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12 Lizards – Measuring Cognition: Practical Challenges and the Influence of Ecology and Social Behaviour

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Species Description

Anatomy

While lizards span a great variety of body shapes, sizes and anatomical features, the majority of species are relatively small and of a size that is amenable to maintaining in the lab. Lizards on the small end of the scale can perch on the tip of a matchstick (e.g. leaf chameleons), while the largest are the varanids (e.g. Komodo dragon), which can be 3 m in length. Their anatomy is strongly indicative of their ecology and behaviour. For example, many lineages have experienced limb reduction and retain only vestiges of their appendages that are non-functional. These species are often sand-swimmers, living in fine sands just below the surface, in desert or coastal areas. Similarly, other species live in grasslands, where a serpentine form improves locomotor performance. These species move more like a snake, and also frequently have tails much longer than their body. Conversely, arboreal lizards have digits capable of greater traction on vertical surfaces (e.g. geckos), or long hind-limbs that allow them to sprint faster on broad surfaces or short stubby limbs for grasping thin perches such as bushes (e.g. bush anoles). A wide range of species live on rock surfaces and are either dorso-ventrally flattened, to allow them to squeeze into narrow crevices to escape from predators, or they may have digits and limbs that facilitate grip or adhesion to rocky surfaces (e.g. geckos). As we would predict, lizards exhibit a tight link between morphology and habitat use, and this needs to be taken into account in cognition studies. Species that are secretive and/or highly specialized may be more challenging to study in the wild, and will require a set-up in the lab that caters for their specific habitat requirements. For example, some active foragers have high rates of movement and high field body temperatures. These species are particularly challenging to keep in the lab and equally challenging to study in the field. For an excellent overview of lizard diversity that documents links between morphology, ecology and behaviour, see Pianka and Vitt (2003).

The vast range of body sizes in lizards also presents a practical challenge. No studies have been attempted on ‘miniature’ species, such as some of the leaf chameleons or dwarf geckos. However, we have conducted learning trials with small baby skinks (*Bassiana duperreyi*, snout–vent length, SVL = about 25 mm; Clark *et al.*, 2014), training them to move coloured caps from food wells. For other small species, an alternative is to position a cue behind a food well sunk into a block of wood, which

would require the lizard to make an active choice by approaching and looking into a well. Larger lizards such as varanids (monitor lizards) are challenging for the opposite reason – many species are quite large and therefore difficult to keep in the lab. However, many zoos have captive animals and they are frequently amenable to collaborative studies of their learning ability. There are many anecdotal reports of monitor lizards being particularly 'intelligent', but besides a single study (Manrod *et al.*, 2008), in which eight individuals rapidly solved a motor task, this is largely unsubstantiated. In Australia, monitor lizards frequently occur near picnic areas, where they are habituated to people. These individuals could be suitable candidates for cognition studies in the wild.

Perception

Visual and chemosensory modalities are strongly developed in lizards, and are the primary means by which individuals perceive stimuli from their environment. Hearing in lizards is generally not well understood, and is thought to be less important, although some geckos use acoustic communication (Marcellini, 1977). In terms of vision, lizards are tetrachromatic and can rely on a broad spectrum of colour-based visual cues (Fleischman *et al*., 1993, 2011). In addition, lizards tongue-flick to sample chemicals from their environment, and this information is extracted in the vomero-nasal organ in the roof of the mouth, before being processed in the brain (Halpern, 1992; Cooper, 1994a). Ambush foragers rarely tongue-flick in the absence of social cues and typically rush out from an ambush post to capture prey, being far more visually oriented than actively foraging species. Conversely, active foragers will move through the landscape, repeatedly tongue-flicking the ground in search of hidden prey (Cooper, 1994b). Active foragers use their forelimbs to move aside leaves and debris that might be sheltering arthropod prey. They also dig up arthropods that are buried below the surface (Reilly *et al.*, 2007).

Cognitive studies to date have taken advantage of lizards' well-developed visual and chemosensory modalities in designing cognitive tasks. Cognitive tasks using chemosensory modalities can be quite difficult to design for certain species, as they require a greater degree of control and a basic understanding on the chemical composition of cues that are likely to elicit behavioural responses. Nonetheless, creative experimentation has been shown to be powerful in understanding, for example, individual recognition (Carazo *et al.*, 2008). Given these difficulties, it is therefore not surprising that most cognitive tasks make use of visual stimuli for training and learning. However, in reality, lizards most likely use a combination of cues, and there are studies that nicely illustrate this. For instance, Pérez-Cembranos and Pérez-Mellado (2015) make use of both visual and chemosensory modalities in the design of experiments. It is therefore important to think carefully about the specific cues in any cognitive task early in the design stages (see below for more details).

Life Cycle

With respect to life cycle, lizards are either oviparous (egg-laying) or viviparous (live-bearing), and this is commonly linked to climate, where colder areas favour viviparity

(Shine, 2014). For the majority of species, there is no parental care, and offspring either disperse after birth or hatching. However, there are species that brood their eggs and protect them from predators (e.g. *Mabuya longicaudata*; Huang, 2006), and in many family-living species there is parental care through association with parents, whereby the risk of infanticide is significantly lowered (While *et al.*, 2014). Age, sex and reproductive state can also influence cognitive ability. Sexual selection can be intense in many species of lizards (Stamps, 1983), and this is reflected in hormone profiles that are dependent on social status and season (Husak *et al.*, 2009). In spring, males are frequently establishing territories or searching for mates, and they typically have higher levels of testosterone than at other times of the year (Moore, 1988; Wack *et al.*, 2008). Likewise, females become receptive and later pregnant, resulting in significant changes in oestrogen and progesterone levels (Jones, 2011). Furthermore, some females become more aggressive during pregnancy (Sinn *et al*., 2008). Changes in hormone profiles could impact performance in cognition tests, and for studies incorporating sex differences, timing could play a significant role. For these species, it may be prudent to conduct cognitive experiments outside of the breeding season, unless the study question links to some aspect of reproductive biology or breeding behaviour.

Longevity in lizards is quite variable and ranges from being seasonal to long-lived. In the case of the annual chameleon *Furcifer labordi*, there is a point in their life cycle where all individuals exist as eggs beneath the ground, following the death of the adult stage after only a 4–5-month life span (Karsten *et al.*, 2008). Conversely, the sleepy lizard and members of the *Egernia* group, such as the Cunningham's skink, live to at least their fifties (Whiting and While, 2017). Longevity is linked to a range of covarying life-history traits, such as length of activity season, age at first reproduction, foraging mode, growth rate and frequency of reproduction (Dunham *et al.*, 1988). Collectively, these life-history traits are under strong selection and likely influence constraints on cognition.

Social Characteristics

Lizard social systems can be viewed as components of three broad categories: social organization, social structure and mating systems (Whiting and While, 2017). The majority of what we know about sociality has come from studies of social structure and mating systems (e.g. on aggression, dominance and territoriality), largely because many species are easy to observe and frequently interact. Furthermore, dyadic contests can easily be staged in the lab. Relative dominance is thought to influence information use. For example, subadult but not adult male eastern water skinks (*Eulamprus quoyii*) use social information to solve a task, and although this was hypothesized to be the result of social feedback (Noble *et al.*, 2014), subsequent tests of this hypothesis found no effect of dominance on social learning ability (Kar *et al.*, 2017). Some species of lizards also live in family groups, and this system of complex sociality offers a unique opportunity for understanding how complex sociality may influence cognition. Social learning, for instance, has been documented in female adult tree skinks (*Egernia*

striolata; Whiting *et al*., in review), and studies on related species with different levels of sociality are currently underway in our lab.

State of the Art

Cognitive studies using lizards have experienced a resurgence over the last 10 years, owing to the need for a greater understanding of the degree of behavioural flexibility and underlying cognitive mechanisms across taxonomically diverse groups (Wilkinson and Huber, 2012). Despite this, studies using lizards are still relatively rare compared to work with mammals and birds, and mainly attempt to address three major types of questions: (1) Can lizards learn novel cognitive tasks? (2) What is the diversity of cognitive tasks that can be undertaken and the mechanisms through which individuals learn? (3) What drives variability in learning among individuals, populations and species? Lizard cognition studies have applied both well-established protocols, developed in the bird and mammal literature (e.g. radial arm, Barnes and T-mazes: Mueller-Paul *et al.*, 2012; Bezzina *et al.*, 2014; LaDage *et al.*, 2017), or have adopted modified and/or species-specific operant or associative learning tasks, which attempt to quantify learning under more ecologically relevant contexts for a given species (e.g. spatial associative learning: Noble *et al.*, 2012; Carazo *et al.*, 2014; Dayananda and Webb, 2017). Much of this work has focused on quantifying cognitive abilities of species through changes in behaviour (e.g. proportion of incorrect choices, latency, etc.); however, a number of studies have quantified brain structure across individuals, populations or species, as a more indirect measure of cognitive capacity (Day *et al.*, 2001; Striedter, 2015; LaDage *et al.*, 2016; Hoops *et al.*, 2017).

Despite the different approaches across studies, much of the early lizard cognition research has been focused on characterizing the extent and variability of learning in lizards (Alkov and Crawford, 1965; Krekorian *et al.*, 1968; Davidson and Richardson, 1970; Powell and Peck, 1970; Garzanit and Richardson, 1974; Loop, 1976; Kirkish *et al.*, 1979; Wilkinson and Huber, 2012). This important research continues today, owing to the difficulty in quantifying, and the general lack of understanding of, lizard cognitive ability across species (Manrod *et al.*, 2008; Gaalema, 2011; LaDage *et al.*, 2012; Leal and Powell, 2012; Noble *et al.*, 2012). Until recently, lizards (and reptiles more generally) were considered to have limited capacity to learn novel problems (LaDage *et al.*, 2012; Leal and Powell, 2012; Noble *et al.*, 2012; Kis *et al.*, 2015). However, this has largely stemmed from the application of inadequate cognitive tasks, contrived situations or a poor understanding of the ecology for a given species. For example, it is not always clear what a useful reward-based stimulus is for ectotherms – one that both stimulates learning in standardized tasks and maintains motivation throughout a task. Indeed, these factors can be dependent on the test temperatures (Krekorian *et al.*, 1968). For food-based rewards, this can also depend on a species' foraging mode, with active foragers often responding more strongly to food rewards and more readily removing a lid from a dish than an ambush forager. Furthermore, active foragers will use prey/food chemicals to detect food rewards hidden from view, so that chemical cues need to be controlled for in an experiment. Nevertheless, many lizards will simply use their snout to manipulate a

simple task, such as a lid covering a well (Leal and Powell, 2012; Clark *et al.*, 2014; Noble *et al.*, 2014) or even a sliding door (Kis *et al.*, 2015). Lack of learning may also be related to the artificial nature or ecological relevance of a task (Day *et al.*, 1999a; Noble *et al.*, 2012). Indeed, many preconceived ideas, such as the limited use of social learning in lizards due to their 'non-social' nature, have recently been shown to be wrong (Noble *et al.*, 2014; Kis *et al.*, 2015; Pérez-Cembranos and Pérez-Mellado, 2015). When considering the ectothermic nature, ecology and motivational differences among species, tasks that attempt to quantify cognitive ability can be more easily designed. Researchers can then begin to address the mechanistic processes underlying learning and memory, the drivers of cognitive differences among individuals and their relationship to fitness.

Mechanistically, lizards have been shown to use a variety of visual cues when learning and navigating environments, with most studies testing animals under a spatial learning paradigm (Day *et al.*, 2003; Paulissen, 2008, 2014; Leal and Powell, 2012; Wilkinson and Huber, 2012; Carazo *et al.*, 2014; Clark *et al.*, 2014; Riley *et al.*, 2017). Selection on spatial learning is thought to be strong in lizards because of the need to locate food or prey patches, safe refuges or mates. Compared to sit-and-wait foragers that are mostly immobile, active foragers move through the landscape in search of food and are required to have knowledge of key resources over a potentially greater area, and should thus perform better in spatial tasks. This was tested in two closely related species: *Acanthodactylus boskianus*, an active forager, and *A. scutellatus*, an ambush forager (Day *et al.*, 1999a). Surprisingly, there was no difference between the two species in their performance on a spatial memory task. However, the active forager performed better in a visual discrimination task, which was a non-spatial task reversal. This result was counter to the predicted spatial adaptation model for vertebrates and led to the development of the pliancy model: active foragers experience selection for behavioural flexibility, which favours complex associations between unpredictably changing stimuli and reinforcers (Day *et al.*, 1999a). Interestingly, when the authors examined the brains of these two species, they found a larger dorsal (DC) and medial cortex (MC), two putative reptilian hippocampal homologues, in the active forager. This is consistent with the prediction for selection on brain regions in relation to demand, but was not borne out in the spatial learning trials (Day *et al.*, 1999b).

In many lizard species, males may defend territories or adopt alternate reproductive tactics, whereby they search for females and sneak copulations (Noble *et al.*, 2013). This is thought to create a greater selective pressure on spatial memory in males than females. Indeed, in the eastern water skink (*Eulamprus quoyii*), males performed better than females in a spatial task (Carazo *et al.*, 2014). In the side-blotched lizard (*Uta stansburiana*), the size of the DC and MC is correlated with spatial demands, such that strongly territorial male morphs have the largest DC and MC, followed by those that defend small territories, and finally, individuals that are floaters and do not defend territories (LaDage *et al.*, 2009). Side-blotched lizards have also been used to confirm the existence of spatial memory, using a Barnes maze, a common test for mammals (LaDage *et al.*, 2012). Finally, the size of the MC and DC are strongly dependent on the environment in which the animals develop, and their development is constrained by simplified environments, such as those in a captive setting (LaDage *et al.*, 2016).

Interestingly, territorial males in *Uta stansburiana* appear to be more sensitive to changes in their environment and experience greater levels of neurogenesis when shifting to more complex environments, highlighting the importance of the environment for brain development (LaDage *et al*., 2013, 2016).

Behavioural flexibility (i.e. the ability of animals to solve novel problems or use different methods to solve the same problem) was recently demonstrated in lizards, which solved multiple tests across different cognitive domains, including reversal learning (Leal and Powell, 2012). Since then, other studies have commonly demonstrated reversal learning and an ability to solve a multitude of tasks, although they might not specifically reference behavioural flexibility. We are currently using a set-shifting paradigm to test for behavioural flexibility in the *Egernia* group of lizards, which have a wide range of sociality (solitary to family living) and mating systems (monogamy to promiscuity). Set-shifting, or task-switching, is an executive function and a form of cognitive flexibility that involves the ability to shift attention between one task and another, and has been successfully used across taxa (e. g. Roberts *et al.*, 1988; Birrell and Brown, 2000; Colacicco *et al.*, 2002; Weed *et al.*, 2008; Titulaer *et al.*, 2012). One constraint for lizard studies is the length of time required to conduct set-shifting experiments, because lizards are hard-pressed to do more than two trials a day because of their energetic needs. Nevertheless, these experiments are likely to be highly informative and will allow a comparison across a diversity of taxa.

Lizards are also an ideal model to study the role of cognitive processes and brain structure in fitness, and whether these traits are heritable (Croston *et al.*, 2015), because they typically show a range of variation necessary for natural selection, and they can be brought into the lab for measurement of cognitive traits and then released back into a population in which they can be tracked. Unfortunately, we are only aware of one study that has explicitly linked cognitive performance to fitness in the wild (Dayananda and Webb, 2017). In this study, velvet gecko (*Amalosia lesueurii*) eggs were incubated at hot or cold temperatures, and offspring from the colder temperature had higher learning scores and higher survival in the wild.

All for One and One for All

Box 12.1 Snake Cognitive Abilities

Michael S. Grace

The cognitive abilities of snakes are poorly understood relative to those of other reptiles, in part because snake anatomical and physiological adaptations may be incompatible with some of the traditional methods used to study cognition in other reptilian and vertebrate taxa. However, these same adaptations (including limblessness, poor visual accommodative ability, lack of eyelids and external ear openings and, in many snake species, the infrequent consumption of massive meals) make snakes fascinating subjects for the study of sensory biology, behaviour and evolution.

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Early studies of snake cognition yielded mixed results. Takemasa and Nakamura (1935) found that snakes could learn to efficiently escape confinement, and Kellogg and Pomeroy (1936) found that watersnakes (*Nerodia fasciata*) demonstrated learning capability in a water maze using heat as a positive reinforcer. Conversely, Wolfe and Browne (1940) saw no evidence of diamondback watersnake (*Nerodia rhombifera*) learning in a terrestrial environment, using potentially noxious heat as a goal or electric shock as an aversive stimulus. Crawford and Bartlett (1966) found no evidence that grey ratsnakes (*Pantherophis obsoletus*) learn to locate food and water following 7 days of food and water deprivation. The methods used in many of these studies suggest the importance of employing experimental paradigms and conditioning stimuli that are appropriate to snake physiology and behaviour. Thermal targets beyond the preferred temperature range of a given species likely make poor goals; aquatic and semi-aquatic snakes may perform more poorly in terrestrial versus aquatic set-ups; and short-term food and water deprivation may have little motivating effect in snakes that efficiently conserve water and may go months without eating.

However, more recent studies employing conditions and testing paradigms more appropriate to snake physiology and behaviour clearly demonstrate that snakes have the ability to learn, and in some cases to perform complex series of novel tasks. Plains garter snakes (*Thamnophis sirtalis*), for example, used novel airborne odorants as cues to learn to navigate a simple maze, in order to gain access to food rewards (Begun *et al.*, 1988). Corn snakes (red ratsnakes, *Pantherophis guttata*), like many snake species, actively seek out shelter in the form of holes and crevices. Holtzman and colleagues (1999) took advantage of this behaviour by setting up a spatial learning arena, in which one of eight hide holes was open and thus available as a retreat. These snakes rapidly learned to navigate the arena, significantly decreasing the latency to find the goal and the distance travelled to reach the goal over the course of only 4 days of trials. In the most complex behavioural challenge yet, Emer and colleagues (2015) trained wild-caught Burmese pythons to accept much smaller food items than would normally be consumed and then used very small food rewards to condition snakes to reliably depress an illuminated button in order to gain access to hidden food rewards. These studies demonstrate that snakes can learn even unnatural and somewhat complex tasks. Taken together, they show that in order for learning to be demonstrable, environmental conditions, tasks and rewards must be appropriate to snake anatomy, physiology and behaviour.

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Field Guide

Experimental Set-up and Practical Considerations

A major challenge in cognition studies is ensuring rigorous experimental procedures in the face of logistical constraints. In a captive setting, many cognition tests are food-based, and lizards anticipate a feeding opportunity. While there are no data on lizards that suggest a 'clever Hans' effect, whereby the experimenter inadvertently cues the animal to the reward, it is best practice to eliminate this possibility. Ideally, one experimenter would set up the apparatus and another would place it in the animal's enclosure or testing arena ('blind experimentation'). If this is not possible, a simple alternative is to either usher a lizard into its refuge, and then place the apparatus in the enclosure (with trials beginning when the lizard emerges), or have a separate chamber connected to the animal's enclosure, which can be opened once the apparatus has been inserted. Both alternatives have the advantage of starting the animal at a constant distance from the experimental apparatus.

For all cognition tests in the lab, we advocate using a camera system (e.g. CCTV) to remotely record the animal's behaviour and performance in all trials (Figure 12.1). By doing so, lizards are not distracted or influenced by the presence of an investigator. In field studies, depending on the circumstances, this may not always be possible. If this is the case, the investigators should first habituate animals to their presence, wear bland clothing and move as little as possible during a trial. If possible, we suggest using a camera that links to a remote monitor and thereby removes the experimenter. In the event that an investigator is present, it may be best not to include measures of latency, in case it is influenced by the experimenter's presence. Video scoring usually involves collecting data on the choices (i.e. whether the lizard chooses correctly/number of incorrect choices or mistakes) and the latency to choose (i.e. the time from the start of the trial to the execution of the task). Videos should be scored blind when possible

Figure 12.1. A typical set-up for cognitive studies, in which lizards perform trials in their home enclosures in plastic tubs. CCTV cameras are mounted on bars to the side of each rack (between two lights), allowing the filming of trials (multiple tubs can be filmed by one camera). We create a thermal gradient using heat tape below the tubs (not pictured). Additional warmth is provided by a spot lamp, which also illuminates the enclosure for CCTV filming. For husbandry purposes, lights and heat cables can be connected to a timer and turned off at night. Each camera connects to a DVR and a monitor for remote viewing. At the end of each trial or at the end of the day, all trials can be backed up to an external USB drive that connects to the DVR. More sophisticated systems allow a connection via Wi-Fi and remote back-ups.

('blind scoring'), even if 'choice' for many tasks is rather unambiguous (e.g. removing a lid from a well), or at least partially recoded by a second observer.

Two well-known constraints in cognition studies are colour preference and lateralization, which may result in a side bias. For example, lizards may have a preference for red, because it is the colour of ripe fruit (Whiting and Greeff, 1997). This is not necessarily a problem if the question of interest is a comparison across treatments (e.g. social learners versus a control with the same stimulus). However, it is always better to split the treatment group in half and use two different colours. Another option is to test lizards a priori for a colour preference, by placing equal amounts of food in two coloured dishes and presenting them over a set number of trials (e.g. 20), alternating between left and right sides. As lizards are tetrachromats with good visual acuity and can cue in on either luminance or hue, it is important to use the same source of paint to ensure that colour cue cards are identical, manipulating hue and luminance with an optic spectrophotometer, if needed (Kemp *et al*., 2015). Because lateralization may be common in lizards, it is possible that some species or individuals may have a side bias. This can be tested before the start of any cognitive trial by presenting two equal dishes/food rewards on the left and right side of the test arena. Depending on the proportion of individuals with evidence of a side bias, we see three viable options. First, if the proportion of animals is small (e.g. < 5–10 per cent), these animals could be excluded and a clear statement about this made within the study. Second, if the proportion of animals is moderate (~50 per cent), then this could be dealt with in the analysis stages: assuming sufficient numbers are present across treatments, individuals

could be dummy-coded ('1' – left bias; '0' – right bias; see below). Lastly, if a high proportion of animals exhibit a side bias, they could first be trained to learn the reward from the opposite of their preferred side. However, care should be taken when interpreting these data, and it should also be made explicit that such biases exist. This will also affect the interpretation of any reversal tasks that should also be discussed. Indeed, it may be the case that the real cognitive question involves understanding laterality itself, if the proportion of individuals with a side bias is high (i.e. decide a priori the proportion of animals with a particular side bias that is acceptable).

Quantifying Learning

Learning in lizards is usually quantified by testing for a change in behaviour across time, in response to an unconditioned stimulus. Ideally, data should be collected consistently during the learning period on all lizards and within each treatment. However, this may be logistically challenging, and one may need to either compare groups of individuals through time (i.e. across every trial) and estimate their learning rates, or to compare individuals exposed to different stimuli at two time points. Our experiments, for example, usually take 2–3 months to complete, and one experiment took 9 months (Szabo *et al.*, unpubl. data). Our tasks generally involve running trials in the morning and afternoon (i.e. twice per day, 10/week). We suggest running trials in 5-day blocks and scoring videos on the same day, once trials are complete, to avoid trial 'fatigue' and also because lizards are ectotherms, and thus have lower food intake requirements.

For studies of social learning, treatments often involve quantifying the change in behaviour for a group of individuals that are able to observe or interact with conspecifics, as compared to a control group of experimental lizards that do not observe conspecifics performing the tasks (Noble *et al.*, 2014; Kis *et al.*, 2015; Pérez-Cembranos and Pérez-Mellado, 2015; Kar *et al.*, 2017). The control group is essential in disassociating any effects of social feedback on learning (Noble *et al.*, 2014; Kar *et al.*, 2017). Depending on the question of interest, one can allow observers or experimental animals to interact with conspecifics (i.e. obtain visual, chemosensory and social feedback), or restrict experimental lizards to particular sensory modalities (e.g. visual or chemosensory only). Depending on the experimental design chosen, any inferences drawn from such studies need to be related to the specific sensory domain being tested. However, practically speaking, it will almost always be necessary to separate demonstrating and experimental lizards. As lizards are extremely visual, this can be done using video stimuli or trained demonstrators that are viewed behind a transparent divider (Noble *et al.*, 2014; Kis *et al.*, 2015; Pérez-Cembranos and Pérez-Mellado, 2015; Kar *et al.*, 2017; Figure 12.2). For example, in the bearded dragon (*Pogona vitticeps*), lizards observed a video demonstrating the bidirectional opening of a sliding door (opening either left or right, but not both ways) or a control video consisting of a blank screen. This is a highly effective design, because it tests for imitation and excludes the possibility of goal emulation or local enhancement (Kis *et al.*, 2015).

The incubation environment also has major effects on reptile learning and other cognitive traits (Amiel and Shine, 2012; Amiel *et al.*, 2014, 2017; Clark *et al.*, 2014;

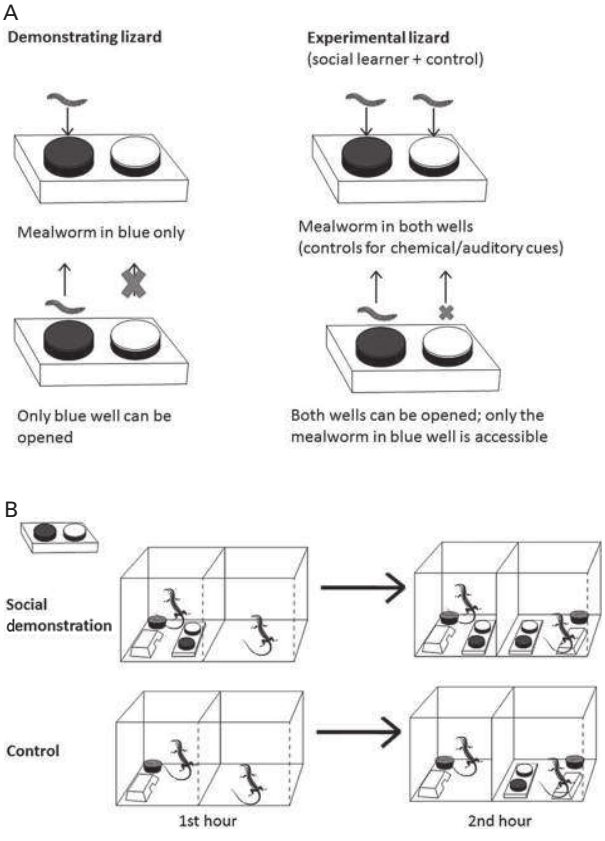

Figure 12.2. Social learning experimental set-up, involving a demonstrator with a social learning treatment compared to a control. (A) The reward (in this case a mealworm) is only available to the demonstrator in the correct well. The lid on the incorrect well is sealed to the well. In the treatment and control groups, the reward is in both wells; however, while both lids can be removed, the reward in the incorrect well is covered by mesh and not accessible to the lizard. (B) The first hour is an observational period, during which the 'learner' watches a demonstrator extract a food reward from the correct dish, while the control 'learner' observes another lizard in the absence of the task. In the second hour, an opaque divider is inserted and the focal lizards are given the task. We suggest two trials per day with at least 1.5 h between trials. For more details, see Noble and colleagues (2014).

Dayananda and Webb, 2017) and is likely a key source of environmental variation driving cognitive differences among individuals (Noble *et al.*, 2018). Comparing learnt behaviour for multiple tasks can be done for multiple treatment groups (i.e. different incubation temperatures: 20°C versus 27°C; social environments: small versus large social groups) to ascertain how the developmental environment impacts cognitive ability. There are now excellent protocols developed for combining an understanding of changes in brain structure (e.g. Herculano-Houzel *et al.*, 2015; Hoops, 2015), with detailed behavioural experiments for lizards that are likely to pave the way for a more integrative examination of environmental effects on cognition in reptiles.

Practical Considerations for Analysis: Learning Curves, Learning Criteria and Statistical Power

Analysing data collected from learning experiments is by far one of the most challenging aspects of any cognitive experiment, depending on the question at hand, the sample size and the intended power of an experiment (Nakagawa and Foster, 2004). This is especially problematic for most lizard cognition experiments, given that there are often major logistical constraints in measuring large numbers of individuals (Nakagawa and Hauber, 2011). Power analyses prior to experimental design can be conducted using published estimates taken from similar studies in the literature, which can help guide the sample size required while planning an experiment. Moreover, expert advice and pilot studies can inform in this area.

In addition, it is often important to control for covariates such as sex (Carazo *et al.*, 2014), body size (Clark *et al.*, 2014) and temperature (Krekorian *et al.*, 1968), as these factors can impact learning. Experimental designs should carefully balance these factors, and we suggest pseudo-randomizing levels of a covariate to each treatment in as balanced a manner as possible. For example, if both male and female lizards will be collected for a social learning experiment, then it is important to balance the sex of the observers across control and social learning treatments as evenly as possible, but also in a randomized fashion. This ensures that sufficient sample sizes are present in each treatment to test for sex effects and that covariates are not confounded by treatment effects. Confounding effects can be problematic for experiments involving incubation temperature, as sex is often affected by temperature in many lizards (Clark *et al.*, 2014). The need to control for covariates, whether these be biological (sex, size, mass) or methodological (batch effects), requires careful planning during the experimental design stages, but also requires some foresight on how such data will be analysed. The low power of many cognition studies means that simplifying assumptions are often necessary. For example, testing treatment effects on a small sample of lizards, where males and females are likely to differ, means there may be a limited sample size for testing sex effects. In these circumstances, it may be better to increase the sample size of one sex, and only focus on one sex, to ensure there is sufficient power to test a given question (e.g. Noble *et al.*, 2012, 2014).

Data collected on all individuals for every trial, whether the individuals are part of multiple treatments or not, can be analysed in a number of different ways. Often, it is useful to organize these data in an easy to view format, which does not necessarily match formats needed for analysis. We provide a link with an example file of how we format our data while running experiments, and we have also written some simple functions (should this format be followed) that can then convert these files to analysis-ready format, saving a substantial amount of time. This can be downloaded (https://github.com/daniel1noble/cogdat) as a package that can be used in the free analysis software package R (R Core Team, 2017).

Theoretically, all these different analyses should support one another, but this will not always be the case, given the different assumptions inherent to the alternative statistical tests and their varying levels of precision. Therefore, care should be taken in establishing whether experimentalists have sufficient data for a given type of analysis, and

Table 12.1. Basic equipment used in the study of lizard cognition.

Equipment	Function
DVR and camera system	Remote filming of trials. Compared to stand-alone cameras, allows monitoring of many animals simultaneously
External hard drives	Used for daily backup from DVR
Camera and tripod	Used for field studies and lab studies requiring higher definition
Plastic tubs or reptile cages	Lizards can be maintained in large plastic tubs while doing trials, open top. Lizards need a refuge and water bowl, can be given objects for enrichment (e.g. bark, leaves, twigs)
Outdoor enclosures	Lizards can be maintained in outdoor enclosures, if species perform better in a more natural environment
Heating, lighting	Under-cage heating wire creates a thermal gradient. A spot lamp can be provided for basking in some species. UV lamps may be necessary for longer studies
Stimulus reward	Depending on species: mealworms, crickets, dog food or baby food (fruit). Wet dog food and baby food can be measured more accurately for quantity
Spray paint, plastic wells, black tape, fine mesh, wooden platforms, putty	For association trials, we use small Petri dishes and reduce the lip of the lid, to allow easier removal by the lizard. Dishes can be mounted on a wooden platform with putty, to allow easy removal. Lids are spray-painted and sides of dishes taped to render them opaque. Avoid painting lids red, because lizards have a strong preference for red. Mesh is used in control well and prevents lizards accessing food (controls for chemical cues). For some species, dishes can be left open, but placed atop a wooden ramp with a cue card
Wood, plastic, Perspex, spray paint, standard-sized cue cards	General materials necessary for constructing a maze, puzzle box, cognitive apparatus, set-shifting set-up

general power analyses can be used to assess this. Analyses largely fall into three different types that statistically compare: (1) group- or treatment-level differences in means, trials to criterion or latencies, preferably at two time points; (2) group- or treatment-level learning or survival curves (survival analysis); or (3) learning curves at both the within- and between-individual levels for different groups or treatments (multilevel, mixed models or generalized estimating equations).

Analyses often take means of a given statistic for a set of independent individuals (e.g. 'average' number of incorrect choices made, average number of trials to criterion for an individual). The binomial probability or simulations can be used to assess the probability of obtaining specific outcomes by chance, when testing learning. Individual choice data and latencies across trials can also be analysed, using powerful mixed-modelling or survival analysis approaches (Gallistel *et al.*, 2004; Noble *et al.*, 2012, 2014; Kar *et al.*, 2017; Riley *et al.*, 2017). These can explicitly account for within- and between-level variability in learning rates and make use of the maximal amount of data from a given experiment (Figure 12.3). Choice data can often be modelled assuming a

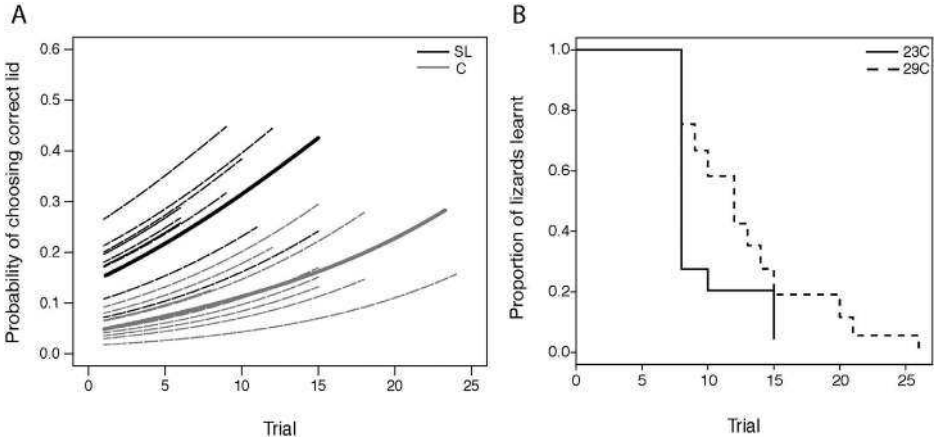

Figure 12.3. Example of presenting figures for cognitive analysis. (A) Modified from Noble and colleagues (2014) with kind permission of the Royal Society, an example of individual based-learning curves for two treatments (control versus social), for a social learning experiment on *E. quoyii*, which provides evidence for age-dependent social learning. Small solid lines are predictions of individual learning 'rates', whereas solid lines are population-average lines for the two treatment groups. (B) An example of a survival analysis for two groups of lizards, using time to event (in this case, time to learn or reach criterion).

Bernoulli or multinomial probability distribution, and, after log-transformation, latency data can often be modelled assuming normality. While there is some debate about whether learning curves are gradual or step-like (Gallistel *et al.*, 2004), we believe that this depends on the variable of interest and the type of task. In our experience, assuming fairly standard error distributions common to many statistical software packages is usually sufficient. Nonetheless, these models are data-hungry and rely on a sufficiently large number of independent animals for models to converge properly, so this should be considered in their use. In Resources, we further provide the link to some simulated data and analysis code that can be downloaded for learning.

Wild vs. Lab

Box 12.2 Cognition Outside the Box

David S. Steinberg and Manuel Leal

As referenced in the current chapter, the unique metabolic requirements and relatively low activity rates of ectotherms impose serious logistical constraints that often hamper laboratory studies of lizard cognition, by limiting sample size and the power of statistical analyses (Burghardt, 1977). These traits also often preclude the use of traditional cognitive paradigms (but see Leal and Powell, 2012), which have been developed largely for organisms with high activity rates. One way to alleviate some of the problems associated with lab-based research (see Shettleworth, 1998; Morand-

(*cont.*)

Ferron *et al*., 2015) might be to investigate the cognitive abilities of lizards in nature. By studying free-ranging lizards in the wild, scientists can potentially evaluate many more individuals, without having to provide adequate or appropriate artificial testing conditions (e.g. in terms of space, temperature and humidity). Instead, researchers must simply transport a testing apparatus from individual to individual in their natural habitat. The most obvious and serious obstacle that must be overcome in this sort of field-based research of cognition is reliably relocating focal subjects over an extended period of time, because most studies of cognition are both long-term and serial. Studying species that are fiercely territorial or exhibit considerable site fidelity, which is common in lizards, can alleviate this problem. Alternatively, modern tracking technologies (e.g. Croston *et al*., 2016; Morand-Ferron *et al*., 2015), including satellite-based or radiotelemetry tags, might be useful in finding lizards that are non-territorial, wide-ranging, or simply cryptic and difficult to spot.

Field-based research of cognition in lizards, as well as in other taxa, has many benefits beyond simply avoiding the common husbandry-related drawbacks of laboratory investigations. First, studies of cognition in natural contexts avoid the need to place subjects in artificial and alien environments, which might generate stress responses that mask, alter or in any way distort cognitive function (Shettleworth, 1998; Toxopeus *et al*., 2005; Jacobs and Menzel, 2014). Although the exact effects of displacement to a laboratory setting on cognition in lizards are unknown, behaviour in general and cognitive functions in particular are strongly influenced by external stimuli. Second, conducting an experiment within the natural habitat of a species requires the experimenter to consider the natural history of the organism in question (Leal and Losos, 2015), thereby allowing the experimenter to design the most appropriate tests or tasks for a given species. Furthermore, these experiments provide an opportunity to measure cognition under the conditions where the costs and benefits of these traits are normally experienced. Finally, studying individuals in the field can prevent the repeated use of the same individuals in multiple cognitive experiments. The repeated use of subjects is a problem common to many studies of cognition in vertebrates and assumes that experimental experience will not bias future experiments. Such an assumption is likely to be unfounded.

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Resources

- Instructional video and PDF published by the *Journal of Visualized Experiments* on how to assess spatial learning and memory in small squamate (snake and lizard) reptiles, using a dry land Barnes maze (LaDage *et al.*, 2017). An empirical study by the authors was separately published (LaDage *et al.*, 2012): www.jove.com/video/55103/assessing-spatial-learning-and-memory-in-small-squamate-reptiles
- Video showing a social learning experimental set-up in the eastern water skink (*Eulamprus quoyii*): the lizard makes an incorrect choice and is not able to access the food reward (Noble *et al.*, 2014).
- Example data sheet for running cognitive experiments, and functions for helping reformat cognition data sheets to analysis-ready format: https://github.com/daniel1noble/cogdat
- Analysis code for running mixed models with individual choice data: https://github.com/daniel1noble/cogAnalysis

Profile

As a behavioural ecologist, Martin has been increasingly drawn to the influence of cognition on lizard behaviour and its potential role in fitness. He is particularly interested in links between sociality, social complexity, cognition and brain size. He is also interested in how a lizard's developmental environment, which is so dependent on environmental conditions such as temperature, can affect a lizard's learning ability and ultimately, survival and fitness.

Daniel is a behavioural ecologist interested in understanding how behavioural traits evolve and are shaped by the environment. He moved to Australia to do his PhD on the charismatic water skinks. They turned out to be an excellent model species for exploring

the capacity of lizards to solve novel problems. Since then, Daniel has worked closely with Martin to explore behavioural flexibility across different lizard species, in an attempt to understand how the environment shapes variation in cognition.

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