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ORIGINAL ARTICLE

Long-term spatial memory in *Vespula germanica* social wasps: the influence of past experience on foraging behavior

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Abstract Social insects exhibit complex learning and memory mechanisms while foraging. *Vespula germanica* (Fab.) (Hymenoptera: Vespidae) is an invasive social wasp that frequently forages on undepleted food sources, making several flights between the resource and the nest. Previous studies have shown that during this relocating behavior, wasps learn to associate food with a certain site, and can recall this association 1 h later. In this work, we evaluated whether this wasp species is capable of retrieving an established association after 24 h. For this purpose, we trained free flying individuals to collect proteinaceous food from an experimental plate (feeder) located in an experimental array. A total of 150 individuals were allowed 2, 4, or 8 visits. After the training phase, the array was removed and set up again 24 h later, but this time a second baited plate was placed opposite to the first. After 24 h we recorded the rate of wasps that returned to the experimental area and those which collected food from the previously learned feeding station or the nonlearned one. During the testing phase, we observed that a low rate of wasps trained with 2 collecting visits returned to the experimental area (22%), whereas the rate of returning wasps trained with 4 or 8 collecting visits was higher (51% and 41%, respectively). Moreover, wasps trained with 8 feeding visits collected food from the previously learned feeding station at a higher rate than those that did from the nonlearned one. In contrast, wasps trained 2 or 4 times chose both feeding stations at a similar rate. Thus, significantly more wasps returned to the previously learned feeding station after 8 repeated foraging flights but not after only 2 or 4 visits. This is the first report that demonstrates the existence of long-term spatial memory in *V. germanica* wasps.

Key words foraging behavior; learning; long-term memory; relocating behavior; social wasps

Introduction

The location of resources in natural environments may be uncertain, given that contexts constantly change. Accordingly, behavioral flexibility is linked to diverse learning and memory processes (e.g., D'Adamo & Lozada, 2007, 2008, 2009, 2011; Lozada & D'Adamo, 2009, 2011, 2014; Geva *et al.*, 2010; Wilson-Rankin, 2014, 2015). The successful establishment and possible spread of invasive species into new territories may be due, among other factors, to behavioral plasticity when facing situations of change (e.g., Beggs *et al.*, 2011; Lozada & D'Adamo, 2014). *Vespula germanica* is a social wasp that has invaded several regions around the world, including Argentina, over the last decades (Beggs *et al.*, 2011). Previous studies have demonstrated that this wasp species displays diverse cognitive abilities while foraging, which could contribute to its invasiveness (D'Adamo & Lozada, 2007, 2008, 2009, 2011; Lozada & D'Adamo, 2009, 2011; Moreyra *et al.*, 2012).

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When a *V. germanica* forager finds an abundant food source it makes several foraging flights between the resource and the nest, where larvae are fed. During this behavior, the wasp associates food with spatial, odor and visual cues (e.g., D'Adamo & Lozada, 2003, 2008, 2009, 2011, 2014; Moreyra *et al.*, 2006; Lozada & D'Adamo, 2009, 2011, 2014). These learning processes enable them to return to a plentiful resource that can suffer unpredictable position changes over consecutive visits; it is common to see dogs and birds of prey, among other large animals, displace or remove resources which wasps are foraging on (Lozada & D'Adamo, 2011, 2014). Moreover, when a wasp is collecting food in an anthropic scenario (e.g., picnics, barbecues), such food and contextual cues (e.g., cutlery, glasses) often change location (D'Adamo & Lozada, 2014; Lozada & D'Adamo, 2014). Previous studies have shown that when a wasp learns to collect food from an experimental plate, located at a certain microsite, it revisits this location (D'Adamo & Lozada, 2003, 2007, 2008, 2011; Lozada & D'Adamo, 2006, 2009, 2011, 2014; Moreyra *et al.*, 2014). However, if food is removed, the wasp continues to visit the feeding station for a certain period of time, until it eventually stops searching (Lozada & D'Adamo, 2006). The length of time the wasp revisits the experimental plate depends on the number of former rewarding experiences. Furthermore, when food is displaced, the *V. germanica* forager continues looking for it in the previously learned location even when the resource has been placed nearby (Lozada & D'Adamo, 2011; D'Adamo & Lozada, 2014). These findings illustrate how past experience can delay detection of more favorable foraging sites (Lozada & D'Adamo, 2011). These previous investigations demonstrate the existence of memory and learning processes in *V. germanica* while relocating food sources in the natural contexts they inhabit.

Associative learning can result in short-term memory (STM) which lasts a matter of minutes, and long-term memory (LTM) that lasts at least 24 h (e.g., Menzel, 2009). Long-term memory processes related to foraging behavior have been demonstrated in hymenopterans such as honeybees (e.g., Menzel, 2009), ants (e.g., Josens *et al.*, 2009), bumblebees (e.g., Chittka, 1998), parasitic wasps (e.g., Hoedjes & Smid, 2014; Hoedjes *et al.*, 2015), and *Vespula vulgaris* social wasps (Santoro *et al.*, 2015). For example, in *Apis mellifera*, 3 conditioning events lead to LTM that can be retrieved after 72 h and may last for the bee's entire life (Menzel, 1999). Similarly, a single conditioning trial in the parasitic wasp *Nasonia vitripennis*, where a female wasp associates an odor with the reward of finding a host, results in the formation of LTM (Hoedjes & Smid, 2014). However, to our knowledge, LTM processes in *Vespula germanica* wasps have been little studied. Recently, it has been shown that foragers of *Vespula vulgaris* learn to associate chemical cues to food from their nestmates, and use these associative memories when choosing resources the following days (Santoro *et al.*, 2015).

Previous investigations in *V. germanica* have demonstrated STM processes while foraging (Lozada & D'Adamo, 2006, 2011; D'Adamo & Lozada, 2014). When a wasp collects food 3 or 4 consecutive times from a certain microsite, it searches over the previously learned location for a longer period of time than a wasp that collected food only once. This result shows that an established memory is recalled even after a single learning trial, although extinction is observed after 1 h (Lozada & D'Adamo, 2006). The contextual training condition in said study remained intact and only food was removed during the testing session. Wasp behavior was analyzed from the moment that food was removed until the wasp no longer returned (Lozada & D'Adamo, 2006). Lozada and D'Adamo (2006) proposed that wasp memory is extinguished after 60–200 min, since after this period of time, wasps do not return to the experimental area. However, in the experimental design used in the aforementioned work it was not possible to determine what would have happened if 2 baited plates had been presented close to each other. In another investigation, short-term memory in *V. germanica* was evaluated when both the food and the array were completely removed for 1 h. Results showed that on the replacement of the array, wasps with 4 feeding experiences remembered and collected food from the previously learned feeding station more frequently than wasps that had collected food only once (Moreyra *et al.*, 2014).

In this study, we further explore how past experience influences future decision making, affecting wasp foraging behavior 24 h later. During this period of time, the array and food were removed, and then re-installed after 24 h in the same place; on this occasion a second baited plate was offered, as in Moreyra *et al.* (2014). We recorded wasp behavior and assessed long-term memory in a discriminative test after 24 h. We hypothesize that the number of previous foraging experiences will affect collecting behavior from the learned feeding station. Thus, our prediction is that after 24 h, wasps which experience more feeding trials will collect food from the learned experimental plate more frequently than those that collect food after fewer trials.

Materials and methods

Experimental locations

The experiments on *V. germanica* wasps were carried out in natural environments near San Carlos de Bariloche

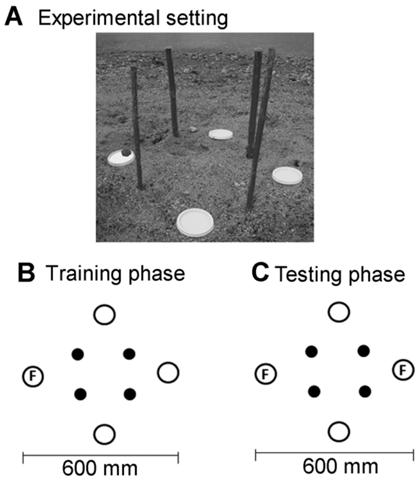

Fig. 1 Photo from experimental setting (A). Diagram of the experimental setting used for the training and testing phases. (B) Training phase: black spots represent yellow cylinders (2 cm × 60 cm), white circles represent plates with potential food, and F = food. Each wasp collected food 2, 4 or 8 consecutive times depending on each single treatment. (C) Testing phase: experimental array used 24 h after training. The 2 "F" plates represent 2 options of feeding stations.

(41°S, 71°W), Rio Negro, Argentina, during their most active period (February to April) in 2012, 2013, 2014, and 2015. The experiments were conducted in areas close to lake shores, in similar weather conditions (sunny and quiet). Although the experiments were performed in different years, all experimental treatments were carried out each year at the same time of the year in order to control temporal variation.

Experimental array and marking procedure

In all the experiments carried out in this work, an individual forager wasp was allowed to collect food from a white plastic experimental plate (diameter = 7 cm) containing 20 g of minced meat. Four yellow cylinders (as landmarks), 2 cm in diameter and 60 cm in high, were arranged in the corners of a square of 30 cm side length, and 4 plates were placed along the 4 edges of the square. Only one of these plates contained food (feeding station) and the other 3 were empty (Figs. 1A and B). The position of the feeding station was randomized during the experiments. An observer sat close to this array, at a distance of 0.5 m. When a wasp arrived at the plate and was collecting food, it was clearly marked with a dot of washable paint on the abdomen or thorax for further identification. This marking procedure caused minimal disturbance to wasps, as they were not captured. Any other wasp visiting the plate was removed in order to work with only 1 individual per experiment, thus, avoiding conspecific recruitment. To record different wasps from different colonies, we conducted each experiment at different geographical positions, more than 200 m apart as in Wilson-Rankin (2014), that is, the number of different locations was equal to the number of tested individuals. During each feeding experience (training phase), the target wasp collected food from the plate, then departed and returned a few minutes later. An individual wasp was used for only a single experiment and a single treatment.

Treatments

We designed 3 treatments in which wasps collected food (foraging trial) from the feeding station: 2 (treatment 1), 4 (treatment 2) or 8 consecutive times (treatment 3) (n = 53, 43, and 54, respectively). We chose 2 and 4 repetitions, given that an earlier work demonstrated that wasps which had collected food only once did not recall a previously learned plate, whereas wasps which had collected food 4 times remembered the location after 1 h (Moreyra *et al.*, 2014). Therefore, we tested whether 2 or 4 visits could promote LTM. We arbitrarily chose 8 trials so that wasps could experience more feeding visits, thus, inducing LTM processes. Each feeding experience involved 1 discrete visit by a wasp to the plate with food, followed by the wasp's departure from the experimental area. Therefore, wasps which returned 2, 4, or 8 consecutive times during training were taken into account for each treatment. However, wasps returning 3 or 5–7 times were not counted.

Experimental steps

After training, the experimental array was completely removed and exactly 24 h later, during the testing session, the array was re-installed in the same location, but this time, a second baited plate was placed opposite the first (discriminative test) (Fig. 1C). That is, the reinstallation of the experimental array was carried out at the same time the following day. As evaluated in Moreyra *et al.* (2014), this method guarantees that wasps which remember earlier experiences will choose the previously learned feeding station. In addition, this methodology helps attract free flying foragers to the experimental array. In the testing phase, we waited for a cut-off period of 60 min for the marked wasp to land at any plate, that is, within the 60-min period if no wasp returned to the array the experiment was cancelled, but if a wasp returned, we analyzed the wasp behavior by recording which plate it collected food from. A landing episode occurred when the marked wasp touched the meat with all 6 legs. In each treatment, we recorded the rate of marked wasps that returned to the array after 24 h, and the rate of foragers that collected food from either the previously learned feeding station or the nonlearned one, with 2, 4, or 8 foraging trials.

Statistics

We analyzed differences in the rate of returning and nonreturning wasps to the experimental array using the χ^2 test. We evaluated differences in the rate of wasps that collected food from the previously learned feeding station after 2, 4, or 8 trials by means of a contingency table (χ^2 test). In each treatment, paired comparisons between foragers' choice (i.e., food collection from the learned vs. nonlearned feeding station) were performed by using the Binomial test.

Results

Most wasps which had been trained with 2 foraging experiences (treatment 1) did not return (78%) to the array 24 h later, that is, we found a higher rate of wasps that did not return than those that returned (22%) to the experimental area ($\chi^2 = 9.98$, $n = 53$, $P < 0.001$). However, nonsignificant differences were observed between the rates of returning and nonreturning foragers with 4 or 8 collecting visits. We found that 49 % of wasps trained with 4 trials (treatment 2) did not return to the experimental area ($\chi^2 = 0.02$, $n = 43$, $P > 0.05$), and 59 % did not return with 8 feeding experiences (treatment 3) ($\chi^2 = 1.85$, $n = 54$, $P > 0.05$) (Fig. 2A).

When analyzing the returning foragers during the testing session after 24 h, significant differences were observed in the number of wasps that chose the previously learned feeding station (experimental plate) with 2, 4 or 8 consecutive visits ($\chi^2 = 6.87$, $n = 59$, $P < 0.03$). The rate of wasps trained with 8 feeding experiences that collected food from the previously learned feeding station was significantly higher (82%) than those that did from the nonlearned one (18%) (Binomial test $n = 22$, $P < 0.0001$). Nevertheless, when wasps had collected food during 4 consecutive visits, returning foragers chose the learned (60%) and nonlearned (40%) feeding station in a similar proportion (Binomial test $n = 22$, $P < 0.12$). Similarly, wasps trained with 2 feeding experiences also collected food from the learned (59%) and nonlearned (41%) feeding station in a similar proportion (Binomial test $n = 15$, $P < 0.14$) (Fig. 2B).

Discussion

This study demonstrates that *Vespula germanica* wasps remember the location of a previously learned feeding station after 24 h. The results show that after 8 feeding experiences which occur 24 h before the testing phase, wasp foragers choose to collect food from the previously learned feeding station when the experimental array is reinstalled and 2 plates with food are offered. Interestingly, this behavior is not observed in the wasps that collected food 2 or 4 consecutive times on the previous day. These findings illustrate that foragers' decision making is dependent on experience, indicating that a higher number of rewarding situations (e.g., 8 trials) promotes long-term memory, whereas less repetitions (i.e., 2 or 4) do not. That is, the intensity of past experience seems to impact on wasps' behavior differently when 2 options are presented 24 h later.

It has been widely demonstrated that repetition of a rewarding experience influences future behavioral responses (e.g., Hall, 1994; Gallistel *et al.*, 2004). In the current study we have found that after 24 h, 8 feeding trials were sufficient for wasps to remember the previously learned feeding station. In contrast, 2 or 4 foraging flights do not seem enough to elicit a significant preference for the learned plate. However, future experiments conducted with few trials could further elucidate whether there is a tendency to choose either plate. Previous investigations have shown how experience impacts future decision making in *V. germanica* wasps (Lozada & D'Adamo, 2006, 2011; D'Adamo & Lozada, 2014). A single foraging experience generates immediate wasp return to the previously visited site, and when food is displaced nearby, foragers continue to search in the previously learned location (Lozada & D'Adamo, 2011; D'Adamo & Lozada, 2014). Nevertheless, when food is completely removed wasps revisit the plate for a limited period of time until they eventually stop searching. This period of time depends on the number of visits formerly experienced (Lozada & D'Adamo, 2006). The influence of past experience in *V. germanica* has also been observed in a recent study, using the same experimental design as the current investigation, where the training session consisted of 1 or 4 foraging visits, and wasps' behavioral response was assessed after 1 h, that is, the memory of past experience was evaluated over a shorter time interval (Moreyra *et al.*, 2014). Wasps trained with 4 feeding events collected food from the previously learned feeding station more frequently than wasps trained only once (Moreyra *et al.*, 2014). Considering that wasps trained with 1 foraging trial did not remember the previously learned feeding station after 1 h, the present study analyzes whether wasps with more feeding experiences are capable of recalling an established association after 24 h. In this case, we found that wasps that had collected food 2 or 4 consecutive times did not prefer the previously learned feeding station, whereas

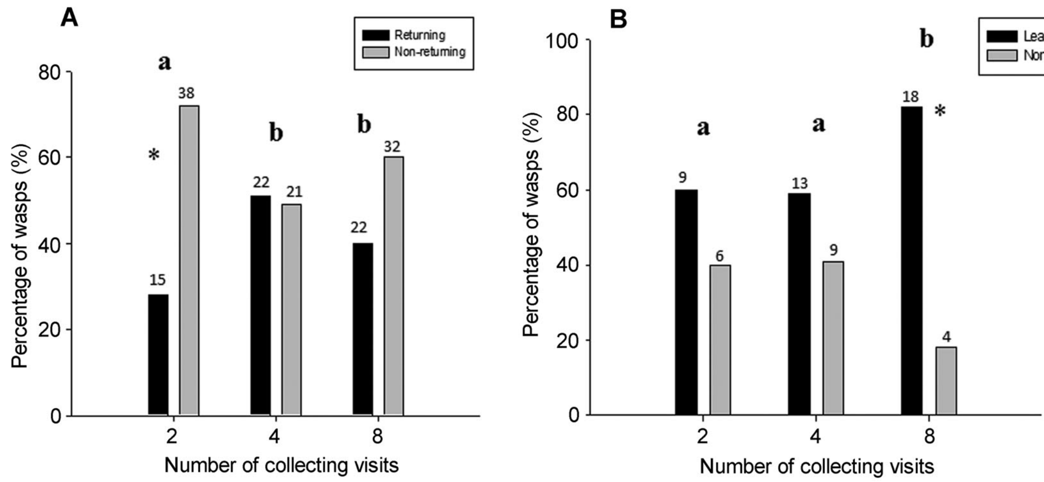

Fig. 2 (A) Rate of returning and nonreturning wasps to the experimental area 24 h after training. (B) Rate of wasps that collected food from the learned and nonlearned feeding station after 2, 4, or 8 trials. *Illustrates differences within each treatment. Letters depict differences between treatments for returning rates with 2, 4, or 8 trials (A) and for food collection rates from the learned feeding station (B). Numbers above the bars indicate the number of wasps.

wasps with 8 foraging trials did show this preference. Therefore, 4 feeding trails promoted the occurrence of short-term memory processes after 1 h (Moreyra *et al.*, 2014); more feeding experiences, however, are necessary for long-term memory after 24 h. Reinstallation of the array with 2 baited plates could have favored memory retrieval in wasps that had more feeding experiences, but not in wasps with less than 5 foraging events. Future studies could further evaluate whether 5–7 training experiences might also promote LMT. Moreover, it would be interesting to analyze how long a single wasp would continue visiting the same feeding station, and at what point the individual would cease to visit the plate.

The fact that the establishment of long-term memory processes demands multiple learning experiences has also been found in other insects such as bees (e.g., Menzel, 1999, 2009), ants (e.g., Guerreri *et al*, 2011), bumblebees (Chittka, 1998). On the other hand, in other hymenoptera species, LTM formation can occur after a single experience, for example, in the parasitic wasps *Lariophagus distinguendus* (Collatz *et al.*, 2006) and *Nasonia vitripennis* (Hoedjes & Smid, 2014). To our knowledge, this article is the first report about long-term spatial memory in *Vespula germanica.*

Regarding the proportion of wasps which returned to the array 24 h after training, it is interesting to highlight that a high rate of wasps did not return to the experimental area in all treatments, even the foragers with 8 collecting visits. These results could be associated with wasp foraging behavior in other patches, sites or resource types that do not illustrate memory processes. For this particular reason, memory rate was analyzed by means of a dual choice procedure. In line with this, a previous work showed that the return rate to the experimental site after 1 h was much higher than after 24 h (Moreyra *et al.*, 2014). This could suggest that diverse foraging strategies might be at work 24 h following the rewarding experience.

In conclusion, this study demonstrates that *V. germanica* wasps are capable of remembering the location of a previously learned feeding station after 24 h. Our findings indicate that past experience conditions wasp choice even 24 h following the rewarding experience. The current work provides new insights into wasp learning and memory processes during the resource relocation, which is a highly frequent foraging behavior of this invasive social species. Our results could be useful in the management and control of this deleterious pest.

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Disclosure

Mariana Lozada, Paola D'Adamo and Sabrina Moreyra have no involvement in anything that could bias their work. Their research does not respond to any external interests other than looking for authentic answers to the questions they investigate.

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