Insect pupae are generally considered inactive and quiescent, but some of them generate air-borne sounds and/or substrate-borne vibrations.1-4 In 1948, Hinton reviewed the mechanisms of sound/vibration production in the pupae of Lepidoptera,2 and suggested a defensive function against predators. Since then, the functions of pupal and larval vibrations/sounds have been examined intensively in ant-associated lycaenid butterflies. Larvae of the common imperial blue Jalmenus evagoras offer nutritious secretions to Myrmicinae ants, and in return the ants protect larvae and pupae of this butterfly from parasitoids and other enemies. The larvae and pupae of J. evagoras generate vibrations, which induce higher levels of ant attendance.5 Likewise, sounds produced by larvae and pupae of the mountain Alcon blue Maculinea rebeli mimic the sounds produced by queen ants, which can elicit additional attention and favor by worker ants.4 To our knowledge, the function of pupal sounds/vibrations in other groups of insects has remained unexplored.

Recently, we revealed a deterring function of pupal vibrations against conspecific larvae in a group-living Japanese rhinoceros beetle Trypoxylus dichotoma (Coleoptera, Scarabaeidae, Dynastinae).5 They construct their own oval pupal cells around their pronotum on the inner wall of pupal cells in response to attracting larval vibrations.5 In a previous paper, we reported that pupae emitted vibrations (Fig. 2B) by drumming their pronotum on the inner wall of pupal cells in response to approaching larvae.5 We tested if the pupal vibrations function as deterring signals to larvae. Pupal cells harboring a live pupa were less likely to be broken by larvae than those harboring a dead pupa.7 When pupal vibrations were played back near to vacant artificial pupal cells, these cells were rarely disturbed by the larvae.5 These results suggest that pupae generate vibrations to deter conspecific larvae, thereby preventing damage to the cells.

In the present study, we hypothesized that the larval response to pupal signals is derived from stereotyped anti-predatory responses such as startle and freezing, which are induced upon detecting vibratory noises produced by predators. If this is true, pupal signals are mimics of predator noises. We therefore examined if larval responses to pupal signals are mimics of predator noises. We chose moles (Mogera immaizumi) as common predators of T. dichotoma in the field. In 27% (3/11) of microhabitats examined (Tamakyuryo Hill), the marks of moles’ foraging on T. dichotoma larvae were found, and in these microhabitats, few surviving larvae were found. Vibratory noises produced in association with moles’ foraging have been recently characterized.6 The mole vibrations and pupal signals are similar in that both predominantly contain low-frequency components (mostly below 500 Hz) and successions of pulses at intervals of a few seconds (Fig. 2B). When the mole vibrations were played back near to vacant artificial pupal cells (see Methods), these cells were never disturbed by the larvae (Fig. 2C). This result clearly shows the deterring effect of mole vibrations on larvae. Pupae may have exploited a preexisting larval response to predatory noises by emitting deceptive signals for their own benefit. The larval response is likely to increase the chance of their survival, considering that various fossorial mammals detect noises associated with foraging of moles, the most common predators of T. dichotoma. Mole vibrations played back in laboratory experiments deterred larval approaches in the same way as pupal signals. These findings suggest that to deter conspecific larvae, pupae of T. dichotoma may have exploited a preexisting response of larvae to predator vibrations by emitting deceptive signals.
prey using vibrations associated with their activity.7 Larvae probably incur a fitness cost as well by responding to ‘false’ predatory cues from pupae (e.g., restriction of activity). However, given that adult females lay dozens of eggs at one site and sibs live close together, the cost may be partially compensated for by avoiding the ‘killing’ of sibs (i.e., increased inclusive fitness).8

How have pupal signals evolved in *T. dichotoma*? Pupae of some other coleopteran species such as ladybird beetles and mealworms are reported to show behavioral defenses such as abdominal rotation and intersegmental pinching behavior.9,10 When pupae of *T. dichotoma* produce vibratory signals, drumming of the cell wall is achieved by rotating their abdomen.5

Figure 1. Pupae and pupal cells of two Scarabaeidae species. (A) A male pupa of *Trypoxylus dichotoma* (Dynastinae) with a long head horn residing in a fragile cell. (B) A pupa of *Dicondylorhina derbyana* (Cetoniinae) residing in a hard cell. Note that humus surrounding the cells was removed. Scale bar = 1 cm.

Figure 2. Interaction between pupae and larvae of *Trypoxylus dichotoma*. (A) A pupa in a pupal cell and a larva. The average distance between pupal cells and larvae was 6.4 cm in their natural habitat. (B) Oscillograms of vibrations produced by a male pupa in a pupal cell (upper) and vibrations produced by a foraging mole recorded by KC Catania (lower).6 Arrows indicate the range used for playback experiments. (C) Proportion of larvae that broke artificial pupal cell(s) when pupal vibrations, background noise, or mole vibrations were played back. Sample sizes are shown in parentheses. NS: Not significant. ***p < 0.0003 by χ² test with Bonferroni correction. Figure 2(A) and (B) (upper) are reproduced with kind permission of Springer Science+Business Media.
Therefore, it is possible that production of vibratory signals in *T. dichotoma* evolved from defensive behaviors against predators under the selective force imposed by conspecific larvae. The fragility of pupal cells may also be associated with the evolution of pupal signals in this species. We examined the abdominal rotation behavior in pupae of Cetoniinae species (e.g., *Platynota orientalis*, *Pseudotorynorhina japonica* and *Dicondyla teretipes*), which belong to the same Scarabaeidae as *T. dichotoma* (Dytiscinae). Like *T. dichotoma*, the larvae of Cetoniinae species feed on humus and show a highly clumped distribution. However, in contrast to *T. dichotoma*, the pupae of Cetoniinae species construct a very hard pupal cell from fecal pellets and humus (Fig. 1B). We found that they rarely show abdominal rotation in response to contact or vibratory stimuli. It is probable that vibratory defense is unnecessary for Cetoniinae pupae because the hard cells protect them from disturbances. Thus, vibratory communication in *T. dichotoma* may have evolved along with some anti-predation characteristics, such as defensive behavior and structure of pupal cells.

**Methods**

For playback experiments, we used a previously described experimental system, where vibrations were played back near to vacant artificial pupal cells. In brief, a plastic container (30 cm × 7 cm × 7 cm) was filled with humus soil and two artificial cells were prepared by briefly thrusting a test tube (3 cm in diam.) into the soil. The container was suspended with a plastic string from the ceiling of a one-side-opened soundproof box. The tips of a U-shaped gadget connected to a vibration exciter (type 4809, Briel and Kjær) were placed under the artificial cells through holes made at the bottom of the container. For pupal vibrations, we clipped a sequence (ca. 4.5–7 sec) of vibrations containing 3–7 pulses.[5] For mole vibrations, we clipped two sequences of vibrations containing several pulses (ca. 5 sec in total) from a recording file provided in reference 6 (Fig. 2B). The vibrations were originally obtained from a field-collected mole (*Scalopus aquaticus*) by using a geophone and contained a broad frequency range with a peak near 200 Hz.[6] For controls, the background noises recorded along with the vibrations were used. A larva was placed at the center of the container, and the vibrations were repeatedly played back at intervals of 10 sec for 60 min. The acceleration of played vibrations was 0.25 m/s² at 10.5 cm from the vibratory source, nearly equal to that of natural pupal vibrations. At the end of trials, it was checked whether a larva in the humus had broken artificial pupal cells.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

We thank Dr. Kenneth C. Catania for audio files used in our playback experiments. We also wish to thank Dr. Frantisek Baluska and the two anonymous referees for invaluable comments on the manuscript. Permission for use of figures from Springer Science+Business Media is acknowledged.

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