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Lethal attack of *Schizotetranychus brevisetosus* Ehara (Acari: Tetranychidae) on predatory midge larva

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Spider mites (Acari: Tetranychidae) display an anti-predatory strategy by constructing a web using silk threads secreted from the palp tips to distract predators. Several studies have shown the defensive effects of the web itself as well as web-associated ovipositional behaviours (Mori *et al.* 1999, Oku *et al.* 2003, Horita *et al.* 2004, Lemos *et al.* 2010, Yano 2012). Conversely, alternative behavioural strategies, such as counterattack against predators, have been only reported in *Stigmaeopsis* spp. (Saito 2010). *Stigmaeopsis* spp. densely weave nests over depressions on the surface of the host leaves (known as WN species; Saito 1983, 1985), and several generations overlap in the same nest among species that make larger nests. In *S. longus* (Saito) that live on the *Sasa* bamboos, adults attending to their offspring increase the survival rate of the offspring by driving out or killing the phytoseiid larvae that enter the nest (Saito 1986, Mori & Saito 2004). Behaviours such as these are also reported in *S. miscanthi* that inhabit the Chinese silver grass (Saito *et al.* 2011, Yano *et al.* 2011) and *S. nanjingensis* (Ma and Yuan) that reside on the Moso bamboo (Saito & Zhang 2017). One possible factor allowing the evolution of this somewhat risky behaviour in these species is the high genetic relatedness among the nest members in the colony. This relatedness is because the individuals develop in the same nest where they were born and end up mating with their siblings or even their parents and such inbreeding can be repeated for several generations (Saito 2005, Saito 2010, Saito *et al.* 2013). Although the extent of genetic relatedness has not been directly measured in any WN species, if the relatedness is very high, similar counterattack behaviour may evolve in other WN species as well. However, there have been no reported observations of counterattack behaviours since the findings in *S. longus* more than 30 years ago (Saito 1986).

This is a brief report of a new type of attacking behaviour in *Schizotetranychus brevisetosus* Ehara. This species (subtype WN-s) inhabits the bottom surface of leaves of the evergreen oak *Quercus glauca* Thunb. (Ehara 1989), builds a dense web near the junction of leaf veins and deposits faeces on the nest roof (Saito 1983). Recent phylogenetic studies revealed that *S. brevisetosus* and the other WN-type congeneric species inhabiting evergreen oaks consist of a separate clade, and this clade is distinct from the monophyletic clade of the genus *Stigmaeopsis* (Matsuda *et al.* 2014, Matsuda *et al.* 2018). Thus, it is believed that the former species independently evolved the WN life type from *Stigmaeopsis* spp. In *S. brevisetosus*, multiple adult females co-operatively make a nest and reproduce in a group. As a result, *S. brevisetosus* might have a primitive form of sociality (Y. Saito, personal communication). The sex ratio at maturity is highly female-biased (Tamura & Ito 2017) where males are observed to be engaged in lethal combat for mating precedence (Masuda *et al.* 2015). Therefore, the nest members comprise of many females and a few males that are occasionally in a harem (consisting of one male per nest) as in *S. miscanthi* (Saito 1990). Curiously, some of the adult females in the nest community venture out, walk around the nest and return home as if they were patrolling. These individuals sometimes limp legs or vigorously spin threads (identified by shaking their anterior body). However, the meaning of these behaviours is unknown.

To perform the first set of observations, a matured *Q. glauca* leaf infested by approximately 100 individuals was collected at the Kochi Forest Synthesis Centre in Tosayamada, Kami, Kochi, Japan (N33°37'59", E133°42'29", WGS84) on 10 June 2013. The leaf was placed on water-soaked cotton pads in an insect breeding case (Insect Breeding Dish; SPL Life Sciences, Gyeonggi-do, Korea). A first instar predatory gall-midge larva (or an egg) of *Feltiella* sp., most likely *F. acarisuga* (Vallot) (Abe *et al.* 2011), was unintentionally included on the leaf surface. The dish was maintained at 25°C under 16:8 h light:dark conditions. Four days later, the leaf was observed using the stereomicroscope SZX7 (Olympus, Tokyo, Japan). The movement of the midge larva and mites on the leaf surface was filmed for approximately 1 h using a digital video camera recorder (NEX-VG20H, Sony, Tokyo, Japan). The precise larval stage was uncertain, because the larva rapidly grows at moderate temperatures without conspicuous morphological changes (the larval period is approximately 4 days at 22°C; Kawano 1969).

The midge larva was standing upright on the posterior spiracle approximately 2 cm from the nest of *S. brevisetosus* and was slowly swaying its anterior body in search of mites. Many individuals, mostly adult females and nymphs, were vigorously running over the leaf surface without returning to the nest or even making a new one. The first observation involved an adult female of *S. brevisetosus* that ran towards the midge larva, held it between a pair of legs and pinched its abdominal skin with the digits present at the palp tips (Fig. 1A). The female stung the larval body with a stylet and stripped off a piece of skin with its digits (Fig. 1B), resulting in rapid writhing of the larva. Further, the female held the midge larva again and sucked its body fluids, which was indicated by the piston movement of the stylophore and the movement of the gut, though the larva was still alive. During the process, the larva wriggled and crawled for a distance of 1 mm dragging the clinging female. After approximately 1 min, the larva jerked the anterior body of the female and pierced the hysterosoma (the anterior body) with its mouthpart. The larva began to suck the body fluids of the female to death and then crawled back to the original place and resumed foraging. The second observation involved a deutonymphal female that attacked the larva after 20 min of the first sting (Fig. 1C) and sucked its body fluids. One minute after the start of this attack, the midge larva pierced the hysterosoma and killed it. However, the larval body gradually shrivelled to die. Lastly, 3 h after the onset of the second sting, another deutonymphal female began to suck the body fluids of the midge larva for 13 min until the larva dried up (Fig. 1D).

The next set of observations conducted on 4 October 2018 was as follows. A midge larva was collected from the mulberry weed infested by *Tetranychus phaselus* Ehara using a fine brush. This larva was introduced onto a *S. brevisetosus* web nest, consisting of approximately 150 individuals, on the leaf collected from the same site as mentioned above. The introduced larva was monitored using the same equipment as above for more than 40 min. The first individual (adult female) attacked the larva within 5 min of placement; following this, another adult female attacked. After 12 min of the first attack, a larva of *S. brevisetosus* began to attack the midge larva. Four immature individuals, which included larvae, deutonymphs and adult females, attacked the midge larva in turns (Fig. 2A). The attack continued for 35 min until the larva dried up. The observations made using differential interference contrast microscopy of mounted specimen of the attacked larva revealed at least four pores on the cuticle made by the stylet (Fig. 3).

These observations indicated that *S. brevisetosus* has evolved unique defensive behaviours independent of several species of the phylogenetically distant genus *Stigmaeopsis*. The attack behaviour of *S. brevisetosus* is initiated by individuals walking outside the nests, followed by other individuals, including larvae and nymphs, attacking in mass. This attacking pattern is distinct from the counterattack reported in *S. longus*, wherein adults drive out or kill a phytoseiid mite only when it enters their nest, although *S. brevisetosus* can also attack in a similar manner. It would be interesting to know why other species do not attack in the same manner. Moving outside the nest

carries the risk of being predated, for example, it is frequently observed that the large dwelling mite *Anystis baccarum* (Linnaeus) (Acari: Anystidae) captures individuals walking outside the nest (Saito 2010). In addition, movement outside the nest for longer periods might decrease the time and energy for reproduction. The benefits (e.g. ingesting nutrition from body fluids) and costs of the attack are important topics for further studies in addition to determining if there exists an association of this type of an attack with the WN life type.

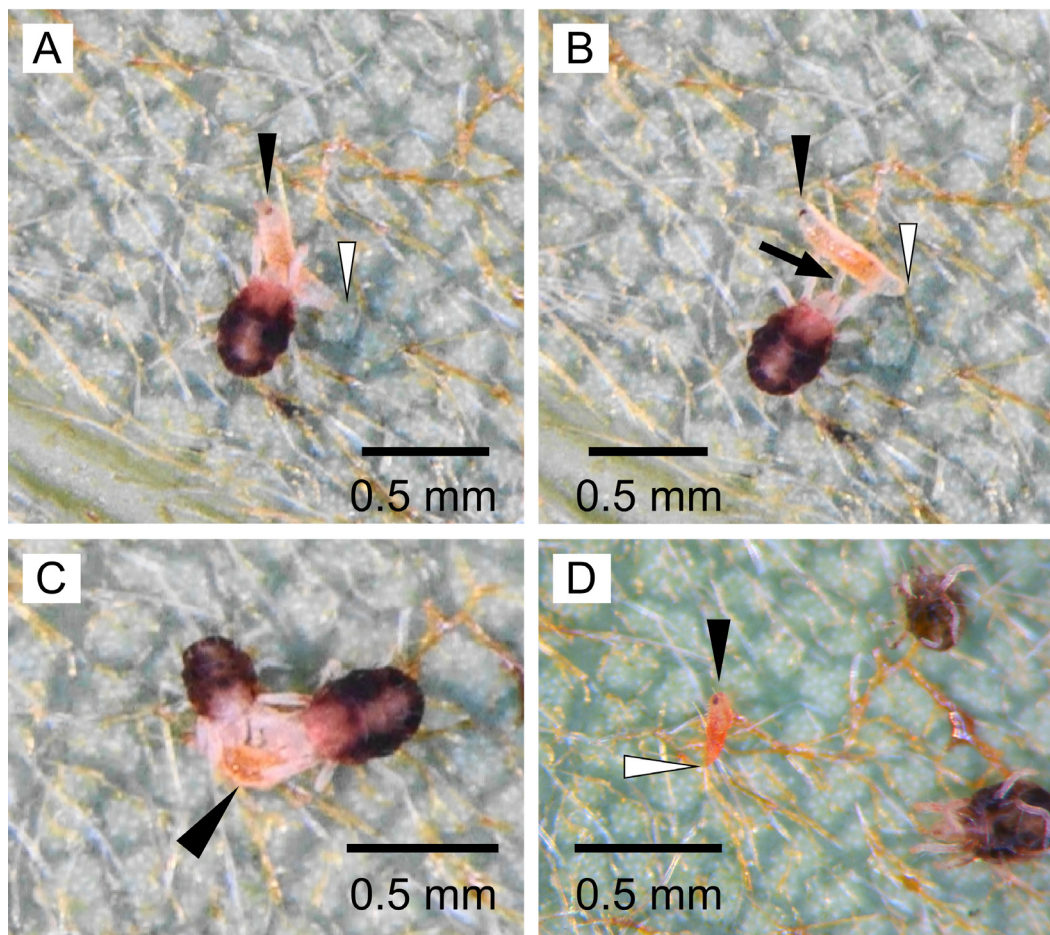


FIGURE 1. A *Feltiella* gall-midge larva waiting for the prey on the leaf surface near the nest of *S. brevisetosus*. (A) A larva searching for preys on the leaf surface and an approaching female. (B) The attacking female having striped off the skin of the larva (arrow). (C) The deutonymphal female (left) attacking the larva. The adult female (right) almost dying due to a previous counterattack by the larva. (D) The larva having dried up by the mite attack, and the deutonymph (upper) and adult female (lower) having been killed in the battle. The position of the larva is indicated by filled triangles, and the position of the posterior end is shown by open triangles. (the bar represents 0.5 mm (A–D)).

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FIGURE 2. Four immature individuals [2 larvae (right) and 2 nymphs (left)] attacking midge larva.

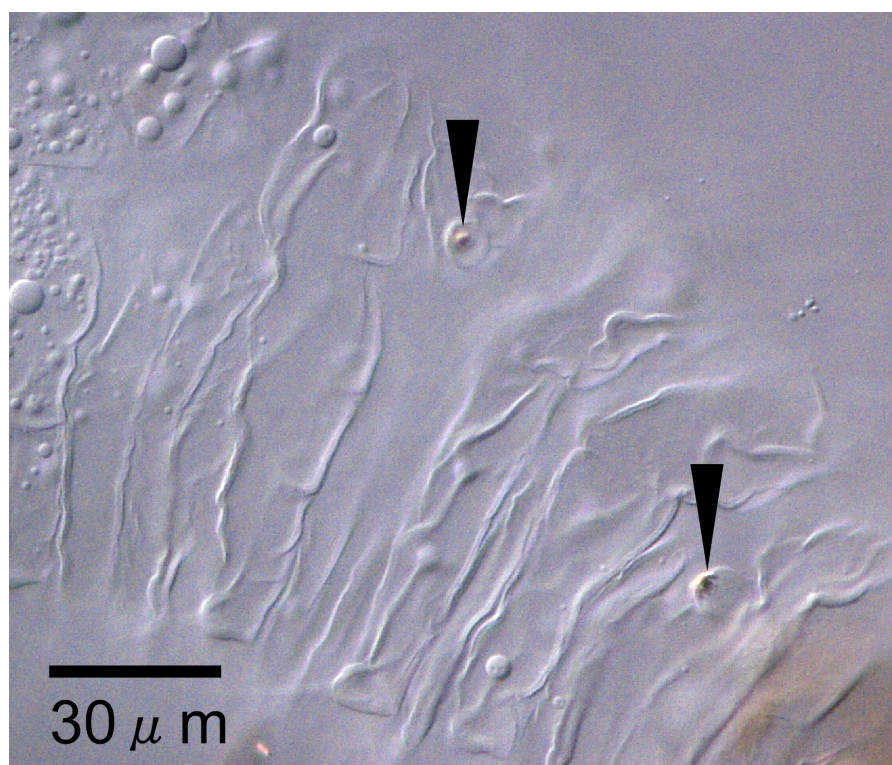


FIGURE 3. Microscopic image (×200) of pores on the cuticle of midge larva stung by mites (*filled triangles*).

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