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Honey bee social collapse arising from hornet attacks

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With 3 figures

Abstract: Collective defense is constrained by co-evolution with the predator such that species typically have effective defenses against sympatric, but not allopatric, predators. The honey bee, *Apis mellifera*, is allopatric with the predatory hornet, *Vespa velutina auraria*, and does not have effective defenses, unlike *A. cerana*, which is sympatric with this hornet. However, the reasons behind the decline of attacked *A. mellifera* colonies remain to be fully elucidated and are relevant given that invading *V. velutina* contributes to the decline of *A. mellifera* in multiple European countries. In southwest China, we show that *A. mellifera ligustica*, unlike the native *A. cerana cerana*, severely reduced foraging, and experienced higher hornet predation of foragers when attacked by native *V. velutina auraria*. Attacks resulted in reduced queen egg production, fewer pupae, and fewer workers and led to *A. mellifera* colony death. Decline began after only one week of hornet attacks, with *A. mellifera* queens significantly reducing their egg-laying even though the number of colony workers had not significantly fallen. In contrast, hornet attacks did not lead to declines in the same proxy measures of colony fitness for *A. cerana*. We suggest that, in addition to direct predation, predator-induced stress may contribute to *A. mellifera* colony declines, an intriguing possibility that accords with a growing body of research demonstrating that such stress can directly harm reproduction and thereby reduce fitness in multiple animals.

Keywords: Asian hornet, *Vespa velutina*, *Apis mellifera*, *Apis cerana*, fear, stress, colony

1 Introduction

Predators impose strong selective pressures through direct predation (Preisser et al. 2005), but can also provide indirect pressures by stressing prey and contributing to the "ecology of fear" (Brown et al. 1999; Creel et al. 2007). Such stressors can indirectly affect survival and reduce prey reproduction (LaManna & Martin 2016; Suraci et al. 2016). For example, playbacks of predator sounds resulted in song sparrows producing 40% fewer fledglings (Zanette et al. 2011). Other factors such as predator hyper-activation of defenses may also be detrimental and contribute to stress experienced by the individual or the collective. In social insects, such as honey bees, collective defense of the nest is essential given the valuable resources that the nest can provide to the predator (Fuchs & Tautz 2011). However, the potential cascading effects of predator attack upon social insects – directly reducing the number of colony members and, indirectly, stressing the colony to reduce its reproduction – are not well understood.

Asian honey bees (*Apis* species) have co-evolved with predatory Asian hornets (*Vespa* species) (Fuchs and Tautz 2011), and have evolved multiple counter-strategies (Mattila et al. 2020), including a strong heat-balling defense (Ono

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et al. 1995) and an "I See You (ISY)" signal that effectively deters nest-attacks (Tan et al. 2012). Heat-balling consists of bees surrounding a hornet attacker and generating heat and carbon dioxide to kill it (Ono et al. 1995; Sugahara & Sakamoto 2009). Heat-balling is a classic co-evolutionary example because it typically occurs and is effective between sympatric hornet predators and their honey bee prey (Ono et al. 2003; Papachristoforou et al. 2007; Baracchi et al. 2010; Tan et al. 2016). In contrast, there is variation in the defensive responses of *A. mellifera ligustica*, but this species typically forms heat balls against allopatric *V. velutina* that are approximately 85% smaller than the defensive heat balls of *A. cerana cerana* (Dong et al. 2018). The defenses of *A. mellifera ligustica* are less effective against hornet attacks, and as a result, this species suffers in areas where *V. velutina* has invaded (Laurino et al. 2020). Severe losses of up to 50% of honey bee colonies have been reported in some European regions despite the work of beekeepers to generate new colonies and actions taken to control hornets by exterminating their nests and using biological controls (Laurino et al. 2020).

A. mellifera colonies can respond to attacks by *V. velutina* hornets by creating a dense "bee carpet" consisting of a large number of bees gathered at the nest entrance (Arca et al. 2014; Monceau et al. 2018). However, this defense is not always effective. Requier et al. (2019) reported that hornets captured returning bee foragers and thereby increased bee homing failures. The same study also found that hornet predation contributed to bee foraging paralysis, defined as the relative flight activity of a bee colony when hornets were near its entrance as compared to the colony's maximum flight activity when hornets were not present. Modeling simulations suggest that such foraging paralysis could contribute to winter colony declines. Leza et al. (2019) reported that honey bee workers from apiaries in which *V. velutina* were present had significantly higher expression of oxidative stress-related genes than worker bees where these hornets were not present.

The ecology of fear may therefore play a role in the demographic collapse of *A. mellifera* colonies under attack by *V. velutina*, particularly with respect to reproduction (LaManna & Martin 2016). Based on our observations of *V. velutina auraria* attacking *A. cerana cerana* and *A. mellifera ligustica* at apiaries, we hypothesized that the allopatric *A. mellifera* suffers from an ineffective defense against *V. velutina* and is reproductively stressed by such attacks. We measured hornet attacks and honey bee colony proxies of fitness (number of eggs, pupae, and workers) in apiaries with both bee species but with and without hornets and quantified fitness effects over seasons in the presence and absence of hornets. We predicted that hornet presence would harm *A. mellifera* more than *A. cerana* colonies because of their different exposure to *V. velutina* over evolutionary time.

2 Materials and methods

We studied 60 full-sized colonies (30 colonies of *A. cerana cerana* and 30 colonies of *A. mellifera ligustica*, four combs per colony) at four apiaries in Yunnan over three field seasons. Our *A. cerana* and *A. mellifera* colonies were bred from standard stocks maintained for beekeeping in Yunnan. All bee colonies were healthy and queen-right, and were *Varroa*-free, as determined by standard inspection techniques (Dietemann et al. 2013). Our experimental methods were performed in accordance with all relevant regional guidelines and regulations. Because we did not use insects that are endangered or restricted, ethics committee approval was not required.

2.1 Experiment 1A: effect of hornet attacks over time

In this experiment, we used 20 colonies (10 of each species), equally divided into two apiaries: Wuding County (experiment 1, June-August 2017, 25°5279'N, 102°4031'E) and the Eastern Bee Research Institute in Kunming (experiment 2, April and August of 2018, 25°1258'N, 102°7509'E). There were five colonies of *A. cerana cerana* and five colonies of *A. mellifera ligustica* at each apiary. At both the Wuding and Kunming apiary sites, there were approximately 20 nearby colonies of *V. velutina auraria* that we visually identified in the eaves of buildings and in trees within 1 km of the apiary. *Vespa velutina auraria* is native to Yunnan (Akre & Davis 1978).

At the beginning of this experiment, all colonies were set up to be approximately equal in size. They were then given four days to settle down before we began the experiment (Imdorf et al. 1987). We placed a transparent plastic sheet (36 cm × 26 cm with a 1 cm grid) in front of each comb, and photographed both sides of all combs with a digital video camera (Sony™ HDR-PJ790). From the images, we counted bees (experiment 1A,B) and pupae (experiment 1B, see below) following the established Liebefelder estimation method (Imdorf et al. 1987; Dainat et al. 2020) of which details are available at this website: https://www.agroscope.admin.ch/agroscope/en/home/topics/livestock/bees/biologie/volksentwicklung/bienen-schaetzmethode.html.

To measure egg-laying, we placed an empty comb of worker-sized cells that had been cleaned by workers and was ready for egg-laying and confined the queen onto this comb with a queen excluder cage that covered the entire side of one comb (Harbo 1986; Ribbands 1953). The cage mesh size allowed the smaller workers to easily pass through to tend the queen. After 24 h (between 16:00 and 18:00), we carefully removed the cage, released the queen back into her colony, took away the comb, brushed off the workers into the colony, and counted the number of eggs laid. Because eggs were never laid in all the available cells, the queen cage size did not limit the number of eggs laid.

From 10:00–14:00 of each day, we observed hornet attacks at colony nest entrances because hornets often attacked bee colonies during this time period and it encompassed, based upon our prior observations, the period of maximum hornet predation (Tan et al. 2007). We also choose these times because bee workers did not perform orientation flights – the classic looping orientation flight behavior (Winston 1987) – during this period. We were therefore able to define bees flying out of or returning to the nest as foragers. For *A. mellifera*, such foraging continued, albeit at a lower rate, even when a colony formed a bee carpet in response to hornet attacks. We placed a camera 50 cm away from the colony entrance (a distance that did not disturb the bees based on visual observations) and videotaped the nest entrance. The observer was positioned directly behind the camera. Over a 15 min trial, we counted the number of foragers that were attacked by hornets at each bee colony nest entrance. Counts were primarily made by the observer, and we used the video footage used to verify these counts, especially in cases where there were multiple simultaneous attacks.

When a hornet closely approaches an *A. cerana* or *A. mellifera* colony (≤4 cm), guard bees can attack the hornet to form a heat-ball (Ono et al. 1995; Dong et al. 2018). However, in the few cases in which bees attempted to heat-ball hornets, the hornets usually immediately retreated, and thus we did not score these rare attempts. We defined guard bees as individuals that directly attacked the hornet or moved towards the nest entrance and remained on this entrance, often gaping their mandibles when hornets approached.

We made measurements at the beginning of each experiment (week 0), then repeated these measurements each week for six weeks for a total of seven time points (Fig. 1). Each week, we followed the same schedule. On the first day of each week, we photographed combs to count workers and counted forager attacks. On the second day of each week, we inserted the empty comb to measure egg laying and returned to count these eggs 24 h later.

2.2 Experiment 1B: comparing sites with and without hornets

To control for hornet presence, we replicated experiment 1A at two apiary sites (one with and one without hornets) in 2020 (from July to August). The Malong County apiary (25°2706’N, 103°3794’E) had no *V. velutina* colonies or foragers as observed daily over the entire experiment. In contrast, the Xinan Center of Biodiversity apiary in Kunming (25°1386’N, 102°7441’E, 100 km away) had over 20 colonies of *V. velutina* nesting within 1 km, and multiple hornets attacked apiary colonies every day. At the start of this experiment, we moved five *A. cerana* and five *A. mellifera* colonies to each apiary from an apiary in Jiuxian County (25°3820’N, 103°4128’E) during a period in which the Jiuxian County apiary had almost no hornet activity. Thus, Jiuxian apiary bees were not stressed by hornets prior to being moved to the experimental apiaries. These colonies were all requeened five days before the move date.

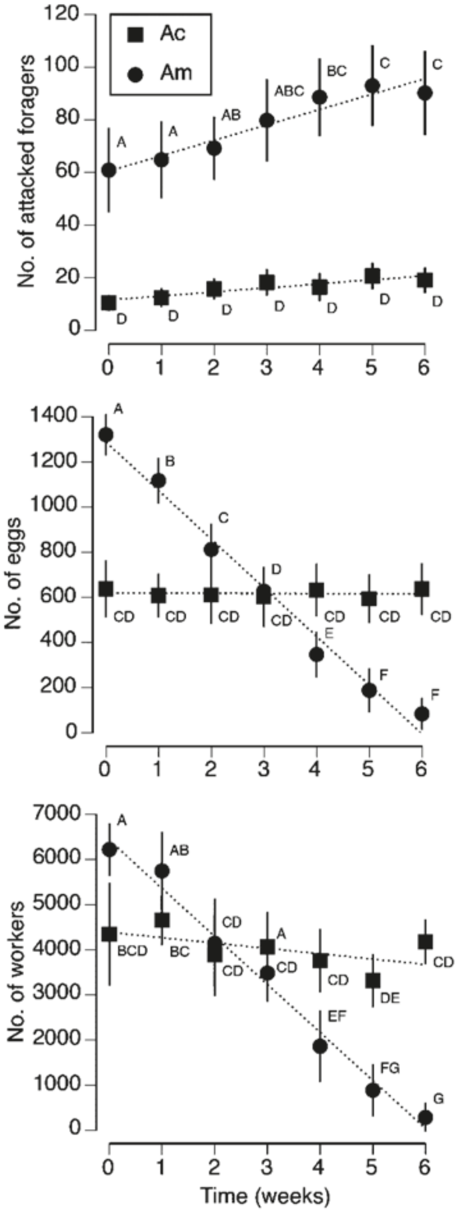

Fig. 1. Effect of hornets on correlates of fitness of Ac (*A. cerana*) and Am (*A. mellifera*) colonies over time (experiment 1A). We used two apiaries (Wuding and Kunming), both with approximately 20 *V. velutina* nests within 1 km of each apiary and found that Am declined over time when under hornet predation, but not Ac. All Am colonies had died by week 6. The numbers of eggs laid during a 24 h period in an empty comb placed inside each colony are shown. Plots show means, and error bars represent the 95% confidence intervals. Dashed linear regression lines are shown to better differentiate the time trends per species. Different letters indicate significant differences (Tukey HSD tests, $P < 0.05$).

The colonies were then given four days to settle down before we began the experiment, in which we counted the number of adult workers and pupae, using the same photographic technique as in experiment 1A (see above). Because queens had been released only a few days earlier, there were no pupae at week 0, and we began our pupae counts at week 1. We also halted this experiment earlier, at week 4 instead of week 6, because three (60%) *A. mellifera* colonies at the apiary with hornets had died by week 4.

2.3 Experiment 2: seasonal effect of hornets on the number of foraging bees

In experiments 1A and 1B, we compared the effects of hornet attacks at multiple locations at times when hornets were actively hunting bees. However, we also wished to determine the effects of hornet attacks on the two bee species when housed at the same location, the Kunming apiary, in a common garden experiment. We therefore we ran a second experiment in which two seasons were compared: the hornet inactive season (April) when there were no hornet foragers because hornet queens were in winter diapause or at the incipient stage of colony formation and the hornet active season (August) when foraging hornets were abundant. We used 10 colonies of *A. mellifera ligustica* and 10 colonies of *A. cerana cerana*. In this seasonal experiment, we measured bee behaviors for 5 min from 11:00 to 13:00 on clear, sunny days when the temperature was approximately 21°C. Tan et al. (2007) found that *V. velutina auraria* attacked honey bees from approximately 9:00 h until 17:00 on clear, sunny days. In the hornet active season, colonies were usually under attack by 10:00 and thus had reached a steady state response to hornet attacks by 11:00. As in experiment 1, we chose this time because it preceded the typical times that bees performed orientation fights, and thus nest entrance flight activity primarily represented foraging. On each observation day, we monitored colonies of both honey bee species.

During every 5 min trial, we placed a camera 50 cm away from the colony entrance and videotaped the nest entrance. The observer was positioned directly behind the camera. We subsequently counted the number of hornets attacking the colony entrance and the number of guard bees at the nest entrance. Counts were primarily made by the observer, with the video footage used to verify these counts, particularly in cases where there were multiple simultaneous attacks and many guard bees. *A. cerana* and *A. mellifera* guard bees were defined as workers that exhibited defensive behaviors, particularly orienting towards and opening their mandibles (gaping) when hornets approached (Fuchs & Tautz 2011; Winston 1987).

At the nests, we also observed bees producing stop signals, vibrational signals that can be associated with danger at a food source or attacks by hornets at the nest entrance, and that can inhibit foraging activity (Tan et al. 2016). Previously, we had detected these signals only inside the nest (Tan et al. 2016), but the stop signals recorded outside the nest were within the same range of frequencies and durations as stop signals recorded inside the nest. These signals were also similar to the antipredator signals observed by Matilla et al. (2021) when *A. cerana* colonies were attacked by *Vespa soror*. We used similar methods to those described in Tan et al. (2016), scanning the nest entrance with a hand-held electret microphone and counting the number of stop signals produced when colonies were attacked with a single *V. velutina* hornet tethered with soft wire to a 1 m wood rod. After the 5 min hornet attack count trial described above, we waited for a pause in hornet attacks on the focal nest and then attacked the nest entrance with the tethered hornet to count stop signals. Typically, such a pause would occur within 10 min after the end of the 5 min trial. Each attack count trial was therefore paired with a stop signal count trial. We used a different tethered hornet for each trial.

2.4 Statistical analyses

We used JMP Pro v15.0.0 (SAS Institute, Inc.) and report means and their 95% Confidence Intervals. Because we had very large counts, we used a Repeated Measures Mixed Model ($\mathrm{RM}_{\mathrm{Mixed\ Model}}$, REML algorithm) with colony (random effect) as the repeated measure nested within species (experiment 1A) to determine the effects of hornet attacks on the number of eggs, pupae, and workers of each bee species (dependent variables, three separate models run). Species was an independent variable. Time was an ordinal independent variable. For experiment 1B, we used the same Repeated Measures Mixed Model with colony (random effect) as the repeated measure nested within treatment (hornets present or absent, an independent variable). Species and time (ordinal variables) were fixed effects. We separately analyzed the number of pupae (log transformed) and the number of workers. For these models, we conducted Tukey Honestly Significant Difference (HSD) tests to make corrected pairwise comparisons between all-time points.

In the seasonal effects experiment, the counts of attacking hornets were relatively low and we therefore used a Repeated Measures Generalized Linear Model ($\mathrm{RM}_{\mathrm{GLM}}$) with colony as the repeated measure (nested within species) for the number of hornets attacking (normal distribution, identity link, and overdispersion correction), guard bees (exponential distribution and reciprocal link), stop signals produced (Poisson distribution, reciprocal link, and overdispersion correction), and foragers (Poisson distribution, log link, and overdispersion correction). We ran four separate models. Colony and hornet season were independent variables. The numbers of attacking hornets, guard bees, stop signals, and foragers were dependent variables. We report our results as Likelihood Ratio (L-R) Chi-Square tests. For each of the dependent variables, we also ran post-hoc contrast tests to compare between bee species within each season.

3 Results

3.1 Hornet attacks significantly reduced *A. mellifera*, but not *A. cerana* colony fitness

In experiment 1A, hornets attacked significantly more *A. mellifera* than *A. cerana* foragers (RM $_{\text{Mixed Model}}$: species effect: $F_{1,98} = 55.55$, $P < 0.0001$, $R^2 = 0.88$, Fig. 1). There was a significant effect of time (RM $_{\text{Mixed Model}}$: $F_{6,108} = 7.61$, $P < 0.0001$) and a significant interaction of species × time (RM $_{\text{Mixed Model}}$: $F_{6,108} = 2.77$, $P = 0.015$) because more hornets attacked *A. mellifera* as the weeks progressed (significantly elevated attacks in week 4 as compared to week 0, Tukey HSD test, $P < 0.05$), but not *A. cerana* (no significant differences at any time point, Tukey HSD test, $P > 0.05$). There were 148% more hornet attacks on *A. mellifera* in week 6 as compared to week 0.

A. mellifera colonies, unlike *A. cerana* colonies, declined rapidly under hornet attacks. The number of eggs laid by *A. mellifera* queens, but not by *A. cerana* queens, significantly decreased over time (RM $_{\text{Mixed Model}}$: interaction species × time: $F_{1,118} = 864.53$, $P < 0.0001$, model $R^2 = 0.94$, Fig. 1). There was a significant effect of time (RM $_{\text{Mixed Model}}$: $F_{1,118} = 868.61$, $P < 0.0001$) but not species (RM $_{\text{Mixed Model}}$: $F_{1,18} = 0.19$, $P = 0.67$), although the species differences are seen in the interaction effect. *A. cerana* egg laying did not significantly decline over time (Tukey HSD test, $P < 0.05$) whereas *A. mellifera* egg laying significantly decreased (beginning at week 1 as compared to week 0) by 96% over 6 weeks (Tukey HSD test, $P < 0.05$).

The numbers of workers in *A. mellifera*, but not *A. cerana*, colonies decreased over time (RM $_{\text{Mixed Model}}$: interaction species × time: $F_{1,118} = 186.48$, $P < 0.0001$, $R^2 = 0.83$, Fig. 1). There were significant effects of time (RM $_{\text{Mixed Model}}$: $F_{1,118} = 292.02$, $P < 0.0001$) and species (RM $_{\text{Mixed Model}}$: $F_{1,18} = 6.54$, $P = 0.02$). For *A. mellifera*, the number of workers significantly declined beginning at week 3 (as compared to week 0, Tukey HSD test, $P < 0.05$). However, for *A. cerana*, the only change was a slight decrease in workers in week 5 as compared with week 1. This slight decrease may have been a random fluctuation because worker numbers had recovered by week 6 (Tukey HSD test, $P < 0.05$). By the end of experiment 1A, 100% of *A. mellifera* colonies, but no *A. cerana* colonies, had died.

We next conducted experiment 1B to compare the effect of hornet presence and absence (treatment) during the same time period. At the Xinan apiary with hornet predators, *A. mellifera* pupae and worker populations declined but *A. cerana* pupae and worker populations did not.

For pupae (overall model $R^2 = 0.94$), there was no significant interaction of species × treatment (RM $_{\text{Mixed Model}}$: $F_{1,64} = 0.0001$, $P = 0.99$), but all other interactions (species × time, treatment × time, and treatment × time × species) were significant (RM $_{\text{Mixed Model}}$: $F_{3,48} \geq 9.69$, $P < 0.0001$). Species and treatment (RM $_{\text{Mixed Model}}$: $F_{1,64} \leq 0.2.27$, $P \geq 0.14$) were not significant effects, but time was significant (RM $_{\text{Mixed Model}}$: $F_{3,48} = 20.15$, $P < 0.0001$). In the three-way interaction (species × treatment × time), all pairwise comparisons showed that the number of *A. cerana* pupae did not significantly change over time or between treatments (Tukey HSD test, $P \geq 0.05$). However, analysis of this three-way interaction showed that the number of *A. mellifera* pupae were constant over time in the no-hornet apiary, but significantly declined beginning in week 3 at the hornet apiary, an average decrease of 98% over four weeks (Fig. 2). Between species, the major difference occurred in week 4 at the apiary with hornets, when the number of workers in *A. mellifera* colonies was significantly lower than the number of workers in *A. cerana* colonies (Tukey HSD test, $P < 0.05$).

For the number of workers (overall model $R^2 = 0.94$), there were significant effects of species (RM $_{\text{Mixed Model}}$: $F_{1,28} = 70.66$, $P < 0.0001$) and time (RM $_{\text{Mixed Model}}$: $F_{4,64} = 68.95$, $P < 0.0001$). There was no significant effect of treatment (RM $_{\text{Mixed Model}}$: $F_{1,28} = 3.71$, $P = 0.06$). However, the interaction species × treatment was significant (RM $_{\text{Mixed Model}}$: $F_{1,28} = 5.04$, $P = 0.03$) as were all other interactions (species × time, treatment × time, and treatment × time × species: RM $_{\text{Mixed Model}}$: $F_{4,64} \geq 15.54$, $P < 0.0001$). In detail, analysis of the three-way interaction revealed that the number of *A. cerana* workers did not change over time or with treatment (all pairwise comparisons, Tukey HSD test, $P < 0.05$). The number of *A. mellifera* workers, in contrast, sharply declined over time in the apiary with hornets such that *A. mellifera* colonies in the apiary with hornets were significantly smaller than *A. mellifera* colonies in the apiary without hornets during weeks 3 and 4 (Tukey HSD test, $P < 0.05$). A key difference between species occurred in week 4 at the apiary with hornets, when the number of workers in *A. mellifera* colonies was significantly lower than the number of workers in *A. cerana* colonies (Tukey HSD test, $P < 0.05$). *A. mellifera* colonies attacked by hornets experienced severe worker declines, an average decrease of 88% over four weeks.

3.2 Hornet attacks elicited more guarding and stop signaling from *A. mellifera* than from *A. cerana* (seasonal comparisons)

Here, we compared the effects of hornet absence or presence across different seasons at the same apiary site. As expected, there were no hornets present and therefore no hornet attacks during the hornet inactive season (season effect: L-R Chi-Square = 283.4, 1 df, $P < 0.0001$, Fig. 3A). During the hornet active season, in response to hornet attacks, both bee species significantly increased the number of guard bees at their nest entrances, but this response was stronger for *A. mellifera* than for *A. cerana* (Fig. 3B). *A. mellifera* created the bee carpet observed in other studies, but *A. cerana* did not. With respect to the number of guard bees, there were significant effects of bee species (L-R Chi-Square = 33.3, 1 df,

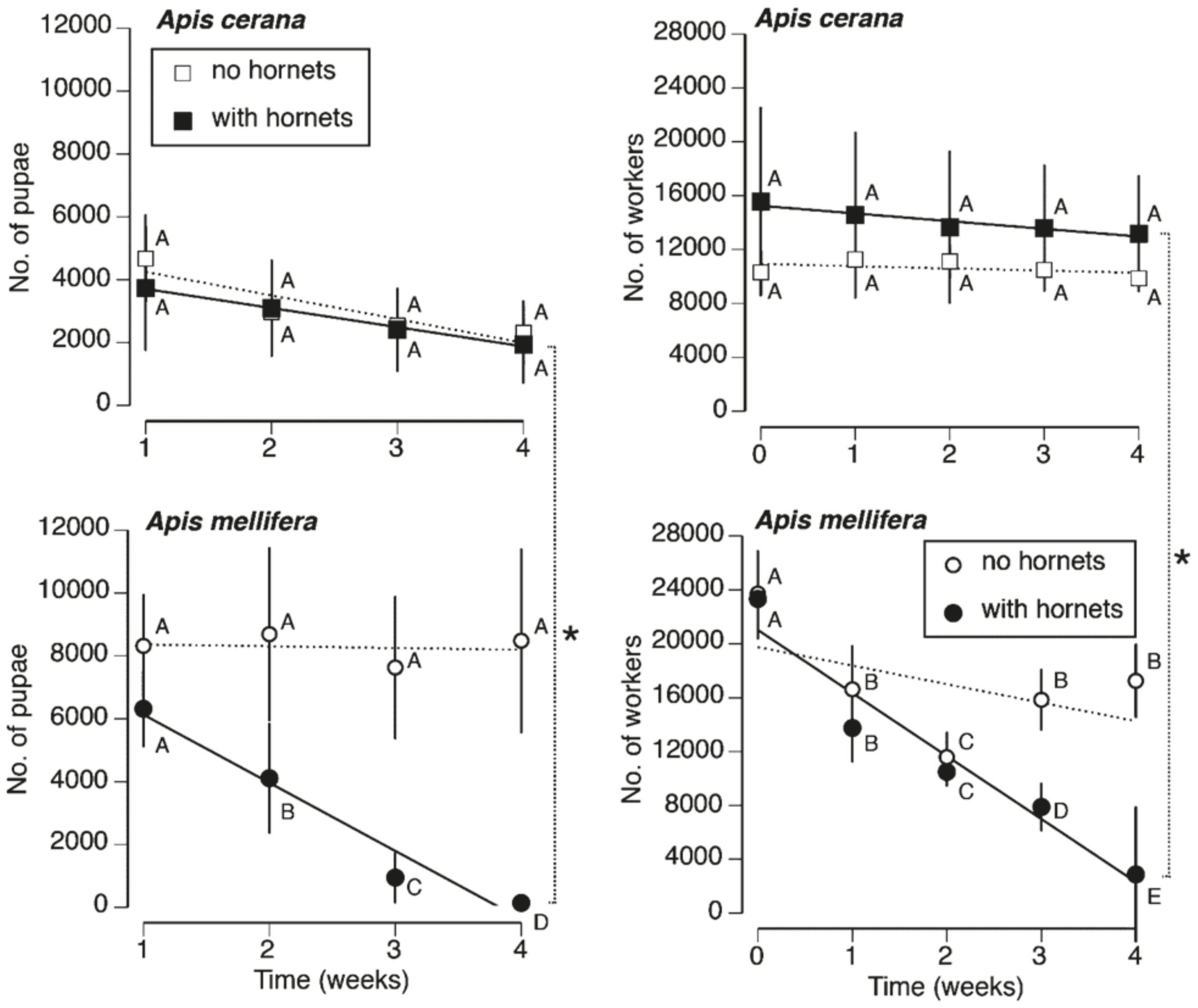

Fig. 2. The effects of hornet presence (Malong apiary with hornets) and absence (Xinan apiary with no hornets) on the fitness of Ac (*A. cerana*) and Am (*A. mellifera*) colonies over time (experiment 1B). This experiment ended at 4 weeks because 60% of Am colonies had died at the apiary with hornets by this time point. Pupae counts only begin at week 1 because all colonies were created with new queens five days before the colonies were moved to the Malong and Xinan apiaries. The data were analyzed as one statistical model per life stage (pupae and, separately, workers) with species, treatment (no hornets or with hornets) and time, but are divided here by species to better illustrate trends. Different letters indicate significant differences (Tukey HSD test, $P < 0.05$) per plot based upon one Tukey HSD test on the interaction species × treatment × time. The vertical dashed lines with stars show significant differences between species at the apiary with hornets from the same Tukey HSD test ($P < 0.05$). Plots show means and error bars represent the 95% confidence intervals. Dashed linear regression lines are shown to better differentiate the time trends per species.

$P < 0.0001$), season (L-R Chi-Square = 445.4, 1 df, $P < 0.0001$), and the interaction species × season (L-R Chi-Square = 31.3, 1 df, $P < 0.0001$), because there were no hornet attacks in the inactive season. In the hornet colony growth season, hornet attacks resulted in 400% more *A. mellifera* than *A. cerana* guard bees coming to colony entrances (Contrast test, L-R Chi-Square = 33.6, 1 df, $P < 0.0001$).

When attacked by hornets, *A. mellifera* produced far more stop signals at the nest entrance than *A. cerana* (Fig. 3C). With respect to the number of stop signals, there were significant effects of bee species (L-R Chi-Square = 10142625, 1 df, $P < 0.0001$) and season (L-R Chi-Square = 87.5, 1 df, $P < 0.0001$) since guards did not produce stop signals during the hornet inactive season. Both bee species produced more stop signals when attacked as compared to the control treatment (Contrast tests, L-R Chi-Square ≥33.3, 1 df, $P < 0.0001$), but *A. mellifera* colonies, on average, produced significantly more stop signals (34-fold more signals) in comparison to *A. cerana* (Contrast test, L-R Chi-Square = 880.9, 1 df, $P < 0.0001$).

In the hornet inactive season, *A. mellifera* had significantly higher foraging activity than *A. cerana* (Fig. 3D).

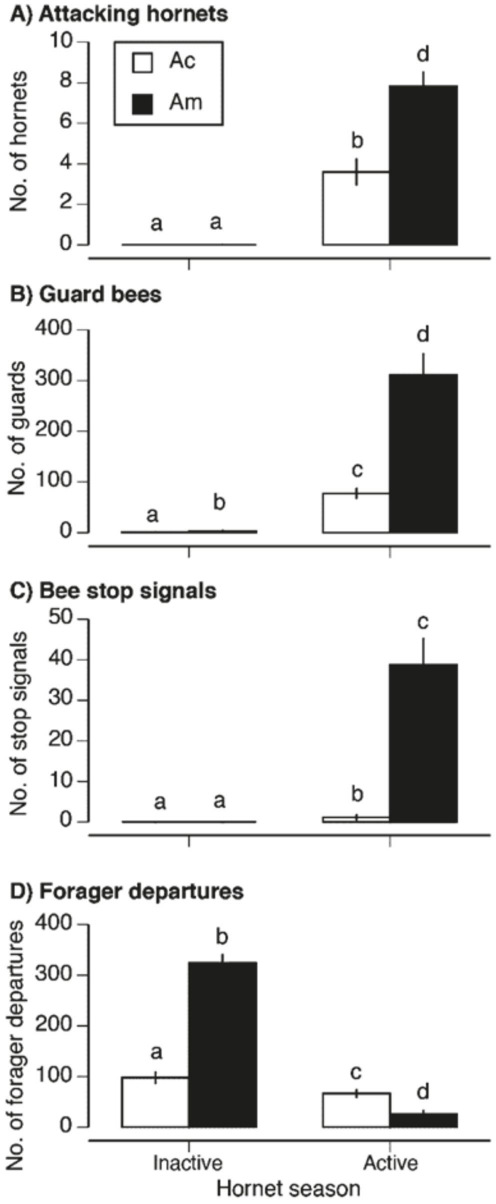

Fig. 3. Effect of season on bee colonies of both species (experiment 2). Per colony in a 5 min trial, the number of **(A)** attacking hornets (Contrast tests, L-R Chi-Square ≥108.4, P <0.0001), **(B)** guard bees (Contrast tests, L-R Chi-Square ≥36.4, *P* <0.0001), **(C)** stop signals recorded outside the nest (Contrast tests, L-R Chi-Square ≥63.8, *P* <0.0001), and **(D)** forager departures (Contrast tests, L-R Chi-Square ≥31.2, *P* <0.0001) are shown. Different letters indicate significant differences, plots show means, and error bars represent the 95% confidence intervals.

This trend reversed when hornets were actively attacking bee nests (Contrast tests, L-R Chi-Square ≥31.2, *P* <0.0001). During the hornet active season, *A. mellifera* had significantly lower foraging activity than *A. cerana* (Contrast tests, L-R Chi-Square ≥31.2, *P* <0.0001, Fig. 3D).

4 Discussion

At an apiary with both bee species under hornet attack, a common garden experiment, *A. cerana cerana* foragers continued to forage and their queens maintained normal, steady egg laying. Typical colony activity essential for fitness was largely unaffected. In contrast, in *A. mellifera ligustica* colonies under attack, egg laying and colony population levels dropped until all *A. mellifera* colonies had died by week 6. Our results make sense given prior work showing that *A. cerana*, but not *A. mellifera*, have behaviors that can deter and halt hornet attacks: the deterring “I See You” (ISY) signal (Tan et al. 2012) and more efficient heat-balling (Dong et al. 2018).

In the next experiment, we examined the effects on the two bee species at apiaries with and without hornets and obtained similar results. At the apiary with no hornets, both bee species maintained pupal and worker populations that declined slightly with time, perhaps because nearby food sources were more limited at this location. However, at the apiary with hornets, *A. cerana* pupal and worker population were not affected by hornets, but *A. mellifera* pupal and worker numbers showed a strong hornet effect, with 60% of *A. mellifera* colonies dying within only four weeks. Although hornets managed to capture some *A. cerana* workers, none of the studied *A. cerana* colonies died and their worker populations did not decline because of hornet presence (Fig. 2). In contrast, all *A. mellifera* colonies died in this experiment.

The effects of hornet predation upon honey bee colonies may vary depending upon colony size, health, and genetic background. There is also natural variation in the defensiveness of *A. mellifera ligustica* and *A. cerana cerana* colonies against *V. velutina auraria*. However, in Yunnan, we have found consistent differences between the honey bee species: *A. cerana cerana* produced heat balls with an average of over 6-fold more attacking bees than the heat balls produced by *A. mellifera ligustica* (Dong et al. 2018).

Comparing one apiary with hornets with one apiary without hornets is a limitation of experiment 1B because the differences observed between sites could reflect environmental differences apart from hornet predation. However, we replicated the effects of hornet predation upon both honey bee species at three different sites (Wuding, Kunming, and Malong). At each of these sites, bees experienced the same weather conditions and food availabilities, and *A. mellifera* colonies showed significantly greater declines than *A. cerana* colonies.

Prior work has shown that the ability of *A. cerana* to perform the ISY signal (Tan et al. 2012) and to heat-ball hornets, if they come too close (Ono et al. 1995), provide effective deterrence and defense, respectively. In contrast the bee carpet created by *A. mellifera* colonies did not deter hornet attacks and likely provided hornets with more predation opportunities. In experiment 2, *A. mellifera* colonies had, on average, 4-fold more guards at the nest entrance (creating the “bee carpet”) than *A. cerana*, but hornets captured guards forming the bee carpet, as described in Tan et al. (2012). On average, *V. velutina auraria*, hornets can capture approximately 100 *A. mellifera* workers per day at each colony entrance (Ken et al. 2005).

We considered the possibility that *A. mellifera* colonies could compensate for the loss of workers to direct hornet predation because an *A. mellifera* queen can lay more than thousand eggs a day (Harbo 1986; Ribbands 1953). However, within one week, queen egg production declined by 16%, double the 8% rate of worker population decline. This reduced egg production likely contributed to colony population collapse.

There are multiple potential reasons for the decrease in egg laying. Workers clean and prepare cells for egg laying and thus their decline could affect queen egg laying. However, we inserted clean comb that was ready for egg laying without further worker preparation. A smaller workforce to feed the queen should eventually reduce egg production, but colonies still had ample food stores in the first week, and care and feeding of the queen is normally prioritized. When food is available, colonies can rapidly increase their population sizes, even with a relatively small number of workers (Winston 1987). We suspect that the stress of constant hornet attacks and the reallocation of worker labor to ineffectively defend the nest entrance could have contributed to decreased colony productivity. These defenders are drawn from a large reservoir of workers between 7–35 days old and, as a result, deploying a large number of such bees for colony defense likely reduced their ability to perform other tasks inside the nest or, for older bees, to forage (Breed et al. 1990).

In addition to hornet predation on adult bees, stop signals may have contributed to colony declines because stop signals can reduce foraging. On average, *A. mellifera* colonies produced 34-fold more stop signals outside the nest when under hornet attack than *A. cerana* colonies under attack. Based upon studies of *A. cerana* stop signals produced inside the nest (Tan et al. 2016), we predict that stop signals produced outside the nest by both bee species may keep foragers that are exiting the nest from leaving the nest and flying into harm's way. If so, the far larger number of stop signals produced by *A. mellifera* than *A. cerana* could have contributed to declines in *A. mellifera* foraging and fitness. Alternatively, stop signals produced outside the nest could have a previously undocumented function such as helping to coordinate nest defense, a possibility that deserves further exploration, particularly given the recent observations of frenetic antipredator piping by *A. cerana* workers attacked by *V. soror* (Mattila et al. 2021). It is quite possible that the signals recorded by Matilla et al. (2021) and the ones we observed are the same. Further studies on the effects of such signals are needed.

The stressors imposed by predator presence are known to reduce prey reproduction (Allen et al. 2022). Fear of predators can influence multiple aspects of prey population regulation and dynamics (Creel & Christianson 2008; Creel et al. 2007). Such fear can influence population sizes, the dynamic stability of predator-prey interactions (Yamamichi et al. 2019), and have cascading food web effects (Suraci et al. 2016). Our results suggest that a largely ineffective defense such as the *A. mellifera* bee carpet response can contribute to population collapse in a social group. Intriguingly, such ineffectual defensive responses are found at multiple levels of biological organization, including intracellular and intercellular responses such as inflammation and overactive immune responses to pathogens (Braciale et al. 2012).

Data statement

All data is freely accessible at Zenodo.org via this link: http://doi.org/10.5281/zenodo.7838842

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