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A scientific note on hive entrance smearing in Japanese *Apis cerana* induced by pre-mass attack scouting by the Asian giant hornet *Vespa mandarinia*

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Apis cerana / *Vespa mandarinia* / plant materials / propolis / defensive strategy

Vespa mandarinia is one of the most important natural enemies of Japanese *Apis cerana* (Ono et al. 1995). *V. mandarinia* attacks the nests of *A. cerana* to feed its brood in autumn, as do other species of wasps such as *Vespa simillima xanthoptera*. A well-known colony-level defence of *A. cerana* against *Vespa* attack is the formation of a hot defensive ball, a huge aggregation of worker bees whose collective body heat (Ono et al. 1987, 1995), combined with increased carbon dioxide from respiration, kills the wasps (Sugahara et al. 2012).

Another *A. cerana* behaviour thought to be a defensive strategy to prevent predation by *V. mandarinia* is ‘entrance smearing’ with unknown materials, as suggested by Okada (1997) and Sasaki (1999). The use of plant materials other than pollen or nectar by *A. cerana* for non-nutritional purposes has not yet been demonstrated; *A. cerana* has been reported to gnaw leaves at the centre of green head lettuce (Yokoi 2005, 2015) in autumn. Here, we report the plant-gnawing behaviour of *A. cerana* attributable to the collection of plant materials used to smear around the hive entrance, leaving dark-coloured stains similar to those described by Okada (1997) and Sasaki (1999).

We hypothesized that the smearing of collected plant materials is part of a colony-defence strategy that evolved specifically to counter the foraging attacks of

V. mandarinia. To test this hypothesis, we (1) tracked plant-gnawing bees to reveal whether they brought plant materials they collected back to the hive; (2) evaluated the seasonality of entrance smearing in total 20 hives to show that the behaviour occurs only in autumn, when *Vespa* attack is common; and (3) performed a series of attack simulation experiments using *V. mandarinia*, *V. simillima xanthoptera*, and *Vespa analis* to confirm that the induction of entrance smearing was a specific response to *V. mandarinia* scouting.

We evaluated *A. cerana* colonies introduced into observational hives in a rural landscape near Ichinoseki City in Iwate Prefecture (apiary A: 38°92′77.14″N, 141°03′31.76″E; apiary B: 38°93′77.22″N, 140°99′00.49″E), northern Japan. Over 5 years of observations and ‘attack simulation’ experiments, we observed in total 12 colonies in apiary A and in total 8 colonies in apiary B, which are separated by 4 km.

We tracked plant-gnawing bees in early October 2011. We individually marked ten worker bees visiting a *Persicaria nepalensis* (Meisn.) H. gross patch in the vicinity of four colonies (apiary A), using coloured paint, and then monitored the hive entrances and, later, the same *P. nepalensis* patch.

We found that the bees gnawed the leaves, calyxes, petals, and buds of the plants (Figure 1a) and then returned to their hives to smear the collected plant materials around the entrances (Figure 1b, c). However, we were unable to determine whether all the staining materials were of plant origin. Most of the smeared materials eventually darkened, turning black or dark brown.

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Figure 1. A worker gnawing *P. nepalensis* near the hive. **a** Multiple bite marks on the leaves. **b** Ventral view of a worker bee holding plant material between its mandibles and proboscis. **c** Smearing the gnawed material on the hive entrance.

To investigate the seasonality of smeared plant materials, we observed patches (>0.5 mm in diameter) around the entrances of a total of 20 colonies by eye and by video recording at least once a month during the annual *A. cerana* active season (April–October) from 2011 to 2015. Dark-coloured stains, presumably from the discoloured, smeared plant materials, were much more frequent in September and October (autumn). Most stains were concentrated within 15 cm of the hive entrance, and the number of stains increased with time during autumn.

To simulate *Vespa* attack, we divided six honeybee colonies into three pairs and subjected each pair to experiments in 2015 with one of the three hornet species: *V. mandarinia*, the suspected inducers of entrance smearing, along with *V. simillima xanthoptera* and *V. analis*, which are sympatric with *A. cerana* and

sporadically attack honeybee hives (Ono et al. 1987). We carried out attack simulation experiments using each *Vespa* species to simulate the scouting behaviour of the respective species. In the *V. mandarinia* experiments, we forced the experimental hornet fastened by its slender petiole to a fine wire (Tan et al. 2012) to fly around the bee hive for 30 s and then to walk around the hive entrance for 30 s. Each treatment consisted of five cycles of hornet flight/walking, and the treatment cycles were repeated four times for each bee colony: twice on July 21 and once each on July 22 and 23. In the *V. simillima xanthoptera* or *V. analis* experiments, the hornets were forced to hover near the entrance of the hive, which was achieved by allowing them to flap their wings freely for 1 min. As above, bee colonies were subjected to four replicates of five treatment cycles with these hornet

Table 1. The results of a series of attack simulation experiments performed from July 21 to July 23 with three hornet species.

<i>Vespa</i> species	Apiary	Colony no.	Date						
			18 July 2015 Before experiment	19 July 2015	20 July 2015	21 July 2015 After experiment	22 July 2015	23 July 2015	28–29 July 2015 Additional experiment
<i>Vespa mandarinia</i>	Apiary B	Colony 1	×	×	×	○	○	○	○
		Colony 2	×	×	×	○	○	○	○
<i>Vespa simillima xanthoptera</i>	Apiary A	Colony 3	×	×	×	×	×	×	×
		Colony 4	×	×	×	×	×	×	○
<i>Vespa analis</i>	Apiary A	Colony 5	×	×	×	×	×	×	○
		Colony 6	×	×	×	×	×	×	○

Additional *V. mandarinia* experiments were performed on July 28–29

○ detection of entrance smearing, × no detection

species on the same dates as the *V. mandarinia* experiments. We checked the presence or absence of entrance smearing and staining 30 min and 3 days after each treatment via naked-eye observations and video. Five-six days after the attack simulation experiments, i.e. on July 28–29, all experimental bee colonies were subjected to an additional attack simulation experiment using *V. mandarinia*, also comprising five cycles.

Before and after the attack simulation experiments with *V. mandarinia*, we randomly collected 100 workers with a butterfly net from each of the two colonies to determine if they had ingested plant materials. These experiments were conducted twice on 28–29 July. We used Fisher's exact probability test to detect significant differences in the percentages of workers that brought back collected plant materials before and after the attack simulation experiments by *V. mandarinia*.

Only *V. mandarinia* attack induced entrance smearing in the experimental bee colonies. Smearing started 15–30 min after attack simulation began, irrespective of the exposure date and time. Neither plant-collecting nor plant-smearing responses were observed when the other colonies were exposed to simulated attack from *V. simillima xanthoptera* or *V. analis* (Table 1). The number of workers that brought plant materials increased after the experimental exposure to *V. mandarinia* (Fisher's exact probability test $P < 0.01$). In colonies in which no entrance smearing was observed in the first experiment with *V. simillima xanthoptera* and *V. analis*, smearing was induced by additional exposure to *V. mandarinia* scouting (Table 1).

Overall, our study revealed a close relationship between the previously reported leaf-gnawing behaviour of *A. cerana* (Yokoi 2005, 2015) and the occurrence of dark-coloured stains around the entrances to the hives of these bees (Okada 1997; Sasaki 1999). The purpose of this behaviour is presumably to prevent mass attack by giant hornets. How the smeared materials counteract *V. mandarinia* attack remains unknown, but they may alter the activity or recognition of the hornet's forage site-marking pheromone (Ono et al. 1995). *A. cerana* did not practise entrance smearing against *V. simillima xanthoptera* and *V. analis*, presumably because these species are less powerful enemies (Tan et al. 2013) and do not use recruitment pheromones.

Similar entrance smearing has been described for *A. cerana* in Vietnam and Korea, where *V. mandarinia* and its different subspecies are sympatric (Sasaki and Fujiwara unpublished data). Therefore, among Asian honeybees, this type of entrance smearing seems to be a common counteraction against Asian hornets of the genus *Vespa*.

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Note scientifique sur un comportement de l'abeille japonaise, *Apis cerana*, déclenché par la première approche d'un frelon asiatique *Vespa mandarinia* et consistant à appliquer autour de l'entrée de la ruche une "bouillie végétale"

Eine wissenschaftliche Notiz zum Beschmieren des Stockeingangs mit Pflanzenmaterial bei *Apis cerana* in Japan, ausgelöst durch Kundschafter der asiatischen Riesenhornisse *Vespa mandarinia*

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