

# Deceptive vibratory communication: pupae of a beetle exploit the freeze response of larvae to protect themselves



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It is argued that animal signals may have evolved so as to manipulate the response of receivers in a way that increases the fitness of the signallers. In deceptive communication, receivers incur costs by responding to false signals. Recently, we reported that pupae of the soil-inhabiting Japanese rhinoceros beetle *Trypoxylus dichotoma* produce vibratory signals to deter burrowing larvae, thereby protecting themselves. In the present study, monitoring of vibrations associated with larval movement revealed that *T. dichotoma* larvae remained motionless for ca 10 min when pupal vibratory signals were played back transiently (freeze response). Furthermore, pupal signals of *T. dichotoma* elicited a freeze response in three other scarabaeid species, whose pupae do not produce vibratory signals. This indicates that the freeze response to certain types of vibration evolved before the divergence of these species and has been evolutionarily conserved, presumably because of the fitness advantage in avoiding predators. Pupae of *T. dichotoma* have probably exploited pre-existing anti-predator responses of conspecific larvae to protect themselves by emitting deceptive vibratory signals.

**Keywords:** sensory trap; receiver bias; conflict; altruism; scarab beetle; deceptive communication

## 1. INTRODUCTION

It is argued that animal signals have evolved so as to manipulate the behaviour of receivers in a way that increases the fitness of the signallers [1,2]. According to the ‘sensory trap’ (or ‘sensory bias’) model, signallers exploit sensory and behavioural responses of receivers that have evolved independently of the signalling situation (e.g. predator–prey interaction) and elicit a particular response of receivers for their own benefit [2]. If the receivers incur a cost by responding to the signals, selection should favour the evolution of the receivers’ ability to distinguish between the original cues and mimic signals, which in turn, will drive the evolution of more elaborate signals [3]. However,

receivers may not evolve the ability to distinguish between original cues and mimic signals if failure to respond to the original cues imposes a high cost. In such a situation, deceptive communication occurs where only signallers benefit, while receivers do not [4].

Recently, we reported intraspecific interaction between larvae and pupae of a group-living beetle, *Trypoxylus (Allomyrina) dichotoma* (Coleoptera, Scarabaeidae, Dynastinae), which live in humus soil [5]. The last-instar larvae of this beetle construct their own pupal cells indispensable for normal pupation and eclosion by compacting a mixture of faecal pellets and humus; however, these cells are fragile and subject to damage from burrowing larvae because the pupae and larvae live close together [5]. We found that the pupae produce vibratory signals to deter approaching larvae, thereby preventing damage to the cells [5]. We also found that vibrations generated by moles, a common predator of the beetle, deter the approach of larvae as well [6]. These findings led us to hypothesize that pupae have exploited larval anti-predator responses [6]. In the present study, we examined the effect of the pupal signals of *T. dichotoma* on the larvae of three scarabaeid species distantly related to *T. dichotoma*, whose pupae do not produce vibratory signals. We monitored movements of larvae by recording associated substrate vibrations. If pupae of *T. dichotoma* exploit a pre-existing behavioural response of conspecific larvae by emitting deceptive vibratory signals, the same signals are likely to elicit a freeze response as well from the larvae of allied species that have no intraspecific vibratory interaction.

## 2. MATERIAL AND METHODS

### (a) Insects

We used last-instar larvae of *T. dichotoma* (Dynastinae) and three other scarabaeid species, *Prototia orientalis* (Cetoniinae), *Dicronorhina derbyana* (Cetoniinae) and *Anomala albopilosa* (Rutelinae). The phylogenetic relationships among Dynastinae, Cetoniinae and Rutelinae have been clarified (figure 1, [7]). *Trypoxylus dichotoma* shows a highly aggregated distribution in humus in the field [5]. Pupae of this species produce vibratory signals by drumming their pronotum against the inner wall of the pupal cell when larvae approach them ([5], electronic supplementary material, movie S1 and figure S1). The two Cetoniinae species, *P. orientalis* and *D. derbyana*, have a mode of life similar to *T. dichotoma*: the larvae live in groups and feed on humus (W. Kojima 2011, personal observation). However, the pupae of Cetoniinae species are protected by hard cocoons and do not produce vibrations ([6], electronic supplementary material, movie S2). As for *A. albopilosa* (Rutelinae), larvae of this species are solitary (W. Kojima 2011, personal observation) and feed on roots of grasses and vegetables [8]. Pupae are enveloped by an exuvia (old larval integument) and do not produce distinct substrate vibrations although they gently move their abdomens upon stimulation (electronic supplementary material, movie S3 and figure S1). The presence/absence of vibratory interaction in the four species tested is summarized in figure 1.

The larvae of *T. dichotoma*, *P. orientalis* and *A. albopilosa* were offspring of adults field-caught in the suburbs of Tokyo, Japan. The larvae of *D. derbyana* were commercially obtained from breeding stock (Tanzania in origin, imported legally) in a Japanese pet shop. The larvae of all species were reared at 25°C in a container filled with humus. For the larval diet, we used humus for *T. dichotoma*, *P. orientalis* and *D. derbyana*, and a piece of carrot for *A. albopilosa*.

### (b) Monitoring of larval behaviour

We examined whether the larvae of the four species stopped moving when they received vibrational signals from *T. dichotoma* pupae. The movement of larvae was monitored by the vibrations generated in association with the movement, following the method of Mankin *et al.* [9] with a slight modification. A single last-instar larva of each species was transferred to a plastic jar (8 × 12 cm in diameter) filled with humus. The jar was fully filled with humus in experiments using *T. dichotoma*, while it was only half-filled in the case of the three

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2012.0386> or via <http://rsbl.royalsocietypublishing.org>.

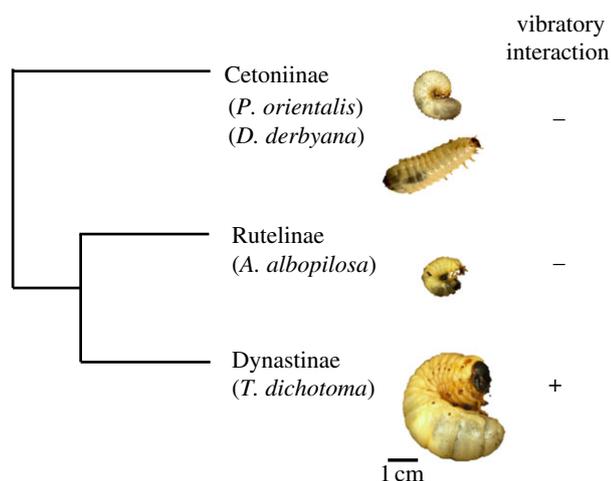


Figure 1. Phylogenetic relationships of Dynastinae, Cetoniinae and Rutelinae (Scarabaeoidea) (modified from Smith *et al.* [7]). Photographs of the last-instar larvae of the four species tested are shown. Among the species, only the pupae of *T. dichotoma* produce vibrations.

other species because the intensity of vibrations derived from these smaller larvae was much weaker than that from *T. dichotoma*. The jar was suspended with a plastic string from the ceiling of a one-side-opened soundproof box (90 × 90 × 70 cm). The vibration reed (5 × 25 mm in diameter) of a vibration exciter (type 4809, Brüel & Kjær, Denmark), placed on a desktop vibration isolator (UM-0405, Nippon Boushin, Japan), was thrust into the soil in the jar through a hole (8 mm in diameter) in the bottom. In order to habituate the introduced larva, the jar was left undisturbed for 20 min. We confirmed beforehand that this conditioning period is sufficient for habituation. For monitoring the movement of the larva, we recorded substrate vibrations using a piezoelectric charge accelerometer (type 4381, Brüel & Kjær) with a screw fixed on it, following the method of Kojima *et al.* [5]. The accelerometer was set on the surface of the soil with the screw pushed into the soil. Twenty minutes after the start of recording, pre-recorded pupal vibrations [5] were played back with the vibration exciter. The acceleration of played vibrations was 300 mm s<sup>-2</sup> at 10 cm from the vibratory source, nearly equal to that of pupal vibrations [5]. We used two trains of pupal vibrations from different individuals; one contained five pulses (6 s in total) from a male pupa and the other contained six pulses from a female pupa (8 s in total). Either of the two was played back twice at an interval of 10 s. The recording of larval vibrations was continued for at least 20 min after the playback. The signals from the accelerometer were processed as described previously [5]. After the recording, we counted the number of pulses whose peak acceleration was greater than the threshold of 15 mm s<sup>-2</sup>, the lowest level at which we can clearly distinguish signals from the background noise (figure 2a). We tested eight larvae for *T. dichotoma* and six larvae each for the three other species. These experiments were conducted in the dark at 25–26°C.

### 3. RESULTS

Vibrations associated with larval activity were frequently detected during the first 20 min of monitoring in all species tested (*T. dichotoma* and the three other scarabaeid species; figure 2a,b, electronic supplementary material, figure S2). However, the vibrations associated with larval activity ceased completely when the vibratory signals of *T. dichotoma* pupae were transiently played back, and the silence continued for *ca* 10 min. Subsequently, the number of vibratory pulses gradually returned to the normal level. All tested individuals of all species showed a freeze response for 3–22 min without exception. The average duration of freeze (i.e. latency to reappearance of larval vibrations after the playbacks) was 11 min in *T. dichotoma* ( $n = 8$ ), 9.1 min

in *P. orientalis* ( $n = 6$ ), 12.6 min in *D. derbyana* ( $n = 6$ ) and 7.8 min in *A. albopilosa* ( $n = 6$ ). No significant difference in the duration was found among the four species (Kruskal–Wallis test;  $H = 4.0$ ,  $p = 0.26$ ).

### 4. DISCUSSION

In the present study, we found that larvae of *T. dichotoma* remain motionless for 3–22 min after receiving pupal vibratory signals (freeze response). Considering that pupal signals of *T. dichotoma* played back near an artificial pupal cell protected the cell from being broken by a burrowing larva [5], the larval freeze response to pupal signals is responsible for ‘rescuing’ pupae. Since a freeze response to pupal signals of *T. dichotoma* was observed in three scarabaeid species whose pupae do not produce vibratory signals, the long-lasting freeze response [10] of *T. dichotoma* larvae to the pupal signals would have existed prior to the evolution of the signals. Overall, our results indicate that pupae of *T. dichotoma* have exploited pre-existing larval behaviour to protect themselves by emitting deceptive vibratory signals.

Freeze responses are observed in numerous animal taxa such as fish [11], insects [10], birds [12] and mammals [13]. Such responses are thought to be an adaptation for avoiding predation, because many predators are unable to detect motionless prey. The presence of a freeze response in Dynastinae, Rutelinae and Cetoniinae in scarabaeid beetles (figures 1 and 2) suggests that the freeze response to certain types of vibration has been evolutionarily conserved presumably because of the fitness advantage in predator avoidance. Important predators of scarabaeid larvae include insects (e.g. elaterid and asilid larvae) and vertebrates (e.g. moles, hogs and skunks) [14]. Predation of *T. dichotoma* by moles (*Mogera imaizumi*) is common in Central Japan [6]. Digging moles generate vibrations [15], and the movement of *T. dichotoma* larvae is suppressed not only by pupal vibrations but also by vibrations generated by foraging moles [6]. For scarabaeid larvae, it might be better to stop moving rather than try to escape upon detection of a predator because they are unable to move rapidly in soil. Considering that various fossorial mammals detect prey using vibrations associated with their activity [16], the freeze response of prey may increase their chance of survival.

Given that pupae of *T. dichotoma* manipulate larval behaviour with deceptive signalling, the larvae probably incur a fitness cost through restrictions to their own behaviour. This may lead to the counter-selection of larvae that can identify and disregard the deceptive signals [3,4]. At present, however, larvae appear not to distinguish pupal signals from signals associated with predators. The counter-adaptation of larvae may be hindered by a frequent predatory stimulus (constrained co-option hypothesis [17]): larvae risk being attacked by predators in cases of misidentification. In addition, kin selection may favour a larval response to pupal signals (synergistic co-option hypothesis [17]). If larvae can avoid the ‘killing’ of siblings by responding to pupal signals, the cost imposed by pupae is expected to be partly offset [5]. The exploited response of larvae may be evolutionarily maintained in

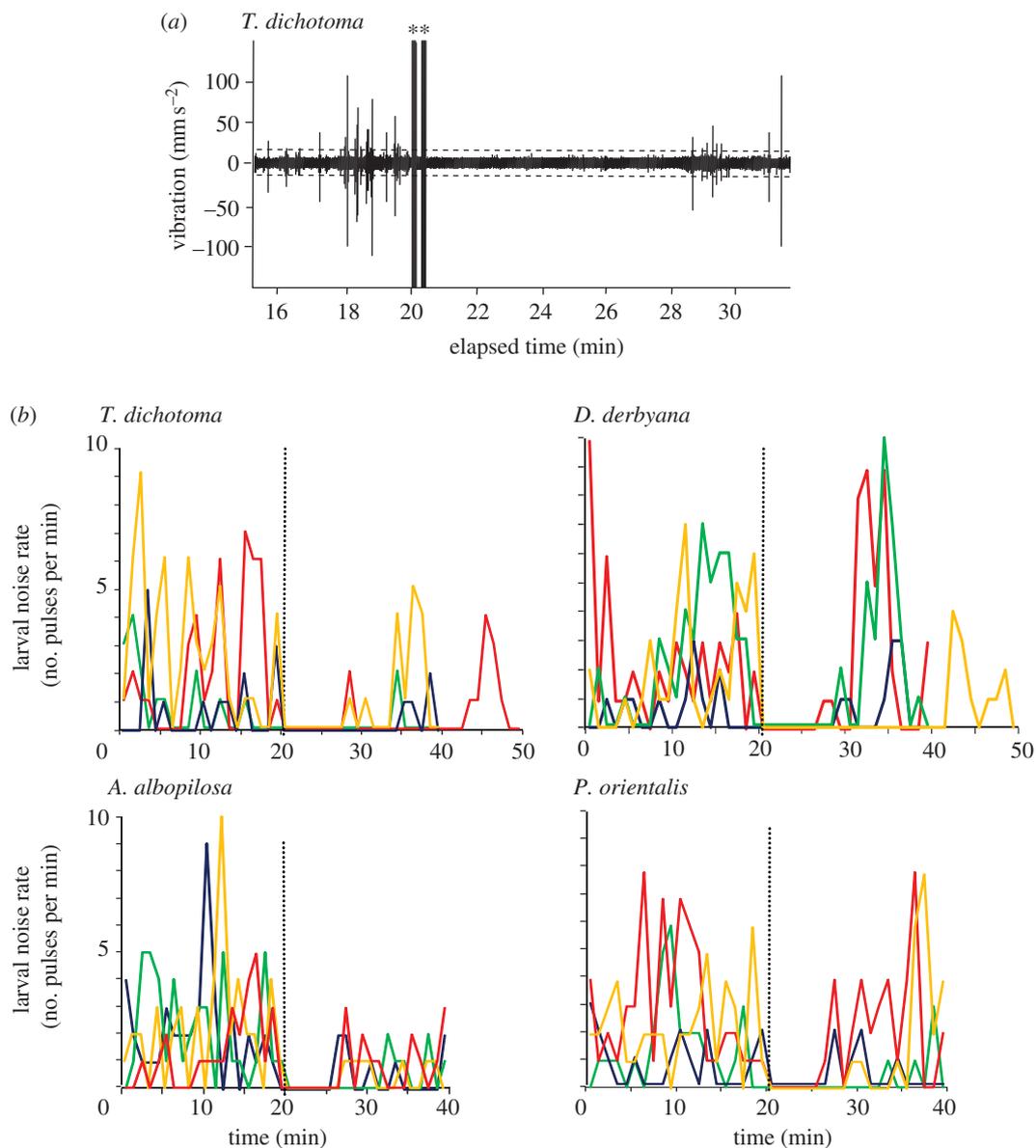


Figure 2. Monitoring of vibrations associated with larval movement. (a) An oscillogram of vibrations associated with activity of a *T. dichotoma* larva. Pulses exceeding a threshold of  $\pm 15 \text{ mm s}^{-2}$  (shown by dashed lines) were counted as those associated with larval activity. Twenty minutes after the start of recording, pupal signals ( $300 \text{ mm s}^{-2}$ ) were played back twice at an interval of 10 s (signals are clipped at  $150 \text{ mm s}^{-2}$  and indicated by asterisks). An absence of vibrations at 20–28 min indicates that a larva remained motionless (freeze response). (b) The rate of vibratory pulses (no. pulses per min) emitted from larvae of the four species. Pupal signals of *T. dichotoma* were played back at 20 min (indicated by dotted lines). All four species clearly showed a freeze response. Representative data from four individuals per species are shown with different colours. Data from the remaining individuals are shown in electronic supplementary material, figure S2.

*T. dichotoma* along with pupal signals in the balance of counteracting selection pressures.

We thank R. Nakano and two anonymous reviewers for helpful comments on the manuscript. This work was supported by Grants-in-Aid for Scientific Research (T.T., Y.I.) and Research Fellowship for Young Scientists (W.K.) from the Japan Society for the Promotion of Science.

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