# Considerations towards a better practice in the study of learning in non-avian reptiles

# What can the past teach us to improve the study of learning in non-avian reptiles

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## Abstract

## Introduction

In his book section “Learning processes in Reptiles” Gordon Burghardt (1978) summarised, for the first time, the knowledge on reptile learning that was available so far. He gave a detailed description of the methods used and what insights were gained from these studies. Since the start of the 21st century, more and more researchers are willing to tackle the unique challenges associated with research into reptile learning (Figure 1; Whiting & Noble, 2018). Now, the field is slowly advancing towards a more structured approach reflected in some recent reviews by Wilkinson and Huber (2012) or Whiting and Noble (2018). We conducted a systematic online literature search and identified over 80 studies investigating learning in reptiles not included in Burghardt (1978). Although a small number of these studies has been included in previous work (Whiting & Noble, 2018; Wilkinson & Huber, 2012), they are important contributions to understanding the full extent of the current knowledge and are therefore included in this review.

Modern non-avian reptiles are the third most species rich group of vertebrates. Many, in other vertebrates well established learning concepts such as sex differences in spatial learning or executive function, have yet to be tested in non-avian reptiles. Reptiles inhabit a wide range of different habitats, show a range of mating systems (monogamy to polygynandry), feeding ecology (herbivorous, insectivorous or omnivorous), social structure (solitary to groups of over 20 individuals) and differ substantially in their reproductive tactics (parthenogenesis, oviparity or viviparity) and behaviour (Whiting & While, 2017). For example, many reptiles exhibit oviparity without parental care representing a unique opportunity to study questions relating to developmental effects on cognition which cannot be answered using mammal or bird species (Matsubara, Deeming, & Wilkinson, 2017). Furthermore, reptiles diverged from lineages leading to mammals about 280 million years ago (Macphail, 1982). Their brain exhibits several features with prototypic phenotypes (Nomura, Kawaguchi, Ono, & Murakami, 2013), reptiles have, however, experienced similar selective pressures since the split (Wilkinson & Huber, 2012). Investigating learning in these vertebrates might give insight into a more prototypic vertebrate cognitive ability as well as reveal convergent evolution based on similar selective pressures.

The aim of our systematic literature review is to first, give a comprehensive overview of the research conducted over the last 40 years. Second, to linking the gathered results and highlight methodological innovations and shortcomings, the following sections are therefore organised to be able to link results between species (some studies fall under more than one subheading). Lastly, we feel that it is of great importance for the field to move forward by paying closer attention to the ecological background of model reptilian species. After reviewing the current knowledge we want to shortly summarise best practise and give some directions to improve future research design to test learning in a more ecologically relevant context.



**Figure 1.** Number of studies from before 1960 to 2017 (in 10 year steps) split between the four main orders of Reptilia: Corocodilia, Rhynchocephalia, Squamata and Testudines. Superimposed as a line is the cumulative number of studies.

## Avoiding aversive stimuli

Animals need to know what to eat, when and where to hide and which conspecifics to avoid. Avoiding potentially harmful stimuli encountered in the environment aids survival (Shettleworth, 2009). Reptiles perform no different from other vertebrates when tested on their avoidance learning. Groups of brown anoles (*Anolis sagreis*), for example, were tested on their shock avoidance learning. Group one received a shock whenever their tail touched a grid and group two received a shock whenever group one was shocked. Animals in the first group learnt to avoid shock by lifting their tails and retained this behaviour for six hours after training; animals in group two did not. After cycloheximide injection in the basal forebrain, however, lizards failed to show any shock avoidance (Punzo, 1985).

Another important skill when it comes to survival is to know which food to avoid because it might be toxic or have a noxious effect. Male green anoles (*Anolis carolinensis*) were tested on their ability to discriminate neutral, bitter and sweet tastes. Crickets were coated with either quinine hydrochloride (QHCl; bitter), Equal® (sweet) or were untreated and received a coloured dot to increase discriminability. Lizards generally rejected bitter tasting prey but were not able to do so when the vomeronasal organ was blocked (Stanger-Hall, Zelmer, Bergren, & Burns, 2001) highlighting these animals strong reliance on olfactory cues. Similarly, A few days old hatchlings of the oriental garden lizard (*Calotes versicolor*) learnt to associate dish colour with prey taste. Independent cohorts of hatchlings with the same basic experience (non-painted dish – neutral taste, orange dish – sweet taste from coating in sucrose and green dish – bitter taste from coating in Chloroquine phosphate) were simultaneously confronted with either the same combinations or with new colour and taste combinations. The results show that hatchlings choice of prey was affected by dish colour (Shanbhag, Ammanna, & Saidapur, 2010). Furthermore, when a novel food was paired with an Lithium chloride (LiCl) injection (to make them sick), *Basiliskus vitattus*, B. *basiliscus* as well as *Eumeces schneideri* and *Mabuya mutifasciata* avoided the same novel food item a week later after experiencing illness. A second novel control food, however, was readily accepted one week after a saline injection (Paradis, & Cabanac, 2004).

Conditioned taste aversion can also be used to teach animals to avoid invasive unpalatable pray. Australian blue-tongue lizard (*Tiliqua scincoides scincoides* and *T. s. intermedia*) presented with cane toad meat (*Rhinella marina*, an invasive toxic toad) paired with a LiCl injection or meat mixed with LiCl still avoided the meat seven weeks after the first encounter, a control group eating meat treated with saline, however, showed no aversion (Price-Rees, Webb, & Shine, 2011). Similarly, hatchling Australian freshwater crocodiles (*Crocodylus johnstoni*) rapidly learnt to avoid freshly metamorphosed cane toads. Treatment crocodiles were more likely to reject toads compared to naïve individuals (Somaweera, Webb, Brown, & Shine, 2011).

Two experiments used a more naturalistic approach testing taste aversion learning. Wild Bonaire island whiptail lizards (*Cnemidophorus murinus)* learnt to distinguish palatable from unpalatable artificial fruit place along their natural habitat (sponges soaked in sweet tomato juice versus toxic quinine hydrochloride) based on visual (colour) and spatial cues (Schall, 2000). A more recent study investigated how populations of juvenile fence lizards (*Sceloporus undulates*), either invaded by toxic fire ants or uninvaded reacted when simultaneously presented with a choice between unpalatable fire ants and an alternative prey (crickets) on two consecutive days. Juveniles from both populations did not learn to avoid this toxic prey but instead increased ant consumption over time (Robbins, Freidenfelds, & Langkilde, 2013). Some species might be more apt to learn to avoid unpalatable or toxic prey, the methodology used, however, might also play an important role.

Undoubtedly, avoiding unpalatable food and adverse environmental conditions are important skills and can potentially increase fitness. Escaping predators is, however, no less important. When threatened, male eastern fence lizards (*Sceloporus undulates)* escape into hiding. Escape behaviour was linked to corticosterone: Compared to control animals, who showed an increase in flight initiation distance and decreased hiding time, males treated with a corticosterone blocker showed no change in these behaviours and no retention 24h later (Thaker, Vanak, Lima, & Hews, 2010). Moreover, little brown skinks (*Scincella lateralis*) used patterns to find a safe refuge but performed better when presented with vertical compared to horizontal lines, presumably because of the nature of sheltering sites which are at the base of trees (Paulissen, 2014). Lizards are not just able to use pattern to find a ‘safe’ refuge, they are similarly skilled at using colour and location cues when escaping a threat. Male delicate skinks (*Lampropholis delicate*) learnt to escape a simulated predator attack into a ‘safe’ refuge using both location and colour alone to find the safe hide (Chung et al., 2017). The same methodology was used to compare performance of male delicate skinks from urban and natural areas. A greater proportion of lizards learnt when both colour and location stimuli were available than when only colour stimuli indicated which refuge was safe. Furthermore, more of the lizards from more naturalistic habitats were successful learners (Kang, Goulet, & Chapple, 2018). Besides population effects on escape behaviour, developmental conditions can also alter escape behaviour. Hatchling White’s skins (*Liopholis whitii*) whose mothers received less food (low resource treatment) were more likely to avoid threat and learn to locate a ‘safe’ refuge compared to hatchlings from mothers receiving more food (high resource treatment; Munch et al., 2018). Furthermore, In their natural habitat, female curly-tailed lizards (*Leiocephalus schreibersii*) rapidly learnt to avoid capture. They did so faster than males and after only one capture event (Marcellini, & Jenssen, 1991).

Summarise results in short paragraph

## Discriminating between stimuli that indicate food and those that don’t

Two-choice discriminations with a food reward

## Quality and quantity discrimination

To understand if and how animals count can help better understand if our own numerical abilities are a unique result of our ability to use language or if there is a conserved neural basis present in all vertebrates. Many species have been shown to be able to discriminate between different reward quantities and qualities (e.g. Agrillo & Bisazza, 2018; Benson-Amram, Gilfillan, & McComb, 2018; Shettleworth, 2009), reptiles are no exception. Red footed tortoises (*Chelonoidis carbonaria*), for example, were first trained to associate a given stimulus with a specific reward quality or quantity, after which they were given preference tests by simultaneously presenting two stimuli with different values. Tortoises discriminated between preferred and less preferred foods as well as large and small quantities of food and retained this knowledge for 18 months (Soldati, Burman, John, Pike, & Wilkinson, 2017). The speed with which Chinese pond turtle (*Geoclemys reevesii*) moved down a runway was contingent on the amount of reward provided at the end. Turtles in the large reward group, receiving 24 pellets each trial, moved faster than turtles receiving only two pellets. During extinction trials, with no food present, similar results were obtained (Papini, & Ishida, 1994).

In lizards, ruin lizards (*Podarcis sicula*) were tested on a trained numerosity and size discrimination. Numerosity was tested using artificial stimuli depicting 1 versus 4, 2 versus 4 and then 2 versus 3 yellow disks. Size discrimination was tested with similar stimuli; yellow disks differing in sizes. Lizards were able to discriminate between 1 versus 4 and 2 versus 4 but not between 2 versus 3. None of the lizards, however, were able to discriminate between two different sizes of stimuli (ration 0.25; Petrazzini, Bertolucci, & Foa, 2018). Taken together, these studies demonstrate that reptiles have a sense of reward quality, size and quantity. Artificial and naturalistic stimuli might be used to test for numerosity in animals, both, however, pose some benefits and limitations; for further reading see Agrillo & Bisazza (2014).

## Reacting to change

Reversals and extra-dimensional shifts

## Solving novel problems

Many animals are well known to be good problem-solvers. New Caledonian crows (*Corvus moneduloides*) bending wires into hooks to reach a reward (Weir, Chappell, & Kacelnik, 2002), or rats (*Rattus rattus*) developing a new technique to feed on pine cones when little other food is available (Zohar & Terkel, 1991) are just two examples of innovation and problem solving skill. Only a few study, so far, looked at non-avian reptiles ability to learn a novel foraging technique. Painted turtles (*Chrysemys picta picta*) as well as sub-adult Burmese pythons (*Python molurus bivittatus*) could be trained to push response keys to gain access to a reward (Blau & Powers, 1989; Cranney & Powers, 1983; Emer, Mora, Harvey, & Grace, 2015; Grisham & Powers, 1989; 1990; Powers et al., 2009; Reiner & Schade Powers, 1978; Reiner & Powers, 1980; 1983; Yeh & Powers, 2005) and five different studies successfully trained lizards to open lids covering food wells to access a reward. Anolis evermanni, A. *pulchellus and A. cristatellus* all removed lids to gain access to a reward, *A. cristatellus,* however, were less successful compared to the other two species (Leal, & Powell, 2012; Powell, 2012). Hatchling three lined skink (*Bassiana duperreyi*) also learnt to remove lids from food wells, however, incubation treatment affected this ability. Only lizards incubated at ‘hot’ temperatures were able to learn this behaviour (Clark, Amiel, Shine, Noble, & Whiting, 2014). Furthermore, both water skinks (*Eulamprus quoyii*) and tree skinks (*Egernia striolata*) were able to learn how to remove a lid from a food well. Neither age (young and old; Noble, Byrne, & Whiting, 2014) nor rearing environment (social or solitary; Riley et a;., 2018) affected performance in these two species, respectively. Although these studies used training procedures, they provide impressive examples of how reptiles can acquiring a novel behaviour to solve a problem. Not all species, however, were able to learn lid opening. Training White’s skinks (*Liopholis whitii*), another Australian skink species, on this task has, so far, proven unsuccessful (personal conversation). Why some reptile species are able to learn novel behaviours and others are not and what techniques these species use to solve these novel problems should be investigated in more detail in the future.

Only a single study has attempted to test a reptile in a more complex task using a puzzle box. Presented with an artificial fruit type task, black-throated monitors (*Varanus albigularis albigularis*) were able to open a plastic tube to retrieve a reward within 10 minutes of the first presentation showing their problem solving ability. They even decreased their time to solve this novel task during the second and third trial (Manrod, Hartdegen, & Burghardt, 2008). Varanids are considered as ‘smart’ lizards and in Australia, monitor lizards can be frequently found in picnic areas (Whiting & Noble, 2018) represent future opportunity to study Varanids on similar puzzle boxes or more complex arrangements in the wild.

## Spatial cognition

### Radial arm maze

### Morris water maze

### Open Arena

## The non-social social learning paradox

Social learning in general refers to learning from other individuals. These might be conspecifics, animals from the same species or even animals from a different species (Shettleworth, 2009). Most social learning studies have been conducted on animals that aggregate into social groups but less research has looked at how less social species use information provided by other animals. Especially in reptiles, we know little about their social behaviour, however, recent work has shown that even those ‘unsocial’ animals can learn from their conspecifics (Doody, Burghardt, & Dinets, 2013).

Wilkinson et al., 2010; Wilkinson & Huber, 2012

Bearded dragons (*Pogona vitticeps)*, a solitary, Australian lizard species, for example,learnt to open a sliding door in the same direction as a demonstrator by watching a video of a conspecific performing the task. A control group watching the door open on its own was not able to learn (Kis, Huber, & Wilkinson, 2015). Furthermore, incubation treatment affected hatchlings performance on the same task. Cold incubated lizards opened the door significantly faster than hot incubated lizards. There was, however, no significant difference in the number of successful door openings between the incubation treatments (Siviter, Deeming, van Giezen, & Wilkinson, 2017).

In the water skink (*Eulamprus quoyii*) age affected the ability of lizards to learn a two-choice discrimination task from a trained conspecific, dominance status, however, influenced lizards social information use only marginally. Both old and young observers learnt to discriminate between two differently coloured lids, no matter if they watched a demonstration or not. Young males, however, used the social information more efficiently than older males; observers learnt significantly faster than control lizards (Noble, Byrne, & Whiting, 2014). When dominance was taken into account, both observers and control lizards learnt the task, dominant social lizards, however, learnt faster than subordinate social lizards. In a subsequent reversal task, no effect of dominance was apparent (Kar, Whiting, & Noble, 2017). Although considered not social, water skinks are often found in high densities around water bodies in urban areas (REF); socially provided information is therefore readily available.

The only non-lab study was conducted on wild *Podarcis lilfordi.* Lizards were presented with two choices between food with and without conspecifics including static copper models. When feeding, wild *Podarcis lilfordi* prefer locations with conspecifics present showing social enhancement in their feeding behaviour, social attraction was also observed when no food was present. *Podarcis lilfordi* occur in high densities, are generalist foragers and experience low predation pressure. In this system, conspecifics can act as a reliable source of information regarding foraging opportunities (Perez-Cembranos & Perez-Mellado, 2015).

The *Egernia* group is a group of Australian skink species includes species varying in their degree of social complexity (Whiting & While, 2017). Two monogamous species from this group have been the focus of social learning. In the White’s skink (*Liopholis whitii*), familiarity plays an important role during reversal learning but not in a simple discrimination task. Observers in three treatment groups were tested: Individuals observing their mating partner, an unfamiliar conspecific, or a control lizard providing no social information. No significant differences were found during the initial discrimination. During reversals, lizards observing a familiar mate learnt significantly faster than control lizards (Munch, Noble, Wapstra, & While, 2018). Contrary to White’s skinks, the closely related tree skink (*Egernia striolata*) does not use social information to learn a similar task. Furthermore, rearing treatment (social or solitary) did not affect learning a discrimination or reversal task (Riley et al., 2018).

Although generally considered as solitary animals, reptiles show a variety of social structures from simple social tolerance to group living (Whiting & While, 2018). So far, some studies indicate that both more solitary species such as the bearded dragon (Kis et al., 2015) or red footed tortoise (Wilkinson et al., 2010; Wilkinson & Huber, 2012) can still use socially provided information to solve different tasks. When sociality was taken into account such as in the White’s skink, familiarity affected learning especially in a more complex task such as a reversal significantly (Munch et al., 2018). In the closely related tree skink no performance increase through social information use was detected. These studies provide a first glimpse into how reptiles use information provided by conspecifics and future studies could potentially reveal some important new insights.

## Memory

Memory, the retention of information through time can be important for survival especially if information stays relevant for extended amounts of time and a good memory is energetically cheaper compared to relearning (Shettleworth, 2009). Turtles are generally long lived and proved to be good models to study long-term memory. The Florida red-bellied cooters (*Pseudemys nelsoni*), red footed tortoises (*Chelonoidis carbonaria*), common box turtles (*Terrapene carolina*) and pond sliders (*Trachemys scripta*) can remember learnt stimuli or rules for up to 36 months after initial training (Davis, & Burghardt, 2007; 2011; 2012; Leighty et al., 2013; Soldati et al., 2017). In lizards studies specifically investigating memory retention are rare. Crevice spiny lizards (*Sceloporus poinsettii*) were tested on their associative learning and memory ability. Animals were trained to find food in four specific locations within an arena. Test trials were divided into two phases: First none of the dishes provided food and the location each lizard inspected first was recorded. In the second phase, food was provided in only one dish. During phase one, lizards preferentially visited the location food was provided the day before (phase 2) indicating a memory for cues associated with successful foraging (Punzo, 2002).

## Conclusions and future directions

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