavior using the artificial pheromone and compared larval growth and behavior among the following three treatments: 1) the begging treatment in which larvae were induced to beg by artificial 2-phenoxyethanol, 2) the non-begging treatment in which 2-phenoxyethanol was introduced into a bioassay device but larvae were not directly subjected to 2-phenoxyethanol, so as to not elicit begging behavior, and 3) the control treatment in which there was no pheromonal stimulus. Before the experiment, we measured the wet weight of each of the newly hatched larvae. Subsequently, the five larvae were placed on a whole-body mouse carcass that had been prepared by the parents ( $4.0 \pm 0.5$  g) in the bioassay device (Fig. 4A) for each replication of each treatment (5 larvae/replication  $\times$  10 replications). In this experiment, we prepared two pieces of 10-mm-diameter filter paper. Each paper was impregnated with 20  $\mu$ L of hexane solution containing 200 ng of 2-phenoxyethanol, or the same amount of solvent. After the solvent had been volatilized for around 3 min, the filter papers were introduced into the bioassay device; one was dangled from the lid using a metal wire, and thereby suspended over the larvae (paper A), and the other was placed next to the small dish containing the larvae and carcass (paper B). Paper B was introduced through a slit ( $13.5 \times 2.5$  mm) on the side of the device 8.5 mm above the bottom using a pair of tweezers. In the begging treatment, paper A was treated with 2-phenoxyethanol and paper B was treated with hexane. In the non-begging treatment, paper A was treated with hexane and paper B was treated with 2-phenoxyethanol. In the control treatment, both papers were impregnated with hexane. The papers were kept suspended over the larvae for 5 min and then new ones were substituted every 15 min. At intervals of 0, 4, 8, 12, 16, 20, and 24 h ( $\pm$  10 min), we observed the larval behavior for 1 min under a red light, and recorded the number of living larvae, the number of larvae that exhibited begging, and the weight of each living larva (measured to the nearest 0.1 mg). We did

not identify individual larva. A larva was defined as dead when it had stopped moving or walking.

GLMMs were used to investigate the cost of begging in terms of larval growth. Larval body weight was treated as a response variable assuming a Gaussian distribution. The treatments, the time measured from when the larva was placed on the carcass, and the interaction between the treatments and the measurement time were treated as explanatory variables. Replication IDs were treated as random effects. Multiple pairwise-comparisons by log-rank test were performed for *post hoc* comparisons of the interaction between treatments and time, and a sequential Bonferroni correction was applied to evaluate pairwise significance. Larval survival was estimated using the Kaplan–Meier method and compared by multiple pairwise-comparisons using the log-rank test with sequential Bonferroni correction. Survival was measured as the time from when the larva was placed on the carcass until death. Larvae that were alive at 20 h were censored. A two-sided significance level of  $P < 0.05$  was set. For the begging treatment, the probability that a *N. quadripunctatus* larva would exhibit begging behavior was calculated by dividing the number of begging larvae by the number of surviving larvae. All statistical analyses and generation of graphs were performed using R software v.3.3.3 (R Core Team, 2016).

### **Data and Software Availability**

The dataset reported in this paper is available in Mendeley data (doi: 10.17632/d94z3f5yy7.1).

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