Miniature spiders (with miniature brains) forget sooner

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Miniature animals have tiny brains and should therefore face cognitive limitations. There is little supporting evidence for this expectation, however. We focused on memory information content and retention time, which likely subtend a broad range of cognitive abilities. Our study species, a web spider, allowed us to use a simple assay of working memory: how spiders search for prey they have captured and lost. We used an ontogenetic approach, taking advantage of variation in body size and the concomitant variation in brain size across instars in a single species. This approach eliminates possible confounding variation from species differences in ecology. Small spiders were the most highly motivated to search for lost prey and made the clearest discrimination of prey size. However, when we introduced a delay between memory formation and memory use, search time decreased more steeply in small spiders than in large spiders. Small spiders also performed less additional searching after their primary bout. Thus, the retention of working memory, but not its content, was limited in small spiders with small brains. We suggest that animals that evolve miniature sizes sacrifice not the ability to perceive and acquire information, but rather the ability to retain information over time in working memory. This may, in turn, limit their ability to relate behavioural decisions to their consequences.

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When animals evolve miniature body sizes, they become extreme in two ways. First, their body sizes reach astonishingly small proportions. There are, for example, lungless salamanders as small as a wasp, and parasitoid wasps as small as a *Paramecium* (Hanken, 1985; Polilov, 2012). Second, an increasingly large proportion of their body mass is taken up by brains (Haller's rule; Rensch, 1948). Across mammals, for instance, brain mass varies 20 000-fold, and relative to body mass the brains of the smallest species are over 600 times larger than those of the largest species (Striedter, 2005). In miniature arthropods, the brain accounts for up to 16% of body mass (compared to 2% in humans), and in some cases it protrudes into the thorax or legs (Eberhard & Wcislo, 2011, 2012; Quesada et al., 2011).

The evolution of miniature animals with absolutely smaller, but relatively larger, brains presents a problem. It seems straightforward to understand the advantage of reduced body size (e.g. smaller animals have lower absolute energy requirements and quicker time to maturity; Peters, 1983). By contrast, reduced brain size seems likely to bring manifold disadvantages. Neural tissue is energetically expensive (Attwell & Laughlin, 2001; Niven & Laughlin, 2008; Rolfe & Brown, 1997; Striedter, 2005), and as

animals decrease in size and carry proportionally larger brains, a greater percentage of their energy must be spent maintaining their central nervous system. This means that, when it comes to brain size, small animals pay relatively more for absolutely less. Furthermore, smaller brains have smaller and/or fewer neurons. Smaller neurons have slower transmission rates, noisier signals and fewer connections with other neurons (Faisal, White, & Laughlin, 2005; Perge, Niven, Mugnaini, Balasubramanian, & Sterling, 2012; Purves & Lichtman, 1985). Additionally, having fewer neurons may diminish certain abilities like parallel processing and memory storage (Chittka & Niven, 2009; Spaethe, Tautz, & Chittka, 2006). Either way, tiny animals are expected to face neurological constraints in sensory acuity, decision making and cognitive and behavioural capabilities (Eberhard & Wcislo, 2011; Niven & Farris, 2012).

This is the problem of miniaturization: Does miniaturization reduce an animal's cognitive or behavioural capabilities? And if not, how do miniature animals escape such limitations? These questions are fundamental for understanding the evolution of brain size and cognitive function (Eberhard, 2007, 2011; Eberhard & Wcislo, 2011). Answering them requires identifying appropriate measures of cognitive and behavioural capability. Measures of general intelligence, behavioural complexity or size of behavioural repertoire may be too vague or hard to compare across species (Chittka & Niven, 2009; Eberhard, 2007; Healy & Rowe, 2007). The clearest

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evidence to date comes from an artificial selection experiment that specifically targeted brain size relative to body size in guppies and found that small-brained fish performed less well than large-brained fish in a numerical learning test (females) and a maze learning test (males) (Kotrschal et al., 2013, 2015). However, the detected difference may reflect an advantage of evolving larger brains (for which evidence is more straightforward; e.g. Benson-Amran, Dantzer, Stricker, Swanson, & Holekamp, 2015; Day, Westcott, & Olster, 2005; Madden, 2000; Sol, Duncan, Blackburn, Cassey, & Lefebvre, 2005), rather than a disadvantage of evolving miniature brains, especially as the range of brain sizes was limited (9–14% difference) compared to the orders of magnitude that miniaturized animals and their ancestors may span (Eberhard & Wcislo, 2012; Striedter, 2005). Furthermore, the limitation detected may represent difficulty in keeping track of numbers (for females) or in learning. Ideally, studies should use measures that pinpoint specific cognitive capabilities as directly as possible and that are comparable across different species and groups.

Here we test for cognitive limitations in miniature animals with an assay that measures variation in the information content and retention time of memory. Memory is a basic capability that subtends many other cognitive processes such as learning and decision making (Shettleworth, 2010; Squire & Kandel, 2009).

We assayed the behaviour of web spiders while searching for prey that they had captured and then lost. This type of assay of memory content has revealed that a broad variety of web spiders form memories about the prey they have captured and that those memories include details about their size and numbers (Kilmer, Havens, & Rodríguez, 2018; Rodríguez et al., 2013, 2015; Rodríguez & Gamboa, 2000; Rodríguez & Gloudeman, 2011). This searching assay is also easy to quantify and yields continuous measures that allow for fine-scaled comparisons. Searching times for lost prey range from a few seconds to under 1 h, and thus likely reflect short-term or working memory (Shettleworth, 2010).

We used an ontogenetic approach, taking advantage of variation in body size and the concomitant variation in brain size across juveniles and adults of a single species of web spider. This approach eliminates potentially confounding variation that might arise from comparisons across adults of different species, where differences in size are typically accompanied by differences in ecology and life history (Agrillo & Bisazza, 2017; Kilmer et al., 2018). However, it has the potential problem that comparisons across instars might be confounded by differences in energetic reserves, metabolic rates, locomotor costs, risk aversion, sensory perception, and so on. A key feature of web spider biology helps to reduce this problem. Starting at a very young age, juvenile web spiders are independent, spinning individual webs and essentially foraging as adults (Foelix, 2011), while presenting a large range of variation in size (Quesada et al., 2011). Furthermore, in a prior study we searched for differences across instars that might confound our tests, finding that any such variation would oppose, rather than force, the findings we present here (Kilmer et al., 2018; see below). We did not measure brain size, but relied on its predictable scaling with body size in web spiders (Quesada et al., 2011) because our questions pertain to miniature animals per se.

We tested two hypotheses that posit two nonexclusive ways in which miniature spiders could be limited in their memory of captured prey. The first hypothesis posits limits to the information content of memory in miniature spiders. This hypothesis predicts that smaller spiders will (1) spend less time searching or be less likely to search for prey that they have captured and lost (signs that they do not remember their captured prey very well) and (2) show little distinction in search behaviour between large and small prey — a sign that they do not remember the features of their prey very well, given that web spiders search longer for prey of preferred

sizes (Rodríguez & Gamboa, 2000; Rodríguez & Gloudeman, 2011; Rodríguez et al., 2013, 2015), including our study species (Kilmer et al., 2018). These predictions were rejected in a prior study (Kilmer et al., 2018). Across a five-fold difference in brain size (estimated from a 50-fold difference in body size across instars), it was the smallest spiders that searched the most for lost prey and that showed the greatest prey size discrimination in their memory. Thus, miniature web spiders were not limited in memory content by their small brains. Note that this result also rejects any potential confounds due to noncognitive limitations in the juveniles, such as motor or energetic constraints (Kilmer et al., 2018).

The second hypothesis posits limits to the retention time of the spiders' working memory of the lost prey. This hypothesis makes predictions for searching after a delay between memory formation (prey capture) and memory use (searching). With a long enough delay, search time for all spiders should drop to zero, regardless of brain size. However, this hypothesis predicts that (3) the search time~delay function will drop more steeply for small spiders than for large spiders. In other words, it should take less of a delay to see any given drop in search time for small spiders than for large spiders. We tested this prediction with an experiment in which we experimentally varied such a delay, or 'retention interval' (Shettleworth, 2010), for large and small spiders. Once spiders had ceased searching, we also asked whether we could induce them to resume their search by simulating the presence of prey on their web. Here, this hypothesis predicts that (4) small spiders should be less likely to resume searching, or search for less time, than large spiders and that (5) small spiders should show less prey size discrimination than large spiders.

A potential concern with our assay of memory content and retention is that it may be confounded by noncognitive influences, such as differences in motivation and hunger between spiders. Indeed, prior work with our study species shows that motivation to search and searching efforts vary across instars in our study species (being highest in the smallest, youngest spiders; Kilmer et al., 2018). We therefore ran an additional experiment to gauge the relative effects of proximate noncognitive motivational cues (e.g. hunger) versus information acquisition in our searching assay.

METHODS

We worked with *Pholcus phalangioides* cellar spiders (Araneae: Pholcidae), a widespread synanthropic species. We collected spiders near the University of Wisconsin-Milwaukee (UWM) campus in the summers of 2014–2016. We housed spiders in our laboratory in individual clear plastic shoe boxes (30 × 17 × 10 cm) in a walk-in environmental chamber (Percival Scientific, Inc., Perry, IA, U.S.A.) with a 14:10 h light:dark cycle and a temperature held at 24.6 ± 0.9 °C (mean $\pm$ SD). Before testing, we allowed each spider time to build a full web in its plastic box, which generally took 2+ days.

All experiments shared a core set of methods, as follows. We used small and large spiders (i.e. of different instars), and gave them small and large prey. We used commercially acquired *Gryllodes sigillatus* crickets as prey for all experiments (with one exception noted below). We allowed the spiders to begin the prey capture sequence, then removed the prey and monitored the spiders' behaviour as they searched for the lost prey.

We measured spider size in two ways. We noted their mass on the day we collected them and again after trials before preserving them in 75% EtOH (both mass measures were highly correlated: $r = 0.97$, $P < 0.0001$). After preservation, we measured the width of each spider's sternum under a dissecting scope (this measure was also highly correlated with both mass measurements: $r = 0.90$ and 0.91, respectively, $P < 0.0001$). We also took

both types of measure for prey sizes (mass, body length). Note that our categorization of small and large spiders encompasses a range of variation in sternum width and mass, which was broader for large spiders than for small spiders (Fig. 1). We therefore size-matched prey size to the spiders' size for the treatments of relatively small and large prey for each experiment (details below). We used the initial measures of spiders' mass (the only available measure at the time) to size-match relatively small and large prey for them. We report the final measures of spiders' mass (taken right after trials) (Fig. 1) and used them to approximate their brain size (according to the allometric regressions in Quesada et al., 2011). We used the linear size measurements in statistical analyses, as they are less often subject to incidental variation (e.g. due to differences in hydration levels).

In all experiments we standardized the spiders' satiation levels before the trials (details below). Then, to begin a trial, we gave each spider a cricket. We attached the cricket to a hook at the end of a thread suspended from a small crane and spooled it down onto the web (details in Kilmer et al., 2018). Sometimes the spider would respond immediately to the arrival of the cricket, but most of the time we had to draw the spider's attention by vibrating the prey (to simulate struggling) with a tuning fork (for the memory retention experiment; see below) or an electric toothbrush (for search resumption and handling time experiments; see below).

We allowed the spider a full 2 min (unless specified otherwise below) to subdue and secure the cricket. We then induced the spider to move away from the cricket by puffing it with air from a 2 ml plastic pipette. The spider would retreat to a corner of its box, leaving behind the cricket, which was tethered in place by the crane's string. We then removed the cricket by raising it up off the web and used a hot soldering iron to cut any strands that kept it attached to the web.

As soon as the cricket was removed, we began our observation of the spider's behaviour. We videorecorded all trials from start to finish and annotated the spiders' behaviour during the trial. Like many spiders, *P. phalangioides* have poor eyesight and rely instead on sensing vibrations in their web. When they search for lost prey, they move around their web, tugging the threads every several seconds, effectively sending out vibrations to feel for objects caught in the web (Kilmer et al., 2018). This behaviour is unique to this context (spiders do not initiate prolonged periods of frequent tugging spontaneously) and is similar to the searching behaviour of other species of web spiders tested to date, including araneids, linyphiids, tetragnathids, theridiids and uloborids (Kilmer et al.,

2018; Opell, 2001; Rodríguez & Gamboa, 2000; Rodríguez & Gloudeman, 2011; Rodríguez et al., 2013, 2015; C. Sergi, personal observation). We tracked every instance of tugging and counted every sequence of tugs broken by no more than 20 s as a bout of search behaviour. Sometimes spiders drop down from their web to search the nearby ground for lost prey, so we also took note of every time a spider made exploratory descents to the bottom of its box (Kilmer et al., 2018). Our criterion for ending observations was when the spider had gone 5 min without tugging; or, if the spider did not tug at all, we observed it for a full 15 min after removing its prey.

We ran all of our statistical analyses (described below) in JMP Pro 13.0.0 (SAS Institute Inc., Cary, NC, U.S.A.).

Testing for Variation in the Search Time~Delay Function

To test prediction (3) (that smaller spiders should have shorter working memory retention times), we ran an experiment in which we imposed a delay between memory formation (prey capture) and memory use (searching for prey) for large and small spiders (Fig. 1a). There was a nearly seven-fold difference in mean body mass for the spider size categories (Fig. 1a), corresponding to a ca. 2.3-fold difference in brain mass according to the allometric slopes in Quesada et al. (2011). To standardize satiation levels, we fed each spider 5 days before testing (one *Drosophila melanogaster* fruit fly to small spiders, and two *D. melanogaster* to large spiders).

We tested each spider twice: once with a relatively small cricket (mean $\pm$ SD = 0.68 $\pm$ 0.19 times the spider's mass) and once with a relatively large cricket (1.36 $\pm$ 0.40 times the spider's mass). After the trials were complete, we took linear measurements of both spiders and crickets for a more precise measure of relative prey size to use in our analysis. (In some trials, we used *Acheta domesticus* crickets instead of *G. sigillatus*. We tested for statistical differences in spider behaviour between the two prey species and we found no difference, so we combined them into a single data set). The trials were 2 days apart and conducted in random sequence. Each trial was as described above with the addition of an imposed delay between when the cricket was removed and when the spider was allowed to search for the cricket. We randomly assigned spiders to one of six delay treatments: 0 (no delay), 1, 2, 4, 8 and 16 min. To prevent spiders from searching during the delay, we kept them confined to their retreat position — if they started moving before their assigned delay was complete, we gave them a small puff of air with our pipette. Each spider only
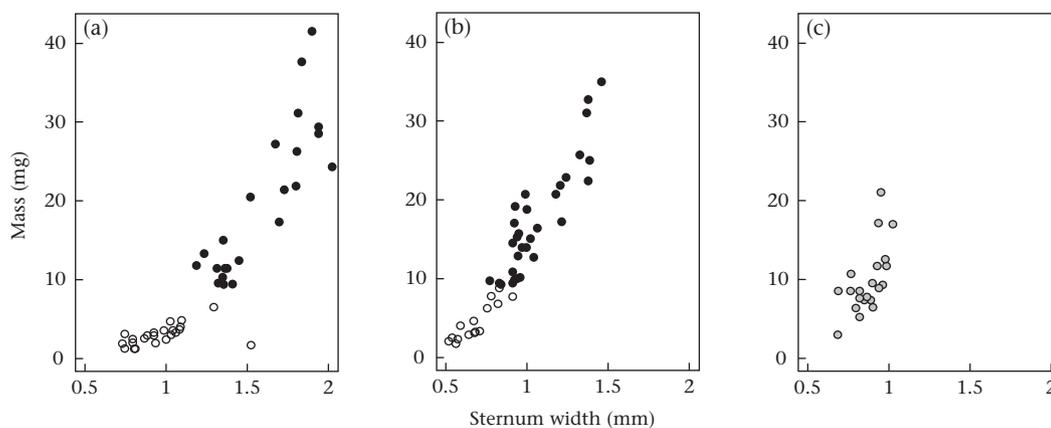
Figure 1. Ranges of final body size measurements (sternum width, final mass) for *P. phalangioides* spiders used in our experiments. (a) Large (filled symbols; *N* = 23) and small (open symbols; *N* = 24) spiders used in the memory retention experiment. (b) Large (filled symbols; *N* = 29) and small (open symbols; *N* = 16) spiders used in the search resumption experiment. (c) Spiders used in the hunger versus handling time experiment (*N* = 22).

received the minimum amount of disturbance that was required to keep it from searching, and these puffs did not significantly affect search time (below). Once the delay treatment was complete, the spider was free to search its web for the lost prey, and we observed its behaviour as above.

We estimated the spiders' memory retention with their 'total search time', which is the length of time starting from when we allowed the spider to start searching for prey and ending with the last bout of search behaviour in the trial. Our rationale for this measure is that as long as a spider is still searching, its memory of the lost prey is still active in its mind.

We analysed variation in total search time using a linear mixed model with the following terms: spider identity (a random effect to account for each spider being tested twice), spider size category (large, small), length of delay (with linear and quadratic terms), the interaction of spider size with the linear and quadratic delay terms, and relative prey size (cricket body length/spider sternum width) (Kilmer et al., 2018). The interactions of spider size with the linear and quadratic delay terms test for differences in the steepness of the search time~delay functions. We included the quadratic term because total search time is bound on one side by zero and we were interested in looking at curvature in the function. To visualize these functions (Fig. 2) we used nonparametric cubic spline regressions fitted with the program 'PFunc' (Kilmer et al., 2017). Preliminary versions of the model included both trial sequence and number of puffs administered to enforce the delay, but neither of these effects were significant, so we removed them for statistical power.

Testing for Variation in Search Resumption

We tested predictions (4) and (5) (that small spiders should be less likely to resume searching after their main bout of search behaviour, or search for less time than large spiders; and that prey size should have less of an effect on search time for small spiders than for large spiders) in a separate experiment. Trials were as above with the following modifications. We randomly assigned

large and small spiders (Fig. 1b) to one of two groups. For one group, when spiders reached our usual stopping criterion, we continued to observe them for at least an additional 5 min. If the spiders tugged at all during this additional time, we continued watching until 5 min passed since the last tug. For the second group, when spiders reached our usual stopping criterion, we attempted to reactivate their search behaviour by gently vibrating the web with an electric toothbrush to simulate the presence of struggling prey. When we did this, spiders readily charged towards the vibration the same way they would attack prey. We removed the toothbrush before they reached it, and, as with the first group, we continued to observe until a further 5 min passed without any tugging. There was an approximately four-fold difference in mean body mass for the spider size categories (Fig. 1b), corresponding to a ca. 1.9-fold difference in brain mass according to the allometric slopes in Quesada et al. (2011). To standardize satiation levels, we fed each spider one cricket 4 days before testing, aiming to give each spider a cricket weighing one-fourth of its capture mass (Fig. 1b). We tested each spider twice with large and small prey in random order.

For each spider, we calculated 'active search time' (not to be confused with the above total search time), which is the sum of all the periods in which the spider was actively performing search behaviours (i.e. tugging in quick succession or exploring the bottom of its box) and it excludes periods in which the spider was not searching (e.g. when it was maintaining its web or resting). Active search time is a measure of effort exerted over the course of the trial, whereas total search time is a measure of the length of time that a memory remains active in the brain. We used this measure because it gives us information about the value that the spider put on its lost prey.

We also determined the percentage of spiders that resumed searching in the additional observation period and the active search time of this additional phase. We analysed the data with a linear mixed model, using active search time from the additional phase as the dependent variable and the following fixed effects: spider size
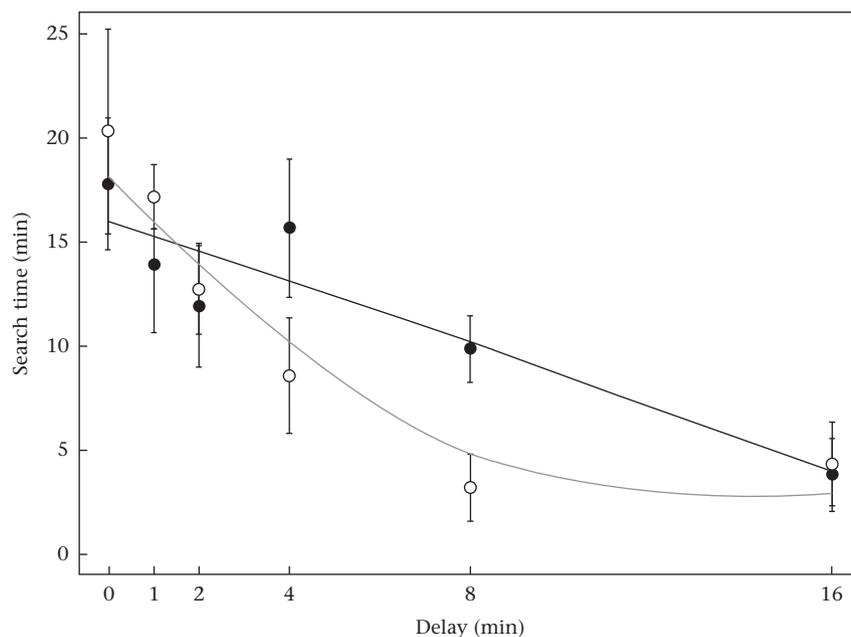
Figure 2. Effects of delay on total search time in *P. phalangioides*. We imposed delays of varying length between memory formation and memory use. We measured total search time as the length of time from the end of the delay until the end of the spider's search behaviour for small spiders (open circles; *N* = 24) and large spiders (filled circles; *N* = 23). Each spider was tested twice at a single delay value—once with large prey and once with small prey (as reported in Table 1, but not shown here). Error bars represent standard error. Corresponding statistics reported in Table 1.

(large or small), cricket size (large or small) and spider size*cricket size. We also included spider identity as a random term. Finally, we asked whether the application of a vibration made any difference. To determine this last point, we ran a mixed model in which the dependent variable was the active search time of the additional observation period, the fixed effect was vibration treatment, and spider identity was included as a random variable, because each spider was tested twice.

Testing Motivation Against Information Acquisition

To assess the relative contributions of proximate motivation and information acquisition on search time, we ran a separate experiment with full-factorial design, manipulating both spider hunger and prey handling time. We used juvenile spiders that covered the species' midrange of body sizes (Fig. 1c). To standardize initial satiation levels, we fed each spider one cricket 4 days before testing, aiming to give each spider a cricket weighing one-fourth of its capture mass (Fig. 1c). We then randomly assigned each spider to a high-hunger or low-hunger experimental group. The low-hunger spiders were tested 2 days after their standardizing meal, and the high-hunger spiders were tested 8 days after their standardizing meal. Each spider was tested once with a cricket that was half the spider's mass. We allowed the spider to wrap up its prey for a set amount of time, according to a randomly assigned treatment group — either short handling time (30 s) or long handling time (120 s). Once the handling time was up, we removed the prey and observed the spider's searching behaviour (as described above).

We analysed our results with a standard least squares model in which the dependent variable was active search time and the main effects were hunger level (high, low), handling time (long, short) and a hunger level * handling time interaction. We also included terms for spider size (measured as sternum width) and prey body size relative to spider body size.

Ethical Note

All our procedures adhered to the ASAB/ABS Guidelines for the use of animals in research, the legal requirements of the U.S.A. and all UWM guidelines.

RESULTS

Smaller Spiders Had a Steeper Search Time~Delay Function

As we increased the delay between memory formation and use, search time decreased, and more quickly so for small spiders than for large spiders (Fig. 2, Table 1). The effect size of this difference in

Table 1
Variation in the retention of memory of lost prey in *P. phalangioides*

Fixed effects	F	ndf, ddf	P
Spider size	**4.89**	1, 40.75	**0.033**
Delay	**21.46**	1, 40.04	**<0.0001**
Spider size*delay	**4.26**	1, 40.68	**0.045**
Delay^2	**4.38**	1, 40.05	**0.043**
Spider size*delay^2	**5.20**	1, 40.05	**0.043**
Prey size	0.84	1, 84.59	0.36
Random effect	Variance comp.		Wald P
Spider identity	92247 (44% of total)		**0.011**

The dependent variable was total search time and the fixed effects were spider size (large or small), delay imposed before searching, the spider size*delay interaction, the quadratic term for delay (indicated as 'delay^2'), the spider size*delay^2 interaction and prey size relative to spider size. We also included a random term, spider identity, because each spider was tested twice (once with each prey size). Significant terms indicated with bold text.

steepness was large; for instance, to see a 50% reduction in search time, it would take a delay of approximately 3 min in small spiders, but approximately 11 min in large spiders. This effect was most likely caused by the delay itself and not by the methods used to enforce the delay: the number of puffs it took to prevent spiders from searching was not a significant term in our initial model; spiders only received as many puffs as necessary to keep them from searching; and all spiders were administered similar puffs of air when we drove them away from their prey during the prey removal phase of each trial, and these had no apparent effect. We found no significant effect of prey size.

Smaller Spiders Were Less Likely to Resume Searches

When we extended observations beyond the usual cutoff (regardless of whether we vibrated the web or not), small spiders searched less compared to large spiders, and they showed virtually no discrimination between prey sizes, while large spiders showed significantly higher (three-fold) search times for larger prey (Fig. 3, Table 2; significant spider size and spider size * prey size terms). Mainly, however, spiders were unlikely to resume searching. There was no resumption in nearly two-thirds of trials (56 of 90, with each spider going through two trials). Thus, our criterion of ending trials after 5 min of no searching was mostly sufficient for capturing the behaviour. In the 34 trials in which spiders did resume searching, they did so only briefly: 50% had an active search time under 30 s, and only in four trials did spiders actively search longer than 2 min. Adding prey cues (in the form of a vibration) affected neither the percentage of spiders that resumed searching (36.4% of the spiders in the vibration group, compared to 37.5% in the no-vibration group) nor the length of search time (LS means ± SE: no vibration: 21.0 ± 5.7 s, N = 23 spiders, 46 trials; vibration: 14.7 ± 5.8 s, N = 22 spiders, 44 trials; $F_{1,43}$ = 0.60, P = 0.44).
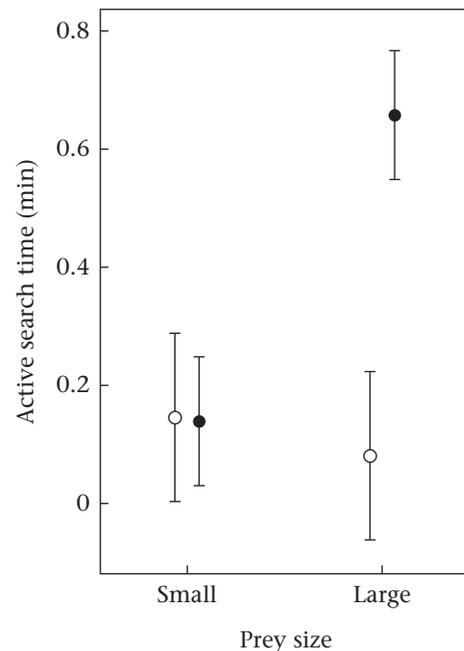
Figure 3. Search resumption in *P. phalangioides* spiders. We measured active search time for small spiders (open circles; N = 16) and large spiders (filled circles; N = 29) as they searched for large and small prey. Each spider was tested twice, once with each prey size. The data shown here are means ± SE of search behaviour that occurred in the additional observation periods — that is, after the normal criterion for ending observations had passed. Statistical results presented in Table 2.

Table 2
Variation in resumed searches for lost prey in *P. phalangioides*

| Fixed effects	$F_{1,43}$	P
Spider size	**4.16**	**0.048**
Prey size	3.20	0.081
Spider size*prey size	**5.48**	**0.024**
Random effect	Variance comp.	Wald P
Spider identity	104 (8% of total)	0.592

The dependent variable was active search time, and the fixed effects were spider size (large or small), prey size (large or small) and spider size*prey size. We also included a random term for spider identity, because each spider was tested twice (once with each prey size). Significant terms indicated with bold text.

Information Acquisition Was More Important Than Hunger

When we measured the relative contributions of hunger versus handling time on search behaviour, we found that a 90 s difference in the opportunity to gather information had an effect that was six to seven times larger than a 6-day difference in hunger (Fig. 4, Table 3). This was the case regardless of prey size and spider size.

DISCUSSION

Testing over an estimated two-fold difference in brain size across juvenile and adult *P. phalangioides* spiders, we find that the retention time of working memory, but not its content, is limited in the smaller instars (Kilmer et al., 2018; this study). Smaller spiders searched similarly or longer than larger spiders for prey that they had captured and lost. Thus, memory formation and content are not limited by brain size; and even very small spiders can be highly motivated and both physically and cognitively capable of forming memories of captured prey and using those memories to regulate searching efforts. By contrast, in two separate experiments, smaller
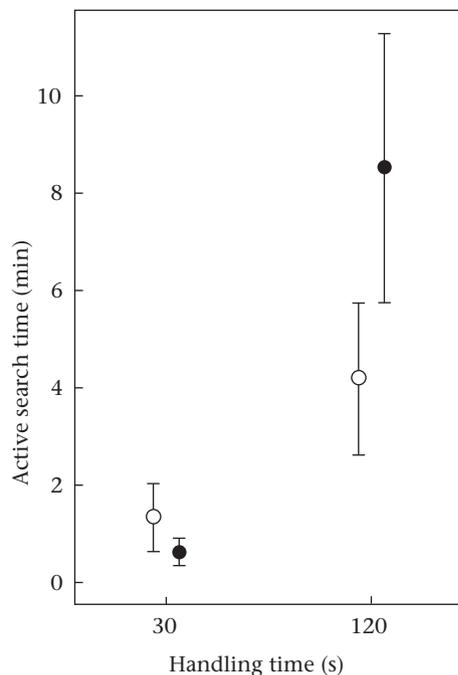
Figure 4. Effects of hunger (physiological motivation) and handling time (information acquisition; 30 s versus 120 s) on active search time for *P. phalangioides*. Spiders in the low-hunger group (open circles; $N = 6$ and 6 for low and high handling time, respectively) went 2 days without food before the trial, while spiders in the high-hunger group (filled circles; $N = 5$ and 5 for low and high handling time, respectively) went 8 days without food. Statistical results presented in Table 3.

Table 3
Effects of motivation and information acquisition on the time that *P. phalangioides* spiders searched for lost prey

Effects	$F_{1,21}$	P
Hunger	0.25	0.625
Handling time	**12.50**	**0.003**
Hunger*handling time	2.59	0.127
Spider size	0.71	0.413
Prey size	1.17	0.296

The dependent variable was active search time, and the fixed effects were hunger (2 days or 8 days since last meal), handling time (30 s or 120 s subduing and wrapping prey), hunger*handling time, spider size (sternum width) and prey size (prey body length/spider size). Significant terms indicated with bold text.

spiders showed a steeper decline in searching when we imposed a delay between memory formation and memory use; i.e. they had worse performance on a delayed memory task. Furthermore, in resumed searches, large spiders discriminated prey size but small spiders did not. Finally, for spiders of similar size, a 90 s difference in information gathering influenced search times much more markedly than 6 days without feeding. Some days without feeding do not starve a spider (Foelix, 2011), but the relative weakness of this effect compared to less than 2 min worth of handling indicates that our searching assay largely reflects information acquired by the spiders about their prey. Thus, we interpret our results as indicating that working memory retention time is limited by brain size in a way that memory content is not.

Our approach took advantage of within-species variation in body and brain size over developmental instars. This may risk confounding the effect of brain size with age and maturation. Perhaps small spiders are not limited in memory retention but in energy supplies, or physical capabilities, or experience, for instance, and in resumed searches perhaps small spiders decided sooner to give up their search efforts. Our data reject this possibility, however. Young *P. phalangioides* were just as capable and motivated as larger, older individuals to remember and search for lost prey as long as no delays occurred between memory formation and its use (Fig. 2, at delay = 0), whereas their searches declined more steeply with such delays. Indeed, it was spiderlings on their first ever foraging experience, having never experienced prey capture and feeding before in their lives, that searched the longest for lost prey, and there was no evidence of an effect of experience (Kilmer et al., 2018). Thus, our results identify a cognitive limitation on memory retention time in smaller brains. Additionally, a separate experiment assessed the relative contribution to search times of information stored in memory versus motivation (hunger), finding that the effect of memory was nearly an order of magnitude stronger.

Two main features of the present study helped us assess cognitive and behavioural capabilities across brain sizes. First, using within-species variation in size across juvenile and adult instars allowed us to compare animals greatly varying in body and brain size with no confounds arising from potential species differences in ecology (cf. Agrillo & Bisazza, 2017). Second, our searching assay directly reflects the content and use of memory in the regulation of behaviour, and it is easily quantifiable. We suggest that integrating the ontogenetic approach and behavioural assays will enhance comparative work on the evolution of cognition.

Why should the retention time but not information content of working memory be limited in animals with miniature brains? Perhaps it is energetically cheaper or neuronally simpler to form memories than to retain them. The mechanisms of forgetting will be key to address these questions. To the extent that our results reflect a passive-decay model of forgetting (e.g. Brown, 1958; Ricker, Vergauwe, & Cowan, 2016), small brains may simply be less capable of holding on to memories. Alternatively, under an

interference model of forgetting (e.g. Lewandowsky, Oberauer, & Brown, 2008), small brains may instead be limited in their ability to hold attention on a given mental object or aim and be more susceptible to distraction. We emphasize, however, our interpretation of the results as a limitation in the small spiders, rather than as adaptive forgetting. This is because the rate of adaptive forgetting should track the rate of change of the relevant resource in the environment (Shettleworth, 2010), which did not differ for spiders of different instars in this study. (If anything, the longer search times that we observed were excessive, as prey that have not been recaptured within the first several seconds of effort are probably unlikely to be recaptured.)

Such limitations in the retention of working memory in miniature animals are likely to have downstream consequences for other abilities. Learning, for instance, may be limited if the interval between an event and its consequence exceeds the retention of the memory of the event. This may be part of the explanation for the poor performance in numerical learning tasks of small-brained guppies, even if their numerosity per se is not compromised (Kotrschal et al., 2013). On the flip side, forgetting itself may be an adaptation that allows for adjusting behaviour and decision making in the face of change (Shettleworth, 2010; West-Eberhard, 2003). Even here, though, a limited retention time would constrain the range over which miniature animals could modulate learning and forgetting.

The scarcity of evidence in the literature for limitations in small brains is not for lack of effort — biologists have been curious about brain size for decades (e.g. Cole, 1985; Eberhard, 2007; Eberhard & Wcislo, 2011; Eisenberg & Wilson, 1978; Garamszegi, Eens, Erritzøe, & Møller, 2005; Harvey, Clutton-Brock, & Mace, 1980; Kotrschal et al., 2015, 2013; Rensch, 1956). Part of the difficulty may be explained by the discovery that some cognitive abilities are not as neurologically complex as initially thought (Chittka & Niven, 2009; Roper, Fernando, & Chittka, 2017; Skorupski, MaBouDi, Dona, & Chittka, 2017). Simulations of neural networks have shown that seemingly complex cognitive functions, like selective attention or visual categorization, can each be accomplished with fewer than 20 neurons (Beer, 2003; Garamszegi et al., 2005; Goldenberg, Garcowski, & Beer, 2004). Some abilities, like pattern recognition, may actually be adaptations that allow brains to process information more efficiently with fewer neurons, rather than being complex functions that require large neural machinery (Srinivasan, 2006). Perhaps larger brains do not add new functions so much as enhance existing abilities, such as memory capacity, sensory resolution and parallel processing (Chittka & Niven, 2009). Additionally, there are some areas in which small animals with small brains are truly not limited. Miniature orb web spiders, for instance, suffer no limitation in their movement precision and error correction (Eberhard, 2007, 2011; Hesselberg, 2010). A dramatic case of lack of limitation occurs in some jumping spiders, which in spite of their small size perform feats of planning and navigation that far surpass the capabilities of larger spider species, and even many vertebrates (Cross & Jackson, 2016; Tarsitano & Jackson, 1997).

Doubtless other factors besides brain size are important as cognitive capabilities evolve to meet the challenges offered by the environment of different species (Shettleworth, 2010). In some cases, within-species variation in cognitive ability tracks population differences in the level of risk entailed in foraging (Jackson, Pollard, Li, & Fijn, 2002), suggesting extraordinary levels of fine-tuning by selection on cognition. Against this backdrop, we interpret our results with caution and we refer specifically to within-lineage reduction in brain size. We suggest that, in such cases, animals may sacrifice working memory retention (albeit not memory information content), or require novel ways to preserve it

or compensate for its loss (e.g. evolving new heuristic rules to regulate behaviour; Hesselberg, 2015; Hutchinson & Gigerenzer, 2005). Limits to working memory retention may, in turn, influence other variables, such as the time window over which miniature animals have the opportunity to learn relationships between their behaviour and its consequences. Alternatively, selection against these limits may also help explain why miniature animals retain relatively large brains.

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