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Defensive behaviour of *Apis mellifera* against *Vespa velutina* in France: Testing whether European honeybees can develop an effective collective defence against a new predator⋆

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ABSTRACT

We investigated the prey–predator interactions between the European honeybee, *Apis mellifera*, and the invasive yellow-legged hornet, *Vespa velutina*, which first invaded France in 2004 and thereafter spread to neighbouring European countries (Spain, Portugal and Italy). Our goal was to determine how successfully honeybees are able to defend their colonies against their new predator in Europe. Experiments were conducted in the southwest of France—the point of entry of the hornet in Europe—under natural and semi-controlled field conditions. We investigated a total of eight apiaries and 95 colonies subjected to either low or high levels of predation. We analyzed hornet predatory behaviour and collective response of colonies under attack. The results showed that *A. mellifera* in France exhibit an inefficient and unorganized defence against *V. velutina*, unlike in other regions of Europe and other areas around the globe where honeybees have co-evolved with their natural *Vespa* predators.

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1. Introduction

Many species of hornets (Vespinae) are serious enemies of honeybees (Morse, 1990; Mishra et al., 1989; Abrol, 1994). Hornets cause considerable damage to honeybee colonies which provide them with a valuable source of both protein (bees and larvae) and carbohydrates (nectar and honey) (Matsuura and Yamane, 1990). The recent establishment of the invasive yellow-legged Asian hornet, *Vespa velutina*, in Europe has raised many questions about its potential impact on the local honeybee, *Apis mellifera*, and consequently about whether the native honeybees have the capacity to defend themselves against this new predator.

V. velutina is widespread in the north of India and China—the area from which it originates. It is distributed in areas that differ widely in their annual temperature range. In the Kashmir region of northern India and in China, *V. velutina* is a major enemy of the native *Apis cerana* as well as the introduced European *A. mellifera* colonies (Shah and Shah, 1991; Abrol, 1994; Ken et al., 2005; Tan et al., 2007) and attacks can cause colony losses of up to 30%

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(Sakagami and Akahira, 1960; Ken et al., 2005). *V. velutina* was discovered for the first time in France in 2004 and has rapidly become a problem for French beekeepers (Monceau et al., 2014). The invasive hornet recently extended its range of distribution to the north of Spain (Castro and Pagola-Carte, 2010; López et al., 2011), Portugal (Grosso-silva and Maia, 2012) and Italy (Demichelis et al., 2013). In 2012, its estimated range covered an area of about 360,000 km^2 in Europe (Rome et al., 2013). Like many other invasive species, the population size of *V. velutina* in its native range is controlled by predation and competition. However, the effects of predation and competition on the population dynamics of *V. velutina* have never been investigated in its new range. Due to the size of their colonies—at least three times larger than those of the European hornet *Vespa crabro*—*V. velutina* exerts a stronger predation pressure on European apiaries than does the native hornet (Villemant et al., 2006).

Many studies have described several coordinated and massive defence tactics performed by the honeybee colonies when attacked by hornets. These studies were conducted in Asia on *A. cerana* and *Vespa mandarinia* (Ono et al., 1995; Ken et al., 2005; Sugahara and Sakamoto, 2009; Tan et al., 2012, 2013; Sugahara et al., 2012), in Cyprus with *Apis mellifera cypria* and *Vespa orientalis* (Papachristoforou et al., 2007, 2008, 2011) and in Italy with *Apis mellifera ligustica* and *V. crabro* (Baracchi et al., 2010). It has been proposed that the native honeybee in Asia, *A. cerana*, employs a defensive strategy called "thermo-balling" to kill its natural enemy, the Asian giant hornet, *V. mandarinia* (Ono et al., 1995; Ken et al., 2005). However, research on the thermo-balling phenomenon revealed that heat alone is not sufficient to kill an engulfed hornet; a high concentration of CO_2 and relatively high humidity interact with temperature to kill *V. mandarinia* inside balls of *A. cerana* workers (Sugahara and Sakamoto, 2009; Sugahara et al., 2012). In Cyprus, the defensive behaviour of the native honeybee *A. mellifera cypria* against its natural predator, the oriental hornet *V. orientalis*, also involves a balling behaviour (Papachristoforou et al., 2007), but since its lethal thermal limit is higher than that of *V. mandarinia* and similar to the thermal limit of *A. m. cypria*, honeybees cannot kill hornets by thermo-balling. Instead, they adopt another strategy called "asphyxia-balling" (Papachristoforou et al., 2007) in which they efficiently block the movement of the abdominal segments (tergites) of the trapped hornet. This action limits the functioning of the predator's respiratory system and, combined with the increase of temperature and CO_2 concentration in the insect haemolymph, causes the death of the insect. In Italy, the native honeybee *A. m. ligustica* exhibits the balling behaviour against its natural predator, *V. crabro* (Baracchi et al., 2010). This behaviour is assumed to work primarily by increasing the hornet's body temperature, but other contributing factors such as asphyxia, CO_2 level or humidity must be still investigated. Regardless of the specific factors that cause hornet death during honeybees' defence, it appears that the key element that determines bees' successful defence against hornets is whether defenders are able to form a ball to fully engulf the predator.

Honeybee colonies can also use defensive strategies that do not require physical contact with their enemies, including intimidation behaviours or physical barriers. Examples include colony aggregation on the beehive platform in a manner called "bee-carpet behaviour" (Baracchi et al., 2010; Papachristoforou et al., 2011), synchronized abdominal shaking known as shimmering (Butler, 1954; Kastberger et al., 2008), emitting an alarm sound defined as hissing (Papachristoforou et al., 2008), and building walls of propolis to prevent hornets from getting into the hive (Papachristoforou et al., 2011).

It is believed that such behavioural differentiations result from co-evolution and adaptation to environmental pressures and variable predation tactics among species (DeGrandi-Hoffman et al., 1998; Papachristoforou et al., 2011). Although some studies show the influence of genetic (Breed et al., 2004; Hunt et al., 2007) and neurophysiological (Ugajin et al., 2012) factors on the development and evolution of such anti-predator behaviours, more studies are required to better understand these processes. In that respect, comparison of such behaviours with those observed in other species of *Apis* and *Vespa* should provide useful insights to understanding this evolutionary arms race.

The objective of this study is to better understand how *A. mellifera* defends its colonies against the new predator, *V. velutina*, by addressing the following questions: (i) how do the two species, *A. mellifera* and *V. velutina*, interact at the entrance of the colony? (ii) Can honeybees develop an effective balling behaviour against the new predator? (iii) Are honeybees able to kill the hornet, and if so, how? To answer these questions, we have conducted experimental field studies to assess the defensive behaviour of honeybees under natural and semi-controlled conditions.

2. Materials and methods

2.1. Description of apiaries

Observations were carried out in August–October 2008 and August–September 2010 when the hornet population was at its maximum (Monceau et al., 2013a, 2013b). Eight apiaries and 95 colonies were selected in a 12,000 km^2 area in south-western France (Fig. 1) (mean number of colonies per apiary: 7.3 ± 2.4). These apiaries were selected according to exposure duration (corresponding to the number of years since hornet activity was first detected at the apiary) and according to the range of predation pressure. Each colony was assigned one of the two levels of predation pressure: zero, when no natural attack was spotted on the tested colonies during the experiment, or one, when the tested colonies were naturally attacked during the experiment. We considered the predation pressure from the European hornet *V. crabro* was negligible, because its natural attacks were very rarely observed in the studied apiaries.

2.2. Simulated hornet attacks

We observed *A. mellifera* defensive behaviour under both natural conditions and simulated hornet attacks; observations were conducted as described by Papachristoforou et al. (2007), with some modifications. Hornets were collected *in situ*, anaesthetized by CO_2 and fixed to a 10 cm transparent nylon thread. Once the hornets had fully recovered, they were brought to the beehive flight board and exposed to colony guards. Hornets were free to move and fly within a range of 10 cm. The test duration was 5 min and consisted of 20 steps of 15 s: at step T_0, we observed the colony activity in natural conditions (no hornet in front of the hive); at step T_1, we positioned the hornet 15 cm from the hive entrance (observations before hornet attacks); at steps T_2–T_{20}, we positioned the hornet on the hive's flight board (observations during hornet attacks). The procedure was video-monitored using a Sony digital camera placed 50 cm from the hive's flight board.

Hive activity, measured as the number of honeybees leaving the hive's flight board, was analyzed only during the first minute after the hornet was introduced in front of the flight board, as we observed that this corresponded to the time required by honeybees to respond. The recorded sequences, each of 1-min duration, were analyzed in slow motion (1/5 of normal speed) by interactive event recording software (LabWatcher, ViewPoint, France).

Video recordings at time T_0 (before attack), T_{10} (after 2 min 30 s) and T_{20} (after 5 min) were transformed into pictures by capturing screenshots in Windows 7. We analyzed pictures with

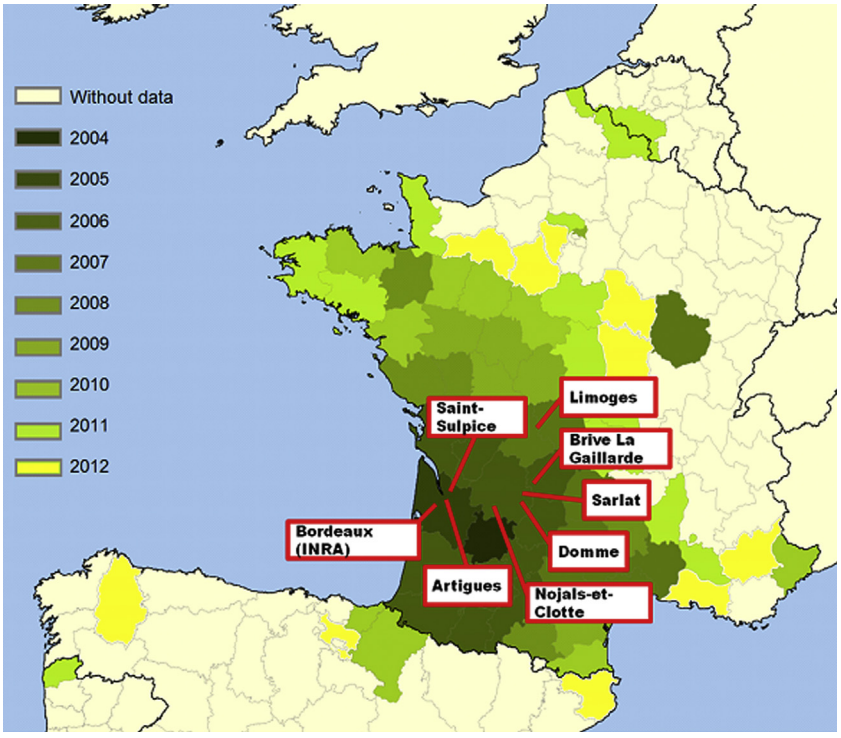

Fig. 1. Map of the locations of apiaries. Green indicates the known range of *V. velutina* in Europe (Rome et al., 2013). (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

ImageJ software and counted the number of honeybees on flight boards during attacks. We carried out these counts at T_0, T_{10} and T_{20} because the guarding behaviour continued to increase for at least 2 min and sometimes took 5 min to reach its maximum. We evaluated the honeybees' ability to form a ball around the hornet by estimating the number of honeybees based on the size of the ball and classifying this estimation as an ordinal value ranging from 0 (no ball) to 4 (more than 30 honeybees in the ball).

We also recorded whether or not the hornets survived to the end of the observation period and the presence/absence of honeybee stings. We used direct visual observation to qualitatively evaluate prey–predator interactions under natural conditions.

2.2.1. Statistical analysis for simulated hornet attack experiment

The activity of the colony, measured as the number of honeybees leaving the nest, and the carpet behaviour, measured as the number of honeybees on the flight board and on the vertical walls near the entrance, are count variables. They were modelled as Poisson variables in a generalized linear model using R statistical software (R Development Core Team, 2010). Five parameters were used as predictors for both count variables independently: (1) the *apiary* in which the experiment occurred, (2) the *colony* (considered as different repeated experiments from an apiary), (3) the *year* when the experiment was performed (2008 or 2010, encoded as a qualitative variable), (4) the *month* of the experiment, and (5) the *time sequencing exposure*, which is the time since the hornet was introduced to the tested colony (encoded as T_0 before the presentation and then increasing by one unit every 15 s).

The significance of the contribution of these five explanatory variables was then tested through a stepwise selection model. Furthermore, the influence of each predictor in the final model (selected by the stepwise process) was tested by comparing the final model with the same model minus the tested predictor. Comparisons were tested in an analysis of deviance computed by chi-square tests.

Both models showed over-dispersion, indicating that they failed to fit all components of the variation of activity and bee-carpet behaviour. The over-dispersion was far more pronounced for bee-carpet (estimated dispersion: 55.7) behaviour than for activity (estimated dispersion: 2.7). Consequently, the final model and the analyses of deviance were performed a second time using a quasi-likelihood approach to account for the over-dispersion and to

Table 1
Vespa velutina survival status after experiments inside the hive.

Colony ID	Experiment duration				Max. temperature in the ball			
	15 min	20 min	30 min	1 h	Mean	s.d.	Lowest	Highest
B1	66.67% (3)	NT	NT	100% (2)	43.8	1.0	42.3	44.4
B2	100% (3)	50% (2)	100% (1)	100% (3)	44.4	0.6	43.5	45.1
B3	0% (3)	0% (2)	0% (1)	100% (3)	43.9	1.4	42.9	44.9
B4	100% (3)	100% (2)	0% (1)	100% (3)	43.6	1.2	41.6	44.4
B5	100% (3)	50% (2)	100% (1)	100% (3)	44.3	0.6	43.5	45
B6	33.33% (3)	NT	NT	100% (2)	43.6	1.2	41.8	45.1
B7	100% (3)	100% (2)	100% (1)	100% (3)	42.8	2.4	40.1	45.4

Percentage of dead hornets per trial. The number of tested hornets appears in parentheses. NT: not tested colony.

confirm whether the selected predictors contributed significantly to the model.

2.3. Balling of hornets

2.3.1. Survival of V. velutina engulfed inside the hive

To test if the honeybees were able to form a ball around and ultimately kill *V. velutina*, we introduced a hornet, prepared as described above, into a hive. This stimulates recruitment of more workers than does the simulated hornet attacks in front of the hive entrance. It also makes it difficult for the hornet to escape. The transparent nylon thread on which the hornet was fixed was limited in length so the hornet was only able to move in a range of 1–2 cm. In total, seven colonies (B1–B7) and 55 hornets were studied in the INRA apiary, for a total of 55 trials (Table 1). To measure the time needed for honeybees to kill the hornet, the experiment was repeated with different durations: 15 min, 20 min, 30 min and 1 h (see Table 1 for experiment duration and number of replicates for each colony studied).

At each time, we recorded the temperature variation inside the hive using two 2 mm micro-probes connected to a highly accurate (±0.1 °C) digital thermometer (YCT RS-232 thermometer). The first probe was attached to the hornet's thorax and the second about 15 cm from the hornet. We recorded the temperature every 30 s (Table 1 shows the means and standard deviations of the maximum temperature recorded) and we determined the survival status of hornets at the end of each trial.

2.3.2. Testing if the highest temperature recorded inside the bee-ball was sufficient to kill the hornets

To test if the highest temperature recorded inside the bee-ball was sufficient to kill the hornets, we conducted an experiment in an incubator. The incubator temperature varied according to a temporal progression similar to the temperature found inside the hives in the previous experiment, up to a maximum of 45 °C—the highest recorded temperature (Table 1). Hornets were captured on site, at the INRA apiary. Each hornet was then transferred into a ventilated transparent cage and placed in the incubator. We conducted four experiments with 27 hornets.

3. Results

3.1. Observations of the natural interactions between honeybees and V. velutina

We observed interactions between honeybees and hornets under natural conditions in both August–October 2008 and August–September 2010, and qualitatively described the general trends of both species' behaviour.

3.1.1. Honeybee response

In response to *V. velutina* the presence around the hive, colonies exhibited three different behavioural patterns. (1) In 42% of observed colonies, a large number of honeybees gathered on the flight board and on the vertical walls near the entrance to form a cluster or a bee-carpet (Fig. 2). (2) In 20% of observed colonies, honeybees on the flight board exhibited a bee-carpet and a coordinated behaviour, with individuals clinging together in groups and following the hornet's movements by turning their body in its direction. We did not observe any shimmering in front of the hive entrance, but we did observe frequent hissing. The level of coordination between honeybees varied among colonies and apiaries. (3) The remaining colonies (38%) did not exhibit any coordinated behaviour; instead, the honeybees dispersed on the flight board and on the front wall of the hive. During our observations under natural conditions, honeybees rarely abandoned the bee-carpet formation

Fig. 2. A honeybee colony under *V. velutina* predation pressure. The foraging activity has totally stopped and a large number of honeybees cover the bee-hive flight board to form a bee-carpet.

to attack the hornet. Only one colony, from the Saint-Sulpice-et-Cameyrac apiary, constructed a propolis wall.

3.1.2. Hornet response

For those colonies that were defended with a coordinated behaviour, hornets never attempted to land on the flight board; instead, they hovered at about 15 cm from the entrance of the hive and tried to catch foraging honeybees returning to and leaving the hive. Hornets are hovering in front of the hive (tended to attack returning foragers loaded with pollen—probably because they were heavier and therefore slower and easier to catch. During our observations at the INRA apiary, we only observed six instances in which a hornet accidentally came into contact with the bee-carpet; of these six instances, guard bees only once engulfed and transported the hornet inside the hive. We never observed a dead hornet in front of the hives. In those hives without any coordinated colony defence, we observed that individual bees were sometimes isolated on the flight board and on the wall of the hive. In such situations, the hornet showed particular interest in the lone bees. It would monitor the isolated bees closely (Fig. 3) and eventually catch them with its legs. When the colony activity was very low and no bee-carpet formed at the hive entrance, hornets entered the hive to steal pollen and honey stores, as well as larvae and pupae. The honey was consumed on site and exchanged with other hornets by trophallaxis. Trophallaxis is the only cooperative behaviour that we observed among

Fig. 3. *V. velutina* attacking isolated honeybees.

Table 2
Test to indicate if the highest temperature recorded inside the bee-ball is sufficient to kill the hornets.

Test	No hornets	Dead at 45 °C	Dead at the end of the test	Alive at the end of the test
1	6	2	6	0
2	9	1	9	0
3	5	1	4	1
4	7	1	7	0

The table gives the number of hornets tested at each experiment in the incubator, the number of hornets dead at 45 °C, the number the hornet dead at the end of the test, the number of hornets alive at the end of the test and the test duration.

hornets in front of the hive. Though patrolling behaviours have been recorded (Monceau et al., 2013b; Monceau et al., in press), *V. velutina* generally operates individually when preying on hives. Here, we observed a number of aggressive interactions between attacking hornets, which probably arose because the hornets came from different colonies, though this cannot be confirmed.

3.2. Simulated hornet attacks

To estimate colony activity, we counted the number of honeybees leaving the hive. The tested colonies showed a common trend: colony activity dropped dramatically from time T_0 (no hornet at the hive entrance) to time T_1 (a hornet positioned at the hive entrance) (predictor *time sequencing exposure* in Table 3).

In addition to the direct effect of the experiment, colony activity was also influenced by all predictors tested. The effects of year and month partly reflected long- and medium-term consequences of hornet predation. Bees tested in October had experienced a much longer period of predation pressure than bees tested in August. Similarly, bees tested in 2010 were exposed for a longer time to predation pressure than bees studied in 2008. Therefore, one might predict reduced activity in the later months, and less activity in 2010 than in 2008. Surprisingly, the impact of month and year on activity appeared to contradict this prediction—activity is lower in the later month in both years but is higher in 2010 than in 2008. This apparent contradiction indicates that there is some correlation between predictors included in the generalized linear model. Tested alone, year and month had the predicted effects on activity; honeybee activity was influenced by both long-term and short-term predation in a similar way. Finally, colony activity varied significantly both between apiaries and between colonies within apiaries (*P*-value: <0.0001).

3.2.1. Bee-carpet behaviour during simulated hornet attacks

Honeybee colonies deployed a bee-carpet when a hornet was either brought close to or placed on the flight board. Interestingly, the number of honeybees involved in defence remained constant after 2–5 min of hornet attacks (Fig. 4; $P > 0.01$, Friedman's test, post hoc multiple comparison). The number of bees on the flight board and on the vertical walls near the entrance increased between T_0 and T_{20} by 30–60% in 14 colonies; by 60–80% in 17 colonies; and by over 80% in 48 colonies. In the remaining 16 colonies, the number of bees did not increase between T_0 and T_{20}—five of these colonies formed a bee-carpet at the beginning of the experiment and the remaining 11 appeared to have slightly increased defensive behaviour and decreased foraging activity.

Bee-carpet behaviour as a colony activity is influenced by predictors other than experimental exposure to hornets. In this case, all predictors had a significant influence (Table 3). However, contrary to the short-term effect of time sequencing exposure (Table 3), the number of guards on the flight board declined with long-term predation, as shown by the impact of month and exposure duration. Furthermore, despite significant variation observed between

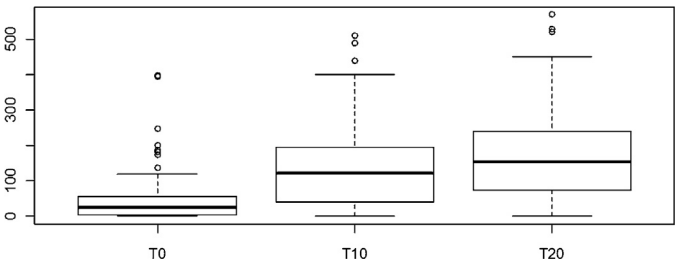

Fig. 4. Box plots of the number of guards on the bee-hive flight board in response to simulated hornet attacks. The box shows the 25th–75th percentile of the number of guards. The horizontal bar inside the box shows the median value. The whiskers represent values within 1.5 times the interquartile range greater than or less than the upper or lower quartile, respectively. The open circles represent values greater than 1.5 times the interquartile range.

apiaries and colonies, no clear relationship emerged between bee-carpet behaviour and colony activity; there were apiaries with high activity and low bee-carpet behaviour, low activity and high bee-carpet behaviour or both low activity and low bee-carpet behaviour.

3.3. Balling of hornets

3.3.1. Balling outside the hive

At T_1, when the hornet was approached at 15 cm from the hive entrance, we never observed balling behaviour. However, honeybees did engulf the hornet in a ball when the hornet was placed directly on the flight board (68 colonies, or 72%, exhibited this strategy; Fig. 5). When the hornet was moved away from the hive entrance, only a small group of honeybees continued to form a ball around the predator. The size of this ball varied: less than 10 bees in 31 colonies (33%); between 10 and 20 bees in 20 colonies (21%); between 20 and 30 bees in 15 colonies (16%); and more than 30 bees in only one colony.

Nine of the hornets (9.5%) used to simulate an attack were killed by bees, and only two of those were stung.

3.3.2. Balling inside the hive

Results showed that honeybees were much more efficient at killing hornets that were introduced inside the hive than those placed on the flight board during simulated hornet attacks: 42 (76.4%) out of the 55 hornets tested inside the hive were found dead (Table 1). Of the 42 dead hornets, only one was stung.

Some colonies appeared to be more effective than others at killing the hornets. For example, colony B7 killed the hornet in all seven repeated experiments. In contrast, colony B3 killed only three

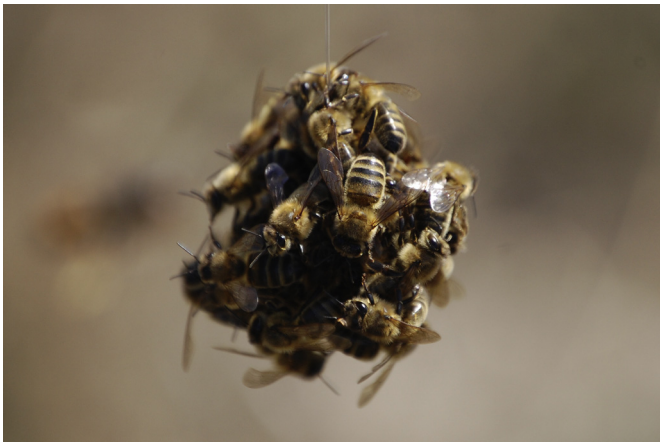

Fig. 5. Balling behaviour of *A. mellifera* in simulated *V. velutina* attacks.

Table 3
Contribution of the predictors to the variation in activity and bee-carpet behaviour.

		Activity			Guarding behaviour		
		P-value	Sign.	Impact	*P*-value	Sign.	Impact
Predictors	Time sequencing exposure	0.0001	***	↘	0.0001	***	↗
	Apiary	0.0001	***		0.0001	***	
	Year	0.91			0.0001	***	↗[a]
	Month	0.0001	***	↘	0.0001	***	↘
	Colony	0.0001	***		<0.0001	***	

The table gives the *P*-values associated with the tests used to compare the global model with all predictors and the model with all predictors except the one tested. Impact indicates whether activity or bee-carpet size increased or decreased when numerical predictors increased. It corresponds to the sign (+ or −) of the coefficient affected by the predictors in the generalized linear model.

[a] Note that the raw effect of the year, not taking into account other predictors, is reversed; bees were less active, on average, and formed smaller bee-carpets in 2010 than in 2008.

of the 9 tested hornets, and took 1 h to do so. Colonies B3 and B7 were of similar strength (based on observation of the colonies after the end of the experiments) so this variability did not seem to be related to colony strength (Table 1).

The success rate at killing hornets also depended on the experimental duration: in 15 min experiments, 75% of hornets were killed; in 20 min experiments, 60% of hornets were killed; in 30 min experiments, 33.3% of hornets were killed. After 1 h inside the hive, 100% of tested hornets were killed.

3.3.3. *Temperature inside the honeybee ball*

The temperature inside defensive honeybee balls increased rapidly. After the first 5–6 min, the temperature remained stable for about 15 min and then decreased slightly, but it never returned to its initial value. On average, the maximum internal ball temperature was 44.0 ± 0.9 °C (Table 1). The rank of ball temperature for each colony, from the highest to the lowest average maximum temperature, was: B2, B5, B3, B1, B4 and B6, B7. The maximum internal ball temperature alone could not explain hornet death. In fact, the lowest maximum value was recorded in colony B7 (40.1 °C), which was able to kill the hornet in all experiments. In contrast, colony B3 (with an average temperature of 43.9 °C) ranked third in its ability to kill hornets.

The mean temperature of the colony recorded by the second micro-probe was 23.8 ± 2.7 °C while the mean environmental temperature during the experiment was 22.8 ± 2.0 °C.

3.3.4. *Hornet death under elevated temperature conditions*

When hornets were placed inside an incubator, only five (18%) died when the temperature reached 45 °C (Table 2). Only one hornet was still alive after 1 h at 45 °C (Table 2).

4. Discussion

In France, when the *V. velutina* population is at its maximum in late summer and early autumn, honeybees are severely impacted by predation (Monceau et al., 2013a, 2013b). *In situ* observations revealed that the predatory hornet adopts stationary flight around and near the entrance until it can intercept a forager returning to or leaving the hive (Monceau et al., 2013b). This strategy, called *beehawking*, has been described in *V. velutina* across its natural range (Ken et al., 2005; Tan et al., 2007).

In response to the presence of the new predator, and in order to reduce predation pressure, the European honeybees have adopted a short-term response that appears to be inefficient. This strategy usually involves reducing colony activity then coordinating a tight group of workers on the flight board or on the vertical walls near the entrance, forming a bee-carpet. The same strategy has been observed in response to natural predators such as *V. crabro* and *V. orientalis*, though it is an efficient defence against natural predators (Baracchi et al., 2010; Papachristoforou et al., 2011).

Colony defence via reduction in foraging activity and formation of the bee-carpet is a general trend that could be explained by the recruitment of foraging honeybees for defence, as proposed in a comparative study of the defensiveness of *docile* and *super-aggressive A. mellifera* colonies (Kastberger and Thenius, 2009).

On the other hand, the effect of predation on foraging activity and bee-carpet behaviour appears to change over the medium- and long-term; both behaviours decrease when predation pressure increases over months within a year and over years. Fewer bees were involved in the bee-carpet in colonies tested in September and October, when predation pressure was higher, compared to those tested in August, when predation pressure was lower (Monceau et al., 2013a, 2013b). Similarly, colonies tested in 2010 (data from apiaries exposed to hornets for a long time) had fewer guards on the flight board than those tested in 2008 at the same of year (data from apiaries exposed to hornets for a shorter period). These opposing effects of long/medium-term and short-term predation may be due to different factors. The decreased response to predation pressure could be related to a specific strategy in which colonies retreat into the hive to defend their nest (Kastberger and Thenius, 2009; Papachristoforou et al., 2011). The low colony activity recorded in September and October could also be linked to lower food availability; forager activity may be lower at this time of the year due to flowering phenology. It could also be linked to a natural decrease in the bee population size in autumn or to a weakening of the colony after continuous predation by hornets in the preceding months. However, to distinguish between these different possible reasons for the apparent reduced defensive response over time, it would be interesting to know the strength and survival status of the colonies after several months of hornet predation. Unfortunately, it is difficult to obtain such information because beekeepers often replace lost colonies with new swarms—a practice that is not well documented.

However, *A. mellifera* defensive behaviour against the invasive predatory hornet *V. velutina* appears to be less efficient than the behaviour observed when prey and predator are sympatric (Abrol, 1994; Kastberger and Sharma, 2000; Ken et al., 2005; Abrol, 2006). When *V. velutina* approach clusters of guard bees, the bees seemed unable to seize and hold the engulfed hornet. We observed only three instances of honeybees leaving the cluster in an attempt to capture the attacking hornet. Over many days of observations in several apiaries and under variable environmental conditions, we observed no instances of balling behaviour, although this defence has been described in response to other predator species (Ono et al., 1987, 1995; Papachristoforou et al., 2007; Baracchi et al., 2010). In France, defensive balling behaviour occurred only in response to experimentally simulated *V. velutina* attacks. However, the number of bees that engulfed predatory hornets was much lower than has been observed in *A. cerana* (Ono et al., 1987, 1995; Ken et al., 2005; Abrol, 2006), *A. m. cypria* (Papachristoforou et al., 2007) and *A. m. ligustica* (Baracchi et al., 2010). However, it was similar to

that observed for *A. mellifera* against *V. velutina* in China by Ken et al. (2005). Only 9.5% of the hornets tested were killed by balling within the 5 min of simulated attacks while about 22.2% died of a sting—a defensive strategy that is absent among sympatric honeybees and hornets. Nevertheless, the honeybees were only able to kill the hornet when it was artificially introduced inside the hive over a long period. We demonstrated that *A. mellifera* in France produced a maximum temperature that could be sufficient to kill the hornet. However, while incubator experiments indicate that the temperature could be lethal, other possible reasons in natural conditions cannot be excluded, like the production of CO_2 (Sugahara and Sakamoto, 2009) or limits to the function of the hornet's respiratory system (Papachristoforou et al., 2007) or the bee stings and the toxic action of the venom (Baracchi et al., 2010). Furthermore, the time required to kill the new predator at high temperature is more than 30 min in the incubator. The experiments contacted in the incubator were preliminary. Future experiments have been designed in order to determine the exact time of death of each hornet at each temperature. Furthermore, factors other than temperature, or in combination with temperature, should be investigated further in the future. Indeed, colonies of the same strength and that exhibited similar temperatures inside the ball differed in their ability to kill hornets.

When foraging activity and number of honeybees involved in the bee-carpet were considered, honeybee defensive behaviour varied among apiaries and colonies. Some colonies invested more in foraging activity and less in defence, while other colonies did the opposite. Such variation may be explained by differences in colony strength and stores or different climatic conditions among apiary locations—these should be addressed in future studies. It could also be related to the genetic composition of the different colonies (e.g., at the subspecies level). Indeed, in France, *A. m. mellifera* (lineage M) is the native subspecies, but *A. m. ligustica* and *A. m. carnica* (lineage C) are also widely used (Rortais et al., 2007).

Many studies (Rothenbuhler, 1960; Stort, 1974; Collins, 1980; Collins et al., 1988; Moritz et al., 1985; Guzmán-Novoa and Page, 1993) have demonstrated the importance of genetics in honeybee aggressiveness and defensiveness, with some strains being more aggressive than others (Ruttner, 1988; Breed et al., 2004). European races of *A. mellifera* are the most widely used honeybees, and genetic selection programmes usually favour calmness. Selection for less aggressive bees—the inability to attack bee-hawking hornets in front of the hive entrance and to rapidly recruit a sufficiently large number of honeybees for balling behaviour—seems to at least partly explain the ineffective defence.

Direct attacks on hornets by honeybees are also ineffective because of *V. velutina*'s highly effective predatory behaviours. In contrast to other well described predatory habits observed in other *Vespa* species (Ono et al., 1995; Papachristoforou et al., 2007; Baracchi et al., 2010), *V. velutina* only attempted to land on the honeybee hive flight board and enter the hive after the colony had become too weak to react; instead, they tended to maintain stationary flight and catch foraging honeybees at safe distance.

Clearly, *V. velutina* has proven to have high predatory potential against the major prey encountered in its novel habitat. Its predation strategy appears to be very costly for the honeybee colonies. Contrary to honeybee species that are sympatric with *V. velutina*, *A. mellifera* in France did not develop an efficient defensive strategy at the colony level. The constant presence of the hornet in stationary flight in front of the hive entrance reduced foraging activity and stimulated a bee-carpet defensive behaviour that incorporated large number of honeybees. Future studies should focus on the progressive weakening of colonies in response to predation pressure from *V. velutina* to better understand if this weakening is the result of reduced flow of food from the environment. It may either result from an indirect reduction in overall foraging behaviour in response to predation risk or from a direct reduction in the number of available foragers because of wasp predation. Furthermore, additional studies should also measure the relationship between predation and honeybee colony survival.

V. velutina, therefore, appears to be an additional cause of the progressive decline in *A. mellifera* colonies; its expansion across Europe represents a potential future threat to European honeybees.

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