Spatial orientation of *Eublepharis macularius* (Reptilia: Squamata) in a Morris Water Maze task

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Abstract. The spatial orientation of mammals and birds has been intensively studied for many years, but the cognitive mechanism of spatial orientation used by squamates remains less well understood. In our study, we evaluated the learning abilities of leopard geckos, *Eublepharis macularius* (Blyth, 1854) in an adapted Morris Water Maze (MWM) with access to proximal and distal cues. We experimentally manipulated the number of reliable arena cues and one distinct room cue. The animals learned during the training phase as well as during the cue manipulation phase. No manipulation with proximal or distal cues caused loss of orientation for the entire group. The animals were able to switch between navigation strategies and showed individual preferences for the different cues. The individual geckos attend to a combination of proximal and distal cues and flexibly adjust their strategy when some of the cues are missing, which is indicative of allothetic orientation. However, the motivation of geckos for solving the task and the quality of learned spatial information were highly individual.

Key words. Allothetic orientation, Morris Water Maze, navigation, Squamata.

INTRODUCTION

Spatial orientation is crucial for the survival and performance of an individual. An animal's ability to navigate in the environment makes it possible to quickly and efficiently find required resources, e.g., when looking for a sexual partner, food, and water sources. It is also connected with antipredatory behaviour (e.g., escaping and finding a shelter), which is more effective in animals that are well oriented in space (Punzo & Madragon 2002). Most of research on spatial orientation and learning concerns endotherms (e.g., Wiltschko & Wilstschko 1996, Burt et al. 1997, Bingman & Cheng 2005, Jensen et al. 2005, Blokland et al. 2006, Muheim et al. 2016). In ectothermic tetrapods, studies are scarcer, but occasionally appear (e.g., Lohmann et al. 1999, Punzo & Mandragon 2002, Noble et al. 2012, Heuring & Mathis 2014, Liu et al. 2016, LaDage et al. 2017, Gutnick et al. 2020, Papet et al. 2020). Nevertheless, spatial cognitive abilities in snakes and lizards (Squamata) are still not fully understood (for review see Mueller et al. 2012, Szabo et al. 2021).

Squamate reptiles can use several types of navigation. The simplest (and probably also very often used) is cue-based navigation (Day et al. 2003). In cue-based spatial learning, the animal uses a characteristic landmark in space as a kind of beacon. However, this cue must be distinctive and usually as close to the goal as possible (Wilkinson et al. 2009). Jenssen (2002) showed that the Puerto Rican crested anole, *Anolis cristatellus* (Duméril et Bibron, 1837), is able to navigate back to the original capture site after being relocated (homing), uses allothetic navigation, and

is probably able to create a cognitive map (can remember the spatial relationships between the original place and the place where they were released). Not many studies focus on this type of navigation, but other species of squamate reptiles are likely to be able to use this strategy as well (e.g., *Pantherophis guttatus* (Linnaeus, 1766); Holtzman et al. 1999). Homing was also demonstrated in desert iguana, *Dipsosaurus dorsalis* (Baird et Girard, 1852), (Krekorian 1977), spiny lizards, *Sceloporus jarrovi* Cope, 1875 (Ellis-Quinn & Simony 1991), and sleepy lizard, *Tiliqua rugosa* (Gray, 1825) (Freake 2001). Several studies focused on learning the location of artificial refuges inside an experimental arena (Amiel & Shine 2012, Noble et al. 2012, Carazo et al. 2014, Dayananda & Webb 2017) according to access to local (i.e., beacons) and/or distal, extra-maze cues. In recent study on a lacertid lizard, *Podarcis liolepis* (Boulenger, 1905), place learning ability was demonstrated by Font (2019). The authors claimed that this type of experimental design effectively rules out the use of egocentric and response-based strategies in ecologically relevant task (refuge seeking behaviour).

Reptiles frequently use also compass navigation (e.g., *Python molurus bivittatus* Kuhl, 1820; Pittman et al. 2014). It is a type of navigation using the position of the sun (*Podarcis sicula* (Rafinesque-Schmaltz, 1810); Foà et al. 2009), moon, and stars (*Pogona vitticeps* Ahl, 1926; Nishimura & Fukushima 2009) or using light gradients of celestial bodies. Most often, it is a gradient of polarized light (*Podarcis sicula*; Beltrami et al. 2010). Reptiles with well-developed parietal eye can perceive the intensity and direction of this light (Freake 1999). Perhaps a more complex type of navigation is allothetic navigation (Mittelstaedt & Mittelstaedt 1980). Allothetic navigation is defined as navigation in space using proximal as well as distal cues, the relationships between multiple cues and the position of the subject (Gallistel 1990). These cues can take diverse forms, be it visual (Wilkinson et al. 2007), olfactory (Gagliardo 2013), magnetic (local changes in the magnetic field, Cochran et al. 2004) or acoustic (Diego-Rasilla & Luengo 2007). This type of navigation has the advantage that it can still be successfully employed even in the absence of some of the cues. The ability to navigate allothetically has been demonstrated in turtles (López et al. 2000).

Studies of allothetic orientation in squamate reptiles are not so common, but the results of some studies suggest that they are able to navigate allothetically. The corn snake (*Pantherophis guttatus*) was able to find a hidden chamber stored under a 16-hole hole-board arena (Holtzman et al. 1999). The snakes learned to orient themselves, indicating the use of allothetic or cue-based navigation. However, it is not clear what mechanism they used. Homing was tested in the tree dtella gecko, *Gehyra variegata* (Duméril et Bibron, 1836), (Gruber & Henle 2004). These geckos were able to return to a tree from which they were moved to one of the neighbouring trees. Possible involvement of allothetic navigation in some experiments with lizards was discussed, although the authors themselves did not test it directly (*Anolis cristatellus*; Jenssen 2002; *Scincella lateralis* (Say, 1822); Paulissen 2008). Moreover, little is known about whether squamate reptiles prefer proximal cues, distal cues, or a combination of both.

In contrast to squamate reptiles, turtles are the most examined ectothermic tetrapods when it comes to spatial abilities. There are plenty of studies describing their spatial orientation and well-developed methodologies (Roth & Krochmal 2015). López et al. (2000) demonstrated the use of allothetic navigation in the yellow-bellied slider, *Trachemys scripta* (Thunberg, 1792), in a four-armed maze. Their results suggest that the turtles were able to orient themselves by distal visual landmarks and did not use another type of navigation in this experiment. Many studies dealing with navigation are focused on sea turtles (Lohmann et al. 2004, 2008, Lohmann & Lohmann 2019). The complex navigation mechanisms of the loggerhead sea turtle, *Caretta caretta* (Linnaeus, 1758), were elucidated by Lohmann et al. (1999). The experiments showed that they employ three mechanisms for navigation. When searching for the sea, the turtles navigate

visually towards the brighter sky above the sea or according to the light of the Moon (use of the Moon as a single beacon, or navigation using celestial bodies or navigation using a light gradient). They also navigate using idiothesis and are able to detect the direction of the waves (Lohmann & Lohmann 1996). When swimming in the sea, they make use of a magnetic compass as well (Irwin & Lohmann 2005). It is very likely that squamate reptiles also combine several types of navigation (Brothers & Lohmann 2015, Szabo et al. 2021).

Eublepharis macularius (Blyth, 1854) belonging to the family of eyelid geckos (Eublepharidae), is a long-lived animal native to the desert and semi-desert areas of Pakistan, Afghanistan, Iran, and NW India (Seufer et al. 2005). In the wild, it favours dry clay and rocky habitats, where it hides in crevices and burrows or digs shelters (Frynta et al. 1997). It is routinely bred in captivity and is becoming increasingly popular as a lizard model in laboratory experiments concerning growth (Frynta et. al. 2018), hybridization (Jančúchová-Lásková et al. 2015), physiology and brain functions (Crews et al. 1997, Sakata et al. 2000) as well as spatial orientation during movement (Kutílek et al. 2015). As with most squamate reptiles, no information is available about its ability to navigate allothetically.

Research shows that reptiles are capable of learning to solve spatial problems, however details on the cognitive mechanisms involved are missing (e.g., López et al. 2000). Although the original Morris water maze was designed primarily for rats (Morris 1984), its modifications can also be used with many other animal species including reptiles (Foà et al. 2009, Beltrami et al. 2010). This type of maze can successfully test both non-spatial discriminant tasks and various spatial tasks (Morris 1984). The great advantage of the Morris water maze lies in the easy and effective motivation of animals and the absence of olfactory cues. This is particularly useful when dealing with reptiles. Due to their slow metabolism, they can be otherwise difficult to motivate by food rewards or access to shelter. During flash floods in the wild, it has been observed that some desert squamate species can swim (pers. obs., D.F.), making the MWM a suitable task for them. We were able to successfully implement a variation of the MWM task for the leopard gecko, *Eublepharis macularius*, to test allothetic navigation in detail and help fill the gap in knowledge about spatial orientation in squamate reptiles.

Our study had several aims and predictions concerning the spatial cognitive abilities of leopard geckos. Specifically, we wanted to test: (1) the ability of leopard geckos to learn the position of the platform; (2) what type of cues/frame (arena frame vs. room frame) they preferentially use, and (3) which navigation strategy (allothetic vs. cue-based) geckos individually prefer.

MATERIALS AND METHODS

Experimental animals

We studied the spatial orientation of 42 adult individuals (1 male, and 41 females) of leopard geckos (*Eublepharis macularius*, Eublepharidae). Animals were 2–5 years old and the mean snout-vent length and body mass were 11.99 cm (range 10.74–12.95 cm) and 45.11 g (range 32.15–64.19 g), respectively. Leopard geckos have temperature-dependent sex determination (Viets et al. 1993), and incubation temperature may also affect behavioural traits in this species (cf. Flores et al. 1994, Sakata & Crews 2003). To avoid the effects of incubation temperature and shifts of the sex ratio of experimental animals towards non-territorial females, we set the incubation temperature to 28.5±0.5 °C, which is close to the temperature (approximately 29 °C) preferred by female *E. macularius* for egg-laying (Bull 1987, Bragg et al. 2000). The experimental animals included individuals from a laboratory population as well as descendants (first and second generation) of wild-caught animals from Pakistan. For further details on the parental animals see Jančúchová-Lásková et al. (2015) and Frynta et al. (2018). Recent study of wild-caught and pet-trade samples of *E. macularius* suggested close relatedness (Agarwal et al. 2022).

Animals were housed individually in the same room with windows and a natural light mode. The room temperature ranged from 26 to 30 °C. Animals had access to food (crickets and larvae of the beetle *Tenebrio molitor*) and water. The insects were dusted with vitamins and minerals (Nutri Mix); vitamins AD3 and E were provided weekly.

Experimental procedure

The spatial orientation of leopard geckos was tested in the Morris water maze (MWM, Morris 1984) modified for small-sized reptiles. A similar experimental design was used in another study (Foà et. al. 2009) to explore compass and other orientation mechanisms in the ruin lizard *Podarcis sicula*. For the study arrangement see Supplementary Information (SI 1 and SI 2). Water in the arena was heated to 28±1 °C. Before the experiment (for half an hour), we preheated the animals to 28±1 °C. This temperature is a bit higher than the preferred temperature 25.8 °C (Werner et al. 2005). Nevertheless, it is common to use higher temperatures in ectotherms when testing cognitive abilities (Whiting & Noble 2018) or performance (Fuller et al. 2011) to allow them to reach optimal physiological function, which can affect motivation and response time. The height of the water level was set so that the platform was 2 cm under the surface (the total height of the water column was 21.5 cm). In all phases of the experiment, the sessions were recorded with a camera placed on a rack attached to the ceiling. The camera was centred above the arena unless the arena was shifted. The experimental animals were weighed and measured every three weeks (13 times in total) before the testing. Moreover, the tail thickness at the widest point was measured. Leopard geckos store fat and fluids in their tails. The thickness of the tail is therefore directly related to their body condition (for assessing body condition in leopard geckos see Landová et. al. 2013). Here we calculated body condition as the ratio of third square root of the body weight to the snout-vent length (Ricker 1975).

Pre-training

The first part of the experiment was a pre-training to select individuals that actively swim in the MWM. For the initial pre-training, we used 140 healthy adult individuals of both sexes (121 females and 19 males). Such skewed sex ratio is a common result of incubation temperature of this TSD gecko population (Viets et al. 1993, Jančúchová-Lásková et al. 2015). Each of the geckos was once placed in the arena from the southeast direction, heading towards the platform. We recorded whether the gecko found the platform. When the gecko did not actively swim, it was removed from the arena and the session was terminated. The maximum duration of the session was 8 min. If the animal did not find the platform during that time, it was gently placed on the platform and the session was terminated. Some animals were passively floating on the water surface. When that happened, they were gently poked by a wooden stick at the base of the tail (simulated
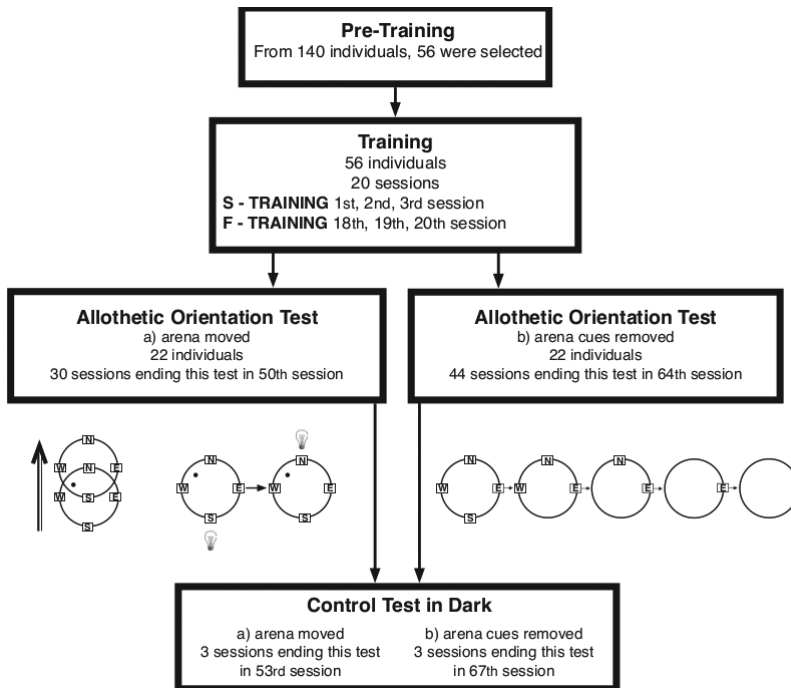

Fig. 1. The diagram of experimental design with detail about number of animals, session names and numbers, and manipulation with proximal and distal arena cues.

predator attack, Bonine & Garland 1999, Punzo & Madragon 2002, Paulissen 2008). The number of these touches during the session was recorded. Out of the 140 animals, 56 were able to swim actively during most of the experiment and found the platform. These animals proceeded to the next training phase.

Training
The second step of the experiment was training (see Fig. 1 for the schedule of the experiment). We tested whether animals were consistently able to find the platform and whether the time and path needed to do so decreases while reducing the number of necessary touches. We used 56 individuals (5 males and 51 females), but not all animals were able to finish the experiment.

During the whole experiment, we provided the animals with various types of navigation frames, four proximal arena cues, and distal room cues. Thus, the geckos were able to use a combination of proximal and/or distal cues (allothetic orientation), and/or one cue (cue-based navigation). We placed four artificial markers (later called arena cues) at the edge of the arena (Fig. S1). Markers consisted of combinations of simple monochrome geometric shapes and represented the cardinal directions (N – north, S – south, E – east, and W – west). Room cues (distal cues) were also visible to the animals. These consisted of the features of the room (walls, doors, light, etc.) and were farther from the animal (but not more than two meters). Each tested gecko underwent 20 sessions (maximum one 8-minute session/per week). Animals were released into the arena at a semi-random start position (four directions: $5\times N$, $5\times W$, $5\times S$, $5\times E$). One concern about the start positions is that they are not equidistant from the goal (platform), creating short (N and W) and long (S and E) paths to the goal. Therefore, the start position (short/long) was accounted for in the analysis. If the animal floated, it was again gently poked with a wooden stick. The time needed to find the platform and the numbers of touches were recorded. In this phase, we excluded 10 individuals, who repeatedly failed to actively swim and were thus unable to find the platform. We also excluded occasional sessions with more than 30 touches per individual during one experiment. Those sessions were repeated next time. For further statistical evaluation, we grouped the first three training sessions as S-training (Start, session 1–3) and the last three training sessions as F-training (Finish, session 18–20). The criterion for learning task was a significant difference in latency and/or path-length between S-training and F-training.

Allothetic orientation test
In the third part of the experiment, we reduced or removed the spatial information provided by the proximal and distal cues by (1) successively removing the arena cues; (2) moving the whole arena so that the absolute position of the platform within the room remained the same, but its position relative to the arena cues changed; (3) manipulation with the position of the most prominent distal cue (the light source).

We used the animals that successfully passed the previous step (42 individuals) and divided them randomly into two groups (Arena cues removed group and Arena moved group). Each gecko was usually tested twice per week for a max of 8 min per session. For the diagram of the experimental design, see Fig. 1.

In the first group (Arena cues removed), we tested if the geckos could find the platform using either allothetic navigation (the relations among multiple cues are essential) or by a simple cue-based (beacon) strategy. We gradually removed the proximal arena cues. After the removal of one or two cues, individuals that use allothetic orientation can still make use of the relationship between the remaining two cues. After the removal of three cues, the animal can use one cue (cue-based navigation) or distal room cues. In the final test, there were no arena cues available, meaning the geckos were able to use only the distal room cues. The difference in the time to find the platform and/or the path taken between the final training phase and the cue manipulation phase gives us some insight into which navigation frame of reference was important for each individual. The total number of sessions in this part of the experiment was 44 (the phase ended at the 64th session) and 20 animals completed this phase. The animals started with four sessions that were the same as in the training phase (no modification, 4 cues). Then, we removed the proximal arena cues one by one: in the 25th session we removed S (first modification, 3 cues), in the 35th session W (second modification, 2 cues), in the 45th session N (third modification, 1 cue), and in the 55th E (fourth modification, 0 cues), so only the distal cues were available for orientation at the end. After removing the last arena cue, the animals completed 10 sessions where they had to find the platform without any cues placed close to the goal. We released the animals into the arena from different directions in equal proportions ($11\times N$, $11\times W$, $11\times S$, $11\times E$), heading towards the mark on the wall of the arena.

In the second group (Arena moved), we tested whether the geckos preferred the distal room cues for navigation. We moved the arena to a different position (but the platform remained in the original position relative to the room), so the arena cues could no longer be relied upon to determine the position of the platform (see Fig. 1). The total number of sessions in this part of the experiment was 30. In all sessions, proximal cues were present on the wall of the arena. In the first manipulation (session 21–35), we moved the arena to the north, but the position of the platform relative to the distal room cues remained the same. Movement of the arena makes proximal arena cues uninformative, while the animals could still rely on distal cues for allothetic navigation. In the second manipulation (session 36–40), the arena was moved back to the original position. This manipulation makes the new arena cues uncertain again. In the last manipulation (session 41–50), the arena remained in the original position, but we moved one of the most prominent distal cues (the light source, power of light: 60 W bulb) from south to north. This enables us to test the possibility of navigation using one very distinct

distal cue – the light source. If the geckos use this distal cue as the only cue for orientation, their performance in finding the platform should decline in this phase. However, if they use multiple cues (or a different cue), their performance should remain stable during this phase. Before moving the light, the illumination of the individual cues on the walls was measured using a lux meter. After moving the light source to the opposite side of the room, this source was manipulated to achieve approximately (+/– 1 lx) the same illumination of the MWM, but from the opposite direction. When the light was in this new position, a reflection appeared on the recordings, sometimes making the path-length impossible to evaluate. We tested 22 females to find which cues (arena cues, room cues, both) they preferentially use to navigate and how the cue preferences change when the arena cues are not informative of the platform location (the first and the second movement of the arena).

Control test in the dark
After finishing the allothetic orientation tests, both groups went through three control sessions in the dark (max 8 min/session). Geckos from the group with arena cues removed finished this phase in session 67 and geckos from the arena moved group in session 53. This test aimed to check if the animals can use cue navigation to find the platform in the dark meaning that visible cues were not available. Thus, geckos can hardly navigate allothetically. Nevertheless, they can use alternative cognitive (idiothetic navigation and/or magnetic compass) or non-cognitive (motoric) strategies.

We turned the light off to reduce the visibility of distal cues. We tested the animals in a closed room without windows. In this test, we used an infrared light source (THIXIN SX-812) with intensity less than 5 lx and the session was recorded using an infrared-sensitive camera. The infrared light source was centred above the arena and could not be used as a navigational cue. No other source of illumination was present. In these sessions, the animals were again released into the arena heading towards the arena wall, in the presence of all cues and from randomly selected directions (W, E, S). Before the start of the dark test, the illumination of the individual cues on the arena wall and illumination above the platform was measured using a lux meter. Due to the low lighting conditions during the dark test, the path-length was not always available for analysis.

Behavioural analysis
In all sessions, we noted whether the gecko succeeded in the finding the platform and we measured the latency (in seconds) to reach the platform using a stopwatch and the number of touches needed if the gecko was not actively swimming. We calculated the path-length (cm) using the tracking software Ethovision XT 11.5 (Noldus, Wageningen, Netherlands). The high dynamic brightness model was the best to use (for this purpose animals were always identified with a non-toxic white colour on the head). Ethovision was mainly used to calculate the path-length (distance moved in cm) and measure the time to reach the platform (s). However, the time estimated in Ethovision did not always agree with the stopwatch time (r=0.611), because sometimes the program did not stop measuring the time when the gecko reached the platform. After manual curation of the data from Ethovision, the correlation was much better (r=0.8921). The correlation of path-length with latency was not so tight (r=0.723) because the latency to reach the platform may be higher in animals that adopted the floating strategy or shorter in fast moving animals. Thus, we assume that the path-length is more suitable for
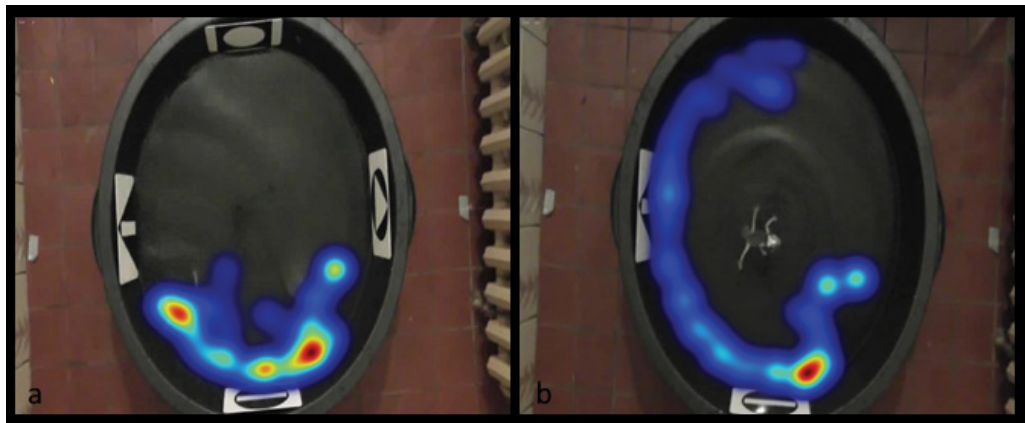

Fig. 2. Movement of leopard gecko in MWM as revealed by heat maps from Ethovision. Reduction of spatial information provided by the proximal cues, which were consecutively removed in Arena cues removed group of geckos; (a) all cues were present; (b) two cues removed. Red colour marks the parts of the arena, where the animal spent the most time.

evaluation of the process of learning in our experiment (visible also from the heat maps, Fig. 2). Nevertheless, we used both variables in our analysis as the main dependent variables. We also calculated the speed (cm/s) as the path-length divided by the latency and included it in supplementary analysis (for the results see Supplementary Information SI 2).

All performed experiments were approved by the Ethical Committee of the Charles University, Czech Republic, and by the Ethical Committee of the Ministry of Education, Youth and Sports of the Czech Republic (license no. 8388/2011-30).

Statistical analysis

The values of path-length, latency, and speed were log-transformed to ensure normality of the residuals. The data were analysed using linear mixed-effects models as implemented in the functions lme in the package nlme (Pinheiro et al. 2015), and estimated marginal means were used for post hoc comparisons between groups in the package emmeans (Lenth 2021) in R 4.0.5 (R Core Team 2020).

The initial full models always included following predictors: the start position (short/long), number of touches, body weight, and the training phase as fixed effects and animal ID as a random factor. The full models were reduced using the Akaike's Information Criterion (AIC). A comparison of the full and reduced models was performed by the *F* test. As an effect size measure, we report the Likelihood Ratios (LR) as implemented in nlme package (Pinheiro et al. 2015). All experimental data are available in Supplementary Information (SI 3).

For evaluating the spatial task, we used path-length, latency and speed as the dependent variables, above mentioned predictors completed with the group membership (Arena cues removed/Arena moved). We compared the initial (S-training) and final (F-training) training phases.

For examining the use of allothetic navigation (group comparison between Arena cues removed/Arena moved), we used the path-length and latency as the dependent variables, the above mentioned predictors completed with the manipulation phase (all cues, 3 cues, 2 cues, 1 cue, no cues for the Arena cues removed group; and arena moved, arena back, and light moved for the Arena moved group, see Fig. 1). The reference level for the comparison of the manipulation phases was the F-training phase (end of training).

In addition, we evaluated the effects of manipulation with proximal and distal cues separately for each individual, as it was obvious that each animal chose its navigation frame (e.g., arena cues, room cues, one cue, or a combination of proximal and distal cues) individually. We employed a linear model with log-transformed path-length as the dependent variable and the above mentioned predictors. We compared the different phases of manipulation to the reference value, which was F-training and all cues present in the Arena cues removed group, and F-training in the Arena moved group.

<div align="center">RESULTS</div>

The stopwatch time was correlated with the distance travelled (r=0.7351, see above). The mean speed was 7.93 cm/s (range 0.14–72.13 cm/s). The mean speed in the dark phase was higher (15.02 m/s, range 0.29–63.05 m/s), which indicates increased motivation to search for the platform under lower visibility of cues.

Spatial memory tests

We found that geckos were able to learn the spatial task. The path-length was shorter at the end of training (F-training) in comparison to the start of training (S-training, Fig. 3). For the model with path length as the dependent variable, group and body weight were not significant (p>0.05) and were not included in the final model. The significant variables were the start position (F=6.759, df=207, p=0.01), the number of touches (F=135.668, df=207, p<0.0001) and phase of the training (F=12.727, df=207, p<0.0001). The model with touches had LR=154.71 (p<0.0001) against the null model, model with touches and phase of the training had LR=90.44 (p<0.0001) against the model with touches. The number of touches was positively associated with the path-length. In contrast to path-length, the latency of initial phase of training (S-training) was not significantly longer than the final phase (F-training) of experiment. It means that geckos did not reach the platform faster, while the path-length decreased during the learning. For the model with latency as the dependent variable, the group, start position, and body weight were not significant (p>0.05) and were not included in the final model. The significant variable was only the number of touches (F=129.506, df=208, p<0.0001), but not the phase of training (F=0.326, df=208, p=0.5686). The number of touches had a positive effect on the latency to reach the platform meaning that some geckos were less motivated to swim actively and adopted the floating strategy across the experiment. Analysis

with the velocity as the dependent variable revealed that the speed was lower at the final phases of training (see result section in Supplementary Information SI 2 and Fig. S2). We hypothesise that the path length is more accurate parameter for cognitive performance as it better reflects the learning abilities of geckos in MWM spatial task. As the latency was not shorter and the path-length decreased during the training, it suggests that animals did not adopt active alternative motoric strategy. Still, the latencies are highly influenced by the motivation of tested animals.

Allothetic navigation

Arena cues removed
We did not find any common effect of cue manipulation on path-length and latency for the whole group of geckos. It does not mean that the removal of cues did not affect the cognitive ability to find the platform and motivation of the animals. Nevertheless, we hypothesise that the cognitive effect was more individual as each gecko can use own combination of cues for navigation (see part called Evaluation of individual strategies and Discussion). For the model with the path-length as the dependent variable, the cue manipulation, body weight and the interaction between the start position and cue manipulation were not significant (p>0.05) and were not included in the final
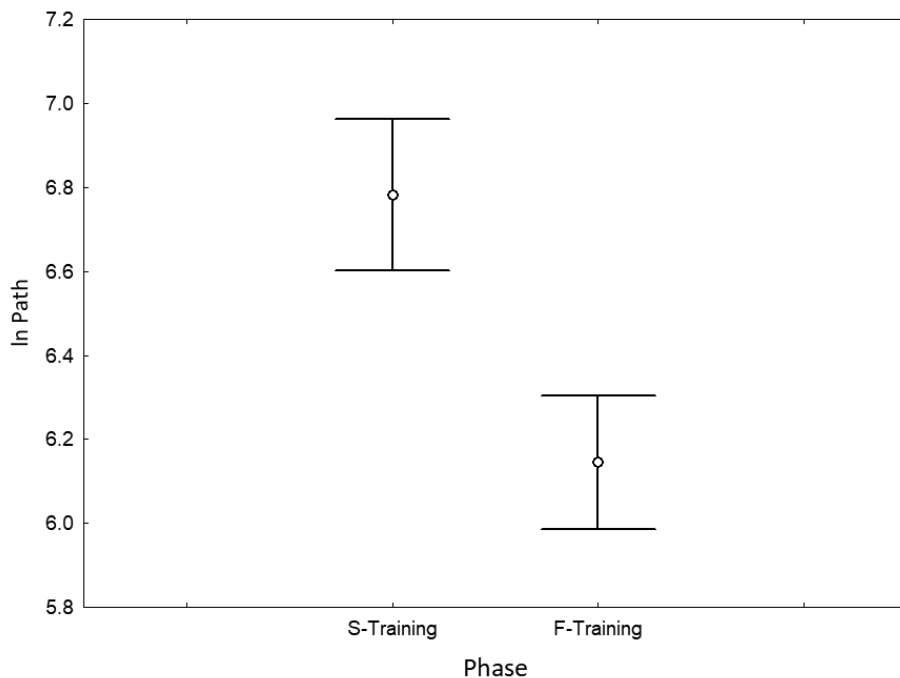

Fig. 3. The comparison of mean path-lengths of leopard geckos across the phases of learning the spatial task in Morris water maze. The log-transformed mean path-lengths was significantly shorter in the final part of training (F-training) in comparison to the initial training (S-training). (means and 95% confidence intervals).

Table 1. Coefficients from the LME model with the path-length as the dependent variable, the start position, number of touches, arena manipulation, and the interaction between the number of touches and manipulation as fixed effects and animal ID as a random factor

	value	95% CI (LL, UL)	S.E.	DF	t-value	p-value
(intercept)	5.6818	(5.377, 5.986)	0.1550	669	36.6460	<0.0001
start position	−0.1101	(−0.228, 0.007)	0.0598	669	−1.8420	0.0659
touches	0.1240	(0.080, 0.168)	0.0224	669	5.5390	<0.0001
arena back	0.3519	(0.011, 0.693)	0.1737	669	2.0258	0.0432
arena moved	0.4556	(0.149, 0.762)	0.1562	669	2.9173	0.0036
light moved	0.1235	(−0.188, 0.435)	0.1584	669	0.7793	0.4361
touches × arena back	−0.0414	(−0.098, 0.015)	0.0286	669	−1.4452	0.1489
touches × arena moved	−0.0126	(−0.059, 0.035)	0.0241	669	−0.5242	0.6003
touches × light moved	0.0537	(0.004, 0.103)	0.0253	669	2.1207	0.0343

model. The significant variables were the start position (F=32.510, df=915, p<0.0001) and the number of touches (F=682.884, df=915, p<0.0001). The model with start position had LR=5.69 (p=0.017) against the null model, and the model with start position and touches had LR=310.28 (p<0.0001) against the model with touches. For the model with latency as the dependent variable, the results were similar. The cue manipulation, body weight and the interaction between the start position and cue manipulation were not significant (p>0.05) and were not included in the final model. The only significant variables were the start position (F=19.914, df=915, p<0.0001) and the number of touches (F=665.400, df=915, p<0.0001). The model with touches had LR=214.10 (p<0.0001) against the null model, model with touches and start position had LR=7.42 (p=0.0064) against the model with touches. The number of touches was positively associated with the path-length and the path-length was shorter for the short type of start position. The different phases of cue manipulation were compared to the reference value (all cues present).

Arena moved
We found that after the first two manipulations with distal room cues (Arena moved, Arena back), animals from Arena moved group were uncertain in navigation to the platform. The path-length was longer, while the latency not. Nevertheless, the latency was shorter after the last manipulation (Light moved). For the model with path-length as the dependent variable, body weight and the interaction between the start position and arena manipulation were not significant (p>0.05) and were not included in the final model. The significant variables were the start position (F=15.632, df=669, p=0.0001), the number of touches (F=388.378, df=669, p<0.0001), arena manipulation (F=5.214, df=669, p=0.0015) and the interaction between the number of touches and arena manipulation (F=8.856, df=669, p<0.0001). The model with start position had LR=4.36 (p=0.0367) against the null model. The model with start position and touches had LR=205.91 (p<0.0001) against the model with start position. The model with start position and touches in interaction with arena manipulation had LR=41.25 (p<0.0001) against the model with start position and touches. The number of touches was positively associated with the path-length and the path-length was shorter for the short type of start position. After arena manipulations, the path-length was longer than in the F-training phase (for model coefficients see Table 1). The different phases of arena manipulation were compared to the reference value reflecting the final phase of the initial training (F-Training).

For the model with latency as the dependent variable, the body weight and the interaction between the start position and the manipulation were not significant (p>0.05) and were not

Table 2. Coefficients from the LME model with the latency as the dependent variable, the start position, number of touches, arena manipulation, and interaction between the number of touches and the manipulation as fixed effects and animal ID as a random factor

	value	95% CI (LL, UL)	S.E.	DF	t-value	p-value
(intercept)	3.9888	(3.733, 4.245)	0.1305	692	30.5583	<0.0001
start position	−0.1115	(−0.204, −0.019)	0.0473	692	−2.3581	0.0186
touches	0.1243	(0.089, 0.159)	0.0180	692	6.9026	<0.0001
arena back	−0.0939	(−0.368, 0.180)	0.1396	692	−0.6728	0.5013
arena moved	0.0059	(−0.239, 0.252)	0.1251	692	0.0474	0.9622
light moved	−0.2734	(−0.523, −0.024)	0.1271	692	−2.1516	0.0318
touches × arena back	0.0163	(−0.029, 0.062)	0.0231	692	0.7061	0.4803
touches × arena moved	−0.0067	(−0.045, 0.031)	0.0193	692	−0.3463	0.7292
touches × light moved	0.0492	(0.009, 0.089)	0.0203	692	2.4209	0.0157

included in the final model. The significant variables were the start position (F=24.376, df=692, p<0.0001), number of touches (F=690.866, df=692, p<0.0001) and the interaction between the number of touches and the manipulation (F=7.334, df=692, p=0.0001). The model with start position and touches had LR=319.54 (p<0.0001) against the model with start position. The model with start position, touches and arena manipulation had LR=0.53 (p=0.9105) against the model with start position and touches. The model with start position and touches in interaction with arena manipulation had LR=18.01 (p=0.0004) against the model with start position, touches and arena manipulation. The number of touches was positively associated with the latency and the latency was shorter for the short start position. Manipulation with the light was negatively associated with the latency (for model coefficients see Table 2). Again, the different phases of manipulation were compared to the reference value (F-Training).

Dark test
We focus also on the ability to navigate without any visible cue. The latency was shorter under dark conditions, nevertheless, the path-length was significantly longer. For the model with latency as the dependent variable, the start position, group, and body weight were not significant (p>0.05) and were not included in the final model. The significant variables were the number of touches (F=164.301, df=208, p<0.0001) and manipulation (F=3.905, df=208, p=0.0495). The model with touches had LR=97.40 (p<0.0001) against the null model. The model with touches and manipulation had LR=3.65 (p=0.0561) against the model with touches. The number of touches was positively associated with the latency and surprisingly, the latency was shorter for the dark phase compared to the final phase of initial training (F-training; for model coefficients see Table 3). Results for the model with path-length as the dependent variable were similar (see Table S1).

Table 3. Coefficients from the LME model with the latency as the dependent variable, the number of touches and manipulation with proximal and distal arena cues (removal) due to the dark conditions as fixed effects and the animal ID as a random factor

	value	95% CI (LL, UL)	S.E.	DF	t-value	p-value
(intercept)	3.9112	(3.727, 4.095)	0.0934	208	41.882	<0.0001
touches	0.1261	(0.107, 0.146)	0.0099	208	12.795	<0.0001
dark	−0.178	(−0.355, −0.001)	0.09	208	−1.976	0.0495

Table 4. Summary of the coefficients (S.E. in parentheses) for individual strategies from the Arena cues removed group. Bold italics and italics indicate a significant difference in the log-transformed path-length ($p<0.05$ and $p<0.1$, respectively) in this individual in a particular manipulation setting compared to the session with all cues present. The interaction between the number of touches and the manipulation can be interpreted as a change in motivation to actively swim and seek out for the platform. In several cases, there was no interaction (–) and a model without interaction was used. ID with asterisk was a male

ID	intercept	cue 3	cue 2	cue 1	cue 0	touches	start position	cue 3*touches	cue 2*touches	cue 1*touches	cue 0*touches
1000	5.621 (0.233)	0.100 (0.252)	0.174 (0.254)	0.077 (0.252)	0.350 (0.254)	***0.118*** ***(0.016)***	–	–	–	–	–
8081	5.865 (0.388)	0.518 (0.523)	0.152 (0.446)	-0.679 (0.473)	-0.943 (0.471)	0.117 (0.080)	–	-0.015 (0.124)	0.037 (0.094)	***0.228*** ***(0.106)***	***0.270*** ***(0.109)***
8120	5.508 (0.295)	0.220 (0.319)	0.240 (0.322)	0.151 (0.343)	0.320 (0.326)	***0.174*** ***(0.023)***	–	–	–	–	–
8122	5.743 (0.222)	–	–	–	–	***0.151*** ***(0.022)***	-0.573 (0.296)	–	–	–	–
8218	5.647 (0.311)	0.357 (0.361)	0.236 (0.363)	0.121 (0.364)	***0.825*** ***(0.366)***	***0.346*** ***(0.064)***	–	–	–	–	–
8236	5.952 (0.295)	-0.482 (0.357)	-0.279 (0.358)	-0.511 (0.361)	0.116 (0.361)	***0.175*** ***(0.027)***	–	–	–	–	–
8276	5.720 (0.176)	0.178 (0.232)	0.060 (0.246)	-0.060 (0.231)	0.411 (0.228)	***0.161*** ***(0.019)***	–	–	–	–	–
8340	5.250 (0.356)	0.102 (0.372)	0.554 (0.374)	***0.890*** ***(0.378)***	***1.075*** ***(0.389)***	***0.278*** ***(0.049)***	–	–	–	–	–
8354	5.215 (0.285)	0.374 (0.335)	0.466 (0.336)	-0.123 (0.337)	0.156 (0.335)	***0.176*** ***(0.025)***	–	–	–	–	–
8363*	6.061 (0.268)	0.527 (0.319)	0.499 (0.325)	0.028 (0.317)	-0.024 (0.323)	***0.101*** ***(0.021)***	–	–	–	–	–
8397	5.349 (0.299)	0.420 (0.327)	0.403 (0.330)	0.448 (0.340)	0.279 (0.351)	***0.202*** ***(0.034)***	–	–	–	–	–
8516	5.794 (0.310)	0.542 (0.359)	0.155 (0.369)	0.034 (0.361)	0.102 (0.362)	***0.215*** ***(0.036)***	–	–	–	–	–
8517	6.359 (0.216)	–	–	–	–	***0.201*** ***(0.040)***	–	–	–	–	–
8551	6.59 (0.355)	-0.143 (0.450)	-0.481 (0.503)	-0.011 (0.435)	***-0.908*** ***(0.432)***	0.054 (0.074)	–	0.107 (0.095)	0.201 (0.123)	0.054 (0.085)	***0.495*** ***(0.168)***
8580	5.596 (0.382)	0.210 (0.462)	0.616 (0.463)	-0.125 (0.463)	0.173 (0.467)	***0.208*** ***(0.063)***	–	–	–	–	–
8619	5.781 (0.259)	–	–	–	–	***0.142*** ***(0.037)***	***-1.049*** ***(0.336)***	–	–	–	–
8673	5.899 (0.277)	-0.310 (0.342)	0.078 (0.317)	-0.199 (0.333)	-0.042 (0.375)	***0.159*** ***(0.045)***	–	0.092 (0.065)	-0.052 (0.049)	*0.141* *(0.082)*	0.066 (0.075)
8722	5.402 (0.308)	***1.055*** ***(0.378)***	0.607 (0.376)	***0.877*** ***(0.378)***	-0.067 (0.388)	***0.164*** ***(0.027)***	–	–	–	–	–
10022	6.038 (0.414)	***0.945*** ***(0.457)***	0.715 (0.452)	0.530 (0.457)	-0.113 (0.458)	0.091 (0.045)	***-0.605*** ***(0.269)***	–	–	–	–
10046	5.966 (0.344)	–	–	–	–	***0.148*** ***(0.030)***	–	–	–	–	–

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Table 5. Summary of the coefficients (S.E. in parentheses) for individual strategies from the Arena moved group. Bold italics and italics indicate a significant difference in the log-transformed path-length ($p<0.05$ and $p<0.1$, respectively) in this individual in a particular manipulation compared to the F-training phase. The interaction between the number of touches and the type of manipulation can be interpreted as a change in motivation to actively swim and seek out the platform. In several cases, there was no interaction (–) and a model without interaction was used

ID	intercept	arena moved	arena back	light moved	touches	start position	touches*arena moved	touches*arena back	touches*light moved
1113	5.423 (0.516)	0.337 (0.577)	1.002 (0.625)	0.673 (0.545)	**0.076** **(0.019)**	–	–	–	–
1125	5.806 (0.422)	0.371 (0.482)	0.134 (0.529)	0.316 (0.451)	**0.157** **(0.036)**	–	–	–	–
8145	4.786 (0.616)	0.665 (0.672)	*1.524* *(0.692)*	*1.316* *(0.654)*	0.236 (0.123)	–	–0.071 (0.128)	–0.187 (0.125)	–0.093 (0.126)
8157	6.489 (0.682)	–0.200 (0.738)	0.234 (0.820)	–0.746 (0.717)	–0.003 (0.037)	–	–	–	–
8158	5.909 (0.373)	–0.150 (0.383)	0.193 (0.421)	0.245 (0.364)	**0.137** **(0.021)**	–0.203 (0.209)	–	–	–
8177	5.684 (0.506)	0.682 (0.634)	0.099 (0.599)	0.059 (0.556)	**0.287** **(0.124)**	–	–0.195 (0.129)	–0.104 (0.128)	0.022 (0.140)
8183	4.842 (0.416)	0.570 (0.407)	*1.356* *(0.438)*	*0.913* *(0.386)*	**0.175** **(0.025)**	–0.345 (0.214)	–	–	–
8228	6.818 (0.758)	0.054 (0.768)	0.086 (0.850)	–0.919 (0.782)	0.149 (0.090)	–	–	–	–
8247	9.033 (1.419)	**–3.061** **(1.457)**	**–3.234** **(1.520)**	**–3.085** **(1.433)**	–0.566 (0.315)	–	*0.768* *(0.322)*	*0.857* *(0.334)*	0.585 (0.316)

8308	6.442 (0.317)	−0.528 (0.359)	0.174 (0.393)	−0.254 (0.340)	**0.086 (0.039)**	–	–	–	–
8337	5.777 (0.560)	−0.256 (0.624)	−0.215 (0.684)	0.133 (0.590)	**0.127 (0.028)**	–	–	–	–
8480	4.825 (0.559)	0.212 (0.560)	0.825 (0.617)	0.549 (0.544)	**0.185 (0.045)**	–	–	–	–
8496	6.089 (0.629)	0.031 (0.589)	0.179 (0.650)	−0.352 (0.569)	**0.122 (0.036)**	−0.489 (0.335)	–	–	–
8620	6.237 (0.355)	−0.371 (0.416)	−0.207 (0.447)	−0.183 (0.394)	**0.168 (0.029)**	–	–	–	–
8652	5.233 (0.332)	0.463 (0.391)	**1.013 (0.422)**	0.593 (0.369)	**0.145 (0.026)**	–	–	–	–
8745	5.101 (0.555)	0.315 (0.533)	0.941 (0.589)	0.607 (0.508)	**0.207 (0.037)**	**−0.763 (0.305)**	–	–	–
8776	5.394 (0.771)	**1.678 (0.804)**	**2.460 (0.828)**	1.324 (0.823)	0.261 (0.262)	–	−0.221 (0.272)	−0.454 (0.309)	**−0.660 (0.294)**
8819	5.763 (0.318)	0.289 (0.357)	0.450 (0.385)	0.219 (0.335)	**0.148 (0.023)**	–	–	–	–
9513	5.145 (0.373)	0.853 (0.437)	**1.201 (0.464)**	**0.955 (0.399)**	**0.094 (0.025)**	–	–	–	–
10012	5.459 (0.310)	**0.965 (0.323)**	**0.782 (0.356)**	**0.836 (0.302)**	**0.112 (0.015)**	–	–	–	–
10025	5.599 (0.534)	0.683 (0.557)	0.654 (0.614)	0.372 (0.532)	**0.111 (0.037)**	−0.627 (0.305)	–	–	–
2010007	3.661 (0.387)	**1.143 (0.379)**	**1.920 (0.425)**	**1.919 (0.370)**	**0.254 (0.029)**	–	–	–	–

Nevertheless, the path-length was longer during the dark phase, which suggests that the navigation was somehow distorted.

Evaluation of individual strategies

As the results of close and distal cue manipulation were inconsistent, maybe due to varying individual navigational strategies, we focused more on individual level of evaluation. In both groups (Arena cues removed/Arena moved) it was apparent that individual animals vary in the strategy used to orient in the MWM. For the model with path-length as the dependent variable, the start position, number of touches and type of manipulation with proximal and distal arena cues were included in the final models. For both groups, the number of touches, manipulation phase and its interaction were the most important variables. The interaction can be interpreted as a change in motivation to actively swim and seek out for the platform. Tables 4 and 5 summarize the results.

During the whole experiment, we observed the body condition, which can affect the cognitive performance. We calculated that the body condition of the tested animals was not affected by the experiments ($F_{(1,494)}$=0.4219, p=0.5163), while their body weight ($F_{(1,494)}$=9.83, p=0.0018) slightly decreased over the course of the study. We interpret this result to mean that the animals did not consume a substantial amount of fat reserves, but that the performance during the training was energetically demanding.

DISCUSSION

Spatial learning in the leopard gecko

Studies that experimentally tested spatial learning in reptiles provided mixed results. While some found that certain species make use of complex visual cues for spatial learning (reviewed in Szabo et al. 2021), others reported no such learning (Bezzina et al. 2014). In this study, we demonstrated that another species of squamates, the leopard gecko, can learn to solve a spatial task in the MWM over 20 trials. The animals improved their performance over the training phase, taking a shorter path to locate the platform at the end of training.

Allothetic orientation – switching between proximal and distal cues

Recent papers investigating the spatial cognition of turtles, tortoises, snakes, and lizards revealed that these reptiles use complex visual cues and can choose between those which are currently most useful to navigate to the goal (Szabo et al. 2021). The specific features of the environments that were used by the tortoise and other tested species, however, remain rather unclear (Wilkinson et al. 2009, see also Introduction). Our results show that leopard geckos use both proximal arena cues and distal room cues to navigate in the MWM. We infer that geckos used close cues, as the path-length was longer when the close cues became suddenly unreliable (due to arena shift). Nevertheless, we cannot recognize which particular close cue and/or their combination was important for them, as there was no effect of one by one cues removal on path-length. The geckos usually depend on multiple proximal cues and learn the important cues individually (see below). When the proximal cues were manipulated (either removed one by one or the arena was shifted to a new position), the geckos were able to switch to the distal room cues. This is in line with the results of an experiment with blotched lizards, *Uta stansburiana* Baird et Girard, 1852, which also used extra-maze cues to find one correct hole out of ten in a circular Barnes maze (LaDage et al. 2012). We found that some individuals were fixed on the most prominent extra-maze cue of the room, the directed light source. When we changed the position of the light source, these individuals failed to navigate directly to the platform. It corresponds with multiple studies on squamates and turtles that demonstrated orientation in space with respect to the most apparent light sources. Italian wall lizards *Podarcis sicula* used the position of the sun (Foà et al.

2009), and leatherback sea turtle hatchlings used the moon (or moonlight reflecting on the water surface) or artificial light sources (Bourgeosis et al. 2009). Leopard geckos display some cognitive flexibility, as certain individuals are able to switch between the two navigational frames (arena cues and room cues), depending on which is available and reliable for locating the goal. Many species of squamates use multiple visual cues for orientation (Szabo et al. 2021). Squamates can also use a geomagnetic sense in the absence of all visual cues or as an additional source of spatial information (Nishimura et al. 2010, Diego-Rasilla et al. 2017). In reptiles, the dominant role of magnetic compass for orientation was reliably demonstrated mainly over much larger distances, e.g., in transoceanic migration of loggerhead turtles (Lohmann et al. 2001, 2004, Fuxjager et al. 2011) or during spontaneous magnetic alignments (Landler et al. 2015, Diego-Rasilla et al. 2017). Interestingly, learning to navigate in adapted 4-armed (plus) water maze according to the alignment with magnetic north, that was experimentally changed between the trials was very efficient in mice (Phillips et al. 2013). The animals learn the magnetic direction of the submerged platform only after two training trials. However, in these experiments no apparent visual cues were in disposal for orientation and mammalian species was tested. As the sensitivity to low frequency magnetic field was confirmed in agamid lizard (Nishimura et al., 2010, 2020) and homing behaviour of displaced Phillippine bent-toed geckos, *Cyrtodactylus philippinicus* (Steindachner, 1867), was related to the local earth's magnetic field (Marek et al. 2010)*, we cannot rule out that in our experiment the geckos used local properties of magnetic field together with visual cue for navigation. However, the importance of each navigational cues (visual vs. magnetic) for geckos' allothetic navigation requires further research effort. A preference for visual or geomagnetic cues could be examined in another experimental design that would manipulate not only visual cues but also the characteristics of the magnetic field.

How animals choose the visual cue for orientation and how they switch between them is highly individual: two animals failed to locate the platform in the allotted time when the first proximal cue was removed, two when three proximal cues were removed, and three animals failed when all proximal cues were removed. The rest of the group used the distal cues in the room to navigate or adopted an alternative non-cognitive motoric strategy to find the platform. We cannot rule out an alternative non-cognitive strategy in some geckos, especially in the dark phase when visual orientation was difficult for them, and they swam more actively and quickly. Specifically, they swam a longer path in this phase, but they found the platform sooner. Some animals also showed a change in motivation to swim actively after the manipulation with arena cues or when the arena was moved; the number of touches required to encourage them was significantly higher in these phases of the experiment. Some other squamates, such as the jewelled lizard, *Timon lepidus* (Daudin, 1802), (Mueller et al. 2012) or two species of skinks of the genus *Lampropholis* (Bezzina et al. 2014), have been shown to commonly use response-based strategies rather than allothetic orientation using complex visual cues. The individual differences in navigation strategies and alternative non-cognitive tactics are a likely explanation for the lack of significant effect of manipulation with arena cues on the group level. However, both groups of geckos (Arena cues removed, Arena moved) were able to learn to reach the goal during the training – they were better at the end of training compared to the initial stage of training in the MWM. These results are consistent with previous studies using various methodologies, all showing individual variation in learning abilities in squamates (Leal & Powell 2012, Carazo et al. 2014, Noble et al. 2014, Chung et al. 2017, Font 2019).

Sources of individual variability potentially generating individual strategies in allothetic orientation

We cannot exclude that incubation temperature affects individual learning performance of the geckos. Incubation temperature has a significant impact on the animal and long-term consequences. Manipulating the incubation temperature of the embryo can influence the phenotype of the animal, i.e., sex, size, and behaviour (Booth 2006, Amiel et al. 2014). Particularly in reptile species with temperature-dependent sex determination (TSD), such as *E. macularius*, thermal regimes in the nest may influence the hormones responsible for gonadal differentiation and development of the brain (Sakata et al. 2000). Thus, thermal effects on hormone levels during incubation can induce structural changes in parts of the brain that control cognitive processes such as learning, which may result later into sex differences in cognitive and performance tasks. We cannot rule out the sex differences in learning in our experimental geckos. Nevertheless, the number of males was too low to allow statistical testing. It will be necessary to perform another study with specific design to control the effect of sex on spatial orientation (see Limitation of the Study).

We are aware that the phenotype of the leopard gecko is influenced by many genetic as well as environmental factors (West-Eberhard 2003). Even though all embryos were incubated in the same condition (and most of the animals were normally behaving, non-aggressive females), still, we can suppose that small changes in incubation temperature may generate part of individual variability in our experiment. Consistent individual differences can be heavily influenced by environmental factors like temperature in TSD reptiles (see above), but still, they have also substantial underlying genetic component (Laine & van Oers 2017). As heritability is assessed by partitioning variance between environmental and genetic factors, we can use it to estimate influence of both on individual variability in behavioural traits. The heritability of cognitive traits highly varies among taxa (various species of mammals, birds and fishes were studied) and cognitive tasks ranging from 0.02 to 0.96 (reviewed in Croston et al. 2015, Boogert et al. 2018), but heritability of cognitive traits have never been studied in reptiles. However, there are many studies reporting heritability of performance traits in squamates (reviewed in Garland & Losos 1994, Waters et al. 2017) that can potentially influence cognitive performance of leopard geckos in MWM. For example, among other performance traits, speed endurance can mirror the endurance of individual animal in MWM. Some squamate species exhibit high heritability of endurance: 0.59 in Fence lizards *Sceloporus occidentalis* Baird et Girard, 1852 (Berkum & Tsuji 1987), 0.58 in Garter snakes *Thamnophis sirtalis fitchi* Fox, 1951 (Garland et al. 1990). The boldness of the individual, usually measured as consistent differences in antipredatory behaviour, could influence the cognitive results of geckos in MWM. For example, close association between boldness tested as behaviour after predatory attack and spatial orientation performance was confirmed for individuals with extreme variability in this trait (both high and low boldness) in Eastern water skink, *Eulamprus quoyii* Duméril et Bibron, 1839 (Carazo et al. 2014). The heritability of individual differences in antipredatory behaviour was measured in several species of snakes of the genus *Thamnophis* reaching: 0.45 for *T. radix* (Baird et Girard, 1853), (Arnold & Bennett 1984), 0.49–0.66 for *T. ordinoides* (Baird et Girard, 1852), (Brodie 1993), 0.42 for *T. sirtalis* (Linnaeus, 1758), (Garland et al. 1990). Thus, we can expect, that individual variability in endurance, antipredatory behaviour and cognitive performance, all have substantial genetic component of the variability that influenced cognitive performance of geckos in MWM.

This is consistent with our findings that only a third of the animals used allothetic orientation and even the geckos with allothetic orientation differed greatly in their individual strategies for finding the platform (see Table 4 and 5). It would be necessary to conduct a similar experiment with contrasting incubation temperatures with at least two generations of geckos to uncover the effect of incubation and heritability on cognitive abilities in leopard geckos.

It would also be interesting to check the differences in learning abilities across ontogeny, as it has been demonstrated over the last few decades that reptiles continue to add neurons in many

regions of the adult brain at a high rate (Font et al. 2001, LaDage 2020). An increase in adult brain size is in contrast with determinate body growth demonstrated recently in the majority of squamate reptiles (Frýdlová et al. 2017, 2019, 2020). Adult neurogenesis in the medial cortex of leopard geckos has been demonstrated recently (McDonald & Vickaryous 2018). Nevertheless, a detailed study of neuron number changes during ontogeny in the Madagascar ground gecko, *Paroedura picta* (Peters, 1854), revealed that young adults do not have significantly lower numbers of neurons than fully grown adults, except for the telencephalon (Kverková et al. 2020). There might be a difference in cognitive abilities between young and adult animals due to the continuous neurogenesis, but our study should not be impacted by neurogenesis as we used a homogenous group of sexually mature animals.

Limitations of the study

It was not possible to perform experiments more often as we know that our geckos could have problems with skin under more intensive training in water environment. Water is not problem for geckos for a short time, nevertheless after longer time the skin become softer and vulnerable for mechanic injuries. This was the reason to perform only one training per day with longer period (several days) between trials. Thus, the task was cognitively more demanding (one or two trials per week) in comparison to the routine in laboratory rats (four trials per day). For the confirmation of learned spatial information, we did not use removal of the platform (probe test), as we want to prevent the loss of gecko's motivation. Instead, we used the dark test (all cues invisible for the geckos) to reveal alternative cognitive and non-cognitive strategies. Moreover, our results are only indicative of female's spatial orientation and learning due to the skewed sex-ration. It is widely discussed the suitability of MWM design in reptiles due to the poor ecological relevance. Nevertheless, the knowledge of spatial orientation of this squamate model species is crucial. We believe that we employed proper experimental design for this species, which is slow, nocturnal, rather lazy and hard to motivate. To highlight the advantages of MWM design in reptiles we declare the absence of chemtrails and temperature gradient, and increased motivation. Even though we slightly adapted MWM experimental design for ecological requirements of the leopard geckos, we mostly followed general procedure typical for rodents. This allows us finally to interpret the navigation frames and cues and thus gecko´s navigational strategies on the similar bases as in rodents.

CONCLUSIONS

In conclusion, we evaluated the learning abilities of leopard geckos in a modified Morris Water Maze. The geckos were able to learn the position of the platform relative to proximal as well as distal cues. The strategy and frame of reference for navigation were highly individual. The individual geckos attend to both proximal and distal cues, and flexibly adjust their strategy when some of the cues are missing, which is indicative of allothetic orientation. We hypothesise that some animals combined cognitive and alternative non-cognitive motoric strategies to find the platform.

SUPPLEMENTARY INFORMATION

Supplementary Information is available at Figshare: https://doi.org/10.6084/m9.figshare.22284526
Supplementary Information 1. Video file. Video depicting the study arrangement and the course of experiment.
Supplementary Information 2. Additional informations concerning Material and methods and Results.
Supplementary Information 3. Dataset. Raw data from MWM experiment with leopard geckos.

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