Can a tortoise learn to reverse? Testing the cognitive flexibility of the Red Footed tortoise (*Geochelone carbonaria*)

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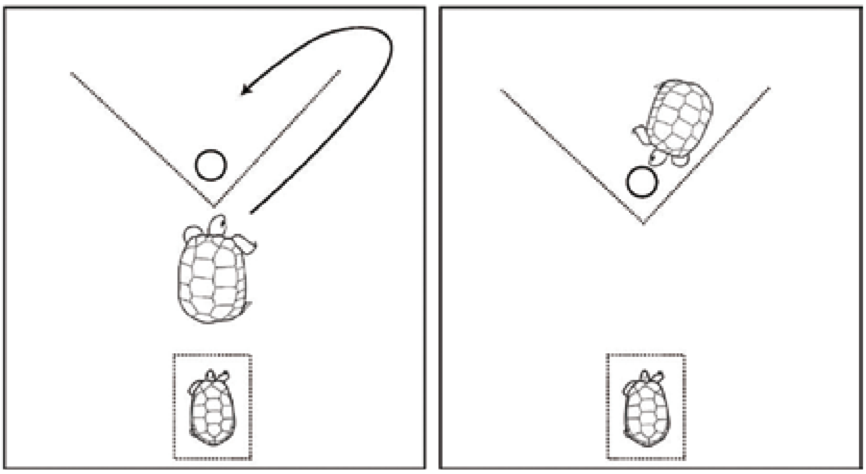
Introduction

Learning in reptiles

Amniotes include all mammals, reptiles and birds whose embryos are surrounded by a membrane known as the amnion (Ridley 2011). These classes evolved from a common amniotic ancestor who lived 280 million years ago (Wilkinson et al. 2007). Due to this, these three animal classes may share many behavioural and morphological traits (Wilkinson & Huber 2012). Despite this, the amount of research into the learning and cognition of these classes is not balanced, with a large emphasis on Mammalian and Avian cognition.

Before the 1970's research into reptilian cognition was very popular, a large proportion of the work was done by Bitterman and colleagues (Burghardt 1977) who investigated areas of cognition including T-mazes (a two choice task where the subject has to enter one of two arms for a reward), and partial reinforcement (a procedure where the subject is rewarded less than 100% of the time for a correct response) in several chelonian species (Boycott & Guillery 1962). However unlike the veritable explosion of work in mammals and birds (Wilkinson & Huber 2012), research in reptiles dwindled, due to poor performance through testing in inappropriate conditions.

Fig. 1
Red-footed tortoise in the xerophytic forest in Paraguay. Photo: Thomas & Sabine Vinke



Graphic 1
Red-footed tortoises are able to learn how to navigate a detour to gain a reward by watching a pre-trained tortoise. Graphic nach Wilkinson et al. (2010a)

Studies into spatial cognition have highlighted the ability of the Red-Footed tortoise (*Geochelone carbonaria*) to successfully navigate a radial arm maze (Wilkinson et al. 2007). The same individual was also capable of switching strategies, changing from using visual room cues to avoid entering previously visited arms, to using a turn-by-one-arm strategy when visual cues were removed or manipulated (Wilkinson et al. 2009); this demonstrated the first evidence that this species was able to switch its behaviour when the information around it had changed.

This group was also the first to test for social learning in reptiles (Wilkinson et al. 2010a). Red-Footed tortoises had to navigate a 'V-shaped' fence to gain a reward, which involved initially walking away from the reward in order to navigate the detour (see graph 1); half of the subjects had to learn through individual learning, resulting in the failure of each individual. However for those subjects who watched a pre-trained demonstrator tortoise successfully navigate the fence, each subject was able to reach the reward, with two tortoises performing successfully on their first attempt. This demonstrated that social learning may be an attribute which was conserved amongst the amniotes, leading to the assumption that perhaps other areas of cognition had been conserved across mammals, birds and reptiles.

Wilkinson et al. (2010b) looked into gaze following in the Red-Footed tortoise and they were able to exhibit the first evidence of gaze following in reptiles, with the majority of the tortoises following the gaze of a conspecific. Again this illustrates that reptiles can perform well in tasks dominated by mammals and birds and demonstrates another form of cognition which may have been conserved in these classes. Therefore it is possible that other forms of cognition have been conserved across the amniotes.

Cognitive flexibility

Cognitive flexibility is the ability to shift from one problem-solving strategy to another (Voytko 1999); to correctly perform cognitive flexibility tests involves the possession of a higher level of cognitive functioning (López et al. 2003) as it is dependent on three aspects which affect an animal's ability to deal with rapid changes.

Firstly animals that live in permanent social groups and display a wide

Fig. 2
Red-footed tortoises have to navigate an environment which is diverse and lacking in shelters. But must they be cognitively flexible and able to reverse what they have learned before? Photo: Thomas & Sabine Vinke

range of social behaviours should face greater demands for flexibility than more solitary species (Bond et al. 2007). Secondly complex environments are thought to select for greater levels of flexibility as these animals are exposed to increased unpredictability (Bond et al. 2007). Thirdly flexibility is favoured in animals that exploit diverse food sources (Leal & Powell 2011) as they must exhibit a win-stay, lose-shift strategy in order to deal with the changing resources (Davey 1989).

One method of investigating cognitive flexibility is reversal learning. In this task the subject is typically presented with two stimuli, each with different reward contingencies (D'Cruz et al. 2011), one stimulus is positive and the subject is rewarded for choosing this stimulus, and the other is regarded as negative and does not result in a reward.

The task is composed of an acquisition phase in which the subject learns to choose the positive stimulus over multiple trials, once this has been learnt the reinforcement contingencies are switched without warning (D'Cruz et al. 2011), therefore the stimulus-reward associations are reversed so that the previously rewarded stimulus is no longer rewarded and the previously negative stimulus is now rewarded. Successful reversal learning requires the suppression of the response initially learned in the acquisition phase, whilst learning a new competing response during the reversal phase (Watson & Stanton 2009).

In serial reversals, subjects are exposed to a long series of reversals (Shettleworth 1998), to determine if the subject can solve subsequent reversals in fewer trials, culminating in one-trial learning (Bonney & Wynne 2004) where the animal can reverse after making just one error, this epitomises the win-stay, lose-shift strategy.

Reversal learning in reptiles

VPrevious reversal learning studies have delved into comparisons between reptile species (Day et al. 1999, Gaalema 2007); however none have yet taken two populations of the same species to examine the effect of habitat on cognitive flexibility. The Day et al. article (1999) tested the reversal abilities of two species of male lizards (*Acanthodactylus boskianus*, an active forager and *Acanthodactylus scutellatus* a sit and wait predator). They were able to demonstrate that there were no differences in acquisition stage between the two species, however the active forager completed the single reversal in fewer trials than the sit-and-wait predator, but both species took more trials to criterion for the reversal than the acquisition. This suggests that the foraging strategy of a species may have an influence on their cognitive flexibility; this may be due to the increased complexity of having to source 'distribute mobile prey'.

Fig. 3
View into the habitat from the tortoises' point of view. Because red-footed tortoises live in groups in Paraguay they should have a level of cognitive flexibility. Photo: Thomas & Sabine Vinke

Fig. 4a–b
The capability to identify different forms can be useful when searching food. Photos: Thomas & Sabine Vinke

Gaalema (2007) investigated visual reversal learning in the Komodo dragon (*Varanus komodoensis*) and rough-necked monitor lizards (*Varanus rudicollis*). All three subjects successfully reached criterion for the acquisition and two reversals, they also completed the second reversal in the minimum time possible (two sessions), therefore showing an improvement from the first reversal. This implies that, for these species at least, reptiles are able to solve and improve upon a reversal, suggesting a high level of cognitive flexibility.

It is suggested that animals with highly fluctuating environmental circumstances should be more adept to reversal learning paradigms than animals which occupy a more stable environment (Bonney & Wynne 2004); this is because a stable environment is less likely to change and therefore the necessity for cognitive flexibility is less demanding. Additionally, it is thought that those animals that inhabit a stable environment would depend more upon static long-term memory than on behavioural flexibility (Bonney & Wynne 2004). This should mean that an animal from a habitat with variable environmental conditions should perform better in a reversal learning task than an animal from a stable habitat.

Therefore we examined whether the Red-Footed tortoise was capable of learning a reversal task and to show improvement across subsequent reversals to determine if this species is cognitively flexible. We also wished to see if there is an effect of habitat on this species by comparing two different populations, a captive bred population and a semi-wild population from Paraguay, on the reversal problem.

Experiment 1

A group of four Red Footed tortoises were given a two-choice, visual discrimination, serial reversal learning paradigm, consisting of an acquisition and four reversals where the reward contingencies were switched with each consecutive reversal.

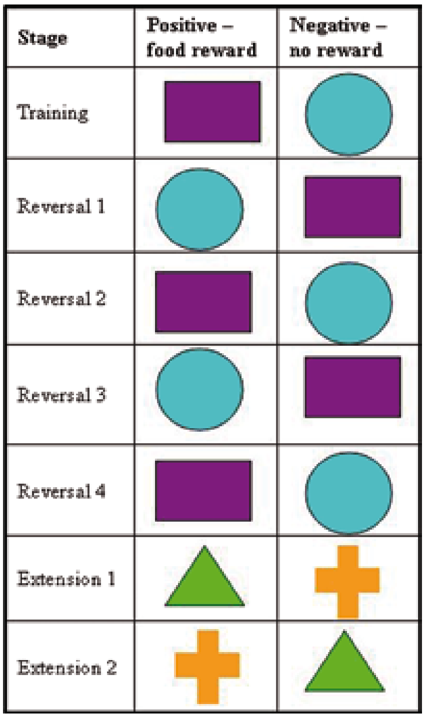
It is hypothesised that the Red Footed Tortoises will be able to learn a serial reversal task, and also that each reversal should take fewer sessions to reach criterion than the previous reversal.

Following the completion of the fourth reversal, the tortoises were presented with a set of novel stimuli and given an acquisition and one reversal with these new stimuli.

It is hypothesised that the Red Footed tortoises will be able to generalise the reversal paradigm to a set of novel stimuli, resulting in them being able to solve the acquisition and reversal stages in fewer sessions than was necessary for the original stimuli.

Name	Sex	Length (cm)	Experimental status	Training stimulus	Extension stimulus
Moses	Female	18	Experienced	Blue Circle	Green Triangle
Wilhelmina	Female	19	Experienced	Purple Square	Orange Cross
Russell	unknown	9	Naive	Blue Circle	Orange Cross
Margot	unknown	8	Naive	Purple Square	Green Triangle

Table 1
Basic information for the Riseholme tortoise population.



Graphic 2
Visual representation of the reversal learning paradigm. Graphic: Emma Smith

Experiment 2

The Paraguayan, semi-wild population of the Red Footed tortoises were given an identical reversal learning procedure in order to identify if they are able to learn a reversal paradigm faster (in fewer sessions), than the captive bred population at Riseholme as they should have a higher level of cognitive flexibility.

It is hypothesised that the Paraguayan tortoises will solve the reversal learning paradigm in fewer sessions to criterion than the Riseholme population of tortoises.

Experiment 1

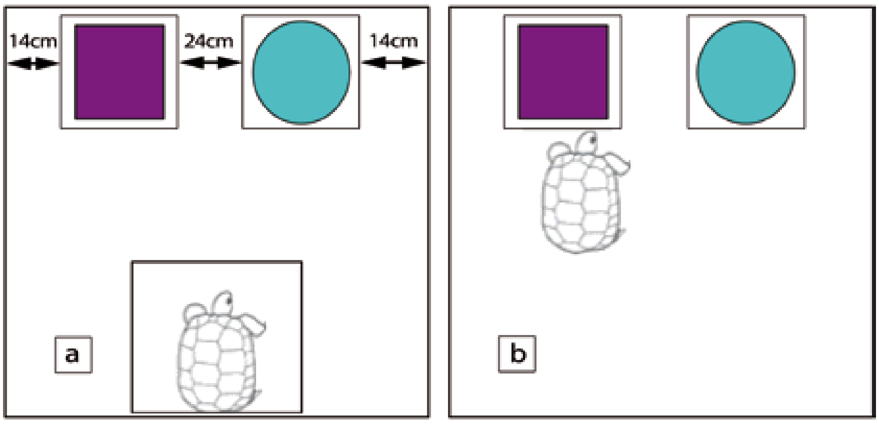
Materials and Methods

Subjects

Four Red-Footed tortoises (*G. carbonaria*) participated (see table 1) and were housed in two groups in a heated room (maintained at an average of 22 °C), with added heat mats and lamps on a cycle (12hL:12hD).

Although two of the participants, Moses and Wilhelmina, were experi-

Fig. 5a–b
Each tortoise was habituated to the arena along with allocated food (a). Later they had been habituated to feed from tweezers (Riseholm) or a little stick (Paraguay) (b).
Photos: Emma Smith



Graphic 3
Visual representation of the experimental set-up: The starting set up, demonstrating the position of the tortoise within the holding pen opposite the two stimuli, positioned upright, so that the subject could see both stimuli clearly before being released (a). The tortoise, having been released from the holding pen, is now free to approach either stimulus within the two minute time limit. In this example the tortoise has approached the purple square (b). Graphic: Emma Smith

mentally experienced, they had never taken part in a reversal study. Their experience included social learning (Wilkinson et al. 2010a), gaze following experiments (Wilkinson et al. 2010b), and radial arm maze studies (Wilkinson et al. 2007, Wilkinson et al. 2009).

Procedure

All training took place in the room in which the tortoises were housed, in a 100×100 cm arena constructed from 12 mm polycarbonate sheeting, covered in sticky-back plastic to ensure that the tortoises could not see through the sides of the arena. The floor was lined with newspaper and covered in bark chips, to match that of the home enclosure so that the tortoises were not distracted by novelty. All stimuli were laminated and trimmed so that they were identical sizes (24 ×21.5 cm), then mounted upon clear plastic presentation tables placed 14 cm in from the corners of the back panel of the arena. This measurement was marked with black tape to ensure consistency throughout. The stimuli were placed 24 cm apart, which was again marked with black tape. A food reward (a piece of a preferred food, including kiwi and tomato) was delivered upon a correct choice being made, using a pair of plastic tweezers. Opposite the stimuli was a metallic holding pen (measuring 30×30 cm and placed 40 cm in from the corners of the arena) in which the tortoises were placed prior to being released for each trial. The subjects participated in one to four sessions per day, each session consisting of ten trials.

Habituation

Prior to training, each tortoise was habituated to the arena. They were individually placed into the arena for three sessions of 20 minutes along with five pieces of dandelion leaf, during this time they could explore the arena and eat the scattered food. So that it was clear that each animal was comfortable working in the arena, they had to explore all four corners and the centre in order to consume all of the food within this 20 minute period. They were each also habituated to the tweezers used to deliver food by having the tweezers present in the arena by a piece of the food. If they did not eat all the dandelion pieces within the 20 minutes, or if they withdrew into their shells, or refused to move, then the tortoise was considered as having not habituated and they were removed and given another session the following day.

Two of the participants were experimental experienced, but they had never taken part in a reversal study

Pre-training phase

The tortoises were required to undergo two stages of pre-training before the experimental section of the study so that they all had an equal minimum level of experience before the testing began. They firstly needed to learn to approach a stimulus for food; this was trained using a yellow star. Tortoises were initially taught to approach the star from a short distance for a food reward, gradually building up to them having to approach the star from the opposite side of the arena. Once this had been learnt, the tortoises were then required to choose between two stimuli and picking the correct one would result in the reward being delivered. For this stage a black arrow was used as the second, non-reinforced stimulus and the tortoises were required to always approach the yellow star. Once the tortoises had learnt to reliably approach the correct stimulus to a pre-set criterion of a minimum of 30/40 on the last four sessions (75%) then they were moved onto the experimental phase of the study.

Experimental phase

For the experimental phase, the test stimuli were used, these were a purple square (with an area of 152 cm²) and a blue circle (with an area of 125 cm²).

The experimental phase consisted of 5 stages; the acquisition stage followed by 4 reversals, where the contingencies of which stimuli was the positive was alternated with each successive reversal (see graph 2, p. 5 for details).

The bark in the arena was redistributed before every session to reduce the possibility of scent cues. The stimuli were then attached to the presentation tables and placed in position and the holding pen located on the opposite side. The tortoise was then positioned in the holding pen until they looked at both stimuli; this

Fig. 6a–f
As pre-training the tortoises had to learn that approaching one stimulus caused a reward and the other did not.
Video capture: Thomas Vinke

Stage	Sessions to criterion for each tortoise				
	Moses	Wilhelmina	Russel	Margot	Group average
Acquisition	6	6	4	6	5,50
Reversal 1	18	12	17	10	14,25
Reversal 2	12	24	14	10	15,00
Reversal 3	11	12	9	20	13,00
Reversal 4	9	11	17	18	13,75
Extension 1	6	4	6	N/A	5,33
Extension 2	9	6	10	N/A	8,33

Table 2
The resulting sessions to criterion for each of the tortoises and the group average data. Margot failed to move onto the generalisation phase; therefore the group averages for these stages include the data from the other three individuals.

was done to ensure that they did not just go to the first stimulus that they saw. They were then released from the holding pen and given a time limit of two minutes to make a decision and approach one of the stimuli (see graph 3, p. 6). An approach was defined as being within 5 cm of the stimulus whilst looking directly at it. If a correct choice was made a food reward was given and the tortoise was then removed from the arena and given a one minute inter-trail-interval (ITI). If an incorrect choice was made then the tortoise was immediately removed from the arena and given the ITI. If the tortoise did not choose a stimuli within the allotted two minutes, or they went into either of the back corners, then this was taken as a 'no choice' and the tortoise was removed from the arena and the trial was repeated after the ITI. If the tortoise made three consecutive 'no choices', they were then removed from the arena and tested in the afternoon or the following day.

Fig. 7
During the experimental phase new stimuli had been used, which at first had to be learned and were then reversed after fulfilling the criterion (compare graph 2).
Video capture: Thomas Vinke

Upon reaching criterion for the training stage, the tortoises were then able to move onto the first reversal, which was identical in its procedure and set up, to the acquisition stage except that the reward contingencies were reversed; this ensured that the tortoises had no cues that a reversal had taken place. The same level of criterion had to be reached in each reversal stage in order for the tortoises to progress through each reversal.

Generalisation

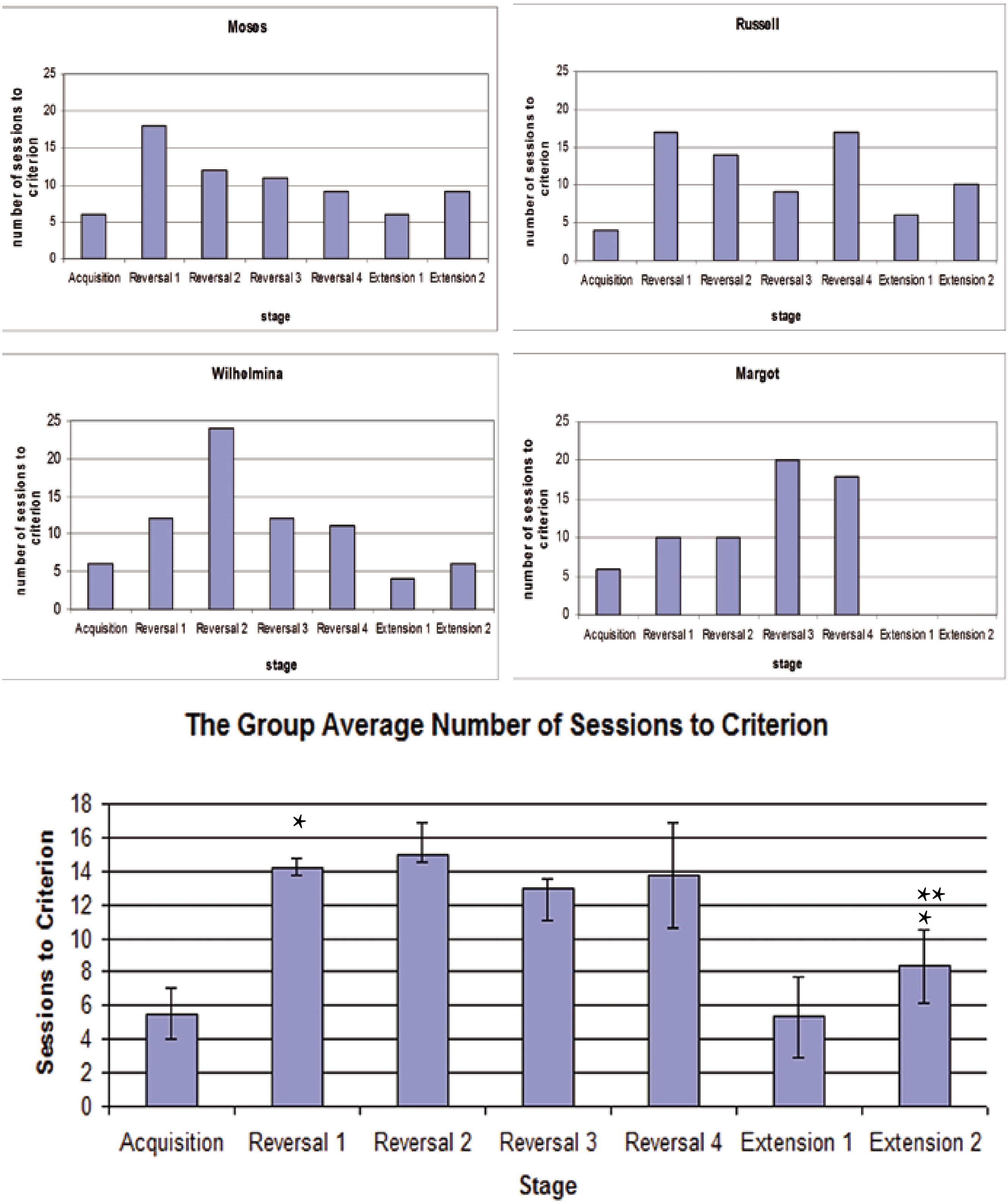
For this part of the study two novel stimuli were introduced, these were a green triangle (with an area of 66 cm^2) and an orange cross (with an area of 65 cm^2). The same methodology was used as in the experimental phase of the study; however there was only the acquisition and one reversal with these new stimuli (see graph 2, p. 5).

Results

We first investigated if the Red-Footed tortoises could learn a serial reversal. All four subjects were able to reach criterion for the acquisition and the four reversals which is the first evidence of reversal learning in this species.

One tortoise, Margot, only completed the acquisition and reversals for the original stimuli and did not progress to the novel stimuli. Therefore the results for extension 1 and extension 2 (the generalisation phases) are based on the remaining three tortoises (see table 2, graph 4).

Secondly we wanted to assess if the tortoises were able to follow the pattern of improving over successive reversals. To determine this, a one-way repeated-measures analysis of variance (ANOVA) was used to analyse differences between the four reversals. We found there to be no



* Indicates significance between Acquisition and Reversal 1 and between Extension 1 and Extension 2.
** indicates the significance between Extension 2 and Reversal 1 inclusive of the Bonferroni correction.
Error bars indicate +/- Standard Error (SE).

Graphic 4a-e
Graphs representing the individual data for the sessions to criterion for the four tortoises across all stages of experiment 1 (a-d). The group data for the number of sessions to reach criterion for each stage in the experiment; data for the two extension stages are based on the results of Moses, Wilhelmina and Russell as Margot did not complete this part of the investigation (e). Graphic: Emma Smith

Fig. 8 a–b
To avoid a preference the tortoise was put in the holding pen, they were released after looking straight at both stimuli (a), not when the head was moved to one side (b). Video capture: Thomas Vinke

statistically significant differences in the performances of each reversal, (Reversal1: M=15.67, SD=3.22, Reversal2: M=16.67, SD=6.43, Reversal3: M=10.67, SD=1.53, Reversal4: M=12.34, SD=4.16, Wilks' Lambda=0.03, $F(2, 1)=16.27$, $p= 0.173$).

This therefore means that the tortoises did not show an improvement over successive reversals.

Generalisation

To see if the tortoises were able to generalise the reversal learning paradigm to the novel set of stimuli, a paired-samples t-test was used to identify differences between the acquisition and first reversal with the original stimuli and the novel stimuli.

It was discovered that there were statistically significant differences for the acquisition and first reversal performances between the original discrimination (Acquisition: M=5.50, SD=1.00, Reversal1: M=14.25, SD=3.86, $t(3)=3.955$, $p=0.029$) and the novel discrimination (Extension1: M=5.33, SD=1.16, Extension2: M=8.33, SD=2.08, $t(2)=5.196$, $p=0.035$). In addition to this, there was also a significant difference between the performance of Reversal 1 and Extension 2 ($t(2)=8.315$, $p=0.014$). This difference was still statistically significant when using a Bonferroni correction so that the p value limit was dropped to 0.017.

Fig. 9a–c
The food reward was given immediately after approaching the correct stimulus. Video capture: Thomas Vinke

Fig. 10a–b
If an incorrect choice was made (a) then the tortoise was immediately removed from the arena (b). Video capture: Thomas Vinke

Fig. 11a–b
If the tortoise did not choose within two minutes, the trail was repeated, independently whether she was close to a stimulus without looking to it (a) or stopped in either of the back corners (b). Video capture: Thomas Vinke

Fig. 12a–b
The experiments had been documented in written form (a). For further analysis and documentation purposes all tests had been documented additionally by video. Video capture: Thomas Vinke

Therefore the tortoises were able to generalise what they had learnt from the reversal procedure and apply it to the set of novel stimuli resulting in them reaching criterion for Extension 2 much faster than they did for Reversal 1.

Name	Sex	Length (cm)	Experimental status	Training stimulus
Number 1	Female	27	Naive	Purple Square
Number 8	Female	29.5	Naive	Blue Circle
Number 9	Male	30	Naive	Blue Circle

Table 3
Basic information for the Paraguayan tortoise population.

Discussion

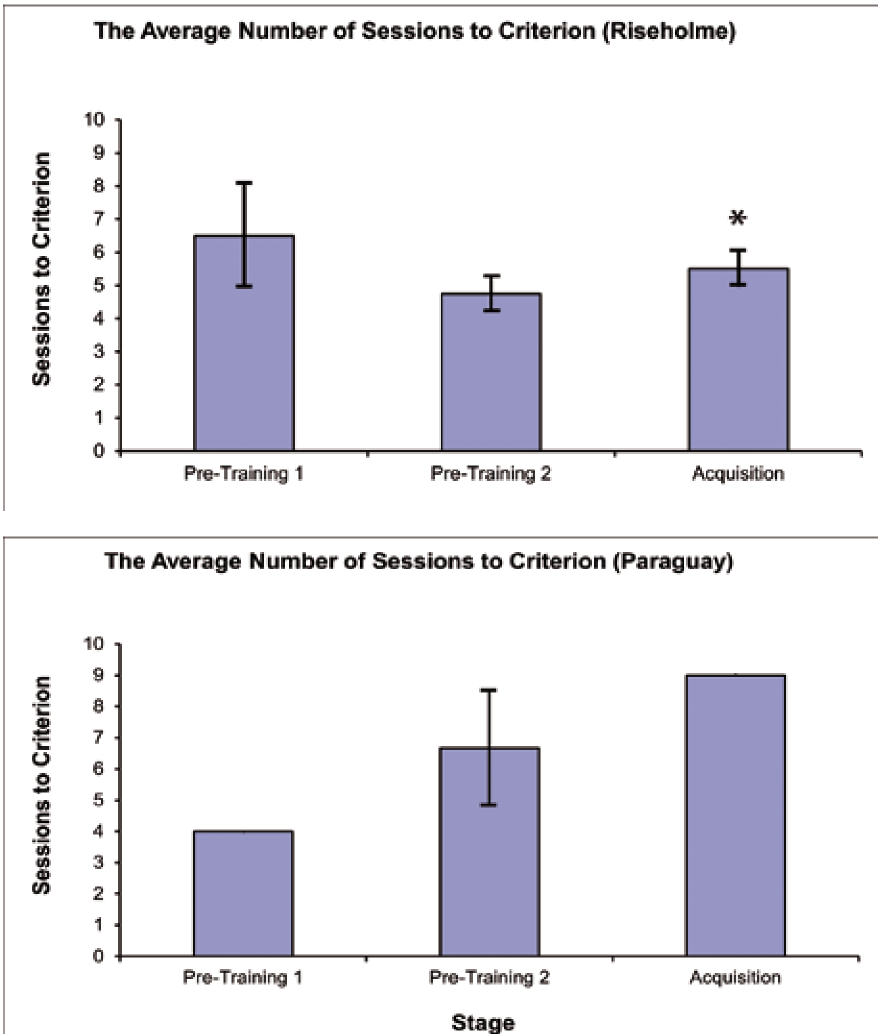
The main findings in this study are that the Red-Footed tortoise can learn a reversal task (all subjects reached criterion for the acquisition and the four reversal stages), secondly they do not appear to improve across subsequent reversals. This reflects the idea that these tortoises do not possess a high level of cognitive flexibility.

A two-choice reversal learning paradigm was used consisting of an acquisition discrimination and four reversals in which the reward contingencies were subsequently switched.

After reaching criterion for any stage, the first trial of a new problem is a matter of chance, if the subject is rewarded then that item should always be chosen (reflecting a win-stay strategy), however if the subject is not rewarded then that item should be subsequently avoided (a lose-shift strategy, Bonney & Wynne 2004). This win-stay, lose-shift response pattern is essential for reversal learning (Davey 1989), and it appears that the tortoises did not rapidly adopt this strategy for solving the reversal. D'Cruz et al. (2011) states that the ability to shift choice patterns under changing reinforcement contingencies represents a type of flexibility that is essential for daily living. Therefore because the Riseholme tortoises do not require cognitive flexibility for their daily life, they were perhaps unable to improve over successive reversals due to the inability to change strategies.

Graph 4a demonstrates that Moses was the only individual who followed the expected pattern of reversal learning, taking less sessions for each subsequent reversal. It is believed that given more reversals Moses would have reached the optimum one-trial learning, as chimpanzees were shown to reach one-trail learning only after 50–100 reversals (Schusterman 1964). This implies that improvement over successive reversals is within the capacities of this species, however not all individuals adopt this strategy.

MacPhail (1982) states that during the first reversal, subjects must choose between a neutral stimulus



* indicates significance between the acquisition stages of the two populations.
Error bars indicate +/- Standard Error (SE).

Graphic 5a–b
Comparison of the group data of the Riseholme population and the Paraguayan population of Red Footed tortoises. Graphic: Emma Smith

whilst learning to avoid the negative stimulus and that in the second reversal they must pick between a stimulus which they deem aversive (but is now rewarded) and the stimulus which they should avoid. Therefore in the third and subsequent reversals subjects have learnt to avoid both stimuli and should therefore show a strong tendency to switch between them. While this pattern is suggested for Moses, it is uncertain why this did not occur in the other three tortoises; Russell appeared to follow this pattern until the final reversal, whereas Wilhelmina seemed to adopt this pattern after the second reversal, which is suggestive of an improved performance.

Margot's pattern was the most unexpected, taking ten sessions for the first two reversals, followed by two reversals in which it took her 20 sessions to reach criterion. This may be because after the second reversal she deemed both stimuli as aversive and it interfered with her learning that one stimulus was being consistently rewarded. Another possibility for Margot's sudden increase in sessions is that it occurred once the other tortoises had completed their training, so Margot's training was increased from two to four daily sessions. In addition, the introduction of four new tortoises and two students may have repressed her ability her learn the serial reversals due to the levels of novelty in the environment and also her motivation to avoid the negative stimulus may have decreased as she had more access to food in the home enclosure.

Margot's pattern was the most unexpected

With regards to the tortoise's ability to successfully generalise a learning strategy to a set of novel stimuli, they were able to learn the novel reversal stimuli significantly faster than the first reversal of the original stimuli. This implies that the tortoises have taken the learning strategy used to distinguish between the original stimuli and applied it to the novel stimuli, this may not be a win-stay, lose-shift approach as the results suggest that this was not used to solve the reversals, however their performance did improve with the new stimuli. Based on MacPhail's theory (1982), a win-stay, lose-shift strategy is unnecessary until after the second reversal when subjects are required to readily switch between stimuli, implying that the tortoises did not necessarily need this strategy to successfully generalise their learning to the new stimuli.

Fig. 13
Does the capability of learning and/or cognitive flexibility change by age? Photo: Thomas & Sabine Vinke

Fig. 14
The tortoises of the Riseholm population learned faster; Moses during another experiment. Photo: Anna Wilkinson

With regards to future research, it may be interesting to see how the tortoises' perform on a spatial reversal. Leal & Powell (2011) suggest that spatial reversals are an easier cognitive task than reversals based on visual cues. However chelonians are known to have good visual perception (Casteel 1911, Mynarski 1951, Quaranta 1952, from Boycott & Guillery 1962) and therefore it was not anticipated that this would be a problem. Successful performance on a visual reversal would also imply a higher level of flexibility, as this is deemed more difficult to solve. In this particular case due to the large volume of literature into spatial cognition, we wished to probe further into visual perception and therefore a visual reversal was chosen.

Experiment 2

Materials and Methods

Subjects

A group of three semi-wild (not born in captivity but taken from the wild and rescued) Red-Footed tortoises (*G. carbonaria*) from the Chaco-Alto region of Paraguay were used (see table 3). The ages and the origin of these subjects were unknown however based on the measurements of their plastrons, they are all older than the Riseholme group. All of the Paraguayan tortoises were experimentally naive.

The animals were housed in a large natural outdoor enclosure, which consisted of many native trees and three purpose built underground shelters to mimic those in their natural habitat. They were exposed to natural sunlight and temperatures with no artificial light or heat sources.

Procedure

The Paraguayan tortoises were tested using the identical procedure as with the Riseholme tortoises in experiment 1; however the food rewards predominantly used were papaya and apple. All habituation and training took place in the home enclosure, therefore outside, and testing was carried out with the same arena as used in experiment 1.

Results

We investigated whether three Paraguayan tortoises could solve the reversal task to compare their performance with the Riseholme population.

Not one of the Paraguayan tortoises made it past the acquisition stage with the original stimuli and onto the reversals, therefore it is not possible to compare their levels of cognitive flexibility.

Despite this, we can determine if there is a difference in their levels of basic visual learning as both populations completed the acquisition discrimination.

An independent-samples t-test was conducted to compare the number of sessions to criterion for the acquisition stage between the two populations. There was a statistically significant difference in the number of sessions to criterion for the Riseholme population (M=5.50, SD=1.00) and the Paraguayan population (M=9.00, SD=0.00; $t(4)=4.667$, $p=0.010$).

Therefore surprisingly the Riseholme population appear to perform better in a visual discrimination task then the Paraguayan population as they reached criterion at a significantly faster rate.

Diskussion

The results show that there was a significant difference between the acquisition discrimination of two populations, in that the Riseholme tortoises reached criterion in fewer sessions than the Paraguayan group. This suggests that differing habitats may have a negative influence on solving a simple visual discrimination. It may imply that the novelty of participating in cognitive tests hindered the Paraguayan tortoises' ability to learn. Also this group were deemed to be older than the Riseholme group and studies with rats (Mizoguchi et al. 2010) have shown that aged individuals do not perform as well on an acquisition than younger individuals, which may reflect their poorer performance.

Due to the Paraguayan tortoises' only reaching criterion to the acquisition stage, there is not enough data to compare the cognitive flexibility of the two populations. We can imply that differences in the learning abilities of these populations may be based upon their different habitats. This is because, reflecting on the three characteristics which can influence cognitive flexibility; sociality of the species, changing environmental conditions and the diversity of food resources, we may be able to eliminate the first and third characteristics by using two populations of the Red-Footed tortoise.

The Red-Footed tortoise is solitary by nature and although this species interacts with conspecifics for mating opportunities, they do not naturally form permanent social groups (Wilkinson et al. 2010b), which would suggest that they may not be required to be highly cognitively flexible.

The environmental conditions of the two populations differ significantly, with the Paraguayan population exposed to temperatures of over 40 °C in the summer and just 1 °C in the winter. This teamed with tropical storms where the rainfall can be over 60 mm in one day, means that these tortoises have to cope with a large amount of variation. In contrast to this, the Riseholme population are kept in heated enclosures maintained at an average temperature of 22.5 °C and therefore they never experience such diverse conditions, this may be enough to have caused a difference between the learning abilities of the two groups, however that fact that the Riseholme population performed the acquisition better than the Paraguayan group is still surprising. Although it must be remembered that being able to successfully learn the acquisition discrimination is not a predictor how well an animal can perform in reversals (DeBartolo et al. 2009) and it is the reversals which reflect flexibility.

In addition to this the fact that both of the populations of tortoises had a restricted range of food sources means that this is unlikely to have had an impact on the results. The Riseholme population were fed at similar times each day, by the same people, as were the Paraguayan group.

Fig. 15
One should eradicate the prejudice that tortoises are stupid creatures, which are able to act exclusively by using instinctive capabilities. Photo: Thomas & Sabine Vinke

Also, the fact that they are two populations of the same species who therefore should use the same foraging techniques means that there should be no differences between the groups.

The data collected in this experiment although is interesting and shows a comparison between the groups on visual discriminations, it can only be pilot data for a more in depth study on the influence of habitat on the cognitive flexibility of the species as the Paraguayan tortoises did not complete the reversals. It may also be interesting to see if this group can generalise a learning strategy to novel stimuli, like their captive bred counterparts.

General Discussion

Reflecting on the previous literature, Day et al. (1999) demonstrated that an active forager lizard could complete a reversal task in fewer trials than a sit-and-wait predator lizard; illustrating the method of foraging does influence different levels of cognitive flexibility. So if two populations of the same species were tested (as in this current study), then it should exclude diversity of food resources as a differing characteristic. This investigation presented their subjects with just one reversal (as does the Leal & Powell 2011 lizard study), meaning that the lizards did not have to adopt a typical reversal learning strategy to solve this discrimination. Therefore although this study suggests differing levels of flexibility, without serial reversals we must imply that there may only be different levels of general learning.

Gaalema (2007) shows levels of cognitive flexibility in lizards by presenting them with two reversals, in which all subjects completed the second reversal in the shortest possible time, implying that reptiles do possess the capacity for successful reversal learning. It is results like these which we should hope to achieve in an attempt to exhibit the flexibility of a species.

Our tortoises are not the only species to struggle with improved performance in serial reversal tasks, Pigeons are known to struggle following the second reversal, making more irrelevant responses and tending to show side biases on reversal 3 (MacPhail 1982). This implies that pigeons find it difficult to choose the positive stimulus once both stimuli have had a negative association. This may again suggest a different learning strategy for our tortoises as Moses, Wilhelmina and Russell all improved on their third reversal.

> The Paraguayan tortoises showed a surprising result

With regards to comparisons between groups of the same species, the mammalian literature is focused on age differences (Lai et al. 1995), pharmaceutical studies (Watson & Stanton 2009) and lesion studies (Clarke et al. 2008) rather than learning abilities. Endo et al. (2011) tested C57BL/6 mice at three different institutions using identical IntelliCage apparatus to test for behavioural flexibility. They showed that all groups of mice could learn the reversal and that the group who were presented with eleven reversals rather than four, demonstrated a rapid adaptation to the reversal task, they adopted the "win-stay, lose-shift" reversal rule. This suggests that perhaps given more reversals, the Red-Footed tortoises may have improved over successive reversals, especially as Moses' pattern of results reflects this strategy already.

Conclusions

In conclusion this investigation has revealed four findings: firstly that the Red Footed tortoise can learn reversal discrimination, secondly that they fail to improve their performance across subsequent reversals. Thirdly we found that they do generalise their learning strategies and apply it to novel stimuli, and finally that habitat appears to influence the ability to perform a visual discrimination.

This suggests that although these tortoises do not seem to possess a high level of cognitive flexibility, as they don't improve across reversals, they do possess high levels of visual perception and that habitat may have an influence in the performance between two populations of the same species.

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Fig. 16
Anna Wilkinson, a pioneer in researching cognition of tortoises.
Photo: Thomas & Sabine Vinke

Fig. 17a–b
The author with the tame capybara, called Bubi. Photo: Thomas & Sabine Vinke

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