



Memory of prey larders in golden orb-web spiders, *Nephila clavipes* (Araneae: Nephilidae)

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Abstract

Nephila clavipes spiders accumulate prey larders on their webs. We conducted a field experiment to ask if the spiders search for larders that have been pilfered (experimentally mimicking the potential effect of kleptoparasites), and to ask if the spiders vary their search efforts according to the size of the larder. All spiders searched for larders removed from their web, and spiders that lost larger larders (i.e., consisting of more prey items) searched for longer intervals. We thus suggest that *N. clavipes* form memories of the size of the larders they have accumulated, and that they use those memories to regulate recovery efforts when the larders are pilfered. The content of those memories may include discrete prey counts or the accumulation of a continuous variable correlated with counts, such as the total mass of captured prey. We discuss the adaptive significance of this ability in the framework of costs related to kleptoparasites and the ecology of food hoarding.

Keywords

cognitive ecology, food hoarding, invertebrate, numerosity, orb-weaver.

1. Introduction

Variation in resource abundance is a fact of life for the great majority of animals, as is competition for resources. These challenges take many forms in nature, and animals have evolved varied ways to deal with them. Many species store food in hoards or caches, saving it for periods of scarcity or when competitors are less likely to be looking (Dally et al., 2006). Food store defense takes a variety of forms, from concealing scattered hoards and memorizing their locations, to aggressively excluding competitors from

centralised larders (Vander Wall, 1990; Dally et al., 2006). The cognitive requirements for these strategies likely differ; e.g., scatterhoarding may involve greater cognitive requirements than other strategies (Healy & Rowe, 2010). However, our ability to draw general conclusions about the evolution of hoarding and its associated cognitive abilities is limited — it is clear that hoarding is widespread across animals (Vander Wall, 1990), but broader exploration of the diversity of ways in which animals create and defend food stores is required (Healy & Rowe, 2010).

Here we study a sit-and-wait predator, a web spider, that hoards its food in a centralised larder. Some web spiders accumulate prey larders on their web, and save them either for later consumption by themselves (Eberhard, 1967; Robinson & Mirick, 1971; Vander Wall, 1990; Champion de Crespigny et al., 2001; Griffiths et al., 2003) or as provisioning for their young (Barrantes & Weng, 2007). Such larders may be vulnerable to pilferage (Robinson & Mirick, 1971; Griffiths et al., 2003). Consequently, keeping track of the presence and size of larders, and modifying behavioural responses to pilferage accordingly, may be important for web spiders.

We explored the ability of the spiders to keep track of the size of their prey larders, and to use this information to regulate efforts to recover pilfered prey. Rather than memory of the physical distribution of scattered hoards, this would involve information about the quality or quantity of resources accumulated in a centralised larder. Spiders often form memories about their environment and experiences, and use them in tasks such as prey capture and web building. Jumping spiders, for instance, memorise circuitous routes to localise hard-to-reach prey (Tarsitano & Jackson, 1997). Web spiders, too, use memories of their own movements or of their web's features in the process of web-building (Eberhard, 1988; Eberhard & Hesselberg, 2012), and to relocate web landmarks (LeGuelte, 1969). There is also evidence that web spiders form memories of items on their web, such as egg sacs or captured prey (Baltzer, 1924; Rodríguez & Gamboa, 2000; Opell, 2001; Rodríguez & Gloudeman, 2011), including items that have been only briefly on the web (Nakata, 2007).

Our study species was the golden orb-web spider, *Nephila clavipes* (Araneae: Nephilidae). These spiders use a line of silk to attach relatively large prey items to the hub of their web — by contrast, small prey are simply held with the mouthparts as the spiders feed on them. When a series of relatively large prey are intercepted by their web in quick succession, *N. clavipes*



Figure 1. *Nephila clavipes* female consuming one prey while a larder of three other prey items hangs from its web. In front of the spider's left leg III there is one *Argyrodes* kleptoparasite. Prey were grasshoppers used in preliminary trials conducted on Barro Colorado Island, Panamá. This figure is published in colour in the online edition of this journal, which can be accessed via <http://booksandjournals.brillonline.com/content/1568539x>.

interrupt feeding on the current prey item to secure each new item to the hub, thereby accumulating a larder at the hub of their web (Robinson & Mirick, 1971; this study; see Figure 1). Additionally, *N. clavipes* have been shown to search when prey are removed from their web — either naturally by kleptoparasites or experimentally by researchers (Rodríguez & Gamboa, 2000). These features of the biology of *N. clavipes* allowed us to create experimental manipulations of the size of accumulated larders, and to test the effect on searching behaviour elicited by removing the larders from the webs.

We tested the hypothesis that memory allows *N. clavipes* to track the size of their prey larders. We used a field experiment in which we varied the size of the larders that spiders accumulated, and then removed those larders to elicit searching behaviour. If *N. clavipes* store information about larder size in their memory, then spiders that have accumulated larger larders (consisting of higher prey counts) should search for longer intervals than spiders with smaller larders (consisting of lower prey counts). The rationale for this

prediction comes from observations of the behaviour of spiders that have lost items from their webs. If items such as prey or egg sacs are removed from the web, the spiders search for them (Baltzer, 1924; Rodríguez & Gamboa, 2000; Opell, 2001; Rodríguez & Gloudeman, 2011). This searching behaviour varies with the features of the lost items; e.g., spiders search for longer intervals when they have lost larger prey, indicating that their memories include details about prey quality or quantity (Rodríguez & Gamboa, 2000; Rodríguez & Gloudeman, 2011).

Our goal was to ask if *N. clavipes* in the field behave in a way that, in effect, allows them to keep track of the size of their larders and to regulate their recovery efforts accordingly. Our test cannot completely rule out non-cognitive mechanisms for the spiders' behaviour — nor can it determine whether the spiders' memory might include counts or an integration of associated variables such as accumulated prey mass (see Discussion). Nevertheless, we were able to rule out some potential confounding factors. We used the time that spiders took to begin searching as an indication of the influence of such factors, according to the following rationale: Search times represent an investment by a spider in an effort to recover lost prey. As such, search times reflect the spiders' valuation of a prey larder as stored in their memory. But search times may also be influenced by confounding non-cognitive factors, such as individual variation in hunger. We expect such confounding factors to be reflected in the time that it takes spiders to begin searching, with delays representing negative influences (e.g., due to the disturbance provided by experimental procedures) and earlier starts representing positive influences (e.g., hunger). Of course, earlier starts likely also reflect memory content, but differences between the patterns for begin and search times would allow distinguishing these factors. We were, thus, able to test three alternative hypotheses about potential influences on the spiders' behaviour, as follows: (i) Spiders given larger larders may be more motivated to search, but not because of their memory of the larder; instead, they may search more because they spent more effort in accumulating larger larders or became hungrier. If so, spiders that accumulated larger larders should begin to search sooner. (ii) Conversely, spiders given larger larders may be less motivated to search because of the greater disturbance they experienced. We attempted to minimise and standardise disturbance across treatments (see below), but we can also test for this possibility: Spiders that accumulated larger larders should exhibit longer delays before they begin to search. (iii) Finally,

in field studies there are many potential differences between test individuals (e.g., in hunger) that may introduce confounding variation. We expect that such differences would introduce noise into our tests rather than bias the patterns in line with our treatments. Nevertheless, we also tested whether such individual differences contribute to variation in the spiders' searching behaviour: if so, there should be a correlation between the delay to begin searching and the time over which the spiders searched.

2. Materials and methods

We conducted the experiment during August 2009 at La Selva Biological Station, Costa Rica. We tested spiders where we found them in the field, mainly at the edges of forest clearings and along trails. Our sample consisted of 18 female spiders; 15 individuals were adult or penultimate instars, and 3 were earlier instars. Excluding the latter did not alter our results (see below) and we retained them in our analyses. We tested each individual only once. This relatively small sample size reflects challenging field conditions during the brief opportunity we had to conduct this study. Nevertheless, our results show that we had adequate statistical power to detect effects of the magnitude required to test our hypotheses and to prevent spurious results (see below).

2.1. Experimental procedure

We randomly assigned spiders either to a control group (capture and sham removal of 1 prey), or to one of three larder-size treatment groups (accumulation of larders of 1, 2 or 4 prey followed by removal of the entire larder). Sample sizes were of 4–5 spiders for each group (see Figure 2). The early-instar individuals (see above) were spread evenly among the larder-size treatments.

Prey were 4-week-old *Acheta domesticus* cricket nymphs (ca. 1 cm body length). While crickets do not correspond to the most common natural prey for *N. clavipes* — which are mainly small to medium flying insects (Robinson & Mirick, 1971; Nentwig, 1985; pers. obs.) — the crickets were approximately the right size and were readily accepted by the spiders. Importantly, using these crickets allowed us to offer prey items of standard size and palatability.

To give prey to the spiders, we lightly dropped each cricket on the sticky spiral of the web, below the hub. We allowed the spider to subdue and wrap

the cricket, return to the hub of the web, and secure the cricket to the hub. We then allowed the spider to feed for ca. 30 s, so it would settle into its normal behaviour. For the treatments of 2 and 4 prey, we repeated this procedure until the desired larder size was reached — recall that an *N. clavipes* spider that is feeding on a prey item attached to the hub of its web will interrupt feeding when another prey item is intercepted by its web in order to capture it and attach it to the hub (see above).

In removing prey larders, we tried to minimise the disturbance experienced by the spiders. We induced each spider to retreat from the hub of the web by lightly touching her on the abdomen and legs with a forceps. Spiders retreated to an upper corner of the web, and varied by a few seconds in the disturbance required to induce them to retreat. We then held each prey item with a forceps and cut the silk line attaching it to the hub with a scissors. For control spiders, we lightly pulled on the prey to simulate cutting its silk line. These procedures left the web intact.

We then waited for the spiders to return to the hub of the web. At this point, control spiders found their larder intact, whereas spiders in the treatment groups found the entire larder to be absent.

We used a chronometer to measure the time between larder removal and the spiders' return to the hub. This is the spiders' 'return time'. Return times largely reflect how long it took the spiders to decide to return to the hub — they also encompass the time it took the spiders to traverse the web to get to the hub, but this was a very small fraction of the total return time.

For those spiders that searched, we measured their search time. Whenever a spider searched, there was no delay between arriving at the hub and the beginning of searching. Our criterion to decide when a spider stopped searching was when it had remained without moving for 3 min. This criterion is based on preliminary observations showing that when spiders had been still for that interval they remained so even when observed for longer, whereas with shorter intervals spiders often resumed searching. Thus, a spider that returned to the hub and remained still for 3 min was scored as 'not searching'; and a spider that searched for x min, then remained still for 3 min, was scored as searching for x min.

2.2. *Statistical analysis*

We analysed the data with one-way ANOVAs, using Welch ANOVAs when variances were unequal. Post-hoc comparisons for these ANOVAs would

have low power, since for each group the sample was 4–5 spiders. We, therefore, adopted an effect size ($0 < r < 1$) criterion to describe differences between groups (Nakagawa & Cuthill, 2007). We also described the effect size of the overall relationship between search times and larder size with Spearman's rank correlation coefficient. Finally, we assessed the relationship between return and search times with Pearson's product–moment correlation. We performed all tests in JMP 7.0.1 (SAS Institute, Cary, NC, USA).

3. Results

Upon returning to the hub, spiders performed their normal behaviour of attaching a security silk line to the hub and tugging on the web with their legs

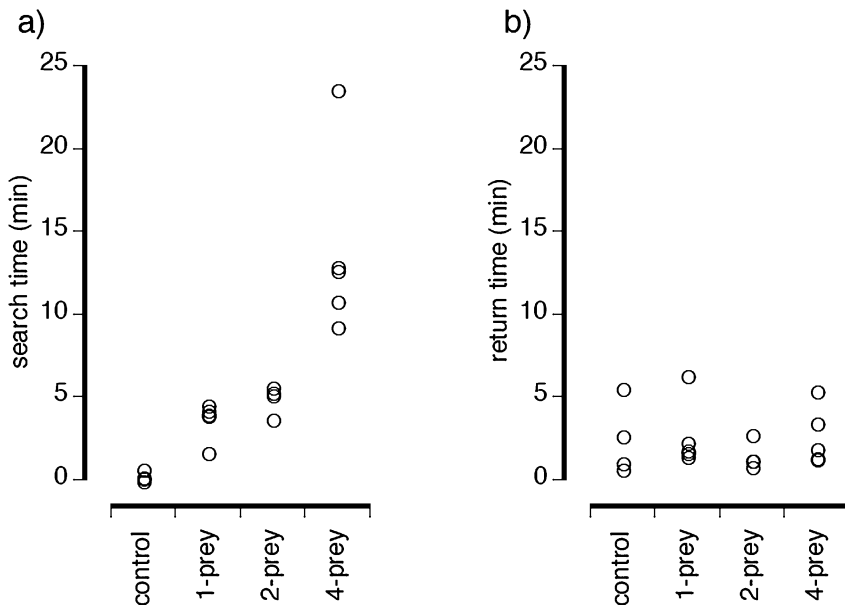


Figure 2. *Nephila clavipes* behaviour after the removal of prey larders varying in size from their web. (a) Search times. Spiders searched for lost prey larders, and they searched for longer intervals when they were searching for larger larders. Note that the sample size for the control group is 4 spiders, 3 of which did not search and, thus, have search time = 0. We introduced slight deviations from 0 for those spiders, so that the data points could be appreciated visually. (b) Return times. The time it took spiders to return to the hub and begin searching did not vary between treatments.

one time. Whenever searching occurred, it began at this point. All spiders that accumulated and lost prey larders searched for them (Figure 2a). By contrast, three of the four control spiders did not search upon returning to the hub and performing the above routine. The fourth control spider did search briefly (35 s) — a burst of wind had moved the prey from its position, and she stopped searching as soon as she relocated the prey. We conservatively retained this spider in the analyses that follow (Figure 2a).

Spiders that accumulated and lost larger prey larders (consisting of more prey) searched for longer intervals (Welch ANOVA; $F_{3,6.6826} = 44.95$, $p < 0.0001$; Figure 2a). This effect remained after excluding the control group from the test (Welch ANOVA; $F_{2,6.8492} = 7.94$, $p = 0.016$). Removing the three early-instar individuals also did not alter these results (test including the control group: Welch ANOVA; $F_{3,4.6108} = 26.52$, $p = 0.0024$; test excluding the control group: Welch ANOVA; $F_{2,5.0156} = 6.96$, $p = 0.036$).

All pairwise differences between treatment groups were of large effect size ($r > 0.5$): control vs. 1-prey: $r = 0.90$; 1-prey vs. 2-prey: $r = 0.53$; 1-prey vs. 4-prey: $r = 0.78$; 2-prey vs. 4-prey: $r = 0.74$. We also described the strength of the relationship between search times and larder size with Spearman's rank correlation coefficient: conservatively excluding the control group, this gives a large effect size ($r = 0.86$, $p < 0.0001$, $N = 14$). Thus, these results support the hypothesis that *N. clavipes* form memories about the size of the prey larders that they have accumulated.

By contrast, return times did not vary significantly among control and treatment groups ($F_{3,14} = 0.43$, $p = 0.74$; Figure 2b). This lack of effect remained after excluding the 3 early-instar individuals ($F_{3,11} = 0.73$, $p = 0.56$).

Finally, the relationship between return times and search times was negligible (Pearson's product-moment correlation; $r = 0.03$, $p = 0.89$; Figure 3).

4. Discussion

Nephila clavipes spiders searched for prey larders that were experimentally removed from their web, and they engaged in longer searches when they had accumulated and lost larger larders (consisting of more prey). The increase in search time with larder size did not appear to level off between one and four prey items (Figure 2a). This suggests that the spiders may be able to keep track of larders consisting of even higher prey counts. Return times

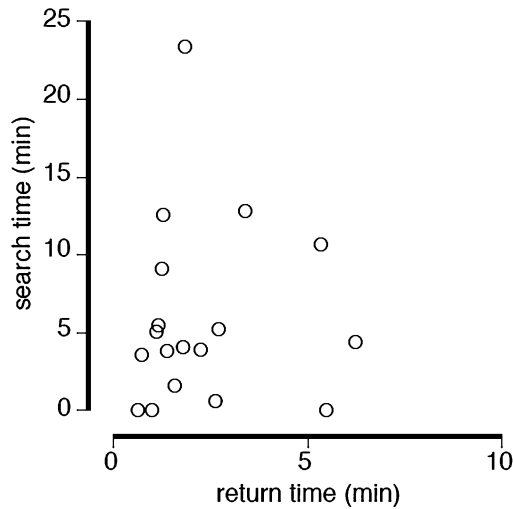


Figure 3. Relationship between return times and search times for *Nephila clavipes*, following removal of prey larders varying in size. Note that the range of variation in return times was nearly five times smaller than the range of variation in search times.

did not vary with larder size and were not correlated with search times, suggesting that confounding non-cognitive effects (arising, e.g., from our experimental manipulations or from between-individual differences in the field) did not have strong effects in this study. We, therefore, interpret our results as suggesting that *N. clavipes* formed memories of the size of their larders, and that they used those memories to regulate their efforts to recover the larders after pilfering. Search times ranged from 1.6 min to 23 min, offering an estimate of the time over which the spiders retained memories of the lost larders; this is likely an underestimate, because our experiment cannot distinguish between forgetting and giving up due to energetic costs.

In terms of the content of the spiders' memories, our findings are equally consistent with memory of prey counts (i.e., with the spiders possessing a sense of numerosity; Dehaene, 1997), as with memory of a continuous correlated variable, such as prey mass. Further work should aim to distinguish between these possibilities. With evidence that the sense of numerosity is present not only in vertebrates (Dehaene, 1997; Shettleworth, 2010) but also in some insects (e.g., Dacke & Srinivasan, 2008; Carazo et al., 2009), it seems interesting to explore the set of ecologies and brain architectures that can endow animals with the ability to keep track of counts.

Our findings add one dimension in which cognitive abilities may be important in the ecology and evolution of food hoarding. Even animals with centralised larders may use memory of larder contents to help regulate defense or recovery efforts. For *N. clavipes*, selection on memory and searching behaviour may be strongly influenced by some of their natural enemies — i.e., by the kleptoparasites that remove prey items from their webs, and which can inflict heavy costs (Robinson & Mirick, 1971; Rypstra, 1981; Grostal & Walter, 1997; Agnarsson, 2003).

More broadly, we suggest that web spiders can help expand the framework for testing ecological and evolutionary hypotheses about memory and food hoarding, and give researchers the ability to use simple behavioural observations to test their predictions. These advantages offer exciting opportunities to study how web spiders evolve to acquire and use information about their environments and experiences.

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