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Factors That Influence the Performance of Complex Behaviors in a Terrestrial Vertebrate: Variability in the Kangaroo Rat Evasive Leap

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Factors That Influence the Performance of Complex Behaviors in a Terrestrial Vertebrate: Variability in the Kangaroo Rat Evasive Leap

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of the requirements for the degree of

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in

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by

Grace Freymiller

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ABSTRACT OF THE DISSERTATION

Factors That Influence the Performance of Complex Behaviors in a Terrestrial Vertebrate: Variability in the Kangaroo Rat Evasive Leap

by

Grace Freymiller

Doctor of Philosophy, Graduate Program in Evolutionary Biology
University of California, Riverside and San Diego State University, June 2021
Dr. Rulon Clark and Dr. Timothy Higham, Co-Chairpersons

Physical performance inextricably ties variation in physiology, morphology, and behavior to fitness. By studying how morphology is linked to performance, and the consequences of performance ability on survival and reproduction, evolutionary biologists can understand the selection pressures driving the evolution of particular morphological characteristics. However, many studies of performance are conducted in the lab in order to control external factors, allowing for detailed analyses of a few potentially influential variables but reducing the overall ecological realism of the results. The capacity for field studies to capture natural levels of variability helps to elucidate important ecological factors that influence locomotor performance and therefore drive individual fitness. This dissertation explores the evolution of bipedalism in rodents and the factors that affect their jump performance using a field-based approach. To do this, I investigated (1) the natural jump performance of kangaroo rats (*Dipodomys*) by filming their escapes from rattlesnake strikes, (2) the conditions under

which they utilize these impressive jump escapes, (3) the relationship between hindlimb morphology and jump performance, and (4) how jump performance compares between bipedal and quadrupedal rodents. From these studies, I was able to determine their natural levels of performance, the ecological contexts that place selective pressure on jump performance, how those pressures on performance have shaped the evolution of their hindlimb morphology, and whether bipedality indeed confers improved jump performance when compared to quadrupedal rodents.

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INTRODUCTION

Since Aristotle first began viewing animals as machines through studies of their anatomy and movement, humans have had a keen interest in understanding the biomechanics of animal locomotion. Most animals rely on locomotion at some point during their lives, and their ability to perform affects foraging success, predator evasion, and whether they find mates and suitable habitat. Performance is generally defined as the ability to execute an ecologically relevant act (Arnold 1983) and it inextricably ties variation in physiology, morphology, and behavior to fitness (Arnold 1983, Garland & Losos 1994). For example, performance plays an important role in evolution: differing performance abilities that arise through local adaptation to new environments have been suggested as a mechanism for population divergence and speciation (Higham et al. 2016). Additionally, studies of courtship displays in Anna's hummingbirds (*Calypte anna*) demonstrate how behavioral ecology and performance interact by revealing that female preference is capable of driving the evolution of extreme physical performance (Clark 2009). Furthermore, understanding performance limits may provide insight into demographic patterns and other ecological measures (Costa et al. 2004). Physiology underlies most aspects of performance as muscle function, for example, is highly temperature-dependent (e.g. Ranatunga 1982). Due to these various links, studying performance often requires an integrative, multi-disciplinary approach, and the results have unequivocal impacts for numerous fields of biology.

One of the major goals within evolutionary biology is to understand why organisms look the way they do and the role natural selection plays in driving the diversity of body plans observed in nature. Addressing these questions often involves detailed examination of performance and biomechanics as natural selection does not act directly on the form of an animal, but rather on the performance capabilities that specific morphologies confer (Arnold 1983, Jayne & Bennett 1990, Garland & Losos 1994, Strobbe et al. 2009). Morphology therefore indirectly influences fitness by directly affecting the physical performance of an animal. By studying how morphology is linked to performance, and the consequences of performance ability on survival and reproduction, evolutionary biologists can understand the selection pressures driving the evolution of particular morphological characteristics.

Studies of locomotor performance are clearly pivotal in furthering our understanding of how organisms interact with the environment and how evolution creates diversity, they have come a long way in linking behavior, physiology, morphology, and evolution. However, there is still a need for studies that examine performance from both field-based and evolutionary perspectives. Studies of wild animals often find that performance is strategically altered based on the conditions surrounding an interaction; however, many studies of performance are conducted in the lab in order to control external factors, allowing for detailed analyses of a few potentially influential variables but reducing the overall ecological realism of the results (Irschick & Garland 2001, Domenici et al. 2011*a,b*). The capacity for field studies to capture natural levels of variability helps to elucidate important ecological factors that influence locomotor performance and therefore drive individual fitness. Likewise, utilizing evolutionary approaches to study performance, such as multi-species comparisons, can uncover selective processes that underlie morphological evolution, which would be unattainable with only one species.

This dissertation explores the evolution of bipedalism in rodents and the factors that affect their jump performance using a field-based approach. There are four extant lineages of bipedal rodent, making it an important example of convergent evolution (Berman, 1985). Understanding why selection favored this morphology multiple times throughout the evolutionary history of rodents is therefore of particular interest to comparative and evolutionary biologists. Unlike other bipedal hopping mammals, rodents gain no energetic benefit from this mode of locomotion (Thompson et al. 1980), and there is no evidence to support the hypothesis that bipedalism allowed for forelimb specialization in these groups (Price 1993). The most likely advantage of bipedalism for these animals is an ability to evade predators with a rapid, powerful jump (Wu et al. 2014; hypotheses reviewed in McGowan & Collins 2018). In order to explore this hypothesis, I investigated (1) the natural jump performance of kangaroo rats (*Dipodomys*) by filming their escapes from rattlesnake strikes, (2) the conditions under which they utilize these impressive jump escapes, (3) the relationship between hindlimb morphology and jump performance, and (4) how jump performance compares between bipedal and quadrupedal rodents. From these studies, I was able to determine their natural levels of performance, the ecological contexts that place selective pressure on jump performance, how those pressures on performance have shaped the evolution of their hindlimb morphology, and whether bipedality indeed confers improved jump performance when compared to quadrupedal rodents.

References

Arnold SJ. 1983. Morphology, performance, and fitness. *American Zoologist* 23:347-361.

Berman SL. 1985. Convergent evolution in the hindlimb of bipedal rodents. *Journal of Zoological Systematics and Evolutionary Research* 23:59-77.

Blob RW, Higham TE. 2014. Terrestrial locomotion—where do we stand, where are we going? An introduction to the symposium. *Integrative and Comparative Biology* 54:1051-1057.

Clark CJ. 2009. Courtship dives of Anna’s hummingbirds offer insights into flight performance limits. *Proceedings of The Royal Society of London B: Biological Sciences* 276:3047-3052.

Costa DP, Kuhn CE, Weise MJ, Shaffer SA, Arnould JPY. 2004. When does physiology limit the foraging behaviour of freely diving mammals? *International Congress Series* 1275:359-366.

Domenici P, Blagburn JM, Bacon JP. 2011*a*. Animal escapology I: theoretical issues and emerging trends in escape trajectories. *Journal of Experimental Biology* 214:2463-2473.

Domenici P, Blagburn JM, Bacon JP. 2011*b*. Animal escapology II: escape trajectory case studies. *Journal of Experimental Biology* 214:2474-2494.

Garland T, Jr., Losos JB. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, PC and Reilly, SM, eds. *Ecological morphology: integrative organismal biology.* Chicago: University of Chicago Press, 240-302.

Higham TE, Rogers SM, Langerhans RB, Jamniczky HA, Lauder GV, Stewart WH, Martin CH, Reznick DN. 2016. Speciation through the lens of biomechanics: locomotion, prey capture and reproductive isolation. *Proceedings of The Royal Society of London B: Biological Sciences* 283: rspb.2016.1294.

Irschick DJ, Garland, T, Jr. 2001. Integrating function and ecology in studies of adaptation: investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics* 32:367-396.

Jayne BC, Bennett AF. 1990. Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* 44:1204-1229.

McGowan CP, Collins CE. 2018. Why do mammals hop? Understanding the ecology, biomechanics and evolution of bipedal hopping. *Journal of Experimental Biology* 221: jeb161661.

Price MV. 1993. A functional-morphometric analysis of forelimbs in bipedal and quadrupedal heteromyid rodents. *Biological Journal of the Linnean Society* 50:339-360.

Ranatunga KW. 1982. Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *The Journal of Physiology* 329:465-483.

Strobbe F, McPeek MA, de Block M, de Meester L, Stoks R. 2009. Survival selection on escape performance and its underlying phenotypic traits: a case of many-to-one mapping. *Journal of Evolutionary Biology* 22:1172-1182.

Thompson SD, MacMillen RE, Burke EM, Taylor CR. 1980. The energetic cost of bipedal hopping in small mammals. *Nature* 287:223-224.

Wu S, Zhang F, Edwards SV, Wu W, Ye J, Bi S, Ni X, Quan C, Meng J, Organ CL. 2014. The evolution of bipedalism in jerboas (rodentia: Dipodoidea): origin in humid and forested environments. *Evolution* 68:2108-2118.

Escape dynamics of free-ranging desert kangaroo rats (Rodentia: Heteromyidae) evading rattlesnake strikes

Running title: Kangaroo rat escape dynamics

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Abstract

Many animals exhibit morphological specializations driven by the extreme selective pressure of predation, and understanding how such specializations shape escape behaviors can elucidate the evolutionary context of these morphologies. We examined the kinematics of the evasive leaps of desert kangaroo rats (*Dipodomys deserti*) during strikes from sidewinder rattlesnakes (*Crotalus cerastes*) to understand the potential importance of predator evasion in shaping bipedalism in desert rodents. We found that kangaroo rats escaping from snake strikes rely on rapid response times to initiate effective evasions. During jumps, their enlarged hindlimbs propel vertical leaps that were multiple body lengths into the air, and these leaps were often accompanied by mid-air kicks and other maneuvers that deterred snakes. Although we found high levels of variability in kinematic factors, all kangaroo rats which successfully evaded attacks escaped in a path away from the snake and thus did not have random/protean escape trajectories. In general, our findings support the idea that bipedalism, which has evolved independently in several desert rodent lineages, may be favored because it allows for rapid and powerful vertical leaps that are crucial for avoiding ambush predators such as vipers and owls.

Introduction

Physical performance inextricably links morphology and fitness. Performance is generally defined as the ability to execute an ecologically relevant act (Arnold 1983) and is frequently used to characterize the locomotor abilities of animals as they either flee from a threat or pursue prey. Locomotor performance is often examined in the lab to control external factors; this context allows for detailed analyses of a few potentially influential variables, but comes at the cost of reduced ecological realism of the results (Irschick & Garland 2001, Domenici et al. 2011*a*,*b*). For example, sprinting is used in a variety of contexts, and sprint speed varies depending on whether the animal is fleeing from predators, chasing prey, or simply moving from one location to another (Irschick & Losos 1998). Lab-based measurements of performance also frequently focus on "maximal performance" and animals in their natural environments may only occasionally (or never) perform at maximal levels (Hertz et al. 1988, Husak 2006, Combes et al. 2012, Wilson et al. 2018). Trade-offs that are commonplace in nature may be lacking in controlled environments, such as high energetic costs of maximal performance (Taylor et al. 1980) or speed-maneuverability trade-offs in complex habitats (Alexander 1982, Wynn et al. 2015), either of which may prevent animals from performing at maximal capacity in the wild. Alternatively, animals in natural environments may exhibit enhanced performance if they are motivated by factors that do not exist in the laboratory (Moore et al. 2017*b*). Thus, although it is clear that quantifying some aspects of performance requires controlled laboratory settings, measuring performance in free-ranging animals complements laboratory studies by providing a better understanding of the ecological relevance of the morphology and physiology underlying performance.

In situ measures of performance and kinematics (i.e., quantitative measure of motion) are particularly important for predator-prey interactions given the high costs for prey associated with predation and the motivation to perform maximally. The strong selective pressure to detect or avoid predators has produced some of the fastest sensory responses and motor actions in nature; these mechanisms often result from changes to morphology (Dayton et al. 2005) and fine-tuning of physiological systems (Domenici & Blake 1997, Jacobs 1995). Therefore, the aspects of an organism's morphology that enhance performance are intimately linked to survival and fitness. For example, crucian carp (*Carassius carassius*, Linnaeus) exhibit intraspecific variation in body shape, where the deep-bodied phenotype (elongated dorso-ventrally) improves escape performance compared to the shallow-bodied phenotype (Domenici et al. 2008). Instances such as this, where organisms are morphologically specialized for predator evasion, provide model systems to explore how performance links morphology to fitness.

Kangaroo rats (*Dipodomys* spp.) are an abundant and widespread radiation of bipedal rodents common throughout arid environments of North America, and they exhibit several specialized adaptations for avoiding predatory attacks. First, they have enlarged auditory bullae which allow them to hear low-frequency sounds often produced by the sudden attack of an ambush predator (e.g. rattlesnake striking or owl swooping) which is key in predator evasion (Webster 1962, Webster & Webster 1971). Second, their enlarged hindlimb muscles and thick tendons are important for producing and withstanding high acceleration during the rapid and forceful jumps used to evade predator attacks (Biewener & Blickhan 1988). Bipedality evolved four times independently within Rodentia (kangaroo rats, jerboas, springhares, and jumping mice), presumably as an adaptation for vertical leaping and predator evasion (McGowan & Collins 2018). Although there are some recent studies of how jerboa locomotion and predator evasion is influenced and driven by this unique morphology (Moore et al. 2017*a*,*b*) and a series of studies exploring the sensory basis for predator avoidance in kangaroo rats (Webster 1962, Webster & Webster 1971), no field-based studies have quantitatively analyzed the biomechanics of the forceful evasive jumps made by bipedal rodents leaping away from ambush predators.

The goals of this study were to examine how kangaroo rats avoid the rapid strike of rattlesnakes during natural encounters and quantify several aspects of these evasions to better understand how the kangaroo rat's specialized morphology may underlie its extraordinary performance during predator evasion. Using 3D high-speed videos, we analyzed several key kinematic details of the evasive jumps used by free-ranging desert kangaroo rats (*Dipodomys deserti,* Stephens) that successfully escaped rattlesnake (*Crotalus cerastes,* Hallowell) strikes in order to determine how these leaps vary under natural conditions and provide new insights into predator evasion by bipedal rodents. We predicted that desert kangaroo rats would exhibit greater evasive jumping abilities compared to other small mammals. We also predicted that they would display truly protean (i.e., random) escape trajectories by occasionally escaping towards/over the snake because 1) other rodents have been described as having protean escape paths (Domenici et al. 2011*b*) and 2) escape trajectories should, in theory, be highly variable during rapid predator-prey interactions such as those between rattlesnakes and their prey (Domenici et al. 2011*a*). This study is an important next step towards understanding why bipedalism convergently evolved in desert rodents as it elucidates how this morphology aids in avoiding ambush predators and provides information necessary to quantify the fitness consequences of high levels of performance.

Methods

Study Site

Our study took place on the southwestern side of the Barry M. Goldwater Range in Yuma, Arizona, USA (32° 22' 13.508" N, 114° 22' 23.783" W) which is managed by the United States Marine Corps. The site is bisected by a dirt road, separating the site into two distinct habitats: wind-blown sand dunes to the west and creosote scrub (*Larrea tridentate,* Coville) to the east (Malusa & Sundt 2015). Data were collected from mid-May to early August in 2016. All interactions were recorded between sunset and sunrise, as both rattlesnakes and kangaroo rats are nocturnal at this time of year.

Study Animals

All procedures were approved by the San Diego State University Institutional Animal Care and Use Committee (APF 16-08-014C). We first located sidewinder rattlesnakes by following the distinct tracks they leave in the sand. Adults were captured and surgically implanted with temperature-sensitive radio transmitters following the methods of Reinert & Cundall (1982). While anesthetized for surgery, we measured mass to the nearest gram, sex, snout-vent length, tail length, head length and width, and the width of each rattle segment, all to the nearest mm. Once normal activities were resumed, snakes were released at the site of capture.

Kangaroo rats were trapped using Sherman live traps baited with black oil sunflower seed placed adjacent to *D. deserti* burrow systems. We marked kangaroo rats with 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. During the marking process, we recorded sex, mass to the nearest gram, and snout-anus length, tail length, and hind foot length all to the nearest mm. All individuals were processed in the field and released immediately at the site of capture.

Filming interactions

We used a modified version of the methods used by Whitford et al. (2017) and Higham et al. (2017) to record natural interactions between free-ranging sidewinders and desert kangaroo rats. Rattlesnakes with transmitters were tracked at least once nightly via radio telemetry. When a telemetered snake was found hunting on the surface, we moved recording equipment to the snake's location. Two tethered high-speed cameras (Edgertronic, model SC1) recording at 500 Hz and four to six infrared lights were positioned approximately 3 m away from the ambushing snake. The cameras were connected to laptop computers via 100 ft Ethernet cables, which allowed the observers to remain a minimum of 20 m away from the snake. Observers watched the live video feed on the laptops until either an interaction with a kangaroo rat occurred or the snake abandoned ambush. Immediately after snake strikes, observers triggered cameras to save the preceding 10 seconds of footage. We encouraged the aboveground movements of kangaroo rats at our site by sprinkling small amounts of black oil sunflower seed (5-10 g), thereby increasing kangaroo rat foraging activity in the area. Although placing seed increases the foraging movements of kangaroo rats, we do not believe this impacts their ability to perceive and escape snake strikes. In our previous studies of natural encounters between rattlesnakes and kangaroo rats (Clark et al. 2016, Whitford et al. 2017, Higham et al. 2017) we found that kangaroo rats respond to strikes similarly regardless of the presence of supplemental seed in the vicinity. Furthermore, our experimental study of kangaroo rat escape performance (Freymiller et al. 2017) found that head position (i.e., down/foraging or up/alert) does not alter kangaroo rat escape kinematics. Due to the high density of desert kangaroo rats at this field site, most of the kangaroo rats that interacted with snakes were not marked despite intensive trapping efforts. However, unmarked kangaroo rats are highly likely to be unique individuals since our recording locations were typically hundreds of meters apart and desert kangaroo rat home ranges are approximately 120 m wide (Langley 1994). Following recording of interactions, we calibrated the video frames with a large object of known dimensions (metal rods screwed in place and fixed to a 30 x 25 cm metal plate) placed in the space where the strike occurred.

Video and statistical analyses

All videos were calibrated and digitized in MATLAB (R2016b) using the software DltDataviewer, version 5 (Hedrick 2008). In order to measure velocity of the kangaroo rats, we digitized a point on the back of the head in between the ears, then applied a quintic spline to the raw data using the package "pspline" in RStudio (version 0.99.473). We used a generalized cross validation smoothing parameter (Walker 1998) to avoid introducing bias with hand-selected smoothing parameters and to ensure our results were reproducible. We then took the first derivative of the splined data to obtain velocity. Due to the high levels of noise in the data and the amplification of noise with each derivative, our field data was not suitable for calculating acceleration values.

We calculated take-off angle as the angle between a point on the foot immediately prior to toe-off, the back of the head 60 ms after toe-off, and a point on the ground. We then used the coordinates for those points to calculate the three-dimensional jump angle. We chose to use the back of the head instead of the center of mass as this is a more easily distinguishable landmark on the body, and we chose 60 ms after toe-off instead of the highest point of the jump because kangaroo rats often jumped off screen by the height of the jump. The ground point was placed such that the kangaroo rat's body was between the snake and the ground point, so jumps away from the snake would have angles <90°.

We recorded reaction time as the amount of time between the first movement of the snake's strike and the first movement of the kangaroo rat's evasion. We also recorded the amount of time the kangaroo rat remained airborne by measuring the amount of time between toe-off and touch down. Ground contact time was measured as the time between the kangaroo rat's first visible reaction and toe-off. As kangaroo rats often jumped out of the camera view during the highest point of the evasive leap, we calculated jump height using the time spent in air with the following equation:

$$Height\ (m) = \frac{(9.81\ m \cdot s^{-2}\ \times\ \frac{time\ in\ air\ (s)}{2})^2}{2\ \times\ 9.81\ m \cdot s^{-2}}$$

Lastly, we quantified escape trajectory as the angle between the path of the kangaroo rat's evasion and the strike trajectory in the horizontal plane (i.e., only two dimensions). To do this, we created two vectors: one between the snake's head and the kangaroo rat's head in the frame of first kangaroo rat reaction (strike trajectory), and one between the kangaroo rat's head in the frame of the first reaction and the kangaroo rat's head from either the frame of landing or, if it landed off screen, the last frame in which the kangaroo rat's head was visible (kangaroo rat's evasion). We tested escape trajectory randomness across a 360° circle using a Rao's spacing test of uniformity (Pewsey et al. 2013). All values are reported as mean ± standard error.

Results

We recorded 32 strikes, 15 "hits" (rattlesnakes contacting and biting kangaroo rats), and 17 "misses" (snakes did not physically contact kangaroo rats). As the snakes often made contact with the kangaroo rat prior to toe-off and this contact influenced the kangaroo rats' movements, we excluded hits from our analyses. We also removed six misses from quantitative analyses for the following reasons: one miss was removed because the cameras moved slightly between the strike and calibration and therefore we could not make accurate measurements. Two misses were removed because the kangaroo rats' evasion maneuvers were so extreme that the body could not be digitized properly. Two additional misses were removed because the kangaroo rats were not in strike range and did not perform evasive maneuvers (i.e., snakes struck prematurely and reached maximum extension well short of kangaroo rats). Lastly, one miss was removed because the kangaroo rat immediately jumped off screen, preventing analysis. Thus, we retained 11 misses in our analyses.

Kangaroo rats avoiding snake strikes exhibited a remarkable ability to move their bodies rapidly out of the initial strike trajectories. Reaction times were highly variable and ranged from 38 ms to 150 ms, with an average of 81 ± 8.7 ms. Mean maximum velocity was 3.5 ± 0.2 $m \cdot s^{-1}$ (range: 2.7 – 4.4 $m \cdot s^{-1}$) and always occurred within 10 ms of toe-off, but was not consistently achieved either before or after toe-off (before toe-off in 45%, after toe-off in 55%). Ground contact time ranged from 28 ms to 46 ms (average: 37.8 ± 2.1 ms). The leaps of kangaroo rats were typically near-vertical and propelled them high into the air to evade the strikes. Successful kangaroo rat evasions had take-off angles ranging from 56° to 97°, with an average of 80 ± 4°. Successful evaders also jumped an average of 0.39 ± 0.05 m in the air (range: 0.16 m – 0.82 m) and spent 0.55 ± 0.04 s airborne (range: 0.36 s – 0.82 s). Lastly, kangaroo rats always jumped in a path away from the snake (Fig. 1.1). Escape trajectory angles varied from 138° to 244°, with an average of 187°. As no kangaroo rats ever evaded towards/over the snake in the horizontal plane, the escape trajectory was not random (i.e., not uniformly spread) across 360° (U=221.3, $p < 0.001$).

Qualitatively, another important aspect of the evasive jump was the high degree of maneuverability displayed by most kangaroo rats, consisting of kicks, flips, twists, body contortions, and other rapid mid-air movements. Torquing of the body in the air appeared to rely on movements of their long tails (Table 1.1). We were unable to quantify these aspects of the evasions, however, as many of the points on the kangaroo rats' bodies would frequently go in and out of frame, preventing more detailed three-dimensional motion analysis.

Discussion

Kangaroo rats that successfully evaded snake strikes exhibited incredible performance, jumping over six body lengths vertically into the air with an average maximum velocity of over 27 body lengths per second, and reacting 3.5 times faster than the average human response time to visual stimuli (Marshall et al. 1998). Despite the obvious importance of evasive jumping as a predator avoidance mechanism, there have been surprisingly few studies of the biomechanics of such maneuvers. Past studies that quantify kangaroo rat and jerboa locomotion have focused mainly on "richochetal" locomotion (i.e., a series of hops used to move between locations in the environment), rather than the "single-shot" explosive escape jumps used to avoid surprise attacks (but see Biewener & Blickhan 1988 and Moore et al. 2017*b*).

Although this study provides the first detailed quantitative analysis of the vertical evasive maneuvers made by a bipedal rodent during natural predatory attacks, we are aware of several studies that measure basic kinematics of escape jumps in other rodent species. The quadrupedal jumping mouse (*Zapus trinotatus,* Rhoads), which exhibits morphological specializations for jumping, is capable of attaining take-off velocities ranging from 1.2 to 3.5 $m \cdot s^{-1}$ (Harty & Roberts 2010). Due to the high power outputs calculated in the study, they found support for the utilization of elastic energy during jumping. Studies of bipedal rodents (Lesser Egyptian jerboas, Moore et al. 2017*b*; banner-tailed kangaroo rats, Biewener & Blickhan 1988; desert kangaroo rats, Schwaner et al. 2018), however, have not found evidence for power amplification via elastic energy storage; these species rely solely on power production by their enlarged hindlimb muscles during jumps. Because of this, bipedal rodents are capable of faster jumps with higher force production and accelerations. Our team estimated maximum velocity of *Dipodomys merriami* (Mearns) in Higham et al. (2017) using the same methodology employed here and found a velocity range of 1.5 to 4.5 $m \cdot s^{-1}$, comparable to the values in this study of 2.7 to 4.4 $m \cdot s^{-1}$. These comparisons indicate that, although all five species have been shaped by natural selection into specialized jumpers, bipedal rodents show specializations that favor high force and acceleration for faster, more controlled jumps whereas the quadrupedal jumping mice favor power amplification via elastic mechanisms to compensate for their relatively smaller hindlimb muscle mass.

The remarkable reaction time of kangaroo rats appears to be the crux of their evasion strategy. In Whitford et al. (*in revision*), we compare various performance variables of both snakes and kangaroo rats that potentially influence the outcome of these interactions and found that kangaroo rat reaction time was the main determinant of whether or not a strike would make contact. Reaction times of kangaroo rats that evaded strikes (i.e., the jumps we analyze here) were typically faster than those of kangaroo rats that were bitten, regardless of the distance between the kangaroo rat and the snake. Thus, the effectiveness of a kangaroo rat escape maneuver is largely determined by their ability to initiate a response as rapidly as possible. Interestingly, our estimates of kangaroo rat reactions times to rattlesnake strikes are markedly slower than those we measured in a previous study examining kangaroo rat evasions to an uncoiling metal spring designed to simulate a snake strike (Freymiller et al. 2017). As kangaroo rats likely rely predominantly on acoustic cues to evade predators (Webster 1962), we assume that noise associated with our simulated strike was different form the noise made by an actual rattlesnake strike, due either to mechanical noise associated with the triggering mechanism or a difference in the bow wave of air moved toward the kangaroo rat during the forward motion of the device.

Escape trajectory

The optimal escape path for evading a rattlesnake strike is predicted to be a relatively vertical path that is perpendicular to the oncoming strike (Freymiller et al. 2017, Higham et al. 2017), which was seen in this study. Truly protean escape behaviors should result in a random mixture of escape trajectories, including occasional escapes towards predators (Domenici et al. 2011*a,b*). While ostensibly riskier than escaping away from a predator, this risk would be mitigated when dealing with "single-strike" predators that cannot launch a second attack quickly; additionally, moving towards a predator gives the predator less time to make major adjustments to the attack path, especially if it is moving quickly and must overcome a higher moment of inertia to maneuver (Shifferman & Eilam 2004). As such, we expected that kangaroo rats escaping rattlesnakes (a classic example of a rapid, single-strike predator) would have occasional escapes towards the snake's position. However, we found that in the horizontal plane, kangaroo rat jumps were almost always directed away from the snake and thus were not random. Kangaroo rats do appear to use random, zig-zagging trajectories in the horizontal plane when being chased (Djawdan & Garland 1988), suggesting that bipedal rodents may utilize different escape trajectories depending on the hunting mode of the predator. The strike path of a viper is inherently limited by the body length of the snake and we have never observed rattlesnakes to strike more than once when attacking prey under natural conditions (Clark 2006, Clark et al. 2012, Clark et al. 2016, Putman et al. 2016, Whitford et al. 2017, Whitford et al. *in revision*), whereas cursorial pursuits often take place over longer distances. Previous studies have also noted that kangaroo rats use rapid vertical leaps to escape owls, another common single-strike predator (Webster 1962). Thus, movement into the vertical plane is likely more important when escaping a single-strike, sit-and-wait ambush predator, whereas unpredictability in the horizontal plane may be more important for escaping pursuit predators.

Role of bipedalism in predator evasion

Several studies have found evidence that bipedal rodents are better at predator evasion when compared to their quadrupedal counterparts (Kotler 1985, Longland & Price 1991). Conversely, another study found no difference in predation rate between bipedal and quadrupedal rodents (Kotler et al. 1988). Although very limited data exist for comparison, a series of past studies from our group have recorded various species of quadrupedal small mammals jumping away from rattlesnake strikes, including ground squirrels (*Otospermophilus beecheyi,* Richardson), gray squirrels (*Sciurus carolinensis,* Gmelin), chipmunks *(Tamias striatus,* Linnaeus), woodrats (*Neotoma lepida,* Thomas), field mice

(*Peromyscus* sp.), and voles (*Microtus* sp.) (Clark 2006, Clark et al. 2012, Putman et al. 2016); qualitatively, the evasive jumps of kangaroo rats are unique among these species. Kangaroo rats exhibit more forceful jumps that propel individuals much further and faster from snakes, and we have not observed other species exhibit the mid-air kicks and turns employed by kangaroo rats (see Whitford et al. *in revision* for details of kicking snakes away). Additionally, using an experimental approach Freymiller et al. (2017) found desert kangaroo rats far outperformed California ground squirrels in the speed and force of their evasive jumps. Thus, we hypothesize that bipedalism and enlarged, powerful muscles provide kangaroo rats with a unique ability to 1) produce rapid, vertical evasions, 2) narrowly evade capture via extreme levels of maneuverability, and 3) reduce the likelihood of envenomation by kicking away snakes attempting to embed their fangs.

Vipers present a significant risk in desert environments across the globe as they can occur in high abundances (see Nowak et al. 2008) and specialize on killing and consuming small mammals with rapid envenomating strikes. In order to successfully evade these predators, kangaroo rats employed complex, intricate, and variable maneuvers. We found a high degree of variability between leaps in all of the factors we measured. Some individuals, for example, jumped almost 90° into the air while others jumped at more acute angles away from the snake's body. Although we focused on different forms of evasion, studies of jerboas bounding away from pursuit predators also found a high degree of variability in evasions (Moore et al. 2017*a*). Both kangaroo rats and jerboas execute predominantly muscle-powered leaps: by not having to load tendons for power amplification, these bipedal rodents are capable of performing more rapid and complex maneuvers (Biewener & Blickhan 1988, Moore et al. 2017*b*). When taken together, this evidence supports the idea that powerful and variable jumping to evade predators makes bipedal rodents more difficult to capture and may be a key reason why this unique morphology has evolved independently multiple times in rodents.

Although our video setup was not sufficient for accurately quantifying maneuverability during these evasions, we observed many kangaroo rats narrowly evade the strikes by quickly moving different parts of their bodies out of the strike path. For example, one kangaroo rat rotated its body upside down, kicked the snake's head away from its body, then righted itself before bounding away (Fig. 1.2). All of our evasion sequences exhibited unique elements, underscoring the high degree of maneuverability and acrobatics used to avoid snake strikes. Analyses of these complex maneuvers will be undertaken in the future with a larger number of synchronized cameras to allow for a more intricate examination of the remarkable maneuverability of these rodents. From the qualitative observations, we noted that much of this maneuverability also may stem from their specialized morphology. As they were still at risk from being hit after initiating the jump (see Whitford et al. *in revision*), individuals would frequently rotate and twist their bodies mid-air, potentially with the aid of their long tail (Table 1.1). Lastly, we observed the kangaroo rats using their large hindlimbs to physically kick the snakes away from their bodies and prevent envenomation (Table 1.1). It is also worth noting that bipedal jumping and kicking may be critical for other facets of kangaroo rat life history (Bartholomew & Caswell 1951, Eisenberg 1963, Kenagy 1976); anecdotal recordings of intraspecific interactions which we opportunistically recorded show that jumping and kicking play central roles in antagonistic encounters (Table 1.1).

Conclusion

This study is the first, to our knowledge, to describe the kinematics of evasive leaps by bipedal rodents avoiding actual attacks from predators. In order to successfully evade rattlesnakes, kangaroo rats combine highly enhanced auditory senses with morphological specializations for jumping and kicking, resulting in remarkable levels of physical performance in natural escape maneuvers. Lab-based studies of bipedal rodents have elucidated the relative importance of various muscles and tendons to jumping, and they provide a framework for understanding why bipedalism has evolved several times in desert rodents. Taking these studies into the field (an “ecomechanical” approach) further underscores the importance of predator evasion in the evolution of bipedalism in small desert mammals.

References

Alexander RM. 1982. *Locomotion of animals*. London: Blackie & Son Limited.

Arnold SJ. 1983. Morphology, performance, and fitness. *American Zoologist* 23: 347-361.

Bartholomew GA, Jr., Caswell HH, Jr. 1951. Locomotion in kangaroo rats and its adaptive significance. Journal of Mammalogy 32: 155-169.

Biewener AA, Blickhan R. 1988. Kangaroo rat locomotion: design for elastic energy storage or acceleration? *Journal of Experimental Biology* 140: 243–255.

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, Putman BJ. 2016. Activity cycles and foraging behaviors of free-ranging sidewinder rattlesnakes (*Crotalus cerastes*): the ontogeny of hunting in a precocial vertebrate. *Zoology* 119: 196-206.

Clark RW, Tangco S, Barbour MA. 2012. Field video recordings reveal factors influencing predatory strike success of free-ranging rattlesnakes (*Crotalus* spp.). *Animal Behaviour* 84: 183-190.

Combes SA, Rundle DE, Iwasaki JM, Crall JD. 2012. Linking biomechanics and ecology through predator-prey interactions: flight performance of dragonflies and their prey. *Journal of Experimental Biology* 215: 903-913.

Dayton GH, Saenz D, Baum KA, Langerhans RB, DeWitt TJ. 2005. Body shape, burst speed and escape behavior of larval anurans. *Oikos* 111: 582-591.

Djawdan M, Garland, Jr. T. 1988. Coexistence of bipedal and quadrupedal rodents: relevance of maximal running speeds. *Journal of Mammalogy* 69: 765-772.

Domenici P, Blake RW. 1997. The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* 200: 1165-1178.

Domenici P, Turesson H, Brodersen J, Brönmark C. 2008. Predator-induced morphology enhances escape locomotion in crucian carp. *Proceedings of the Royal Society of London B: Biological Sciences* 275: 195-201.

Eisenberg JF. 1963. *The behavior of heteromyid rodents*. Oakland: University of California Press.

Freymiller GA, Whitford MD, Higham TE, Clark RW. 2017. Recent interactions with snakes enhance escape performance of desert kangaroo rats (Rodentia: Heteromyidae) during simulated attacks. *Biological Journal of the Linnean Society* 122: 651-660.

Harty TH, Roberts TJ. 2010. Jump energetics and elastic mechanisms in the pacific jumping mouse (*Zapus trinotatus*). Unpublished D. Phil. Thesis, Oregon State University.

Hedrick TL. 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration & Biomimetics* 3, 034001.

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.

Husak JF. 2006. Does survival depend on how fast you can run or how fast you do run? *Functional Ecology* 20: 1080-1086.

Irschick DJ, Losos JB. 1998. A comparative analysis of the ecological significance of maximal locomotor performance in Caribbean *Anolis* lizards. *Evolution* 52: 219-226.

Jacobs GA. 1995. Detection and analysis of air currents by crickets. *BioScience* 45: 776-785.

Kenagy GJ. 1976. Field observations of male fighting, drumming, and copulation in the Great Basin kangaroo rat, *Dipodomys microps*. Journal of Mammalogy 57: 781-785.

Kotler BP. 1985. Owl predation on desert rodents which differ in morphology and behavior. *Journal of Mammalogy* 66: 824-828.

Kotler BP, Brown JS, Smith RJ, Wirtz, II WO. 1988. The effects of morphology and body size on rates of owl predation on desert rodents. *Oikos* 53: 145-152.

Langley CM. 1994. Spatial memory in the desert kangaroo rat (*Dipodomys deserti*). *Journal of Comparative Psychology* 108: 3-14.

Longland WS, Price MV. 1991. Direct observations of owls and heteromyid rodents: can predation explain microhabitat use? *Ecology* 72: 2261-2274.

Malusa, J, Sundt P. 2015. Vegetation of the Barry M. Goldwater Range West, Marine Corps Air Station—Yuma, Arizona. *Report submitted to USMC under Cooperative Agreement DACA87-05-H-0018,* doi: 10.13140/RG.2.1.2058.7681

Marshall R, Ming C, Withington DJ, Turner DL. 1998. Visual cues initiate different latency reaction times in finger and respiratory-related motor responses in humans. *Journal of Physiology, London* 511: 102-103.

McGowan CP, Collins CE. 2018. Why do mammals hop? Understanding the ecology, biomechanics and evolution of bipedal hopping. Journal of Experimental Biology 221: [doi: 10.1242/jeb.161661].

Moore TY, Cooper KL, Biewener AA, Vasudevan R. 2017*a*. Unpredictability of escape trajectory explains predator evasion ability and microhabitat preference of desert rodents. *Nature Communications* 8: 440.

Moore TY, Rivera AM, Biewener AA. 2017*b*. Vertical leaping mechanics of the Lesser Egyptian jerboa reveal specialization for maneuverability rather than elastic energy storage. *Frontiers in Zoology* 14: 32.

Nowak EM, Theimer TC, Schuett GW. 2008. Functional and numerical responses of predators: where do vipers fit in the traditional paradigms? *Biological Reviews of the Cambridge Philosophical Society* 83: 601-620.

Pewsey A, Neuhäuser M, Ruxton GD. 2013. *Circular statistics in R*. Oxford: Oxford University Press.

Putman BJ, Barbour MA, Clark RW. 2016. Foraging behavior of free-ranging rattlesnakes (*Crotalus oreganus*) in California ground squirrel (*Otospermophilus beecheyi*) colonies. *Herpetologica* 72: 55-63.

Reinert HK, Cundall D. 1982. An improved surgical implantation method for radio-tracking snakes. *Copeia* 1982: 702-705.

Schwaner MJ, Lin DC, McGowan CP. 2018. Jumping mechanics of desert kangaroo rats. Journal of Experimental Biology 221: [doi: 10.1242/jeb.186700].

Shifferman E, Eilam D. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl (*Tyto alba*) attack. *Journal of Avian Biology* 35: 111-116.

Taylor CR, Heglund NC, McMahon TA, Looney TR. 1980. Energetic cost of generating muscular force during running: a comparison of large and small animals. *Journal of Experimental Biology* 86: 9-18.

Walker JA. 1998. Estimating velocities and accelerations of animal locomotion: a simulation experiment comparing numerical differentiation algorithms. *Journal of Experimental Biology* 201: 981-995.

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. 1971. Adaptive value of hearing and vision in kangaroo rat predator avoidance. *Brain, Behavior and Evolution* 4: 310-322.

Whitford MD, Freymiller GA, Clark RW. 2017. Avoiding the serpents tooth: predator-prey interactions between free-ranging sidewinder rattlesnakes and desert kangaroo rats. *Animal Behaviour* 130: 73-78.

Wynn ML, Clemente C, Nasir AFAA, Wilson RS. 2015. Running faster causes disaster: trade-offs between speed, manoeuvrability and motor control when running around corners in northern quolls (*Dasyurus hallucatus*). *Journal of Experimental Biology* 218: 433-439.

Figures & Tables

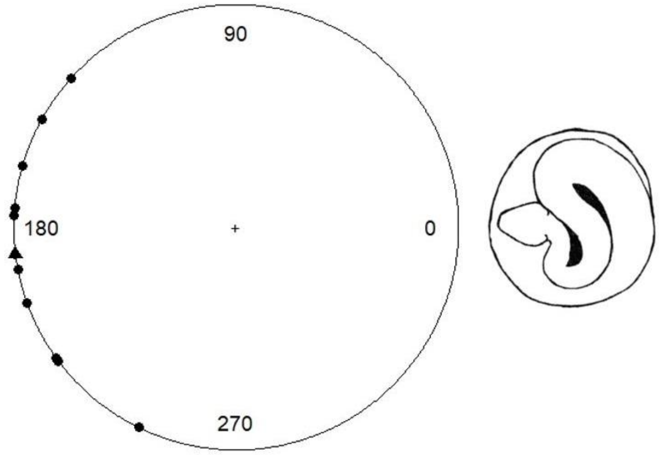

Figure 1.1 Circle plot showing the escape trajectory angles for all eleven kangaroo rats that successfully evaded rattlesnake strikes. Strikes come from 0° and escape trajectory angle (in degrees) for each individual kangaroo rat is plotted along the circumference. No individual ever jumped over/towards the snake, which would be expected if escape trajectories were protean/random. Triangle shows mean escape trajectory angle.

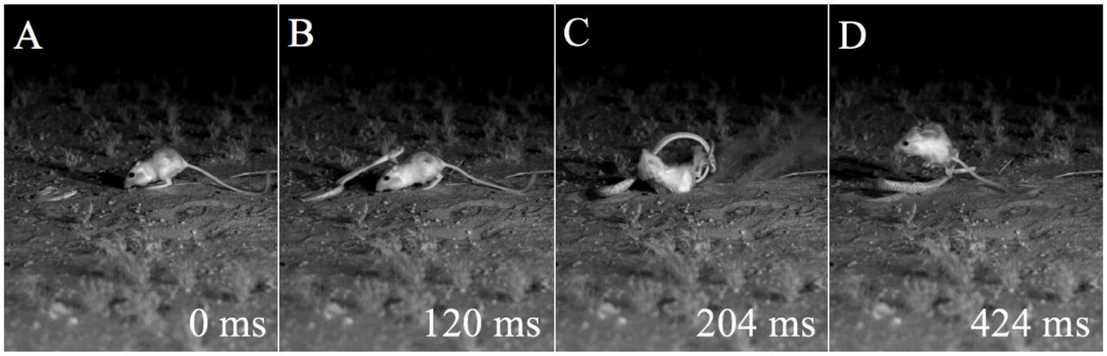

Figure 1.2 Panel of stills showing the extreme maneuverability of desert kangaroo rats during evasions from snakes. A) Snake initiates strike. B) Kangaroo rat begins reaction. C) Kangaroo rat flips upside down and kicks snake away. D) Kangaroo rat rights self before landing. Time (in ms) shown in lower right corner of each image with 0 ms being the moment of strike initiation. Video footage of this interaction viewable at https://youtu.be/y8O5wJIl2sE.

Table 1.1. Video examples of kangaroo rat evasions. All videos filmed at 500 Hz using dual Edgertronic SC-1 cameras.

Description	**Video Link**
Kangaroo rat uses tail to torque body during jump	https://youtu.be/za9rayMFTN8
Kangaroo rat uses hind legs to kick away snake mid-air during leaps	https://youtu.be/og20xrzxugk
Kangaroo rats using mid-air kicks during antagonistic interactions with conspecifics	https://youtu.be/mBSRzN6Pmoo

Recent Interactions with Snakes Enhance Escape Performance of Desert Kangaroo Rats (Rodentia: Heteromyidae) During Simulated Attacks

Running title: Vigilance and anti-predator performance of small mammals

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Abstract

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 behavioral 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 behavioral state can have major effects on performance during predatory encounters. Therefore, under some conditions, laboratory studies of evasive anti-predator maneuvers may underestimate performance in the wild.

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 behavior. Animals often exhibit defenses, 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 maneuvers 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 (Domenici & Blake 1997). This is likely 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 antelope (*Antilocapra Americana,* Ord) are capable of reaching 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 the coevolved 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 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 (Combes et al. 2012, Wilson et al. 2015, Irschick & Losos 1998). 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 behaviors 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 behavior that is underappreciated in the literature on anti-predator behavior. 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 necessitate 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 hindlimbs that have apparently evolved in response to selection favoring 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 *in press*). Kangaroo rats are also known to exhibit a series of stereotyped anti-snake behaviors when in states of heightened vigilance (Randall 1993, Clark et al. 2016), which may include (depending on the species): orienting toward 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 behavioral 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 behaviors 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 maneuvers faster than low vigilance kangaroo rats during a simulated strike, demonstrating that behavioral state can significantly affect prey performance during predator evasions.

Materials and methods

Study Site

Our study site was approximately two miles southwest of the Desert Studies Center in San Bernardino County, California, 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 mm. 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, likely 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 neighbor 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 neighbor 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 toward 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 three seconds. 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 behaviors/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 behavior 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 (Clark et al. 2016, Randall & Boltas King 2001) 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 behaviors (Putman & Clark 2015). When the kangaroo rat returned, we recorded the occurrence of any anti-snake behaviors 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 behavior

We recorded the occurrence of vigilance and anti-snake behavior to determine the degree of vigilance expressed by individuals in each treatment and compare their effects on kangaroo rat escape behavior. Three main anti-snake displays have been described for desert kangaroo rats: foot drumming, sand kicking, and jump backs (Clark et al. 2016, Randall & Boltas King 2001, Whitford et al. *in press*). We also recorded the occurrence of a fourth behavior, 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 behavior in which the kangaroo rat momentarily stops moving and stands bipedally for at least one second 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 behaviors and derive sufficient data to test our hypotheses.

All values are reported as mean ± 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 maneuver. 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 recording) between the frame in which we saw the first movement of the evasive maneuver 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/s^2)

$$\text{Velocity}_\text{i} = \sqrt{\text{Velocity}_\text{h}{}^2 + \text{Velocity}_\text{v}{}^2}$$

$$\text{Jump angle (°)} = \text{atan}\left(\frac{\text{Velocity}_\text{v}}{\text{Velocity}_\text{h}}\right) \text{X} \frac{\pi}{180}$$

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-m^2 grid of 20 cm x 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, and 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 AIC 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 behavior, 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.

Results

Vigilance behavior

In the recent snake treatment, 57% of individuals exhibited at least one of the three main anti-predator displays (Table 2.1, Video 1). Including head-up scanning, 86% of individuals in the recent snake treatment exhibited heightened vigilance behavior. 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. 2.1). 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 x 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. 2.1). 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 ± 2.8°; no snake = 38.0 ± 3.6°; $F_{1,45}$=26.7, p < 0.0001; Fig. 2.2). 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. 2.2). 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 x head position: $F_{1,41}$=03.02, p=0.09; distance x head position: $F_{2,41}$=2.39, p=0.10).

Discussion

After interacting with a rattlesnake, kangaroo rats exhibited higher rates of vigilance behavior when returning to the location of the interaction, often including conspicuous anti-predator displays (Table 2.1, Video 2). Individuals that had recently interacted with a snake out-performed individuals that had not in both reaction time and body displacement time (Fig. 2.1). 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 maneuvers in the high-vigilance treatment are similar to those elicited by natural strikes of free-ranging sidewinder rattlesnakes (Whitford et al. *in press*), 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 2.1, 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 ms 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 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. *in press*), 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 maneuverability 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 likely 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 hindlimbs 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 likely 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 likely 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 maneuver. 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 likely 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 likely 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 likely 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 likely exist. 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, Nelson, & Jenkinson 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. *in press*). 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.

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? *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*. DOI: http://dx.doi.org/10.1093/jmammal/gyw137

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. *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.

Herrel A, Huyghe K, Oković P, Lisičić D, Tadić Z. 2011. Fast and furious: effects of body size on strike performance in an arboreal viper *Trimeresurus* (*Cryptelytrops*) *albolabris*. *Journal of Experimental Biology* 315:22–29.

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. *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.

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*. DOI: 10.1038/srep40412

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, Nelson CE, Jenkinson NM. 1998. Effects of testosterone on locomotor performance and growth in field-active northern fence lizards (*Sceloporus undulates 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, Dill LM. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* 68:619-640.

Lima SL, Bednekoff PA. 1999. Back to the basics of antipredatory vigilance: can nonvigilant animals detect attack? *Animal Behavior* 58:537-543.

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*. DOI 10.1098/rsbl.2016.0011.

Poppinga S, Weisskopf 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*. DOI 10.1093/aobpla/plv140.

Putman BJ, Clark RW. 2015. The fear of unseen predators: ground squirrel tail flagging in the absence of snakes signals vigilance. *Behavioral Ecology*. DOI 10.1093/beheco/aru176.

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. *Veterinary Record* 171:87–94.

Steiner UK, Pfeiffer T. 2007. Optimizing time and resource allocation trade-offs for investment into morphological and behavioral defense. *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. 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.

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.

Figures and Tables

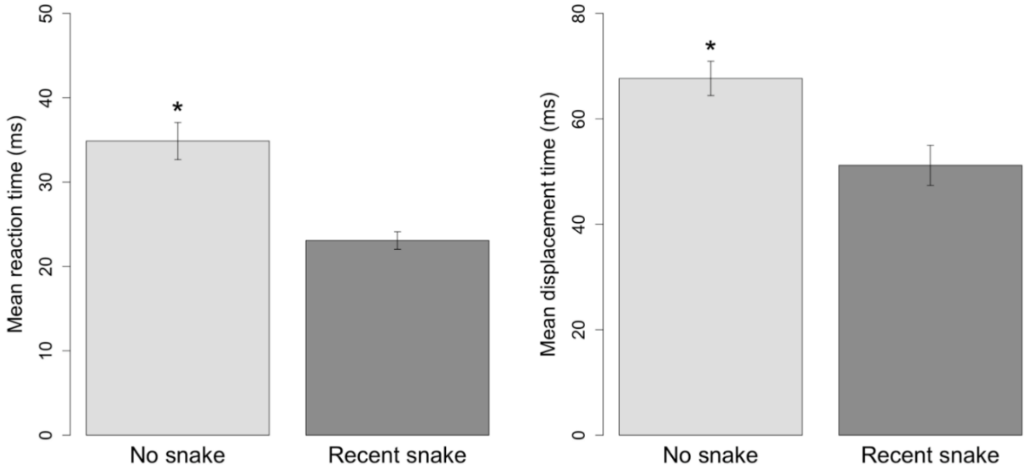

Figure 2.1 Comparison of reaction time and body displacement time between kangaroo rats in the no snake and recent snake treatments. Individuals in the recent snake group reacted sooner and moved out of the 'strike' trajectory faster than those in the no snake group. Error bars indicate standard error.

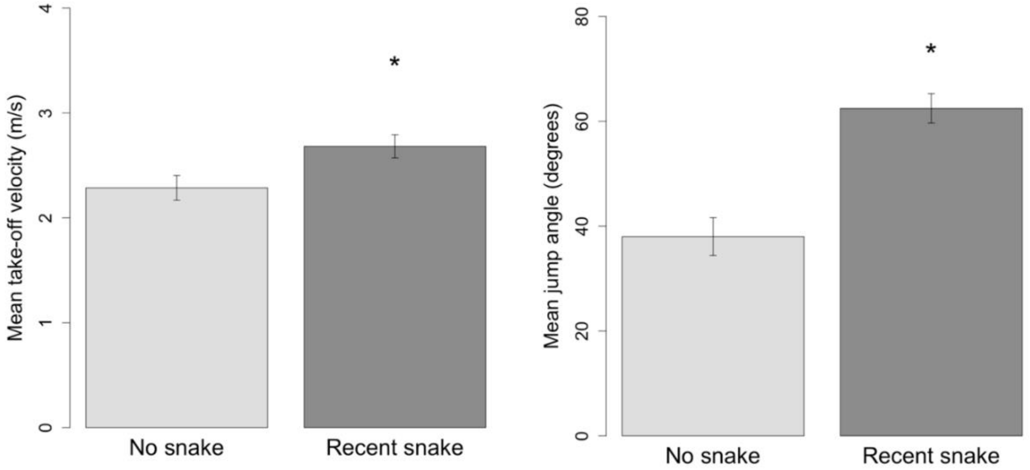

Figure 2.2 Comparison of jump angle relative to ground and take-off velocity between kangaroo rats in the no snake and recent snake treatments. Individuals in the recent snake treatment tended to jump with a greater take-off velocity and at a greater angle relative to the ground, therefore spending more time airborne. Error bars indicate standard error.

Table 2.1 Videos of described kangaroo rat behaviors.

Behavior	**Video Link**
Anti-snake and vigilance head-up behaviors	https://youtu.be/2-iUoSmKLd4
Comparison of pre- and post-snake interaction behavior to RSS	https://youtu.be/qctTWLidtvU
Comparison of evasion between low vigilance and high vigilance rats	https://youtu.be/2IfLsqyl63I

Comparative analysis of *Dipodomys* species indicates that kangaroo rat hindlimb anatomy is adapted for rapid evasive leaping

Running title: Morphology and performance of kangaroo rats

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Abstract

Body size is a key factor that influences antipredator behavior. For animals that rely on jumping to escape from predators, there is a theoretical trade-off between jump distance and quickness (i.e., acceleration) as body size changes at both the inter- and intraspecific levels. Assuming geometric similarity, acceleration will decrease with increasing body size due to a smaller increase in muscle cross-sectional area than body mass. Smaller animals will likely have a similar jump distance as larger animals due to their shorter limbs and faster accelerations. Therefore, in order to maintain quickness (i.e., acceleration) in a jump across different body sizes, hind limbs must be disproportionately bigger for larger animals. We explored this prediction using four species of kangaroo rats (*Dipodomys* spp.), a genus of bipedal rodent with similar morphology across a range of body sizes (40–150 g). Kangaroo rat jump performance was measured by simulating snake strikes to free-ranging individuals. Additionally, morphological measurements of hind limb muscles and segment lengths were obtained from thawed frozen specimens. Overall, jump acceleration was constant across body sizes and jump distance increased with increasing size. Additionally, kangaroo rat hind limb muscle mass and cross-sectional area scaled with positive allometry. Hind limb segment length scaled isometrically, with the exception of the metatarsals, which scaled with negative allometry. Overall, these findings support the hypothesis that kangaroo rat hind limbs are built to maintain jump acceleration rather than jump distance. Selective pressure from single-strike predators, such as snakes and owls, likely drives this relationship.

Introduction

Predation is a fundamental evolutionary force that shapes the behavior and morphology of animals. Factors that influence the dynamics of predator-prey interactions vary from system to system, but physical performance is often a crucial component of a successful antipredator strategy. Performance is directly affected by morphology, so predation thereby shapes morphology through its selective pressure on performance (Arnold, 1983). For example, Bronze Frog tadpoles (*Lithobates clamitans*) exhibit variation in morphology based on the dominant predators in the local habitat; those morphological differences relate directly to fast-start escape performance and presumably the tadpoles' ability to escape from the respective dominant predators (Johnson et al., 2015). Therefore, in order to understand why organisms look the way they do, and the role natural selection plays in driving the diversity of body plans observed in nature, an integrative approach combining detailed examinations of performance and morphology is necessary.

One fundamental aspect of morphology that shapes prey performance is body size (Domenici & Blake, 1993; Martín & López, 1995; Dangles et al., 2007; Landberg & Azizi, 2010). In a variety of comparative studies, relative prey body size can affect both the encounter rate and the capture rate by predators (Kotler et al., 1988; Osenberg & Mittelbach, 1989; Holmes & McCormick, 2009; Asquith & Vonesh, 2012). For animals that rely on jumping to escape from predators, there is a theoretical trade-off between jump distance and quickness (i.e., acceleration) as body size changes. Therefore, the selective pressures on jump escape maneuvers can be inferred by comparing jump performance across individuals of different body sizes at the inter- and/or intraspecific level.

A.V. Hill (1950) laid the foundation on which many studies have tested the competing demands of jump distance and acceleration. In theory, if an animal maintains geometric similarity (isometry) in morphology as it gets larger, the acceleration of the body will be lower; mass (which is proportional to volume) increases more quickly than area, and the force produced by a muscle is proportional to its cross-sectional area (CSA) (Powell et al., 1984). Therefore, larger animals produce proportionally smaller forces, which in turn leads to overall slower accelerations (Hill, 1950). However, larger animals can accelerate for a longer period because they also have longer legs, so both large and small animals achieve similar velocities at the time when the limbs leave the ground (take-off velocity) and therefore jump similar distances. If the purpose of an animal's jump is to put as much distance as possible between predator and prey (i.e., long distance jumps), the capacity of a small animal to jump as far as a larger animal would be most beneficial. On the other hand, if the purpose of the jump is to accelerate out of a predator's attack trajectory as fast as possible (i.e., quick jumps), then muscle CSA should scale with positive allometry, resulting in the maintenance of acceleration across a range of body sizes as well as longer jumps as body size increases. Using this logic, Hill developed a set of hypotheses in which to predict whether the evolution of a species' morphology favors acceleration or distance: animals that rely on acceleration should exhibit positive allometry in muscle CSA in order to maintain acceleration across a range of body sizes, whereas animals that rely on distance should exhibit isometry in limb morphology in order to maintain distance across a range of body sizes. This framework has served as the backbone of numerous studies of jumping in animals, primarily in anurans or invertebrates (e.g. Emerson, 1978; Katz & Gosline, 1993; Wilson et al., 2000; Ryerson, 2013).

Kangaroo rats (*Dipodomys* spp.) are a group of bipedal rodents that exhibit notable variation in body size: the smaller species, such as Merriam's kangaroo rats (*Dipodomys merriami*) average approximately 40 g, whereas the giant kangaroo rat (*Dipodomys ingens*) can reach 160 g. In general, bipedality in rodents appears to have evolved as a means of enhancing predator evasion (McGowan & Collins, 2018; Freymiller et al. *in review*), and kangaroo rats perform impressive, acrobatic leaps that aid in their escape from predators (Webster, 1962; Higham et al., 2017; Whitford et al., 2019). During these escapes, kangaroo rats rely on their large hindlimbs to jump up to a meter into the air with maximal velocities exceeding 4 m/s, which is equivalent to 27 body lengths per second (Freymiller et al., 2017; Freymiller et al., 2019). That said, it is not clear if kangaroo rats are morphologically adapted to optimize jump distance or acceleration.

Kangaroo rats use evasive jumps as their primary means of avoiding the attacks of single-strike ambush predators, such as snakes or owls. Given that attacks from these predators occur in less than a second and over small spatial scales (often less than a meter), we expect that kangaroo rats maximize their ability to displace their body from the attack trajectory as quickly as possible (i.e., acceleration). For example, rattlesnakes can reach their prey in as little as 54 ms once they initiate a strike (Whitford et al. 2019), giving prey very little time to evade the strike, especially after factoring in the prey's reaction time (for kangaroo rats, however, the reaction time can be as short as 8-16 ms; Freymiller et al., 2017; Freymiller et al., *in review*). Furthermore, rattlesnakes rarely initiate a second strike immediately following a first attempt (Kardong & Bels, 1998; Hayes, 2003; Clark, 2006), so a jump maneuver that removes the kangaroo rat from an attack trajectory quickly would be more beneficial than a jump which trades off speed to move the kangaroo rat further from the rattlesnake. Similarly, owls can reach their prey in approximately 1.13 seconds (Ilany & Eilam, 2007) and their swoops typically cover short distances (Edut & Eilam, 2004). Unlike rattlesnakes, owls are capable of initiating a second attack when their first attempt misses, but the window of time between attacks provides prey with an opportunity to escape to cover; therefore, dodging the initial attack as quickly as possible then fleeing to cover would likely increase survival probability more so than jumping far from the owl. As all kangaroo rat species (regardless of body size) are preyed on by snakes, owls, and other ambush predators, it is crucial that larger species can displace their bodies from a predator's attack path just as quickly as smaller species.

In this study, we compared the jump performance and hindlimb morphology of four species of kangaroo rat. We utilized well-established methods to elicit startle responses from kangaroo rats in the field, coupled with morphological analyses of hindlimb muscle mass and segment length. Since kangaroo rats primarily use jump maneuvers when escaping single-strike predators, we hypothesized that kangaroo rat hindlimbs maximize jump acceleration rather than jump distance. Thus, we predicted that the cross-sectional area of kangaroo rat hind limb muscles would scale with positive allometry and the functional segment lengths would scale isometrically, resulting in a maintenance of average acceleration during jumps and an increase in jump distance as body mass increases. We also predicted that positive allometric scaling would be biased towards the proximal muscles, as they produce a large portion of the work during jumps (Schwaner et al. 2018).

Methods

Study Sites and Animals

All methods were approved by the San Diego State University Institutional Animal Care and Use Committee [APF 16-08-014C]. We focused on four species of kangaroo rat: the desert kangaroo rat (*Dipodomys deserti*, DIDE), Merriam's kangaroo rat (*Dipodomys merriami,* DIME), Dulzura kangaroo rat (*Dipodomys simulans,* DISI) and banner-tailed kangaroo rat (*Dipodomys spectabilis,* DISP). These species were chosen as they encompass the relatively large variation in body size among kangaroo rats (average mass range of 40–150 g). Field measurements of performance took place at several locations throughout southwestern North America. Data were collected from mid-May through early August in 2018 at Rodeo, New Mexico, USA (21 DISP, 15 DIME) and in Animas, New Mexico, USA (13 DISP). We collected data from June to July of 2019 in the Mojave Desert of California at a site south of the California State University's Desert Studies Center located in Zzyzx, California, USA (20 DIDE and 15 DIME). Lastly, we attempted to collect data for Dulzura kangaroo rats from mid-March to early May of 2020 in the Rancho Jamul Ecological Reserve, California, USA. However, we were unable to gather performance data for this species, so they were only retained in the morphological analyses.

Kangaroo rats were captured using Sherman live traps baited with sterilized black oil sunflower seeds. Traps were set between sunset and sunrise near burrows. Trapped individuals were sexed and measured (mass, snout-anus length, tail length, and hind foot length), then marked with fingerling ear tags (National Band and Tag #1005-1) and a unique fur dye mark using Nyanzol dye. The fur dye allowed individuals to be visually identified without recapture. Individuals were processed in the field and immediately released at the site of capture. Additionally, two to five specimens for each species were collected from each population for morphological analyses, providing a total of 5 DISP, 6 DIME, 5 DIDE, and 2 DISI. Specimens were either salvaged from incidental mortalities during field data collection or euthanized with isoflurane. Specimens were immediately frozen to preserve the muscles for morphological analyses.

Performance experiments

All performance experiments were conducted in the field with free-ranging individuals. Once a marked kangaroo rat was relocated, an experimental set-up was placed in the vicinity of the known individual's location. The set-up consisted of a rattlesnake strike simulator (RSS), infrared lighting outside of the rodents' visual spectrum (850 nm wavelength), a GoPro video camera (Hero 4 Black) retrofitted with an IR-sensitive lens (Peau Productions, 2.97mm f/4.0 90d HFOV 5MP, no IR filter) and recording at approximately 240 frames per second (fps), and a second IR-sensitive video camera (Sony Handycams, model SR-65 or SR-300) recording at 30 fps. The RSS consists of a one-inch diameter PVC pipe containing a compressed spring that projects a cork toward a target with a peak velocity of 2.8 m/s, approximately the same velocity as a rattlesnake strike (Penning et al., 2016; Higham et al., 2017; Whitford et al 2019). The spring was held in a compressed state with monofilament nylon line that was tied on one end to the spring and on the other end to a camera tripod operated by an observer 3-5 m away.

At the beginning of a trial, the kangaroo rat was encouraged to feed near the RSS by baiting it with sunflower seed. Trials were not conducted on individuals that behaved apprehensively around the RSS (e.g., through anti-predator displays), as vigilance affects jump performance (Putman & Clark, 2015; Freymiller et al., 2017). Once the kangaroo rat left to cache, more seed was placed in a small pile directly in front of the cork. When the kangaroo rat began to feed from the seed pile, the monofilament nylon line was cut which released the spring, and the kangaroo rat's response was recorded. If the kangaroo rat jumped, the jump distance (distance between the take-off and landing positions, measured in meters) was immediately measured in the field with a measuring tape using the video playback of the landing location for guidance. Trials in which the individual did not jump were excluded from analyses. No individual was ever tested twice to prevent the possibility that learning would affect the response to the RSS. All trials were recorded between sunset and sunrise.

In order to calculate average acceleration from the videos, we used the jump distance measured in the field, the amount of time spent airborne (amount of time, in seconds, between take-off and landing), and the contact time (amount of time, in seconds, between the kangaroo rat's first visible movement and toe-off) with the following equations:

$$\mathrm{Velocity_h}\ (\mathrm{m/s}) = \frac{\text{jump distance}}{\text{time spent airborne}}$$

$$\mathrm{Velocity_v}\ (\mathrm{m/s}) = g\left(\frac{\text{time spent airborne}}{2}\right)$$

$$\text{Take off velocity}\ (\mathrm{m/s}) = \sqrt{\mathrm{Velocity_h}^2 + \mathrm{Velocity_v}^2}$$

$$\text{Average acceleration } (\mathrm{m/s^2}) = \frac{\text{Take off velocity}}{\text{Contact time}}$$

Where *g* = acceleration due to gravity (9.8 $\mathrm{m/s^2}$). Video image quality was not sufficient to determine the frame of toe-off and/or the frame in which the kangaroo rat initiated its response for 26 trials out of 84 trials, and therefore we omitted these trials from the acceleration analyses.

Morphological data

Overall body mass and tail length were recorded for each specimen. Muscle and segment length data were collected from the left hindlimb of each specimen. Measurements were made for the major extensors of the hindlimb. Hip extensors included the biceps femoris, semitendinosus, and semimembranosus. Knee extensors included the rectus femoris, vastus lateralis, and vastus medialis. Ankle extensors included the plantaris and the medial and lateral heads of the gastrocnemius. For each individual muscle, we measured the wet mass (g) using an analytical balance (0.001-g precision) and the overall length using digital calipers (0.01-mm precision). For pennate muscles (rectus femoris, vastus lateralis, vastus medialis, medial and lateral gastrocnemius, and plantaris), we also measured the fiber length (mm) with digital calipers and pennation angle (°). Pennation angle was measured by slicing the muscle belly with a scalpel and visually inspecting the fiber directionality, then using a small ruler and protractor to measure the fiber angle. Cross-sectional area (CSA) was calculated in $\mathrm{cm^2}$ for each muscle using the following equation:

$$CSA = \frac{m\ cos\theta}{\rho\ l}$$

Where m is the mass (g), θ is the pennation angle (radians), ρ is the muscle density (assumed value of 1.06 g cm^{-3}; Méndez, 1960) and l is the fiber length (cm). Functional segment lengths (mm) were measured for the femur, tibia, metatarsals, longest toe (phalanx), and calcaneus. We also calculated the lever arm ratio about the ankle by dividing the sum of the metatarsal length and half the phalanx length (assuming that the middle of the toes is the center of pressure during a jump) by the length of the calcaneus.

Statistical Analyses

All statistical analyses were performed in R (version 4.0.3). Scaling relationships were determined by regressing each variable against body mass. For the performance analyses, scaling coefficients (a) and exponents (b) were obtained for both pooled species and each species individually using model II regression analyses (with the package 'lmodel2') on log-transformed data. For the morphological analyses, scaling coefficients and exponents were obtained for only pooled species using model II regression analyses on log-transformed data (due to the relatively small number of specimens for each species, we could not conduct individual scaling analyses for each species). For the muscle mass analyses only, we examined each muscle individually as well as each functional group of muscles (i.e., hip extensors, knee extensors, and ankle extensors). Scaling exponents were determined as significantly different from the expected value under isometry if the 95% confidence interval did not include the isometric value (b = 1.0 for mass, b = 0.67 for area, and b = 0.33 for length). Based on our *a priori* hypothesis that kangaroo rats maintain acceleration during jumps, we tested the obtained exponents from the performance regressions against the expected exponents under the morphological isometry model (b = -0.33 for acceleration, b = 0 for distance; Emerson, 1978). In other words, we tested the slopes to see if they significantly differed from the expected values under the null hypothesis that distance is relatively constant across body sizes and acceleration decreases as body size increases.

Results

Jump performance

Body mass for all individuals in the performance analyses ranged from 21–155 g (Table 1). Average acceleration did not show a significant relationship with body mass for pooled species (R^2 = 0.01, P = 0.40; Fig. 1A; Table 2) nor for each individual species (DIME *n* = 27, R^2 = 0.06, P = 0.22; DIDE *n* = 18, R^2 = 0.02, P = 0.61; DISP *n* = 13, R^2 = 0.13, P = 0.22). Additionally, the estimated scaling exponent for acceleration differed significantly from the expected isometric exponent (b = -0.33) in the pooled species comparison, but not in any of the individual species comparisons (Table 2).

Jump distance exhibited a significant positive relationship with body mass for pooled species (R^2 = 0.06, P = 0.03; Fig. 1B; Table 2). Additionally, the scaling exponent differed significantly from the expected isometric exponent (b = 0). However, there was no relationship between body mass and jump distance in the individual species comparisons (DIME *n* = 30, R^2 = 0, P = 0.80; DIDE *n* = 20, R^2 = 0, P = 0.85; DISP *n* = 34, R^2 = 0, P = 0.81; Table 2).

Hindlimb morphology

Body mass for all individuals in the morphological analyses ranged from 25–127 g (DIME: 25–47 g; DISI: 63–65 g; DIDE: 86–118 g; DISP: 89–127 g). Muscle CSA scaled with significant positive allometry in the biceps femoris, semitendinosus, vastus lateralis, and plantaris (Table 3). Total muscle mass for all functional groups scaled with significant positive allometry (Fig. 2). Individual muscle mass scaled with significant positive allometry in the biceps femoris, semitendinosus, rectus femoris, vastus lateralis, and plantaris (Table 3). Thus, there was greater positive allometry in muscle mass for the proximal muscles (hip and knee extensors), and less pronounced positive allometry in the distal muscles (ankle extensors). Fiber length was only significantly allometric for the vastus lateralis, which scaled with negative allometry (Table 3). Therefore, positive allometry in muscle CSA was driven primarily by changes in muscle mass.

With the exception of the metatarsals, which scaled with significant negative allometry, all the other segment lengths scaled isometrically with body mass (Table 4). Although they were insignificant, the femur, tibia, and phalanges had positive allometric exponents, while the calcaneus had a negative allometric exponent. The lever arm ratio about the ankle did not show any relationship to body mass ($R^2 = 0.02$, $P = 0.61$).

Discussion

Average acceleration of kangaroo rat jumps was maintained in larger-bodied species, suggesting that kangaroo rat morphology favors quickness rather than distance in jump escapes. This is supported by the finding that kangaroo rat hindlimb muscle cross-sectional area generally scales with positive allometry such that the muscles of larger kangaroo rat species are capable of producing more force (Powell et al., 1984), resulting in higher accelerations than expected under isometry. The increase in cross-sectional area was driven by positive allometric scaling of muscle mass as most fiber lengths scaled isometrically. As a result of the disproportionately large muscle mass, the larger kangaroo rat species also jumped farther. A larger proportion of proximal muscles exhibited positive allometric scaling, which is expected considering that the proximal muscles produce a substantial amount of the work that is exerted at the ankle during jumps (Schwaner et al., 2018).

The large variation in acceleration and distance resulted in relatively low R^2 values in each performance analyses (Fig. 1, Table 2). It should be noted that the jumps elicited here are not necessarily representative of maximal performance; kangaroo rats in a baseline state of vigilance (such as the kangaroo rats in this study) exhibit slower reaction times and take-off velocities, do not jump as high, and take longer to displace their bodies from an attack trajectory when compared to kangaroo rats that have recently interacted with a rattlesnake and are in a state of enhanced vigilance (Freymiller et al., 2017). Thus, it is possible that body mass would be more tightly correlated with performance if all the animals in this study were performing closer to their maximal abilities and the variation in jump performance was reduced. This may also explain the large variation in acceleration scaling exponents (Table 2) and why jump acceleration is maintained without any direct effects on jump distance in the intraspecific comparisons, which does not fit into the framework proposed by Hill (1950).

With the exception of the metatarsals, all hindlimb segments scaled isometrically (Table 4). Although the metatarsals scaled with negative allometry (i.e., larger kangaroo rats have disproportionately shorter metatarsals), this difference does not appear to be functionally significant due to the slight positive allometry in the phalanges and slight negative allometry of the calcaneus (Table 4). As a result, the lever arm ratio about the ankle showed no relationship to body mass, indicating that the mechanical advantage is similar between large and small species. Therefore, the force applied to the ground from the ankle extensors is similar across body size regardless of the significant negative allometry in metatarsal length. We would expect larger species to have longer contact times because the absolute length of the distal segments is longer for larger species, yet desert kangaroo rats had a shorter average contact time than the smaller Merriam's kangaroo rats (Table 1). It is possible that kangaroo rats exhibit slight postural shifts as body size changes, which could cause the observed discrepancy between contact time and limb length; a more detailed kinematic analysis examining joint angles at the onset of the jump would be necessary to address this.

Kangaroo rats are one of four extant groups of bipedal rodent, and their bipedal morphology enhances their ability to escape predators (Kotler, 1985; Longland & Price, 1991; Pierce et al., 1992; McGowan & Collins, 2018; Freymiller et al. *in review*). When escaping cursorial predators, bipedal rodents rely on erratic, unpredictable escape paths rather than rapid, powerful jumps (Djawdan & Garland, 1988; Djawdan, 1993; Moore et al., 2017). These predators would therefore place little selective pressure on jump performance. However, when evading single-strike ambush predators such as rattlesnakes and owls, they utilize impressive jump escapes (Webster, 1962; Higham et al., 2017; Freymiller et al., 2019; Whitford et al., 2019). Escaping from such predators requires a quick jump that rapidly moves the body out of the trajectory of the attack; if they are able to dodge the initial strike, the predator cannot immediately launch a fully-coordinated second attack, thus giving the kangaroo rat time to escape (Kardong & Bels, 1998; Shifferman & Eilam, 2004). Furthermore, because these attacks occur within relatively small spatial scales compared to attacks from pursuit predators, jump distance may be less important. Therefore, predation pressure from single-strike predators likely explains why kangaroo rat morphology favors the ability to quickly displace the body from a predator's attack trajectory (i.e., acceleration).

Conclusion

Evolution of large body size (average mass greater than 90 g) has occurred independently at least twice in the *Dipodomys* genus (Alexander, 2003). For rodents, increasing body size can provide advantages in foraging (Muñoz & Bonal, 2008) and interspecific fighting/dominance and territory defense (Bartholomew & Caswell, 1951), but it comes with certain limitations as well (e.g. Kotler et al., 1988). For rodents which rely on jumping to escape predators, and jumping animals in general, a theoretical disadvantage of larger body sizes is a reduction in jump acceleration. This can be circumvented if the morphology of the limbs used to propel the jump scales with positive allometry (Hill, 1950). Here we show that, as kangaroo rat species increase in body mass, morphological changes favor rapid jumping rather than jump distance. Hindlimb muscle cross-sectional area, particularly of the proximal muscles, generally scales with positive allometry such that larger species produce more force than expected based on their overall body size. This results in a maintenance of average jump acceleration, which allows larger species to jump away from single-strike predators just as quickly as their smaller counterparts.

References

Alexander LF. 2003. *Evolutionary and biogeographic histories in a North American rodent family (Heteromyidae)*. Ph.D. dissertation, Las Vegas, University of Nevada.

Arnold SJ. 1983. Morphology, performance and fitness. *American Zoologist* 23:347-361.

Asquith CM, Vonesh JR. 2012. Effects of size and structure on predation and inter-cohort competition in red-eyed treefrog tadpoles. *Oecologia* 170:629-639.

Bartholomew GA, Caswell HH, Jr. 1951. Locomotion in kangaroo rats and its adaptive significance. *Journal of Mammalogy* 32:155-169.

Berman SL. 1980. *Convergent evolution in the hindlimb of bipedal rodents*. Ph.D. dissertation, Pittsburgh, University of Pittsburgh.

Clark RW. 2006. Post-strike behavior of timber rattlesnakes (*Crotalus horridus*) during natural predation events. *Ethology* 112:1089-1094.

Dangles O, Pierre D, Christides JP, Casas J. 2007. Escape performance decreases during ontogeny in wild crickets. *Journal of Experimental Biology* 210:3165-3170.

Djawdan M. 1993. Locomotor performance of bipedal and quadrupedal heteromyid rodents. *Functional Ecology* 7:195-202.

Djawdan M, Garland T, Jr. 1988. Coexistence of bipedal and quadrupedal rodents: relevance of maximal running speeds. *Journal of Mammalogy* 69:765-772.

Domenici P, Blake RW. 1993. The effect of size on the kinematics and performance of angelfish (*Pterophyllum eimekei*) escape responses. *Canadian Journal of Zoology* 71:2319-2326.

Edut S, Eilam D. 2004. Protean behavior under barn-owl attack: voles alternate between freezing and fleeing and spiny mice flee in alternating patterns. *Behavioural Brain Research* 155:207-216.

Emerson SB. 1978. Allometry and jumping in frogs: helping the twain to meet. *Evolution* 32:551-564.

Freymiller GA, Whitford MD, Higham TE, Clark RW. 2017. Recent interactions with snakes enhance escape performance of desert kangaroo rats (Rodentia: Heteromyidae) during simulated attacks. *Biological Journal of the Linnean Society* 122:651-660.

Freymiller GA, Whitford MD, Higham TE, Clark RW. 2019. Escape dynamics of free-ranging desert kangaroo rats (Rodentia: Heteromyidae) evading rattlesnake strikes. *Biological Journal of the Linnean Society* 127:164-172.

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.

Hill AV. 1950. The dimensions of animals and their muscular dynamics. *Science Progress* 38:209-230.

Holmes TH, McCormick MI. 2009. Influence of prey body characteristics and performance on predator selection. *Oecologia* 159:401-413.

Ilany A, Eilam D. 2007. Wait before running for your life: defensive tactics of spiny mice (*Acomys cahirinus*) in evading barn owl (*Tyto alba*) attack. *Behavioral Ecology and Sociobiology* 62:923-933.

Johnson JB, Saenz D, Adams CK, Hibbitts TJ. 2015. Naturally occurring variation in tadpole morphology and performance linked to predator regime. *Ecology and Evolution* 5:2991-3002.

Kardong KV, Bels VL. 1998. Rattlesnake strike behavior: kinematics. *Journal of Experimental Biology* 201:837-850.

Katz SL, Gosline JM. 1993. Ontogenetic scaling of jump performance in the African desert locust (*Schistocerca gregaria*). *Journal of Experimental Biology* 177:81-111.

Kotler BP. 1985. Owl predation on desert rodents which differ in morphology and behavior. *Journal of Mammalogy* 66:824-828.

Kotler BP, Brown JS, Smith RJ, Wirtz WO, II. 1988. The effects of morphology and body size on rates of owl predation on desert rodents. *Oikos* 53:145-152.

Landberg T, Azizi E. 2010. Ontogeny of escape swimming performance in the spotted salamander. *Functional Ecology* 24:576-587.

Longland WS, Price MV. 1991. Direct observations of owls and heteromyid rodents: can predation explain microhabitat use? *Ecology* 72:2261-2274.

Martín J, López P. 1995. Escape behaviour of juvenile *Psammodromus algirus* lizards: constraint of or compensation for limitations in body size? *Behaviour* 132:181-192.

McGowan CP, Collins CE. 2018. Why do mammals hop? Understanding the ecology, biomechanics and evolution of bipedal hopping. *Journal of Experimental Biology* 221: jeb161661.

Méndez J. 1960. Density and composition of mammalian muscle. *Metabolism* 9:184-188.

Moore TY, Cooper KL, Biewener AA, Vasudevan R. 2017. Unpredictability of escape trajectory explains predator evasion ability and microhabitat preference of desert rodents. *Nature Communications* 8: 440 (2017).

Muñoz A, Bonal R. 2008. Are you strong enough to carry that seed? Seed size/body size ratios influence seed choices by rodents. *Animal Behaviour* 76:709-715.

Osenberg CW, Mittelbach GG. 1989. Effects of body size on the predator-prey interaction between pumpkinseed sunfish and gastropods. *Ecological Monographs* 59:405-432.

Penning DA, Sawvel B, Moon BR. 2016. Debunking the viper’s strike: harmless snakes kill a common assumption. *Biology Letters* 12: 20160011.

Pierce BM, Longland WS, Jenkins SH. 1992. Rattlesnake predation on desert rodents: microhabitat and species-specific effects on risk. *Journal of Mammalogy* 73:859-865.

Powell PL, Roy RR, Kanim P, Bello MA, Edgerton VR. 1984. Predictability in skeletal muscle tension from architectural determinations in guinea pig hindlimbs. *Journal of Applied Physiology* 57:1715-1721.

Putman BJ, Clark RW. 2015. The fear of unseen predators: ground squirrels tail flagging in the absence of snakes signals vigilance. *Behavioral Ecology* 26:185-193.

Ryerson WG. 2013. Jumping in the salamander *Desmognathus ocoee*. *Copeia* 2013:512-516.

Schwaner MJ, Lin DC, McGowan CP. 2018. Jumping mechanics of desert kangaroo rats. *Journal of Experimental Biology* 221: jeb186700.

Shifferman E, Eilam D. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl *Tyto alba* attack. *Journal of Avian Biology* 35:111-116.

Webster DB. 1962. A function of the enlarged middle-ear cavities of the kangaroo rat, *Dipodomys*. *Physiological Zoology* 35:248-255.

Whitford MD, Freymiller GA, Higham TE, Clark RW. 2019. Determinants of predation success: how to survive an attack from a rattlesnake. *Functional Ecology* 33:1099-1109.

Wilson RS, Franklin CE, James RS. 2000. Allometric scaling relationships of jumping performance in the striped marsh frog *Limnodynastes peronii*. *Journal of Experimental Biology* 203:1937-1946.

Figures and Tables

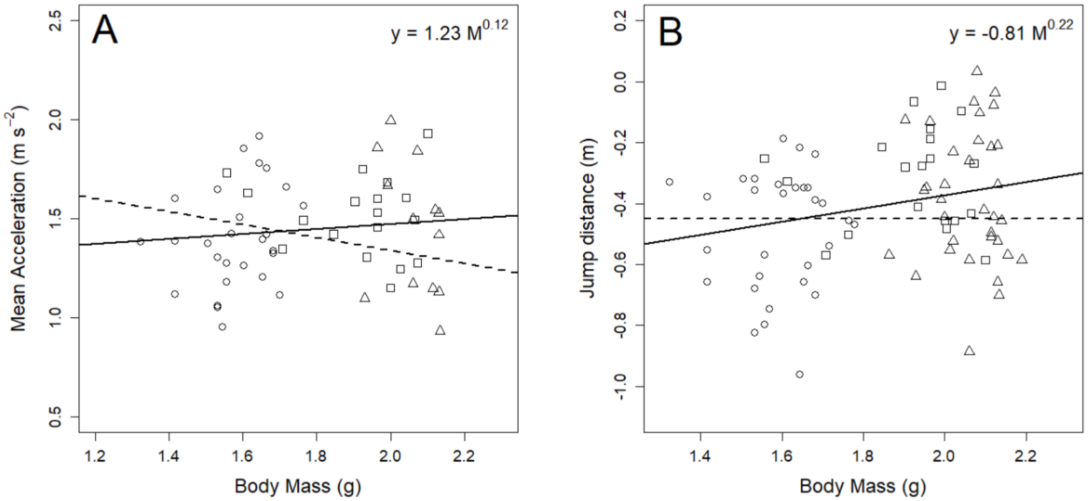

Figure 3.1 Log-log plots of jump performance with body size for pooled species comparisons. Average acceleration (A) shows no relationship with body mass (P = 0.4), whereas jump distance (B) shows a significant positive relationship with mass (P = 0.03). Open circles represent DIME, open squares represent DIDE, and open triangles represent DISP. Solid lines show obtained regression line for equation in top right corner, and dashed line shows the expected trend based on morphological isometry (b = -0.33 for acceleration, b = 0 for distance).

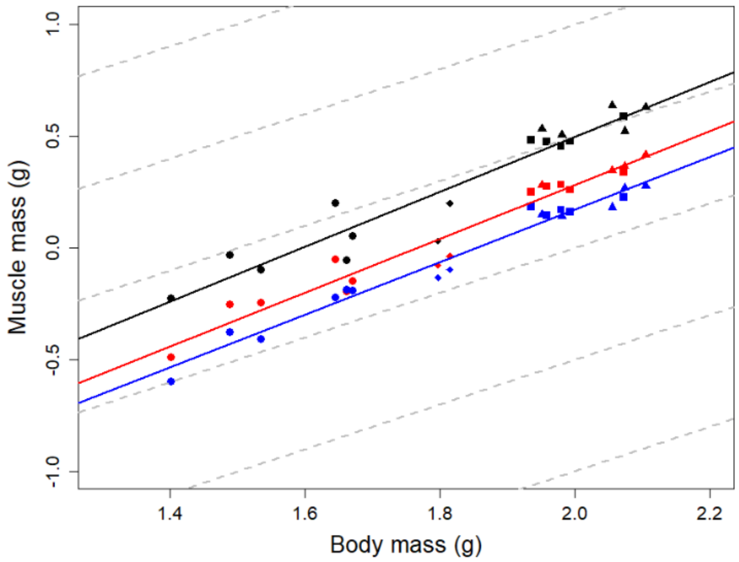

Figure 3.2 Log-log plot of scaling relationships for hindlimb muscle group mass (black = hip extensors, red = knee extensors, and blue = ankle extensors). All functional groups scaled with significant positive allometry. Circles represent DIME, squares represent DIDE, triangles represent DISP, and diamonds represent DISI. Solid lines show regression results and grey dashed lines show expected trend based on isometry (b = 1.0).

Table 3.1 Summary statistics from performance experiments. Results are mean ± standard error (min–max).

Species	Mass (g)	Contact time (ms)	Average Acceleration (m s^{-2})	Jump distance (m)
DIME	40 ± 2 (21–60)	90 ± 9 (25–197)	30 ± 4 (9–83)	0.35 ± 0.03 (0.11–0.65)
DIDE	91 ± 5 (36–126)	83 ± 7 (29–139)	39 ± 4 (14–85)	0.54 ± 0.04 (0.26–0.97)
DISP	117 ± 3 (73–155)	99 ± 14 (21–210)	36 ± 8 (9–99)	0.47 ± 0.04 (0.13–1.08)

Table 3.2 Results from performance scaling analyses. Equations are in the form $y=aM^b$. Bold values indicate significant difference from isometric exponents under the morphological isometry model (acceleration = -0.33, distance = 0).

	a	b	R^2	±95% CI	P
Pooled species					
Avg. Acceleration (m/s^2)	1.23	**0.12**	0.01	0.29	0.40
Jump distance (m)	-0.81	**0.22**	0.06	0.19	0.03
DIME					
Avg. Acceleration (m/s^2)	0.46	0.60	0.06	0.97	0.22
Jump distance (m)	-0.63	0.09	0.00	0.71	0.80
DIDE					
Avg. Acceleration (m/s^2)	1.83	-0.16	0.02	0.67	0.61
Jump distance (m)	-0.41	0.05	0.00	0.55	0.85
DISP					
Avg. Acceleration (m/s^2)	4.97	-1.70	0.13	2.9	0.22
Jump distance (m)	-0.14	-0.12	0.00	0.99	0.81

Table 3.3 Constants from muscle scaling equations. Equations are in the form $y=aM^b$. Bold values indicate significant difference from isometric exponents (1.0 for mass, 0.67 for area, and 0.33 for length).

	Muscle Mass (g)				***Muscle CSA (cm^2)***				***Fiber length (mm)***			
	a	**b**	**R^2**	**±95% CI**	**a**	**b**	**R^2**	**±95% CI**	**a**	**b**	**R^2**	**±95% CI**
Hip extensors	-1.96	**1.23**	0.91	0.20								
BF	-2.52	**1.39**	0.81	0.35	-2.55	**1.09**	0.72	0.35	1.01	0.31	0.86	0.06
ST	-2.78	**1.19**	0.94	0.15	-2.87	**0.90**	0.94	0.12	1.07	0.29	0.72	0.10
SM	-2.10	1.01	0.76	0.30	-2.10	0.70	0.63	0.28	0.97	0.31	0.66	0.12
Knee extensors	-2.12	**1.20**	0.95	0.14								
RF	-2.83	**1.32**	0.95	0.17	-2.01	0.93	0.81	0.26	0.40	0.24	0.17	0.32
VL	-2.38	**1.17**	0.96	0.13	-2.24	**1.06**	0.92	0.17	0.77	**0.13**	0.16	0.16
VM	-2.74	1.14	0.83	0.27	-2.46	0.99	0.71	0.36	0.62	0.17	0.14	0.24
Ankle extensors	-2.18	**1.18**	0.97	0.10								
LG	-2.54	1.14	0.94	0.15	-1.85	0.80	0.74	0.26	0.24	0.34	0.38	0.24
MG	-2.54	1.13	0.93	0.16	-1.96	0.91	0.72	0.33	0.32	0.24	0.17	0.30
PL	-3.12	**1.33**	0.98	0.11	-2.52	**1.10**	0.84	0.30	0.36	0.21	0.16	0.31

Muscle abbreviations: BF= biceps femoris, ST= semitendinosus, SM= semimembranosus, RF= rectus femoris, VL= vastus lateralis, VM= vastus medialis, PL= plantaris, MG= medial head of gastrocnemius, LG= lateral head

Table 3.4 Constants from segment length scaling equations. Equations are in the form $y=aM^b$. Bold values indicate significant difference from isometric exponents ($b = 0.33$).

	a	**b**	**R^2**	**±95% CI**
Femur	0.70	0.36	0.94	0.05
Tibia	0.95	0.35	0.93	0.05
Metatarsals	0.93	**0.24**	0.90	0.04
Phalanx	0.47	0.37	0.77	0.10
Calcaneus	0.19	0.30	0.66	0.11

Springing into action: comparing jump performance between bipedal and quadrupedal rodents

Running title: Jump performance of rodents

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Abstract

Bipedalism is a unique mode of locomotion for mammals, and its occurrence in divergent rodent lineages is a classic example of convergent evolution. The most well-supported hypothesis is that bipedalism in rodents evolved as a means to enhance predator evasion via powerful vertical jumps, but no studies have directly compared the jumping performance of bipedal and quadrupedal rodents. We used simulated predator attacks to compare the evasive jumping ability of bipedal kangaroo rats (*Dipodomys*) to that of three quadrupedal rodent groups—pocket mice (*Chaetodipus*), woodrats (*Neotoma*), and ground squirrels (*Otospermophilus*). Jumping performance of pocket mice was remarkably similar to that of kangaroo rats, which may be driven by their shared anatomical features (such as enlarged hindlimbs) and facilitated by their relatively small body size. Woodrats and ground squirrels, in contrast, almost never jumped as a startle response, and they took longer to perform evasive escape maneuvers than the heteromyid species (kangaroo rats and pocket mice). Among the heteromyids, take-off velocity was the only jump performance metric that differed significantly between species. These results support the idea that bipedal body plans facilitate vertical leaping in larger-bodied rodents as a means of predator escape.

Introduction

One of the goals of evolutionary biology is to understand why organisms look the way they do (i.e., their morphology) and the role natural selection plays in driving the diversity of body plans observed in nature. Addressing these questions often requires detailed examination of performance (the ability to execute an ecologically relevant task; Arnold, 1983) and comparative biomechanics. Natural selection acts directly on performance, such as the ability to sprint from a predator or successfully complete a courtship display; morphology therefore influences fitness by directly affecting the physical performance of an animal (Arnold, 1983; Jayne & Bennett, 1990; Garland & Losos, 1994; Strobbe et al., 2009). By studying how changes to morphology alter performance, and the consequences of performance ability on survival and reproduction, evolutionary biologists can understand the selection pressures driving the evolution of specialized morphological characteristics.

Bipedalism is a unique mode of locomotion for mammals, and as such it is accompanied by specialized morphological features such as enlarged hindlimbs and reduced forelimbs. Within rodents, bipedalism has independently evolved in four extant lineages: kangaroo rats in North America (Heteromyidae), jerboas in Asia and northern Africa (Dipodidae), springhares in central and southern Africa (Pedetidae), and hopping mice in Australia (Muridae) (Berman, 1985). Why has selection favored this morphology multiple times throughout the evolutionary history of rodents? The best supported hypothesis is that bipedalism evolved as a means to enhance predator evasion (reviewed in McGowan & Collins, 2018). Although bipedal rodents have radiated into arid, open habitats, bipedality appears to have originated in ancestral rodent species that occupied forested habitats (Voorhies, 1975; Wu et al., 2014), likely as an adaptation for vertical jumping to avoid predators. In both forested and arid habitats, escaping predators via vertical leaps is an important tactic that would be enhanced by a bipedal morphology.

Several studies have suggested an advantage of bipedality in extant species during predator-prey interactions. Bipedal kangaroo rats are found in owl pellets less frequently than expected based on population densities (Kotler, 1985), and they are less likely than some quadrupedal rodents to be captured by owls (Longland & Price, 1991) and rattlesnakes (Pierce et al., 1992; Whitford et al., 2019) when attacked. Both rattlesnakes and owls are single-strike ambush predators, meaning they rely on short, rapid attacks and do not chase prey down. Therefore, if prey evade the initial attack, they will have a chance to escape before the predator can capture it in a second attempt (Kardong & Bels, 1998; Shifferman & Eilam, 2004). Because these attacks occur rapidly and on relatively small spatial scales, kangaroo rats rely on rapid vertical leaps to evade both snakes and owls (Webster, 1962; Higham et al., 2017; Freymiller et al., 2019; Whitford et al., 2019). These observations support the hypothesis that bipedalism enhances vertical jumping for predator evasion, but the particular mechanisms of how bipedality could enhance escape maneuvers are not clear.

To date, studies that directly compare the kinematics and performance of rodent escape maneuvers have focused solely on running ability. Jerboas and kangaroo rats utilize zig-zagging patterns when running away from a simulated predator attack, making their escapes more erratic and less predictable (Djawdan & Garland, 1988; Djawdan, 1993;

Moore et al., 2017). Additionally, compared to quadrupedal rodents, kangaroo rats reach higher maximum speeds (Djawdan & Garland, 1988) and have higher running endurance (Djawdan, 1993). While these differences in running ability are important for escaping cursorial predators such as coyotes and foxes, they do not fully explain the documented differences in owl and snake predation rates (Kotler, 1985; Longland & Price, 1991; Pierce et al., 1992). Thus, to date we have only indirect evidence in support of the hypothesis that bipedalism evolved as an adaptation for evasive antipredator jumping.

We compared the evasive jumping ability of kangaroo rats (*Dipodomys* spp.), bipedal rodents common throughout North America, to that of three sympatric, quadrupedal rodents: pocket mice (*Chaetodipus* spp.), woodrats (*Neotoma* spp.), and ground squirrels (*Otospermophilus* spp.). All of these species are common prey of ambush-hunting rattlesnakes (*Crotalus* spp.), one of the most abundant predators of small mammals in arid environments. Kangaroo rats are well-known for their impressive evasive antipredator leaps, which have been the focus of several recent kinematic studies (Higham et al., 2017; Freymiller et al., 2019; Whitford et al., 2019; Schwaner et al., 2018; Schwaner et al., *in review*). Although pocket mice are quadrupedal, they are also heteromyid rodents like kangaroo rats, and they share some gross anatomical features such as enlarged hindlimbs, reduced forelimbs, and enlarged auditory bullae (Hatt, 1932; Bartholomew & Cary, 1954; Webster & Webster, 1980). However, their evasive jumping ability has never been studied experimentally (but see Bartholomew & Cary, 1954 for qualitative descriptions). Woodrats have also never been studied in terms of evasive jumping abilities, and to our knowledge, evasive jumping of ground squirrels has been examined in only one instance (Putman & Clark, 2015) with no direct comparisons to bipedal rodents. We thus predicted that kangaroo rats, when compared to quadrupedal rodents also predated by rattlesnakes, would execute faster, more vertical jumps away from a simulated snake strike, and that pocket mice would perform best among the quadrupedal rodents given their similarity to kangaroo rats. We also predicted that heteromyids would have faster reaction times compared to non-heteromyid rodents due to their enlarged auditory bullae facilitating rapid detection of auditory cues from predator attacks (Webster, 1962; Webster & Webster, 1971).

Materials & Methods

Study Sites and Animals

All methods were approved by the San Diego State University Institutional Animal Care and Use Committee [APF 16-08-014C]. We targeted three species of kangaroo rat: the desert kangaroo rat (*Dipodomys deserti*, Stephens; DIDE), Merriam's kangaroo rat (*Dipodomys merriami,* Mearns; DIME), and banner-tailed kangaroo rat (*Dipodomys spectabilis,* Merriam; DISP). These species were chosen as they encompass the relatively large variation in body size seen among kangaroo rats. Additionally, data were collected for the desert pocket mouse (*Chaetodipus penicillatus,* Woodhouse; CHPE) and the white-throated woodrat (*Neotoma albigula,* Hartley; NEAL). Data for California ground squirrels (*Otospermophilus beecheyi,* Richardson; OTBE) were obtained from a previous study from our research group examining the effect of vigilance on squirrel escape responses using the same methodology (detailed below). Our study took place at several sites throughout southwestern North America. Initial data were collected from mid-May through early August in 2016 at a site located within the Barry M. Goldwater Range outside of Yuma, Arizona, USA (n = 5 DIDE). In mid-May through early August of 2018, we collected additional data at a site in Rodeo, New Mexico, USA (31 DISP, 25 DIME, and 12 CHPE) and at a nearby site in Animas, New Mexico, USA (15 DISP). We collected data from June to July of 2019 in the Mojave Desert of California at a site south of the California State University's Desert Studies Center located in Zzyzx, California, USA (22 DIDE and 22 DIME). Lastly, we revisited the Rodeo site from mid-June to early August in 2020 (1 DIME, 10 CHPE, and 14 NEAL).

Rodents were trapped using Sherman live traps baited with heat-sterilized black oil sunflower seeds. Traps were set between sunset and sunrise near burrows or middens. Trapped individuals were sexed and measured (mass, snout-anus length, tail length, and hind foot length), then marked with fingerling ear tags (National Band and Tag #1005-1) for long-term identification and a unique fur dye mark using Nyanzol dye for short-term identification. Fur dye marks ensured that rodents could be reliably identified during experiments and to prevent retesting of individuals. Individuals were processed in the field and immediately released at the site of capture.

Experimental procedure

We used a modified version of the methodology detailed in Freymiller et al. (2017) & Putman & Clark (2015) to record rodent evasive leaps. Once a marked rodent was reliably relocated (i.e., home burrow identified or an individual was found in the same area at least twice via traps and/or visual surveys), an experimental set-up was placed in the vicinity of the known individual's location. The set-up consisted of a rattlesnake strike simulator (RSS), infrared lighting (850 nm wavelength), and a GoPro video camera (Hero 4 Black) retrofitted with a lens that allowed the camera to pick up infrared light (Peau Productions, 2.97mm f/4.0 90d HFOV 5MP, no IR filter) recording at 240 frames per second (fps). A second video camera (Sony Handycams, model SR-65 or SR-300) recording at 30 fps was used to record the entirety of the trial and observe the animal during baseline feeding but was not used to collect videos for analysis. The RSS consists of a one-inch diameter PVC pipe housing and a compressed spring that projects a cork toward a target at 2.8 m s^{-1}, approximately the same velocity as a rattlesnake strike (Penning et al., 2016; Higham et al., 2017; Whitford et al., 2019). To hold the spring while compressed until the trial was ready to begin, we attached a piece of monofilament nylon line to the end of the spring, then tied the other end to a camera tripod manned by an observer 3-5 m away.

At the beginning of a trial, the target individual was allowed to approach and inspect the RSS. They were encouraged to feed near the device by baiting it with sunflower seed. Most rodents did not appear disturbed by the presence of the RSS, but the trial was immediately ended if an individual behaved apprehensively (e.g., through anti-predator displays), as vigilance/alertness can affect jump performance (Putman & Clark, 2015; Freymiller et al., 2017). Once the rodent left to cache, more seed was placed in a small pile approximately 14 cm in front of the cork. We began recording with both cameras when the rodent returned. When the individual approached the seed pile, the monofilament line was cut which released the spring and cork, and the rodent's response was recorded. If the rodent jumped (Table 4.1, Video 1), the horizontal displacement, defined as the distance (in meters) between the take-off and landing positions, was immediately measured in the field with a tape measure using the video playback for guidance. Horizontal displacement was not measured if the rodent did not jump (in which case the trial was classified as a "scramble", Table 4.1, Video 2), or if the rodent jumped out of the frame of the video camera (n = 3 trials). These trials were retained for reaction time and body displacement time calculations (see Video and Statistical Analyses below) but were not used in performance analyses. No individual was ever tested twice to prevent the possibility that learning would affect the response to the RSS. All trials were recorded between sunset and sunrise. As light levels at night vary widely based on the moon phase and could affect the rodents' ability to see the cork (and therefore influence reaction time), ambient light was measured with a digital light meter (Extech LT300, minimum sensitivity of 0.01 lux) immediately after every trial.

In 2016, two paired high-speed cameras (Edgertronic, model SC1) recording at 500 fps and connected to laptop computers via 100 ft Ethernet cables were used to record the evasive jumps instead of a single GoPro camera. These videos were calibrated with a large object of known dimensions (metal rods fixed to a 30 x 25 cm metal plate) for three-dimensional analyses. To make these videos comparable to the GoPro recordings, the frame rate was reduced by converting the videos to a series of still images using the "magick" package in R (version 4.0.3) and eliminating every other frame to recreate a 250 fps video. Using the three-dimensional calibration, the horizontal displacement of the jump was extracted by digitizing a point on the toes in the frame of toe-off and in the frame of landing using the software DltDataviewer, version 7 (Hedrick 2008) in MATLAB (R2018b). This horizontal displacement was then used in the jump performance calculations the same way that we used the displacement values measured in the field for the other trials.

Incorporation of data from previous study

In order to allow for a broader comparison of rodent performance, we incorporated data from Putman & Clark (2015), which was conducted by our research group and utilized similar methods to those outlined above. California ground squirrels were tested with the RSS at a site approximately 20 miles east of San Jose, CA, USA from May-August in 2012 and 2013 (n = 23 OTBE). In this study, squirrels were tested under three treatments (snake present, recent snake, and no snake) based on whether they were exposed to a tethered rattlesnake placed by the device immediately before or during a trial. As we did not incorporate a tethered snake into the current study, we only used ground squirrel data from the “no snake” treatment of that study. The methods between that study and the present study differ in several other ways as well. First, horizontal displacement was not measured for OTBE trials, so we could only use this data for the reaction time and body displacement time analyses. Second, the rodents’ responses were filmed at 120 fps instead of 240 fps, so the measurements of reaction time and body displacement time are at a courser timescale than the measurements for the other five species (i.e., each frame is 8.3 ms in the squirrel videos and 4.2 ms in the other videos). However, as the error rate in these values is ±2 frames, this difference only affects measurement differences less than 5 frames. Third, the length of the device in the present study was about half as long as the one used for the ground squirrels. However, because the cork was always tied back so that it aligned with the edge of the PVC pipe in both studies, and because the velocity of the devices were very similar (3.1 m s^{-1} and 2.8 m s^{-1}), we do not expect this difference to affect the analyses. Lastly, because ground squirrels are strictly diurnal, all OTBE trials were recorded during the day.

Video and Statistical Analyses

We used the GoPro video recordings to quantify several variables associated with the rodents' evasive maneuvers, including reaction time, body displacement time, take-off velocity and angle, and jump height. Reaction time was measured as the time between the first movement of the cork and the first visible movement of the rodent's reaction. If the rodent did not react until after the cork made contact with the individual (i.e., they were hit with the cork, n = 20 trials) or they seemed to react before the cork started to move (n = 2 trials), the reaction time was not measured. Body displacement time was measured as time between the first visible movement of the rodent's reaction and the frame immediately preceding toe-off. Thus, body displacement time does not include reaction time but rather is a measurement of how quickly the animal can move its body from the path of the cork once it starts to react.

Using the horizontal displacement measured in the field and the amount of time spent airborne (measured as the number of seconds between the take-off and landing frames), we calculated take-off velocity (m s^{-1}), take-off angle (°), and jump height (m) using the following equations:

$$\mathrm{Velocity_h} = \frac{\text{horizontal displacement}}{\text{time spent in air}} \quad (1)$$

$$\mathrm{Velocity_v} = g\left(\frac{\text{time spent in air (s)}}{2}\right) \quad (2)$$

$$\text{Take off velocity} = \sqrt{\mathrm{Velocity_h}^2 + \mathrm{Velocity_v}^2} \quad (3)$$

$$\text{Jump angle} = \operatorname{atan}\left(\frac{\mathrm{Velocity_v}}{\mathrm{Velocity_h}}\right) \mathrm{X}\ \frac{180}{\pi} \quad (4)$$

$$\text{Jump height} = \frac{\mathrm{Velocity_v}^2}{2 \times g} \quad (5)$$

Where g = acceleration due to gravity (9.8 m s^{-2}).

All statistical analyses were performed in R (version 4.0.3). Because individuals were only tested once, repeated-measure techniques were not used. We analyzed species differences in jump performance using a PERMANOVA from the R package ‘vegan’, with take-off velocity, take-off angle, and jump height as the dependent variables, and species and body mass as independent variables. We used Euclidean distance on standardized performance variables and 999 permutations. To further tease apart the differences among species, individual models were used for each performance variable. A Welch’s one-way ANOVA and Games-Howell post-hoc test were used on log-transformed (natural log) velocity values to address issues of normality and heteroskedasticity. Individual linear models were used to test species differences with respect to jump height and take-off angle. Phylogenetic comparative methods were not utilized in any of the analyses due to the small number of species in the study (Garland & Adolph, 1994).

Reaction time and body displacement time were each analyzed with separate linear models with species as the only independent variable, and a Tukey's HSD test was used for pairwise species comparisons. We were unable to include ambient light in the model of reaction time as there was little variation in our recorded values (the light meter had a minimum sensitivity of 0.01 lux, and only 20% of trials occurred at levels above 0.02 lux). We also explored the relationship between reaction time and the probability a rodent would jump using a logistic regression. Lastly, we used a linear model to compare body displacement times between individuals within a species which scrambled and those which jumped. Only Merriam's kangaroo rats were included in these last two analyses as they were the only species with a large enough sample size for both jumps and scrambles.

Results

Sample sizes and jump frequency

We collected data for a total of 48 Merriam's kangaroo rats (DIME), 46 banner-tailed kangaroo rats (DISP), 27 desert kangaroo rats (DIDE), 22 desert pocket mice (CHPE), and 14 white-throated woodrats (NEAL), and data were incorporated for 23 California ground squirrels (OTBE). The large difference in overall samples for each species is due to differences in the propensity to jump; species which jump less frequently required more trials to reach an adequate sample size of jumps. Merriam's kangaroo rats jumped 63% of the time (30/48 trials), and banner-tailed kangaroo rats jumped 74% of the time (34/46). Comparatively, desert kangaroo rats jumped 93% of the time (25/27) and desert pocket mice jumped 91% of the time (20/22). Ground squirrels only jumped in 9% of trials (2/23), and woodrats only jumped 7% of the time (1/14), so it was not feasible to gather a large enough sample of jumps for either of these species. Because we could not always collect each variable of interest from every trial due to variation in video quality or rodent behavior (e.g. if the rodent was struck with the cork, we could not measure reaction time but we could measure jump performance variables), we report the final sample sizes for each analysis below.

Jump performance

After we removed trials in which individuals scrambled, jumped off screen, or the video quality was too poor to extract the necessary information, we ended with the following sample sizes for each species for jump analysis: 29 DIME, 27 DISP, 25 DIDE, and 19 CHPE. Woodrats were completely excluded because only one individual jumped and it landed off screen, preventing statistical analyses. Ground squirrels were also excluded because only two individuals jumped, and the horizontal displacement values were not measured in that dataset so performance metrics could not be calculated. Overall jump performance was significantly different among the species retained ($F_{3,95} = 2.4$, $P = 0.03$), so we used individual models to determine how the species varied with respect to each dependent variable. Body mass did not significantly affect jump performance ($F_{1,95} = 0.1$, $P = 0.9$) so it was not included in the individual analyses. Take-off velocities of desert kangaroo rats were significantly faster than both Merriam's kangaroo rats and desert pocket mice, and banner-tailed kangaroo rats had intermediate velocities that were not significantly different from any of the other species ($F_{3,52.7} = 6.9$, $P = 0.001$; Fig. 4.1A).

Neither jump height nor take-off angle significantly differed among any species (height: $F_{3,96} = 1.4$, $P = 0.25$; angle: $F_{3,96} = 1.4$, $P = 0.25$; Fig. 4.1B,C).

Reaction time & body displacement time

After we removed trials in which the individual was hit with the cork, the animal began an evasive maneuver before the cork began to move, or the video quality was not sufficient to see the first movement of the animal, we had the following sample sizes for the reaction time analyses: 39 DIME (27 jumps, 12 scrambles), 24 DISP (19 jumps, 5 scrambles), 24 DIDE (23 jumps, 1 scramble), 21 CHPE (19 jumps, 2 scrambles), 13 NEAL (1 jump, 12 scrambles), and 21 OTBE (2 jumps, 19 scrambles). Based on the logistic regression with Merriam's kangaroo rats, there was no relationship between the probability of jumping and reaction time (mean scramble reaction time 26 ms, mean jump reaction time 24 ms; odds ratio = 1.00, P = 0.97). Therefore, we included all trials (i.e., both scrambles and jumps) for all species in the final model. There were significant differences among species in reaction time: desert kangaroo rats and woodrats reacted faster than the other species, and ground squirrels reacted significantly slower than all the other species ($F_{5,136} = 16.1$, $P < 0.001$; Fig. 4.2). Merriam's kangaroo rats, pocket mice, and banner-tailed kangaroo rats all had intermediate reaction times.

For the body displacement time analyses, we had to remove trials in which the video quality was not sufficient to determine the frame of toe-off and/or see the first movement of the animal, leaving the following sample sizes: 38 DIME (27 jumps, 11 scrambles), 20 DISP (18 jumps, 2 scrambles), 24 DIDE (23 jumps, 1 scramble), 20 CHPE (19 jumps, 1 scrambles), 13 NEAL (1 jump, 12 scrambles), and 19 OTBE (2 jumps, 17 scrambles). Pocket mice had the fastest average displacement time, and non-heteromyid quadrupeds had significantly slower displacement times than the heteromyid rodents ($F_{5,128} = 12.2$, $P < 0.001$; Fig. 4.3). The heteromyids had generally similar displacement times, with only pocket mice and Merriam's kangaroo rats exhibiting significantly different mean values.

Merriam's kangaroo rats had significantly slower body displacement times when they scrambled compared to when they jumped (mean scramble displacement time 126 ms, mean jump displacement time 88 ms; $F_{1,36} = 6.7$, $P = 0.01$). Therefore, jump maneuvers produce a significantly faster escape from the attack trajectory. This is supported by the finding that woodrats and ground squirrels had the overall slowest displacement time because they almost never jumped, and the fact that Merriam's and banner-tailed kangaroo rats, the two kangaroo rat species which were most likely to scramble rather than jump, had the slowest displacement times among heteromyids (Fig. 4.3).

Discussion

The rodents in this study showed highly variable startle responses and escape abilities. The stark differences in jump probability and body displacement time suggest that bipedality in heteromyid rodents functions to enhance rapid jumping in larger-bodied species as a means of predator escape. Only the heteromyid rodents were prone to jumping when startled, so we were unable to compare the evasive jump kinematics (velocity, height, and angle) between all quadrupedal and bipedal rodents. Among the heteromyids, the jump performance of the quadrupedal desert pocket mouse and the bipedal kangaroo rat species was similar. This suggests that quadrupedal heteromyids are adapted for powerful evasive jumping to some degree, which is plausible considering that pocket mice share many basic anatomical features with kangaroo rats, such as enlarged hindlimbs and reduced forelimbs, and have been considered a morphological intermediate between bipedal and quadrupedal forms (Hatt, 1932; Bartholomew & Cary, 1954). Indeed, pocket mice have been recorded escaping rattlesnake strikes with jumps that are qualitatively similar to those of kangaroo rats (Table 4.1, Video 3). Ongoing analyses of hindlimb morphology of these species will help determine if bipedal and quadrupedal heteromyids share similar muscle and bone morphology.

Overall, heteromyids were much more likely to rely on jumping as a startle response to a high speed “attack” from our strike simulator than non-heteromyids. This propensity to jump seems to translate to a more effective escape from ambush predators, as both quadrupedal and bipedal heteromyid rodents are less likely than non-heteromyid rodents to be captured when attacked by owls (Kotler, 1985; Kotler et al., 1988; Longland & Price, 1991). The heavy reliance of heteromyids on jumping as a general escape maneuver, regardless of whether they are bipedal or quadrupedal, is likely driven by a combination of body posture and size. When jumping, animals must align their center of mass over the line of action of the propulsive force (i.e., over the hindlimbs) to avoid excessive torque on the body as it moves through the air. The bipedal posture of kangaroo rats, as well as their elongated tails, keep their center of mass over their hindlimbs during normal locomotion, allowing them to rapidly and smoothly execute a jump maneuver. Quadrupedal rodents, on the other hand, must first use their forelimbs to pitch their bodies over the hindlimbs before propelling themselves into the air. Both the pitching maneuver and the acceleration of the body takes less time and effort for a smaller-bodied quadruped, such as a pocket mouse, which may explain why they were more likely to jump than woodrats and ground squirrels. There may also be a link to the specialized hearing of heteromyids, as auditory cues are processed relatively quickly by the central nervous system (Davis, 1984; Nicolas, 1997), which would facilitate a more rapid jump.

It should be noted that woodrats and ground squirrels are capable of jumping—we recorded one jump from a woodrat when it was physically struck by the cork (Table 4.1, Video 1), and ground squirrels jump more frequently when they are in a high-vigilance state (Putman & Clark, 2015). However, unlike heteromyids, neither species appears to jump readily when in a "baseline" vigilance state. Given that both groups can jump, and jumping appears to be a more effective means of predator escape based on predator diet and capture studies, why don't non-heteromyid rodents rely more on jumping as a generalized escape response? Aside from the potential difficulty for these large-bodied quadrupeds to adequately align their bodies quickly enough for a jump and subsequently accelerate their bodies, there may be other factors at play. Injury risk could also contribute to the observed differences in jump probability, as kangaroo rat hindlimbs are well-built for the rapid acceleration and force associated with their evasive leaps compared to more typical rodents (Biewener & Blickhan, 1988; Rankin et al., 2018; Schwaner et al., 2018; Javidi et al., 2019). Kangaroo rats (and presumably pocket mice) are also capable of using their long tails to reorient mid-air (Schwaner et al., *in review*), allowing them to always land on their hindlimbs and eliminating some of the need to plan their trajectory before jumping. If woodrats and ground squirrels cannot control their trajectory and body position as effectively during the aerial phase of an escape, jumping would be more risky as a general response to any threat. However, it should be noted that when ground squirrels do jump (e.g., when in a high-vigilance state), they also appear to use tail movements to control their mid-air body position (see supplementary videos in Putman & Clark, 2015). Lastly, it's possible that venom resistance may drive differences in escape response, specifically to a simulated snake strike. Both ground squirrels and woodrats are known for their physiological resistance to rattlesnake venom (Biardi, 2008; Robinson et al., *in review*), whereas heteromyids are not known to possess any resistance. Therefore, there might be an interaction between venom resistance and behavioral responses to rattlesnake stimuli among small mammals. Incorporating a wider variety of quadrupedal rodents in future studies can tease these explanations apart.

Body displacement time

When examining body displacement time, quadrupedal rodents outside of the heteromyid family take longer to move their bodies out of the path of an attack (Fig. 4.3). This pattern is driven by the fact that the quadrupedal rodents need to turn and reorient their bodies before scrambling out of the trajectory of the RSS (Table 4.1, Video 2). The negative effect of reorienting on body displacement time is even seen among the heteromyids: Merriam's kangaroo rats that scrambled had significantly slower displacement times compared to Merriam's kangaroo rats that jumped because they took more time to orient their bodies onto an escape path. Furthermore, the two heteromyid species that were less likely to jump

(Merriam's and banner-tailed) had slower displacement times than the two heteromyid species that had the highest propensity for jumping (desert kangaroo rats and desert pocket mice; Fig. 4.3). In the few instances when the woodrats and ground squirrels did jump, their displacement times were noticeably faster: the quickest displacement time we recorded for woodrats was from the one individual that jumped (46 ms), and the next fastest displacement time took almost three times as long (122 ms). Thus, heteromyid rodents are able to displace their bodies from the trajectory of a threat faster because they jump more readily, and jumping does not require the individual to reorient prior to initiating an escape. If non-heteromyid rodents jumped more frequently, it's likely that their average displacement times would be much faster.

As body displacement time in this study is a measure of how quickly an animal moves its body from the vector of an oncoming attack, it has important consequences for escape success. Scrambling adds significant time to the escape maneuver, which could be detrimental during a real rattlesnake or owl strike, as even small increases in displacement time could give these high-speed ambush predators the advantage. Rattlesnakes can extend their coiled bodies to bite prey within approximately 135 ms, on average (range of 54 to 308 ms; Whitford et al., 2019). Woodrats had an average displacement time of 153 ms and ground squirrels had an average displacement time of 158 ms, whereas the Merriam's kangaroo rats, which had the slowest displacement time among heteromyids, had an average displacement time of 103 ms (Table 4.2). Therefore, a kangaroo rat would be more likely to successfully escape when compared to their quadrupedal counterparts. The extra time that woodrats and ground squirrels take to evade could provide a rattlesnake with just enough time to close the gap between itself and its prey, highlighting the positive consequences of jumping on the likelihood of escaping the rapid attack of a single-strike predator.

Jump performance

Because we were unable to include jumps from non-heteromyid quadrupeds, it is not clear if bipedality influences the kinematics of the jumps, or if it mainly serves to make jumping more rapid. For the species we were able to examine, overall differences in jump performance were driven primarily by differences in take-off velocity as there were no significant differences in jump height nor take-off angle. On average, all species made low jumps that were more horizontal than vertical (Fig. 4.1B & C), but there was substantial variation in velocity. Desert kangaroo rats had average take-off velocities that were significantly faster than Merriam's kangaroo rats and pocket mice, with banner-tailed kangaroo rats having intermediate take-off velocities (Fig. 4.1A). This increased speed resulted in desert kangaroo rats reaching greater jump heights, which could increase the necessity of specialized mechanisms for airborne body reorientation during an escape jump (see Schwaner et al., *in review*).

Pocket mice had take-off velocities that ranged from 1.5–3.1 m s^{-1}, which is similar to recorded take-off velocities for the jumping mouse (*Zapus trinotatus*), another quadrupedal rodent which is morphologically specialized for jumping (Harty & Roberts, 2010). Jumping mice utilize power amplification via elastic energy storage to execute their jumps, but it is not clear if pocket mice do as well. Kangaroo rat tendons do not provide significant power amplification during jumps (Biewener et al., 1981; Schwaner et al., 2018), so it is likely that pocket mice do not rely on elastic energy storage to power their jumps either, but this has yet to be determined.

It is not clear what is driving the species differences in take-off velocity, as body size was not a significant factor, and there does not appear to be a link with bipedality. One important consideration is that the jumps in this study were a general escape response and not necessarily reflective of the maximal capacity of these animals. Previous studies with desert kangaroo rats have found that they can jump almost a full meter into the air when alerted to the presence of a rattlesnake (Freymiller et al., 2017; Freymiller et al., 2019), whereas the average jump heights for the desert kangaroo rats in this study were a small fraction of that (often less than 10 cm). Thus, species-level differences in jump performance may become exaggerated when comparing maximal performance, which would help elucidate relationships between body size, bipedality, and jump performance.

Reaction time

Reaction time is an important factor when considering escape ability. In natural interactions between rattlesnakes and kangaroo rats, reaction time is one of the most important factors in determining if a kangaroo rat will successfully evade a strike (Whitford et al., 2019). Kangaroo rats are known for their enlarged auditory bullae which appear to help them detect low-frequency sounds (Heffner & Masterton, 1980; Webster & Webster, 1980), such as those recorded from both snake strikes and owl swoops (Webster, 1962). Thus, it is not surprising that kangaroo rats had extremely fast reaction times, in some instances as quick as 8-16 milliseconds. Pocket mice also show enlarged bullae compared to other small rodents, but not to the same extent as kangaroo rats, which may explain why their average reaction time was slower than some of the kangaroo rats.

The degree of variation among kangaroo rats (Fig. 4.2) is interesting and could be related to unknown anatomical or physiological differences in their system. There does not appear to be any relationship to body mass as the relatively large desert kangaroo rat (average mass of 89 g in this study, Table 4.2) and the smaller Merriam's kangaroo rat (40 g), both had significantly faster reaction times than the banner-tailed kangaroo rat (116 g). The scaling relationships of auditory bullae among kangaroo rat species is not known and should be investigated. If some species have disproportionately small bullae (and less sensitive hearing) it could drive differences in reaction time that would not be apparent from external morphology.

Although woodrats are not known to have extremely sensitive hearing, they had significantly faster reaction times than ground squirrels (and actually faster than many of the heteromyids). This difference between ground squirrels and woodrats could be explained by the dominant sensory systems each species uses to detect predators. Woodrats are nocturnal and, like kangaroo rats, likely respond more strongly to auditory cues, whereas ground squirrels are diurnal and therefore likely rely more heavily on vision. Because auditory processing is more rapid than visual processing (Davis, 1984; Nicolas, 1997), enhanced sensitivity to auditory cues would be expected to result in a faster reaction time, even in the absence of specialized hearing.

We would also caution that a more ecologically relevant interpretation of differences in reaction time among these nocturnal species would require a more ecologically realistic auditory stimulus. The RSS we used does produce noise as the spring uncoils, and it is not at all clear if the sound frequencies produced are similar to the sounds made by common single-strike predators like snakes and owls. Kangaroo rats are especially sensitive to low-frequency sounds associated with owl swoops and snake strikes, and it is possible that the frequencies of the sounds made by the RSS do not faithfully capture those frequencies. However, the only known differences in hearing ability between kangaroo rats and woodrats is that kangaroo rats have better low-frequency hearing (i.e., woodrats do not have better high frequency hearing; Heffner & Masterton, 1980; Heffner & Heffner, 1985), so it is somewhat unlikely that the woodrats have faster reaction times because they are sensitive to sounds that the kangaroo rats cannot hear.

Conclusion

Although bipedalism is not necessary to be a good jumper, as in the case of the pocket mouse, it clearly provides an advantage when escaping from predators. Jumping reduces the amount of time needed to move out of a predator's trajectory (i.e., reduced body displacement time), and bipedal rodents were much more likely to jump as a general startle response than non-heteromyid quadrupeds, which translates into better escape performance during attacks. When considering that pocket mice share important anatomical features with kangaroo rats, it is expected that their ability to jump would be more similar to these species than to other rodents. Therefore, we can conclude that relatively enlarged hindlimbs provide an advantage during predator escape maneuvers by increasing the ability to jump out of the attack trajectory, which is most exaggerated in bipedal rodents. These findings lend further support to the general hypothesis outlined in McGowan and Collins (2018), that bipedalism evolved in response to selective pressures favoring vertical jumping in forested environments and is maintained in extant species as an adaptation for rapid escapes in open, arid environments.

References

Arnold SJ. 1983. Morphology, performance, and fitness. *American Zoologist* 23:347-361.

Bartholomew GA, Jr., Cary GR. 1954. Locomotion in pocket mice. *Journal of Mammalogy* 35:386-392.

Berman SL. 1985. Convergent evolution in the hindlimb of bipedal rodents. *Journal of Zoological Systematics and Evolutionary Research* 23:59-77.

Biardi JE. 2008. The ecological and evolutionary context of mammalian resistance to rattlesnake venom. In: Hayes WK, Beaman KR, Cardwell MD, Bush SP, eds. *The Biology of Rattlesnakes.* Loma Linda: Loma Linda University Press, 557-568.

Biewener AA, Blickhan R. 1988. Kangaroo rat locomotion: design for elastic energy storage or acceleration? *Journal of Experimental Biology* 140:243-255.

Biewener AA, Alexander RM, Heglund NC. 1981. Elastic energy storage in the hopping of kangaroo rats (*Dipodomys spectabilis*). *Journal of Zoology* 195:369-383.

Davis, M. 1984. The mammalian startle response. In: Eaton RC, ed. *Neural Mechanisms of Startle Behavior*. New York: Plenum Press, 287–353.

Djawdan M. 1993. Locomotor performance of bipedal and quadrupedal heteromyid rodents. *Functional Ecology* 7:195-202.

Djawdan M, Garland T, Jr. 1988. Coexistence of bipedal and quadrupedal rodents: relevance of maximal running speeds. *Journal of Mammalogy* 69:765-772.

Freymiller GA, Whitford MD, Higham TE, Clark RW. 2017. Recent interactions with snakes enhance escape performance of desert kangaroo rats (Rodentia: Heteromyidae) during simulated attacks. *Biological Journal of the Linnean Society* 122:651-660.

Freymiller GA, Whitford MD, Higham TE, Clark RW. 2019. Escape dynamics of free-ranging desert kangaroo rats (Rodentia: Heteromyidae) evading rattlesnake strikes. *Biological Journal of the Linnean Society* 127:164-172.

Garland T, Jr., Adolph SC. 1994. Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiological Zoology* 67:797-828.

Garland T, Jr., Losos JB. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: Wainwright, PC and Reilly, SM, eds. *Ecological morphology: integrative organismal biology*. Chicago: University of Chicago Press, 240-302.

Harty TH, Roberts TJ. 2010. Jump energetics and elastic mechanisms in the pacific jumping mouse (*Zapus trinotatus*). Unpublished D. Phil. Thesis, Oregon State University.

Hatt RT. 1932. The vertebral columns of ricochetal rodents. *Bulletin of the American Museum of Natural History* 63:599-745.

Hedrick TL. 2008. Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration and Biomimetics* 3: 034001.

Heffner HE, Heffner RS. 1985. Hearing in two cricetid rodents: wood rat (*Neotoma floridana*) and grasshopper mouse (*Onychomys leucogaster*). *Journal of Comparative Psychology* 99:275-288.

Heffner H, Masterton B. 1980. Hearing in glires: domestic rabbit, cotton rat, feral house mouse, and kangaroo rat. *Journal of the Acoustical Society of America* 68:1584-1599.

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.

Javidi M, McGowan CP, Schiele NR, Lin DC. 2019. Tendons from kangaroo rats are exceptionally strong and tough. *Scientific Reports* 9:8196.

Jayne BC, Bennett AF. 1990. Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* 44:1204-1229.

Kardong KV, Bels VL. 1998. Rattlesnake strike behavior: kinematics. *Journal of Experimental Biology* 201:837-850.

Kotler BP. 1985. Owl predation on desert rodents which differ in morphology and behavior. *Journal of Mammalogy* 66:824-828.

Kotler BP, Brown JS, Smith RJ, Wirtz WO, II. 1988. The effects of morphology and body size on rates of owl predation on desert rodents. *Oikos* 53:145-152.

Longland WS, Price MV. 1991. Direct observations of owls and heteromyid rodents: can predation explain microhabitat use? *Ecology* 72:2261-2274.

McGowan CP, Collins CE. 2018. Why do mammals hop? Understanding the ecology, biomechanics and evolution of bipedal hopping. *Journal of Experimental Biology* 221: jeb161661.

Moore TY, Cooper KL, Biewener AA, Vasudevan R. 2017. Unpredictability of escape trajectory explains predator evasion ability and microhabitat preference of desert rodents. *Nature Communications* 8:440 (2017).

Nicolas S. 1997. On the speed of different senses and nerve transmission by Hirsch (1862). *Psychological Research* 59:261-268.

Penning DA, Sawvel B, Moon BR. 2016. Debunking the viper's strike: harmless snakes kill a common assumption. *Biology Letters* 12: 20160011.

Pierce BM, Longland WS, Jenkins SH. 1992. Rattlesnake predation on desert rodents: microhabitat and species-specific effects on risk. *Journal of Mammalogy* 73:859-865.

Putman BJ, Clark RW. 2015. The fear of unseen predators: ground squirrels tail flagging in the absence of snakes signals vigilance. *Behavioral Ecology* 26:185-193.

Rankin JW, Doney KM, McGowan CP. 2018. Functional capacity of kangaroo rat hindlimbs: adaptations for locomotor performance. *Journal of the Royal Society Interface* 15: 21080303.

Schwaner MJ, Lin DC, McGowan CP. 2018. Jumping mechanics of desert kangaroo rats. *Journal of Experimental Biology* 221: jeb186700.

Shifferman E, Eilam D. 2004. Movement and direction of movement of a simulated prey affect the success rate in barn owl *Tyto alba* attack. *Journal of Avian Biology* 35:111-116.

Strobbe F, McPeek MA, de Block M, de Meester L, Stoks R. 2009. Survival selection on escape performance and its underlying phenotypic traits: a case of many-to-one mapping. *Journal of Evolutionary Biology* 22:1172-1182.

Voorhies MR. 1975. A new genus and species of fossil kangaroo rat and its burrow. *Journal of Mammalogy* 56:160-176.

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. 1971. Adaptive value of hearing and vision in kangaroo rat predator avoidance. *Brain, Behavior, and Evolution* 4:310-322.

Webster DB, Webster M. 1980. Morphological adaptations of the ear in the rodent family Heteromyidae. *American Zoologist* 20:247-254.

Whitford MD, Freymiller GA, Higham TE, Clark RW. 2019. Determinants of predation success: how to survive an attack from a rattlesnake. *Functional Ecology* 33:1099-1109.

Wu S, Zhang F, Edwards SV, Wu W, Ye J, Bi S, Ni X, Quan C, Meng J, Organ CL. 2014. The evolution of bipedalism in jerboas (rodentia: Dipodoidea): origin in humid and forested environments. *Evolution* 68:2108-2118.

Figures and Tables

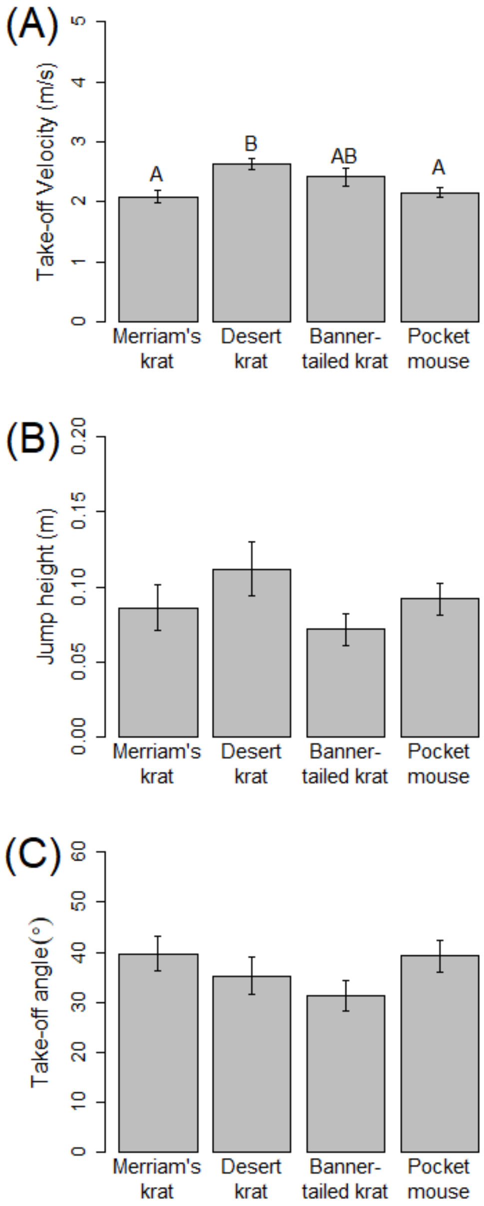

Figure 4.1 Jump performance comparisons among heteromyid rodents in take-off velocity (A), jump height (B), and take-off angle (C). Species were only significantly different from one another in take-off velocity; all species made low, relatively horizontal jumps. Errors bars show standard error, and letters show significant differences (if applicable).

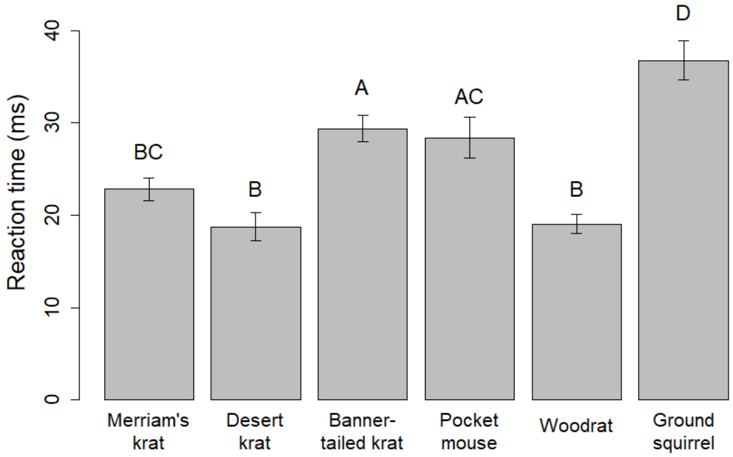

Figure 4.2 Comparison of reaction time among rodent species. Diurnal ground squirrels had the overall slowest reaction time. Interestingly, average woodrat reaction time was faster than many of the heteromyid species. Error bars show standard error, and letters show significant differences.

Figure 4.3 Comparison of body displacement time among rodent species. Non-heteromyid rodent species had significantly slower displacement times than heteromyid species, largely due to the need to reorient and turn before escaping. Error bars show standard error, and letters show significant differences.

Table 4.1 Video examples of rodent escape maneuvers.

Description	Video link
Video 1: examples of rodent jumps	https://youtu.be/j38-ZygU7MM
Video 2: examples of rodent scrambles	https://youtu.be/BZTaqe1G1PQ
Video 3: examples of kangaroo rats and pocket mice escaping rattlesnake strikes	https://youtu.be/piNuJHAM8FU

Table 4.2 Summary statistics and sample sizes of jump variables for each species. Woodrats (NEAL) and ground squirrels (OTBE) did not have jump kinematic metrics because they rarely jumped. Values are mean ± standard error.

	Mass (g)	Reaction time (ms)	Body displacement time (ms)	Take-off velocity (m s^{-1})	Jump height (cm)	Take-off angle (°)
DIME	40 ± 1	22.8 ± 1.2 (*n* = 39)	103.1 ±8.3 (*n* = 38)	2.1 ± 0.1 (*n* = 29)	8.6 ± 1.5 (*n* = 29)	39.7 ± 3.4 (*n* = 29)
DIDE	89 ± 5	18.8 ± 1.5 (*n* = 24)	77.8 ± 6.0 (*n* = 24)	2.6 ± 0.1 (*n* = 25)	11.2 ± 1.8 (*n* = 25)	35.3 ± 3.8 (*n* = 25)
DISP	116 ± 3	29.4 ± 1.4 (*n* = 24)	101.9 ± 13.5 (*n* = 20)	2.4 ± 0.1 (*n* = 27)	7.2 ± 1.1 (*n* = 27)	31.6 ± 3.1 (*n* = 27)
CHPE	18 ± 1	28.4 ± 2.2 (*n* = 21)	58.2 ± 10.5 (*n* = 20)	2.2 ± 0.1 (*n* = 19)	9.2 ± 1.1 (*n* = 19)	39.2 ± 3.2 (*n* = 19)
NEAL	177 ± 21	19.1 ± 1.0 (*n* = 13)	152.5 ± 12.3 (*n* = 13)	--	--	--
OTBE	537 ± 37	36.8 ± 2.1 (*n* = 21)	157.7 ± 12.2 (*n* = 19)	--	--	--

CONCLUSIONS

Kangaroo rats are capable of incredible feats of performance both at the whole-animal level and the sensory level. They have some of the quickest mammalian reaction times observed thus far, which aids in their remarkable ability to evade predators. Additionally, their ability to quickly leap vertically into the air helps them narrowly avoid rattlesnake strikes, and even if they are bitten, they can use their large hindlimbs to kick the snakes away. The primary selective pressure on kangaroo rat jump performance likely stems from ambush predators that rely on sudden, rapid attacks to capture prey in an instant. Kangaroo rats do not appear to rely on these extreme jumps as a general startle response, and they haven't been observed to jump away from cursorial predators. We were only able to elicit extreme jumps from kangaroo rats after exposing them to a rattlesnake, suggesting that the jump performance observed in natural interactions with predators is only utilized when the perceived danger is relatively high. Furthermore, this modulation of jump performance highlights the plasticity in their response and the ability for environmental factors to alter animal performance, a consideration that is often lacking in laboratory-based studies.

Given that ambush predators place such high pressure on the jump performance of kangaroo rats, their morphology has evolved to favor acceleration rather jump distance. The muscles of bigger kangaroo rat species are disproportionately large, allowing them to accelerate more quickly than if their muscles maintained the same geometric proportions as the smaller species. Comparisons of jump performance confirmed this: average acceleration is maintained across a range of body sizes. That said, the most important aspect of the bipedal morphology is that it allows these rodents to utilize rapid jumps more easily than quadrupedal rodents. In comparisons with quadrupeds, kangaroo rats tend to jump more often, and this results in a faster movement of the body out of the attack trajectory of a predator. The only exception to this finding is that pocket mice, which are in the same family as kangaroo rats (Heteromyidae) and share basic anatomical features with them, also tended to jump more frequently than other non-heteromyid quadrupeds. This appears to confer a greater antipredator strategy, as heteromyid rodents are far less likely to be captured by predators. Thus, enlarged hindlimbs, a feature which is exaggerated in kangaroo rats, appears to enhance predator evasion via rapid, vertical jumps.

Future studies comparing the performance of the other three groups of bipedal rodents would bolster the findings of this dissertation in several ways. First, utilizing a comparison of each bipedal group with sympatric quadrupedal rodents would create a stronger study design in which to explore the function of bipedalism in rodents. One would then have four pairs of lineages, each pair having a bipedal and a quadrupedal line, which increases the sample size . Given that bipedalism evolved independently in each lineage, so the evolution of this trait occurred presumably under different circumstances, it is difficult to extrapolate the findings from one group of bipedal rodent to all the others. Second, integrating more species would also permit the incorporation of phylogenetic comparative methods (PCMs). PCMs are useful tools not only for increasing statistical power, but for addressing hypotheses regarding trait evolution that cannot be addressed otherwise. While the analyses presented in this dissertation lend support to the hypothesis that bipedalism is an adaptation for vertical jumping during predator escapes, they lack the ability to consider the evolutionary histories of the species. PCMs would allow us to better understand the factors which drove the evolution of bipedalism in rodents and how this trait has evolved in the different lineages.