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Potential role of bird predation in the dispersal of otherwise flightless stick insects

In response to selection pressures from predators, many animals have evolved striking resemblances to inanimate objects found in their environment (Endler and Mielke 2005). This kind of visual camouflage is typically achieved when body coloration and/or patterning match the background against which the organism is seen, thus reducing the chances of detection by predators. Stick insects (i.e., phasmids; order Phasmatodea), as their name implies, exhibit an extreme example of this strategy, in that they have evolved both their structures and coloration allowing them to closely imitate the appearance of sticks, twigs, or leaves (Bedford 1978). However, despite being well-disguised in their natural habitat, they are still subject to frequent predation by birds (Fig. 1A; Bedford 1978).

Interestingly, besides their notable camouflage, stick insects also exhibit several other remarkable features, such as the morphology and structure of their eggs (Hughes and Westoby 1992, Shelomi 2011). For example, the eggs of many stick insects resemble the sizes, shapes, colors, and textures of seeds. In addition, phasmid egg capsules are diversely sculptured and remarkably hard-shelled, with a distinct layer of calcium oxalate on the outermost coating (i.e., exochorion), which is only dissolved under highly acidic environments (pH 2; Moscona 1950). Although the eggs of some other insects also possess calcium oxalate crystals (Clark 1958), phasmids appear to be unique in producing a distinct layer of the substance.

Accordingly, we were motivated to investigate whether the robustness of phasmid eggs would allow the eggs within gravid female stick insects eaten by avian predators to retain viability as they pass through the digestive tract. Such a phenomenon would not be possible in many other insects, since the eggs of most species are only fertilized just before oviposition, using sperm stored within the seminal vesicle of the female insects after copulation. In contrast, females of some stick insect species are parthenogenic, enabling them to produce viable eggs without fertilization (Suomalainen 1962, Bedford 1978). In which case the predation of gravid females could disperse offspring, analogous to the dispersal of plant seeds by frugivorous birds. Therefore, we determined the viability of stick insect eggs after passing through the digestive tract of an avian predator, in order to estimate the frequency of predation-mediated dispersal.

We used eggs that were excised from three species of stick insects with parthenogenetic reproductive capability (*Ramulus irregulariterdentatus*, *Neohirasea japonica*, and *Micadina phluctaenoides*) in feeding experiments at the Tokyo University of Agriculture and Technology (Fuchu, Tokyo, Japan), along with the brown-eared bulbul (*Hypsipetes amaurotis*), which is one of main avian predators of stick insects in Japan (Fig. 1A). The excised eggs were mixed with an artificial diet and a total of 45, 40, and 60 eggs of *R. irregulariterdentatus*, *N. japonica,* and *M. phluctaenoides* were independently fed to a brown-eared bulbul, which defecated within 3 h of feeding in mid-November 2015. In addition, a total of 70 eggs of *R. irregulariterdentatus* were fed using the same methods in late-October 2017. The fecal pellets were then collected and carefully examined under a stereomicroscope, in order to determine the proportion of intact eggs.

We demonstrated that 5.0%, 8.3%, and 8.9%, respectively, of the *R.irregulariterdentatus*, *N. japonica,* and *M. phluctaenoides* eggs consumed by the brown-eared bulbul remained intact without any mechanical damages in the feeding experiments conducted in 2015. The hatching rate of these stick insects is low even under normal conditions (Funaki and Suetsugu unpublished data), and none of these intact eggs have hatched for two years since the experiments. These results suggested that these eggs could survive through the avian digestive system, while the possibilities remain that other factors such as the chemical and temperature environment the eggs passed through might have annihilated them, in spite of a lack of mechanical damages. In contrast, the feeding experiments conducted in 2017 also showed that 20.0% of the *R. irregulariterdentatus* remained intact. In addition, two out of 14 intact *R. irregulariterdentatus* in the feeding experiments in 2017, successfully hatched by late February 2018 (Fig. 1B, C). As a result, we could obtain direct evidence that at least some eggs of *R. irregulariterdentatus* are viable under the avian predation.

Therefore, it is possible that eggs within consumed female stick insects can be successfully dispersed to new locations. It remains unknown whether the robustness of phasmid eggs has evolved to facilitate avian dispersal or whether they evolved for the other reasons, and that this fortuitously allows for avian dispersal. As it stands, we consider that the robustness of their eggs is an adaptation to reduce parasitization rates because parasitism by wasps is known as an important source of egg mortality in stick insects (Hughes and Westoby 1992, Shelomi 2011).

In any case, since birds are among the most effective dispersal agents, as a result of their ability to traverse vast distances in short periods of time, and given that the brown-eared bulbul can attain average flight speeds of 40–60 km/h, stick insects consumed by the brown-eared bulbul and other similar species have the potential to be dispersed over tens of kilometers. Moreover, the season of egg production in stick insects (September–November) coincides with the migration of Japanese brown-eared bulbuls, which gather in flocks that range in size from ten to several hundred individuals (Nakamura 2008). Thus, bird predation could be an

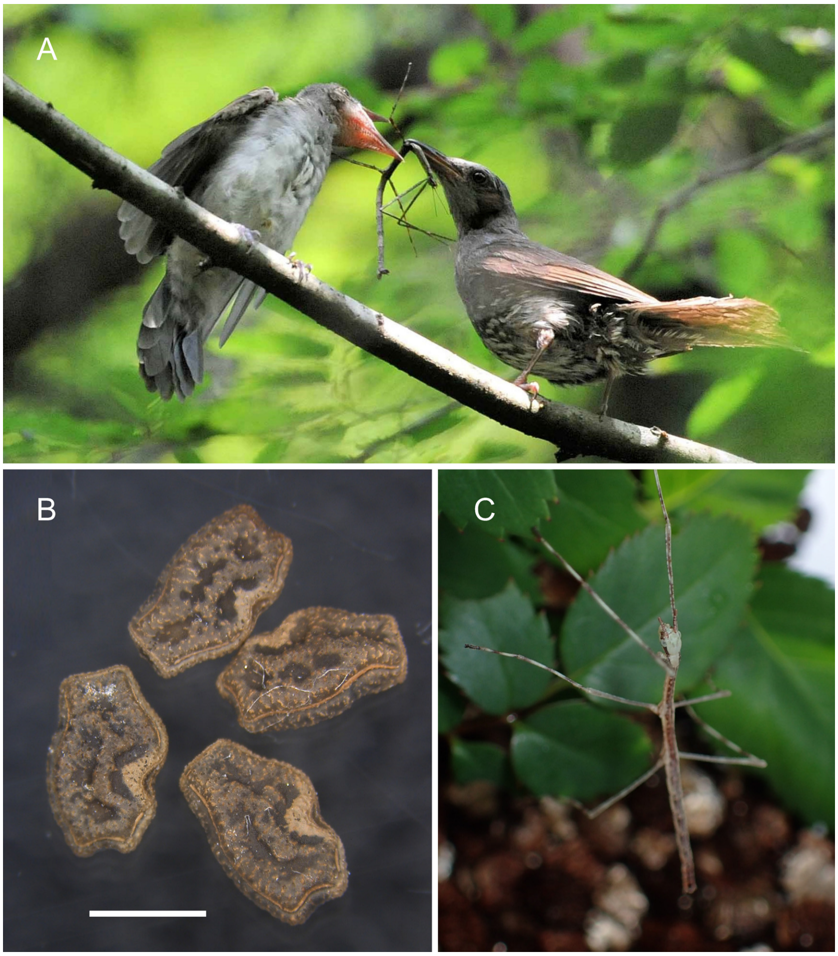

Fig. 1. (A) The parental brown-eared bulbul *Hypsipetes amaurotis* feeding the stick insect *Ramulus irregulariterdentatus* to its chicks. (B) Intact *Ramulus irregulariterdentatus* eggs defecated by the brown-eared bulbul *Hypsipetes amaurotis*. Bar = 2 mm. (C) First instar nymph of *R. regulariterdentatus* hatched from the egg defecated by *H. amaurotis*.

important factor in the long-distance dispersal of stick insects in Japan.

It should be noted that the successful dispersal of stick insect eggs via the predation of adult females would be an infrequent occurrence because adult female stick insects carry a low number of fully developed eggs and only a small percentage of the eggs (ca. 10%) remained intact after being consumed. Nonetheless, considering that stick insects are slow moving and often flightless, with a limited capacity for dispersal (Bedford 1978), the benefits of long-distance dispersal via bird predation should not be underestimated. In addition, while avian predators would not play a significant role in helping the dispersed offspring locate host plants, the females of most stick insect species remain in the foliage and drop eggs from their ovipositor to the ground (Bedford 1978). Therefore, the situation will not differ greatly from the more typical dispersal, whereby young nymphs are also required to independently locate appropriate food plants after hatching.

Overall, we consider that the passive dispersal of stick insect eggs by birds could profoundly affect the distribution, gene flow, and community composition of many species of stick insects. If avian dispersal is important to stick insects, the phylogeographical patterns should reflect occasional long-distance dispersal events (e.g., Miura et al. 2012). In addition, the patterns of spatial genetic structure will differ among stick insects with parthenogenetic reproductive capability (and hence potential avian dispersal) and non-parthenogenetic stick insects. The phylogeographical patterns in these stick insects thus deserves further studies.

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