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Animal behaviour

Continuous and discrete quantity discrimination in tortoises

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The ability to estimate quantity, which is crucially important in several aspects of animal behaviour (e.g. foraging), has been extensively investigated in most taxa, with the exception of reptiles. The few studies available, in lizards, report lack of spontaneous discrimination of quantity, which may suggest that reptiles could represent an exception in numerical abilities among vertebrates. We investigated the spontaneous ability of Hermann's tortoises (*Testudo hermanni*) to select the larger quantity of food items. Tortoises were able to choose the larger food item when exposed to two options differing in size, but equal in numerousness (0.25, 0.50, 0.67 and 0.75 ratio) and when presented with two groups differing in numerousness, but equal in size (1 versus 4, 2 versus 4, 2 versus 3 and 3 versus 4 items). The tortoises succeeded in both size and numerousness discrimination, and their performance appeared to depend on the ratio of items to be discriminated (thus following Weber's Law). These findings in chelonians provide evidence of an ancient system for the extrapolation of numerical magnitudes from given sets of elements, shared among vertebrates.

1. Introduction

Animals use information that is potentially available in their environment in order to survive, find food and reproduce. An example is the ability to discriminate between sets of physical objects (e.g. food items, conspecifics, predators, refuges) making use of discrete (countable) or continuous quantities. Evidence has shown that when animals make non-symbolic quantity judgements, their accuracy is limited by the ratio between the numerical values being compared, as indicated in Weber's Law (reviewed in [1]). For example, accuracy at choosing the larger of two sets is similar when the numerical choices have the same ratio (e.g. 5 versus 10, 10 versus 20 or 50 versus 100: 1.0 Weber fraction) but shows a decrease with lower ratios (e.g. 4 versus 5, 16 versus 20 and 40 versus 50: 0.25 Weber fraction).

This ratio-dependent pattern of success and failure has been documented in warm-blooded vertebrates in a number of species (mammals, e.g. [2–4]; birds, e.g. [5–7], reviews in [8,9]), even if with different experimental designs and procedures. Less clear is the evidence for quantity discrimination in cold-blooded vertebrates. Amphibians [10,11] and fish [12,13] showed quantity judgements that vary as a function of ratio in accordance with Weber's Law. In reptiles, by contrast, ruin lizards (*Podarcis siculus*) proved able to spontaneously discriminate between the surface area of two food items of different size, but failed when food was presented in sets of discrete items differing in numerousness [14]. The lizards also showed very poor performance in experiments involving explicit training: six out of ten discriminated 1 versus 4 items; among these, only one was capable of learning a 2 versus 4 discrimination [15]. This is clear in contrast with evidence collected in fish and amphibians (above) in similar tasks.

In this study, we explored for the first time the numerical competence in chelonians, and particularly in *Testudo hermanni*, in the spontaneous discrimination between quantities differing in number or size. We adopted a similar protocol to that used in the experiment conducted on lizards [14] by presenting tortoises with four different combinations of food items. Each combination represented a choice test between two items and was performed with four proportional differences in magnitude (i.e. ratios: 0.25, 0.50, 0.67, 0.75), both for number and size (see §§2d,e below).

2. Material and methods

(a) Subjects

We collected 25 adult Hermann's tortoises, *Testudo hermanni*, from the naturalistic area of 'Oasi di Sant'Alessio', located roughly 20 km south of Milan, in Northern Italy. In order to determine sexual adultness, we measured carapace length of all turtles using a digital calliper (accuracy $\pm$ 0.1 mm). Sexual dimorphism is noticeable in this species: males are smaller and their plastron is concave, which allows them to mount females during mating [16]. After a short pre-testing phase (see electronic supplementary material, S1, for additional details), in our experiments we selected 16 males and three females (carapace length (mean $\pm$ s.e.), males: 167.7 $\pm$ 4.3 mm, females: 200.0 $\pm$ 26.2 mm). The total sample consisted of 16 subjects for the number discrimination experiment (14 males and 2 females), and 15 subjects in size discrimination experiment (13 males and 2 females). Of these, 12 tortoises (11 males and 1 female) were used in both experiments.

(b) Experimental apparatus and visual stimulus

The tortoises were tested in an outdoor arena consisting of a tunnel that served both as starting zone and approach area to the stimuli and a wider testing compartment where stimuli were presented during the trials. Subjects could observe the stimuli from the start of the trial and throughout the tunnel before entering the testing compartment. Visual stimuli were represented by *Solanum lycopersicum* (San Marzano tomato variety) slices placed on two supports arranged in the testing compartment. Slices were presented in a symmetrical position compared to the longitudinal axis of the tunnel. Since olfactory cues might represent a signal of attraction [14], four tomato slices were included in the experimental compartment and hidden from the sight of the tortoises (see electronic supplementary material, S1).

(c) Experimental procedures

We applied the same general procedure and the same set-up in the two different experiments (number and size experiment; see the electronic supplementary material, S1, for details). A 5-day acclimation period was necessary to allow the tortoises to become familiar with the experimental apparatus, and subsequently be tested. Each tortoise was presented with two food items that were different either in numerousness (number experiment) or in dimension (size experiment). In each testing trial, the subject was allowed a single choice between the two items. We considered the choice made as the first attempt to eat any tomato slice of any group. When the subject approached the slices at the distance of about 1 cm, the choice was considered made. The tortoise was then removed from the arena and placed back into its enclosure. However, in order to avoid any possible learning effect, the tortoises were not allowed to eat the tomatoes [11]. In this way, the subject performed the task in the general context of a spontaneous choice. If after 3 min the tortoise had not approached any stimulus, the response was discarded, and the trial was repeated. The left–right position of the larger stimulus was presented in a pseudo-random sequence to exclude the possible effect of lateralization [17]. All different food item combinations were presented in a mixed sequence in both of the experiments.

(d) Number discrimination experiment

We investigated the tortoises' choices between two groups of items of the same size (circular tomato slices, diameter = 2 cm) but differing in number. We adopted four numerical comparisons: 1 versus 4, 2 versus 4, 2 versus 3 and 3 versus 4, representing four different ratios within each combination (0.25, 0.50, 0.67 and 0.75, respectively). Each tortoise was tested in a total of 60 trials (15 for each discrimination) over 13 days.

(e) Size discrimination experiment

In this experiment, we explored the ability of tortoises to distinguish between food items of different size. We observed subjects in their spontaneous preference between combinations (1 versus 1) of differently sized tomato slices (range from 2 to 8 cm^2), with four size ratios within each combination (0.25, 0.50, 0.67 and 0.75, as in the first experiment). The tortoises underwent a total of 60 trials for each subject (15 for each discrimination) over a period of 13 days.

3. Results

Overall, the tortoises showed a significant preference in each combination they were presented with, both for larger food items and higher numerosity (figure 1). Both the main effects in the mixed model, performed on the index of choice (number of choice for larger item/total number of choice), were significant (combination (ratios): $\chi^2 = 31.21$, d.f. = 3, $p < 0.001$; experiment: $\chi^2 = 4.87$, d.f. = 1, $p = 0.027$) but not their interaction ($\chi^2 = 2.10$, d.f. = 3, $p = 0.55$).

The pattern analysis, performed including the combination as a numerical variable in the model, showed a main effect of the combination ($\chi^2 = 23.88$, $p < 0.001$) and of the experiment ($\chi^2 = 4.87$, $p = 0.027$) but no significant effect of their interaction ($\chi^2 = 1.10$, $p = 0.29$). The analysis conducted separately on the single experiment showed a decline in performance for both number ($\beta \pm$ s.e. $= -0.22 \pm 0.08$, $t = -2.58$, $p = 0.01$) and size experiments ($\beta \pm$ s.e. $= -0.34 \pm 0.07$, $t = -4.55$, $p < 0.001$; figure 2), but their decrease was not different ($t = -1.05$, $p = 0.29$).

Considering the animals tested in both of the experiments (12 subjects overall), we did not find a significant correlation between the number and size experiments for the same ratio (Pearson's product-moment correlation test (lower p-value for 0.25 ratio): $t = 1.70$, d.f. = 10, $p = 0.12$; for this ratio effect size was medium–large: $r = 0.47$). Finally, no laterality effect emerged from the collected data.

4. Discussion

Tortoises showed a remarkable ability to discriminate food items of different numerosity and of different size. Their performance was ratio-dependent and aligns with the ability observed in other groups of vertebrates [8,9].

However, among vertebrates, the class of reptiles has remained largely understudied for numerical competence. Evidence of poor performance in lizards as compared to fish (see §1) has been hypothesized to be owing to some genetic change that took place within ray-finned fish, but after their divergence from the lineage leading to land vertebrates. This

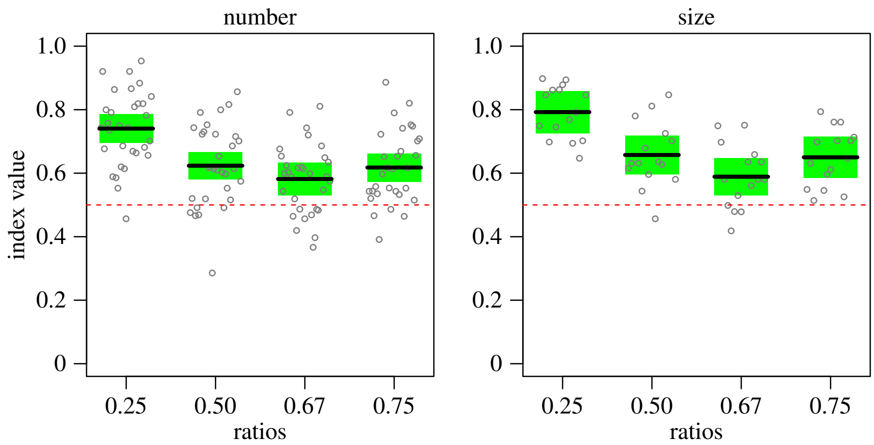

Figure 1. Index expected values and 95% confidence interval bands (green colour) calculated from the mixed models (by bootstrap with 10 000 repetitions), for the separate experiments. Dashed lines represent chance value of the index. (Online version in colour.)

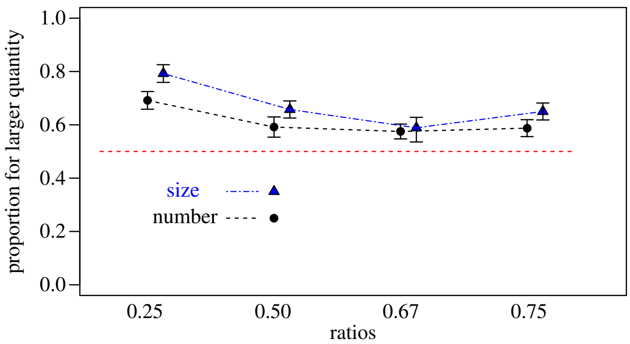

Figure 2. Plot showing the means and s.e. for the index-value (*y*-axis) for all ratios in both experiments. The straight dashed line represents the chance level for choice (index = 0.5).

promoted the appearance of complex cognitive skills in fish, including numerical abilities [14]. Our results with tortoises suggest that this is not the case, because these reptiles show numerical performances comparable to those of fish and amphibians. It seems likely that lizards' difficulties with quantity discrimination may depend on more mundane factors related to motivation, task used or type of reward. Since lizards are known to actively prey on live animals [18], dead *Musca domestica* larvae used in the previous experiment could have failed to properly simulate a fairly intense food stimulus to motivate the interest of the lizards in discriminating larger numerical quantities.

The procedures used with lizards [14] and, in the present study, with tortoises, did not allow disentangling of the specific role played by strictly numerical aspects of the stimuli and those associated with continuous physical variables. Even with discrete items, the discrimination could have been based on some computation of continuous physical variables that covary with numerosity (see [19]). It is interesting to note, however, that the tortoise's performance was different both in the discrete numerical discrimination and the size discrimination (the latter being easier). Moreover, no correlation was observed between the number and size experiments for the same ratio. This could be suggestive of different mechanisms involved.

Among the extant reptile orders, most research in cognition has focused on chelonians [20] and has revealed remarkable abilities in spatial cognition [21,22], visual cognition [23] and acquisition of novel behaviours [24]. Over the past 225 Myr, chelonians seem to have undergone little change and possibly represent an ancient evolutionary solution to cognitive problems such as quantity estimation [20].

Ethics. All tortoises in this study were maintained in outdoor conditions to allow them to adjust their biorhythms according to a natural temperature and photoperiod conditions. Turtles fed and basked regularly and therefore did not show any apparent sign of stress, and at the end of each experimental session were returned to their enclosures. We received permission to use Hermann's tortoises from the 'Oasi di Sant'Alessio', which has permits to hold, breed, capture, manipulate and carry out conservation projects and scientific research on this and many other species found in it.

Data accessibility. The dataset is available from electronic supplementary material, S3.

Authors' contributions. A.G., G.V. and D.P.-R. developed the study concept, contributed to the study design and drafted the manuscript; A.G. and D.P.-R. collected and analysed data. All authors contributed to the revisions of this manuscript, agreed to be held accountable for this work and approved the final version of the manuscript for publication.

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