

Recent interactions with snakes enhance escape performance of desert kangaroo rats (Rodentia: Heteromyidae) during simulated attacks

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When predators rely on high-speed movements to capture prey, prey often exhibit traits that result in correspondingly extreme physical performance. Biomechanical studies of these interactions are typically conducted in laboratory settings, thereby eliminating some of the ecological context. We studied how behavioural state, specifically vigilance level, of kangaroo rats affects evasion performance during simulated rattlesnake strikes. Vigilance levels were manipulated through the presentation of a tethered sidewinder rattlesnake. After predator exposure, we recorded kangaroo rats evading simulated attacks, and the videos were used to extract information about kangaroo rat performance. High-vigilance kangaroo rats (recently exposed to a rattlesnake) significantly outperformed low-vigilance kangaroo rats (not exposed to a rattlesnake) in both reaction time and take-off velocity, and executed steeper jumps. Although our recordings were not adequate for detailed biomechanical quantification, reaction times of high-vigilance kangaroo rats may be among the fastest recorded for mammals, with 36% of these individuals producing a visible response to attacks within no more than 8–17 ms and a group mean of 23.1 ms. This study demonstrates that behavioural state can have major effects on performance during predatory encounters. Therefore, under some conditions, laboratory studies of evasive anti-predator manoeuvres may underestimate performance in the wild.

ADDITIONAL KEYWORDS: anti-predator behaviour – biomechanics – mammalian startle response – predator–prey – vigilance.

INTRODUCTION

Predation is a ubiquitous driver of evolution; with the exception of apex predators, most animals are subject to predation pressures that shape their morphology and behaviour. Animals often exhibit defences, such as weapons or anti-predator displays, that enhance their likelihood of evading or deterring predatory attacks (West, Cohen & Baron, 1991; Steiner & Pfeiffer, 2007). In systems where predators ambush prey with high-speed attacks, prey must develop correspondingly rapid detection and avoidance manoeuvres to survive; anti-predator adaptations in these

systems often result in extreme physical performance of the prey (Bro-Jørgensen, 2013). Some of the fastest sensory responses and motor actions in the animal kingdom are associated with predator evasion. For example, fishes possess Mauthner cells, neurons responsible for fast C-start responses, that allow them to initiate a response to an oncoming predator within 30 ms (Domencici & Blake, 1997). This is probably related to the ability of predatory fish to attack rapidly, with some fish being capable of reaching maximum gape in less than 30 ms (Higham, Day & Wainwright, 2006). Additionally, pronghorn antelopes (*Antilocapra americana*, Ord) are capable of reaching the speeds of 93 kph, a trait that was almost certainly driven by their extinct predator, the American cheetah (*Miracinonyx*) (Sharp, 2012), a relationship similar to

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the co-evolved high-speed performance of the African cheetah (*Acinonyx jubatus*, Schreber) and Thomson's gazelle (*Eudorcas thomsonii*, Günther) (Schaller, 1968). High-speed attack and evasion systems have attracted attention from biomechanical researchers in an effort to better understand how prey are capable of evading extremely fast predators (see below for examples).

Performance of animals in predator–prey interactions (e.g. velocity and acceleration) is often quantified under laboratory conditions due to the logistical challenges associated with recording these interactions in the wild. Laboratory studies using high-speed cameras have undoubtedly expanded our understanding of predator–prey kinematics, providing detailed measures of the relative timing and speed of movements (Hawlena *et al.*, 2011; Stewart *et al.*, 2014; Poppinga *et al.*, 2015). The trade-off, however, is that much of the ecological context surrounding these encounters is lost; it is therefore difficult to understand ecological factors that can impede or enhance performance in the wild (Irschick & Losos, 1998; Combes *et al.*, 2012; Wilson *et al.*, 2015). One such factor often overlooked in laboratory studies of escape performance is the state of wariness or vigilance of the individual.

Vigilance is one of the most well-studied behaviours in vertebrates, and many taxa have been shown to increase vigilance in the presence of predator cues (see Lima & Dill, 1990); performance increases due to vigilance state could therefore be a common behaviour that is underappreciated in the literature on anti-predator behaviour. Why would we expect vigilance to increase performance? Heightened vigilance is frequently associated with arousal of the sympathetic nervous system (Arthur, 1987; Schneiderman, Ironson & Siegel, 2005), which is responsible for the flight or fight response. This response functions to prepare the prey for an impending predator encounter, and it therefore has the potential to enhance prey performance via hormonal responses (Higham & Irschick, 2013). Chronic exposure to these hormones (e.g. cortisol, epinephrine) can, however, have negative impacts on the individual (Romero & Butler, 2007), so balancing the positive and negative effects of the fight or flight response necessitates natural variation in vigilance responses.

Kangaroo rats (*Dipodomys* spp.) are nocturnal rodents found throughout the arid regions of western North America, and they are common prey for owls and rattlesnakes (Webster, 1962; Funk, 1965). Rattlesnakes (*Crotalus* spp.) rely on short bursts of speed (typically less than 0.5 s) initiated from ambush to strike and envenomate their prey (Kardong & Bels, 1998), an attack strategy that may be a driving force in the speed and agility exhibited by kangaroo rats.

Dipodomys have disproportionately long hind limbs that have apparently evolved in response to selection favouring rapid evasions from attacking predators (Biewener & Blickhan, 1988). When ambushed, a kangaroo rat will avoid capture using an extremely rapid jump accompanied by an acrobatic twist that carries it away from the trajectory of the snake strike (Whitford, Freymiller & Clark, 2017). Kangaroo rats are also known to exhibit a series of stereotyped anti-snake behaviours when in states of heightened vigilance (Randall, 1993; Clark *et al.*, 2016), which may include (depending on the species): orienting towards the ambushed snake, foot drumming (stamping the ground with one or both hind feet), sand kicking (using both hind legs to propel substrate behind them), jump-backs (a close approach followed by a rapid backwards leap) and an increased rate of head-up scanning. The kangaroo rat–rattlesnake system thus provides a model system to examine how specialized morphology has led to extreme performance, and the context dependency of that performance.

In this study, we quantified several aspects of the evasive jumps of free-ranging desert kangaroo rats (*Dipodomys deserti*, Stephens). We altered the behavioural state of kangaroo rats by manipulating recent prior exposure to predators in an attempt to determine whether heightened vigilance enhanced performance towards a simulated rattlesnake strike. We used a cryptic, sit-and-wait ambush predator widely abundant in their natural habitat, the sidewinder rattlesnake (*Crotalus cerastes*, Hallowell), to enhance vigilance levels. Desert kangaroo rats exhibit all of the well-defined, snake-specific anti-predator behaviours described above when interacting with, or attempting to detect, snakes or snake-like objects. Based on Putman & Clark (2015), we predicted that the high-vigilance kangaroo rats would perform evasive manoeuvres faster than low-vigilance kangaroo rats during a simulated strike, demonstrating that behavioural state can significantly affect prey performance during predator evasions.

MATERIAL AND METHODS

STUDY SITE

Our study site was approximately two miles southwest of the Desert Studies Center in San Bernardino County, CA, USA (35° 7'7.16"N, 116° 7'5.01"W). The site is composed of low-lying, wind-blown sand dunes with interspersed mesquite patches (*Prosopis glandulosa*, Torrey) and is bordered by creosote scrub (*Larrea tridentata*, Coville) and an alkali sink. Data were collected from mid-May to early August in 2014. All trials and observations were conducted between sunset

and sunrise, as desert kangaroo rats are exclusively nocturnal.

STUDY ANIMALS

All procedures were approved by the San Diego State University Institutional Animal Care and Use Committee (APF 13-08-015C). We trapped desert kangaroo rats using Sherman live traps baited with black oil sunflower seed. We placed baited traps adjacent to conspicuous *D. deserti* burrow systems. We marked the trapped kangaroo rats using fingerling ear tags (National Band and Tag #1005-1) for long-term identification and a unique dye mark using Nyanzol fur dye for short-term identification. We weighed individuals to the nearest gram and measured snout-anus length, tail length and hind foot length to the nearest millimetre. We processed all individuals in the field and released them immediately at the site of capture.

STRIKE SIMULATION EXPERIMENT

We used a modified version of the methods detailed by Putman & Clark (2015) to assess kangaroo rat vigilance. Putman & Clark (2015) used three treatments to assess ground squirrel evasive responses: no snake (squirrels tested without being presented a tethered snake), snake present (squirrels tested while the tethered snake was present) and recent snake (squirrels presented with a tethered snake that was then removed immediately prior to testing evasion). Squirrels in the recent snake treatment exhibited the highest levels of vigilance, probably because they were attempting to detect a snake that was no longer present. Therefore, in this study, we used just two treatments: no snake (individuals with baseline vigilance) and recent snake (heightened vigilance).

We first established bait stations at each burrow system by placing a small amount of black oil sunflower seed at a set location within 0.5 m of an active burrow entrance, as determined by the distinctive tracks these rodents leave in the sand. Due to the highly territorial disposition of desert kangaroo rats, we were then able to target known individuals at specific bait stations. Occasionally neighbour kangaroo rats would visit the bait stations, but they were promptly chased away by the resident individual (see Randall & Boltas King, 2001). The presence of neighbour kangaroo rats did not have any apparent effect on vigilance of the focal kangaroo rat. After a minimum of 3 days of baiting, we began a trial by placing the rattlesnake strike simulator (RSS) adjacent to a bait station. The RSS consists of a one-inch diameter PVC pipe which houses a compressed spring that projects a cork towards a target at approximately 2.8 m/s, a velocity similar to that

achieved by striking rattlesnakes (Penning, Sawvel & Moon, 2016). The cork was held while compressed using fishing line tied to a camera tripod manned by an observer 3–5 m away. Once the RSS was in place, the observer would retreat to the camera and wait for the focal kangaroo rat to explore the RSS. A camera set-up consisted of an infrared light and two video cameras (Sony Handycams models SR-65 and SR-300). One camera was programmed to record at 30 frames per second (fps) and the other at 120 fps. We used the 120-fps camera to obtain more accurate displacement and reaction times (discussed in more detail below), but we could only record at this frame rate for 3 s. Thus, we included the 30-fps camera to capture the entire trial.

At the beginning of every trial, baseline observations were recorded for all individuals to ensure individuals were not already expressing anti-snake behaviours/high levels of vigilance (Clark *et al.*, 2016). We placed sunflower seeds around the RSS and in a small plastic tray affixed approximately 14 cm from the edge of the device to promote exploration by the focal kangaroo rat. All kangaroo rats immediately began normal foraging behaviour and did not appear disturbed by the presence of the RSS. Following the baseline assessment, new seed was placed in the feeding tray when the kangaroo rat left to cache seed. We waited for the kangaroo rats to leave naturally to minimize human–rat interactions. In the no snake treatment, we began recording with the 30-fps camera when the kangaroo rat returned. When the individual approached the feeding tray, the fishing line was cut which released the cork towards the kangaroo rat and the response of the kangaroo rat to the RSS was recorded with both cameras.

In recent snake (high vigilance) treatments, we followed the technique described by Clark *et al.* (2016) to tether rattlesnakes in a semi-coiled position less than 0.5 m away from the RSS. We waited until the kangaroo rat interacted with the tethered snake, exhibiting investigatory approaches, jump-backs, sand kicks and/or foot drums (Randall & Boltas King, 2001; Clark *et al.*, 2016) to ensure that the kangaroo rat was aware of the snake's presence. After the kangaroo rat had interacted with the snake and left the area, we removed the snake and placed more seed at the RSS. Snakes were removed before deploying the RSS so that returning kangaroo rats were unaware of the snake's location, thereby increasing their vigilance and anti-snake behaviours (Putman & Clark, 2015). When the kangaroo rat returned, we recorded the occurrence of any anti-snake behaviours and followed the same protocol for deploying the RSS used during no snake trials. Each individual only received one trial to prevent learning from affecting responses to the RSS. Trials were implemented in a random order.

VIGILANCE BEHAVIOUR

We recorded the occurrence of vigilance and anti-snake behaviour to determine the degree of vigilance expressed by individuals in each treatment and compare their effects on kangaroo rat escape behaviour. Three main anti-snake displays have been described for desert kangaroo rats: foot drumming, sand kicking and jump-backs (Randall & Boltas King, 2001; Clark *et al.*, 2016; Whitford *et al.*, 2017). We also recorded the occurrence of a fourth behaviour, head-up scanning, which has been used in many studies as a way to quantify vigilance levels (see Lima & Bednekoff, 1999). In this study, we define head-up scanning as a behaviour in which the kangaroo rat momentarily stops moving and stands bipedally for at least 1 s and does not appear to be masticating or handling seed.

DATA ANALYSIS

Video analysis

Because we recorded animals under field conditions in physically remote and challenging environments, we were constrained to using low-cost, portable, consumer-grade video equipment for this experiment. Thus, our ability to calibrate distances and extract detailed biomechanical variables is limited compared to experiments run in laboratory situations. However, even with these limitations on recording and extracting data, the methods we detail below allowed us to quantify relative metrics for several key behaviours and derive sufficient data to test our hypotheses.

All values are reported as mean \pm SE. We used video recordings to quantify five key variables associated with evasive jumps away from the RSS. These variables, which we identified *a priori* from a similar experiment with ground squirrels (Putman & Clark, 2015), were reaction time, body displacement time, take-off angle and velocity, and time spent airborne. Using the 120-fps videos, reaction time was the time between the frame in which we first saw movement of the RSS and the frame in which we saw the first movement of the evasive manoeuvre. We added 8.3 ms (the time of one frame) to each reaction time value in order to make our estimates more conservative (i.e. our estimates represent the slowest reaction time possible based on our video frame intervals). To quantify body displacement time, we counted the number of frames (from 120-fps recordings) between the frame in which we saw the first movement of the evasive manoeuvre and the frame in which the kangaroo rat no longer occupied the space before the beginning of the movement. The time spent airborne was the number of frames between when the kangaroo rat's feet left the ground and when the feet made contact (all individuals always landed feet first). As the kangaroo

rats often jumped out of the frame of the 120-fps recordings, we used the 30-fps recordings in VLC media player to measure time spent airborne.

We also measured the angle of the jump relative to the ground and take-off velocity (Velocity_{*i*}). We calculated the angle of the jump and take-off velocity using the horizontal displacement of the kangaroo rat (the distance between the kangaroo rat's location immediately prior to jumping and the kangaroo rat's landing location) and the time the kangaroo rat spent in the air.

$$\text{Velocity}_h = \frac{\text{Horizontal displacement (m)}}{\text{Time spent in air (s)}}$$

$$\text{Velocity}_v = g \left(\frac{\text{Time spent in air (s)}}{2} \right),$$

where g = acceleration due to gravity (9.8 m/s²).

$$\text{Velocity}_i = \sqrt{\text{Velocity}_h^2 + \text{Velocity}_v^2}$$

$$\text{Jump angle (}^\circ\text{)} = \text{atan} \left(\frac{\text{Velocity}_v}{\text{Velocity}_h} \right) \times \frac{180}{\pi}$$

To measure horizontal displacement for the calculation of take-off velocity and jump angle, we calibrated our experimental video frames *post hoc* in the laboratory by recording calibration videos of the RSS device (an object of known size) on top of 9-m² grid of 20-cm \times 20-cm cells from a series of distances and horizontal and vertical angles. From the calibration videos, we were able to select frames that closely matched the position and size of the RSS relative to the camera in the experimental recording. We then used the calibration frame to set size standards and estimate horizontal displacement to the nearest cm using ImageJ (1.49v).

Statistical analysis

We analysed response variables (reaction time, body displacement time, time airborne, angle of jump and take-off velocity) using generalized linear models in the statistical program R (version 3.2.2). All models included the same seven explanatory variables: trial type, weight of rat, distance from front edge of RSS, head position at the time of RSS deployment (up, down or turned), the interaction between trial type and distance, the interaction between trial type and head position and the interaction between distance and head position. Weight was included because this variable can directly influence the power or force that a kangaroo rat is able to generate, but was not used in interactions during analyses as we did not expect it to be related to the other three independent variables. Head position

was included because an individual with its head up could potentially detect the device more quickly than an individual with its head down. We included the distance from the edge of the cannon as some kangaroo rats were closer than others at the time the RSS was fired and this could affect their ability to detect the moving cork. Sexes were pooled as this species does not exhibit any sexual dimorphism that could affect performance abilities, and there were several instances where sex could not be determined with certainty during processing. Because each individual only ever received one trial, no repeated-measure techniques were used. Reaction time was $1/\sqrt{}$ transformed, displacement time was log transformed and take-off angle was squared, as indicated by the Box–Cox method (Osborne, 2010), to satisfy the assumption of equal variances and normal variance. Time airborne and take-off velocity both satisfied the assumption of equal variances and of normality. Stepwise model selection, using Akaike information criterion values, was used to determine the best-fit model for all five response variables.

We recorded 78 trials. We removed three trials because observers appeared to disturb kangaroo rats prior to deploying the RSS. We removed two trials because the kangaroo rat was facing directly away from the RSS. Another trial was removed as the kangaroo rat did not return after interacting with the snake. The 30-fps videos malfunctioned for one of the recent snake treatments so we were unable to score vigilance behaviour, and as such this trial was removed from further analyses. Lastly, we removed one trial due to technical problems with the video files. Thus, we retained 70 trials for analysis: 35 no snake and 35 recent snake trials. We removed different trials from each of the analyses due to camera and software malfunctions, inadequate calibration frames and/or when individuals landed off screen. Final sample sizes for each response variable are stated below.

Table 1. Videos of described kangaroo rat behaviours

Behaviour	Video link
Anti-snake and vigilance head-up behaviours	1. https://youtu.be/2-iUoSmKLd4
Comparison of pre- and post-snake interaction behaviour to RSS	2. https://youtu.be/qctTWLidtvU
Comparison of evasion between low- and high-vigilance rats	3. https://youtu.be/2ifLsqyl63I

RSS, rattlesnake strike simulator.

RESULTS

VIGILANCE BEHAVIOUR

In the recent snake treatment, 57% of individuals exhibited at least one of the three main anti-predator displays (Table 1, Video 1). Including head-up scanning, 86% of individuals in the recent snake treatment exhibited heightened vigilance behaviour. In the no snake treatment, only one individual exhibited anti-predator displays (one jump-back and 11 sand kicks) and no individuals exhibited head-up scanning.

REACTION TIME

Kangaroo rats in the recent snake treatment ($n = 32$) exhibited significantly quicker reaction times than individuals in the no snake treatment ($n = 35$) (reaction time for recent snake = 23.1 ± 1.0 ms; no snake = 34.9 ± 2.2 ms; $F_{1,60} = 25.1$, $P < 0.0001$; Fig. 1). Although the best-fit model for reaction time included the distance, head position and the interaction between distance and head position in addition to treatment, none of the other explanatory variables significantly affected reaction time (distance: $F_{1,60} = 0.57$, $P = 0.46$; head position: $F_{2,60} = 1.37$, $P = 0.26$; head position \times distance: $F_{2,60} = 2.53$, $P = 0.09$).

BODY DISPLACEMENT TIME

The best-fit model for body displacement time included treatment, head position and the interaction between treatment and head position. Body displacement times for kangaroo rats in the recent snake treatment ($n = 30$) was significantly faster than those in the no snake treatment ($n = 33$) (displacement time for recent snake = 51.2 ± 3.8 ms; no snake = 67.7 ± 3.3 ms; $F_{1,58} = 15.0$, $P = 0.0003$; Fig. 1). Head position also significantly affected displacement time (displacement time for individuals with head up = 46.2 ± 6.2 ms; head down = 62.9 ± 2.8 ms; head turned = 83 ± 0 ms; $F_{2,58} = 4.24$, $P = 0.019$). The interaction between treatment and head position, however, was not statistically significant ($F_{1,58} = 2.87$, $P = 0.095$).

TIME AIRBORNE

The only explanatory variable in the best-fit model for time spent airborne was treatment. Kangaroo rats in the recent snake treatment ($n = 28$) spent almost twice as much time airborne than kangaroo rats in the no snake treatment ($n = 27$) (time airborne for recent snake = 456.0 ± 25.8 ms; no snake = 263.0 ± 25.6 ms; $F_{1,53} = 26.5$, $P < 0.0001$).

ANGLE OF JUMP

Kangaroo rats in the recent snake treatment ($n = 27$) jumped at greater angles relative to the ground when compared to individuals in the no snake treatment ($n = 22$) (angle for recent snake = $62.5 \pm 2.8^\circ$; no snake = $38.0 \pm 3.6^\circ$; $F_{1,45} = 26.7$, $P < 0.0001$; Fig. 2). The best-fit model for take-off angle included only treatment and head position, and head position did not significantly affect the angle of jump ($F_{2,45} = 2.62$, $P = 0.084$).

TAKE-OFF VELOCITY

The best-fit model for take-off velocity included treatment, distance, head position, the interaction between treatment and head position and the interaction

between distance and head position. Kangaroo rats in the recent snake treatment ($n = 27$) jumped with a greater take-off velocity when compared to individuals in the no snake treatment ($n = 22$) (velocity for recent snake = 2.7 ± 0.1 m/s; no snake = 2.3 ± 0.1 m/s; $F_{1,41} = 6.26$, $P = 0.016$; Fig. 2). No other explanatory variables were statistically significant (distance: $F_{1,41} = 0.54$, $P = 0.47$; head position: $F_{2,41} = 0.27$, $P = 0.77$; treatment \times head position: $F_{1,41} = 03.02$, $P = 0.09$; distance \times head position: $F_{2,41} = 2.39$, $P = 0.10$).

DISCUSSION

After interacting with a rattlesnake, kangaroo rats exhibited higher rates of vigilance behaviour when

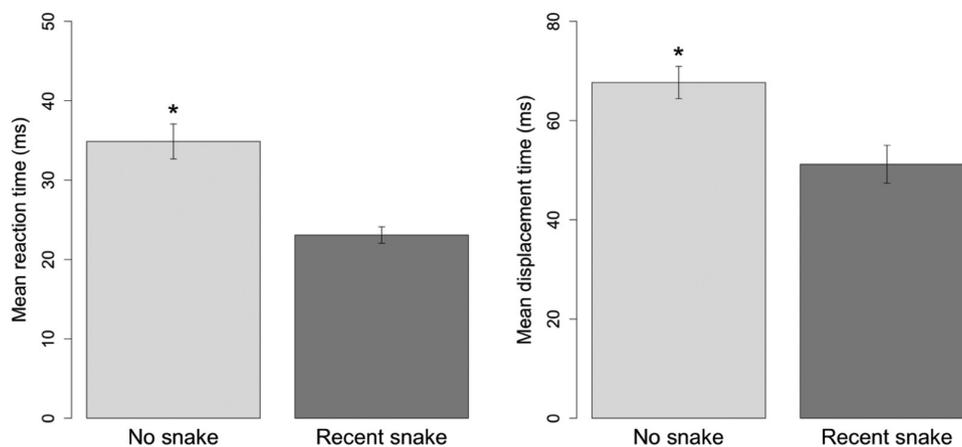


Figure 1. Comparison of reaction time and body displacement time between kangaroo rats in the no snake and recent snake treatments. Individuals in the recent snake group reacted sooner and moved out of the 'strike' trajectory faster than those in the no snake group. Error bars indicate SE, asterisk indicates significant difference.

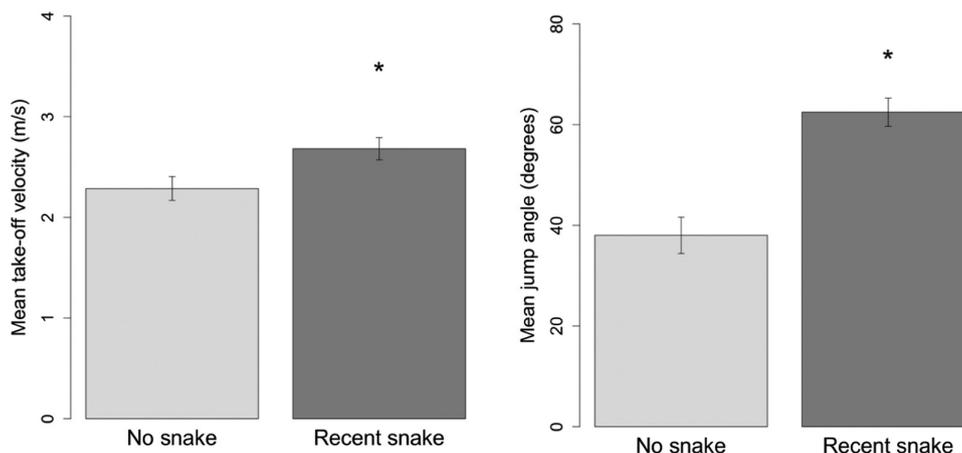


Figure 2. Comparison of jump angle relative to ground and take-off velocity between kangaroo rats in the no snake and recent snake treatments. Individuals in the recent snake treatment tended to jump with a greater take-off velocity and at a greater angle relative to the ground, therefore spending more time airborne. Error bars indicate SE, asterisk indicates significant difference.

returning to the location of the interaction, often including conspicuous anti-predator displays (Table 1, Video 2). Individuals that had recently interacted with a snake outperformed individuals that had not in both reaction time and body displacement time (Fig. 1). Additionally, high-vigilance kangaroo rats spent more time airborne and exhibited a greater take-off velocity than low-vigilance kangaroo rats. These differences were not subtle; the performance of high-vigilance kangaroo rats was often markedly higher than that of low-vigilance individuals, and the statistical differences were highly significant (most P values less than 0.0001). The evasive manoeuvres in the high-vigilance treatment are similar to those elicited by natural strikes of free-ranging sidewinder rattlesnakes (Whitford *et al.*, 2017), suggesting that the kangaroo rats responded to the RSS as they would an actual rattlesnake strike. These results provide compelling evidence for enhanced locomotor performance as a result of heightened vigilance.

Two distinctly different evasive jumps were used when evading the RSS, and the type of jump depended on whether the kangaroo rat had been exposed to a rattlesnake (Table 1, Video 3). Low-vigilance kangaroo rats tended to jump low to the ground away from the oncoming cork. High-vigilance kangaroo rats tended to jump not only more quickly, but also more vertically, at an angle greater than 45° relative to the ground. When considering the characteristics of a rattlesnake strike, the optimal escape strategy would likely be to move as rapidly as possible out of the trajectory of the strike (i.e. a vertical jump perpendicular to the strike path). Once the strike has been initiated, rattlesnakes appear to have limited ability to change the trajectory of the strike (Kardong & Bels, 1998). Additionally, rattlesnakes often remain in a period of post-strike immobility for an extended period (Hayes, 1993; Clark, 2006) during which time they rarely initiate a second strike (personal observation). Thus, it is not as essential for prey to move far from the rattlesnake as it is to move out of the path of initial strike. The tendency to make vertical jumps is seen during natural snake strikes (Higham *et al.*, 2017), suggesting this is the true optimal escape trajectory for evading rattlesnake predators.

COMPARISONS TO OTHER MAMMALS

The performance of high-vigilance kangaroo rats in this study indicate that kangaroo rat reaction times may be among the fastest documented for any mammal. Previous studies have estimated that the latency to initiate a reaction for mammals (i.e. the time between the onset of the stimulus and the first visible reaction of the animal) ranges from an average of 12 to 250 ms (Davis, 1984; Yilmaz & Meister, 2013; Putman & Clark, 2015), with the fastest recorded responses occurring in 10 ms (Davis,

1984). This range in reaction time is likely to be driven by differences in processing time of different stimuli; for example, auditory cues produce startle responses faster than visual cues (Davis, 1984). In the present study, 36% of high-vigilance desert kangaroo rats produced a visible response to the RSS within one frame (thus between 8.3 and 16.6 ms, or 0 and 8.3 without the additional frame correction). Because the reaction and movements of the kangaroo rats are so rapid, we are conducting additional research using higher speed cameras (500 fps) with better resolution so that we can more accurately quantify reaction times in this system (e.g. Higham *et al.*, 2017). The ability of kangaroo rats to rapidly initiate a reaction and subsequently displace their bodies from the trajectory of the RSS corresponds to a remarkable ability to evade natural rattlesnake strikes (Whitford *et al.*, 2017), challenging previous assumptions that snake strikes are faster than the sensory and motor responses of their mammalian prey (Penning *et al.*, 2016). As both speed and manoeuvrability are likely to be important factors affecting the ability of kangaroo rats to avoid snakes (Clemente & Wilson, 2015), our ongoing research will examine both the acceleration of kangaroo rat evasions as well as the use of their tails in achieving mid-air turns.

Putman & Clark (2015) studied the reactions of California ground squirrels (*Otospermophilus beecheyi*, Richardson) during different stages of rattlesnake encounters using the same RSS methods we employed here. While they also found that individuals that recently encountered snakes had faster reaction and body displacement times than those in the no snake treatment, desert kangaroo rats are qualitatively faster, react more quickly and jump farther. For example, the average reaction time for low-vigilance kangaroo rats in this study was 34.9 ms, which is on par with the average reaction time of the high-vigilance ground squirrels in Putman & Clark (2015). These differences probably arise from differences in morphology and sensory acuity. The ankle tendons in kangaroo rats are too thick and stiff to store and contribute a substantial amount of elastic energy during steady locomotion (Biewener, Alexander & Heglund, 1981). Thus, previous studies have concluded that the large tendons in kangaroo rat hind limbs evolved for rapid acceleration (as would occur during predator evasions) rather than for energy-efficient locomotion (Biewener & Blickhan, 1988). This unique morphology may be key in allowing kangaroo rats to displace their bodies faster and farther than more typical mammals such as ground squirrels.

The use of visual, tactile and auditory cues may contribute to the observed differences in kangaroo rat and ground squirrel reaction times. Kangaroo rats are highly sensitive to auditory cues from predators due to their enlarged auditory bullae (Webster, 1962),

whereas ground squirrels are probably more reliant on visual cues due to their diurnal activity patterns (Thorson *et al.*, 1998). Previous research on laboratory rats has shown that auditory and tactile cues from air pressure waves are more important than visual cues for initiating startle responses (Davis, 1984); thus, kangaroo rats may be able to initiate responses more quickly than ground squirrels because of their heightened auditory system. Both species can probably detect tactile cues via the vibrissae, but vibrissae do not appear to play a major role for kangaroo rats in detecting oncoming snake strikes. Webster & Webster (1984) found that kangaroo rats that had their vibrissae physically removed were able to avoid strikes when placed in an enclosure with foraging sidewinders, whereas in an earlier study, deafened kangaroo rats were unable to avoid the strikes (Webster, 1962). The spring inside of the PVC pipe did make noise as the cork was projected, and this could be the cue that the kangaroo rats were using in our experiment to initiate an evasive manoeuvre. Although an artificial sound, kangaroo rats in both high- and low-vigilance treatments responded similarly and does not affect our ability to compare the responses of the two groups.

COSTS OF ENHANCED VIGILANCE

If vigilant kangaroo rats perform better, why are they not always in this state? There are probably costs associated with enhanced vigilance that prevent individuals from being able to execute these intensified evasions at all times. If kangaroo rat heightened vigilance and enhanced performance is caused by the mammalian stress response, the benefits of performance would have to be balanced with the costs of chronic exposure to stress hormones (Romero & Butler, 2007), leading to transitory states of heightened vigilance. In addition to physiological costs, there are ecological costs to increased vigilance, such as decreased foraging abilities: time spent being vigilant takes time away from harvesting food and can cause individuals to make more foraging mistakes (see below). Another cost to consider is the risk of attracting the attention of other, more mobile predators, such as owls and canids (i.e. coyotes and foxes). The higher, more vertical evasive leaps and the anti-predator displays of vigilant kangaroo rats could unintentionally make these individuals more conspicuous, making them only beneficial when dealing with the immediate threat of a rattlesnake. Lastly, kangaroo rats probably experience relatively high forces when landing from a powerful jump, such as those seen in the high-vigilance treatment, which could pose an unnecessary threat of physical damage over time if the kangaroo rats were to utilize only that type of evasion. The risk of injury during one of these

evasive leaps could be an important factor affecting the jump performance of kangaroo rats, just as the relative risk of injury has been shown to influence foraging and patch use in red foxes (Berger-Tal *et al.*, 2009).

Decades of work using game theory models have sought to understand the context dependency of risk management by prey, particularly while foraging (see Lima & Dill, 1990). Prey must harvest enough food to sustain themselves and potentially their young while simultaneously minimizing the risk of predation. Increased vigilance can lead to decreased foraging time and an increase in foraging mistakes (Brown, 1999), but it has been hypothesized that vigilant foragers should also show a marked increase in escape abilities (Kotler *et al.*, 2002). Previous studies have demonstrated a decrease in foraging abilities when animals are vigilant (Kotler, Brown & Bouskila, 2004), and the present study has confirmed that vigilance can indeed make individuals nearly impossible to capture, thus empirically supporting the trade-off that has been theorized for vigilant animals.

While predator-prey foraging games are often examined from the perspective of foraging prey, they can also be applied to predators hunting prey (see Lima, 2002). In natural encounters between sidewinder rattlesnakes and desert kangaroo rats, the snakes will almost never strike at a kangaroo rat that has displayed its awareness of the hunting snake's location (Whitford *et al.*, 2017), possibly because they are adapted to hunting such evasive prey. In this 'game' between predator and prey, the sidewinders use information presented by the kangaroo rats to make optimal hunting decisions; it is probably in the snake's best interest to remain in ambush and wait for unsuspecting prey than to reveal its hidden position for a strike attempt on a vigilant kangaroo rat that it probably will not be able to capture.

PERFORMANCE ENHANCEMENT

Performance can be defined as the ability to execute an ecologically relevant task (Irschick & Higham, 2016). Biomechanical studies seek the mechanistic underpinnings to this maximum performance, yet biomechanical studies are often conducted in laboratory settings. Thus, a mismatch between biomechanical studies and field performance measures probably exists (Irschick, 2003). A relatively well-studied system of performance enhancement is to circumvent the normal limits of the neuromuscular system, typically through power amplification via elastic energy storage (reviewed in Higham & Irschick, 2013). However, the role of hormones has received less attention, apart from literature involving humans (Husak & Irschick, 2009). Indeed, testosterone in non-human vertebrates can enhance performance (e.g. Klukowski, Jenkinson & Nelson, 1998), but the

association with elevated performance via this mechanism in association with vigilance is unclear. That said, elevated testosterone or stress hormones (e.g. glucocorticoids) could be physiologically responsible for the heightened vigilance, as is the case for birds.

CONCLUSION

We provide evidence that differences in behavioural state (vigilance) can lead to major variation in physical performance, an effect which must be considered in future studies attempting to understand the upper limit of performance in animal systems. In high-speed attack and evasion systems, the difference between the prey escaping or not is often a difference of a few milliseconds. Thus, the 33% decrease in reaction time we documented between treatments represents a very biologically meaningful effect. Successful rattlesnake strikes in the wild reach average maximum speeds of 3.5 m/s (Higham *et al.*, 2017), and sidewinders tend to strike at prey when they are about 8 cm away (Whitford *et al.*, 2017). Rattlesnakes can potentially reach the target in approximately 23 ms, which is similar to the average reaction time for vigilant kangaroo rats. The 36% of high-vigilance individuals who responded in less than 23 ms could avoid such an attack, but only 9% of low-vigilance individuals would have. Ignoring the degree to which awareness of predators or increased vigilance may enhance anti-predator performance would have led to a dramatic underestimation of ability.

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REFERENCES

- Arthur AZ. 1987.** Stress as a state of anticipatory vigilance. *Perceptual and Motor Skills* **64**: 75–85.
- Berger-Tal O, Mukherjee S, Kotler BP, Brown JS. 2009.** Look before you leap: is risk of injury a foraging cost? *Behavioral Ecology and Sociobiology* **63**: 1821–1827.
- Biewener AA, Alexander R, Heglund NC. 1981.** Elastic energy storage in the hopping of kangaroo rats (*Dipodomys spectabilis*). *Journal of Zoology* **195**: 369–383.
- Biewener AA, Blickhan R. 1988.** Kangaroo rat locomotion: design for elastic energy storage or acceleration? *The Journal of Experimental Biology* **140**: 243–255.
- Bro-Jørgensen J. 2013.** Evolution of sprint speed in African savannah herbivores in relation to predation. *Evolution* **67**: 3371–3376.
- Brown JS. 1999.** Vigilance, patch use and habitat selection: foraging under predation risk. *Evolutionary Ecology Research* **1**: 49–71.
- Clark RW. 2006.** Post-strike behavior of timber rattlesnakes (*Crotalus horridus*) during natural predation events. *Ethology* **112**: 1089–1094.
- Clark RW, Dorr SW, Whitford MD, Freymiller GA, Hein SR. 2016.** Comparison of anti-snake displays in the sympatric desert rodents *Xerospermophilus tereticaudus* (round-tailed ground squirrels) and *Dipodomys deserti* (desert kangaroo rats). *Journal of Mammalogy* **97**: 1709–1717.
- Clemente CJ, Wilson RS. 2015.** Balancing biomechanical constraints: optimal escape speeds when there is a trade-off between speed and maneuverability. *Integrative and Comparative Biology* **55**: 1142–1154.
- Combes SA, Rundle DE, Iwasaki JM, Crall JD. 2012.** Linking biomechanics and ecology through predator-prey interactions: flight performance of dragonflies and their prey. *The Journal of Experimental Biology* **215**: 903–913.
- Davis M. 1984.** The mammalian startle response. In: Eaton RC, ed. *Neural mechanisms of startle behavior*. New York: Plenum Press, 287–353.
- Domencici P, Blake RW. 1997.** The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* **200**: 1165–1178.
- Funk RS. 1965.** Food of *Crotalus cerastes laterorepens* in Yuma County, Arizona. *Herpetologica* **21**: 15–17.
- Hawlena D, Kress H, Dufresne ER, Schmitz OJ. 2011.** Grasshoppers alter jumping biomechanics to enhance escape performance under chronic risk of spider predation. *Functional Ecology* **25**: 279–288.
- Hayes WK. 1993.** Effects of hunger on striking, prey-handling, and venom expenditure of prairie rattlesnakes (*Crotalus v. viridis*). *Herpetologica* **49**: 305–310.
- Higham TE, Clark RW, Collins CE, Whitford MD, Freymiller GA. 2017.** Rattlesnakes are extremely fast and variable when striking at kangaroo rats in nature: three-dimensional high-speed kinematics at night. *Scientific Reports* **7**: 40412.
- Higham TE, Day SW, Wainwright PC. 2006.** Multidimensional analysis of suction feeding performance in fishes: fluid speed, acceleration, strike accuracy and the ingested volume of water. *The Journal of Experimental Biology* **209**: 2713–2725.
- Higham TE, Irschick DJ. 2013.** Springs, steroids, and slingshots: the roles of enhancers and constraints in animal movement. *Journal of Comparative Physiology, B* **183**: 583–595.
- Husak JF, Irschick DJ. 2009.** Steroid use and human performance: lessons for integrative biologists. *Integrative and Comparative Biology* **49**: 354–364.

- Irschick DJ. 2003.** Measuring performance in nature: implications for studies of fitness within populations. *Integrative and Comparative Biology* **43**: 396–407.
- Irschick DJ, Higham TE. 2016.** *Animal athletes: an ecological and evolutionary approach*. Oxford: Oxford University Press.
- Irschick DJ, Losos JB. 1998.** A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* **52**: 219–226.
- Kardong KV, Bels VL. 1998.** Rattlesnake strike behavior: kinematics. *Journal of Experimental Biology* **201**: 837–850.
- Klukowski M, Jenkinson NM, Nelson CE. 1998.** Effects of testosterone on locomotor performance and growth in field-active northern fence lizards, *Sceloporus undulatus hyacinthinus*. *Physiological Zoology* **71**: 506–514.
- Kotler BP, Brown JS, Bouskila A. 2004.** Apprehension and time allocation in gerbils: the effects of predatory risk and energetic state. *Ecology* **85**: 917–922.
- Kotler BP, Brown JS, Dall SR, Gresser S, Ganey D, Bouskila A. 2002.** Foraging games between gerbils and their predators: temporal dynamics of resource depletion and apprehension in gerbils. *Evolutionary Ecology Research* **4**: 495–518.
- Lima SL. 2002.** Putting predators back into behavioral predator-prey interactions. *Trends in Ecology & Evolution* **17**: 70–75.
- Lima SL, Bednekoff PA. 1999.** Back to the basics of anti-predatory vigilance: can nonvigilant animals detect attack? *Animal Behaviour* **58**: 537–543.
- Lima SL, Dill LM. 1990.** Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**: 619–640.
- Osborne JW. 2010.** Improving your data transformations: applying the Box-Cox transformation. *Practical Assessment, Research, and Evaluation* **15**: 1–9.
- Penning DA, Sawvel B, Moon BR. 2016.** Debunking the viper's strike: harmless snakes kill a common assumption. *Biology Letters* **12**: 20160011.
- Poppinga S, Weiskopf C, Westermeier AS, Masselter T, Speck T. 2015.** Fastest predators in plant kingdom: functional morphology and biomechanics of suction traps found in the largest genus of carnivorous plants. *AoB Plants* **8**: plv140.
- Putman BJ, Clark RW. 2015.** The fear of unseen predators: ground squirrel tail flagging in the absence of snakes signals vigilance. *Behavioral Ecology* **26**: 185–193.
- Randall JA. 1993.** Behavioural adaptations of desert rodents (Heteromyidae). *Animal Behavior* **45**: 263–287.
- Randall JA, Boltas King DK. 2001.** Assessment and defence of solitary kangaroo rats under risk of predation by snakes. *Animal Behavior* **61**: 579–587.
- Romero LM, Butler LK. 2007.** Endocrinology of stress. *International Journal of Comparative Psychology* **20**: 89–95.
- Schaller GB. 1968.** Hunting behaviour of the cheetah in the Serengeti National Park, Tanzania. *African Journal of Ecology* **6**: 95–100.
- Schneiderman N, Ironson G, Siegel SD. 2005.** Stress and health: psychological, behavioral, and biological determinants. *Annual Review of Clinical Psychology* **1**: 607–628.
- Sharp NC. 2012.** Animal athletes: a performance review. *The Veterinary Record* **171**: 87–94.
- Steiner UK, Pfeiffer T. 2007.** Optimizing time and resource allocation trade-offs for investment into morphological and behavioral defense. *The American Naturalist* **169**: 118–129.
- Stewart WJ, Nair A, Jiang H, McHenry MJ. 2014.** Prey fish escape by sensing the bow wave of a predator. *The Journal of Experimental Biology* **217**: 4328–4336.
- Thorson JM, Morgan RA, Brown JS, Norman JE. 1998.** Direct and indirect cues of predatory risk and patch use by fox squirrels and thirteen-lined ground squirrels. *Behavioral Ecology* **9**: 151–157.
- Webster DB. 1962.** A function of the enlarged middle-ear cavities of the kangaroo rat, *Dipodomys*. *Physiological Zoology* **35**: 248–255.
- Webster DB, Webster M. 1984.** The specialized auditory system of kangaroo rats. In: Neff WD, ed. *Contributions to sensory physiology, Vol. 8*. Cambridge: Academic Press, 161–196.
- West K, Cohen A, Baron M. 1991.** Morphology and behavior of crabs and gastropods from lake Tanganyika, Africa: implications for lacustrine predator-prey coevolution. *Evolution* **45**: 589–607.
- Whitford MD, Freymiller GA, Clark RW. 2017.** Avoiding the serpent's tooth: predator-prey interactions between free-ranging sidewinder rattlesnakes and desert kangaroo rats. *Animal Behaviour* **130**: 73–78.
- Wilson RS, Husak JF, Halsey LG, Clemente CJ. 2015.** Predicting the movement speeds of animals in natural environments. *Integrative and Comparative Biology* **55**: 1125–1141.
- Yilmaz M, Meister M. 2013.** Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology* **23**: 2011–2015.