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Long-term memory of relative reward values

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Long-term memory can be adaptive as it allows animals to retain information that is crucial for survival, such as the appearance and location of key resources. This is generally examined by comparing choices of stimuli that have value to the animal with those that do not; however, in nature choices are rarely so clear cut. Animals are able to assess the relative value of a resource via direct comparison, but it remains unclear whether they are able to retain this information for a biologically meaningful amount of time. To test this, captive red-footed tortoises (*Chelonoidis carbonaria*) were first trained to associate visual cues with specific qualities and quantities of food, and their preferences for the different reward values determined. They were then retested after an interval of 18 months. We found that the tortoises were able to retain the information they had learned about the cues as indicators of relative reward values over this interval, demonstrating a memory for the relative quantity and quality of food over an extended period of time. This is likely to impact directly on an animal's foraging decisions, such as the exploitation of seasonally varying resources, with obvious fitness implications for the individual; however, the implications may also extend to the ecological interactions in which the animal is involved, affecting processes such as herbivory and seed dispersal.

1. Introduction

The retention and retrieval of information is essential for a wide range of behaviours [1], including the relocation of key resources like shelter and food [2], and the retention of social information [3]; it can therefore be highly adaptive [1]. Generally, we investigate memory retention in animals by asking subjects to select stimuli that are associated with acquiring food while avoiding stimuli that are not (e.g. [4–6]), and studies using this approach have provided evidence that a number of species are able to retain this sort of information for a substantial amount of time (e.g. [4,6]). However, in nature, decisions are rarely so clear cut, and animals are often required to evaluate the relative benefits of different resources. There is evidence to suggest that animals are able to make these discriminations when given direct comparisons (e.g. [7,8]); however, it is still not clear whether information of this type, i.e. relative salience/value, can be retained over an extended period of time.

Hoarding birds, for example, are able to recall relative reward features such as the size and transience of cached food for up to 100 h [9]. However, for many animals, foraging decisions require retention of information over a much greater time period (e.g. [10]). For instance, fruit often recurs in the same location at long but predictable seasonal periods [11]. It may therefore be adaptive for animals to retain information regarding relative values of different resources, such as the quality (in terms of energetic content and/or preferred taste) and quantity of fruit produced by different trees [12], over many weeks or months.

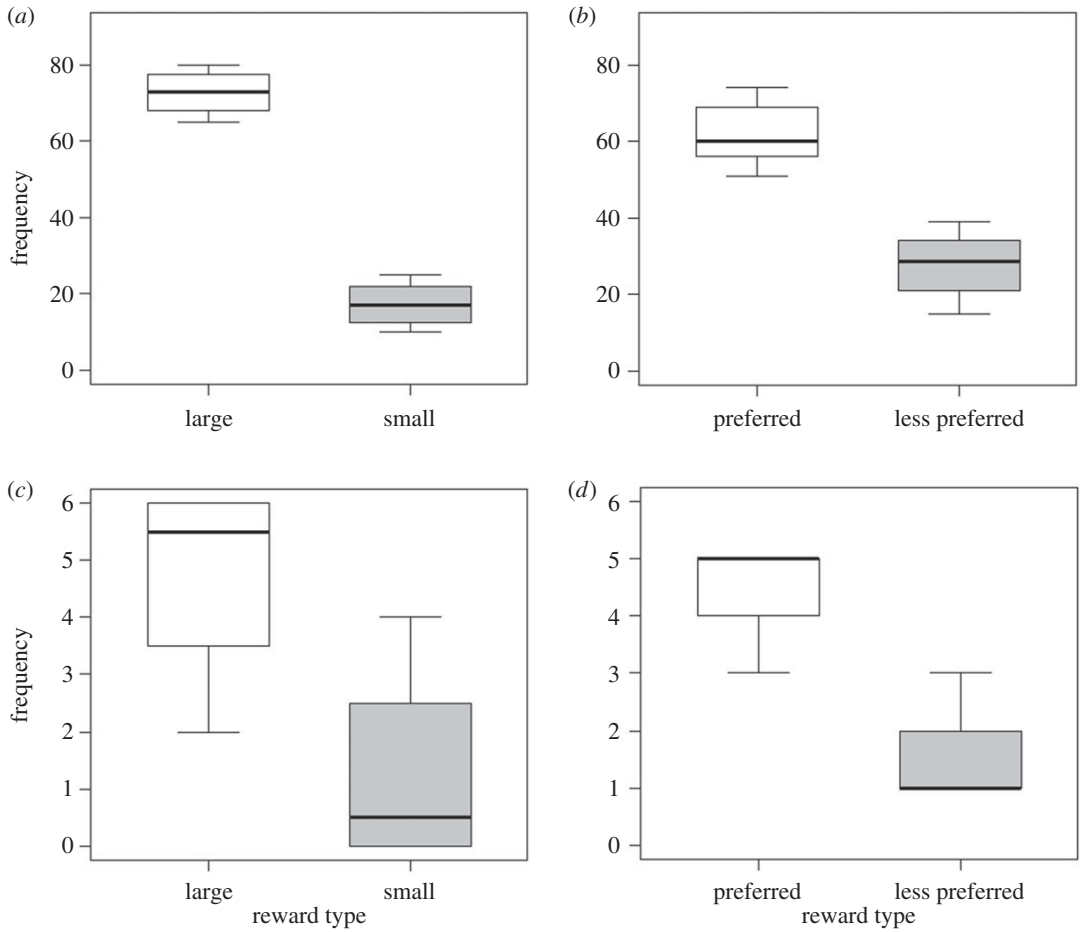

Figure 1. Median, quartiles and range in the frequency of choices for stimuli corresponding to different reward types (large/small or preferred/less preferred) following initial training in the (*a*) quantity and (*b*) quality tests, and after an 18 month retention interval in the (*c*) quantity and (*d*) quality memory tests.

The long-term retention of foraging-related information would be particularly pertinent for long-lived animals as, over the course of their lifetime, the benefits of retaining this information are likely to outweigh costs associated with storing a memory [13,14]. This is especially important in environments where resources are patchily distributed [15]. Here, we experimentally test for long-term memory of relative reward value (quantity and quality), using red-footed tortoises (*Chelonoidis carbonaria*) as a model species. Red-footed tortoises are long-lived omnivores, whose natural diet contains a high proportion of fruit (up to 70%; [16]). They live in spatially complex forest environments [17], and are highly visual [18], are able to learn rapidly [19] and can retain spatial information for more than three months [5]. Animals were first trained to associate visual cues with specific values of reward. We then tested their ability to retain this information for an interval of 18 months, a period that encompasses most of the major temporally separated events, such as plants' fruiting seasons, in their natural environment [17].

2. Methods

(a) Reward values assessment

Initial training was necessary to allow the tortoises to learn associations between visual stimuli and different quantities and qualities of food reward and to confirm that they were then able to use this information to demonstrate preferences for high value rewards; previous experiments exclusively tested whether subjects were able to select a cue that was associated with acquiring food while avoiding stimuli that were not (e.g. [5,6]).

Captive-raised red-footed tortoises were trained and tested in an experimental arena measuring 1×1 m located in a room maintained at 28°C (± 2°C). The tortoises were trained to associate visual cues (laminated coloured sheets, 10.5×14.85 cm) with specific qualities (preferred (mango-flavoured jelly) versus less-preferred (apple-flavoured jelly)) and quantities (large (125 mm^3 jelly) versus small (27 mm^3 jelly)) of food, counterbalanced across individuals. After the completion of 72 training trials, tortoises were tested by presenting the two stimuli simultaneously. When a tortoise approached one of the two stimuli, it was presented with the corresponding reward, and, after consuming the rewards was immediately removed from the arena. No food was present in the arena until after the tortoise made a choice. Each tortoise completed 90 test trials. Please see electronic supplementary material for full experimental details.

(b) Long-term memory test

After an 18-month retention interval, during which the tortoises were not exposed to either the visual stimuli or rewards, we assessed their retention of relative reward value. The memory test took place in the same arena as the previous testing. Tortoises ($n = 4$ for the study involving reward quantity, and $n = 6$ for the study involving reward quality, four common to both studies) were simultaneously presented with the two stimuli used during the training. Stimuli were both presented 60 cm from the starting point, and their positions (left or right) were counterbalanced across trials. Tortoises had 1 min to approach one of the two stimuli. When a choice was made (a tortoise approached the stimulus within 3 cm and oriented its head towards it), the tortoise was removed from the arena without receiving any reward; this was to ensure that no new learning took place. After 2 min the next trial started. Each animal

received six consecutive memory test trials. Between trials the arena was cleaned to remove possible scent trails.

(c) Data analysis

We investigated tortoises' choice of reward, both following initial training and after 18 months, by testing whether preferences for the stimuli associated with the high-quality or high-quantity rewards differed significantly from chance levels (i.e. a relative preference of 0.5) using exact binomial tests (function 'binom.test' in R v. 2.15.1).

3. Results

Tortoises were able to learn and discriminate between visual stimuli representing relative reward values, significantly preferring the stimuli associated with the reward that was greater in quantity ($p < 0.001$; figure 1*a*) or greater in quality ($p < 0.001$; figure 1*b*). This information was successfully retained after an 18 month interval, with animals performing significantly above chance in both cases (quantity: $p = 0.007$; quality: $p = 0.004$; figure 1*c*,*d*).

4. Discussion

The tortoises successfully learned that different visual stimuli represented different reward values and discriminated between them on this basis, preferring the stimulus representing the higher value of reward (in terms of both quality and quantity) when given the choice. Crucially, the tortoises retained this information for a period of at least 18 months. This suggests that tortoises can remember the relative value of a reward, and not just its presence or absence (e.g. [5,6]), for a period spanning seasons and significantly longer than previously found in hoarder species [9,10]. The retention of this information could increase fitness as it would improve foraging efficiency by eliminating the necessity to re-evaluate food sources during each foraging event and reduce the risk of re-visiting inadequate food sources [20]. Red-footed tortoises inhabit environments where the resources are patchily distributed in space and time [17], and this, in combination with their relatively slow locomotion [17], suggests that the benefits gained from retaining information regarding higher value food sources are likely to outweigh the cost associated with memory [15].

Long-term memory of relative values associated with visual stimuli is likely to be highly adaptive for animals. For instance, it may allow them to remember the characteristics of aposematic signals, or allow the comparative assessment of sexual signals between breeding seasons. Recent work [12] suggests that long-term memory might impact on a range of ecological interactions and ecosystem processes. Red-footed tortoises are important seed dispersers in their natural environment [16], and so the retention of the knowledge of food values might affect herbivory or seed dispersal through endozoochory [12,21]. Plant fruiting cycles are usually predictable in time [22], and 18 months is longer than the fruiting interval of the majority of plants species in the red-footed tortoise's habitat [17]. Thus, our findings reveal that tortoises are able to remember information relating to the value of a food source for a duration that is greater than the periods between fruiting periods. This suggests that plants that provide better fruit in terms of quantity or quality may not only receive more visits in a given season, but also receive repeat visits in successive fruiting seasons [23]. As a consequence, the ability to remember the relative value of food sources may have a direct impact on the amount and type of species of seeds dispersed.

Ethics. This experiment had approval from the College of Science ethical committee at the University of Lincoln (reference COSREC-2012-01), and the work was carried out in accordance with the relevant guidelines and regulations of the UK.

Data accessibility. We provide supporting data in the electronic supplementary material.

Authors' contributions. All authors designed the experiment. F.S. ran the experiment. All authors contributed to writing the manuscript. All authors have approved the final version of the manuscript and agree to be held accountable for the contents of this work and approve the final version of the manuscript.

Competing interests. We declare we have no competing interest.

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References

1. Healy SD, Jones CM. 2002 Animal learning and memory: an integration of cognition and ecology. *Zoology* **105**, 321–327. (doi:10.1078/0944-2006-00071)
2. Lohmann K, Luschi P, Hays G. 2008 Goal navigation and island finding in sea turtles. *J. Exp. Mar. Biol. Ecol.* **356**, 83–95. (doi:10.1016/j.jembe.2007.12.017)
3. Boeckle M, Bugnyar T. 2012 Long-term memory for affiliates in ravens. *Curr. Biol.* **22**, 801–806. (doi:10.1016/j.cub.2012.03.023)
4. Vaughan Jr W, Greene SL. 1984 Pigeon visual memory capacity. *J. Exp. Psychol. Anim. B* **10**, 256–271. (doi:10.1037/0097-7403.10.2.256)
5. Mueller-Paul J, Wilkinson A, Aust U, Sturer M, Huber L. 2014 Touchscreen performance and knowledge transfer in the red-footed tortoise (*Chelonoidis carbonaria*). *Behav. Process.* **106**, 187–192. (doi:10.1016/j.beproc.2014.06.003)
6. Davis KM, Burghardt GM. 2012 Long-term retention of visual tasks by two species of emydid turtles, *Pseudemys nelsoni* and *Trachemys scripta*. *J. Comp. Psychol.* **126**, 213–223. (doi:10.1037/a0027827)
7. Addessi E, Crescimbene L, Visalberghi E. 2008 Food and token quantity discrimination in capuchin monkeys (*Cebus apella*). *Anim. Cogn.* **11**, 275–282. (doi:10.1007/s10071-007-0111-6)
8. Krusche P, Uller C, Dicke U. 2010 Quantity discrimination in salamanders. *J. Exp. Biol.* **213**, 1822–1828. (doi:10.1242/jeb.039297)
9. Clayton NS, Dally J, Gilbert J, Dickinson A. 2005 Food caching by western scrub-jays (*Aphelocoma californica*) is sensitive to the conditions at recovery. *J. Exp. Psychol. Anim. Behav. Processes* **31**, 115–124. (doi:10.1037/0097-7403.31.2.115)
10. Balda RP, Kamil AC. 1992 Long-term spatial memory in Clark's nutcracker, *Nucifraga columbiana*. *Anim. Bahav.* **44**, 761–769. (doi:10.1016/S0003-3472(05)80302-1)
11. Fleming TH, Breitwisch R, Whitesides G. 1987 Patterns of tropical vertebrate frugivore diversity. *Annu. Rev. Ecol. Syst.* **18**, 91–109. (doi:10.1146/annurev.es.18.110187.000515)
12. John EA, Soldati F, Burman OHP, Wilkinson A, Pike TW. 2016 Plant ecology meets animal cognition: impacts of animal memory on seed dispersal. *Plant Ecol.* **217**, 1–16. (doi:10.1007/s11258-016-0652-3)

13. Burns JG, Foucaud J, Mery F. 2011 Costs of memory: lessons from 'mini' brains. *Proc. R. Soc. B* **278**, 923–929. (doi:10.1098/rspb.2010.2488)
14. Mery F, Kawecki TJ. 2005 A cost of long-term memory in *Drosophila*. *Science* **308**, 1148. (doi:10.1126/science.1111331)
15. Boyer D, Walsh P. 2010 Modelling the mobility of living organisms in heterogeneous landscapes: does memory improve foraging success? *Phil. Trans. R. Soc. A* **368**, 5645–5659. (doi:10.1098/rsta.2010.0275)
16. Strong JN, Fragoso JMV. 2006 Seed dispersal by *Geochelone carbonaria* and *Geochelone denticulata* in northwestern Brazil. *Biotropica* **38**, 683–686. (doi:10.1111/j.1744-7429.2006.00185.x)
17. Moskovits DK, Bjorndal KA. 1990 Diet and food preferences of the tortoises *Geochelone carbonaria* and *G. denticulata* in northwestern Brazil. *Herpetologica* **46**, 207–218.
18. Wilkinson A, Huber L. 2012 Cold-blooded cognition: reptilian cognitive abilities. In *The Oxford handbook of comparative evolutionary psychology* (eds J Vonk, TK Shackelfor), pp. 129–143. Oxford Township, NJ: Oxford University Press.
19. Wilkinson A, Coward S, Hall G. 2009 Visual and response based navigation in the tortoise (*Geochelone carbonaria*). *Anim. Cogn.* **12**, 779–787. (doi:10.1007/s10071-009-0237-9)
20. Armstrong N, Garland A, Burns KC. 2012 Memory for multiple cache locations and prey quantities in a food-hoarding songbird. *Front. Psychol.* **3**, 1–9. (doi:10.3389/fpsyg.2012.00584)
21. Bracis C, Gurarie E, Van Moorter B, Goodwin RA. 2015 Memory effects on movement behavior of foraging animals. *PLoS ONE* **10**, e0136057. (doi:10.1371/journal.pone.0136057)
22. Momose K. 2004 Plant reproductive interval and population density in aseasonal tropics. *Ecol. Res.* **19**, 245–253. (doi:10.1111/j.1440-1703.2003.00629.x)
23. Di Fiore A, Suarez SA. 2007 Route-based travel and shared routes in sympatric spider and woolly monkeys: cognitive and evolutionary implications. *Anim. Cogn.* **10**, 317–329. (doi:10.1007/s10071-006-0067-y)