

# Predators in training: operant conditioning of novel behavior in wild Burmese pythons (*Python molurus bivitattus*)

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**Abstract** Large pythons and boas comprise a group of animals whose anatomy and physiology are very different from traditional mammalian, avian and other reptilian models typically used in operant conditioning. In the current study, investigators used a modified shaping procedure involving successive approximations to train wild Burmese pythons (*Python molurus bivitattus*) to approach and depress an illuminated push button in order to gain access to a food reward. Results show that these large, wild snakes can be trained to accept extremely small food items, associate a stimulus with such rewards via operant conditioning and perform a contingent operant response to gain access to a food reward. The shaping procedure produced robust responses and provides a mechanism for investigating complex behavioral phenomena in massive snakes that are rarely studied in learning research.

**Keywords** Python · Snake · Reptile · Predator · Behavior · Shaping · Operant conditioning

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## Introduction

Pythons and boas are among the most evolutionarily ancestral snakes (Kluge 1991, 1993; Noonan and Chipindale 2006; Rawlings et al. 2008), and development of an effective operant conditioning paradigm is fundamentally important for experimental analysis of complex behaviors in these apex predators. To date, however, few studies have included large snakes due to ontogenetic and logistical concerns including the space, dietary and thermal needs required to maintain experimental animals, and the fact that they are ambush predators and therefore often sedentary. The work described here demonstrates the feasibility of operant conditioning as an approach to study sensory, neurophysiological and behavioral processes in large snakes.

Snakes possess a variety of highly specialized adaptations that make them considerably distinct from most other vertebrate animals. These predatory animals have a highly elongated and limbless body, and lack external ears (Hartline and Campbell 1969), eyelids and the conventional vertebrate mechanism of accommodation (i.e., visual focus) (Walls 1940). Such anatomical and physiological adaptations make snakes fascinating models for the study of vertebrate evolution, behavior, sensory biology, physiology and memory. Unfortunately, however, operant learning studies have rarely been done using snakes, possibly because many snakes intermittently consume large meals followed by long durations of inactivity (Murphy and Henderson 1997; Wilson 2007; Secor 2008), and because responses of snakes in learning studies have been inconsistent (see below). However, the use of snakes as a platform to investigate behavioral phenomena can be realized with minimal modification of traditional operant conditioning protocols. Pythons use vomerolfactory (Halpern

1992), nasolfactory (Halpern 1992), visual (Walls 1942) and thermal stimuli to effectively localize and acquire prey (Noble and Schmidt 1937), so we proposed that the development of operant conditioning paradigms should be effective in psychophysical tests of behavioral sensitivity to these types of environmental stimuli. We describe the design of an operant training apparatus and report the results obtained following discrimination training of wild Burmese pythons.

Investigators studying learning and motivation in snakes have used many of the same techniques applied to other vertebrate animals, but results have been mixed. Takemasa and Nakamura (1935) demonstrated that snakes could learn to escape from confinement in a box. This early learning model evolved as investigators utilized reinforcement and discriminative stimuli to shape maze learning. Kellogg and Pomeroy (1936) found that snakes in a T-maze filled with cold water made fewer errors in finding a warm-water goal after repeated trials in the maze. On the other hand, Wolfe and Browne (1940) found no evidence of learning when they attempted to train snakes to avoid noxious heat or electric shock. Using positive reinforcement, Crawford and Bartlett (1966) failed to demonstrate decreased latencies to a goal over successive trials. These latter two studies demonstrate the failure of the equipotentiality premise (i.e., not all species can be equally conditioned to the same types of stimuli with the same behavioral response and/or same reinforcement contingencies). Thus, effective conditioning requires a conditioning protocol with sensory stimulus, conditioned response and reinforcement that are suitable for that the species in question. Indeed, several more recent studies indicate that conditioning in snakes is possible under appropriate conditions.

When shaped to contact a wire or a key with their snout to access drinking water, indigo snakes (*Drymarchon couperi*) exhibited response rates comparable to lever pressing by mammals or disk pecking by birds (see Kleinginna 1970). Researchers investigating the olfactory system of the garter snake used pieces of earthworm to reinforce maze learning (Kubie and Halpern 1975; Begun et al. 1988). More recently, Holtzman et al. (1999) demonstrated escape-motivated spatial learning in corn snakes (*Elaphe guttata guttata*), and Almli and Burghardt (2006) demonstrated enhanced problem-solving ability in rat snakes (*Elaphe obsoleta*) in environmentally enriched conditions. Perhaps, the only reported study that used mammalian prey to reinforce a conditioned response was conducted with Montpellier snakes (*Malpolon monspesulanus*, Gavish 1979); here, snakes were said to exhibit decreased strike latencies following illumination of a light within the apparatus, which signaled mouse availability, but no quantitative data were presented.

Nevertheless, snakes in general and large pythons in particular pose specific challenges for the development of effective operant conditioning paradigms. For example, reinforcement may be problematic. While water was used as a reinforcer in one snake study (Kleinginna 1970), snakes generally are highly efficient at water conservation (Gans et al. 1968; Zug 1993; McNab 2002). Because Burmese pythons grow to be very massive, they may be able to withstand very long periods of time without water. Thus, water access may be a poor means of reinforcement.

Furthermore, pythons and boas are often massive animals that are highly effective predators of live homeothermic prey (i.e., mammals and birds). Because of their size, pythons generally consume correspondingly large food items, likely because the energy gain from small prey items is minimal considering the energy required for capture, ingestion and digestion (see Arnold 1993 for a review). Consumption of large food items corresponds with long durations of time between meals—sometimes months (Murphy and Henderson 1997; Wilson 2007; Secor 2008). Thus, it seems that food may be an ineffective positive reinforcer, because feeding on a normal regimen may lead to satiation for long periods of time—too long, perhaps, for conditioning of behavior to be possible. In fact, the diets of wild pythons in south Florida are comprised primarily of large prey, such as raccoons, rabbits, cats, squirrels, large rats and even aquatic birds (Snow et al. 2007). Nevertheless, recent analysis of gut contents from adult Burmese pythons caught in the Everglades indicated that some snakes ingest multiple, relatively small prey items over short periods of time (K. Krysko, personal communication).

Our study was designed to determine (1) whether a robust, effective response within the physical capabilities of Burmese pythons could be identified and shaped (Gould and Gould 1994), (2) whether a physiologically relevant reinforcer could be established and (3) if so, whether very small units of the reinforcer could be used to maintain response levels while concurrently controlling for *modus operandi* (MO) effects. As we report here, gradual modifications of the natural python feeding biology were used to establish patterns of response to reinforcement that are similar to those used in operant-based experiments in other animals, in order to shape an approach and touch response in large, wild snakes.

## Materials and methods

### Subjects

One captive-bred juvenile Burmese python (*Python molurus bivittatus*, subject S75) was used to establish reinforcement procedures for use in larger snakes, which included one captive-bred subadult (subject SA) and four

wild, subadult Burmese pythons (subjects S4, S6, S11 and S12) collected in Everglades National Park (pythons reach sexual maturity at a length of 2.6 m (Pope 1961). The snakes were weighed and measured upon arrival (Table 1) and individually housed in a 25 °C temperature-controlled room on a 12:12 LD light cycle. Water was provided ad libitum in a dish, and after four consecutive once-monthly feedings, the pythons were deprived of food for 2 months prior to the start of experimental trials. The number of trials per experimental session was dependent upon food size and the food intake limit of the maintenance-level diet. That is, food intake during the trials was restricted to provide a maintenance level equal to 7 % of individual body weight per week (Gavish 1979). Food was provided only as reinforcement during conditioning sessions (i.e., no food was available outside of test sessions). Animals were maintained according the Florida Institute of Technology Animal Care and Use Committee (Protocol #070801) and the State of Florida (Permit EXOT-11-79) and remained in captivity for future research.

### Apparatus

During feeding trials using the captive-hatched juvenile python (S75), the snake was relocated from its home container to the experimental apparatus, a custom-built Plexiglas® box (61 cm × 32 cm × 43 cm) within which external visual and thermal stimuli were minimized using black rigid-foam insulation. A small camera (Dynex™ 1.3MP Webcam) connected to a laptop computer permitted observations by the investigator. A shallow food reservoir (5 cm × 5 cm × 2 cm) with an external door allowed for manual addition of food.

During feeding trials with subadult pythons, snakes were transferred to a larger version of the apparatus described above (61 cm × 46 cm × 91 cm, Fig. 1), and a door between the snake and a larger food reservoir (7 cm × 7 cm × 7 cm) was controlled remotely by the experimenter. An illuminating push-button microswitch and a tactile position cue (AstroTurf®) were later added to the larger apparatus used by the subadult pythons (see Fig. 1).

**Table 1** Python demographics

Subject	Mass (kg)	SVL (m)	TL (m)	Sex
SA	5.2	2.0	2.2	F
S4	4.8	1.6	1.8	F
S6	6.6	2.5	2.8	F
S11	2.6	2.1	2.4	M
S12	2.2	1.3	1.4	F
S75	0.08	0.57	0.63	F

Snout vent length (SVL) and total length (TL) are standard measurements used in sexually dimorphic reptiles including pythons

### Procedures

Because the juvenile python (S75) was used as a pilot study, experimentation for the animal was conducted before experimentation with the larger, subadult pythons. Subject S75 was used to determine whether a python could be trained to take dead prey, whether it would accept very small prey, at what rate prey items (reinforcers) would be accepted, and how long such training would take. Initially, live mice (approximately 5 g) were offered, followed by euthanized neonatal mice (approximately 0.5 g), and finally hemisected neonatal mice. Sessions were terminated after the snake had ingested an amount of prey corresponding to the dietary maintenance level. This snake was then trained sequentially in the test chamber to (1) strike live prey in the food reservoir, (2) retrieve dead prey from the reservoir, (3) approach the closed door of the reservoir in order to have the door opened to gain access to food and finally (4) make contact with the door in order to gain access to the food reservoir. Though the reinforcers used for S75 were weanling mice and the apparatus lacked electronic components (remote-controlled door, illuminating push button), the shaping procedure was similar to that used for the large pythons, described below. Training for large pythons was divided into three phases: pre-training, shaping and discriminated operant training.

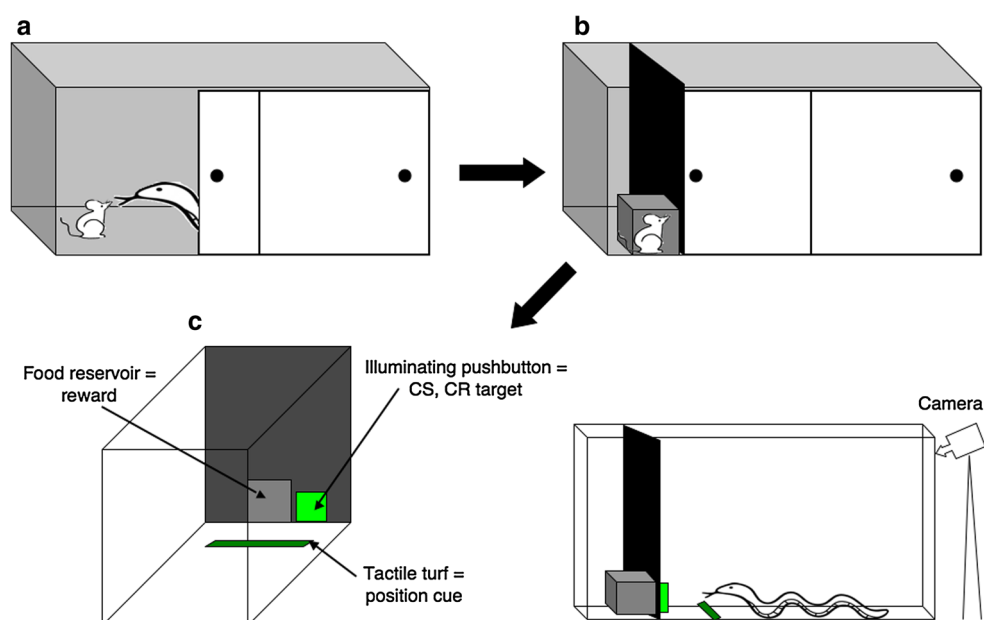
#### Pre-training

Because the subadult pythons were large (i.e., required larger prey) and wild (i.e., inexperienced at feeding in captivity), live, free-roaming adult rats were used during initial weekly sessions. During pre-training sessions with the larger snakes, one live prey item at a time was presented to the snake on the open floor of the test chamber through a side sliding door. Latency to respond with a strike was recorded to the nearest second. When the first prey item was fully ingested, the next prey item was immediately added. Gradually, smaller and smaller rats were used as postcedent stimuli, followed by adult mice. When a snake reliably consumed small prey items, an unrestrained live mouse was presented in the reservoir to familiarize the snake with the location of the food reservoir (i.e., the origin of the food reward). Even when a mouse exited the reservoir and was captured in the area containing the snake, the snake continued to orient toward the food reservoir in subsequent trials.

#### Shaping

The shaping phase of training was conducted after adding a remote-controlled door to the reservoir. A prey item was added to the food reservoir behind the closed door, and

**Fig. 1** Diagram of custom-built operant conditioning apparatus for use with subadult Burmese pythons. Components were gradually added to the early prototype (a) in the following order: food reservoir (b), remote-controlled (RC) door (b), illuminating push button (c), tactile turf (c)



when the snake oriented toward the door, it was opened. The snake then seized the prey and removed it from the reservoir, after which the door was immediately closed. As the snake ingested its prey, another prey item was added to the reservoir behind the closed door. To facilitate ingestion, snakes formed an S-shaped posture with the anterior portion of the body. Thus, latency to approach the door was defined as the time from cessation of the S-posture until re-orientation toward the reservoir door after it had been opened. Live mice were gradually replaced with mice euthanized via CO<sub>2</sub> inhalation. During a given session, four to six trials were conducted, each of which produced a behavioral response. Trials within a session initially alternated between live and euthanized mice in order to firmly establish the relationship between food and reservoir, as well as to establish pre-killed mice as the positive reinforcers of behavior. Euthanized mice were used for subsequent sessions during which snakes were fed progressively smaller prey, until they ultimately consumed a series of euthanized juvenile mice during a session. Thereafter, juvenile mice (approximately 10 g) were used in all trials with large, wild snakes. High-sensitivity infrared videography was used to confirm that the reservoir door blocked thermal cues from the prey item.

#### *Discriminated operant training*

Following sessions during which the snakes reliably consumed six euthanized weanling mice, the number of which varied among individual snakes, an illuminating push button was added. This button served during the discriminated operant training phase as the discriminative

stimulus, which evoked the conditioned response of the snake pushing the button (manipulandum) with its snout to obtain the food reward. When the snake oriented toward the reservoir, the button was illuminated. When the snake contacted the illuminated button (indicated by the “click” of the microswitch), the experimenter raised the door for food access (Online Resource 1). The door was closed once the snake retrieved the food, and as it ingested its prey, another prey item was added to the reservoir behind the closed door. Measured from the moment the snake oriented toward the door, latency to contact the illuminated button was measured for each of a session’s six trials. Each snake was tested every 72 h, except during ecdysis (i.e., shedding of the skin), a process which took, on average, 10 days.

#### *Statistical analysis*

The rank-transformed latencies for S75 to take food from the open reservoir, to approach the door and to make door contact were analyzed for differences using repeated measures analysis of variance (ANOVA) and Tukey’s post hoc analyses. In the subadult pythons (SA, S4, S6, S11 and S12), latencies to take food from the open reservoir, to approach the door and to depress the button (“pre-training,” “shaping” and “discriminated operant training” phases, respectively) were analyzed for differences among training phases and among individual pythons. Repeated measures ANOVA and Tukey’s post hoc analyses were conducted on rank-transformed response latency data using JMP 9 statistical analysis software by SAS.

## Results

Juvenile python S75 reliably switched from larger live mice to smaller dead mice, and the rate at which reinforcers were accepted increased as prey item size decreased (Fig. 2a). The mean number of prey items accepted during a 20-min test session reached four, and the snake accepted as many as six prey items in sequential trials during a single session. The range of means of prey items accepted during individual sessions ( $\pm$ SE) was  $1.9 \pm 0.4$ – $3.9 \pm 0.4$ .

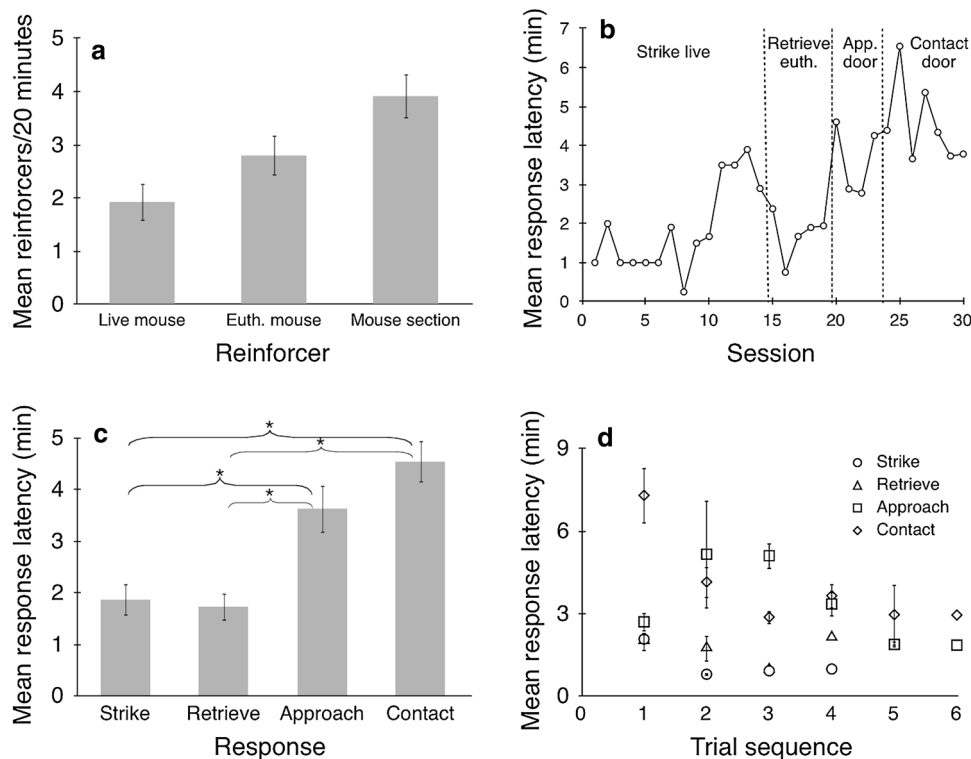
Over the course of 30 test sessions, python S75 underwent shedding twice (between sessions 14 and 15 and sessions 23 and 24) during which time no trials were conducted. Despite the fact that test sessions were normally conducted every second day and the two breaks for shedding lasted 11 days each, S75 resumed its feeding behavior with essentially the same mean response latency as it had in the most recent test session (Fig. 2b), and it furthermore continued accepting multiple small food rewards.

Mean response latency (mean  $\pm$  SE: strike =  $1.9 \pm 0.3$ ; retrieve =  $1.7 \pm 0.3$ ; approach =  $3.6 \pm 0.5$ ; contact =  $4.5 \pm 0.4$  min) increased as tasks became more demanding, (repeated measures ANOVA:  $F_{3, 26} = 11.79$ ,  $P < 0.0001$ ;

Fig. 2c). That is, during the first 19 sessions in which the snake was given access to either live prey items (sessions 1–14) or pre-killed prey items (sessions 15–19), response latency was significantly lower than in later sessions during which the snake was required to approach the reservoir door (sessions 20–23) or make contact with the reservoir door (sessions 24–30) to gain access to the food reward (Fig. 2c).

When response data were analyzed over the course of the trial series for all sessions, no significant change in response latency was observed between the first and last trial within a session, when a session involved only retrieval of food from the open reservoir. However, when the snake was required to make contact with the door to gain food access, the mean response latency across all sessions decreased significantly from the first to the last trial in a series (repeated measures ANOVA:  $F_{3, 11} = 12.68$ ,  $P = 0.0082$ ; Fig. 2d).

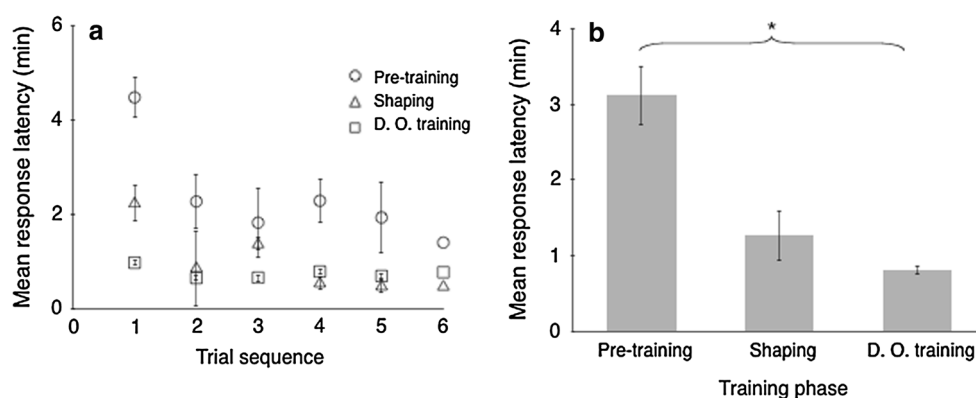
During the first set of pre-training sessions, subadult pythons (SA, S4, S6, S11 and S12) only accepted large, live adult rats ( $\sim 300$ – $600$  g), and, despite an entire 2 months of food deprivation, refused to take live juvenile rats ( $\sim 150$  g) or large adult mice ( $\sim 30$  g). After an average of 12 sessions (range 3–30 sessions) over the



**Fig. 2** **a** Mean number of reinforcers ( $\pm$ SE) acquired in 20 min by a captive-born, juvenile Burmese python (S75); **b** mean latency for each of 30 sessions performed by S75 to respond by striking prey in an open reservoir, retrieving euthanized prey from an open reservoir, approaching a door added to the reservoir or contacting the door; **c** mean ( $\pm$ SE) response latency by S75 to strike prey in an open reservoir, retrieve euthanized prey from an open reservoir, approach a

door added to the reservoir or contact the door, for which response had a significant effect on latency, which differed significantly between early phases (strike, retrieve) and later phases (approach and contact) ( $*P < 0.05$ ); **d** mean ( $\pm$ SE) response latency for each of six sequential (within-session) trials performed by S75 over all 30 sessions





**Fig. 3** **a** Mean response latency ( $\pm$ SE) for each of six sequential (within-session) trials performed by five subadult pythons (SA, S4, S6, S11 and S12) during pre-training, shaping and discriminated operant (D.O.) training phases. Latency decreased between trials one and five and trial one and six ( $P < 0.05$ ), and between pre-training and both shaping and D.O. training ( $P < 0.05$ ). **b** Mean response

latencies ( $\pm$ SE) during pre-training, shaping and D.O. training sessions conducted in subadult pythons (SA, S4, S6, S11 and S12). Training phase had a significant effect on mean response latency, which decreased significantly between pre-training and D.O. training phases ( $*P < 0.05$ )

course of which prey size was gradually reduced, the subadult pythons eventually began reliably accepting euthanized juvenile mice, though never accepted prey less than 10 g, or about 0.2–0.4 % of body weight. Mean response latency (mean range among sessions  $\pm$  SE:  $0.9 \pm 0.1$ – $2.5 \pm 0.6$  min) as well as variability in individual performance (as reflected by the SE values) decreased as training progressed (Online Resource 2).

A decrease in mean response latencies was also observed over the course of sequential trials within sessions (repeated measures ANOVA:  $F_{5, 72} = 3.60$ ,  $P = 0.0058$ ), with significant decreases detected between the first and fifth trials and between the first and sixth trials (Fig. 3a).

Subadult pythons exhibited a decrease in mean response latency (mean  $\pm$  SE: pre-training =  $3.1 \pm 0.4$ ; shaping =  $1.3 \pm 0.3$ ; discriminated operant =  $0.8 \pm 0.06$  min) as training progressed. Specifically, there was an overall significant difference between the pre-training and discriminated operant phases (repeated measures ANOVA:  $F_{2, 8} = 8.83$ ,  $P = 0.0094$ ; Fig. 3b). Pythons did not experience equal times of each training phase, but mean response latency for each phase did not significantly differ among individuals (repeated measures ANOVA:  $F_{4, 8} = 0.93$ ,  $P = 0.4943$ ).

Additional modifications of python behavior were observed. Striking behavior (a rapid lunge of the head and rostral quarter of the body, resulting in prey capture) exhibited with live prey was replaced with “retrieval” behavior when a euthanized mouse was provided (i.e., the snakes did not strike dead prey). Furthermore, before the addition of the remote-controlled door, the pythons typically positioned themselves opposite the food reservoir immediately following ingestion of the previous mouse in anticipation of the next reward. Following installation of the door, the pythons continued to orient toward the door

and often pushed it with their snouts shortly after ingestion of the previous reward.

## Discussion

Operant conditioning is possible in large, wild snakes whose physiology and behavior would seem at odds with developing a robust conditioning paradigm. The Burmese python is a massive, ambush predator of (usually) homeothermic prey, and typically consumes large meals on an infrequent basis. In the current study, investigators used successive approximations to train wild Burmese pythons to accept food items much smaller than they commonly consume in the wild, associate a stimulus with very small food rewards via operant conditioning and perform a contingent operant task to gain access to a food reward.

A variety of animals that exhibit highly specialized strategies to meet physiological needs have been successfully trained to perform operant tasks. Such animals include insectivorous bats, which use ultrasound cues to detect and localize prey while both predator and prey are in flight. In an experiment on auditory sensitivity, big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*) were trained to walk to a platform to observe a stimulus (an unnatural behavior) and then walk to a food reservoir to obtain a mealworm (an unnatural food reward) (Dalland 1970). Furthermore, animals completely lacking appendages appropriate for behaviors like lever pressing can be trained to perform operant tasks. Fish and honeybees, for example, have successfully performed learned tasks using snout-pressing and shuttling behaviors, respectively (Yager and Thorpe 1970; Walker and Bittermann 1985; Walker et al. 1997).

Despite the fact that operant conditioning techniques have been applied to a wide array of vertebrate and invertebrate species, there are very few reports of operant conditioning in snakes, and most of those are limited to small species that perform behavioral responses in mazes and/or escape paradigms. For example, movement in an arm of a Y-maze in response to an odor trail was reinforced with earthworm prey in a small (30–50 g body mass) snake species (Halpern et al. 1997). More recently, Holtzman et al. (1999) demonstrated escape-motivated spatial learning in corn snakes (*Elaphe guttata guttata*). The snakes, which were trained to locate one open hole in an arena containing seven additional, closed holes, exhibited decreased latency and distance traveled to the goal hole following 4 days of training. In a similar study, Almlí and Burghardt (2006) demonstrated in rat snakes (*E. obsoleta*) enhanced ability to locate a goal hole when exposed to an odor trail toward or odor within the goal hole. Both movement and shelter-seeking responses described in these studies are part of the natural behavioral repertoire of snakes. Thus, the behavioral conditioning paradigm described in this study represents the first report of an effective, complex unnatural conditioned response (button-pressing) for a boid snake.

Even though pythons can and often do consume very large meals, plasticity of prey choice and digestive function may make pythons (and other snakes) *particularly* amenable to dietary modification for use in operant conditioning studies. Python gastrointestinal organs are extremely flexible, such that size and function, and hence metabolic rate, can be adjusted rapidly in response to a meal or lack thereof (Starck and Beese 2001; Overgaard et al. 2002). Increased gastrointestinal activity occurs within 24–48 h of a meal and is maintained for several days (Overgaard et al. 2002). Therefore, when food is available every 72 h, gastrointestinal function remains highly active, and the snake may exhibit increased motivation to obtain food. Some snake species may actually prefer smaller meals; when offered an excess of small items or a single large item, water snakes chose smaller meals (Andreadis and Burghardt 2005). Here, we showed that large pythons can be trained to consume prey items much smaller (perhaps 0.3–1.0 % of normal, or a 10-g mouse vs a 1–3-kg raccoon) than they normally would in the wild, which makes these animals more amenable to operant procedures than previously thought.

The dietary modification employed here—gradual transition from large, live, freely moving prey to very small, inanimate food items—overcame potential obstacles involving the sensory cues normally used in predation by pythons. Predatory behavior in these snakes normally involves a combination of visual (including motion detection), thermal, chemical and mechanical cues, most of

which are absent in the small, dead prey items required by the protocol. But, as snakes formed associations between translocation from the home cage to the experimental apparatus, the light stimuli, the behavioral response and the food reward, normal prey cues were unnecessary for initiating consumptive behavior. Initially, snakes refused small, euthanized mice, though successively smaller food items were taken with decreased latencies as trials progressed. It should be noted, however, that there was an apparent lower limit to the size of food rewards accepted (about 10 g, or about 0.2–0.4 % of body weight).

The results presented here furthermore demonstrate that captive-born and wild-caught subadult pythons can be conditioned to use their snout to push an illuminated button. While the response itself is similar to snout responses in fish and beak responses in birds, response rates were much lower in the pythons. The average python rate was one response per 5.5 min, which is much lower than reported previously for fish (4 responses/minute in Siamese fighting fish; Thompson and Sturm 1965) and birds (90 responses/minute in crows; Powell 1972). The rate in pythons is closer to that reported by Kleinginna (1970) for indigo snakes (up to 2 responses/5 min, CRF), whose responses were reinforced with drinking water. Even so, the response rate in pythons demonstrated here was about half that reported for indigo snakes (Kleinginna 1970). The difference may be due to a longer inter-trial interval duration here, which was driven by anatomical constraints that increased food ingestion time, particularly with very small food rewards, compared to the water consumption observed in the indigo snakes. We suggest that while the methods described here utilized the opportunistic hunting behavior of pythons with a continuous reinforcement (CRF) schedule, future experiments could investigate cumulative response rates to a variety of reinforcement schedules in well-trained snakes that have acquired the desired response behavior. Despite the low response rate, there was a decrease in the pythons' latency to respond following either orientation toward the reservoir or light illumination, indicating an improvement in performance with practice.

Because the work presented here could provide the basis for future experiments involving forced-choice procedures, it must be noted that these snakes could have used chemical and/or thermal cues emitted from food rewards to simply guide approaches to the food reservoir. Stimulus control, however, was integral to these experiments as demonstrated by the fact that after training, snakes only performed the operant response after the light was illuminated. That is, the pythons oriented toward and fixed on the discriminative stimulus area, but they did not perform the push-button response until the light was illuminated, indicating that the push-button response was under control of

the visual discriminative stimulus. In addition, later experiments involving a two-alternative forced-choice procedure with prey items in two reservoirs resulted in correct responses to a non-visual discriminative stimulus and equal probabilities of left and right choices (data not shown). Thus, snakes here were not simply approaching an odorant, but rather making conditioned responses to an unnatural stimulus (i.e., illumination of the green light).

Similarly, thermal cues were unlikely to have been guiding snake behavior, even though pythons possess a robust infrared imaging system that can lead to orientation and targeting even in the absence of prey chemical cues. Ball pythons, for example, exhibit strike postures, freeze-and-fix (snake motionless, fixed on target) and fix-and-follow behavior (snake fixed on target and following target movement) in response to an artificial thermal target free of prey chemical cues (Ebert et al. 2007). Furthermore, pit vipers struck at artificial targets that lacked prey odor, and when presented simultaneously with a warm artificial target and a prey-scented cool artificial target, pit vipers always struck at the warm target (Safer and Grace 2004). In the work presented here, however, the door separating the snake from the food reward was insulated, and high-sensitivity infrared videography showed that the insulated reservoir door effectively maintained ambient temperature and eliminated prey-associated thermal cues.

A green light was chosen here as the visual discriminative stimulus because the most common cone photoreceptors in ball python (*Python regius*, the only python for which such data are available) are maximally green-sensitive (Sillman et al. 1999). There are no published behavioral studies on spectral sensitivity of visually guided behavior in snakes, but the use of light as a discriminative stimulus in these experiments provides a foundation for such studies. Furthermore, the conditioning paradigm presented here can be adapted for other, less-studied snake sensory modalities including infrared imaging, which is unique to pythons, boas and pit vipers (Bullock and Diecke 1956; Molenaar 1992; Grace and Matsushita 2007). Infrared imaging plays an integral role in the behavior of these snakes, allowing accurate and precise predatory and defensive targeting, even in complete darkness, as well as the localization of thermal refugia (Haverly and Kardong 1996; Krochmal and Bakken 2003; Grace and Matsushita 2007). The behavioral chain learned by the pythons in the experiments reported here provides a foundation for psychophysical analysis of infrared imaging in pythons and other large IR-sensitive snakes, many of which are massive ambush predators like *P. molurus*.

At various points during the experiments reported here, snakes underwent shedding (“ecdysis”), the process of which lasted 1–2 weeks. Because the shedding process caused the snake’s eyes to become temporarily opaque, interest in feeding to wane, and tactile sensitivity and

defensiveness to increase, snakes were not included in conditioning sessions from the time the snake’s eyes first appeared “opaque” until after shedding was complete. Despite these breaks in data collection, when testing resumed, snakes immediately resumed the conditioned behavior and quickly returned to pre-shedding performance levels, which supports the idea that long-term memories had been formed.

In summary, pythons, like other animals, exhibit behavioral plasticity and a significant capacity for learning and memory, as supported by recent field studies of wild snakes that exhibited experience-dependent anti-predator behavior (Gregory 2013) and homing behavior following translocation (Pittman et al. 2014). Despite the snakes’ previous experience in the wild, the discouragement of use of wild-caught animals (Burghardt and Layne 1995) and the notion that unnatural behaviors (response to lights, lever pressing) are unsuitable for snake experiments (Reiserer 2002), the pythons in this study adjusted well to the captive environment and learned to perform a complex, contingent operant task. The results shown here indicate that pythons (including wild ones) can, like other vertebrate and invertebrate species, (1) be effectively conditioned to perform operant responses to conditioned stimuli, (2) exhibit sensitivity to discriminative stimuli and (3) respond to contingencies of reinforcement using well-established learning theories and practices. That is, when physiologically relevant reinforcement is provided and required response behaviors are within behavioral capabilities, positive reinforcement and effective operant conditioning is possible. Rapid shaping of behavior and similarity of performance among individual snakes provides a foundation for future experiments aimed at testing the ability of snakes to discriminate a variety of environmentally relevant stimuli. Importantly, exotic, invasive Burmese pythons are a serious ecological threat to sensitive ecosystems in south Florida (Dove et al. 2011; Dorcas et al. 2012) and beyond (Reed 2005); a detailed understanding of their capacity for learning new behaviors may prove critically important to mitigating the threat they pose.

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