ORIGINAL ARTICLE

The influence of past experience on wasp choice related to foraging behavior

Moreyra Sabrina, Paola D'Adamo and Mariana Lozada

Laboratory Ecotono, INIBIOMA, Quintral 1250 (8400) Bariloche, Argentina

Abstract Memory has been little studied in social wasps. *Vespula germanica* (Fab.) (Hymenoptera: Vespidae) frequently revisits nondepleted food sources, making several trips between the resource and the nest. In this study, we analyzed this relocating behavior in order to evaluate whether this species is capable of remembering an established association after 1 h. To this end, we trained wasps to feed from a certain array. Then it was removed, setting it up again 1 h later, but this time 2 baited feeders were put in place, one at the original feeding site and the other opposite the first. We recorded the proportion of returning foragers, and their choice of feeder, after either 1 or 4 feeding trials. After 1 h, 78% of wasps trained with 4 feeding trials and 65% trained with 1, returned to the experimental area. Furthermore, during the testing phase, wasps trained with 4 feeding trials collected food from the previously learned feeder significantly more frequently than from the nonlearned one ($P < 0.05$). In contrast, wasps that had been trained only once chose both feeders equally. Thus, memory retrieval could be observed 1 h after wasps had collected food on 4 consecutive occasions, but not after only 1. To our knowledge, this is the first study showing that *V. germanica* is capable of remembering an association 1 h after the last associative event, demonstrating that 1 h does not impair memory retention if 4 feeding experiences have occurred.

Key words cognition, foraging behavior, learning, memory, social wasps, *Vespula germanica*

Introduction

The German yellowjacket (*Vespula germanica* Fab.) is a social wasp that has invaded several regions around the world (Thomas, 1960; Spradbery, 1973; Akre *et al.*, 1989; Tribe & Richardson, 1994). This highly efficient invader became established in north-western Patagonia around 1980, becoming an abundant and problematic insect in the Andean Region (Farji-Brener & Corley, 1998; D'Adamo *et al.*, 2002). Several studies in behavioral mechanisms and cognitive plasticity suggest reasons for its successful

establishment and rapid colony growth in new areas (D'Adamo & Lozada, 2008, 2009, 2011; Lozada & D'Adamo, 2009, 2011; Moreyra *et al.*, 2012).

Associative learning is an ability that occurs throughout the animal kingdom. As a result of experience, it permits the establishment of predictive relationships between contingent events, thus diminishing uncertainty. Honeybee associative learning mechanisms have been thoroughly studied in foraging contexts (e.g., Cheng, 2005; Cheng & Wignall, 2006; Giurfa, 2007; Menzel, 2009). Associative learning, involves the establishment of a temporal or spatial link between 2 stimuli or a stimulus and a response. For example, during foraging, animals learn to associate some food reward (unconditioned stimulus) with certain visual, spatial and odor cues (conditioned stimuli). The recurrence of this experience enables a conditioned

Correspondence: Moreyra Sabrina, Laboratory Ecotono, INIBIOMA, Quintral 1250 (8400) Bariloche, Argentina. Tel/fax: +54 294 4426368; email: sabrimoreyra@hotmail.com

response toward the conditioned stimuli. As the acquisition of a conditioned response is a function of the number of rewarding experiences (e.g., Hall, 1994; Gallistel *et al.*, 2004), learning is expected to be more persistent when more associative events are experienced.

Complex cognitive mechanisms related to foraging behavior have been thoroughly demonstrated in honeybees (e.g., Menzel, 1999, 2009) parasitic wasps (Smid *et al.*, 2007; Hoedjes *et al.*, 2011; van den Berg *et al.*, 2011; Kruidhof *et al.*, 2012) and predatory wasps (Torezan-Silingardi, 2011; Alves-Silva *et al.*, 2013). However, cognition in social wasps has received less attention (Raveret Richter, 2000). Some studies have shown that *Vespula germanica* wasps learn to associate food with certain visual, spatial and olfactory cues (D'Adamo & Lozada, 2003, 2008, 2011; Moreyra *et al.*, 2006; Lozada & D'Adamo, 2011). Social wasps frequently revisit nondepleted food sources by making several trips between the food source and the nest, where larvae are fed (Takagi *et al.*, 1980; Raveret Richter & Jeanne, 1985). This relocating behavior has been studied in order to analyze diverse cognitive abilities and memory mechanisms in this social species (D'Adamo & Lozada, 2003, 2007, 2008, 2011; Lozada & D'Adamo, 2006, 2009, 2011). Once a wasp learns to forage from a certain location, it continues visiting the site, evidencing the existence of memory processes. Although wasps persist in visiting a feeder after food removal, they stop visiting a no longer rewarding site after a length of time which depends on the number of previous experiences (Lozada & D'Adamo, 2006, 2011). Thus, wasps that had fed only once from a certain site searched over the learned location for a significantly shorter period of time than wasps that had fed 3 or 4 times. This indicates that an established memory is recalled even after 1 learning trial, though it is more rapidly extinguished (Lozada & D'Adamo, 2006). In the aforementioned study, although food was removed during testing, contextual training conditions were maintained unaltered (i.e., the array and the experimenter remained), and wasp behavior was analyzed from the moment we took food away until the wasps no longer returned (Lozada & D'Adamo, 2006). However, what would happen if 2 baited feeders were presented close to each other after a time lapse of 1 h? Would wasps choose the previously learned feeder or would they feed randomly from both?

In this study, after training, food was removed along with the array, for 1 h. After this period, the array was reinstalled, this time with 2 baited feeders, and wasp behavior was recorded. We studied memory persistence in an associative learning paradigm by pairing a certain context (i.e., location and array) with food. We analyzed the influence of past experience on wasps' choice in a dis-
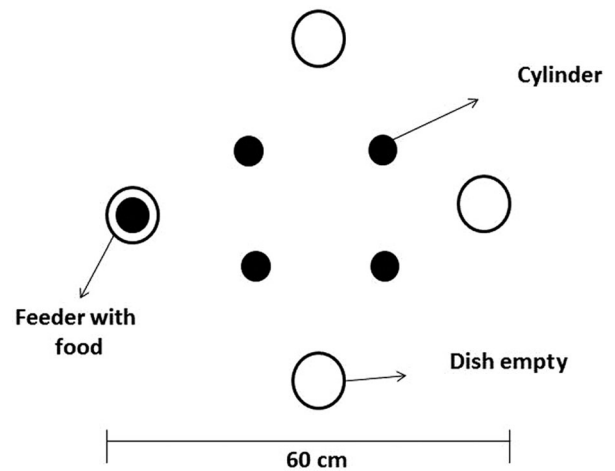

Fig. 1 Experimental array used for training wasps under natural conditions. The array consisted of 4 yellow cylinders and 4 dishes, one of which contained food. Each wasp collected food from the feeder either 1 or 4 consecutive times depending on treatment.

criminative test 1 h after learning. We hypothesized that wasps would successfully relocate the previously learnt food source after having experienced a certain number of foraging experiences. Thus, our prediction was that after 4 learning experiences wasps would relocate and remove food from the learned feeder more frequently than after only 1 learning experience.

Materials and methods

The experiments were carried out under natural conditions near San Carlos de Bariloche (41°S, 71°W), Argentina, during the most active period of *V. germanica* wasps' (February–April) in 2011 and 2013. The experiments were conducted in suburban areas, in similar weather conditions (sunny and still). In all experiments, an individual forager was allowed to collect food from a white plastic dish (diameter = 7 cm) containing 20 g of raw bovine minced meat. Four yellow cylinders, 2 cm in diameter and 60 cm in height, were arranged at the corners of a square of 30-cm side length, and 4 dishes were placed along the 4 edges of the square. One of these dishes contained food (Fig. 1). The position of the feeder with meat was randomized during the experiments. An observer sat close to this array, at a distance of 0.5 m. When a forager arrived at the dish and was collecting food, it was clearly marked with a dot of washable paint on the abdomen for further identification. This marking procedure caused minimal disturbance to wasps, as they were not captured. Any other wasp visiting the dish was removed, in order
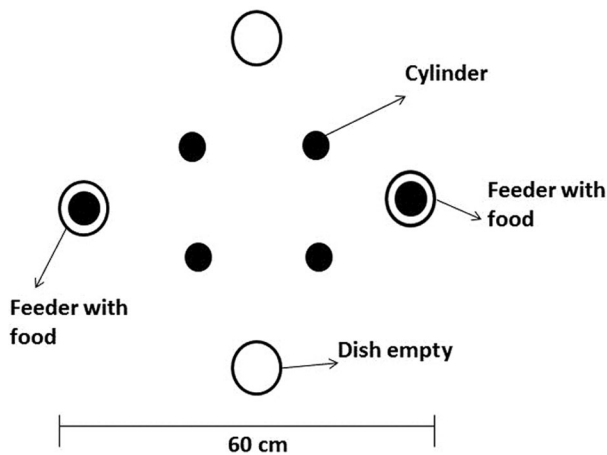
Fig. 2 Experimental array during the testing phase. One hour after training, the array was set up once again in the same location, and 2 baited feeders were put in place, one at the original feeding site and the other opposite to the first.

Fig. 3 Percentage of wasps that either returned or did not return to the experimental area 1 h after finishing the training phase. *denotes statistical differences within each treatment ($P < 0.05$), and numbers above bars indicate number of wasp.

to work with only 1 individual per experiment. To record different wasps from different colonies, we performed each experiment in a different geographic location, that is, separated more than 200 m away from one another. During each feeding experience, the studied wasp collected food from the dish then departed and returned a few minutes later (on average wasps took 8.4 min to return, SE: ± 0.78, $n = 90$). An individual wasp was used for only 1 experiment and 1 treatment. Depending on treatment, 1 group of wasps collected food only once whereas the other group collected food during 4 consecutive times. Each feeding experience involved 1 discrete visit by a forager to the feeding dish, followed by the forager's departure from the experimental area. After training, the experimental array was completely removed, and the observer left the experimental area, leaving no trace. One hour later, the experimenter came back and set the array again at the same location, and 2 baited feeders were put in position, one at the original feeding site and another opposite the first (Fig. 2). We recorded the proportion of marked foragers that returned to the array 1 h later, and the wasps' choice of feeder.

Treatment 1: Each wasp was allowed to collect food 4 consecutive times from the feeder (i.e., 4 feeding trials) (Fig. 1). The entire array was then removed, and 1 h later, we recorded whether the wasp returned to the array, which now contained 2 baited feeders (i.e., the original feeder plus another one located 60 cm in the opposite direction). We waited for the trained wasp for 40 min and if it came, we recorded the feeder from which the wasp collected food.
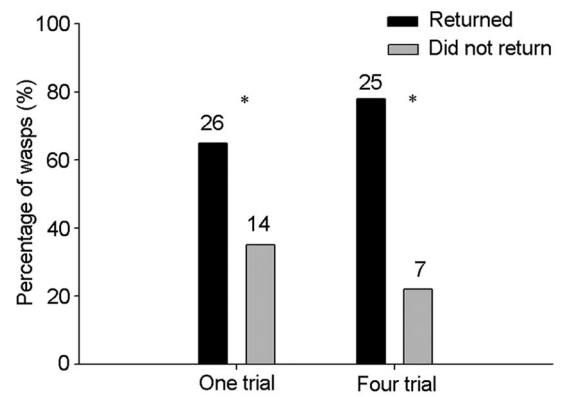
Treatment 2: This experiment followed the same basic procedure, except that each wasp was allowed to feed only once before the array was removed for an hour (Fig. 1).

We evaluated significant differences in the proportion of returning foragers to the array between treatment 1 and treatment 2 by chi-square test. Wasp feeder choice was analyzed by means of the binomial test. Comparison of wasp feeder choice between the different treatments (i.e., wasps feeding from learned or nonlearned feeders with 1 or 4 trials) was done with the Fisher Test.

Results

Most wasps (78%, $n = 32$) which had been trained with 4 feeding experiences (treatment 1) returned to the array 1 h after the training phase, that is, the proportion of wasps that returned was significantly higher than the proportion of wasps that did not ($\chi^2 = 10.12$, $n = 32$, $P < 0.001$). However, when trained with 1 feeding experience (treatment 2), only 65% ($n = 40$) of the wasps returned ($\chi^2 = 3.60$, $n = 40$, $P > 0.05$) (Fig. 3). We found a higher, but nonsignificantly different proportion of returning wasps in the 4-visit treatment compared to the 1-visit treatment ($\chi^2 = 1.48$, $n_{1,2} = 32, 40$, $P > 0.22$).

Considering those wasps that returned, during the testing phase, wasps trained with 4 feeding experiences collected food from the previously learned feeder significantly more frequently (88%) than from the nonlearned feeder (binomial test $n = 25$, $P < 0.05$). However, wasps that had experienced only 1 feeding experience, collected food from both the learned and nonlearned locations in a similar proportion (50%) (binomial test $n = 26$, $P > 0.05$) (Fig. 4). Wasps trained with 4 feeding experiences
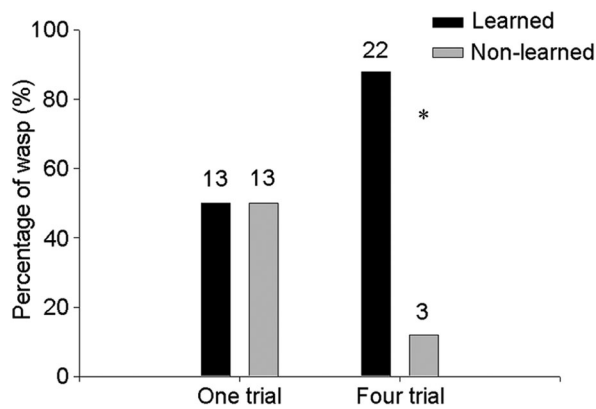
Fig. 4 Number of wasps feeding from the learned and non-learned feeder after 1 or 4 feeding trials. *denotes statistical differences within each treatment ($P < 0.05$), and numbers above bars indicate number of wasp.

chose the learned feeder in a significantly higher proportion than wasps fed with only 1 ($\chi^2 = 8.55$, $n_{1,2} = 25, 26$, $P < 0.003$).

Discussion

This study describes how *V. germanica* wasps relocate a previously learnt feeder after 1 h. To our knowledge, this is the first experimental study to demonstrate that this species is capable of remembering an established association for a relatively long period of time. When offering 2 alternatives 1 h after feeding, wasps preferred the previously rewarded feeder. Thus, although food and the associated array had been unavailable for 1 h, wasps' past experience still influenced their behavior when facing a choice of 2 food sources. This was observed in wasps which had collected food 4 consecutive times but not after only 1 experience.

It has been extensively demonstrated that the repetition of a rewarding experience greatly influences future decision making (e.g., Bitterman *et al.*, 1983; Hall, 1994; Gallistel *et al.*, 2004). In this study, 1 feeding experience had no effect on wasp choice while 4 feeding experiences did. The influence of past experience in *V. germanica* foragers was also observed in a previous study (Lozada & D'Adamo, 2011) which showed that when food was displaced 60 cm, wasps took some time visiting the previously rewarded dish, till detecting the new food location. Thus, after 4 feeding experiences wasps spent more time searching for the novel food source than after 1. Similarly, wasps which had experienced 1 feeding trial searched less time over the previously learned location than wasps that had experienced 3 (Lozada & D'Adamo, 2006). Thus, in

both experiments whether the array was maintained (as in previous work) or removed (the present study), 1 feeding experience had less impact on wasps' future decisions than more experiences. In the previous work, Lozada and D'Adamo (2006) proposed that wasps extinguished memory after 60–200 min because they no longer returned to the experimental area. However, it is not possible to ascertain what would have happened if baited feeders had been reinstalled. The removal and posterior reinstallation of the array with the baited feeders in this study, could have favored memory retrieval in those wasps that had not extinguished this memory. The fact that wasps did not return to a nonrewarding place does not imply that memory was extinguished. To elucidate this, the present experiment was designed to analyze memory retention and wasp choice 1 h after feeding.

When evaluating the proportion of returning wasps, we further analyzed the influence of past experience on the persistence of revisiting a previously rewarded location. This also depended on the number of feeding visits wasps had experienced. When wasps had fed 4 consecutive times, they returned to the rewarded area more frequently than after feeding only once. This could indicate that the frequency of revisiting a certain patch is directly related to the number of rewarding episodes. This has been extensively documented for other hymenopteran species like bumblebees (Dramstad, 1996), honeybees (Collett & Collett, 2002; Menzel, 2009), and ants (Hölldobler & Wilson, 1980; Wehner *et al.*, 2004; Elizalde & Farji-Brener, 2012).

This study provides new evidence of *V. germanica* cognitive processes in relation to foraging in natural contexts, which further contributes to the understanding of the learning abilities of this invasive species. To our knowledge, this is the first study that analyzes whether *V. germanica* wasps are capable of remembering an association 1 h after the last associative event. We can conclude from the obtained results that after 1 h, 4 feeding experiences may lead to more frequent memory retention compared to only 1 feeding experience. It is interesting to highlight the importance of past experience, which conditions wasp choice even 1 h after the rewarding experience. This study sheds new light on memory processes associated with the relocalization of a food source in this social wasp, which could be a relevant factor in their successful global invasion.

Acknowledgments

Disclosure

Mariana Lozada, Paola D'Adamo, and Sabrina Moreyra are not involved 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.

References

Akre, R.D., Ramsay, C., Grable, A., Baird, C. and Stanford, A. (1989) Additional range extension by the German yellow-jacket, *Paravespula germanica* (Fabricius), in North America (Hymenoptera: Vespidae). *Pan-Pacific Entomologist*, 65, 79–88.

Alves-Silva, E., Baronio, G.J., Torezan-Silingardi, H.M. and Del-Claro, K. (2013) Foraging behavior of *Brachygastra lecheguana* (Hymenoptera: Vespidae) on *Banisteriopsis malifolia* (Malpighiaceae): Extrafloral nectar consumption and herbivore predation in a tending ant system. *Entomological Science*, 16, 162–169.

Bitterman, M.E., Menzel, R., Fietz, A. and Schäfer, S. (1983) Classical conditioning of proboscis extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, 97, 107–119.

Cheng, K. (2005) Context cues eliminate retroactive interference effects in honeybees *Apis mellifera*. *Journal of Experimental Biology*, 208, 1019–1024.

Cheng, K. and Wignall, A.E. (2006) Honeybees (*Apis mellifera*) holding on to memories: response competition causes retroactive interference effects. *Animal Cognition*, 9, 141–150.

Collett, T.S. and Collett, M. (2002) Memory use in insect visual navigation. *Nature Reviews Neuroscience*, 3, 542–552.

D'Adamo, P. and Lozada, M. (2003) The importance of location and visual cues during foraging in the German wasp (*Vespula germanica* F.) (Hymenoptera: Vespidae). *New Zealand Journal of Zoology*, 30, 171–174.

D'Adamo, P. and Lozada, M. (2007) Foraging behavior related to habitat characteristics in the invasive wasp *Vespula germanica*. *Insect Science*, 14, 383–388.

D'Adamo, P. and Lozada, M. (2008) Foraging behaviour in *Vespula germanica* wasps re-locating a food source. *New Zealand Journal of Zoology*, 35, 9–17.

D'Adamo, P. and Lozada, M. (2009) Flexible foraging behavior in the invasive social wasp *Vespula germanica* (Hymenoptera: Vespidae). *Annals of the Entomological Society of America*, 102, 1109–1115.

D'Adamo, P. and Lozada, M. (2011) Cognitive plasticity in foraging *Vespula germanica* wasps. *Journal of Insect Science*, 11: 103.

D'Adamo, P., Sackman, P., Rabinovich, M. and Corley, J.C. (2002) The potential distribution of German wasps (*Vespula germanica*) in Argentina. *New Zealand Journal of Zoology*, 29, 79–85.

Dramstad, W.E. (1996) Do bumblebees (Hymenoptera: Apidae) really forage close to their nests? *Journal of Insect Behavior*, 9, 163–182.

Elizalde, L. and Farji-Brener, A. (2012) To be or not to be faithful: flexible fidelity to foraging trails in the leaf-cutting ant *Acromyrmex lobicornis*. *Ecological Entomology*, 37, 370–376.

Farji-Brener, A. and Corley, J.C. (1998) Successful invasions of hymenopteran insects into NW Patagonia. *Ecología Austral*, 8, 237–249.

Gallistel, C.R., Fairhurst, S. and Balsam, P. (2004) The learning curve: implications of a quantitative analysis. *Proceedings of the National Academy of Sciences of the United States of America*, 36, 13124–13131.

Giurfa, M. (2007) Behavioral and neural analysis of associative learning in the honeybee: a taste from the magic well. *Journal of Comparative Physiology A*, 193, 801–824.

Hall, G. (1994) Pavlovian conditioning: laws of association. *Animal Learning and Cognition* (ed. N.J. Mackintosh), pp. 15–43. Academic Press, San Diego.

Hoedjes, K.M., Kruidhof, H.M., Huigens, M.E., Dicke, M., Vet, L.E.M. and Smid, H.M. (2011) Natural variation in learning rate and memory dynamics in parasitoid wasps: opportunities for converging ecology and neuroscience. *Proceedings of the Royal Society B*, 278, 889–897.

Hölldobler, B. and Wilson, E.O. (1980) *The Ants*. Cambridge, Belknap.

Kruidhof, H.M., Pashalidou, F.G., Fatouros, N.E., Figueroa, I.A., Vet, L.E.M., Smid, H.M. and Huigens, M.E. (2012) Reward value determines memory consolidation in parasitic wasps. *PLoS ONE*, 8: e39615.

Lozada, M. and D'Adamo, P. (2006) How long do *Vespula germanica* wasps search for a food source that is no longer available? *Journal of Insect Behavior*, 19, 591–600.

Lozada, M. and D'Adamo, P. (2009) How does an invasive social wasp deal with changing contextual cues while foraging? *Environmental Entomology*, 38, 803–808.

Lozada, M. and D'Adamo, P. (2011) Past experience: a help or a hindrance to *Vespula germanica* foragers? *Journal of Insect Behavior*, 24, 159–166.

Menzel, R. (1999) Memory dynamics in the honeybee. *Journal of Comparative Physiology A*, 185, 323–340.

Menzel, R. (2009) Learning and memory in invertebrates: honeybee. *Encyclopedia of Neuroscience*. (ed. L.R. Squire), pp. 435–439. Academic Press, Oxford.

Moreyra, S., D'Adamo, P. and Lozada, M. (2006) Odour and visual cues utilised by German yellowjackets (*Vespula germanica*) while relocating protein or carbohydrate resources. *Australian Journal of Zoology*, 54, 393–397.

Moreyra, S., D'Adamo, P. and Lozada, M. (2012) Cognitive processes in *Vespula germanica* wasps (Hymenoptera: Vespidae) when relocating a food source. *Annals of the Entomological Society of America*, 105, 128–133.

Raveret Richter, M.A. and Jeanne, R.L. (1985) Predatory behavior of *Polybia sericea* (Olivier), a tropical social wasp (Hymenoptera: Vespidae). *Behavioral Ecology and Sociobiology*, 16, 165–170.

Raveret Richter, M. (2000) Social wasp (Hymenoptera: Vespidae) foraging behavior. *Annual Review of Entomology*, 45, 121–150.

Smid, H.M., Wang, G., Bukovinszky, T., Steidle, J.L., Bleeker, M.A., van Loon, J.J. and Vet, L.E.M. (2007) Species-specific acquisition and consolidation of long-term memory in parasitic wasps. *Proceedings of the Royal Society B*, 274, 1539–1546.

Spradbery, J.P. (1973) *Wasps: An Account of the Biology and Natural History of Solitary and Social Wasps*. University of Washington Press, Seattle, WA.

Takagi, M., Hirose, Y. and Yamasaki, M. (1980) Prey-location learning in *Polistes jadwigae* Dalla Torre (Hymenoptera: Vespidae), field experiments on orientation. *Kontyu*, 48, 53–58.

Thomas, C.R. (1960) *The European Wasp (Vespula germanica Fab.) in New Zealand. New Zealand Department of Science and Industrial Research Information Series No. 27*. New Zealand Department of Science and Industrial Research, Wellington.

Torezan-Silingardi, H.M. (2011) Predatory behavior of *Pachodynerus brevithorax* (Hymenoptera: Vespidae, Eumeninae) on endophytic herbivore beetles in the Brazilian tropical savanna. *Sociobiology*, 57, 181–189.

Tribe, G.D. and Richardson, D.M. (1994) The European wasp, *Vespula germanica* (Fabricius) (Hymenoptera: Vespidae), in southern Africa and its potential distribution as predicted by ecoclimatic matching. *African Entomology*, 2, 1–6.

van den Berg, M., Duivenvoorde, L., Wang, G., Tribuhl, S., Bukovinszky, T., Vet, L.E.M., Dicke, M. and Smid, H.M. (2011) Natural variation in learning and memory dynamics studied by artificial selection on learning rate in parasitic wasps. *Animal Behaviour*, 81, 325–333.

Wehner, R., Meier, C. and Zollikofer, C. (2004) The ontogeny of foraging behaviour in desert ants, *Cataglyphis bicolor*. *Ecological Entomology*, 29, 240–250.