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Defence reactions of *Apis mellifera ligustica* against attacks from the European hornet *Vespa crabro*

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The predatory behaviour of *Vespa crabro* hornets on the honeybee *Apis mellifera ligustica* and the counter strategy of the prey were observed and described in two apiaries of 27 and 41 colonies in Central Italy. Observations were carried out in the second half of October and accompanied by experiments aimed at determining the mechanisms of honeybee defence. We confirmed that *V. crabro* represents a relatively mild predator for *A. mellifera ligustica* but it can have a considerable impact on already debilitated colonies. Hornet predatory strategy is to spot then swoop on returning foragers. Honeybee defence centres on forming packed aggregations of individuals near the hive opening, which probably deters the wasps from entering. Coordinated behaviour by the bees, which cling together in groups, can knock the approaching hornets down, which are then totally covered by the bees. This balling behaviour has the effect of over-heating the predator whose lethal thermal limit is about 44 °C, although other factors, such as carbon dioxide emission and the release of venom by the honeybees, may also contribute to the death of the hornet. Comparing the differences and similarities of this behaviour with those observed in other species of *Apis* and *Vespa* reveals that these two species are an interesting model for the study of the evolutionary arms race.

**KEY WORDS:** *Apis mellifera*, honeybee, *Vespa crabro*, hornet, hot balling, defence behaviour.

**INTRODUCTION**

A honeybee colony is a huge resource of carbohydrates (honey) and proteinic substances (pollen, larvae and adults), making it the target of a large number of potential vertebrate and invertebrate predators, including conspecifics. Among insects, some
hornet species are the most important honeybee predators in the world (Matsuura & Sakagami 1973). The hornets often attack honeybee hives to steal the larvae and some species can easily destroy a bee colony. For example, a few workers of the Asian giant hornet Vespa mandarinia can exterminate a hive with a population of 30,000 bees in just a few hours (Matsuura & Sakagami 1973). Once the hornets have rid the hive of all its defending bees, they will feed on the honey and carry the bee larvae corpses back to their own colony to rear their developing brood. Moreover, different hornet species have been reported to prey on different honeybee species: Matsuura & Yamane (1990) reported V. simillima, V. analis and V. crabro attacking hives of Apis mellifera and Apis cerana in Japan. V. tropica has been seen preying on small colonies of A. mellifera and A. cerana in Thailand (Burgett & Akratanakul 1982). V. orientalis is known to be a serious apicultural pest in Mediterranean countries (Alber 1953; Wafa 1956; Ishay 1964), while in India, Singh (1962) reports attacks by V. velutina, V. orientalis, V. basalis and Koeniger et al. (1994) observed V. affinis assailing drones of A. cerana. V. multimaculata has been observed attacking nests of Apis nuluensis in East Borneo (Koeniger et al. 1996).

In the evolutionary arms race between prey and predator, honeybees have evolved various defence mechanisms against hornet attacks. Ono et al. (1987, 1995) were the first to describe the ability of A. cerana to defend itself against the Asian giant hornet V. mandarinia. In fact, although the small stings of the bees cannot inflict much damage on such a large predator, A. cerana uses a collective defence mechanism against them. As a hornet enters a nest, a large number of bees surround it (“balling behaviour”), raising the temperature to as high as 47 °C, which is lethal for the hornet but not the bees. On the other hand, a very recent paper by SugaHara & Sakamoto (2009) stresses how not only heat but also carbon dioxide generated in the bee balls contributes to the death of the honeybee workers themselves.

Recently, Papachristoforou et al. (2007) reported that A. mellifera cypria uses a balling defence against V. orientalis. In this case, as the lethal temperature threshold is considerably higher in V. orientalis, the honeybees kill the hornets by asphyxia, blocking abdominal pumping and thus indirectly increasing carbon dioxide concentration. Moreover, Papachristoforou et al. (2008) observed that A. mellifera cypria produces a characteristic high-frequency hissing sound during V. orientalis attacks, which could be an alert signal for the colony.

Although the European A. mellifera introduced to Oriental regions are unable to defend their colonies against V. mandarinia attacks (Matsuura & Yamane 1990), Ken et al. (2005) demonstrated that in China, A. mellifera does use “bee balling defensive behaviour” against V. velutina. The aggressive behaviour of A. cerana is, however, more efficient than that of A. mellifera colonies, in terms of both the number of recruited workers and the increase in balling temperature. Moreover, under hornet attack, foraging activity in the former species was significantly reduced compared with that of the latter (Ken & Wang 2004; Ken et al. 2005; Tan et al. 2007).

No detailed studies exist regarding the defence behaviour of A. mellifera against the European V. crabro. V. crabro is the natural sympatric predator of A. mellifera ligustica and its presence in Italy has rapidly increased in the last decade, although information on this subject relies solely on anecdotal data. The alleged spread of the species has probably raised the frequency of hornet attacks on honeybee colonies, and it is by no means a negligible problem for beekeepers. Although V. crabro is unable to completely destroy a bee colony like V. mandarinia, the high predation rate, especially at the end of the summer, can be extremely costly, especially for debilitated colonies. The discovery of dead hornets in front of bee hives suggests that A. mellifera ligustica can perform effective defensive mechanisms against hornets and indeed kill them.
The aim of this study is to investigate the predator–prey relationship between *A. mellifera ligustica* and *V. crabro*. Particular attention was paid to the rate of hornet attacks on beehives, their hunting strategy and the defence reaction of bees, observed in two apiaries in Central Italy.

**MATERIALS AND METHODS**

**Behavioural data of hornet–honeybee colony interactions**

In the second half of October 2009 we studied two apiaries near Florence (site A and site B), consisting of 27 and 41 *A. mellifera ligustica* colonies respectively. Behavioural data regarding the interactions between hornets and honeybees were collected in the apiaries, both directly by observing hornet attacks and indirectly by analysing brief (up to 300 frames/sec) video sequences recorded with a digital photocamera (Casio Exilim EXF1).

**Colony observations**

Each colony at sites A and B was observed for three different days, 10 min per hour (from 10:00 am to 5:00 pm). The number of hornet visits and number of bees seized, foragers and bees at the entrance (including the presence/absence of particular “bee-carpets” formed by tight groups of workers on the beehive platform or on the vertical walls near the entrance) were estimated for each colony through direct observations and 1 minute long video-recording sessions. The environmental temperature was continuously monitored with an electronic thermometer.

For 27 beehives (apiary A), we also calculated approximate colony “strength”, i.e. the size and health of the colonies (number of wood frames covered by bees, number of wood frames with immature brood and number of wood frames with food reserves). Finally, we counted the number of dead hornets found in front of each beehive in apiaries A and B.

**Bee ball formation and balling temperature**

In an experiment conducted on the 27 colonies in site A, a dead hornet, tied to the tip of a 50 cm long thin stick, was introduced 5 cm in front of the beehive entrance. After 10 sec from the onset of bee ball formation, we counted the number of workers involved by putting the bee ball into a nylon bag.

For the “temperature balling experiments” we used 31 colonies from site B. Dead hornets (captured directly in the field and rapidly killed at high temperature) were tethered with a fine fishing-line to the tip of a 5 mm diameter, 35 mm long electronic sensor hanging from a 50 cm thin long stick and connected to a thermometer by means of an electric wire. The hornets were suspended close to the beehive entrance (about 5 cm away) and were soon covered with bees. The rise in temperature in the core of the bee ball, whenever it formed after presentation, as well as environmental temperature, was checked every 30 sec for 10 min. In all experiments the dead hornets were replaced after every three presentations.

**V. crabro lethal thermal limit experiment**

To determine the *V. crabro* lethal temperature threshold, 20 hornets were captured at site B and treated soon after arrival in the laboratory. Each hornet was transferred to a glass vial (15 mm in diameter and 100 mm long). The opening was blocked with an electronic thermo sensor.
The temperature source for the experiments \((n = 10)\) was a lamp bulb placed above the glass vials, and we increased the temperature (in rates ranging from 0.5 to 2.0 °C/30 sec) by bringing the lamp bulb closer to the glass vials. Ten hornets (controls) were left in glass vials at a constant temperature of 20.0 °C.

**Detection of honey bee venom on killed hornets by MALDI-TOF MS analysis**

To ascertain whether bee venom was present on the cuticle of *V. crabro* killed by the bees, three such hornets and an equal number of hornets captured in flight (and subsequently killed by freezing) were washed in 400 μl of methanol for 2 min. Methanol extracts from the entire body of each individual were analysed with a MALDI-TOF/TOF Ultraflex III (Bruker Daltonics, Bremen, Germany). The instrument was operated in positive ion reflector mode. The accelerating voltage and Ion Source 2 were set at 25.0 and 21.9 kv, respectively and delay time was 20 nsec. The matrix for the MALDI-TOF experiments was a solution of α-cyano-4-hydroxycinnamic acid (α-CHCA) (10 mg/ml) dissolved in 70/30 acetonitrile/TFA 0.1%. One microlitre of the sample was mixed with the MALDI matrix (1:1, vol:vol) and the mixture spotted on a stainless steel target; 800 shots were automatically accumulated for each spectrum. External calibration was performed with the Bruker Standard Peptide Calibration kit (m/z 1000–3500) and the peptidic fraction of the samples was acquired in the range m/z 800–4000. Similarly, we prepared and analysed honey bee venom extracted from 10 honeybee workers according to the method described by BARACCHI & TURILLAZZI (2009). Honeybee venom is characterised by well-known peptides (apamin, MCD, melittin) which were sought in the MALDI spectrum of the killed hornet cuticles.

**Data analysis**

The Spearman test was used to check for any correlations between the number of foragers, the presence or absence of “bee-carpets”, the presence of bees at the beehive entrance and the rate of hornet attacks, and the number of bees captured. The same test was also applied for testing possible correlations among environmental temperature, honeybee ball core temperatures and the number of bees forming the balls.

**RESULTS**

**Behavioural data for hornet–honeybee colony interactions**

The hornet hunting strategy includes various tactics: *V. crabro* workers can prey on honeybees (and other insects) whilst engaged in foraging activities, or they can hover near the hive entrance in a search for recently dead, tired and debilitated workers. Rarely, hornets will approach the hive entrances to catch freshly landed foragers, but instances of hornets taking bees from groups of individuals (“bee-carpets”) which sometimes form near the entrance were not observed. More often, hornets catch individual honeybees in flight, hovering in front of the hive entrance and waiting for them to arrive. After catching a bee, the hornet has to take it to a perch for processing before taking it back to its nest. The perch may be a tree branch and, in some cases, it is repeatedly used by the same hornet. The predator grasps the bee with its forelegs and first bites the head, killing it instantly. The processing of the corpse continues with the elimination of the head, legs and wings (Fig. 1). Sometimes, before departing for its nest, the hornet may cut off the bee’s gastrum, after partially chewing it to extract the honey bag. When a hornet approaches in flight, a group of bees on the platform in
Honeybee defence behaviour against hornets

front of a hive survey them and perform a kind of “hola” wave by raising their bodies when the hornet hovers near them. This behaviour has been noted and described before in A. mellifera with the term “body shaking” (Butler 1974) and in other species such as A. cerana (Sakagami 1960), A. nuluensis (Koeniger et al. 1996), A. dorsata (Kastberger et al. 1998) and A. florea (Pirk et al. 2002). Breed et al. (2004) summarised these observations as “body shaking”, the behaviour when “bees massed at the nest entrance or curtaining exposed comb synchronously raise their abdomens, creating a ripple effect that impedes the hornet from landing”. We did not observe any actual abdomen raising, rather a tendency for the bees to turn towards the flying hornet when it hovered near them. The bees which most actively keep the hornet under surveillance are always in contact with other bees in the group (Fig. 2). If the hornet gets closer, the bees try to catch it by waving their anterior legs and keeping their mandibles open. They may hold their mandibles open to disperse the mandibular pheromone heptanone, involved in defence functions and in the recruitment of other bees (Vallet et al. 1991). If some of the defenders succeed in seizing the predator, their rearward mates hold on to them tightly (“clinging” behaviour); thus chains of bees are formed that can knock the hornet down and subsequently cover it in a group (“balling” behaviour). Thus, perfect coordination among the defenders, their spatial proximity and the formation of body chains are all necessary to deal with the predator; “ball formation” certainly implies communicative devices to increase the number of bees involved in the defence (Fig. 3). In fact, if only a few bees are engaged in blocking a hornet, the latter can easily get rid of them and fly away to start hunting again in just a few minutes.

Finally, we did not observe the emission of any particular sounds from a group of defending bees during hornet attacks. However, piping sounds, which could be similar to the “shimmering” first reported by Butler (1974), were audible from various hives which were not under direct attack, when several hornets were flying around the apiaries.
Colony observations

The hornets start to visit the apiaries early in the morning at about 9:00 am, going on until about 5:00 pm and reaching maximum activity during the warmest hours of the day (1:00 pm–3:00 pm). From 11:00 am to 5:00 pm, the number of hornet visits to each colony during the 10 min observation periods was 27.6 ± 23.0 \( (n = 27) \) in apiary A and 6.9 ± 5.6 \( (n = 41) \) in apiary B, while the number of bees caught was 1.4 ± 1.97 and 0.7 ± 0.9 respectively (see also Table 1). We can estimate that on average the beehives in the two apiaries suffer a loss of up to 65 ± 17.1 and 6.3 ± 8.1 bees in a single day respectively, and that the hardest-hit beehives undergo a loss of up to 65 and 38 bees, respectively.

As shown in Fig. 4, hornet visits varied considerably among the hives: many beehives received no visits at all, while others received a high rate of hornet visits, in spite of the fact that all the hives were next to each other in a row. As the hornets were not marked for our observations, we cannot say whether the hornets preferred any particular colony in general (i.e. a lot of different hornets independently plundered the same beehives), or not (i.e a few hornets repeatedly plundered the same colony they recognised).

We did not observe any behaviour by the attacking hornets that could suggest evidence of collaboration between them at either site; on the contrary at site A we observed various aggressive interactions between attacking hornets. In most cases these actions were limited to flight clashes but on two occasions we observed a fierce
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The number of hornet visits to the beehives was strongly correlated with the number of foragers (Spearman correlation test, \( n = 68, r = 0.65, P < 0.001 \)), the number of bees caught (Spearman correlation test, \( n = 68, r = 0.57, P < 0.001 \)) and the size of the “bee-carpets” (Spearman correlation test, \( n = 68, r = 0.45, P < 0.001 \)). Moreover, “bee-carpets” size near the hive entrance was also correlated to the number of foragers (Spearman correlation test, \( n = 68, r = 0.60, P < 0.001 \)). Interestingly, the number of foragers was significantly correlated with colony “strength” (see Materials and Methods) (\( n = 27, r = 0.66, P < 0.001 \)), furnishing a good external estimate of colony quality.

We directly observed nine cases in the two apiaries in which assaulting hornets were emballed and killed by the bees. The balls dissolved after a minimum of roughly 20 min, leaving the wasps dead or dying.

Table 1.
Mean and standard deviation of number of hornet visits, number of caught bees, number of guards and number of foragers counted in the beehives in apiaries A and B.

<table>
<thead>
<tr>
<th></th>
<th>No. of hornet visits</th>
<th>No. of caught bees</th>
<th>No. of guards</th>
<th>No. of foragers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site A</td>
<td>27.67 ± 23.00</td>
<td>1.44 ± 1.97</td>
<td>425.70 ± 206.98</td>
<td>439.48 ± 206.31</td>
</tr>
<tr>
<td>Site B</td>
<td>6.93 ± 5.68</td>
<td>0.68 ± 0.93</td>
<td>148.10 ± 258.32</td>
<td>232.3 ± 151.91</td>
</tr>
<tr>
<td>Total average</td>
<td>15.16 ± 18.14</td>
<td>0.99 ± 1.47</td>
<td>258.32 ± 213.47</td>
<td>314.76 ± 201.94</td>
</tr>
</tbody>
</table>

Fig. 3. — A bee ball forming around a hornet lure suspended from a fishing line. Bees cling to each other to block the hornet.
The number of accumulated dead hornets found in front of the hives varied considerably over the season (\( n = 72 \), median = 1, range 0–16), but there was no correlation between the number of dead hornets and number of hornet visits (Spearman correlation test, \( n = 68 \), \( r = -0.01 \), \( P = 0.97 \)), the number of bees caught (Spearman correlation test, \( n = 68 \), \( r = -0.73 \), \( P = 0.55 \)), “bee-carpet” size (Spearman correlation test, \( n = 68 \), \( r = 0.11 \), \( P = 0.39 \)) or number of foragers (Spearman correlation test, \( n = 68 \), \( r = 0.13 \), \( P = 0.28 \)) recorded during the observation period.

**Bee ball formation and balling temperatures**

When killed hornets were presented at the beehive entrance, guards and other honeybees engulfed them in balls of 14.3 ± 7.4 workers within 10 sec (\( n = 27 \), range 6–38), but in another experiment 135 bees were counted in the ball. This accounts for the great variability in response to a possible threat from different colonies. A similar variability was recorded in the temperatures reached in bee balls formed by different colonies in the experiment with dead hornets mounted on a thermal sensor. On average, at the end of 10 min the maximum temperature recorded at the core of the bee balls was 39.9 ± 7.4 °C (\( n = 31 \)). Nearly 42% of colonies could reach temperatures of over 43.0 °C, while the other 57.9% could not (Fig. 5). The highest temperature measured in the honeybee ball core was 44.4 °C in a single colony. The environmental temperature during the experiment averaged 17.21 ± 2.4 °C, while the difference between the environment and bee balls temperatures averaged 17.7 ± 7.8 (range = 1–30).

The number of worker bees forming the ball around the hornet was strongly correlated to the maximum temperature reached by each colony (Spearman correlation test, \( n = 31 \), \( r = 0.73 \), \( P = 0.02 \)).

Fig. 4. — Difference in hornet visits to the colonies of the two study apiaries.
test, $n = 16$, $r = 0.86$, $P < 0.001$). A strong correlation was also found between the maximum temperature each colony reached and the environmental temperature (Spearman correlation test, $n = 31$, $r = 0.87$, $P < 0.001$).

**Determination of V. crabro lethal thermal limit**

The lethal thermal limit of *V. crabro* determined with our measurements was approximately $44.2 \pm 0.5 \, ^\circ C$ ($n = 10$); different rates of temperature increase did not influence the lethal thermal limit (Spearman correlation test, $n = 10$, $r = 0.009$, $P = 0.98$). The lethal temperature refers to the complete immobilisation of the wasps, yet above $40 \, ^\circ C$ their vital capacities already seemed compromised. In contrast, after 90 min from the start of the experiment, all the hornets used in the control trials ($n = 10$) were completely healthy.

**Use of venom and MALDI-TOF MS analysis**

Fig. 6 reports the spectra ranging from 950 to 4000 Da for the total body methanol extracts of each hornet killed by bee balls in the field ($n = 3$) (Fig. 6A–C), the average spectrum of hornets killed by freezing ($n = 3$) (Fig. 6D) and the average spectrum of the venom of 10 honeybee workers (Fig. 6E) obtained by MALDI-TOF mass spectrometry analysis. The reference spectrum for bee venom is quite simple and is mainly composed of three main peaks: apamin (2027 Da), MCD (mast cell degranulating peptide, 2658 Da) and melittin (2856 Da) (GMACH & KREIL 1995; DE LIMA & BROCHETTO-BRAGA 2003; BARACCHI & TURILLAZZI 2009). All three venom peptides were found in high amounts in the extracts from hornets killed by bee balls (Fig. 6A–C), while they were totally absent in the extracts from hornets killed by freezing (Fig. 6D). All the other peaks of the medium-weight polar compounds present in the extracts of hornets killed by bee balls or killed by freezing belong to the hornets’ epicuticle.
DISCUSSION AND CONCLUSIONS

Field observations carried out in October–November 2009 on apiaries of *A. mellifera ligustica* in Central Tuscany confirmed that workers of *V. crabro* repeatedly attack the beehives. Not all the colonies undergo the same rate of predation, and sometimes adjacent beehives are subjected to very different attack rates during the day. In our observations we did not notice evidence of group predation similar to that described for *V. mandarinia* (Ono et al. 1995), where a mass attack against *Apis* colonies is initiated by a single scout hornet depositing a foraging-site marking pheromone that induces nest mates to congregate and attack the marked beehive. Nevertheless, predation by *V. crabro* can be a serious problem for a colony already weakened by other causes.

Our study demonstrates a strong correlation between the number of hornet visits to a particular beehive and the number of incoming and outgoing foraging bees. This
finding suggests that hornets are attracted by the flying bees and this is in line with our
direct observations on the apiaries. The fact that we did not observe a hornet plunder-
ing a bee from a “bee-carpet” also suggests that this aggregation is a defensive strategy
against these predators, although clusters of bees in the front of the hive may occur for
other reasons, for example induced by particular climatic conditions. “Bee-carpets”
are in fact larger in beehives with higher forager flow (where hornet predation is also
high) but carpet size is not related to any other parameter, including colony size and
environmental temperatures. Direct observation of bees forming the carpets shows
that they are particularly reactive to any disturbance, but do not perform other par-
ticular behaviours such as wing fanning or flying attacks. Further experiments are
necessary to confirm this hypothesis. KOENIGER et al. (1996) describe a very similar
reaction in A. nuluensis to an approaching hornet (V. multimaculata) as the “bees per-
form body shaking behaviour (Korperschuttel) join together in a tight group and
keeping in close contact with each other. When the hornet attacks a group member,
some will immediately cling to their nest mates, while the others rush to surround the
predator with a ball of bees”.

In our observations we did not notice any particular shaking behaviour or expo-
sure of the Nasanov gland, similar to that described in other species: shaking A. dors-
sata and A. nuluensis exposed their Nasanov glands, suggesting a possible function of
the latter in defensive communication (KONIGER et al. 1996; KASTBERGER et al. 1998).

“Shimmering”, on the other hand, is an audible high-pitched hissing sound (per-
haps piping) produced in the case of direct contact with the threat (BREED et al. 2004).
Whilst the alarm pheromone is the primary mode of communication and recruitment
among defensive workers in Apis, shimmering seems to stimulate other workers to
“hiss”. Shimmering serves as an alarm signal in A. florea; the hissing noise alerts work-
ers to the presence of a threat and may deter small predators (SEN SARMA et al. 2002).
A. dorsata workers also “hiss” when the nest is threatened (SEELEY et al. 1982), and in
A. nuluensis tactile contact stimulates shimmering whilst visual cues cause shaking
(KOENIGER et al. 1996). PAPACHRISTOFOROU et al. (2008) also described a high-frequency
sound produced by A. mellifera cypria under attack by V. orientalis but we did not asso-
ciate any definite sound with any particular colony under attack during our observa-
tions. Colonies did produce occasional piping sounds when the hornets were flying
around them during the day: it is possible that colonies under attack for several days
perform this behaviour only when the hornets assault them for the very first time.

Dead hornets found in large numbers under some beehive platforms and direct
observation of events demonstrate that A. mellifera ligustica is able to counteract
V. crabro predatory pressure with a defence strategy. Part of this strategy consists of
knocking down and killing the flying hornets through coordinated honeybee group
activity. As already mentioned, “balling” behaviour is described in A. mellifera against
V. velutina (KEN et al. 2005) and in the subspecies A. mellifera cypria against V. orienta-
lis (PAPACHRISTOFOROU et al. 2007). However, while the latter study shows how the par-
ticular balling behaviour of A. mellifera cypria is adapted to the biological
characteristics of its predator (very high lethal thermal threshold compared with that
of V. crabro), the first study stresses the poorly adapted response of A. mellifera
towards a non-co-evolved predator like V. velutina. Balling behaviour in A. mellifera
ligustica seems quite adequate to counteract V. crabro predatory behaviour, providing
a sign of long co-adaptation in a temperate environment, while it seems totally inade-
quate against other predators such as V. orientalis (cf. an interesting report by a bee-
keeper on a recent invasion of hornets in Sicily which, judging from the illustrations,
evidently refers to V. orientalis rather than V. crabro – CARONIA 2009). Balling bees can
kill a hornet worker in less than 20 min, reaching temperatures that in our experiments approached the lethal thermal limits of the wasp. Considering that the duration of bee balls is far longer than the experimental times, we maintain that over-heating is probably sufficient to cause the death of the predator. This does not happen with *V. orientalis*, which has a much higher lethal thermal limit than *V. crabro* (50.5 °C vs 44.2 °C) (PAPACHRISTOFOROU et al. 2007); however, the concomitance of other factors, such as the increase in carbon dioxide causing asphyxia, cannot be ruled out. It is worth noting the wide variance in this behaviour and that more than half of the colonies were not so reactive to the lures, and in most cases the balls showed only very low increases in temperature: this could be due to the use of dead hornets in the experiment but it could also indicate that not all the colonies can perform successful defence against these predators. Of course, the success of the reaction depends on various circumstances, such as the number of bees available for the defence, environmental temperature, aggressiveness of the predator and the efficiency of the recruiting system in the bee ball. This last aspect is quite important and not completely clear, as in other species: ONO et al. (1995) indicate the alarm pheromone as the principal ball recruiting agent in *A. cerana*, while KEN et al. (2009) observe that the signal for the formation of the ball may be a sudden increase in temperature of the guarding bees. In our study, the use of venom by *A. mellifera ligustica*, which literally covered the body of the “emballed” hornet, could support the hypothesis of ONO et al. (1987) but it also raises some questions concerning the contribution of the venom to the death of the predator. Even if we only observed three cases out of dozens of presentations when the bee stings were thrust into the corpses of the killed hornets, the toxic action of the venom could become decisive for causing the death of the wasp if it penetrates through its spiracula or mouth. Again, further experiments are necessary to test this possibility.

We must stress the fact that counter-predatory group behaviour against hornet attacks is widespread in the genus *Apis* and represents an ancestral character of its social behaviour. Certain behavioural patterns have remained the same over the co-evolution of various species of *Apis* and *Vespa*, such as abdomen shaking, balling, heating, etc. But both prey and predator have adjusted their physiology and behaviour towards each other and, as a result, we now observe wide differences in attack and defence tactics. For this reason the *Apis–Vespa* duo is an extremely interesting system for studying the co-evolutionary arms race.

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